

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS QUÍMICAS



TESIS DOCTORAL

**Funcionalización de superficie de aleaciones de magnesio para
implantes biodegradables**

**Surface functionalisation of magnesium alloys for
biodegradable implants**

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Lara Moreno Turiégano

Directores

Endzhe Matykina
Juan Rodríguez Hernández

Madrid

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***“Suelta la necesidad de control,
Salta a pesar del miedo,
Confía en tus valores”***

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- ❖ **L. Moreno**, M. Mohedano, B. Mingo, R. Arrabal, E. Matykina. Degradation behavior of Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys with bioactive plasma electrolytic oxidation coatings. *Coatings*, 2019, 9(6), 383.
- ❖ **L. Moreno**, K. A. Yasakau, C. Blawert, R. Arrabal, E. Matykina M. Mohedano. As-cast and extruded Mg-Zn-Ca systems for biodegradable implants: characterization and corrosion behaviour. *Journal of Magnesium and Alloys*, 2022. *Submitted*

Chapter V

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- ❖ E. Wierzbicka, B. Vaghefinazari, S.V. Lamaka, M.L. Zheludkevich, M. Mohedano, **L. Moreno**, P. Visser, A. Rodriguez, J. Velasco, R. Arrabal, E. Matykina. Flash-PEO as an alternative to chromate conversion coatings for corrosion protection of Mg alloy. *Corrosion Science*, 2021, 180, 109189.

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ABSTRACT

The most important challenge for tissue engineering is the design and fabrication of biomaterials that fulfil specific functions according to the needs of society. In the Introduction of this Doctoral Thesis emphasis is placed on the literature review of magnesium based biomaterials in terms of their corrosion behavior under physiological conditions similar to those of the human body. Both commercially available materials and materials under development for application in temporary orthopaedic and cardiovascular implants are reviewed. The main limitation of magnesium based alloys is their low corrosion resistance under physiological conditions leading to premature implant failure.

Surface modification of temporary implants is a strategy that enables modulation of surface composition and topography as well as the implant degradation rate according to specific needs. A bibliographic study has been carried out focussing on the advances in hybrid biomaterials achieved through combination of ceramic and polymeric layers, revealing their potential for controlled drug delivery and improved biocompatibility of the implant. The literature review revealed the challenge that loaded drugs can present for the degradation rate of magnesium alloy-based implants. The inhibitory or accelerating effect of the drugs on corrosion behavior of the system is currently overlooked.

In this context, the aim of this Thesis is the design of hybrid coating systems that can be multifunctionalized and improve the original characteristics of the alloy. The main challenge is to develop corrosion resistant coatings combining different surface treatments for Mg alloys with biocompatible alloying elements (Ca, Zn, Ag). Subsequently, the hybrid coating system is optimized, drug-functionalized and the influence of the drug on corrosion resistance of the system is evaluated.

In Chapter IV, Mg-Ca, Mg-Ca-Ag and Mg-Zn-Ca alloy systems processed by casting and/or extrusion were characterised in terms of microstructure and surface potential evolution. A significant effect of the alloying elements, the manufacturing process, and, most importantly, the presence of impurities on the formation of the microstructure, and subsequently on the corrosion mechanisms of the alloys is disclosed. In addition, a reliable routine to screen the short term corrosion behavior of different Mg-Zn-Ca alloys in appropriate physiological medium by means of electrochemical impedance spectroscopy was proposed. The presence of organic compounds in physiological medium was disclosed to reduce the corrosion resistance compared to the medium with

only inorganic compounds. The influence of pH on the degradation resistance of the alloys was also evaluated using two different pH control strategies. The pH control by CO₂ flow, approximating *in vivo* conditions, provided a more realistic idea of the degradation mechanism of the Mg-Zn-Ca alloys.

In Chapter V, different ceramic coatings produced by plasma electrolytic oxidation (PEO) were studied in order to control the degradation rate of the coatings by incorporating chemical species such as Si, P, Ca and/or F from suspension-based and true solution (transparent) electrolytes. Electrolytes with high and low fluoride content were explored in terms of corrosion resistance of the coatings. All fluoride-containing coatings revealed a low corrosion protection due to the easy permeability of the coatings and the low protective capacity of the barrier layer of the coating. In turn, the Mg-Zn-Ca alloys presented problems associated with the presence of Zn-rich partially oxidized particles embedded in the PEO coating, and the formation of crevice at the interface between the PEO coating and the substrate, leading to a more aggressive corrosion environment, and therefore a higher degradation rate of the systems. Among the studied systems, the fluoride-free coating formed in suspension electrolyte on Mg₃Zn_{0.4}Ca alloy revealed a clear improvement of the corrosion resistance due to its lesser permeability and a more protective barrier layer. This coating was selected for further development of hybrid hierarchical coatings systems.

In Chapter VI, hybrid hierarchical coating systems comprising a ceramic PEO layer and a homogeneous polymer top-coat (PLA or PCL) with porous structure were developed using the Breath Figures and Dip coating techniques. Different process parameters were studied in order to evaluate their influence on porosity, pore size, and the thickness of the polymer layer. Following the process parameters optimization, the corrosion behavior of the hybrid systems was evaluated. An improvement in the corrosion resistance of the hybrid material was evidenced for both PLA and PCL top-coats, yielding 10 times greater corrosion resistance in comparison to the stand-alone ceramic coating and the substrate material.

In Chapter VII, the hybrid hierarchical material was functionalised with anti-inflammatory and antibacterial agents incorporated in the porous polymer layer following a preliminary screening of corrosion inhibiting effect of the drugs with respect to Mg alloy. In the first section of the chapter, a proof of drug carrier concept was carried out using a stand-alone polymer film in order to evaluate the influence of drug incorporation

on the porosity, pore size and composition of the polymer layer. For these systems, the drug release was also evaluated. As a result, the optimal top-coat configuration loaded with ciprofloxacin or paracetamol was implemented in the real hybrid hierarchical system evaluating its drug release capacity and degradation rate. It was determined that the incorporation of ciprofloxacin led to two orders of magnitude higher corrosion resistance compared to the non-loaded hybrid system. Paracetamol-loaded system exhibited corrosion acceleration by five orders of magnitude. The results demonstrate that corrosion screening of clinically relevant drugs can help to select an adequate candidate in order to control the degradation rate of the temporary Mg-based implant at the desired level.

The results in Chapters IV to VI revealed the key steps to the selection of different elements of the hybrid material with enhanced corrosion resistance. In Chapter VII, the results demonstrated the suitability of the hybrid hierarchical coating systems for controlled drug delivery as well as the possibility of enhanced degradation rate control via the selected drug. Therefore, the results of this Thesis offer a viable multifunctionalisation route for Mg alloy-based biomaterials for potential applications in temporary orthopaedic and cardiovascular implants.

RESUMEN

El reto más importante para la ingeniería de tejidos es el diseño y fabricación de biomateriales que cumplan las funciones específicas según las necesidades de la sociedad. En la Introducción de esta Tesis Doctoral se enfatiza en la revisión bibliográfica de los biomateriales de base magnesio en términos de su comportamiento a la corrosión en condiciones fisiológicas similares a las del cuerpo humano. Se revisan tanto los materiales comercializados como los materiales en desarrollo para su aplicación en implantes temporales ortopédicos y cardiovasculares. La principal limitación de estas aleaciones de base magnesio es su baja resistencia a la corrosión en condiciones fisiológicas dando lugar a un fallo prematuro del implante.

La modificación superficial de estos implantes es una estrategia interesante que permite modular la composición y la topografía de la superficie, así como la velocidad de degradación del implante según las necesidades específicas buscadas. Se ha realizado un estudio bibliográfico centrado en los avances en biomateriales híbridos mediante la combinación de capas cerámicas y poliméricas, revelando su potencial para la administración controlada de fármacos y la mejora de la biocompatibilidad del implante. La revisión bibliográfica reveló que los fármacos cargados pueden presentar un desafío para la velocidad de degradación de los implantes basados en aleaciones de magnesio. El efecto inhibidor o acelerador de los fármacos sobre el comportamiento de la corrosión del sistema es actualmente ignorado.

El objetivo de esta Tesis es el diseño de sistemas de recubrimiento híbridos que puedan ser multifuncionales y mejorar las características originales de la aleación. El principal reto es desarrollar recubrimientos resistentes a la corrosión combinando diferentes tratamientos superficiales para aleaciones de Mg con elementos de aleación biocompatibles (Ca, Zn, Ag). Posteriormente, se optimiza el sistema de recubrimiento híbrido funcionalizándolo con fármacos y evaluando la influencia del fármaco en la resistencia a la corrosión del sistema.

En el capítulo IV, se caracterizaron los sistemas de aleación Mg-Ca, Mg-Ca-Ag y Mg-Zn-Ca procesados por colada y/o extrusión en términos de microestructura y evolución del potencial superficial. Se revela un efecto significativo de los elementos de aleación, el proceso de fabricación y, sobre todo, la presencia de impurezas en la formación de la microestructura, y posteriormente en los mecanismos de corrosión de las aleaciones.

Además, se propuso una sistema fiable para examinar el comportamiento a la corrosión a corto plazo de las diferentes aleaciones de Mg-Zn-Ca en un medio fisiológico adecuado mediante espectroscopia de impedancia electroquímica. Se reveló que la presencia de compuestos orgánicos en el medio fisiológico reduce la resistencia a la corrosión en comparación con el medio que sólo contiene compuestos inorgánicos. También se evaluó la influencia del pH en la resistencia a la degradación de las aleaciones utilizando dos estrategias diferentes de control del pH. El control del pH mediante flujo de CO₂, que se aproxima a las condiciones *in vivo*, proporcionó una idea más realista del mecanismo de degradación de las aleaciones Mg-Zn-Ca.

En el capítulo V, se estudiaron diferentes recubrimientos cerámicos producidos por oxidación electrolítica por plasma (PEO) con el fin de controlar la velocidad de degradación de los recubrimientos mediante la incorporación de especies químicas como Si, P, Ca y/o F de electrolitos en suspensión y en solución verdadera (transparente). Se exploraron electrolitos con alto y bajo contenido en flúor en términos de resistencia a la corrosión de los recubrimientos. Todos los recubrimientos que contenían flúor revelaron una baja protección contra la corrosión debido a la fácil permeabilidad de los recubrimientos y a la baja capacidad de protección de la capa de barrera del recubrimiento. Por su parte, las aleaciones de Mg-Zn-Ca presentaron problemas asociados a la presencia de partículas parcialmente oxidadas ricas en Zn incrustadas en el recubrimiento de PEO, y a la formación de grietas en la interfase entre el recubrimiento de PEO y el sustrato, lo que provocó un entorno de corrosión más agresivo, y por tanto una mayor velocidad de degradación de los sistemas. Entre los sistemas estudiados, el recubrimiento sin flúor formado en electrolito en suspensión sobre la aleación Mg₃Zn_{0.4}Ca reveló una clara mejora de la resistencia a la corrosión debido a su menor permeabilidad y a una capa de barrera más protectora. Este recubrimiento fue seleccionado para el desarrollo posterior de sistemas de recubrimientos jerárquicos híbridos.

En el capítulo VI, se desarrollaron sistemas de recubrimiento jerárquico híbrido que comprendían una capa cerámica de PEO y una capa superior de polímero homogéneo (PLA o PCL) con estructura porosa, utilizando las técnicas de recubrimiento por aliento y por inmersión. Se estudiaron diferentes parámetros del proceso para evaluar su influencia en la porosidad, el tamaño de los poros y el espesor de la capa polimérica. Tras la optimización de los parámetros del proceso, se evaluó el comportamiento a la corrosión

de los sistemas híbridos. Se evidenció una mejora en la resistencia a la corrosión del material híbrido tanto para las capas superiores de PLA como de PCL, obteniendo una resistencia a la corrosión 10 veces mayor en comparación con el recubrimiento cerámico y el sustrato.

En el capítulo VII, se funcionalizó el sistema híbrido jerárquico con agentes antiinflamatorios y antibacterianos incorporados en la capa polimérica porosa, tras un estudio preliminar del efecto inhibitor de la corrosión de los fármacos con respecto a la aleación de Mg. En la primera sección del capítulo, se llevó a cabo una prueba del concepto de portador de fármacos utilizando una película polimérica independiente para evaluar la influencia de la incorporación del fármaco en la porosidad, el tamaño de los poros y la composición de la capa polimérica. Para estos sistemas, también se evaluó la liberación del fármaco. Como resultado, la configuración óptima de la capa superior cargada con ciprofloxacino o paracetamol se implementó en el sistema híbrido jerárquico real evaluando su capacidad de liberación de fármaco y su velocidad de degradación. Se determinó que la incorporación de ciprofloxacino conducía a una resistencia a la corrosión de dos órdenes de magnitud mayor en comparación con el sistema híbrido sin cargar. El sistema cargado con paracetamol mostró una aceleración de la corrosión de cinco órdenes de magnitud. Los resultados demuestran que el cribado de la corrosión de fármacos clínicamente relevantes puede ayudar a seleccionar un candidato adecuado para controlar la velocidad de degradación del implante temporal basado en Mg al nivel deseado.

Los resultados de los capítulos IV a VI revelaron los pasos clave para la selección de diferentes elementos del material híbrido con mayor resistencia a la corrosión. En el Capítulo VII, los resultados demostraron la idoneidad de los sistemas de recubrimiento híbrido jerárquico para la administración controlada de fármacos, así como la posibilidad de mejorar el control de la velocidad de degradación a través del fármaco seleccionado. Por lo tanto, los resultados de esta Tesis ofrecen una ruta viable de multifuncionalización para los biomateriales basados en aleaciones de Mg para posibles aplicaciones en implantes temporales ortopédicos y cardiovasculares.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

Mg alloys		Techniques and parameters	
Mg-2Al-RE	AE21	Alternative current	AC
Mg-4.9Al-0.45Mn-0.2Zn	AM50	Backscattered electron scanning	BSE
Mg-2.5-3.5Al-0.6-1.40Zn-0.2Mn	AZ31	Secondary electron	SE
Mg-0.4-1.5Zn-0.15Mn-0.1Si	AZ61	Corrosion potential	E _{corr}
Mg-8.3/9.7Al	AZ91D	Current density	I _{corr}
Mg-2.2Nd-0.1Zn-0.4Zr	JDBM	Direct current	DC
Mg-4Li-3.6Al-2.4RE	LAE442	Electrochemical impedance spectroscopy	EIS
Mg-1.7-4.1Y-6%RE-0.4Zr	WE42	Energy dispersive X-ray spectroscopy	EDS
Mg-Y-Nd	WE43	Inductively Coupled Plasma	ICP
Mg4.75-5.55Y-1.5-2Nd-1-2RE0.4Zr	WE54	Open circuit potential	OCP
Mg-1Zn-0.1Zr-0.1RE	ZEK100	Plasma electrolytic oxidation	PEO
Mg-4Zn-RE-Nd	ZE41	Scanning electron microscopy	SEM
Physiological solutions		Scanning Kelvin probe force microscopy	SKPFM
Dulbecco's Modified Eagle's Medium Modified	DMEM	X-ray diffraction	XRD
Fetal bovine serum	FBS	Polymers	
Hank's solution	HBSS	Chitosan	CS
Eagle's Medium	MEM	Layer By Layer	LBL
Simulated body fluid	SBF	Poly (D-, L-lactic) acid	PDLLA
Phosphate buffered saline	PBS	Poly (lactic) acid	PLA
Others		Poly (lactic-co glycolic) acid	PLGA
Bisphosphonate	BP	Poly (l-lactic) acid	PLLA
Calcium phosphate cements	CPC	Poly (vynil acetate)	PVA
Ciprofloxacin	CIP	Poly(adipic anhydride)	PADA
Drug eluting stent	DES	Poly(methyl methacrylate)	PMMA
Foreign body reaction	FBR	Polycaprolactone	PCL
Hybrid Hierarchical Coating	HHC	Polydimethylsiloxane	PDMS
Hydroxyapatite	HA	Polydopamine	PDA
Mesoporous silica nanoparticles	MSNS	Polyethyleneglycol	PEG

Ordered mesoporous	OMMS	Polypropylene fumarate	PPF
magnesium silicate			
Paracetamol	PAR	Polypropyleneimine	PPI
Proteins		Polytetrafluoroethylene	SPTFE
Bovine serum albumin	BSA	Polyurethane	PU
Betamethasone sodium phosphate	BSP-2	Sodium polyacrylate	SPA
Bone morphogenetic proteins	BMP's	Zwitterions	ZPU-12
Epithelial growth factor	EGF		
Fibroblast growth factor	FGF		
Insulin-like GF-I	IGF-I		
Platelet derived GF	PDGF		
Platelet-derived growth factor	PDGF		
Recombinant human bone morphogenetic protein	Rhbmp		
Transforming growth factor beta	TGF- β		
Vascular endothelial GF	VEGF		

1. CHAPTER I- State of the art: Literature review

1.1 Magnesium as biomaterial

1.1.1 Biodegradable materials for temporary implants

Biological materials are used to replace or repair the fractures in humans and improve the quality of humans life. The idea of biodegradable implants was introduced by Kulkarni *et al.* in the late 1960s in search of an alternative materials for functional restoration of damaged bone tissues and healing of fractures without the need for removal of the implant via second surgery. But it was in 1984 when Rokkanto *et. al* used the first biodegradable material for the elaboration of orthopaedic implants. Over the past three decades, the number of biodegradable implants in clinical practice and research has been growing significantly ¹.

Temporary implants are used for a precise period of time; for instance, in orthopaedics, the fractured bone has to re-grow or overcome deformation ^{2,3}. The main advantage of a temporary implant is that no surgery is needed for its removal after the healing process is completed. However, when the fractures are severe and occur in the orthopaedic joints such as hip, knee, spinal cord, shoulder and ankle, it is necessary to use a permanent implant during indefinite periods of time ². Despite this, common still unresolved problems of permanent implants include physical irritation, inflammatory local reactions, thrombogenicity and endothelial dysfunction.

Patients with heart and blood vessel diseases need cardiovascular stents which are also temporary implants ². There are also non-degradable stents, but these stents can cause restenosis which leads to vessel remodelling, a decreased cellularization and an increased extracellular matrix accumulation, among other problems such as physical irritation, long-term endothelial dysfunction/vascularization, inflammatory reaction etc. These problems can be resolved using biodegradable implants.

Polymer implants such as poly(glycol acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolide) (PLGA), collagen, cellulose or silicone have been used in a great number of tissue engineering applications due their unique properties such as lightweight, ductility, biocompatibility and biodegradability. Another reason to use these materials is that they degrade forming non-toxic products that are excreted in the urine ⁴, and their degradation rate is slow and can be controlled. However, a major limitation of

these materials is their low mechanical strength; for this reason, they are used in soft tissue reconstruction and not in low load bearing applications.

Ceramic implants, typically hydroxyapatite (HA) and/or tricalcium phosphate based bone allografts, are considered a promising alternative solution for natural bone repairs, since they present characteristics similar to those of the bone in regards to osteoconduction and mechanical properties ⁵. Due to their porous structure, the bone growth happens on the surface of a ceramic implant via interaction with the pores favouring tissue ingrowth and supplying a way for transportation of nutrients. However, the main limitation of these materials are their brittleness and poor tensile strength ⁶.

Metallic implants are used in load bearing applications where their high mechanical strength and fracture toughness makes them more suitable in comparison to polymers, ceramics and composite materials. The most extensively employed metallic implant materials include stainless steel, cobalt-chrome alloys, titanium and titanium alloys. In case of steel and the cobalt-chrome alloys, their elastic modulus are ten times greater than the bone while the modulus of titanium is five times greater than that of the bone ⁴. The problem associated with the mismatch between the mechanical properties of the implant and the surrounding natural bone tissue is the phenomenon known as stress shielding. Stress shielding occurs when the implant supports most of the load and the surrounding bone tissue supports a reduced load ⁷, which may lead to retarded bone regeneration or bone atrophy. Another problem of metallic implants is that excessive metal ion release from some alloying elements (e.g. Al, V, Co, Cr, RE) into the body, which can cause an inflammatory response of the immune system and the surrounding tissue, reducing the biocompatibility of the implant ⁴.

Among the above mentioned metallic materials, Mg is a metallic element abundant in the human body; a major part of Mg is stored in bone tissues as Mg^{2+} . Therefore, Mg is considered a suitable biomaterial for tissue regeneration because it already participates in human body metabolism. It takes part in many enzymatic reactions as a cofactor, in the reactions involving adenosine triphosphate (ATP), in proteins and nucleoid synthesis, in mitochondrial activity and integrity, in stabilization of the DNA and RNA structures, in ion channel modulation, plasma membrane stabilization, translational processes and other cellular functions ^{2,8}.

Comparison of the mechanical properties of pure Mg with those for others metals **Table 1** shows that with a density of 1.74-2.0 g/cm³ Mg is the lightest metallic biomaterial and its elastic modulus is the closest to that of the bone ⁹. These properties make Mg an appropriate choice of a biodegradable material for orthopaedic implants ^{2,3}.

Table 1. Mechanical properties of different materials for biomedical applications adapted from ⁹.

Properties	Natural bone	Mg	Ti alloys	Stainless Steel	Co-Cr alloys
Density (g/cm ³)	1.8-2.1	1.74-2.0	4.4-4.5	7.9-8.1	8.3-9.2
Elastic modulus (GPa)	3-20	41-45	110-117	189-205	230
Compressive yield (MPa)	130-180	65	758-1117	170-310	450-1000
Fracture toughness (MPam ^{1/2})	3-6	100	55-115	50-200	N/A

However, the principal problem of commercially pure Mg is its high corrosion rate in aqueous environment reducing the mechanical integrity of the implant. This problem can be overcome by the use of (i) biocompatible alloying elements, which additionally can adjust the mechanical properties to those of cortical bone and reduce the negative effect of stress-shielding, and (ii) surface modification of the implant ^{4,10,11}.

1.1.2 Commercial Mg implants

A brief history of commercial biodegradable implants based on Mg alloys included the following milestones (**Figure 1**).

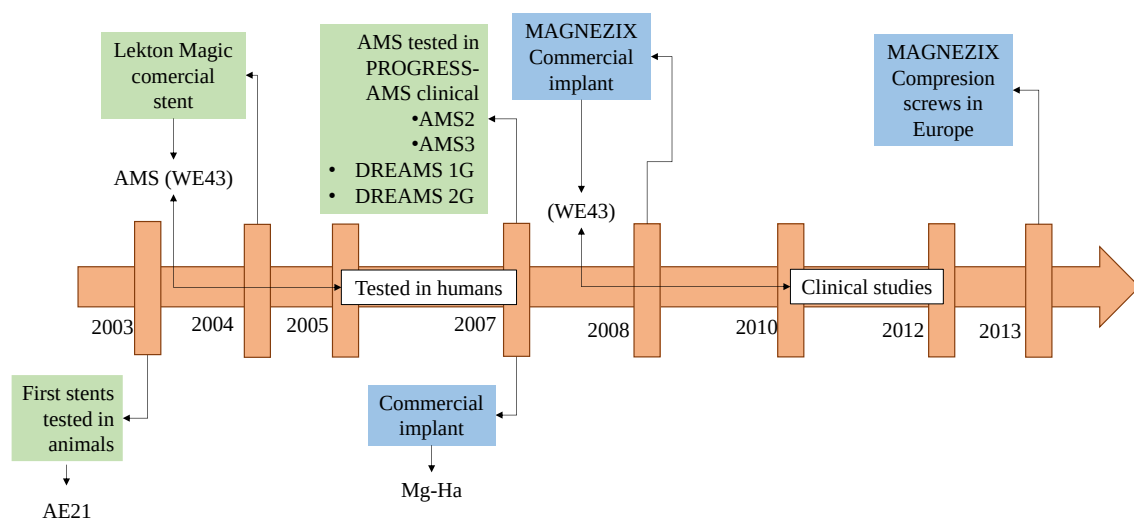


Figure 1. Chronological evolution of stent and orthopaedic implants.

In 2003, Heublein *et al.* were pioneers working on an animal model using stents made of AE21 Mg alloy; their results showed negligible inflammatory response up to 56 days¹². In 2004, Di Mario *et al.* evaluated The Lekton Magic (by BIOTRONIK company) stent made of Mg (93 % Mg and 7 % RE) WE43 alloy, which was the first version of the commercial absorbable metallic stent (AMS1)¹³. This stent presented good mechanical properties, demonstrated a fast endothelialization and positive remodelling.

Also, there was a complete resorption after 5 months but some contraction of stents was observed due to their rapid degradation^{11,14}. After 4 months, the AMS presented restenosis due to negative remodelling and early recoil. Between 2005 and 2007 the implant was tested on humans and, more precisely, in 2007 was tested in a multicenter PROGRESS-AMS clinical trial. These trials uncovered the principal problem of these stents, a complete resorption of the stents at 4 months and a vessel recoil because of the radial strength loss. In order to overcome the radial strength loss, a new AMS2 implant was developed which was the same as the AMS1 but the section of WE43 alloy was square and was refined. After some time, the AMS3 was developed, this version was the same as the AMS2 but coated with a poly(lactic-co-glycolic) acid (PLGA) matrix imbued with paclitaxel drug¹⁵. Later, AMS3 scaffolds were renamed as the DRugEluting AMS first generation (DREAMS 1G) which was tested in BIOSOLVE-I clinical trial and was implanted in 46 patients. DREAMS 1G evolved into DREAMS 2G, a scaffold where polymer matrix was changed from PLGA to poly(L-lactic) acid (PLLA), which was used in cancer therapy^{16,17}. These scaffolds were tested in BIOSOLVE-II and was implanted in 123 patients¹⁸. In June 2016, Magmaris BRS (known as DREAM 2G) received CE approval in Europe as the first bioresorbible metallic scaffold on a rapid-exchange delivery system improving the previous paclitaxel-eluting DREAMS platform¹³.

Velox CD (Transluminal Technologies LLC, Syracuse, USA) is another type of stent made of magnesium alloy (containing magnesium, aluminium and iron) which has been tested in 42 patients. However, this stent was found to dissolve after 24 h and the extracellular part is dissolved after 2 weeks¹⁴.

In 2007, Manfré *et al.* presented a new implant for vertebral compression fracture, with or without trauma. This implant was made of magnesium and hydroxyapatite (Mg-HA). Some years after, MaioRegen (Fin-Ceramica Faenza S.p.A., Faenza, Italy) designed a scaffold composite with collagen (Col) and Mg-Ha which was used to promote tissue

regeneration in case of large and severe chondral/osteochondral lesion ¹⁴. These scaffolds were investigated in 11 European centres with 150 patients.

In 2008, a MgYREZr implant was developed (MAGNEZIX[®]) with a composition similar to that of WE43 alloy but with a 90 wt. % magnesium, instead of 93 wt. %. Between 2010 and 2012 clinical studies were performed, showing good biocompatibility, non-toxicity and osteoconducting properties ^{19,20}. In 2013 these implants were marketed as compression screws in Europe. They are used in reconstructive procedures after fractures, malposition and other pathological bone alterations of the human skeleton, in small bone and joints avulsion fractures, osteochondrosis dissecans and intra-articular and extra-articular fractures.

Recently, Lin *et al.* developed new biodegradable Mg-Nd-Zn-Zr-based alloys (JDBM) for cardiovascular stents and orthopaedic implants at Shanghai Jiao Tong University ²¹. This material has not been commercialized yet but were tested both *in vitro* and *in vivo* ^{22,23}. In addition, Li *et al.* evaluated the vascular biocompatibility and cytotoxicity of JDDB and WE43 stents *in vitro* with human umbilical vein endothelial cells (HUVECs). The results obtained demonstrated that the JDDB offers a more favourable surface for endothelial cell adhesion and spreading of HUVECs ²⁴ than WE43 alloy. Also, the alloy presented an improved of mechanical durability and long-term biocompatibility as no occlusions and neointimal formation were found in the stent.

It is evident that currently the use of commercialized Mg implants is limited to very narrow applications, which justifies the need for further development and functionalization of Mg-based implants in order to improve their corrosion resistance and compatibility with a wide range of tissues.

In the past years, a range of Mg alloy systems had been studied in regard to their suitability for biomedical applications such as Mg-Y, Mg-Zr, WE43, WE54, Mg-Zn, Mg-Zn-Mn, Mg-Bi-Ca and Mg-Zn-Ca, Mg-Zn-Ca-Pd. Recent studies are investigating others alloys such as Mg-Nd-Zn-Zr, Mg-Gd-Zn-Zr, MgNd₂, Mg-Dy, ZEK100, Mg-Sr, Mg-Zn-Sr and Mg-3Sc-3Y, LAE442, Mg-Nd-Zn, Mg-Ca, Mg-Zn ^{22,25} as possibilities for commercial implants.

1.1.3 Role of alloying elements in biomedical Mg alloys

The insufficient strength and high degradation rate of pure Mg ² is being resolved through refinement of the grain structures in order to improve the corrosion resistance, and formation of intermetallic phases that can improve the strength and assist in the manufacture of Mg alloys ^{4,26}.

There are a few considerations for element selection in developing biomedical grade Mg alloys. The first is related to the toxicity of the elements: the degradation products should be non-toxic and absorbable by human body ²⁷. Common alloying non-toxic elements for Mg in biomedical applications include Zn, Ca, Ag, Mn, Al, Rare earths (RE) or Li ¹⁰. The intermetallic compounds formed by these elements contribute to the improvement of mechanical properties and corrosion behavior. The main characteristics of these elements are summarized in **Table 2**.

As mentioned before, the corrosion products of alloys should be non-toxic, absorbable, dissolved by surrounding tissues and excreted ²⁸. However, the main commercial Mg alloys are constituted by Mg-Al (AM50 or AZ91D) ^{29,8} and Mg-RE systems (LAE442 or WE43) ^{22,13}, whose alloying elements are considered as toxic and/or allergic causing harmful effect on the human body ^{4,30,31}.

Nevertheless, Ca, Zn and Ag are of special interest as alloying elements since they are essential nutritional elements involved in numerous reactions in the human body. Ca plays an important role in body since bone is constituted by 99.5 % of calcium, phosphate and magnesium; also, the level of bone mass necessary to support the structure of the body is maintained by Ca. The formation of Mg-Ca intermetallic phases in Mg-Ca alloys helps the refinement of the microstructure and improves the strength and creep properties of Mg alloys.

Over 85 % of all Zn present in human body is contained in bones and muscles; Zn increases osteoblast cell proliferation and takes part in physiological functions such as immune system regulation, protein synthesis and many enzymatic reactions. Interestingly, the harmful corrosive effect of Ni and Fe impurities in Mg alloys is reduced by Zn ²⁸. In addition, Ag is an element in the human body that has excellent antibacterial activity, it is also effective in the treatment of some microbes reducing the probability of eventual nosocomial infections ^{34,13}.

Table 2. Alloying elements used in Mg alloys for biomedical applications adapted from ^{4,32}.

Alloying element	Pathophysiology	Toxicity	Mechanical properties	Daily allowance
Zn	12.4-17.2 $\mu\text{mol/L}$ Involved in physiological function as immune system regulation, protein synthesis etc.	High concentration is neurotoxic	Improves yield stress and elastic modulus similar to bone. Reduces hydrogen gas during corrosion	15 mg
Ca	0.9-0.993 mg/L Abundant in bones and teeth. Stabilizes and activates enzymes	Excessive Ca formation in the kidneys	Improves the corrosion resistance of binary alloys (Mg-Ca)	0.8-1.4 g
Ag	Antibacterial and antifungal agent	Accumulation of Ag causes damage on liver and kidney	Improve the mechanical strength and corrosion resistance	70-88 mg
Mn	<0.8 $\mu\text{g/L}$ Essential to immune system, bone growth, cellular function. Involved in metabolic of cycle of lipids	High concentrations cause neurological disorders	Reduces the harmful effect of impurities and corrosion resistance	4 mg
Al	2.1-4.88 $\mu\text{g/L}$	Can be neurotoxic and is linked to Alzheimer's disease	Acts as passivator and improves corrosion resistance	14 mg
RE	Some of them have anticancerogenic properties	Accumulate in bone and liver	Improve corrosion resistance	4.2 mg
Li	2-4 ng/g Used as medication	Overdose causes central nervous centre disorders and impaired kidney function	Improves corrosion resistance	14.3 $\mu\text{g/kg}$

In the last years, a great number of research groups have investigated binary Mg-Ca and Mg-Zn alloys ^{33,34,35}. The effect of different compositions of Ca (1-3 wt. %) on the increase of the elastic modulus, compressive strength, hardness, corrosion resistance and the decrease of the ductility were evaluated ³⁶. The increase of Ca up to 10 % in Mg-Ca alloys decreases the corrosion rate due to the presence of intermetallic phases, which causes brittleness of the alloy, while low concentrations of Ca (0.5 %) decrease the degradation rate and cytotoxicity of the alloy ³⁷. Mechanical and corrosion behavior were evaluated in cast, rolled and extruded alloys. Low corrosion rates were observed in extruded and rolled Mg-Ca alloys due to the reduction in the formation of Mg_2Ca phases ³⁴. In the case of Mg-Zn alloys, the evaluation of corrosion behavior as a function of different amounts of Zn evidenced that the corrosion resistance decreases with the increase of Zn ³⁸. Mg-Zn alloys support fibroblast cell viability and show no significant toxicity with respect to osteoblasts *in vitro* ³⁹, as well as no adverse effect *in vivo* ^{40,41}. In addition, the antibacterial effect of Mg-Ag alloys has been reported for a wide range of

the microbial spectrum ³⁴. However, high corrosion rate ³⁴ and cytotoxicity ⁴² were observed in these alloys. The cytotoxicity can be decreased if another secondary element, such as Ca, is included in the alloy, while still maintaining the antimicrobial effect ³⁴.

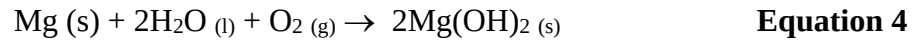
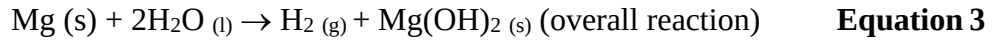
Ternary MgZn_xCa_y alloys were investigated in order to evaluate the effect of micro alloying, manufacturing, mechanical properties and corrosion behavior ^{43,44,45,46}. The addition of 1 % of Zn in these systems was found to increase the hardness and corrosion resistance while the addition of 3 % of Ca increases the volume of intermetallic phases causing higher corrosion rate due to the formation of more anode-cathode sites ^{47,48}. *In vitro* and *in vivo* studies have demonstrated that MgZn_xCa_y alloys present low degradation rate favouring slow hydrogen evolution and stable mechanical behavior, as well as an adequate biocompatibility and osteogenic potential ^{49,50,51}.

Not only the type and amount of alloying elements are crucial for the degradation behavior of Mg alloys; the fabrication process has also been reported to play an important role on the corrosion performance and the properties of Mg alloys ⁵². The majority of the reports of Mg alloys have been produced by conventional casting and extrusion because of the beneficial effects on both mechanical and corrosion properties of these alloys ^{53,54}. It is worth mentioning that casting is the most economical method for processing Mg-based biomaterials, because it is carried out in an inert gas atmosphere and can produce very complex geometries that can be designed directly in the mould for one-step manufacturing ^{28,55}. Although, the extrusion process produces materials with mechanical properties similar to those produced by casting, it has limitations in product size, as only one type of cross-section can be obtained at a time and requires a high cost for initial preparation.

In the last years, additive manufacturing (AM) processes for Mg alloys have been of great interest in the community because of the number of designing possibilities, not achievable by traditional manufacturing, the low cost, the low processing time, and the potential for the development of biodegradable implants. However, the AM of Mg has limitations due to the difficulty of preparing Mg powders and their flammability ⁵⁶. Taking into account this, Mg-Zn-Ca alloys prepared by extrusion and casting will be used in order to carry out the studies in this Thesis. It is expected that the obtained results and knowledge will be useful and applicable for implementation on Mg alloys produced by the additive manufacturing process.

1.2 Degradation mechanisms of Mg alloys - Overview

As above mentioned, the high corrosion rate of magnesium is the main limitation for its uses in orthopaedic and cardiovascular applications. The fast degradation rate is due to its low reduction potential (-2.37 V) and the presence of impurity-enriched secondary phases in the alloy microstructure that form microgalvanic couples ¹⁴. Superficial magnesium oxide (MgO) and magnesium hydroxide (Mg(OH)₂) layers formed when magnesium is in contact with physiological solutions are not protective enough, as Pilling-Bedworth ratio for MgO is 0.87. Therefore, the corrosion product layer is cracked. In addition, there are other factors in the human body such as pH, [Cl⁻], temperature of blood plasma and the presence of certain proteins that affect the corrosion mechanism of magnesium ⁵⁷. The corrosion of Mg in physiological environment can be expressed with the following equations:



The corrosion process of Mg in the body is a complex phenomenon and the main corrosion products are hydrogen gas and Mg(OH)₂. When Mg(OH)₂ is under standard environmental conditions, it forms a partially protective layer on the surface of Mg alloys. The quick Mg substrate dissolution that generates H₂ gas and OH⁻ ions ^{10,26,58}. In addition, there is an increasing evidence that oxygen reduction reaction (ORR) takes place in this process (**Equation 4**). This reaction is not reflected in the total cathodic corrosion process of Mg, although it can be used to compare the corrosion resistance of Mg alloys with similar degradation rates ^{59,60}. Rapid corrosion and generation of hydrogen gas increase the pH around the implant leading to necrosis of the surrounding tissue ¹⁰. Also, the buffering agents such as proteins and amino acids consume the generated OH⁻ and accelerate the corrosion mechanism, hence the implant could quickly lose the necessary mechanical integrity ²⁷. As mentioned before, the temperature is another parameter that can affect the corrosion mechanism ⁶¹. Abin *et al.* ⁶² evaluated the corrosion rate of pure Mg, ZE41, AZ91 and Mg2Zn0.2Mn at 37°C and 25°C in Hank's solution. The results showed that the corrosion rate of pure Mg and Mg2Zn0.2Mn were independent of the temperature but the corrosion rate of ZE41 and AZ91 were 6-9 times higher at 37°C than

25°C. This high corrosion rate at 37°C can be attributed to electrochemical activity between Mg and secondary intermetallic phase⁶³ and the migration and diffusion of aggressive chloride,⁶⁴ which is dependent on the temperature.

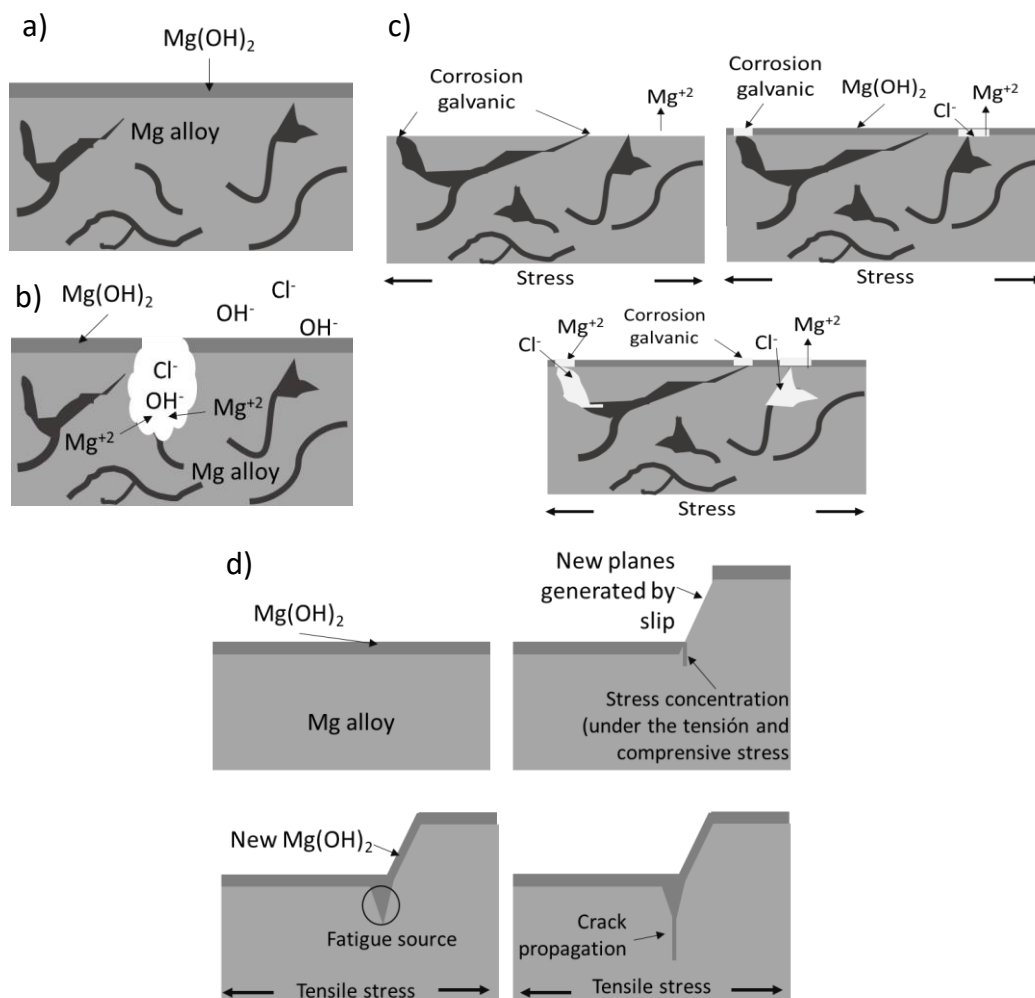


Figure 2. Scheme of different types of corrosion: (a) uniform, (b) pitting, (c) stress corrosion crack and (d) corrosion fatigue crack in body fluid adapted from^{4,65,64}.

Mg alloys may exhibit two main different corrosion modes: uniform and localized⁶⁶. The localized corrosion modes, such as pitting, stress corrosion cracking and corrosion fatigue are more dangerous as it may lead to cytotoxicity and early mechanical failure of the implant (**Figure 2**). The factors that determine the corrosion mode are the presence of impurities, secondary phases and precipitates in Mg alloys. If the secondary phase particles (that usually entrap the impurities) present in Mg alloys are more noble than Mg, they act as cathodes and the Mg matrix acts as an anode resulting in pitting at the sites of galvanic micro-couples². The most common impurities in Mg alloys are Ni, Fe and Cu. These impurities are cathodic with respect to the matrix² causing faster corrosion rate due to the formation of pitting with quickly destruction of the $Mg(OH)_2$ protective layer

⁶⁷. If this does not happen evenly, the material will corrode acting as precursor of stress corrosion cracking (SCC) and corrosion fatigue (CF). The SCC occur when an electrochemical potential is formed between stressed and unstressed regions of the implant under load, leading to the formation of cracks and concentrating stress. The crack grows through the grains and matrix by the strain rate resulting from the implant loading cycle and the presence of H₂ by the corrosion process. However, CF can occur by the presence of porous or pitting from corrosion forming crack nucleation. These cracks are propagated by the combination of cycling loading and the presence of chloride ions in the body fluids. Relation of corrosion and mechanical failure of biodegradable Mg implants in the human body environment are further discussed in detail in several reviews ^{4,68}.

1.3 Corrosion “*In vivo*” and “*In vitro*”

One of the most challenging tasks during the design and performance testing of biodegradable Mg alloys is the control of adequate degradation conditions in order to obtain corrosion rates closely representative to those in aqueous environment of human body ⁴. In order to evaluate the implant corrosion behavior, *in vitro* immersion tests in biological solutions simulating the human body conditions are used. However, many studies have observed that the results obtained *in vitro* are often not reflective of *in vivo* occurrences. This may be due to the fact that *in vitro* studies are influenced by variables such as surface area to volume ratio, temperature fluctuation and buffer selection ⁶⁹. The factors influencing corrosion *in vivo* are additionally complicated by physiological response of living tissue as well as by numerous organic and inorganic components of body fluids and fluid flow rates which make the prediction of corrosion behavior more difficult ⁵⁸. These difficulties are the reason why the majority of the carried out research avoids direct comparison of their *in vitro* results with the results *in vivo*. Some comparative examples of corrosion rates of Mg alloys for biomedical applications obtained *in vivo* and *in vitro* by immersion tests in different physiological solutions are given in **Table 3**.

It is evident from this comparison that the results *in vivo* and *in vitro* can differ by up to one order of magnitude. In most of the cases, corrosion rates *in vivo* tests are lower than *in vitro* test, which may be due to low amount of chloride in the blood plasma and bone ²⁷ and the presence of proteins that are known to decrease the corrosion rate ⁷⁰. The disparity in these values highlight the necessity of comparison between them and

adequate simulation of *in vitro* conditions because the *in vitro* methods should predict correctly what is going to happen in *in vivo* conditions to help an adequate material design and selection.

Table 3. Comparison of corrosion rate in *in vivo* and *in vitro* tests on Mg alloys.

Alloy	Days	Immersion Solution	Corrosion rate (mm/year)		Ref.
AZ31	7, 14, 21 days	Plasma, EBSS, MEM and MEMp	<i>In vivo</i>	1.37	69
			<i>In vitro</i> (MEMp)	1.19	
MgCa			<i>In vivo</i>	0.35	
			<i>In vitro</i> (MEMp)	0.76	
MgZn			<i>In vivo</i>	0.16	
			<i>In vitro</i> (MEMp)	0.82	
MgMn			<i>In vivo</i>	0.25	
			<i>In vitro</i> (MEMp)	0.50	
MgCaZn			<i>In vivo</i>	1.00	
			<i>In vitro</i> (MEMp)	10.04	
MgSr	1-4 weeks	Hank’s	<i>In vivo</i>	1.4	71
			<i>In vitro</i>	0.37	
MgSrCa			<i>In vivo</i>	1.1	
			<i>In vitro</i>	0.24	
MgSrZn			<i>In vivo</i>	0.85	
			<i>In vitro</i>	0.15	
MgZnCa	1, 3, 7, 14, 21 days	SBF	<i>In vivo</i>	2.87 (at week 4)	72
			<i>In vitro</i>	20.15 (at 21 days)	

1.3.1 Degradation *In vitro*

There are different methods to measure *in vitro* corrosion rate. Kirkland *et al.* classified these methods in unpolarized and polarized ones. The unpolarized methods consist in the immersion testing where released Mg ions, weight loss²² or hydrogen evolution^{10,73} are measured, while polarized methods include potentiodynamic polarization and electrochemical impedance spectroscopy. Both methods have one aspect in common which involves the immersion of the material into a solution. In both methods it is very important to take into account various factors such as the composition, pH and temperature of physiological solution^{73,74}, the size of the samples and the flow rate (which depends on the implantation site), because these factors can change the corrosion behavior.

The solution composition should be close to physiological conditions and the simulated body temperature should be at 37°C^{10,74}. Phosphate buffered saline (PBS), simulated body fluid (SBF), Hank's solution (HBSS), Ringer's etc are commonly used for *in vitro* corrosion screening⁷⁵. For the studies of accurate degradation behavior and the corrosion mechanisms involved, cell culture media with fetal bovine serum (FBS) such as Dulbecco's Modified Eagle's Medium (DMEM), Modified Eagle's Medium (MEM) and α -MEM, are recommended to simulate the *in vivo* conditions⁵⁷. For instance, Yunchang *et al.* evaluated the corrosion rate of AZ91 in different physiological solutions, including DMEM, SBF, Hank's solution, PBS and 0.9 % NaCl solutions. The DMEM presented the lowest corrosion rate due to the presence of high concentration of carbonates, which induce fast passivation at severe corrosion sites by rapid precipitation of insoluble carbonates⁷³.

Keeping constant the pH around the material is another critical parameter, since pH in blood flow is regulated by respiration via lungs, plasma protein buffers and a low concentration of phosphates. The increase of pH as a result of corrosion process (**Equation 2**) in the absence of a buffer decreases the corrosion rate due to formation of a protective layer on the alloy surface⁷⁰. Various buffers such as HEPES (4-(2(hydroxyethyl)-1-piperazineethanesulfinic acid), tris-HCl, CO₂/NaHCO₃ or phosphates are typically used in order to get pH constant in *in vitro*⁷⁶. Out of all of them, CO₂/NaHCO₃ are preferable for *in vitro* tests since it maintains the pH neutral in a way closest to natural conditions and does not inhibit the corrosion process^{77,57}. For instance, HEPES has been recently advised against, since it leads to overestimation of corrosion rates⁷⁸. The existence of flow avoids the accumulation of corrosion products, favouring uniform corrosion⁷⁹. At low flow regime conditions (0.9 Pa) a uniform corrosion occurs, while when the flow rate is high (8.8 Pa) uniform corrosion may be accompanied by undesirable localised corrosion^{80,81}.

In order to rule out potential hazardous effect from degradation of the biomaterial, and in spirit with 3Rs requirements (Replacement, Reduction and Refinement) of EU research ethics⁸², it is compulsory to carry out cell culture assays prior to *in vivo* testing. To evaluate degradable Mg alloys, human and animal non-cancerous and cancerous or osteoporotic cell lines are used. Fibroblasts, osteoblasts and osteoclast are the common cell lines used to screen orthopaedic implant materials while endothelial cells are used to test biodegradable stents⁸³. Cell culture can be divided into direct assays where culture

cells are seeded directly on the test material and indirect methods when culture cells are incubated for a specific time in a medium with the studied materials ⁷⁵. Anna *et al.* evaluated direct contact between different cells lines (osteosarcoma cells (MG63, U2OS or SaoS2) and primary human osteoblasts (OB)) and Mg-alloys implants ^{84,85}. They showed that when a differentiation medium is employed, cells count decreased for Mg63, SaoS2 and OB, although, their viability was not influenced significantly; however, the viability of OB decreased for the differentiated cells. Other studies have reported that the presence of Mg^{2+} in the surface coatings on permanent biomaterials, such as hydroxyapatite and Ti, increased the adhesion of fibroblasts and effected the improved osteoblasts response ^{86, 87}.

In addition, Zander *et al.* evaluated the corrosion rate of $MgCa_yZn_x$ ($y= 0.6$ and 1 wt. %; $x=0, 0.8$ and 1.8 wt. %) in PBS and HBSS at $37^{\circ}C$. The results showed that $Mg_{0.6}Ca_{0.8}Zn$ alloy presented the lowest corrosion rate (0.12 and 0.11 mm/y, respectively) compared with other Mg-based alloys ⁴⁶. Also, Zhang *et al.* evaluated the corrosion rate of Mg_1Ca_xZn ($x= 0-6$ wt. %) in Hank's solution at $37^{\circ}C$ and the alloy with 1 wt. % Zn content presented the lowest corrosion rate (2.13 mm/y) due to the formation of $Mg(OH)_2$ and Ca/Mg phosphate protective layer on the Mg surface ⁸⁸.

1.4 Corrosion protection strategies for Mg-based implants

1.4.1 Degradation in vivo

The techniques utilized to measure the corrosion of Mg after *in vivo* implantation are less complicated. These techniques consist in the measurement of the sample degradation during the implantation period by micro-computed tomography, analysis of the remaining cross-sectional area of the sample or the measurement of the weight loss of the sample ^{10,89}.

Different Mg alloys and pure Mg implanted in the femur or tibia of rabbit, rat and guinea pig concluded that the implantation site, did not affect the corrosion rate for the same material (although it was rightly expected to) ⁷⁴, attributing this inconsistency to the different methods and implantation periods used to obtain the corrosion rate. Gu and Zheng have studied different Mg alloys such as LAE442, WE43, AZ31, AZ91, AZ91Ca, AZ61Ca, ZE41, cast and extruded Mg-Mn-Zn, extruded Mg-Zn-Y and cast and extruded Mg-Ca alloys *in vitro* in different physiological solutions and *in vivo*. Although LAE442 and Mg-Mn-Zn presented the lowest of all corrosion rates *in vivo*, the residual magnesium

implant after 18 weeks was not found to retain sufficient mechanical properties for fracture fixation ⁴³. Lin *et al.* investigated Mg-Sr (99.9 wt. % Mg and pure grade of Sr) alloys implanted into femur of rabbit during 5 months and showed it to be non-toxic, with a slow degradation rate (0.6 mm/y) and superior osteogenesis ⁹⁰. Some studies have evaluated the corrosion rate and corrosion behavior in *in vivo* test of JDBM and WE43. JDBM exhibited lower degradation rate *in vivo* than the WE43 (JDBM: 0.34 mm/y and WE43: 0.71 mm/y) showing an uniform corrosion during the degradation process ⁹¹.

In order to obtain a significant improvement in the resistance against corrosion of Mg alloys, biocompatible protective coatings are applied to delay the onset of substrate corrosion process, providing other desirable qualities to the biomaterial, such as antibacterial properties or improvements in bone tissue growth. These strategies will be discussed in the following sections.

1.4.2 Protection methods-Overview

The key factor for interconnection of biomaterial interface with surrounding tissue for engagement and cell attachment is through the design of surface morphology of biodegradable implant ^{92,93}. The surface modification consists in the generation of a protective barrier that isolates the metal from the aggressive environment. This coating must be uniform, adherent and free of imperfections such as cracks, pits and scratches. The approaches to carry out the surface modification can be classified as chemical, physical and electrochemical methods. The physical methods include the treatments that do not generate chemical bonds between the outer surface coating and the substrate, while in the chemical and electrochemical methods the new produced phase is adhered to the substrate by chemical bonds and the native thin oxide layer is removed because it is not protective ⁹⁴. Different types of surface modifications used for Mg and its alloys are summarized in **Table 4**.

Table 4. Types of surface modification on Mg and its alloys adapted from ^{4, 8, 94}.

Surface modification	Types of treatment
Physical	Laser surface processing
	Sputtering
	Ion implantation
	Electron beam treatments
	Physical vapour deposition
Chemical	Sol-gel
	Conversion coating
	Polymeric coatings
	Alkaline passivation
	Immersion in alkaline solution and heat-treatment
	Acid etching
	Layer double hydroxide
	Self-assembled monolayers
Electrochemical	Anodic oxidation
	Microarc or plasma electrolytic oxidation
	Electrophoretic deposition

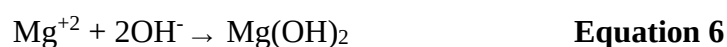
1.4.3 Plasma electrolytic oxidation

Anodic oxidation is one of the most popular and effective methods to decrease the corrosion rate of light alloys (Al, Ti, Mg) due to its low cost and versatility of oxide film compositions, precision of process and coating parameters control. This electrolytic oxidation process generates a thick and stable oxide with barrier or porous morphology, depending on the electrolyte ⁹⁵. However, unlike Al, Mg alloys do not passivate and grow thick, porous films in acid electrolytes; in alkaline electrolytes the low anodizing voltage does not produce barrier films on Mg thick enough to provide sufficient resistance to wear and corrosion. Plasma electrolytic oxidation (PEO) process presents similitude with conventional anodizing in terms of experimental setup that needs an electrolyte, anode and cathode, but there are differences in the type and magnitude of applied electrical signal and the type of electrolyte. The advantages of PEO over conventional anodizing include greater corrosion resistance and improved both wear resistance and surface hardness without the need for extensive surface preparation. **Table 5** shows the main parameters employed in conventional anodizing and plasma electrolytic oxidation (PEO) on aluminium and magnesium alloys.

Table 5. Comparison between conventional anodizing and PEO on Al and Mg alloys adapted from ^{96,97,98}.

Parameters	Anodization		PEO	
	Al	Mg	Al	Mg
Voltage (V)	1-300	3-100	100-600	
Current (mA/cm ²)	3-30	<20	100-500	
Thickness Barrier (μm)	0.01-0.5	<1	1.5	<1
Thickness (μm)	5-50	5-30	1-130	5-200
Electrolyte	Acid		Alkaline	

In PEO process, generation of plasma microdischarges on the metal surface immersed in an alkaline electrolyte occurs under high voltage that causes dielectric breakdown of the oxide material and generated collateral gas. Four stages are recognized during PEO process (**Figure 3**): the first is the partial dissolution of magnesium alloy by oxidation, generating a passive film on the surface (**Equation 5, 6 and 7**), constituted by MgO and Mg(OH)₂ and the migration of the electrolyte species through the layer towards the substrate.



The second stage coincides with commencement of microdischarges accompanied with acoustic and light emission on the surface due to the breakdown of the passive film and the evolving gas; a copious gas evolution can be observed (**Equation 8, 9 and 10**). The characteristics and intensity of the microdischarges range from sparks to micro arcs, which depends on process parameters. Three types of reactions occur simultaneously during this stage which includes electrochemical oxidation, plasma chemical reactions and thermally assisted oxidation which modify the structure, composition and morphology of PEO oxide coatings ^{99,100}. In addition, plasma chemical reaction consisted in the formation of any gas/vapour phase due to ionization of anions and molecules of water ¹⁰¹, which is composed of atomic and single ionised species as O, H, and elements incorporated from electrolyte and substrate ^{102,103}. This reaction includes charge transfer in substrate/electrolyte interfaces, intense ionization and charge transfer between the substrate and over the oxide film with plasma ⁹⁹.



At the beginning of the second stage, the sparks are fine with short lifetimes; as the voltage and treatment time increase, both the size and duration of the discharges increase and the oxide film growth enters the third stage⁹⁷. In the fourth stage, the voltage becomes independent of time and long-lasting large microarcs may develop, which are destructive with the material coating^{97,99}.

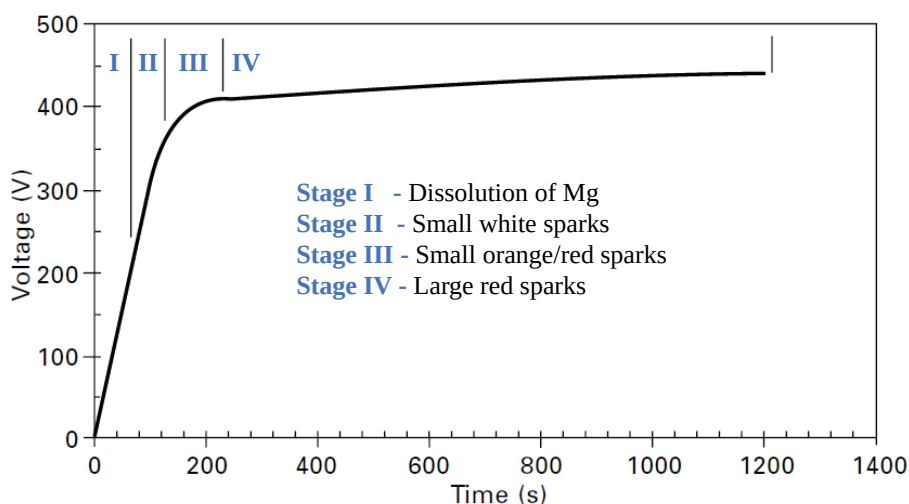


Figure 3. Scheme voltage vs time plot and representation of different step process, adapted from⁹⁷.

The general structure of a PEO coating is constituted by three layers: a porous outer layer, which presents relatively large pores or cavities, intermediate compact layer with lower porosity or microcracking and a thin barrier layer (**Figure 4**).

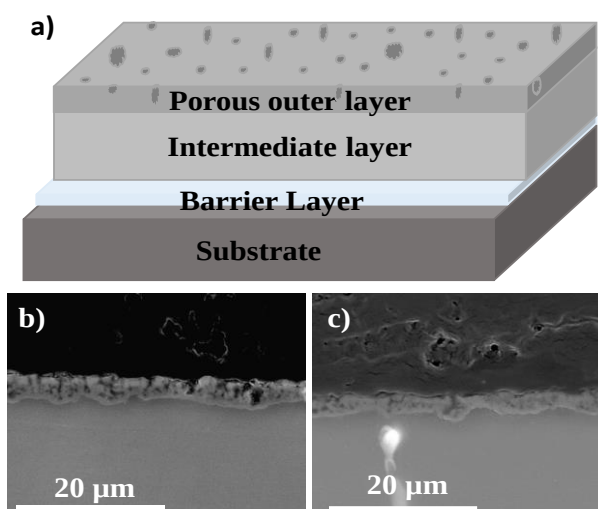


Figure 4. (a) Typical layer structure of plasma electrolytic oxidation, adapted from¹⁰⁴. Cross-sectional SEM image of PEO coating on MgZnCa alloy by (b) AC and (c) DC mode (The Author's unpublished results).

The morphology, composition and properties of the coatings are determined by the composition and concentration of the electrolyte, the voltage and current density and the type of the power (AC or DC mode) ⁸⁴. The three-layered structure is common for AC regimes, whereas in DC regimes the intermediate layer is often underdeveloped and indistinguishable from the outer porous layer, which results in essentially 2-layered structure.

The electrolyte design plays a critical role in the reduction of Mg dissolution, the improvement of the efficiency of PEO process and in the enhancement of biocompatibility. In regards to the latter, positive biological response of bone cells to the implant can be supported by incorporation of bioactive elements such as Ca, P, Si and F ^{105,106,107} into the coatings. Incorporation of P, Ca and F favours the generation of compounds such as hydroxyapatite or fluorapatite, which improves the corrosion resistance of the implant as well as cell adhesion, proliferation and differentiation processes ^{100,108}. Also, the presence of F in the electrolyte enhances corrosion resistance and cell adhesion attributed to generation of MgF_2 in the ceramic coating ^{109,110}. However, other researchers observed low biocompatibility with endothelial cells in fluoride coatings. This may be due to the limited tolerance of cells to released F^- causing high cytotoxicity ¹⁰⁵. The incorporation of Si into electrolytes favours the synthesis of collagen and matrix mineralization when Si is in contact with osteoblasts ¹¹¹. **Table 6** recompiles examples of the effect of PEO coatings formed from Ca-, P-, Si- and F- containing electrolytes on Mg alloys on corrosion behavior. It is showed that the corrosion resistance varies in the following order $MgCaAg < AZ80 < MgCa (SBF) < MgCa < AZ31 < MgZnCaMn < MgZnCa < MgZnCa (SBF)$.

Table 6. Corrosion behavior of bioactive PEO coatings on Mg alloys.

Alloy	Electrolyte	Thickness (μm)	Solution	i_{corr} ($\mu\text{A}/\text{cm}^2$)	Ref.
MgCa	$\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, $\text{KF} \cdot 2\text{H}_2\text{O}$ and KOH in different mass	13.3-22.5	Hank's	2.34 to 0.43	108
MgZnCa	2 g/L KOH, 10 g/L, $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ and 5 g/L KF	5	PBS	1.7	112
MgCa	10 g/L Na_2PO_4 , 1 g/L KOH,	32-35	SBF	0.262 to 0.196	113
MgCaAg	8 g/L NaF and 2.9 g/L CaO			0.13 to 0.043	
MgZnCa	12 g/L, $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, 0-2 g/L $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ (in different mass) and 6 g/L NaOH	5-15	SBF	2.38 to 7.28	114
MgZnCaMn	3 g/L $(\text{NaPO}_3)_6$ and 8 g/L $\text{KF} \cdot 2\text{H}_2\text{O}$	11	SBF	~ 1	115
AZ31	NH_4HF_2 in different mass	1.3-13.9	Hank's	3.97 to 0.853	116
AZ80	HA nanoparticles into 10 g/L K_2TiF_6 sonicated at different time	8-24	SBF	1.23 to 0.087	117

It has been widely evidenced that PEO coatings not only increase the corrosion resistance of Mg alloys, but can also cause an improved osteoblast cell adhesion and proliferation promoting the development of hydroxyapatite and extracellular matrix *in vitro*^{99,118,119,120,121}. *In vivo* tests have demonstrated that PEO-modified implants improve the osseointegration and bone-to-implant contact¹²². As added functionality, the porous morphology of the PEO coatings with typical porosity values ranging from 0.5 to 50 μm have been investigated as drug delivery containers in order to reduce post-surgery infection and inflammatory response^{123,124,125,126,127}. This aspect will be further discussed in Section 1.7.

However, excessive porosity can be identified as a significant limitation of PEO coatings, as it can induce faster degradation of Mg due to the access of aggressive species of physiological medium to the metal/coating interface. This problem can be resolved by sealing pores and cracks, infiltrating them, with polymeric layers known to be resistant

to corrosion, for instance using biodegradable polymers ¹⁰⁰. This alternative will be discussed in the next sections.

1.4.4 Corrosion inhibitors

The majority of post-treatment techniques are intended to retard the degradation rate of Mg alloys sealing the pores, cracks and defects of the PEO coatings. Additionally, they can be combined with integration of corrosion inhibitors.

The corrosion inhibitors may be classified into inorganic and organic compounds. Inorganic compounds may be introduced *in situ* into PEO electrolyte or post-treatment sealing of the PEO coating by sol-gel, layer double hydroxide (LDH), conversion coating, and paint layer ^{128,129,130,131,132,133}. Organic compounds are mainly incorporated by immersion post-treatment ^{134,135,136,137,138}, where corrosion inhibitors block the transfer of species between anodic/cathodic areas and the electrolyte. Additionally, some highly effective organic compounds have the ability to "self-heal" the PEO-coated metals ^{139,140,141}. Recently, Lamaka *et al.* carried out a screening of a large number of inorganic and organic corrosion inhibitors for Mg alloys evaluating their efficiency and toxicity. Some examples of the most efficient inhibitors for selected alloys relevant to biomedical applications are illustrated **Table 7**.

The positive values of efficiency indicated that the corrosion rate was retarded for all cases. Although the majority of compounds showed high inhibiting efficiency for industrial applications, it is important to assess the environmental effect of each inhibitor. It should be noted that even if a chemical is not toxic, carcinogenic or mutagenic, it may still be harmful to the environment. This problem may be resolved using green corrosion inhibitors which are biodegradable and eco-friendly because they do not use metals and toxic compounds.

In the last few decades, pharmaceutical agents containing N, O, S and aromatic rings were identified as suitable corrosion inhibitors ^{142,143,144}. Some reports of their efficiency are illustrated in **Table 8**. The corrosion inhibitor effect of drugs was evaluated by potentiodynamic polarization, electrochemical impedance and weight loss methods. The results showed a considerable corrosion inhibitor effect of many of the assessed molecules. The latter are adsorbed onto the metal surface generating a protective layer preventing and delaying the negative effect of the corrosive environment.

Table 7. Values of inhibiting efficiency and toxicity in 0.5 wt. % NaCl solution containing corrosion inhibitors, adapted from ¹⁴⁵.

Type of inhibitors	Compound and concentration	Efficiency (%)			Toxicity
		WE43	ZE41	Commercial pure Mg	
Inorganic	NaF (0.05M)	55	75	67	Toxic No-identified
	Na ₃ PO ₄ (0.01M)	27	43	93	No-identified
	Na ₃ PO ₄ (0.002M)	33	51	86	
	Na ₂ SO ₄ (0.05M)	68	46	68	
	KNO ₂ (0.05M)	77	70	53	Carcinogenic
	KNO ₃ (0.05M)	97	88	95	Toxic for aquatic life
	Ce(NO ₃) ₃ (0.05M)	92	70	62	No-identified Toxic for aquatic life
	La(NO ₃) ₃ (0.05M)	88	49	45	No-identified
Organic	5-Methylsalicylic acid (0.32M)	72	76	55	No-identified
	3-Methylsalicylic (0.05M)	97	52	75	
	5-Methyl-2-nitrobenzoic acid (0.02M)	65	19	11	
	Quinaldic acid (0.05M)	85	62	21	
	2,6-Pyridinedicarboxylic acid (0.03M)	82	73	71	
	Glycolic acid (0.05M)	65	50	59	
	Quinic acid (0.05M)	59	15	19	

Table 8. Evaluation of the inhibiting efficiency of pharmaceutical compounds.

Agents	Compound	Concentration	Medium (HCl, M)	Efficiency (%)			Ref.
				Mild steel	Bronze	Mg-Al	
Anti-inflammatory	Dexamethasone	0.1-0.5 µg/mL	2	22-83	-	-	146
	Naproxen	200 ppm	1	96.61	-	-	147
	Paracetamol	0.01-1mM	30 % ethylene glycol	-	-	80-96	148
	Streptomycin	200-500 ppm	1	88.5	58.5	-	143,149
Antibiotic	Gentamicin	0.1-1 %		40-92	-	-	150
	Ciprofloxacin	200 ppm		86	56.6	-	151,152
	Doxycycline	200 ppm		95	68.5	-	143,153
	Amoxicillin	0.1-1 mM	Artificial urine	-	-	93.6	125

As observed in **Table 8**, the majority of studies have been carried out on mild steel and in acid environment. Anti-inflammatory and antibiotic molecules disclosed in **Table 8** are often similar in structure (functional groups) to known efficient organic inhibitors for Mg (**Table 7**) therefore, it is reasonable to expect that some of them may reduce the corrosion rate of Mg alloys in neutral mild saline solutions. However, it appears that with respect to Mg alloys, the only two studied pharmaceutical agents were evaluated on AZ91 alloy (with high content of Al, hence not ideal for biomedical applications). For this reason, there is a great scope of unexplored potential in evaluating the effect of these drugs on corrosion of Mg alloys.

The idea of adding a corrosion inhibiting drug incorporated into a protective coating on Mg alloy is attractive. However, given that the selection of an appropriate drug is dictated first and foremost by its clinical justification, it is just as important to disclose the possible corrosion accelerating effect of the drug. In those cases, where corrosion acceleration takes place, a hybrid coating system as a drug-carrier can be a relevant alternative, as it offers a tailoring flexibility with respect to its layers that can compensate for an accelerated implant degradation provoked by a medication of choice.

1.4.5 Polymeric coatings

Another method to improve the corrosion resistance of Mg alloys without altering the bulk properties of the material involves the application of polymeric coatings onto the alloy. Polymeric coatings have been employed not only to reduce the corrosion rate of

Mg and its alloys, but also to provide the system with additional properties such as biocompatibility and biostability¹⁵⁴, thromboresistance, antimicrobial action, dielectric strength, wear properties, and lubricity¹⁵⁵. Also, polymeric coatings have been widely recognized as suitable methods to control and target cell responses as proliferation, differentiation and adhesion¹⁵⁶, making more feasible and desirable their use as medical implants in the body. Biodegradable polymeric layers are employed as antibacterial coatings, surgical sutures, drug delivery systems, fixation devices and tissue replacement components¹⁵⁷. Therefore, polymer coatings on Mg and its alloys can be regarded as excellent candidates to enhance the implant biocompatibility.

1.4.5.1 Types of biodegradable polymers

Over last decades, there has been an exponential increase in the use of biodegradable polymers in the field of biomedicine. The use of biodegradable polymers in orthopaedic implant has been limited because of the load bearing required. In general, polymeric devices have lower strength and stiffness values compared to both cortical bone and the more traditional metal implants¹⁵⁸. However, the combination of Mg alloys with biodegradable polymeric coatings can overcome this issue and offers unprecedented opportunities due to their unique properties such as degradation along with biocompatibility, drug-delivery and osteoinduction just to mention a few of them¹⁵⁹. The main application of polymers and the application of polymers on Mg alloys are illustrated in **Table 9**.

Natural polymers are, in general, biodegradable, non-toxic, and promote the cell adhesion and proliferation achieving an intimate bond between the tissue and the implanted material^{160,161}. However, some natural polymers are susceptible to enzymatic degradation making their use less predictable when inside the human body¹⁶². Synthetic polymers can be designed with tailored properties including mechanical strength, bioactivity and, usually, hydrophobicity¹⁶³. Many biodegradable polymers employed to coat alloys have shown to enhance the corrosion resistance of Mg alloys^{164,165}.

Table 9. The main biodegradable polymers coatings for biomedical application adapted from ¹⁶⁶.

Type	Polymer	Application	Ref.	Application on Mg alloys	Ref.
Synthetic	PLA	Medical devices, injection capsule, microsphere and implants, tissue culture, drug delivery devices, orthopaedic fixation, surgical suture and 3D printing scaffold	167	Bone tissue engineering, drug delivery	168, 169
	PLGA	Drug delivery, tissue grafts, surgical sutures, bone tissue engineering scaffolds and degradation rate modulation	159	Bone tissue engineering, drug delivery	170, 171, 172
	PCL	Drug delivery, bone regeneration, shape memory biomaterials, scaffold for skin, cartilage, bone and cardiac constructs	154, 173, 174	Bone tissue engineering, screw, drug delivery	175, 176, 177
	PDA	Biosensing and drug delivery	178, 179	Orthopaedic implants	180, 181
Natural	CS	Artificial skin, biomaterial for cartilage and drug release carrier	182, 183, 184	Bone tissue regeneration, orthopaedic implants	185, 186
	Collagen	Biomaterials for nerve, bone/cartilage and tendon/ligament	187, 188	Orthopaedic implants, Bone tissue regeneration, dental	189, 190, 191

However, the clinical experiments with these hybrid coated materials, such as Mg-based biomaterials are rare, and the practical clinical application of these hybrid materials is still in its infancy. The following considerations apply when using biodegradable polymers as stand-alone coating systems. For widely reported biocompatible polymers (PLA and PLGA) ¹⁹², the potential acid products of hydrolysis of the polymers may change the local pH value ¹⁹³, which may be to the benefit of Mg implants since they can leverage the alkalinisation caused by corrosion of Mg. Furthermore, the application of PCL directly on Mg may cause insufficient adhesion strength problems, provoking detachment of PCL coating from Mg-based substrates without surface pre-treatment ^{194, 195}. Also, the developed approaches to address the issue by improving the surface roughness of the substrates ¹⁶⁴ introducing another high adhesive strength moiety into PCL are complex ¹⁹⁶. Another limitation is the degradation period of the polymer, which is associated with the polymer type and layer thickness. While a thin layer causes low corrosion protection, a thick coating could lead to cohesive failure. Also, non-uniform coatings in elaborate 3D structures can be formed.

1.4.5.2 Homogeneous layer

In order to obtain uniform coatings enhancing the adhesion stability, different methods are currently being used including electrophoretic deposition, spin coating, painting, spraying and immersion¹⁹⁷. The thickness of the coatings can vary from nanometres, in the case of self-assembled monolayers, up to several hundred microns. Among the methods mentioned above, immersion by dip coating is one of the most extensively used methods because it is simple and cheap. Moreover, uniform coatings with variable thicknesses are obtained by controlling the parameters of the process and it allows the preparation of coatings on wide myriad of materials with variable geometries.

Dip coating is employed to coat specimens by immersing the sample in a polymer solution containing the dissolved absorbent. The substrate is withdrawn, the solvent is evaporated and the substrate is dried at a required temperature (**Figure 5**). The main advantages of this technique include the uniformity of the film formed and the possibility to create coatings on non-planar structures. In order to obtain coatings with controlled thickness and with the required properties, many experimental parameters of the process can be modulated; including the immersion time, number of dipping cycles, withdrawal speed, type of polymer, polymer composition and concentration or the type of solvent¹⁹⁸.

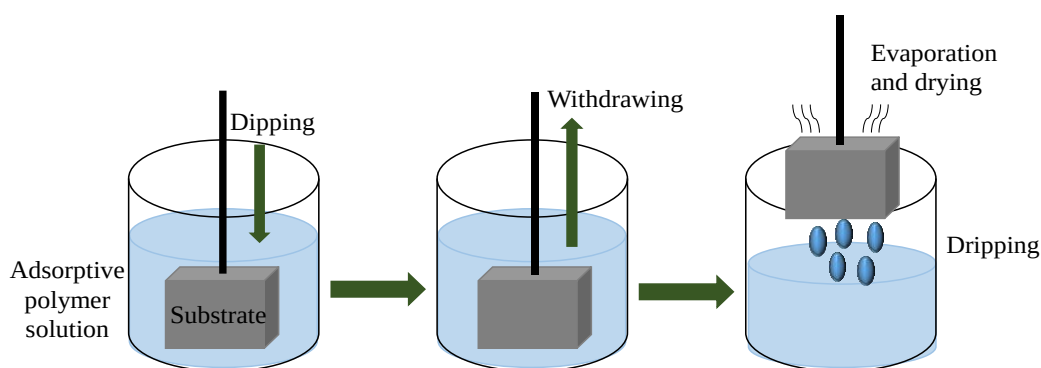


Figure 5. Dip coating technique. Adapted from ref¹⁹⁸.

This strategy has been already employed for the preparation of hybrid coated materials. For instance, Wang *et al.* evaluated the corrosion resistance and the antibacterial properties of different polymer coatings on pure Mg. Homogeneous coatings were obtained with a thickness of 4-15 μm . The corrosion resistance of pure Mg was increased with polyurethanes (PU) and zwitterions (ZPU-12) polymers (pure Mg: 353.8 to 80.38 and 1.42 $\mu\text{A}\cdot\text{cm}^{-2}$, respectively) enhancing the antibacterial properties¹⁹⁹. In addition, three different polymers (PCL, PLA and PLGA) were evaluated on WE43 stents to

enhance the corrosion resistance, biocompatibility and hemocompatibility. The results showed that PLA and PLGA decreased the corrosion rate of WE43 from $1.95 \cdot 10^{-5}$ to $1.36 \cdot 10^{-5}$ and $1.67 \cdot 10^{-5}$ mm/y, respectively. All coated samples showed excellent haemolysis rate but only PLA showed better biocompatibility than the others ²⁰⁰. AbdallaAbdal-hay *et al.* evaluated biodegradable polymer coatings prepared either by electrospinning or dip coating technique on AM50 alloy. The results demonstrated that the coatings obtained by both methods enhance the corrosion resistance of the Mg alloy (E_{corr} : -1.42 to -1.17 and -1.3 V, respectively). The authors, also evidenced excellent cell adhesion, proliferation and differentiation, thus, being promising for orthopaedic and cardiovascular implants ²⁰¹. In order to control the degradation rate and enhance the biocompatibility of Mg alloys, different solvents were evaluated in order to assess their role over the quality of the coating. For instance, the poly(vinyl acetate) (PVAc) coating prepared in tetrahydrofuran and dimethylformamide solvents presented high adherence, proliferation, and survival on the surface ²⁰².

Polymer coatings must be uniform and well-adhered to the substrate in order to act as excellent protective layers. However, polymer coated metals sometimes present filiform corrosion due to the apparition of filamentous corrosion products under the coating ²⁰³. There are several difficulties in preventing corrosion with organic coatings including the difficult of applying a perfectly uniform film, non-uniform crosslink density in the applied film, imperfect film formation and swelling or degradation of the polymer when exposed to various gases or liquids ²⁰⁴. These problems can be minimized by using a multi-layer coating system consisting of an inner layer that must have a good adhesion to the substrate, and a hydrophobic and UV-resistant top polymer layer ²⁰⁴.

1.5 Hybrid coatings obtained by the combination of PEO and polymer layers

The combination of PEO treatments with subsequent deposition of a polymer coating can be a beneficial hybrid coating strategy. PEO coatings present excessive porosity which can compromise the degradation rate of coated Mg, a drawback that can be overcome by sealing of pores and cracks by infiltrating polymers ¹⁰⁰. More interestingly, the polymer layer on top of the PEO coating not only improves the corrosion resistance of PEO/Mg system but also enhances the cytocompatibility ²⁰⁵. In the last years, researchers have been focusing on the design of hybrid coatings in order to improve the

corrosion resistance of PEO coating on Mg alloys by sealing of pores. The **Table 10** illustrates some of the reported examples of such systems.

Table 10. Corrosion behavior of hybrid coatings on Mg alloys.

Alloy	Ceramic coating	Polymer coating	Corrosion behavior	Ref.
AZ31-B	Na ₃ PO ₄ ·12H ₂ O, 0.8 g/L NaF, 1 g/L KOH and 2.9 g/L CaO 30-45 µm	PLA 2.70-114 µm	E _{corr} (Mg) = -1.25 V, i _{corr} = 2 µA/cm ² E _{corr} (Mg/PLA) = -1.21 V, i _{corr} = 7 µA/cm ² E _{corr} (Mg/PEO) = -1.23 V, i _{corr} = 0.065 µA/cm ² E _{corr} (Mg/PEO/PLA) = -1.7 V, i _{corr} = 0.017 µA/cm ²	206
AZ91	4.8 g/L C ₆ H ₈ O ₇ ·H ₂ O, 16 g/L Na ₂ B ₄ O ₇ , 24 g/L Na ₂ SiO ₃ and 20 g/L NaOH 3.5-8 µm	PLA Thickness unspecific	V _{H2} (Mg) = 12 mL/cm ² V _{H2} (Mg/PLA) = 3 mL/cm ² V _{H2} (Mg/PEO) = 8 mL/cm ² V _{H2} (Mg/PEO/PLA) = 1.25 mL/cm ² after 14 days of immersion	207
AZ31	100 g/L Na ₂ SiO ₃ and 100 g/L NaOH	PCL and CS (0-5wt%) Thickness unspecific	Corrosion rate (mg/cm ² ·h) (Mg) = 0.24, (Mg/PEO)= 0.14 (Mg/PEO/PCL+CS)= 0.08-0.01 after 28 day of immersion	208
Mg-Al	500 mM CaEDTA, 500 mmol/L KH ₂ PO ₄	SPA	E _{corr} (Mg) = -1.48 V E _{corr} (Mg/Hap) = -1.495 V E _{corr} (Mg/Hap/SPA) = -1.45 V	209
Mg-Al	20 g/L NaOH, 24 g/L Na ₂ SiO ₃ , 16 g/L Na ₂ B ₄ O ₇ and 4.8 g/L C ₆ H ₈ O ₇ ·H ₂ O	PLA 10 µm	V _{H2} (Mg) = 12 mL/cm ² V _{H2} (Mg/PLA) = 3.5 mL/cm ² V _{H2} (Mg/PEO) = 6.2-6.5 mL/cm ² , V _{H2} (Mg/PEO/PLA) = 0.5 mL/cm ² after 14 days of immersion	210
Mg-Mn- Ce	5 g/L Na ₂ SiO ₃ ·5H ₂ O and 5 g/L NaF 20 µm	SPTFE 1-1.5 µm	E _{corr} (Mg) = -1.56 V, i _{corr} = 53 µA/cm ² E _{corr} (Mg/PEO) = -1.50 V, i _{corr} = 0.078 µA/cm ² E _{corr} (Mg/PEO/SPTFE)= -1.46/-1.27 V i _{corr} = 0.033-0.003 µA/cm ²	211
Pure Mg	0.1 M Na ₂ SiO ₃ ·9H ₂ O and 0.2 M NaF 2 µm	PCL 3.61, 20.5 and 52.6 µm	E _{corr} (Mg) = -1.65 V, i _{corr} (Mg) = 121 µA/cm ² E _{corr} (Mg/PEO) = -1.65 V, i _{corr} (Mg/PEO) = 1.14 µA/cm ² E _{corr} (Mg/PEO/PCL) = -1.72/-1.52 V, i _{corr} (Mg/PEO/PCL)= 0.81-0.005 µA/cm ² R _p (Mg)= 0.41 Ω·cm ² , R _p (Mg/PEO)= 28.60 Ω·cm ² , R _p (Mg/PEO/PCL)= 76.9-997 Ω·cm ²	212

As illustrated in **Table 10**, most of the works are developed on Mg-Al alloys but as it was mentioned in the Section 1.1.3, the Mg-Al alloys are considered cytotoxic. For this reason, Mg-Al alloys in these reports have been exclusively employed as model alloys and limited to in vitro experiments. Furthermore, Mg alloys with hybrid coatings presented 2-3 times orders of magnitude higher corrosion resistance than the substrates in short period of time. The corrosion resistance of hybrid coating systems is improved by 4-6 times compared to stand-alone PEO coatings during the long-period of time. Although only few of the works summarized in **Table 10** are focused on biological performance of hybrid PEO/polymer coatings, they report that a reduced degradation rate is accompanied by an improved of cytocompatibility.

1.6 Control of the surface topography at the micrometric scale: role of the surface properties in improving cell adhesion

1.6.1 Surface structuring: role in the biomaterial-cell interactions

In order to employ a particular material for tissue engineering purposes, some aspects have to be taken into account including biodegradability, biocompatibility, 3D architecture, mechanical properties or the manufacturing technology employed. One of the most important factor in tissue engineering is the biocompatibility. Biocompatibility is the ability of a material to provide an appropriate response to the biological environment in which they are employed. The different interactions that occur at the biomaterial/biological environment interface, in terms of duration and intensity, depend on the biomaterial and the tissue in contact ²¹³.

In order to establish a correlation between at biomaterial and its cell behavior different parameters and characteristics of the biomaterial as topography (porosity and roughness), surface energy etc. require consideration ²¹⁴. Surface topography is one of the most important parameters, as both cells adhesion and proliferation are highly dependent on the surface chemical composition but also on roughness, porosity and average pore size. Surfaces with a certain roughness tend to show greater osseointegration and implant integration compared to smooth surfaces ²¹⁵. In the case of porosity and pore size, high porosity materials showed better diffusion of nutrients and gases towards the cell and removal of secondary products. However, in materials with high porosity with a large average pore size, cell growth and proliferation are interfered preventing cell spreading.

For this reason, pore size and porosity must be considered together to allow cells to proliferate, migrate and adhere to the implant ²¹⁶.

Another important factor affecting cell adhesion is the surface wettability. Hydrophilic surfaces with high roughness reduce the contact angle ($\theta < 90^\circ$) so that the surface is more hydrophilic and the liquid is distributed over the surface of the material ²¹⁷ in comparison to planar surfaces. This phenomenon is because the barrier effect depends on the stiffness of the substrate and the capillary attraction depends on the advancing and receding contact angle ²¹⁸.

1.6.2 Strategies to produce surfaces with controlled surface topography

In order to enhance the adhesion between the cell/material, modification of the implant topography can be carried out by optimising the physical, chemical and biological characteristics of the material. Surface modification is one of the strategies to modulate the characteristics of the material surface. This strategy is considered one of the most promising alternatives as cell/material interactions and biocompatibility are improved without altering bulk properties ²¹⁹. Surface modifications include chemical and topographical modifications that can lead to the fabrication of functional nanostructures and microscopic architectures on a surface using different biocompatible materials as small molecules, metals, inorganic non-metallic materials and functional polymers.

Hybrid alloy/polymer coatings produced by combining PEO treated and polymer-coated alloys have been explored to improve the biocompatibility. In particular, in the last years, the use of functional polymers has attracted increasing attention due to their excellent mechanical and chemical robustness and the high flexibility with different functional groups. Previous studies on the development of hybrid coatings for Mg alloys using dip coating technique have been focused on development of uniform, planar and relatively thick (3-50 μm) polymer top-coats in order to improve the mechanical properties and corrosion behavior of Mg alloys ^{220,221,212,208}. However, few reports focused on the design of hybrid materials on Mg alloys that modulate the surface topography of the polymer by exploiting the use of nanotechnologies and microtechnologies. For example, Gao *et al.* developed hybrid coatings comprising PEO and Propolis/PLA porous top-coat on Mg-Zn-Ca alloys with an average of pore size around 4.5-6.5 μm without employing a high humidity chamber ²²². In addition, some preliminary studies carried out in this Thesis demonstrated that the withdrawal speed also

affect both the topography and thickness of the polymer layer (**Figure 6**). When the polymer concentration is low, a thin polymer layer is formed and the polymer film formed tends to adapt to the PEO coating topography. On the opposite, high withdrawal speed forms planar homogeneous polymer layers.

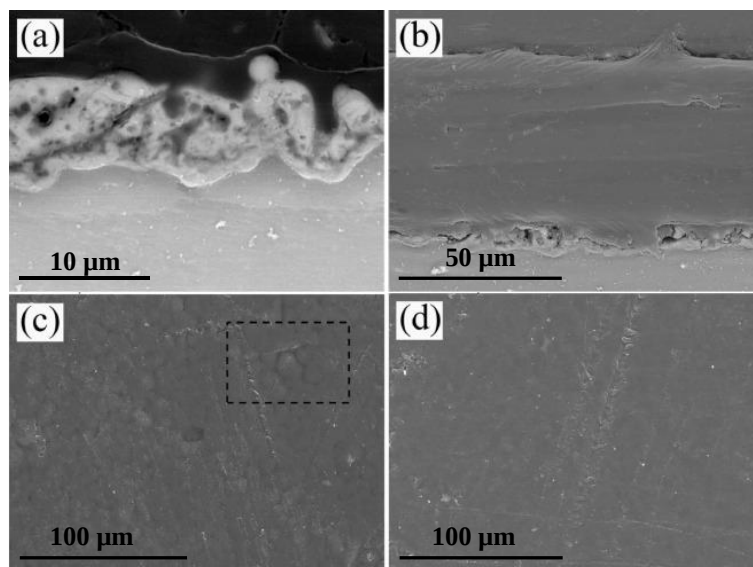


Figure 6. Effect to withdrawal speed on the topography of coatings (a, c) 20 mm/min and (b) 150 mm/min on PEO/AZ31-B alloy adapted from ²²³.

In order to control the topography of thick polymeric coatings different methodologies have been developed. These have been divided into conventional or unconventional techniques. Conventional methods include colloidal lithography, polydimethylsiloxane (PDMS) molding, photolithography, polymer phase separation, electron beam lithography, soft lithography, microfluidics, hydrogels and chemical vapour deposition or electrospinning ^{224,225,226}. Non-conventional methods can be classified into top-down (e.g. photolithography, sputtering, inkjet printing, or molecular beam epitaxy) and bottom-up approaches (building structures atom- by-atom, or molecule-by-molecule) ²²⁷.

1.6.3 Formation of porous layers

One of the most extensively employed techniques for the fabrication of ordered porous on polymers surfaces using non-conventional methodologies is the called *Breath Figures (BFs)* approach. The name Breath Figures comes from the fact that the patterning occurs due to the formation water micro droplets when breathing on cold surface. In the case of the formation of porous polymer surfaces, the breath figures approach occurs by condensation of water vapour onto the surfaces of an evaporating polymeric solution ²²⁸. The main steps of this method are illustrated in **Figure 7**. The polymer is dissolved in a volatile and usually water-immiscible organic solvent and the resulting solution deposited

onto a solid support. Then, the solvent is evaporated under high relative humidity (RH%) in a closed vessel chamber. During the evaporation process, the liquid-air interface is cooled and water vapour start to condense. During this process, water droplets are formed and, eventually, arranged into ordered hexagonal lattice. Upon complete evaporation of the solvent and the condensed water droplets, a porous pattern is formed where the pores reflect the places where the water droplets condensed ²²⁹. Interestingly, different experimental factors can be modulated, affecting the size and distribution of the pores. These include the relative humidity, type of solvent employed, type of polymer, polymer architecture and polymer molecular mass among others. As a result, controlling experimental parameters such as polymer concentration (type, architecture and polymer molecular weight) ^{230,231} or relative humidity amount allow to produce pores with variable shapes and sizes ^{232,233}. For example, BF's formation using low RH% do not produce water condensation and pores are not formed. An increase of the relative humidity can produce regular honeycomb patterns, while a higher humid environment increases the pores size by improving the number of droplets at the polymer surface. However, too high relative humidity can produce pore coagulation and the formation of rather disordered arrays of pores ^{234,235,236,237,238}.

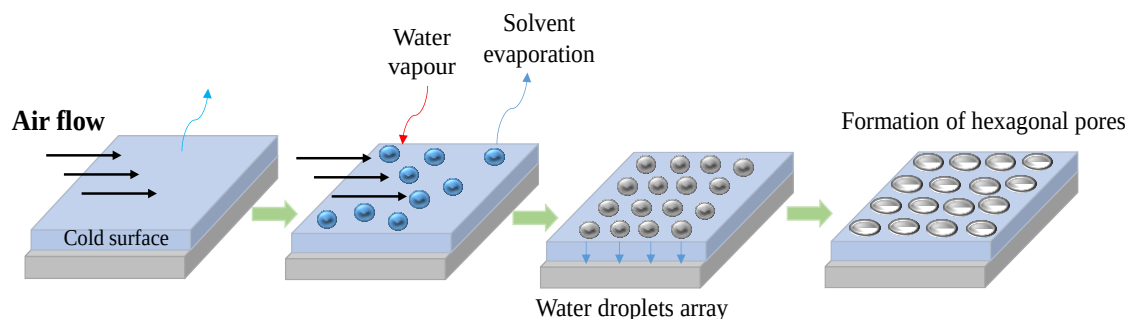


Figure 7. Scheme of Breath Figures mechanism in order to form porous polymer structure.

In general, this process has been carried out by simple solvent casting in a moist closed chamber. However, some alternatives to carry out BF's have been developed. For instance, some researchers used a cold stage to reduce the evaporation rate of the solvent and thus varying the condensation rate producing pores with larger average pore sizes ²³⁹. Other groups proposed to control the evaporation process by using an airflow where the distance, the angle of the air and the airflow are key parameters controlling the pores characteristic and quality of the patterning obtained. Another form to control the pores characteristics and quality of patterning is using different techniques as spin-coating,

emulsion technique or water spread spreading, dip coating etc.^{240,241,242,243,244,245}. Of all of them, the dip coating methodology to produce porous films allows the formation of mono/multilayers with open pores on the entire surface of a 3D part. The gravity plays no important role in the process and it is the humidity of the air and the physico-chemical properties of a solid substrate that allow the formation of the honeycomb topography^{244,245}.

Independently of the approach employed for the preparation of porous films, many different applications have been explored for these patterned surfaces. In particular, in this thesis we will focus on the use of porous polymeric films as scaffolds for cell culture. In effect, the cells can adhere better to the pores surface and improve the initial cell-adhesion. These pores can also act as storage and protective sites for active molecules, growth factors or drug delivery systems (DDSs). Finally, the pores and the rims of the Breath figures can entrap cells that are cultured on the material and mimic important microtopographical features of native ECM. In **Figure 8** is shown the main applications of the breath figures as biomaterial in tissue engineering applications. Based on these applications and the improved cell-response of these microstructured surfaces the use of the BFs approach to prepare polymer coatings is, to our opinion, an excellent alternative to improve the surface properties of Mg based implants.

The micrometer size pores permits the flow of nutrients, oxygen and waste products, which are important requirement for cell survival and function in the cell culture medium. In addition, the polymeric layer can be employed as a reservoir of active compounds. Low solubility drugs can be dispersed into hydrophobic polymer matrices or encapsulated into the porous films, which reduces the burst release and the delivery can be produced for longer periods with a minimum systemic toxicity²⁴⁶. Examples of the use of polymeric microstructured layers as drug containers have been recently reported. Some researchers studied drug loaded porous and non-porous films combining spin coating and BF process using PLGA and polyethylenglycol (PEG)/PLGA as polymers. The release rate of salicylic acid with PLGA and (PEG)/PLGA samples was faster in breath figures morphologies than planar films within five days most probably due to the change of surface topography. A similar situation was observed in ibuprofen with PLGA and (PEG)/PLGA, although release takes place over longer periods that included the formation of pinprick pores during the latter release stages initiating the gradual film breakdown²⁴⁷.

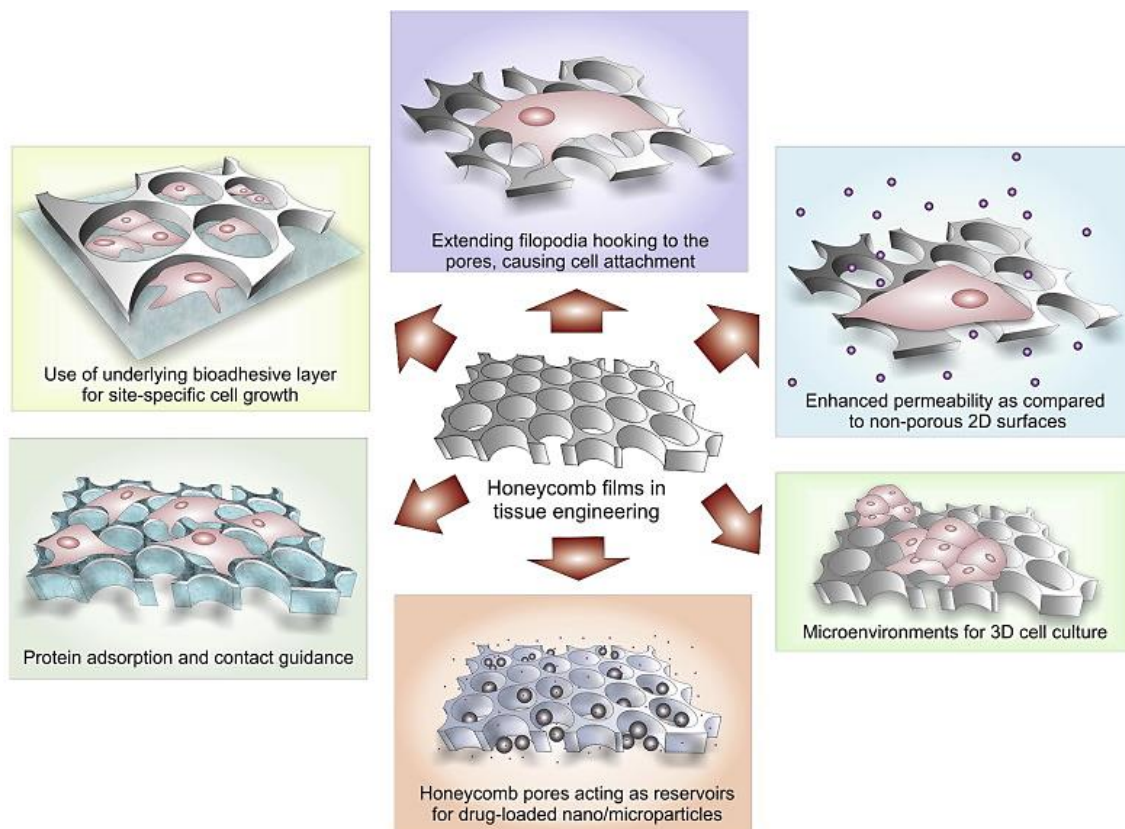


Figure 8. Honeycomb topography in tissue engineering, adapted from ²²⁹.

In order to reduce the degradation rate and add new functionalities to the biodegradable implant based on Mg alloys, the surface modification strategy proposed in this Thesis consists in hybrid Mg/PEO coatings with biodegradable polymer hierarchical top-coats employing PLA, PCL and the combination thereof.

1.7 Surface functionalization for drug delivery

1.7.1 Types of drug eluting system and targets of medicated biomaterials

The drugs are used for diagnosing, cure, treat or prevent diseases, and can be classified depending on their mode of action, pharmacological action and activity. The incorporation of drugs allows for the treatment of infections, inhibition of fever ²⁴⁸ just to mention a few of them.

The introduction of the implant into the body is always associated with the risk of microbial infection, particularly for the fixation of open-fractured bones and joint-revision surgery. After implantation of the biomaterial, the body reacts in a cascade of events leading to foreign body reaction (FBR), and a final stage of wound healing and inflammatory responses. This behavior can be deleterious for the material tissue-integration causing chronic pain and, finally, failure of the implant. In addition,

orthopaedic implants can cause a decrease in bone mass density called osteoporosis ²⁴⁹, which causes substantial pain and suffering, disability, and even death ²⁵⁰. However, the long-term host FBR to cardiovascular implant implantation often results in restenosis ²⁵¹ or lumen encroachment, brought about by the recruitment of inflammatory and smooth muscle cells to the intima with subsequent proliferation and extracellular matrix deposition cardiovascular implant ²⁵². These problems can be avoided or at least significantly reduced, for instance, by using antibacterial, anti-inflammatory, growth factors, immunosuppressant, antiproliferative, antiresorptive or anticoagulant agents (Figure 9).

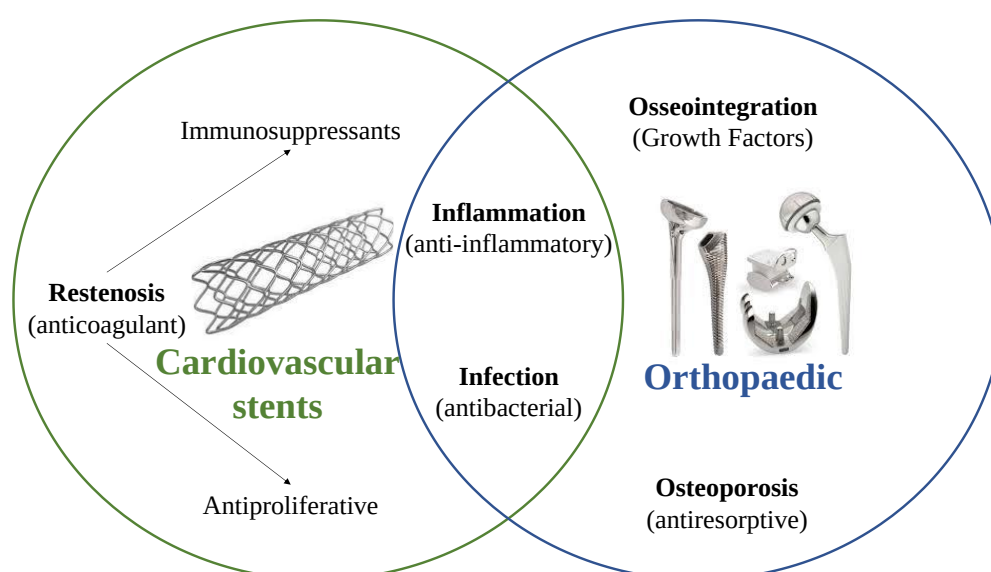


Figure 9. Problems associated with orthopaedic and cardiovascular stents and problem-solving drugs.

Drug delivery by orthopaedic and cardiovascular implants

The infection is associated with adhesion and colonization of micro-organisms in the implantation site coming from the outside such as skin, surgical instruments or the environment, by bacteria on the patient's skin or those present in the body which can become pathogenic. Antibiotic agents such as vancomycin, tobramycin, amoxicillin, gentamicin or antibacterial agents as metallic nanoparticles (Ag, Cu and Zn) have been extensively employed to avoid the above described infection problems ²⁵³. Biodegradable polymer carriers such as Poly(methyl methacrylate) (PMMA), PLGA, PLA, Poly (D-, L-lactic) acid (PDLA) or PEG are used for controlled release of antibiotics ^{254,255}. However, polymer carriers are not bone-active focusing on alternative osteoconductive materials as hydroxyapatite (HA) or calcium phosphate (CaP) coatings, which are excellent candidates due to their ability to support the growth of new bone tissue and for

promoting bone-tissue integration²⁵⁶. Aves *et al.* evaluated the efficiency of gentamicin into HA coating and the results showed that was released 30-40 % of drugs in 3 h, being only drug molecules in the superficial layer²⁵⁷. Stigter *et al.* evaluated the release and efficiency of different antibiotics in PBS into HA coatings on Ti alloys. The antibiotics with carbonyl functional groups presented slower release rates. In the study of Stigter *et al.* the bacterial inhibition test on *Staphylococcus aureus* bacteria showed inhibition of growth for all antibiotics²⁵⁸. In addition, Heakal and Bakry evaluated Mg alloys stents in artificial urine with doses of amoxicillin. They evidenced that amoxicillin has an inhibitory effect on Mg degradation due to the tendency of its molecules to be adsorbed on surface implant¹²⁵. CaP coating on Ti rods showed reduced infection through degradation of the vancomycin eluted coating in an *in vivo* rat model²⁵⁹. Also, Roy *et al.* studied the antibacterial effect of electrodeposited silver, zinc or copper on tricalcium phosphate (TCP) coated titanium substrates to assess the optimal Ag concentration for desired bactericidal effect with no toxicity^{260,261,262}.

The inflammation around the implant occurs because activated macrophages are mobilized to attack foreign bodies and invading bacteria are produced by aerobic respiration and metabolism causing the formation of superoxide and hydrogen peroxide compounds^{263,264}. Also, the pH can drop from 7.4-7.6 to 5.5 due to severe pathological change in surrounding tissues²⁶⁵. Different approaches for the delivery of anti-inflammatory and immunosuppressing drugs such as dexamethasone, superoxide dismutase mimic²⁶⁶, Growth Factors (GF) or cytokine have been described²⁶⁷ incorporated into the implant surface or into matrix material²⁶⁸. PEG hydrogel with surface immobilized immunosuppressive cytokines (TGF- β 1 and IL-10) were reported to significantly decrease bone marrow-derived dendritic cells maturation²⁶⁸. Local delivery of dexamethasone was evaluated from nitrocellulose-based coating on silicon neuronal probes. The results showed a stabilization of the inflammatory response after 4 weeks post implantation reducing neuronal loss²⁶⁹.

Another problem associated with orthopaedic implants is the osteoporosis. This phenomenon is based on loss of bone mass by marked bone microarchitecture deterioration causing multifactorial diseases. The osteoporosis can be avoided by the incorporation of antiresorptive agents as bisphosphonates (BPs) or alendronate into HA or CaP coatings. Adverse secondary effects of oral administration or intravenous injection of BPs, as well as their low bioavailability, have brought more attention to BP's local

delivery. Some researchers evaluated the BPs delivery from HA coatings on Ti implants by plasma sprayed. The results revealed enhancement in mechanical fixation and higher density of the peri-implant bone ²⁷⁰. Garbuz *et al.* investigated the role of CaP coated tantalum soaked in alendronate-phosphate buffer (PBS) showing an increase in new bone formation ²⁷¹.

In order to regulate osteointegration, bone formation, cell activities, and remodel and tackle impaired healing GF are employed. GFs stimulate the intricate biological cascade of bone regeneration and enhance the bone healing rate by promoting cell proliferation and differentiation as well as accelerating osteoclastic resorption. Some of osteogenic GFs are bone morphogenetic proteins (BMPs), recombinant human bone morphogenetic protein (rhBMP), vascular endothelial GF (VEGF), derived GF (PDGF), insulin-like GF-I (IGF-I) ^{272,273}. Soaking and absorbing drugs in the coatings have turned out to be effective in promoting osteointegration. However, their wide and successful application in bone healing is still partially restricted considering the fast diffusion of the proteins from administration sites and difficulties with their controlled release ²⁷⁴. In a research on the osteointegration of Ti implants coated with HA and soaked in nonglycosylated BMP-2 in aged sheep model revealed the positive effect of the released drug phosphate buffered saline (PBS), simulated body fluid (SBF), Hank's solution (HBSS), Ringer's even in aged-compromised individuals ²⁷⁵. In the case of a CaP coated implant, osteoclasts assisted the degradation of the coating promoting control over the delivery compared to the initial burst release that characterizes coatings with superficially absorbed osteogenic agents; this observation highlights the role of delivery mode in order to control the GF efficacy ^{276,277}. Henslee *et al.* employed a porous polypropylene fumarate (PPF) sleeve to contain a solid PPF intra-medullary rod, where rhBMP-2 loaded PLGA microparticles were incorporated into the porous sleeve ²⁷⁸.

The restenosis ²⁷⁹ is one of the most important problems in cardiovascular stents. Inflammatory and infection around of stent can also occur during the restenosis process. The restenosis is caused by the balloon expansion causing an increase in the vascular smooth muscle cells and vessel remodelling (hyperplasia) ²⁸⁰. To prevent the restenosis, a variety of as anticoagulant, immunosuppressant and antiproliferative agents are currently being used, which are included in polymeric coatings on drug eluting stents (DES). The anticoagulants are essential to prevent the thromboembolism especially in patients who are undergoing percutaneous coronary intervention (heparin and its

derivatives), while the immunosuppressant inhibit or prevent the immune system (prednisone, cyclosporine abatacept) and antiproliferative inhibit the cell growth (sirolimus, paclitaxel, and everolimus)^{251,16}. Xu and Yamamoto evaluated PLLA and PCL coating on Mg and the results showed that PLLA had a better adhesion strength than PCL and that more cells were more proliferative on PLLA¹⁹⁵. Interestingly, the early performance of PLLA-coated DES was similar to a bare metal stent, though the long-term performance was not studied²⁸¹.

In conclusion, unlike DES, drug-eluting orthopaedic implants based on Mg alloys are not yet well established, although a variety of inorganic and organic coatings with drug-eluting features for orthopaedic and cardiovascular stent have been studied. The most effective way to release drugs reported up to date on orthopaedic implants is using CaP coatings, on Mg alloys only few studies can be found. Therefore, the investigation of CaP-based coatings produced by plasma electrolytic oxidation on Mg alloys incorporating anti-inflammatory or antibiotic agents appears to be a promising strategy.

1.7.2 Direct PEO loading

Investigations on novel biodegradable immune-interactive biomaterials are focused on modulating the inflammatory response, supporting the implant integration, as well as, improving the healing and tissue regeneration²⁸². However, some researchers design coatings that prevent infection, ignoring bone mineralization. And other authors consider promotion of osteointegration while ignoring infection²⁸³. In summary, both modalities are required for the development of new implant coatings strategies.

PEO coatings are of great interest for local drug delivery because of their intrinsic porosity²⁸⁴. The drug incorporation in this coating is carried out by two different ways: *in situ* or post-treatment^{270,285,286}. **Table 11** illustrates the main progress in development of drug eluting PEO coating for Ti and Mg alloys. In general terms, the drug release from the coatings occurs as a burst during the first hours followed by a gradual and stable release obtaining <80 % of the initial load. However, the main limitation of PEO coating is the low incorporation of the drug into the coating, which leads to loosely bonded drugs and in complete absorption by the pores, which is the cause of a burst release. Also, burst release can have other negative effects such as being pharmacologically dangerous and economically ineffective.

Table 11. Drug-eluting PEO coatings on Ti and Mg alloys adapted from ¹⁶⁶.

Alloy	Ceramic coating	Drug and concentration	Release rate	Release period	Ref.
Ti-6Al-4V	Anodizing 1.0 M H ₃ PO ₄ 1.5 wt. % HF 1.25 g CaCO ₃ Thickness unspecific	Gentamicin 150 and 300 µg	Burst release during the first hour followed by the gradual and stable release (40-80 %)	8 h	287
Ti pure	PEO 0.1-0.15 M GLyCa Thickness unspecific	Simvastatin 25 and 50 µg	Burst release during 24 h followed by the gradual and stable release (80 %)	4 weeks	288
Ti pure	PEO 12 mL HF 40 mL HNO ₃ 5-15 µm	BSA 3750 µg	Burst release after 4h followed by slow release (70 %)	48 h	123
Ti-6Al-4V	PEO 0.042 M Ca(NO ₃) ₂ ·4H ₂ O 0.025 M NH ₄ H ₂ PO ₄ 1.7-1.8 µm	BSP-2 500 ng/mL	Burst release after 3 days followed by slow-release stage (80 %)	35 days	289
Ti pure	PEO 0.08 g /L CaCO ₃ 0.04 g/L KOH 4 µm	Rifampin 1 mg/mL	Burst release during first 10 h followed by gradual and stable release (55 %)	96 h	290
AZ91	PEO 5 g/L K ₃ PO ₄ ·3H ₂ O 2 g/L KOH 19-24 µm	BSP	Burst release first 2 days followed by sustained release for 24 days (70 %)	24 days	127

For this reason, numerous assisted loading strategies have emerged using monolithic layers and dispersed nano/microparticle drug carriers which will be discussed in the following section.

1.7.3 Hybrid and smart drug carriers

In the last decades, nanostructured materials have been employed for drug delivery purposes in order to control the burst release during the first hours of implantation. Nanomaterials are classified by the dimensions of the structure 0D (cluster, filaments, quantum), 1D (bars, wires), 2D (layers, films) and 3D (bulk, polycrystals). They can subsist as single, focused or aggregate into forms from spherical to other irregular shape²⁹¹.

Nanocontainer carriers can be classified as nanoparticles (polymer, ceramic or metallic), nano-capsule and nano-sphere, liposomes, micelles, dendrimers, solid core and mesoporous. These nanomaterials are developed to obtain systems with optimized drug loading and release properties, low toxicity and long shelf-life¹⁴³. The potential interest in orthopaedic and cardiovascular implants is that it is possible to modulate the surface of the nanocontainer carriers with organic molecules controlling the drug release using internal and external stimulus such as pH, temperature, light, redox agents, magnetic and electric fields, ultrasonic, enzymes or glucose^{292,293,294}. Of all of them, the great interest on orthopaedic implant are pH and temperature due to a reaction in the human body against the implant suggesting a change in pH and T. For instance, materials with pH responsiveness can be employed to differentiate the normal tissue (pH 7.4) from the tumour tissue (pH 5.7–7.0)²⁹⁵. Temperature is another stimulus frequently used as external, the heat is applied from the outside, or internal, the T with tumour or inflammation is around 40-42°C²⁹⁶.

However, nanocontainers are designed in order to treat tumours and for gene therapy, the administration is done through an injection which may be directed to the target area. So these drug delivery carriers are not suitable for the elaboration of orthopaedic and cardiovascular implants. For this reason, we will focus on bulk layer materials which will be discussed in the next section.

1.7.3.1 Bulk layer carriers

The bulk layer carrier is placed in a fixed location and the drug release is ensured to the surrounding tissue at the target site, reducing the eventual release and avoiding toxicity to other non-target sites. In addition, bulk layer carrier presents high flexibility, which means versatility for different applications in the body.

Hybrid hierarchical materials

As described above, hybrid hierarchical materials comprising a ceramic layer and a porous polymer coating have a potential to be excellent candidates as drug delivery carriers. These systems can improve the corrosion resistance of Mg alloys maintaining an excellent cell response and present the possibility to load drugs, either in the ceramic or in the polymeric layer that can be released in a controlled manner²⁰⁵

In order to incorporate the drug into a polymer coating, different alternatives can be employed including mixing with a polymer solution and further evaporation^{297,298}, soaking the free-drug into drug solution, site specific binding of the drug to the polymer, pre-loading in nano-microparticles and drug polymer conjugation²⁹⁹. The selection of the most appropriated method depends on the polymer-drug compatibility, the preparation method required, drug stability and the targeted drug release rate. The drug release from polymer coating depends on the thickness and permeability of the polymer film, polymer degradation rate, size and morphology of the pore, and solubility of the drug³⁰⁰. The drug release kinetics can be governed by different physical processes such as desorption, dissolution, diffusion, degradation of matrix, erosion, osmotic pressure and swelling³⁰¹. Another form to modulate the drug release involves the use of stimuli-responsive materials. By response to external stimuli such as pH, temperature, light, redox agents, magnetic and electric fields, ultrasonic, enzymes or glucose^{292,300}, the drug delivery process can be controlled.

Table 12 illustrates illustrative examples of drug release from the hybrid materials on Mg alloys. As it can be observed for some of the examples included in **Table 12**, a burst release is typical during the first few hours followed by a stabilization and slow release over time. This observation is due to the drug entrapment preferentially near the surface of the polymer coating. Compared to stand-alone ceramic coating used as direct loading (**Table 11**), hybrid material reduces significant initial “burst” release, obtaining a nearly linear delivery over time, up to 50 days.

Table 12. Hybrid coatings based on Mg alloys functionalized with polymer coatings adapted from ¹⁶⁶.

Alloy	Ceramic carrier	Polymer carrier	Drug and concentration	Release rate	Release period (days)	Ref.
Mg-Al	Hydrothermal treatment 14 mM Ca(NO ₃) ₂ 8.4 mM NaH ₂ PO ₄ 4 mM NaHCO ₃ 11.26 μm	PAA Multilayer film 21.82 μm	Gentamicin 10 μg/mL	Burst release for first hour (23.1 %) followed subsequent long-term slow release (88.78 %)	15	302
Mg-Al	PEO Na ₂ SiO ₃ ·5H ₂ O NaOH Thickness unspecified	PLLA and PLGA Monolithic layer Thickness unspecified	Paclitaxel 8 wt. %	Burst release from PLGA/PLLA (50:50) after 25 days (80 %) compared to others samples which released (<20 %)	50	303
Mg-Y-RE	PEO Na ₂ SiO ₃ ·5H ₂ O NaOH Thickness unspecified	PLGA nanoparticles and glutaraldehyde 150–300 nm	Paclitaxel -	Burst release from PLGA particles during the first hour followed subsequent long-term slow release (25 %) while slow release was observed on PLGA/glutaraldehyde (<25 %)	45	304
Mg-Al	PEO Na ₂ SiO ₃ ·5H ₂ O NaOH Thickness unspecified	EN- plating and PLGA Monolithic layer Thickness unspecified	Paclitaxel 8 , 10 and 12 wt. %	Burst release from all specimens followed long-term slow release (<20 %)	50	305

As it is seen in **Table 12**, the majority of researchers have focused on evaluate the drug release from monolithic polymer layers, obtaining a burst release during the first hours of immersion and a low drug release at the end of the test. In order to avoid these problems, an interesting strategy would be the drugs release from porous polymer layers in hybrid systems, which has not been studied to date. In addition, the effect of drugs on the rate of implant degradation is also an important challenge for magnesium alloys, because drugs can have an inhibitory or accelerating effect on corrosion.

1.8 Summary

The literature review of the stand-alone and hybrid biomaterials and their main applications in tissue engineering clarifies the focus of this Doctoral Thesis in regards to Mg-based temporary implants as follows. The still remaining challenges, related to the enhanced degradation control, while ensuring the bioactivity and additional functionality of Mg-based implants, the proposed strategies and potential clinical applications are summarized in **Table 13**.

Table 13. Proposed strategies to advance the state of the art of functionalized Mg based implants.

	Material	Challenges	Proposed Strategies	Application
Temporary implants	Mg-Ca Mg-Ca-Ag MgZn _x Ca _y	Degradation control Alternative to commercial (Mg-Al and Mg-RE) implant	Biocompatible alloying elements as Zn, Ca and Ag in the human body	
	PEO PCL /PLA	Degradation control Functionalization of the surface with ceramic and polymeric coatings	Plasma electrolytic oxidation by the functionalization with inorganic species 3D polymeric top-coat layer	Bone regeneration (screws, plates, pins)
	Anti- inflammatory agent and antibiotics	Chronic pain, device rejection and failure	Prototype of selective functionalization	

2. CHAPTER II- Hypothesis and Objectives

2.1 Hypothesis

The research programme of this Thesis is based on the hypothesis that it is possible to fabricate a hybrid hierarchical ceramic/polymer coated system for Al-free and RE-free Mg alloy-based temporary implants, improving on the degradation characteristics of the non-treated alloy. The following aspects will be taken under consideration: 1) enhancement of the degradation rate control and 2) control of the drug-eluting capacity. The hybrid coating system will comprise a porous ceramic bioactive layer and one or more biodegradable polymer layers with micrometer size surface topography. The coating system may be loaded with one or several bioactive pharmaceutical agents and will ensure their controlled gradual release. The selected bioactive pharmaceutical agents may additionally provide a corrosion inhibiting effect. This Thesis aims at design, characterization and evaluation of each key element of the proposed hybrid system individually, as well as of the fully assembled proof of concept.

2.2 Objectives

The specific objectives of the research programme constituting the multifunctionalization steps are:

I. Characterization of the alloys

- Characterization of microstructures, composition and morphology of Mg-Ca, Mg-Ca-Ag and MgZn_xCa_y alloys by Scanning Electron Microscopy, Transmission Electron Microscopy and X-Ray Diffraction. Determination of the role of second phases in corrosion mechanism of the alloys by Scanning Kelvin Probe Force Microscope.
- Evaluation of the corrosion behavior of non-coated magnesium alloys during short-term (electrochemical AC and DC tests) and long-term (hydrogen evolution) immersion in a selected physiological solution at 37°C.

II. Electrochemical surface modification

- Design and fabrication of bioactive and corrosion resistant coatings on selected Mg alloys by means of PEO. Design of stable PEO electrolytes containing bioactive elements, such as Ca, P, Si, or F, by modifying the chemical composition, molar concentration, pH and conductivity of the electrolyte, and adjustment of the PEO process parameters such as voltage, treatment times and current density, in order to obtain homogeneous ceramic coatings with sufficient thickness and content of

bioactive elements. Characterization of the coating morphology, composition and microstructure and surface characteristics.

- Evaluation of the effect of the physiological solution compositions over the degradation behavior of the coated and non-coated alloys. Evaluation of the corrosion behavior of PEO-coated magnesium alloys during short-term (electrochemical AC and DC tests) and long-term (hydrogen evolution) immersion in a selected physiological solution at 37°C. Identification of the PEO coating candidates for further functionalization, based on the corrosion behavior screening.

III. Deposition of polymeric hierarchical structures on PEO coatings

- Design and fabrication of polymeric hierarchical top-coats by BFs methodology optimizing the different parameters of the process in order to obtain surfaces with regular and uniform porosity.
- Evaluation of the corrosion behavior of hybrid hierarchical PEO/polymer coatings during short-term (electrochemical AC and DC tests) and long-term (hydrogen evolution) exposure in physiological solution at 37°C.

IV. Drug-eluting functionalisation of hybrid hierarchical coating systems

- Screening of the corrosion inhibitor effect of the pharmaceutical agents. Incorporation of selected anti-inflammatory and antibiotic agents, into the polymer layers of the hierarchical coating, elucidating the effect of the structured morphology of the polymer top-coat on drug release control.
- Determination of the drug release capacity of the developed hybrid coating systems.
- Evaluation of the corrosion behavior of selected functionalized hybrid hierarchical Mg-alloy based systems during long-term (hydrogen evolution) exposure in a physiological solution at 37°C.
- Determination of the corrosion protection mechanism of the selected functionalized hybrid hierarchical coating systems using local electrochemistry and dissolved gas concentration measurements techniques.

3. CHAPTER III- Materials and methods

3.1 Materials

3.1.1 Mg-Ca and Mg-Ca-Ag alloys

Cast ingots of Mg0.6Ca and Mg0.6Ca2Ag alloys were supplied by Magnesium Innovation Centre (presently, The Institute of Surface Science of Helmholtz-Zentrum Hereon, Geesthacht, Germany). The composition of the alloys is illustrated in **Table 14**. The specimens were cut into $60 \times 6 \times 4 \text{ mm}^3$ bars.

Table 14. Spark OES analysis of the Mg0.6Ca and Mg0.6Ca2Ag alloys composition (wt. %).

Alloy/EDS	Mg	Ca	Fe	Cu	Mn	Ag	Ni	Si	Al
Mg0.6Ca	balanced	0.504	0.0002	0.00156	0.015	0.0005	0.0004	0.001	0.01985
Mg0.6Ca2Ag	balanced	0.504	0.0002	0.0004	0.03715	2.417	0.0005	0.001	0.0002

3.1.2 MgZn_xCa_y alloys

Cast ingots of MgZnCa and Mg3Zn0.4Ca alloys and extruded ingot of Mg0.5Zn0.2Ca alloy were supplied by Magnesium Innovation Centre (presently, The Institute of Surface Science of Helmholtz-Zentrum Hereon, Geesthacht, Germany). The cast ingots were cut into $25 \times 30 \times 4.2 \text{ mm}^3$ while extruded ingot was cut into $3 \times 4.7 \text{ mm}^3$. The compositions of the alloys are presented in **Table 15**. All specimens were ground with SiC abrasive paper to P1200 grit size and cleaned with deionized water and isopropyl alcohol before the PEO treatment.

Table 15. Spark OES analysis of the MgZn_xCa_y alloys composition (wt. %).

Alloy/EDS	Mg	Ca	Zn	Fe	Cu	Mn	Ag	Ni	Si
MgZnCa	balanced	1.47	1.012	0.007	0.002	-	-	-	-
Mg3Zn0.4Ca	balanced	0.4	3.14	0.012	0.002	-	-	-	-
Mg0.5Zn0.2Ca	balanced	0.23	0.47	0.002	0.001	0.0001	0.031	0.001	0.009

3.1.3 Polymeric materials

Poly(ϵ -caprolactone) (PCL) and polylactic acid (PLA) (**Figure 10**) were supplied by Naturplas (France). Poly(ϵ -caprolactone) is a biodegradable polyester with a boiling point of 60°C, a glass transition temperature of -60°C and, the density of 1,145 g/cm³. Polylactic acid is a thermoplastic polyester with a boiling point of 130-180°C, a glass transition temperature of 60-65°C and the density of 1,210-1,443 g/cm³.

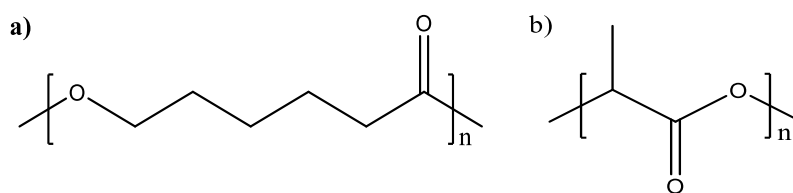


Figure 10. (a) Polycaprolactone and (b) Polylactic acid structures.

3.1.4 Active pharmaceutical agents

3.1.4.1 Anti-inflammatory agents

Paracetamol (4-Acetamidophenol) with 98 % purity and Aspirin (Acetylsalicylic acid) with 99 % purity were supplied by ACROS OrganicsTM. Naproxen (99 % purity) was supplied by FisherScientific.

3.1.4.2 Antibiotic agents

Streptomycin sulfate (99 % purity), Gentamicin sulphate (99 % purity) and Ciprofloxacin (98 % purity) were supplied by ACROS OrganicsTM.

3.2 Surface modification

3.2.1 Plasma electrolytic oxidation (PEO)

PEO treatment was carried out using a set-up comprising a 2 kW regulated AC power supply (EAC-S2000, ET Systems electronic) and a 2 L double jacket thermostatic glass cell with re-circulating cooling system (**Figure 11**) in order to control the temperature of the electrolyte at 20°C. Mg_{0.6}Ca, Mg_{0.6}Ca₂Ag or MgZn_xCa_y, alloy specimen was used as a working electrode and a stainless-steel cylinder mesh (AISI 316L, Ø15 cm) as a counter electrode. Electrical contact with the specimen was made through the threaded hole using an insulated copper rod. A square AC waveform with a 50 % duty cycle was applied during PEO treatment with an appropriate positive-to-negative pulse ratio (**Table 16**). An initial ramp of 60 s was used to reach the pre-set positive and negative voltage amplitude values. The root-mean-square (rms) voltage and current responses were controlled electronically, with a sampling time of 0.1 s, employing a Keithley KUSB-3116 data acquisition card (16 bit, 500 Ks/s) and Labview program (National Instruments). Instantaneous voltage and current values were monitored using a 2-channel Tektronix TDS 2012B oscilloscope at 100 MHz sampling rate. The compositions of used electrolytes, prepared with 99 % purity grade reagents and deionized water are presented

in **Table 17**. The electrolyte design strategy will be discussed in Chapter V (Section 5.1). After PEO treatment, the samples were rinsed in deionized water, isopropyl alcohol and dried in warm air.

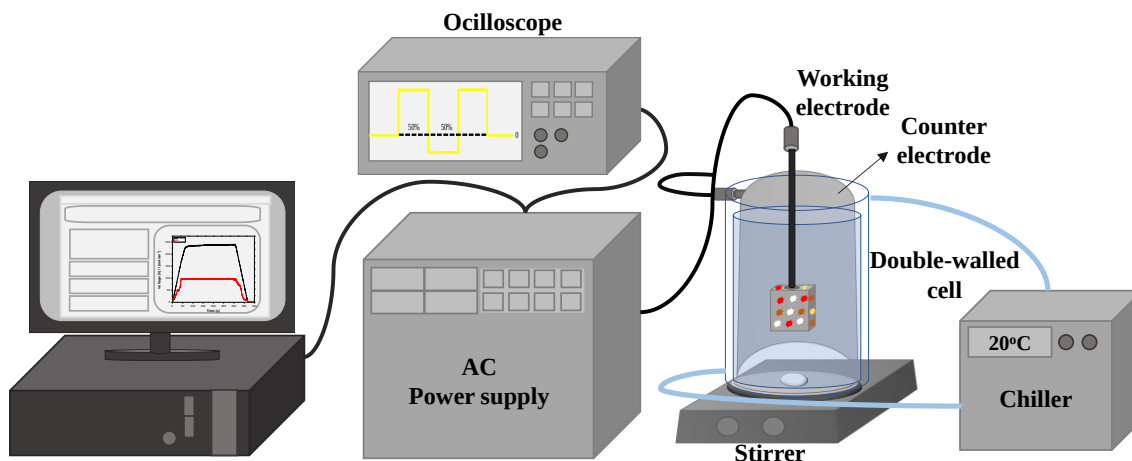


Figure 11. Schematic representation of the electrochemical set-up for the PEO coating process.

Table 16. Parameters of the PEO process.

Parameters/Electrolyte	PEO-8F	PEO-3F	PEO-T-2Ca	PEO-S-3Ca
Positive and negative voltage pulse amplitudes (V)	+440/-50	+350/-50	+350/-50	-350/-50
Current density (mA/cm ²)	138	100	100	
Time (s)	420	300/600/900	300/600/900	300/600/900

Table 17. Chemical composition, pH and conductivity of PEO electrolytes.

Electrolyte (g/L)	PEO-8F	PEO-3F	PEO-T-2Ca	PEO-S-3Ca
Composition	suspension	transparent	transparent	suspension
Na ₃ (PO ₄)·12H ₂ O	10	5	10	10
Na ₂ (SiO ₃)·5H ₂ O	-	5	10	9
KOH	1	8	8	1
GlyCa*	-	2	2	-
CaO	2.9	-	-	2.9
Na ₂ EDTA·2H ₂ O	-	3.67	3.67	-
KF	-	3	-	-
NaF	8	-	-	-
σ (mS/cm ²)	28.9	31.3	40.5	24.6
pH	13.11	13.41	13.45	12.92

*Calcium glycerophosphate, C₃H₇CaO₆P

3.2.2 Polymer Breath Figures

Breath figures allow the preparation of porous surface controlling the size and distribution of the pores; the mechanism of pore formation is illustrated in **Figure 12**. The polymer deposition was carried out by dip coating method (model, VT-04 control unit) using PLA and PCL as polymers and chloroform as solvent at room temperature and a constant relative humidity (>99 % RH), (monitored by a digital hygro-/thermometer, VWR) in a closed chamber. **Figure 13** shows the set-up of the system employed for the deposition of the polymer coating.

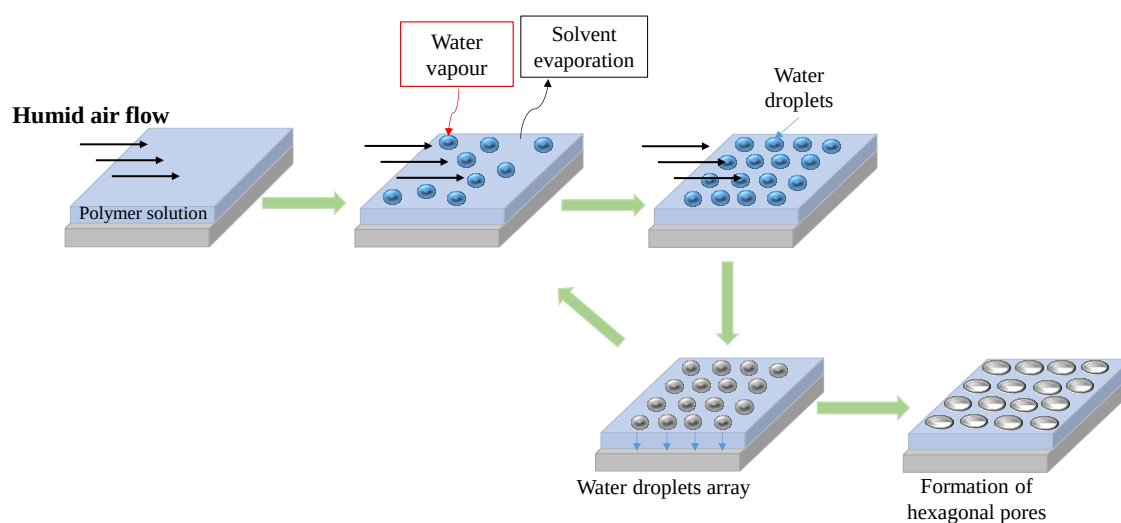


Figure 12. Model of pattern formation by breath figures approach. Adapted from ²³².

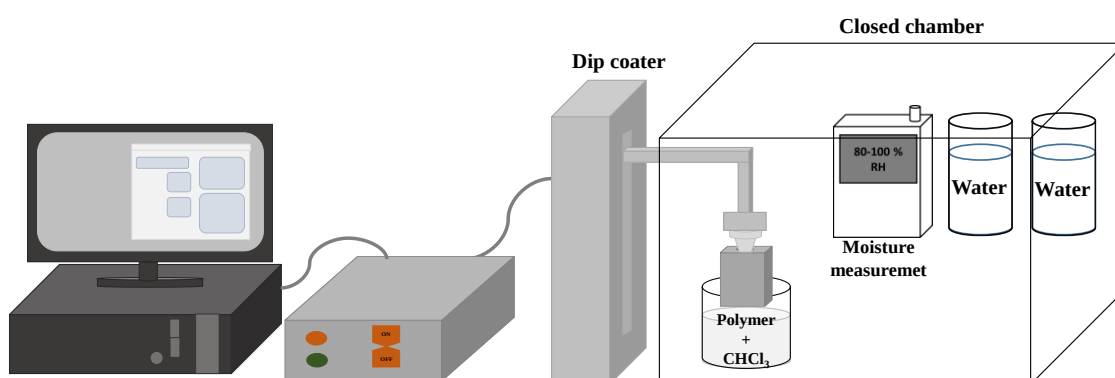


Figure 13. Schematic diagram of the system employed for the polymer deposition onto PEO coatings.

3.3 Surface characterization

3.3.1 Scanning electron microscopy

Scanning electron microscopy (SEM) employs a light microscope of high magnification which has a potential to reveal the microstructure at a micro- and nanoscale by scanning the surface of the sample with a stable electron beam. Electron and photon

emissions are produced as a result of the electron beam hitting the atoms of the material analysed. Imaging is performed by detecting the generated electrons which, depending on their nature, can be classified as secondary or backscattered electrons. Secondary electrons are those resulting from the impact of an electron from the incident beam with an atom of the substrate, removing an electron from it. Backscattered electrons are electrons from the incident beam after it has impacted and bounced off a substrate atom. The compositional (semi-quantitative) analysis is performed by detecting high-energy photons emitted by the material. After the electronic impact, the atoms are excited and, upon electronic relaxation, these photons are emitted. This technique is known as energy dispersive X-ray spectroscopy (EDS).

In this work, the metallographic study of all the substrates, PEO-coated samples and hybrid materials were carried out using JEOL JSM-6400 and JEOL JSM-820 equipment operating at 20 kV, which incorporates an OXFORD LINK PENTAFET 6506 EDS analysis system and a backscattered scanning electron detector (BSE). The cross section of all specimens were prepared for examination by grinding to P1200 finish followed by polishing with 3 and 1 diamond pastes and coated by graphite or gold. The longitudinal section of polymer-coated samples were analysed by using a Philips XL30 microscope operating at 25 kV and the porous surfaces were metallized with gold-palladium (80/20) in order to improve the visualization.

3.3.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is a high-resolution (<0.25 nm) microscopy widely used for the detailed study of the atomic arrangement in crystal structures. The focused electron beam penetrates the samples and the images are produced from both transmitted and deflected electrons. The incident electrons along the beam path can be scattered (i.e., elastic and inelastic) by atoms within the sample. In addition, a diffracted (scattered) and a transmitted (unscattered) beam is produced on the background of the sample. Depending on the position of the objective, three main imaging modes are commonly used: brightfield, darkfield and high-resolution microscopy. Interaction with an atomic nucleus or electron cloud could cause diffraction of the incident beam. The scattering will be higher (both inelastic and elastic) and the image will be darker, the heavier the atom the brighter field imaging mode. The interaction between the incident beam and the crystalline structures is much more important. From this type of interaction,

diffraction patterns can be obtained in order to characterise the phases. In addition to transmitted and diffracted beams, the same signals that are generated in the SEM are generated in the incident electron beam in the TEM: secondary electrons, backscattered electrons and characteristic X-rays.

In this work, a JEM 2100HT JEOL instrument equipped with EDS operated at 200 keV of acceleration voltage was used in order to examine the secondary phases and composition of $\text{Mg}_{0.6}\text{Ca}$ and $\text{Mg}_{0.6}\text{Ca}_{2}\text{Ag}$ alloys. However, MgZn_xCa_y alloys were characterised using TEM JEOL JEM 3000-F, operated at 300kV equipped with an XEDS (OXFORD INCA) for compositional analysis. The PDF-4 + 2019 software containing the ICDD database was used to determinate the crystal system, space group and lattice parameters of the sample. TEM samples (disks with a diameter of 2.5 mm and $<80\text{ }\mu\text{m}$ -thick) were prepared by ion milling using a 691 GATAN PIPs system with a small incident angle until perforation.

In order to examine the microstructure and morphology of PEO coating in more detail, the cross-section of $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}$ specimen was analysed by TEM using JEOL JEM 1400 instrument operated at 200 kV. The sample was assembled in a sandwich structure (diameter 3 mm) and subsequently thinned until $<0.1\text{ mm}$ thick. After that, the specimen was prepared by ion milling in a 691 GATAN PIPs system with a small incident angle until perforation.

3.3.3 Scanning Kelvin Probe Force Microscopy

Scanning Kelvin Probe Force Microscopy (SKPFM), also known as surface potential microscopy, is a scanning technique derived from atomic force microscopy (AFM); it is based on the measurement of electrostatic forces between an AFM tip and the sample under study. The main feature of this technique lies on its ability to simultaneously measure surface potential differences and the topography of different phases with nanometric resolution. Therefore, this technique is especially interesting in corrosion studies as it allows the evaluation of the cathodic/anodic behavior of the different phases present in the alloys. The surface potential measurement is based on the same principle as that used in macroscopic Kelvin probes, although in this case a voltage is applied to the AFM tip with an AC and a DC component. The latter is continuously readjusted by the device in such a way that it matches the Volta potential value also known as contact potential.

In this work, the surface potential maps of the Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys were carried out by NANOSCOPE IIIA Multimode (SKPFM, Bruker, Billerica, MA, USA) equipped with Pt coated Si tip operated in tapping mode. Topographic images and potential maps were obtained with the tip-to-sample distance fixed at 100 nm. The tip to sample distance was kept constant at 100 nm using a two-pass technique, where the height data is recorded in tapping mode during the first pass and the tip lifts above the surface to an adjustable lift height and scans the same line while following the height profile recorded in the second pass. All measurements were made at room temperature with a relative humidity between 40–65 %.

The MgZn_xCa_y alloys measurements were made using a digital SKPFM instrument Nanoscope III (Bruker, USA) equipped with Extender™ electronic module which was operated in the two pass scan mode. The topography of the surface was lifted off the surface to a height 100 nm using two scans. Piezo oscillations were switched off and AC signal with the amplitude of 5 V was applied between the sample and the AFM tip to cause electrostatically induced oscillations of the cantilever. Subsequently the Volta potential difference (VPD) was measured between the sample and the cantilever using the nulling technique. More details related to the theory behind the measurements can be found in these reports ^{306,307}. For all measurements heavily doped Si probes coated with Pr/Cr layers, Budget Sensors, have been used. The measured VPD is presented versus the standard Ni surface which is useful for significantly reducing the error due to wearing out the AFM tip ³⁰⁸. The following typical parameters and conditions were used for the measurements: scan size 50 × 50 μm, scan rate 0.3 Hz, the engage setpoint was maximized to reduce the force between the tip and the surface; temperature was about 25°C and relative humidity about 52 %. Three different places were studied on each of the Mg alloys to ensure reproducibility. MgZn_xCa_y alloys were abraded on SiC papers and then polished using diamond pastes followed by 100 nm SiO₂ non aqueous suspension. The polished samples were cleaned on a soft sponge soaked in 2-propanol, dried and stored in a desiccator before analysis.

3.3.4 Crystalline structure

X-ray diffraction (XRD) is a non-destructive technique based on diffraction phenomena where an incident beam of X-rays is produced on crystalline solid sample. This technique provides information about the crystalline structure of the materials which permits the identification of their phase composition.

A PANanalytical X'Pert-MRD diffractometer (Amsterdam, The Netherlands) with $\text{Cu-K}\alpha = 1.54056 \text{ \AA}$ radiation and 2θ values between 10° and 90° in steps of $0.04^\circ/\text{s}$. was used for X-ray diffraction analysis to characterise the phase components of $\text{Mg}_{0.6}\text{Ca}$ and $\text{Mg}_{0.6}\text{Ca}_{2}\text{Ag}$ alloys and PEO coatings. A PANanalytical X'Pert-MRD Research Edition was used to characterize $\text{Mg}_{0.6}\text{Ca}_{2}\text{Ag}$ using 2θ values between 10° and 30° at steps $0.04^\circ/\text{s}$. PANanalytical X'Pert-MRD diffractometer ($\text{CuK}\alpha = 0.154056 \text{ nm}$, $0.04^\circ/\text{s}$ scanning speed, 2θ range: 10 to 80 or 90°) was used to MgZn_xCa_y alloys with and without PEO coatings. The spectra of all specimens were analysed with X'PertHighScore software and the ICDD PDF4+ database.

3.3.5 Topography

The focus-variation optical profilometry is a technique used to measure piece geometry, roughness and surface texture in large areas and volumes with excellent resolution (0.4 nm lateral resolution and 10 nm vertical resolution). A modulated light beam, which is focused on the sample through a beam splitter creating coaxial illumination is used. The light reflected by the sample is projected onto a high-precision moving digital sensor capable of adjusting the focus. All positions of the sample are measured in focus at a given distance, allowing to obtain extremely fine images to be obtained in which the interference produced by the light arriving from the different optical fields of the entire sample under analysis has been removed. Sequential measurements of different focused planes are taken and three-dimensional images can be reconstructed with this information. In this work, Infinite Focus SL (Alicona GmbH, Austria) was used to evaluate the roughness parameters S_a (arithmetical mean height of the area) and S_{10z} (ten-point height). The topography results were extracted using the IF-Measure Suite software.

3.3.6 Coating thickness

Coating thickness was determined as an average of ten measurements by Fischer ISOSCOPE- FMP10 portable eddy current meter and later confirmed with cross-sectional SEM micrographs.

3.3.7 Porosity

The pore population density and pore size of the coatings were estimated using ImageJ software. Image analysis of the coating surface was carried out using three SEM micrographs taken at arbitrarily locations.

3.3.8 Wettability

FTA1000 Drop Shape Analysis System (First Ten Angstroms, USA) and the accompanying FT32 software was employed in order to determinate the wettability of the PEO-coated and hybrid materials specimens. The drops were recorded in a trigger mode within 25 s from the moment the drop of the droplet is within 1 mm of the surface. Contact angles were measured over 50 frames taken at 0.5 s intervals with an average of three measurements for each surface treatment; the variability of the measurements was within $\pm 10\%$.

In addition, the water contact angle measurements of all loaded-specimen were carried out using a KSV theta goniometer controlling the volume of the droplets to 5.0 μL . The charge-coupled device camera was used to capture the images of the water droplets for the determination of the contact angle values.

3.3.9 Microhardness

The hardness tests were carried out using an AKASHI Vickers microhardness tester model AVK-AII. Due to the very thin thickness of certain ceramic coatings, indentations were made with Vickers indenters using two different loads of 10 g applied for 15 s. The images corresponding to the hardness indentations were taken with a MOTIC digital camera and measured using the AXIOVISION software to calculate the hardness of the coating using the expressions **Equation 11**.

$$H_V = \frac{1.8544 \cdot P}{d^2} \quad \text{Equation 11}$$

HV is the Vickers microhardness, P is the applied load (kg) and d is the average of the footprint diagonal measurements (μm). The hardness was expressed as the average of three measurements made for each specimen.

3.4 Ion release analysis

3.4.1 Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Inductively Coupled Plasma (ICP) is a technique for multi-element analysis by optical emission spectroscopy (ICP-OES), which is mainly used for the analysis of liquid samples or solids dissolved in liquids. Its detection limits range from sub-parts per billion (sub-ppb) to 0.1 parts per million (ppm), which are similar to or better than those provided by flame atomic absorption. Seventy different elements are determined with the possibility of simultaneous measurement of several elements. The method is based on the fact that the atoms and ions of each element in the plasma are excited and emit a characteristic spectrum in the ultraviolet and visible region. The intensity of the light at one of the characteristic wavelengths is proportional to the concentration of that element in the sample. It is measured and related to the concentration of each element by calibration curves.

In this work, PEO-8F coatings on MgZnCa and Mg₃Zn_{0.4}Ca alloys and their substrates with an exposed area of 4.51 cm² were immersed in 25 mL of 0.9 wt. % NaCl solution at 37°C. Mg, Zn, Ca, P and Si ions release was analysed after 1, 3, 5 and 12 days of immersion. In order to dissolve the precipitates, the solution was acidified with 200 µL of nitric acid (65 wt. %). The samples were analysed using a Perkin-Elmer 3300 DV instrument, operating at RF power of 1.3 kW, with a plasma flow of 1 L/min, sample flow rate of 0.8 mL·min⁻¹ and argon (plasma maintaining carrier gas) flow of 0.8 L/min. The blank of 0.9 wt. % NaCl was included into the batch measurements as a reference and the results are cited as an average of three replica measurements.

3.4.2 Fluoride ion selective electrode

Fluoride ions released during immersion of PEO-8F-coated Mg_{0.6}Ca alloy (10.58 cm² of surface area) and PEO-8F coated MgZnCa and Mg₃Zn_{0.4}Ca alloys (4.51 cm² of surface area) specimens in 0.9 wt. % NaCl at 37°C were measured using a fluoride ions selective electrode that consists of a lanthanum fluoride monocrystal membrane doped with europium. The membrane potential difference depended on the concentration of F⁻ in the solution and was measured using a reference electrode of Ag/AgCl and pH meter GLP22 (Crison) every 30 min for the first 2 h of immersion and at 24 h. The calibration curve was obtained using fluoride reference solutions (500 ppb, 1, 2.5, 5 and 10 ppm) prepared from a fluoride standard of 1000 µg/L as (**Figure 14**). Each reference solution

was prepared using 25 mL of a TISAB solution (58 g/L NaCl, 4 g/L $C_{14}H_{22}N_2O_8$ in distilled water, pH adjusted to 5.0–5.5 with 6 M NaOH) in order to normalize the ionic strength.

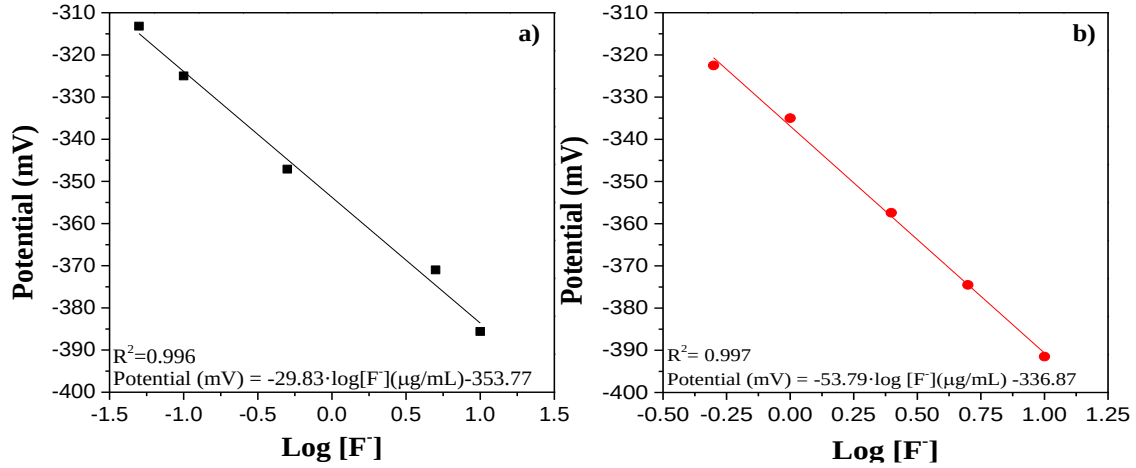


Figure 14. Calibration curve of the F^- standards for as-cast (a) Mg_{0.6}Ca, and (b) Mg₃Zn_{0.4}Ca and Mg₃Zn_{0.4}Ca alloys.

3.5 Corrosion behavior

3.5.1 Electrochemical measurements

The electrochemical behavior of Mg alloys with and without PEO coatings and hybrid ceramic-polymer coatings were evaluated by DC potentiodynamic tests and AC electrochemical impedance spectroscopy (EIS). The corrosion rate, corrosion potential (E_{corr}), corrosion current (i_{corr}) and passive/active potential range were determined by DC potentiodynamic test. The test consists of polarising the sample over a voltage range with respect to the open circuit potential and the current density flowing through the system is recorded. The potential versus current curve is represented from the results obtained. The corrosion occurs at the point where the anodic and cathodic parts of the curve converge. For potentials above E_{corr} (anodic part of the curve) the material is oxidised. For some metals, in constant segments can be found, where no dissolution or a slow dissolution (oxidation) of the material (i_{pass}) occurs and the material is passivated. In the cases when this does not occur, the material is dissolved.

EIS is an alternating current electrochemical technique used to investigate the kinetics and mechanisms involved during the corrosion process of the material. The test consists of applying a sinusoidal voltage signal of a few mV to the working electrode with respect to a reference potential (commonly, an open circuit potential, or OCP) by scanning the total impedance of the system for a frequency range from $\sim 10^5$ Hz to 10^{-2} Hz. Barrier

coatings or processes such as diffusion of active species under an AC voltage represent an impedance that induces a shift between voltage and current, similar to a capacitor in a circuit. The phase shift or phase angle is a time-dependent parameter that changes its magnitude with the frequency of the applied AC voltage. Each process that takes place during testing produces a characteristic time- or frequency-dependent change in the impedance. The experimental impedance can be simulated by means of an equivalent circuit in which each element of resistance and/or capacitance represents an electrochemical process. The capacitances and resistances of the equivalent circuit are calculated to fit the experimental data at each frequency.

3.5.1.1 Electrolyte preparation and electrochemical equipment set-up

Both polarization and EIS test were carried out using a three-electrode cell computer-controlled potentiostat (GillAC, ACM Instrument, **Figure 15** (a)). A double-walled electrochemical cell was connected to a water thermostat in order to control the temperature inside the cell during the tests at 37°C. The specimens for as-cast alloys (surface area 1 cm²) serve as a working electrode, platinum as a counter electrode and silver/chloride electrode (Ag/AgCl, 3M KCl, 0.21 V vs. SHE) as a reference electrode placed inside a Luggin probe. The medium was selected depending on the test.

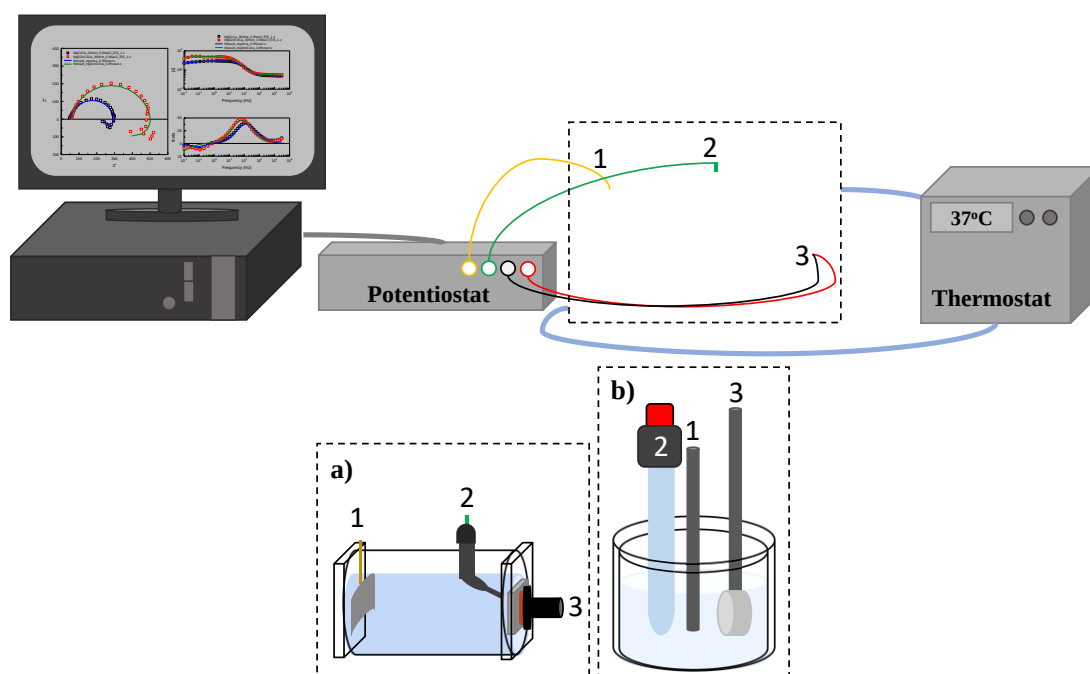


Figure 15. Electrochemical set-up for DC potentiodynamic and EIS test using (a) 1) Pt as auxiliary electrode, 2) Ag/AgCl reference electrode and 3) working electrode. (b) 1) Graphite as auxiliary electrode, 2) Ag/AgCl reference electrode and 3) working electrode for extrude Mg_{0.5}Zn_{0.2}Ca alloy specimens.

Coated and uncoated Mg_{0.5}Zn_{0.2}Ca specimens with an area of 4.26 cm² were carried out in a double-walled electrochemical cell (**Figure 15 (b)**) using graphite as a reference electrode. Potentiodynamic DC polarization was performed in inorganic part (further on termed as inorganic α -MEM) only of an α -MEM (Minimum Essential Medium Eagle: 6.8g/L NaCl, 2.2 g/L NaHCO₃, 0.4 g/L KCl, 0.12 g/L Na₂HPO₄, 0.09 g/L, MgSO₄, 0.2 g/L CaCl₂ (in-house prepared)) solution at 37°C. EIS measurements were performed in inorganic part of α -MEM as well as in a complete α -MEM (containing ribonucleosides, deoxyribonucleosides and sodium bicarbonate, without L-glutamine; supplied by Merck M8042). The OCP after 1 h of immersion was measured before both types of experiments. At least two samples per conditions were employed for both experiments to ensure the repeatability.

3.5.1.2 Potentiodynamic polarization

Polarization measurements were obtained using a scan rate of 0.3 mV/s, potential sweep from -200 mV to +2000 mV relative to the open circuit potential (OCP) and current density limit of 5 mA/cm². The corrosion properties were evaluated from E_{corr} and i_{corr} estimated using cathodic and anodic Tafel slopes.

3.5.1.3 Electrochemical impedance measurements (EIS)

EIS measurements of substrates and PEO coated samples were carried out applying a sinusoidal perturbation of 10 mV amplitude with respect to the OCP and a frequency sweep from 30 kHz to 10 mHz. Zview software was used to analyse the impedance spectra and the equivalent electrical circuit. Where necessary, measurements were carried out following extended immersion periods (e.g. 24 h, 48 h etc).

EIS measurements of hybrid ceramic-polymer and functionalised hybrid ceramic-polymer specimens were carried out using EChem Analyst (Gamry, USA) at Helmholtz-Zentrum Hereon, Geesthacht in inorganic α -MEM at 37°C. The used frequency range was from 100 kHz to 0.1 Hz at an AC perturbation of 5 mV rms with respect to the OCP. In order to prevent the polarisation of the sample, the measurement at low frequencies were avoided. The area exposed to the electrolyte was 4.51 cm² as for the potentiodynamic polarisation tests.

3.5.2 Hydrogen evolution measurements

In order to estimate the corrosion rate of Mg alloys, hydrogen evolution tests of PEO coatings and hybrid coatings were carried out. This method is based on the quantification of H_2 gas released during the corrosion process since this is the main product obtained in the cathodic reaction (see Section 1.4.2, **Equation 6**). Given that, by stoichiometry of the overall corrosion reaction, the oxidation of one mole of Mg generates one mole of H_2 , it is relatively easy to calculate the corrosion rate from the volume of the generated gas. The hydrogen evolution experiments were carried out using two different systems which controlled the pH in the different ways (**Figure 16**).

In **Figure 16 (a)** set up, six samples were immersed in 250 mL of physiological media each one in independent bottles. The samples were localised inside of 25 mL burettes placed in inverted position and filled up with the test medium with the key closed and sealed at the end with Teflon to avoid H_2 loss. This assembly is placed in a thermostatic water bath at 37°C and a water pump was used to agitate and heat up the whole bath. In order to control the pH, the physiological media was replaced every 24 h and the pH was monitored during the test.

In the second set-up (**Figure 16 (b)**) the pH was continuously controlled at 7.4 by CO_2 flowing through the medium. The six samples were testing on a plastic grid immersed in a plastic chamber with 15 L of physiological media; over each sample a 25 mL burette was placed in inverted position. The chamber was placed in a thermostatic water bath at 37°C. A 6 W submersible water pump was used to agitate the media and a drip system was used to renew the chamber medium at a flow rate of 2 mL/100 mL·min (total volume of the medium circulating through the chamber and drip system was 21 L). A pH meter was connected to a solenoid valve controlling the supply of CO_2 in order to maintain pH at 7.4.

With either of the set-ups, the H_2 volume was recorded every 15 minutes for the first hour, every hour of the day-time for the first three days, and then three times a day until the end of the trial. After test, all samples were sprayed with isopropanol and dried, the cross-section of the samples was examined using SEM.

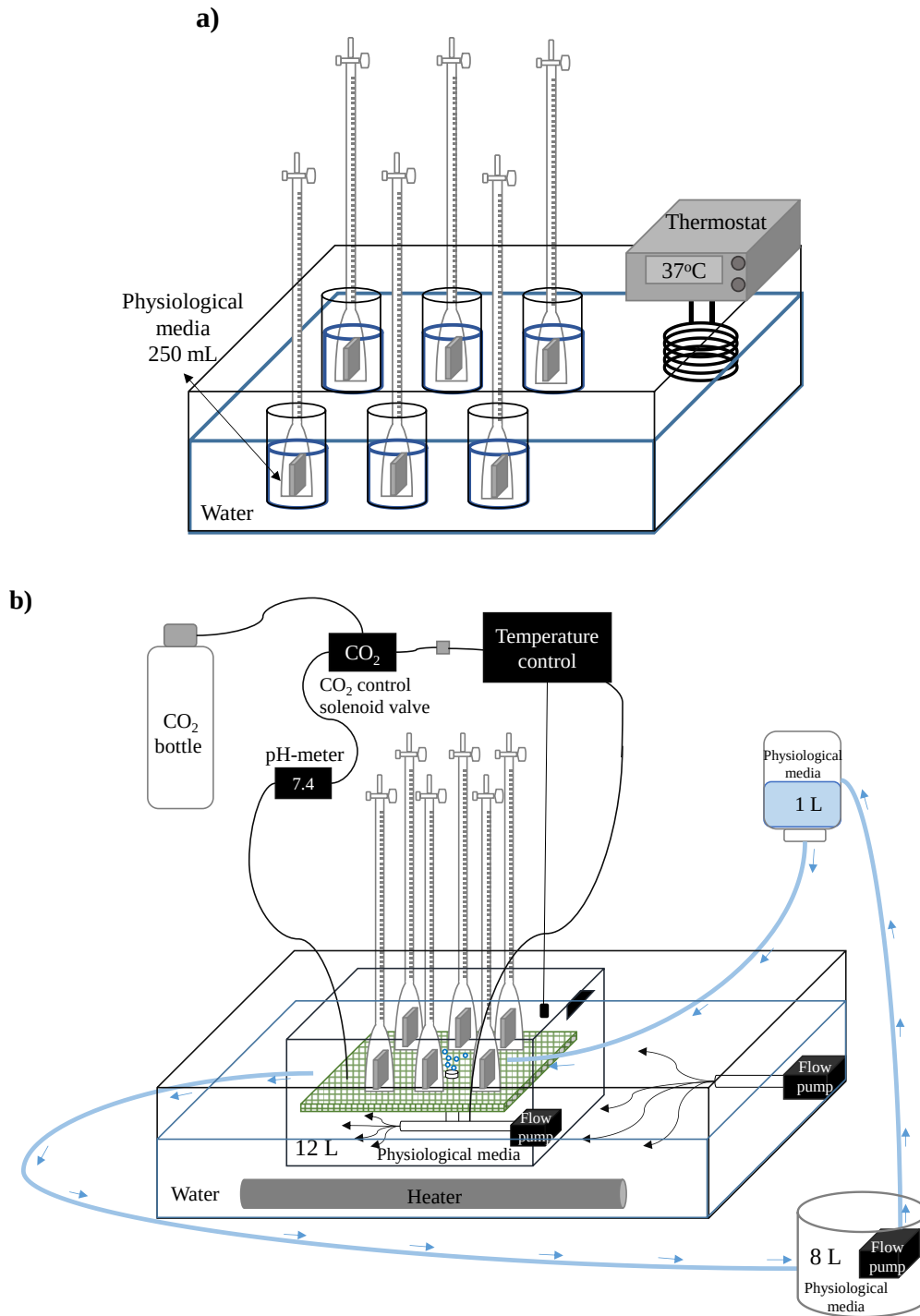


Figure 16. System representation of hydrogen evolution test (a) without CO₂ and (b) under CO₂ flow.

3.5.2.1 Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys

The hydrogen evolution test of Mg_{0.6}Ca, Mg_{0.6}Ca₂Ag with and without PEO-coatings was carried out as represented in **Figure 16** (a) system using m-SBF (**Table 18**) as physiological media for up to 55 days of immersion. In this case, the physiological media was changed every 48 h or when the pH exceeded 8.4.

3.5.2.2 MgZn_xCa_y alloys

In order to evaluate the pH role in the degradation, the corrosion behavior of MgZn_xCa_y alloys was evaluated using both testing systems (**Figure 16**) during 120 h of immersion at 37°C. In the system with periodic replacement of the medium (**Figure 16** (a)), the hydrogen evolution was evaluated in inorganic α -MEM as well as in complete α -MEM. The composition of the physiological media is illustrated in **Table 18**. The medium was changed every 24 h.

In a continuous CO₂ flow set-up (**Figure 16** (b)), the corrosion behavior of MgZn_xCa_y alloys was evaluated in inorganic part of α -MEM. Where necessary, (e.g. for PEO-coated, hybrid ceramic-polymer coated systems with and without pharmaceutical agents) the experiment was extended up to 11 days and 17 days.

Table 18. Composition of the physiological media employed in **Figure 16** set-ups in order to evaluate the hydrogen evolution during corrosion process.

Components	Inorganic	Complete	m-SBF (g/L)
	α -MEM (g/L)	α -MEM (g/L)	
NaCl	6.80	6.80	5.4
NaHCO ₃	2.20	2.20	0.5
KCl	0.40	0.4	0.02
Na ₂ HPO ₄	0.12	0.12	-
MgSO ₄	0.09	0.09	-
MgCl ₂ ·6H ₂ O	-	-	0.31
CaCl ₂	0.20	0.20	0.29
Na ₂ CO ₃	-	-	0.43
K ₂ HPO ₄ ·3H ₂ O	-	-	0.23
Na ₂ SO ₄	-	-	0.07
HEPES	-	-	17.89
Red Phenol	-	0.02	-
Vitamins	-	0.06	-
Amino acids	-	0.97	-
Others	-	1.20	-

3.6 Drug release

3.6.1 UV-visible spectroscopy

Drug release was evaluated by UV-visible spectroscopy. For the drug release optimization and as a probe of concept, a load polymer film was developed depositing 90

μL of polymer/chloroform (30 mg/mL) solution into disc glass (ϕ 12 mm). The films were developed under high humidity inside a closed chamber (80-100 % RH) and also, under low humidity <40 % RH in order to evaluate the surface effect on the drug release. The 1, 2, 5, 10, 20 wt. % of paracetamol and ciprofloxacin were incorporated into PCL, PLA or/and PCL/PLA blends polymers at different proportion 100/0; 25/75; 50/50; 75/25 and 0/100 wt. % In order to incorporate the drugs into the polymer coating, 5 wt. % of drug was dissolved in 75 mg/mL of PCL/chloroform solution. $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}$ was immersed into load polymer solution using dip coating and BF technique.

The drug release from polymer films and hybrid polymer-ceramic materials were measured in inorganic α -MEM solution. The specimens were immersed in 4 mL of inorganic α -MEM solution at 37°C. At regular time intervals, an aliquot of 1 ml was removed from inorganic part of α -MEM solution and the same aliquot was returned in order to not keep constant the concentration of the media. The drug concentration of the solution was measured by its adsorption using UV/VIS spectrometer (PekinElmer instrument, Lambda35) in the range of 200-400 nm in triplicate samples.

3.7 Localized measurements

In order to evaluate the corrosion inhibiting effect of the drug loaded in a hybrid hierarchical coating system, measurements of local changes in current density, pH and dissolved hydrogen concentration in the solution were carried out. The measured samples correspond to $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO}/\text{PCL}$ and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO}/\text{PCL}+\text{CIP}$. Measurements were performed using inorganic α -MEM solution as electrolyte. The specimens were sealed by beeswax, exposing a surface area of roughly $1\text{ mm} \times 4\text{ mm}$. All measurements were carried out in the centre of each sample at 100 μm above the sample surface as it is indicated in **Figure 17**.

The local current density measurements were performed by Scanning Vibrating Electrode Technique (SVET) using an Applicable Electronics™ equipment with 100 μm of distance between the tip and the samples surface on an area of $3\text{ mm} \times 3\text{ mm}$ scanned at a rate of 75 μm per step. The sampling interval at each step was 1 s and the total time for a map (41×41 grid) was approximately 1 h, aiming to move the microelectrodes in that time.

All local pH was recorded by a commercial glass-type pH microelectrode (Unisense, pH-10), together with a mini Ag/AgCl reference electrode and local H_2 concentration was

measured using a H_2 microsensor (pH 10, Unisense, Denmark). Both pH microelectrode and H_2 microsensor were integrated into a commercial SVET-SIET system (Applicable Electronics™) for probe movement. The distance between pH microelectrode and H_2 microsensor were positioned at 100 μm in horizontal planes via micromanipulators. The local pH and H_2 concentration distribution on the surface were scanned at a rate of 100 μm per step and the interval at each step was 3 s.

Local measurements were performed at 37°C under hydrodynamic conditions with a flow rate of 1.0 mL/min controlled by a peristaltic pump (Medorex) using a cell volume of 5 mL, renewing the medium every 5 min. The used medium was discarded and not recirculated. The data was obtained using LV4 software from Sciencewares™ in parallel with PyroOxygenLogger from Pyroscience™.

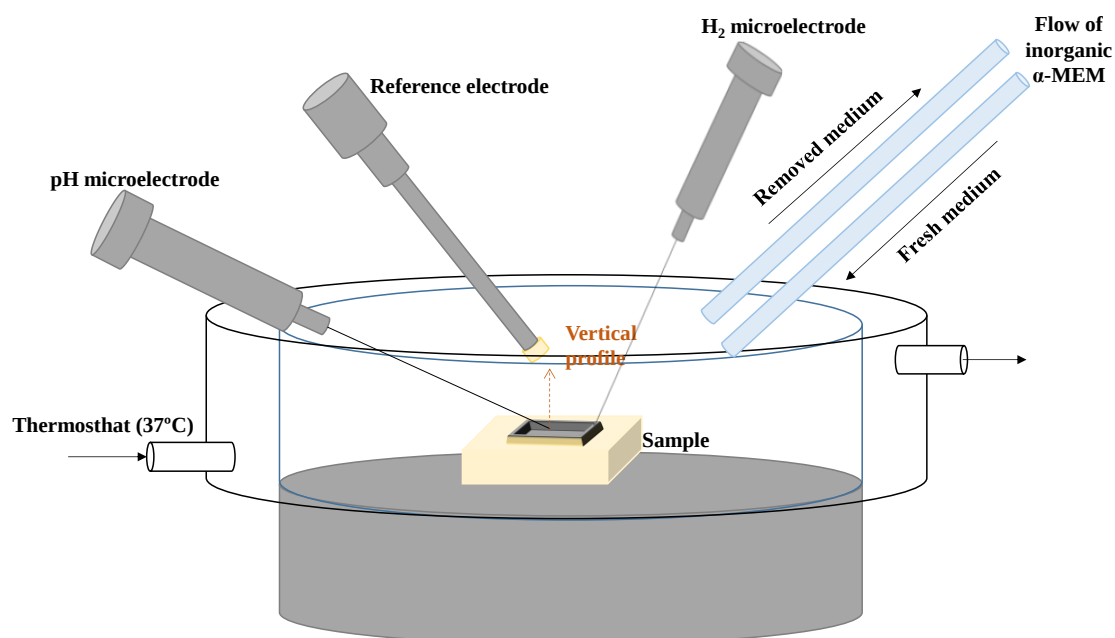


Figure 17. Scheme of setup for local pH and H_2 measurement adapted from ³⁰⁹.

4. CHAPTER IV - Characterization and corrosion of Mg alloys

As reviewed in the introduction (Section 1.1.3), the most used commercial Mg alloy implants are constituted by Mg-Al and Mg-RE systems. The main challenge with these implants is that the degradation products of these systems are toxic and/or allergenic compounds that may cause a harmful effect on the human body ^{30,31}. For this reason, the ongoing efforts in development of potentially non-toxic alloys with suitable corrosion properties and biocompatibility have pinpointed systems such as binary Mg-Ca and ternary Mg-Ca-Ag ³¹⁰ and Mg-Zn-Ca ³³ alloys for tuning in terms of the alloying element content and/or the manufacturing process ⁵².

In this context, this Chapter is focused on the understanding of the corrosion performance fabricated by cast and extrusion, in order to be able to tailor the protection systems accordingly. The structure of the Chapter is divided in two sections: the characterization of i) as-cast Mg-Ca and Mg-Ca-Ag alloys and ii) as-cast and extruded MgZn_xCa_y alloys. The critical goal is to evaluate the effect of the alloy composition and the manufacturing process on the corrosion behavior. In terms of microstructure, the alloys were evaluated by SEM, TEM and XRD. Secondary phases were characterized with special interest in the study of galvanic coupling phenomenon between microconstituents by measuring their Volta potential difference using SKPFM. The corrosion behavior was evaluated by means of electrochemical measurements and hydrogen evolution tests, with a special attention to the medium pH control strategies and selection of the corrosive medium composition. **Figure 18** shows the graphical abstract of Chapter IV.

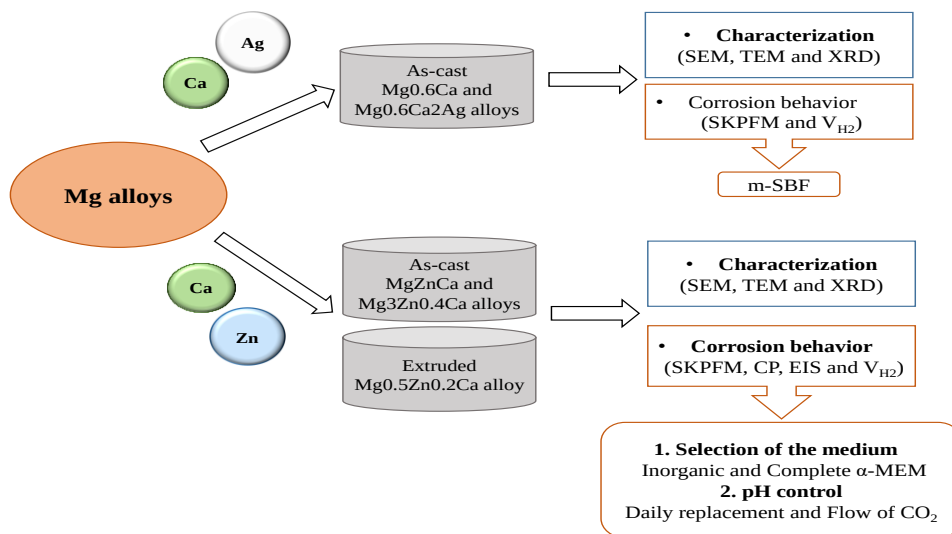


Figure 18. General Scheme of Chapter IV.

4.1 Characterization of Mg-Ca/Mg-Ca-Ag alloys

4.1.1 As-cast Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys

For both materials, dendritic α -Mg grains were revealed (**Figure 19**) with the second phase distributed along the grain boundaries in the form of a discontinuous network and decorating the interdendritic regions (bright contrast regions). The EDS analysis of areas 1 and 4 correspond to the matrix of Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag, respectively (**Table 19**). Points 2 and 3 (**Figure 19** (b)) are located in the second phase of the Mg_{0.6}Ca and reveal a similar amounts of Mg and Ca elements. A considerable amount of Al and Si impurities was detected in Mg_{0.6}Ca alloy. In the case of the Mg_{0.6}Ca₂Ag alloy, two EDS analyses within the interdendritic space (**Figure 19** (d), points 5 and 6) showed the formation of particles with largely different contents of Ag.

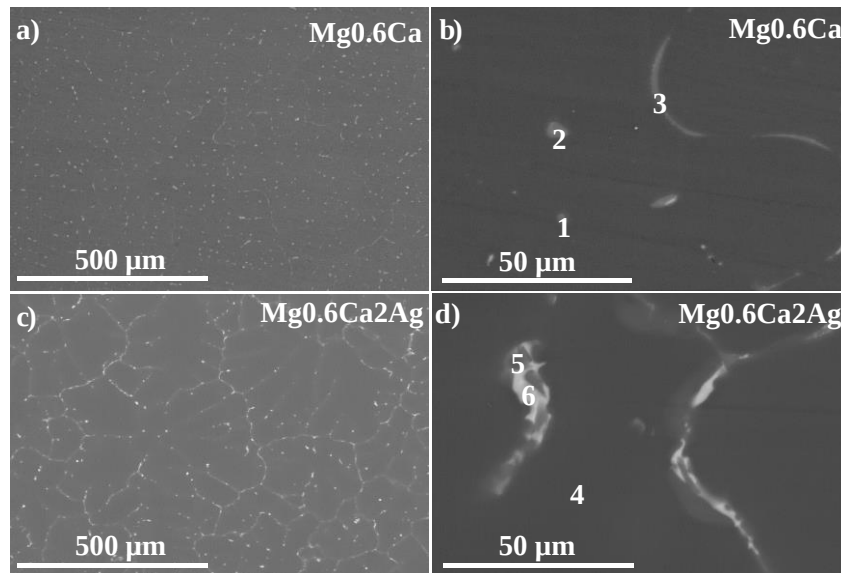


Figure 19. Backscattered electron micrographs of the studied alloys: (a, b) Mg_{0.6}Ca and (c, d) Mg_{0.6}Ca₂Ag.

Table 19. Local EDS point analysis (at. %) as per **Figure 19**.

Alloy	Location	Mg	Ca	Al	Si	Ag
Mg _{0.6} Ca	1	96.8	2.3	0.7	0.2	–
	2	90.2	9.4	0.4	–	–
	3	92.7	7.1	0.3	–	–
Mg _{0.6} Ca ₂ Ag	4	99.4	0.2	–	–	0.4
	5	98.1	0.3	–	–	1.6
	6	76.0	7.5	–	–	15.6

TEM analyses of Mg_{0.6}Ca alloy displayed the secondary phase (**Figure 20** (a)) as an eutectic aggregate with lamellar morphology, probably formed by α -Mg/Mg₂Ca phases in accordance with the EDS analysis (**Table 20**), the Mg–Ca phase diagram and other

works on binary Mg–Ca alloys^{311,33,312,313}. In addition, polygonal shape particles (**Figure 20 (b)**) containing Fe, Al and Ni impurities were found (Point 2, **Table 20**).

In the case of the ternary alloy (Mg0.6Ca2Ag) (**Figure 20 (c) and (d)**), TEM studies were not conclusive as the zones of the ion milled lamella, where particles were located, were too thick for generating an informative electron diffraction pattern. In fact, the available data of TEM studies on Mg alloys containing silver are very scarce and all of them have been conducted on binary Mg–Ag alloys reporting the formation of different intermetallic compounds such as MgAg, MgAg₃ or MgAg₄^{314,315}. The present work revealed the formation of particles with different shapes (**Figure 20 (d)**) and compositions (**Table 20**), some of them were Ag-enriched (**Figure 20**, point 2). The detected impurities included Fe, Ni, Si, Co and Cr.

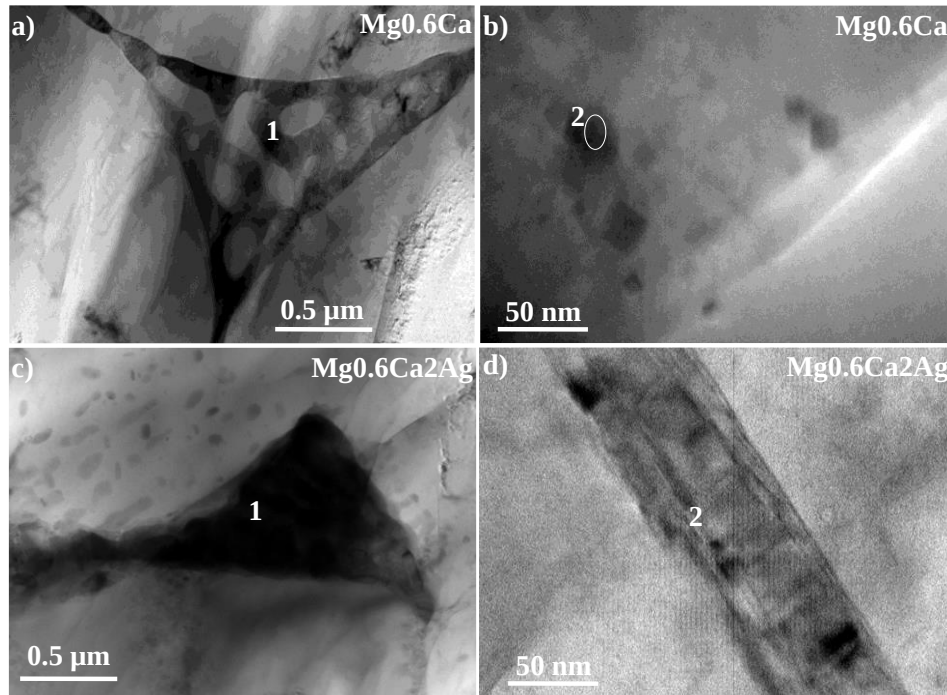


Figure 20. Transmission electron micrographs of the (a, b) Mg0.6Ca and (c, d) Mg0.6Ca2Ag alloy with locations of EDS analysis.

Table 20. Local EDS analysis (at. %) of intermetallic particles as per **Figure 20**.

Alloy	Location	Mg	Ca	Ag	Fe	Al	Ni	Co	Cr	Si
Mg0.6Ca	1	92.6	7.1	–	–	0.3	–	–	–	–
	2	85.0	12.7	–	0.1	1.3	0.9	–	–	–
Mg0.6Ca2Ag	1	74.6	7.3	16.5	0.6	–	0.2	0.5	0.6	0.2
	2	99.1	0.2	0.3	0.2	–	–	0.1	0.2	–

The XRD analyses revealed peaks corresponding to α -Mg for both alloys (**Figure 21**). In the case of Mg0.6Ca, no intermetallic/secondary phases were detected probably due to their negligible amount. Although, for a similar alloy (cast Mg0.8Ca) a formation of Mg_2Ca phase was reported³¹¹. Most of the diffraction peaks of Mg0.6Ca2Ag were the same as those for Mg0.6Ca, except for the small peaks in the low 2θ angles region corresponding to binary and ternary intermetallic phases as Mg_2Ca , MgAg_4 , $\text{Mg}_{54}\text{Ag}_{17}$ ^{316,317} and Ca_2MgAg_3 (**Figure 21**, inset).

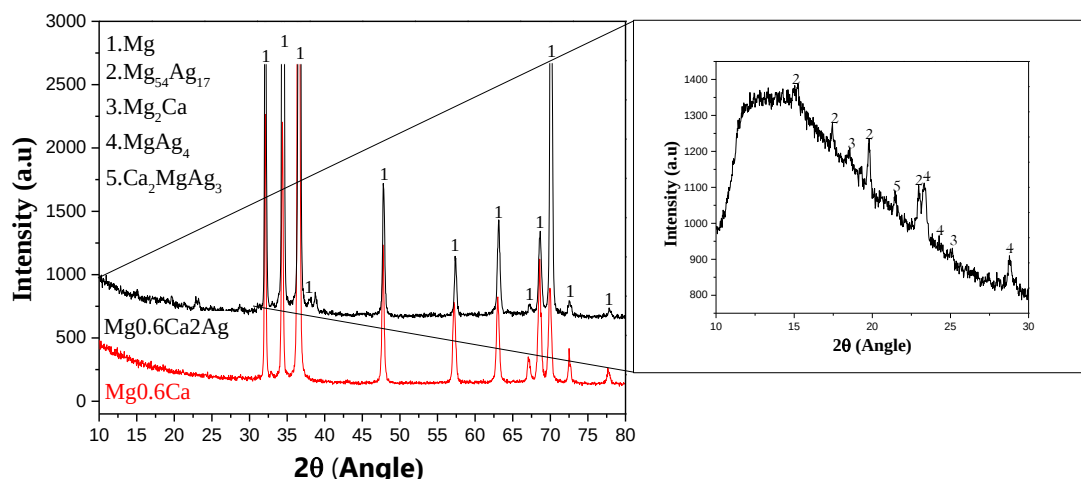


Figure 21. XRD patterns of Mg0.6Ca and Mg0.6Ca2Ag alloys. Inset: slower scan rate at 15 s/step.

4.1.2 Corrosion behavior of Mg0.6Ca and Mg0.6Ca2Ag alloys

4.1.2.1 Scanning Kelvin Probe Force Microscopy (SKPFM)

The local Volta potential difference (VPD) between constituents on a submicron scale was obtained using SKPFM. Surface potential maps and potential profile along with the SEM images of the studied area are displayed in **Figure 22** and **Figure 23** for the Mg0.6Ca and Mg0.6Ca2Ag alloys, respectively. **Figure 22** (a, d) shows the selected region of the Mg0.6Ca alloy where surface potential maps and profiles were acquired. Point 1 corresponds to the Mg_2Ca phase that shows a negative potential with respect to the α -Mg matrix (VPD of ~ -50 mV). It is important to note that the available data regarding the electrochemical behavior of Mg_2Ca is quite limited and controversial results are reported about the anodic⁴⁷, as well as cathodic behavior³⁴ of this phase, which have been mainly attributed to the differences in the composition of the alloy (e.g., presence of impurities) and matrix segregation. Points 2 and 3 (**Figure 22** (d)) correspond to an impurity (point 2) and to the Mg_2Ca phase (point 3, **Table 21**). As it was observed before, the latter shows an anodic behavior (VPD ~ -15 mV) compared to the matrix, whereas the presence of impurities reveals a slight cathodic behavior with respect to the α -Mg

matrix (VPD $\sim +20$ mV) probably due to the presence of a more noble impurity such as Si.

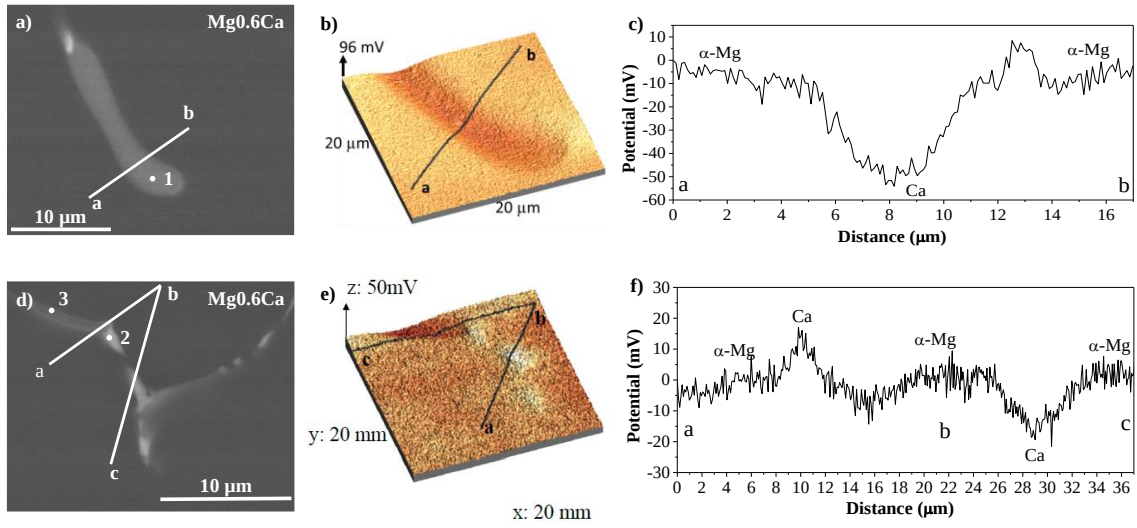


Figure 22. SEM backscattered images (a, d), surface potential maps (b, e) and potential profile (c, f) in selected areas of the Mg0.6Ca alloy.

Table 21. EDS analysis (at. %) of Mg0.6Ca alloy at the locations of SKPFM mapping as per **Figure 22**.

Alloy	Location	Mg	Ca	Si	O	Al
Mg0.6Ca	1	91.3	6.12	-	2.2	0.3
	2	80.4	7.4	10.9	1.3	-
	3	95.7	4.3	-	-	-

For the Mg0.6Ca2Ag alloy, the surface potential profiles measured in the inclusion (**Figure 23**) reveal a different electrochemical response depending on the elemental composition analyzed by EDS (**Table 22**). The area enriched in Ag (Ca/Ag ratio of 0.89) (point 2, **Figure 23** (a)) revealed a cathodic behavior (VPD $\sim +20$ mV) in comparison with the α -Mg matrix, whereas the Ca/Ag ratio of 2.39 (Ca-rich point 1, **Figure 23** (a)) leads to an anodic performance with potential differences around VPD ~ -27 mV. Ben *et al.* studied the electrochemical behavior of magnesium–silver ternary alloys (MgZnAg) and reported a cathodic behavior of MgAg secondary phase ³¹⁸, which appears to be similar to the behavior of the areas enriched with Ag in Mg0.6Ca2Ag alloy of the present Thesis.

Table 22. EDS analysis (at. %) of the Mg0.6Ca2Ag alloy at the locations of SKPFM mapping as per **Figure 23**.

Alloy	Location	Mg	Ca	O	Ag	Ca/Ag
Mg0.6Ca2Ag	1	91.4	5.0	1.4	2.1	2.4
	2	81.7	8.3	0.7	9.3	0.9

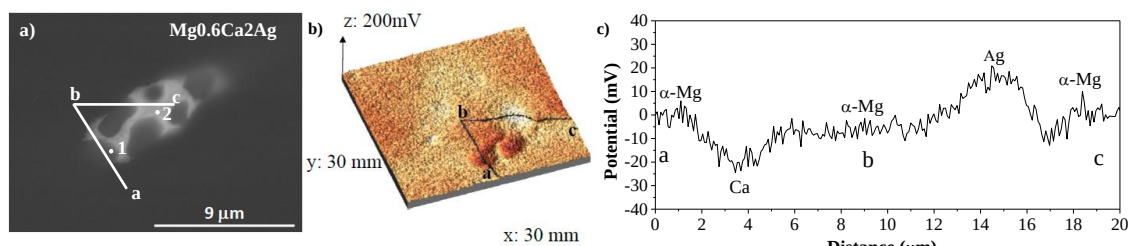


Figure 23. SEM backscattered image (a), surface potential maps (b) and potential profile (c) in selected areas of the Mg_{0.6}Ca₂Ag alloy.

4.1.2.2 Hydrogen evolution tests

Figure 24 shows the hydrogen evolution volume for Mg_{0.6}Ca after 60 days and Mg_{0.6}Ca₂Ag after 4 days of immersion in SBF at 37°C. Insets in **Figure 25** show the corresponding macro degradation photos of the specimens. Mg_{0.6}Ca alloy initially exhibited a relatively high hydrogen evolution rate, 3.86 mL/cm² per week after 1 week of immersion, with progressive decrease to 1.02 and 1.11 mL/cm² per week after 6 and 8 weeks of immersion, respectively. These values are about two times higher in comparison with the degradation rate observed for other Mg–Ca systems in SBF³¹¹. In the present work, the corrosion rate of Mg_{0.6}Ca during the first three weeks of immersion occurred at a rate of 7.85 mm/year.

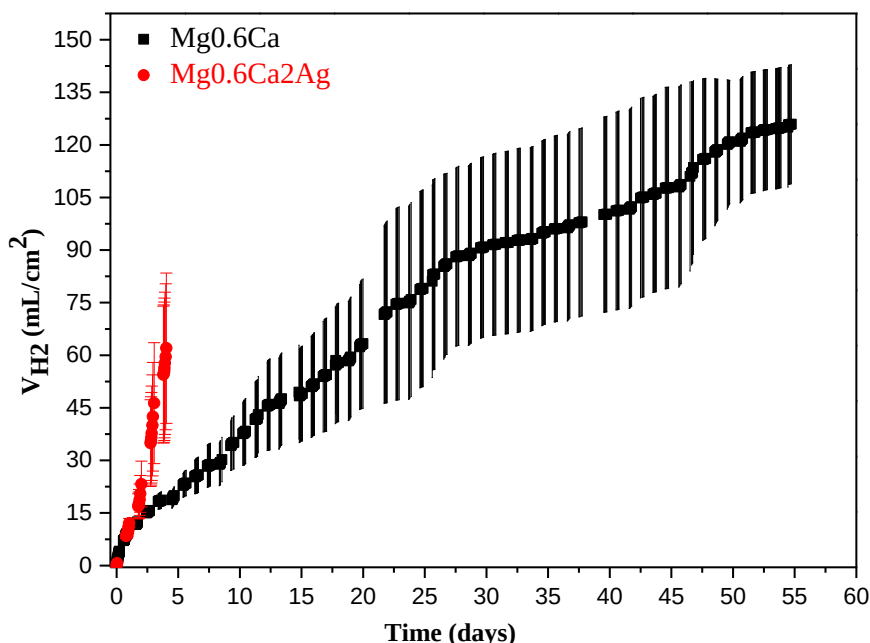


Figure 24. Hydrogen evolution of Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys after 60 and 4 days, respectively, of immersion in SBF at 37°C.

Mg_{0.6}Ca₂Ag exhibited an extremely high degradation rate, corresponding to ~60 mL/cm² and the experiment was stopped after 4 days of immersion. Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys (**Figure 25**) presented a generalized corrosion, but the latter

exhibited a heavily heterogeneous corrosion morphology and the specimen completely disintegrated after about 10 days of immersion.

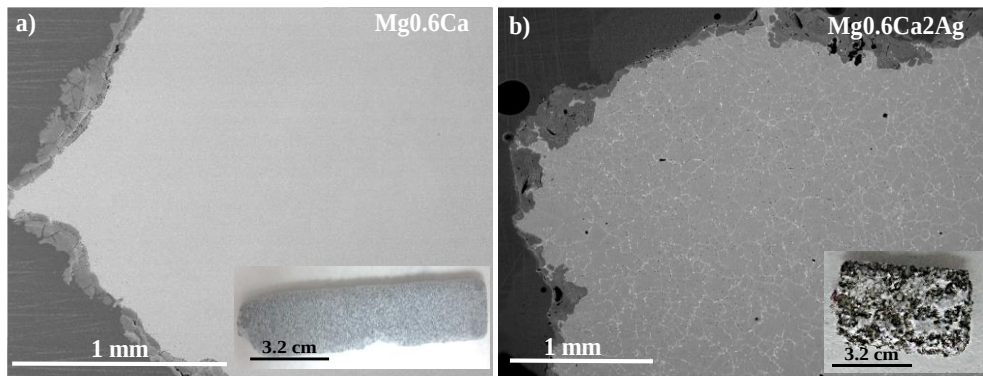


Figure 25. Backscattered cross-section images of (a) Mg0.6Ca after 60 days and (b) Mg0.6Ca2Ag after 4 days of immersion in SBF. Inserts: macroscopic views of the specimens.

Some studies reported that the corrosion process of Mg-Ca systems with Ca content $<1.25\%$ tends to become localized due to severe electrochemical activity⁴⁵. Other studies suggest that when the amount of Ag in MgAg alloys is increased³¹⁹ the corrosion rate does not increase proportionately (e.g. 1.15 mm/year for Mg2Ag to 1.43 mm/year for Mg6Ag) and the Ag-containing second phase does not play an important role in microgalvanic activities, as the responsibility for these phenomena fall to the impurities^{320,321}. However, in the present study the corrosion rate for Mg0.6Ca2Ag was higher, at 31.26 mm/year. Such extremely fast degradation may be explained as illustrated in **Figure 26**.

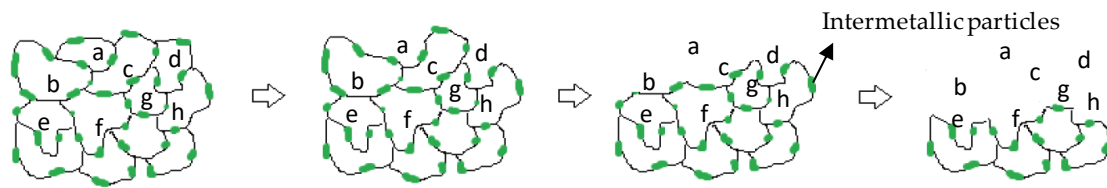


Figure 26. Schematic diagram of corrosion attack at the grain boundary and loss of grains (labelled with letters).

It is evident that the intermetallic particles in Mg0.6Ca2Ag alloy disclosed some regions with active behavior that depended on the Ca/Ag ratio in the region (**Figure 22** and **Figure 23**). Such regions present galvanic micro-couples with a minimum anodic surface compared to a large cathodic surface of α -Mg causing dissolution of the intermetallic particles. It can be clearly appreciated from **Figure 19** (c, d) that in the Mg0.6Ca2Ag alloy the grain boundary network is much more decorated with intermetallic particles than in Mg0.6Ca. Consequently, dissolution of anodic grain

boundaries can lead to an easy fall-out and loss of the cathodic α -Mg grains or clusters of grains (**Figure 26**).

4.2 Characterization of MgZn_xCa_y alloys

4.2.1 As-cast MgZnCa and $\text{Mg}_3\text{Zn}_0.4\text{Ca}$ alloys and extruded $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ alloy

The optical and backscattered SEM micrographs of the investigated alloys are presented in **Figure 27**. Both as-cast alloys (**Figure 27** (a, b, d, e)) showed a microstructure constituted by α -Mg grains with the second phases mainly distributed along the grain boundary (elongated shape) and also located at the interdendritic spaces (spherical and elliptic shape). From the optical micrographs (**Figure 27** (a, b)), slight differences can be found related to the grain size, with average values of $282 \pm 55 \mu\text{m}$ and $205 \pm 48 \mu\text{m}$ for MgZnCa and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$, respectively. The slight grain refinement effect can be ascribed to the higher amount of Zn, as it has been reported for different Mg alloys systems³²². The volume fraction of secondary phases is higher for MgZnCa alloy (8 %) compared with $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ (3.5 %), suggesting that higher Ca amounts could promote the formation of secondary phases at the grain boundary and the interdendritic interstices⁴⁶.

SEM studies of as-cast alloys (**Figure 27** (d, e)) along with the EDS point analyses reveal that the matrix is mainly formed by the α -Mg phase in which Ca and Zn are partially dissolved (**Table 23**, points 3, 7 for MgZnCa and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$, respectively). EDS analyses located at the second phases (**Table 23**, points 1, 2, 4, 5) showed the formation of particles with different contents of Ca and Zn. It is worth mentioning that particles enriched in Zn are more abundant in the $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ alloy. Impurities as Ni, Fe, Al, Si, Ag and Mn are also observed (**Table 23**), the implications will be discussed in more detail in the next section. The intermetallics formed in these type of alloys were identified as Mg_2Ca and $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ in agreement with other works^{323,324}.

In the case of the extruded $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ alloy, the optical and SEM evaluations (**Figure 27** (c, f)) showed a microstructure of α -Mg equiaxed grains and particles distributed along the extrusion direction. This type of alignment is common for the extruded material and could be beneficial for the mechanical properties of the alloy^{325,326}. The grain size ($25 \pm 3 \mu\text{m}$) and the volume fraction of secondary phases (1.41 %) were

lower compared with those for as-cast alloys which is normal for extruded Mg alloys due to the dynamic recrystallization occurred during the manufacturing process^{327,328}.

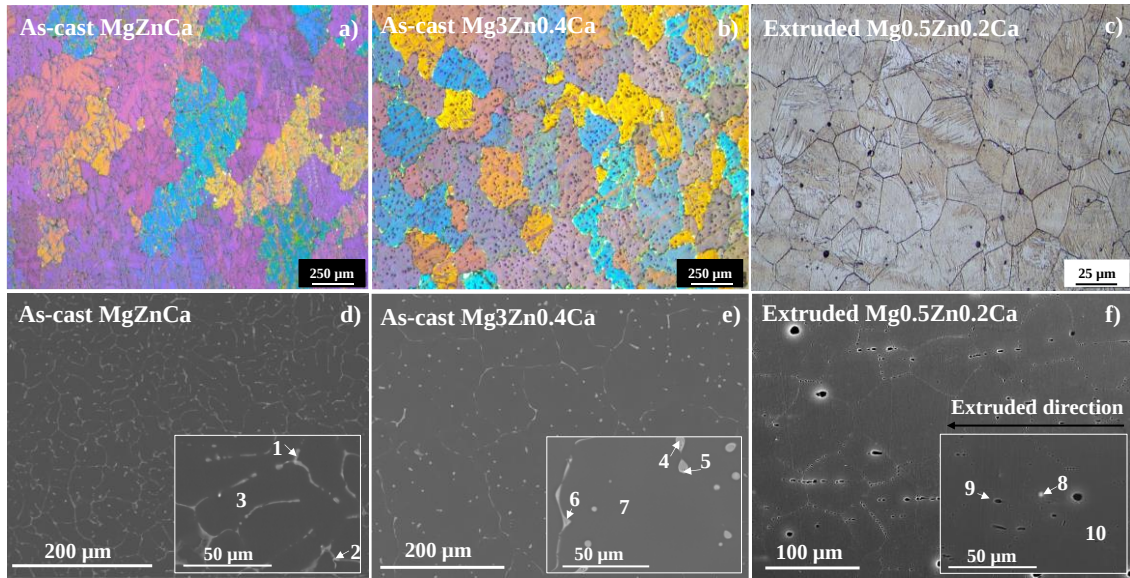


Figure 27. Optical (a-c) and secondary electron (d-f) micrographs of alloys microstructure: (a, d) as-cast MgZnCa, (b, e) as-cast Mg₃Zn_{0.4}Ca and (c, f) extruded Mg_{0.5}Zn_{0.2}Ca alloys.

EDS point analysis of the matrix (**Table 23**, point 10) revealed that Ca and Zn are partially dissolved in α -Mg phase in a lower concentration compared to the as-cast alloys, which was more significant in the case of Zn element. In regards to secondary phases, only particles enriched in Ca were detected by EDS (**Table 23**, points 8 and 9). Zn-enriched particles are not found probably due to their detachment during the metallographic preparation as can be deduced from the presence of small pores in (f).

Table 23. Composition determined by point analyses EDS (at. %) as per **Figure 27**.

Alloy	Location	Mg	Ca	Zn	Al	Fe	Ni	Ag	Mn	Si
MgZnCa	1	87.9	11.6	1.8	0.54	0.02	0.05	-	-	0.04
	2	90.8	4.0	5.0	0.12	-	0.03	-	-	0.02
	3	99.3	0.3	0.3	-	-	-	-	0.01	0.06
Mg ₃ Zn _{0.4} Ca	4	74.9	18.2	6.8	0.23	0.01	0.08	0.03	0.05	0.05
	5	73.4	8.9	17.2	0.30	0.03	0.07	0.01	0.02	0.07
	6	76.4	7.7	13.3	0.3	0.66	0.06	0.01	0.02	1.59
	7	98.9	0.1	0.9	-	0.02	0.03	-	0.02	0.02
Mg _{0.5} Zn _{0.2} Ca	8	88.9	10.6	0.4	0.01	0.01	0.02	-	0.03	0.04
	9	89.1	10.5	0.4	-	-	0.04	-	-	0.04
	10	99.6	0.1	0.2	-	-	0.04	-	0.03	0.02

Both as-cast alloys showed similar hardness values (56.4 ± 2.3) HV and (61.3 ± 2.1) HV for MgZnCa and Mg₃Zn_{0.4}Ca, respectively, whereas the extruded alloy revealed a

slightly lower hardness (51 ± 2) HV. The differences are possibly related to the low amount of Zn and Ca in solid solution in the extruded alloy³²⁹.

The XRD patterns of the three studied alloys are presented in **Figure 28**. All the alloys showed high intensity peaks corresponding to the α -Mg phase and lower intensity peaks ascribed to Mg_2Ca and $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ secondary phases. $\text{Mg}_3\text{Zn}_0.4\text{Ca}$ alloy, that had the highest Zn/Ca ratio, revealed a greater presence of $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ peaks which is in accordance with other studies³³⁰.

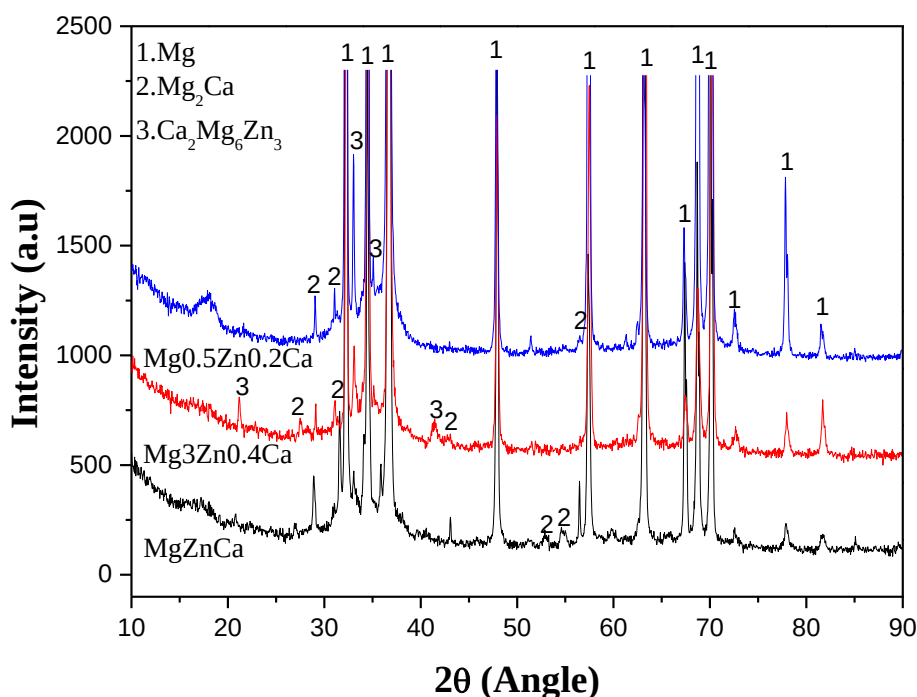


Figure 28. X-ray diffraction patterns of MgZn_xCa_y alloy.

TEM examination of the three alloys revealed particles in the size range of 100 nm-2.3 μm with different concentrations of Zn and Ca (**Figure 29-Figure 30** and **Table 24-Table 25**) suggesting the presence of different phases. It is worth mentioning that only few particles were successfully diffracted, mainly due to the limitations imposed by the excessive thickness of the inclusions. **Figure 29** shows the characterization of four particles along with their diffraction patterns and EDS analyses (**Table 24**). In the case of as-cast alloys (**Figure 29** (a, b)), the particles revealed a sizes in the range of 100-200 nm and corresponded in both cases to $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ inclusions according to the diffraction patterns. The EDS point analyses of these particles (**Table 24**, points 1, 2) revealed an enrichment in Zn. For the extruded alloy (**Figure 29** (c, d)), the $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ and Mg_2Ca phases were identified by electron diffraction analysis and supported by EDS results

(Table 24, points 3, 4). The size of the inclusions was about 200 nm and 50 nm for $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ and Mg_2Ca phases, respectively.

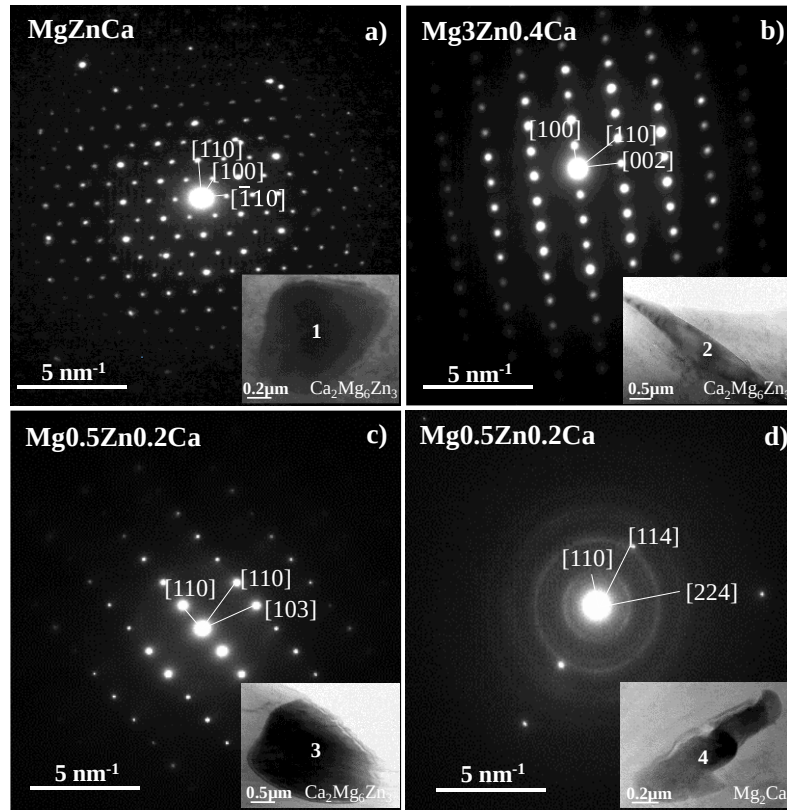


Figure 29. Transmission electron microscopy images and diffraction patterns (a) MgZnCa , (b) $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ and (c-d) $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ alloys.

Table 24. EDS analysis (at. %) of particles inside the α -Mg grains of MgZn_xCa_y alloys, as per **Figure 29** (a-d).

Alloy	Location	Mg	Ca	Zn	Fe	Si	Al	Mn	Ag
MgZnCa	1	82.6	5.2	11.8	0.05	0.04	0.3	0.01	-
Mg₃Zn_{0.4}Ca	2	75.7	10.1	14.0	0.03	0.02	0.2	-	-
Mg_{0.5}Zn_{0.2}Ca	3	74.7	10.0	12.1	-	0.5	1.4	-	0.3
	4	83.4	15.4	0.1	-	0.2	0.8	-	0.1

In **Figure 30** are illustrated different polygonal particles found within the alloys matrices whose composition is presented in **Table 25**. Points 3, 5 and 6 were rich in Ca while points 1, 2 and 4 were rich in Zn. These results suggest that particles inside the grain of all Mg-Zn-Ca alloys are constituted by $\text{Ca}_2\text{Mg}_6\text{Zn}_3$ and Mg_2Ca phases, which is in accordance with SEM and XRD results. Notably, all the examined particles contained a considerable amount of Al and Si impurities (**Table 24** and **Table 25**). Additionally, the particles in the as-cast alloys contained Fe and Mn impurities, while the extruded alloy contained Ag.

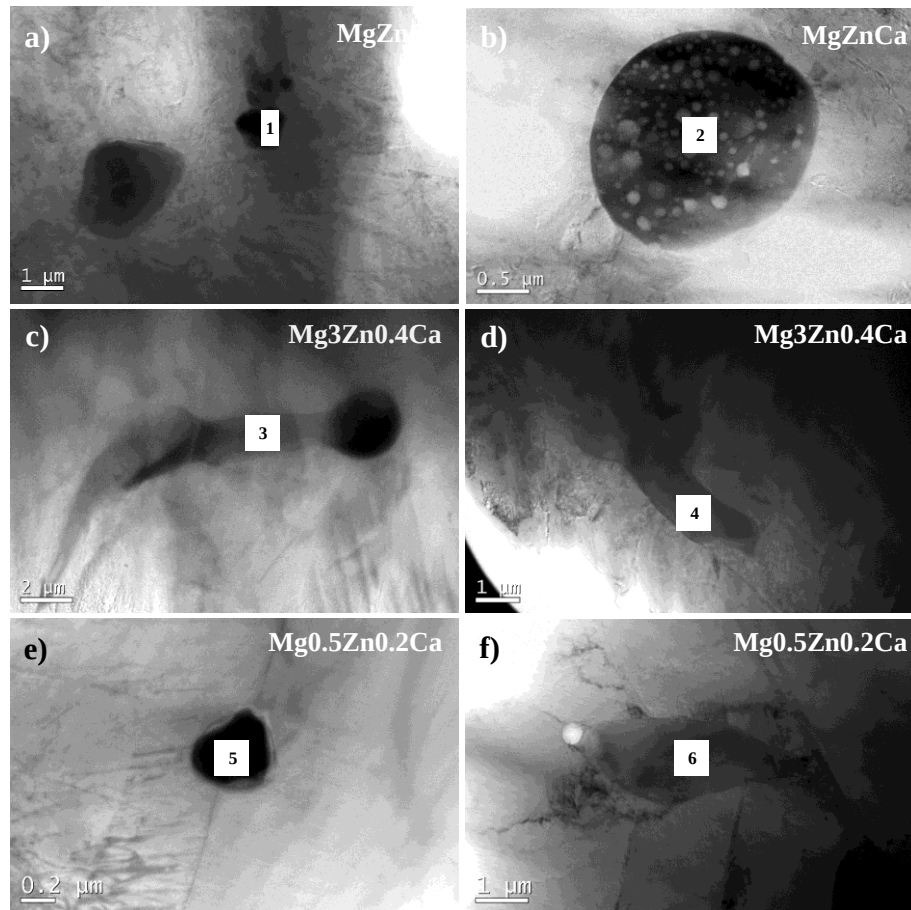


Figure 30. Electron diffraction patterns of the secondary phase particles located inside the grains of (a, b) MgZnCa; (c, d) Mg₃Zn_{0.4}Ca and (e, f) Mg_{0.5}Zn_{0.2}Ca alloys.

Table 25. EDS analysis (at. %) of particles inside the α -Mg grains of MgZn_xCa_y alloys, as per **Figure 30**.

Alloy	Location	Mg	Ca	Zn	Fe	Si	Al	Mn	Ag
MgZnCa	1	54.2	1.3	1.0	31.9	7.3	0.6	0.9	-
	2	54.0	12.2	32.8	0.1	0.1	0.9	0.03	-
Mg ₃ Zn _{0.4} Ca	3	70.0	26.0	2.8	0.05	0.1	1.0	0.01	-
	4	73.8	9.3	16.6	0.04	0.05	0.1	-	-
Mg _{0.5} Zn _{0.2} Ca	5	87.7	11.4	0.04	-	0.2	0.7	-	0.04
	6	49.5	14.5	0.01	-	0.2	35.7	-	0.03

4.2.2 Corrosion behavior of MgZn_xCa_y alloys

4.2.2.1 Scanning kelvin probe force microscopy (SKPFM)

SKPFM analysis of the three alloys was performed both before (**Figure 31**) and after the immersion in inorganic part of α -MEM at 37°C for 1 h (**Figure 32**). Additionally, following the immersion, the analysed areas were cross-examined by SEM with local EDS analyses (**Table 26**).

Figure 31 shows the surface potential map and surface potential profile of the microconstituents at grain boundaries and intermetallics inside the grains in as-cast MgZnCa and Mg₃Zn_{0.4}Ca alloys and extruded Mg_{0.5}Zn_{0.2}Ca alloy before immersion. The intermetallics at the grain boundary of MgZnCa (**Figure 31** (a, b)) showed both lower (around -50 mV) and higher surface potential differences (in the range of 90-200 mV) compared to the Mg matrix which is comparable to other reports ^{331,332}. The higher potential may be related to the presence of impurities as Fe, Al, or Si, as is evidenced in **Figure 30** (a) (point 1, **Table 25**), suggesting the formation of a strong galvanic couple with the matrix. These inclusions could correspond to Zn-rich (ternary) intermetallics corroborated by EDS analysis in **Table 26** (point 2 and 4) which in general exhibit cathodic behavior ^{331,332} and can show a potential difference as high as ~396 mV ³³³. However, the lower potential may be associated to Ca-rich (binary) intermetallics, as it is similar to the Volta potential values of Mg_{0.6}Ca alloy in **Figure 22** (c). The published literature features controversial results about the electrochemical character of Mg₂Ca, which was reported to exhibit anodic ⁴⁷ as well as cathodic ³⁴ behavior. The reported anodic values for Mg₂Ca range from -40 to 100 mV, depending on the composition of the alloy. Some authors reported that due to the anodic behavior of this inclusion, the lamellar eutectic corroded first to protect the matrix ³³⁴. However, other works observed the opposite behavior where Mg₂Ca acted as a weak cathode about 10 mV ³¹¹.

For Mg₃Zn_{0.4}Ca alloy (**Figure 31** (d)), the maximum potential value found for the intermetallic phases was 253 mV, which is slightly higher than those for MgZnCa alloy suggesting a stronger corrosion accelerating effect due to microgalvanic coupling. This could be attributed to the presence of impurities (**Table 25**, point 4) in this Zn-rich (ternary) intermetallics, which is similar to other reports ³³⁵. The intermetallics with a potential of -47 mV compared to the matrix may be associated with Ca-rich (binary) intermetallics, which is similar to the anodic intermetallics in MgZnCa alloy and is in accordance with the EDS analysis of point 3, **Table 25**.

In the case of extruded Mg_{0.5}Zn_{0.2}Ca alloy ((**Figure 31** (f))), the surface potential of the intermetallic phases may act as cathode with a maximum value of 50 mV or an anode with a values of -93 mV, with respect to the α -Mg matrix. These cathodic behavior could corresponded to the particles with impurities, which is similar to that of as-cast alloys. The anodic behavior of this particles may be attributed to Ca-rich (binary) intermetallics. It is worth mentioning that the anodic surface potential values of the intermetallics of

Mg_{0.5}Zn_{0.2}Ca alloy were slightly higher compared to Mg₃Zn_{0.4}Ca and MgZnCa alloys, which may be associated with the differences in the composition of alloys and/or distribution of the secondary phases in the alloy.

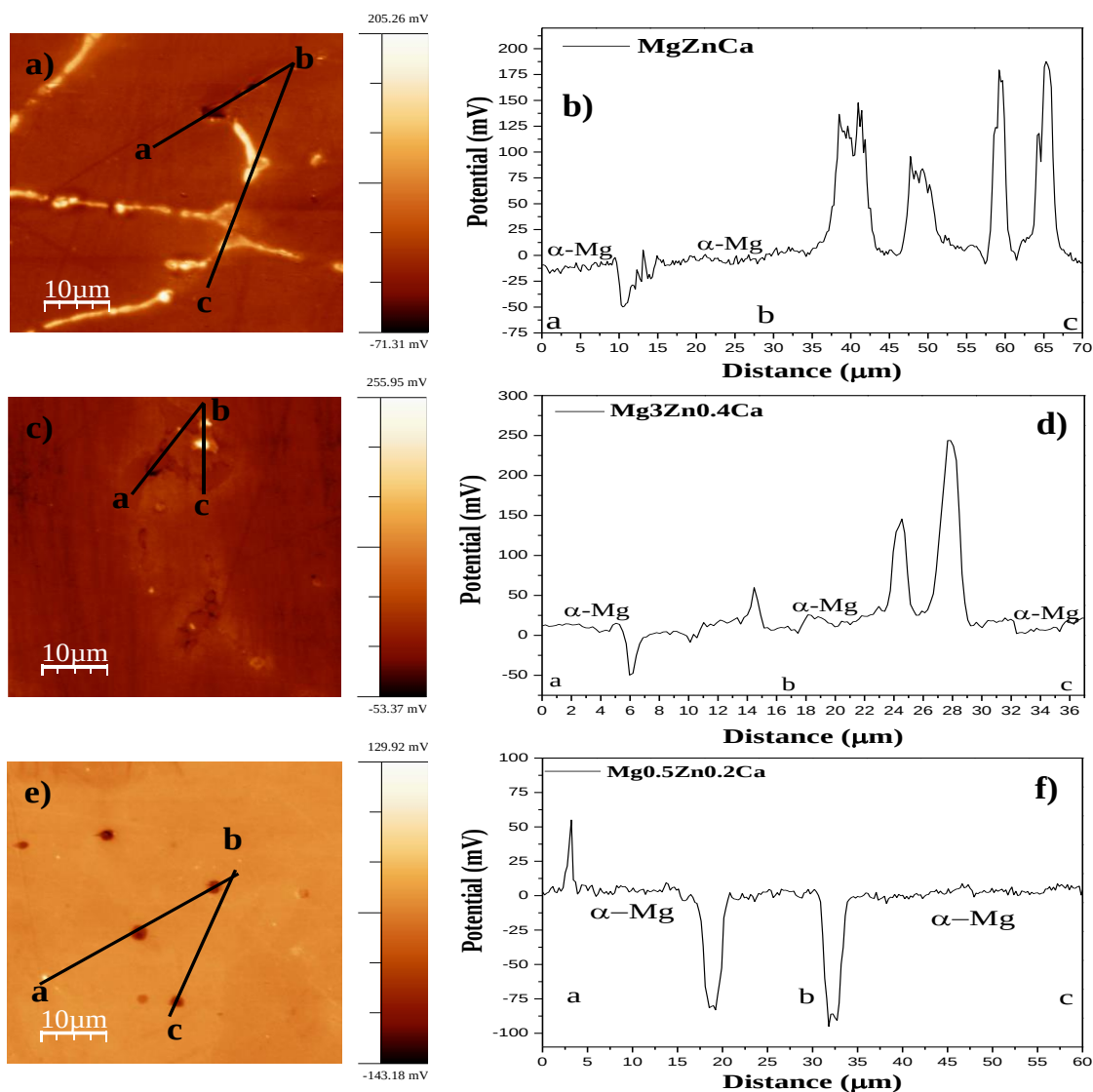


Figure 31. (a, c, e) Surface potential map and (b, d, f) profiles of (a, b) MgZnCa, (c, d) Mg₃Zn_{0.4}Ca and (e, f) Mg_{0.5}Zn_{0.2}Ca alloys.

In order to understand the corrosion behavior of these different intermetallics, an immersion test was carried out during 1 h in inorganic α-MEM solution at 37°C. The surface potential and the potential profiles of MgZn_xCa_y alloys (**Figure 32**) were analysed at the same locations that were presented in **Figure 31** (a, c, e).

Normally, the active intermetallics would reveal their nature after immersion in a corrosive solution. The present study has not showed a visible dissolution of intermetallics as it is illustrated in the topography maps of as-cast alloys (**Figure 32** (a,

d, g)). However, macro photos disclosed a localized corrosion in other areas of the specimens after 1 h of immersion test (**Figure 33** (a, c, e), inset).

For MgZnCa alloy (**Figure 32** (c)), cathodic phases showed a decrease in the VPD by ~ 100 mV while anodic phases showed a slight increase in VPD (-60 mV) compared to the original state, which may be associated with the formation of corrosion products constituted by Ca-P-based compounds that could have contributed to the measured VPD. In the case of the Mg3Zn0.4Ca alloy, a reduction of the VPD for α -Mg corresponds to a large anodic activity, in the area adjacent to the cathodic phase (line b-c, **Figure 32** (f)). However, a reduction of the VPD of cathodic phases could be due to the formation of corrosion products film. For Mg0.5Zn0.2Ca alloy, slightly lower Volta potentials (-30 mV vs the original -90 mV) are observed for Ca-rich intermetallics against the matrix. These intermetallics showed a visible dissolution according to **Figure 32** (g). Overall, the VPD profile of the extruded alloy exhibits greater tendency to anodic “noise”, which is in agreement with the observed generalized corrosion morphology.

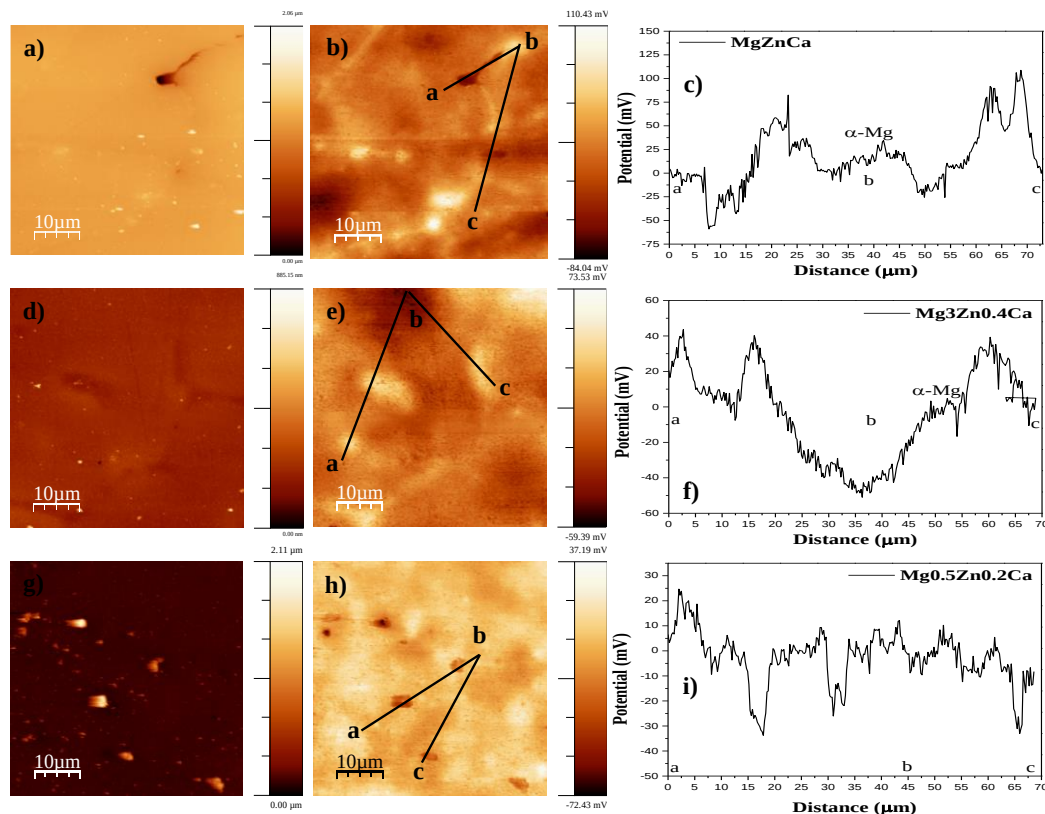


Figure 32. (a, d, g) Topography, (b, e, h) surface potential map and (c, f, i) surface potential profiles of (a-c) MgZnCa, (d-f) Mg3Zn0.4Ca and (g-i) Mg0.5Zn0.2Ca alloys after 1 h of immersion in inorganic α -MEM solution at 37°C.

Figure 33 illustrates the plan views of the examples of corroded specimens of MgZnCa, Mg3Zn0.4Ca and Mg0.5Zn0.2Ca for immersion time of 1 h in inorganic α -

MEM at 37°C. In all cases, localized corrosion is observed around the intermetallic particles inside the grains, which are identified with micro-cracks in **Figure 33** (b, c, f). These particles were analyzed by EDS (**Table 26**, point 2, 5, 8, 9 and 12) and may correspond to Ca-rich (binary) intermetallics due to the high content of Mg and Ca. However, the intermetallics at the grain boundaries (**Table 26**, point 4 and 6) of MgZnCa and Mg₃Zn_{0.4}Ca alloys were mainly constituted by Mg, Ca and Zn that may correspond to these Zn-rich (ternary) intermetallics appeared intact after 1 h of immersion. For all alloys, the corrosion products formed on the surface of the samples were constituted by Mg, O, Ca, P and Cl suggesting that MgO and Ca/P-based compounds have been formed.

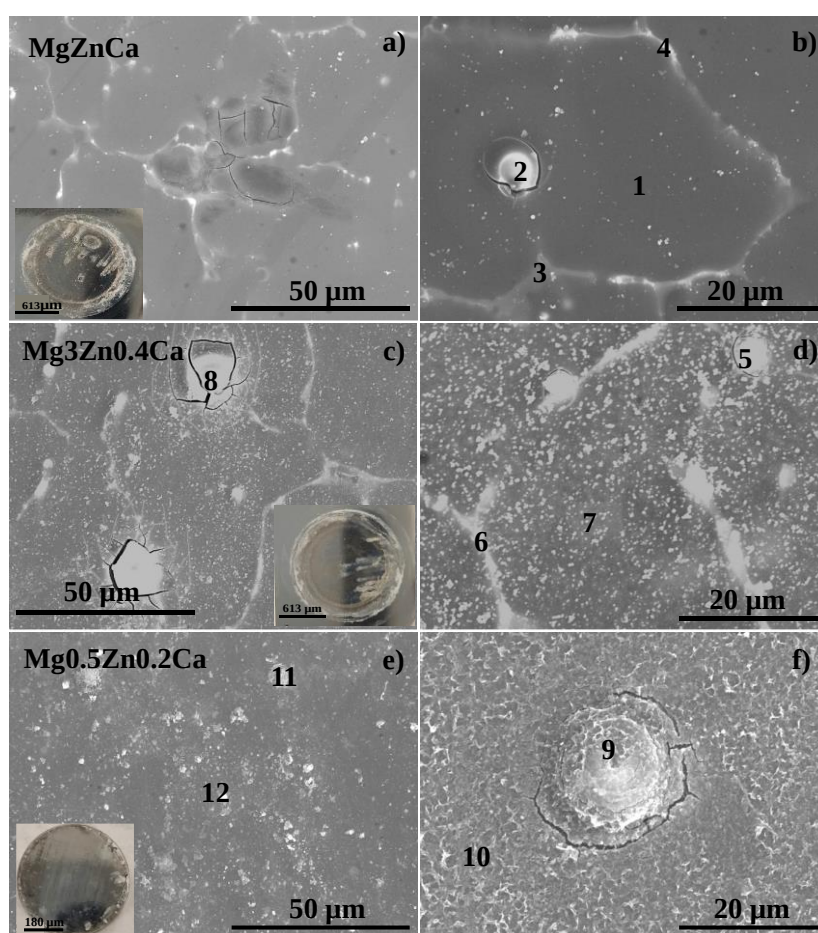


Figure 33. Plan view backscattered images of (a-b) MgZnCa, (c-d) Mg₃Zn_{0.4}Ca and (e-f) Mg_{0.5}Zn_{0.2}Ca alloys after 1 h of immersion in inorganic α -MEM at 37°C.

According to the VPD profiles and SEM images, it is concluded that the corrosion mechanism for these alloys may occur by the micro-galvanic effect between α -Mg matrix and active binary phase and/or between noble ternary phase and α -Mg matrix. It suggested that cathodic intermetallics (with higher VPD) may comprise impurities, thus forming a strong microgalvanic couple and accelerating the corrosion of the matrix. On the other hand, Ca-rich (binary) intermetallics inside the α -Mg grains induce small pits and cracks

in the matrix around the particles as it is illustrated in **Figure 33** (b, c, f). When active intermetallics (e.g. Mg_2Ca) are partially undissolved, the corrosion products formed upon them shift the VPD to the level similar to that of the matrix.

Table 26. EDS (at. %) results for MgZn_xCa_y alloys as per **Figure 33** after 1 h of immersion in inorganic α -MEM solution.

Alloy	Location	O	Na	Mg	Al	P	Cl	K	Ca	Fe	Ni	Cu	Zn
MgZnCa	1	6.4	0.3	92.0	-	0.6	-	-	0.6	-	0.1	-	0.3
	2	27.6	0.7	55.0	0.1	6.9	0.1	-	8.5	0.1	-	-	1.0
	3	9.2	0.5	70.5	0.4	0.4	-	-	15.4	-	0.1	0.1	3.7
	4	10.7	1.4	72.4	0.1	0.8	-	-	3.7	0.1	0.1	0.1	10.6
Mg3Zn0.4Ca	5	38.4	2.4	39.1	-	8.1	1.1	0.1	10.1	0.1	0.1	-	0.4
	6	13.9	1.4	61.1	0.3	1.5	0.3	-	10.2	-	0.2	0.1	10.9
	7	7.3	0.3	90.1	-	1.1	-	-	1.0	0.1	-	0.1	0.3
	8	53.1	2.3	12.4	-	13.4	1.1	0.1	17.4	-	0.1	-	0.2
Mg0.5Zn0.2Ca	9	51.9	0.3	44.2	-	2.2	0.2	-	1.2	-	-	-	-
	10	14.2	0.1	84.3	-	0.8	-	-	0.5	-	0.1	-	0.2
	11	45.1	0.5	49.9	-	2.6	0.2	-	1.8	-	-	-	-
	12	48.4	0.7	45.6	-	3.1	0.2	-	2.0	-	-	-	0.1

4.2.2.2 Potentiodynamic polarization

With the aim to compare the corrosion performance of the three alloys, open circuits potential measurements and potentiodynamic polarization curves were conducted in inorganic α -MEM for 1 h of immersion at 37°C (**Figure 34**). The obtained corrosion parameters (final open circuit potential (OCP), corrosion potential (E_{corr}) and corrosion current density (i_{corr})) are presented in **Table 27**.

Figure 34 (a) shows a rapid increase of the OCP values for both as-cast alloys (up to 500 s) followed by a relative stabilization until they reach the values of ~ -1.65 V. This type of response has been ascribed to fast electrochemical activities at the beginning of the immersion, followed by the formation of a corrosion product layer (mainly oxides and hydroxides of Mg) on the specimen surface leading to nobler potential^{336,337}. The extruded alloy revealed some fluctuation at the beginning of the immersion possibly related to metastable surface breakdown phenomena³³⁸. After 400 s, the OCP increased showing higher values than those for both as-cast alloys, which could be related to the formation of a more protective layer of corrosion products.

Potentiodynamic curves (**Figure 34 (b)**) for as-cast alloys showed a very similar trend with no significant differences in the values of the main parameters (**Table 27**). The extruded alloy presented different features compared to the as-cast alloys: i) a nobler E_{corr} , which is in agreement with the obtained OCP; ii) a smaller semi-passivation range before the pitting potential; iii) the cathodic branch is shifted to higher current densities accelerating the corrosion rate; iv) the anodic branch is shifted to low current densities due to the formation of corrosion products. In particular, these indicated a slightly lower corrosion resistance of the Mg0.5Zn0.2Ca under the tested conditions compared to the as-cast alloys. This could be related to the presence of defects such as dislocations by the extrusion process which is in agreement with other reports^{339,340}.

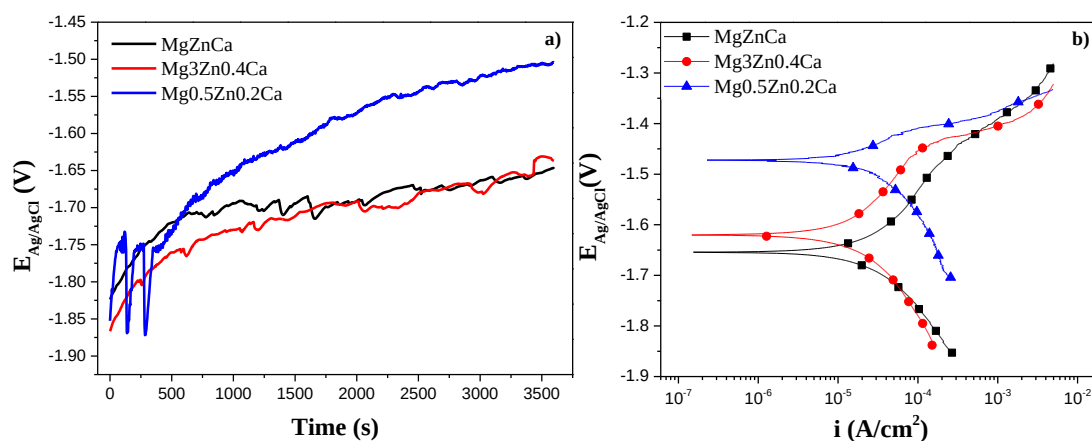


Figure 34. (a) OCP evolution and (b) polarization curves for MgZn_xCa_y alloys in inorganic α -MEM solution after 1 h of immersion.

Table 27. Parameters obtained from the potentiodynamic polarization curves after 1 h of immersion in inorganic α -MEM solution.

Alloy	OCP, E (V)	E_{corr} (V)	i_{corr} ($\mu\text{A/cm}^2$)	E_{pit} (V)
MgZnCa	-1.65	-1.65	27.37	-1.45
Mg3Zn0.4Ca	-1.64	-1.62	30.25	-1.42
Mg0.5Zn0.2Ca	-1.50	-1.47	33.41	-1.40

The i_{corr} of MgZn_xCa_y alloys obtained in the present work were slightly higher compared to those for Mg0.7Zn0.6Ca alloy, tested in DMEM solution ($i_{\text{corr}} = 25.2 \mu\text{A/cm}^2$)³⁴¹, and higher compared to those for Mg1.5Zn0.5Ca and Mg2Zn1Ca alloys, tested in Hank's solution ($i_{\text{corr}} = 12.36$ and $13.8 \mu\text{A/cm}^2$, respectively)^{342,50}. The information available in the literature showed an important influence of alloying elements on the corrosion performance of MgZn_xCa_y systems. In general, for as-cast alloys, it has been reported that low amounts of alloying elements (<1 wt. %) can improve the corrosion

resistance, whereas an increase of their content may lead to the opposite behavior. For instance, the addition of Zn from 0.2 to 1 wt. % (for a constant and low content of Ca) enhanced the corrosion properties measured by DC polarization³³¹. However, the addition of Zn >1 wt. % in the case of Mg_xZn_{0.8}Ca alloy³⁴³ led to an increase in the current density of the systems. Also, in the case of Ca addition, its increase (>1 wt. %) has been shown to increase the degradation rate of Mg₅Zn_xCa alloy⁴⁸.

4.2.2.3 Electrochemical impedance spectroscopy

One of the aims of this Thesis is the development of a reliable, fast and low-maintenance alloy screening process from the corrosion protection point of view, in order to ensure that only the most promising candidates would pass to the *in vitro* bio-validation stage. The basic cell work experiments that determine the cytotoxicity of the biomaterial and cell viability typically takes 4-5 days. Corrosion screening experiments of that duration in full cell culture media and non-sterile conditions, end up almost inevitably in contamination almost inevitably. Therefore, it is important to elucidate the role of the corrosion media composition of over the short term corrosion behavior, and the ability of the accelerate the electrochemical test, in order to discriminate between the protective properties of the different alloys. The EIS is the method of choice, since, unlike PDP, it applies a very small perturbation, close to the steady-state corrosion conditions.

In order to elucidate the role of the corrosion media in the short term corrosion properties, EIS measurements were carried out in both inorganic and complete α -MEM solutions at 37°C after 1 h of immersion. Bode plots along with the equivalent circuit and the fitted results are presented in **Figure 35** and **Table 28**, respectively. Complete α -MEM contains inorganic salts, amino acids, vitamins and glucose (among other organic components) that would also be present in the *in vivo* body fluids and would affect the passivation and degradation of Mg alloys.

Nyquist and Bode plots for the three alloys present three time constants **Figure 35** (a, b). The first constant at high frequencies is commonly ascribed to the resistance and capacitance of deposited products from the media. The constant at middle frequency corresponds to the resistance and capacitance of corrosion products and the constant at low frequencies is referred to the charge transfer resistance and capacitance of double layer. The equivalent circuit used to fit the EIS results (**Figure 35** (c)) includes: (i) R_{elec} - the solution resistance, (ii) R_1/CPE_1 - the response ascribed to the capacitive and resistance

behavior of the deposited products on the surface of the specimens, (iii) R_2/CPE_2 - the capacitive and resistance behavior of the corrosion products layer and (iv) R_{dl}/CPE_{ct} - the electrochemical activity at the substrate/electrolyte interface associated to the double layer and charge transfer phenomena. The surface roughness and heterogeneity are accounted for constant phase elements (CPE) instead of ideal capacitors. The reported numerical CPE values correspond to admittance at the radial frequency $\omega=1$ rad, in accordance with $Z=1/(CPE(j\omega)^n)$ where j is the imaginary number and n is $0 \leq n \leq 1$. This equivalent circuit is in concordance with reported studies on the corrosion performance of Mg alloys in a medium similar to α -MEM⁵¹. The total resistance of the system ($R_{total} = R_1 + R_2 + R_{ct}$) is shown in **Figure 35** a summary of the overall corrosion performance of the studied materials.

The total resistance of the system was higher in inorganic α -MEM, up to 2-6 times ($2 \cdot 10^3 \Omega \cdot \text{cm}^2$) compared to the results obtained for complete α -MEM ($0.4 \cdot 10^3 \Omega \cdot \text{cm}^2$). Lower values of R_1 and R_2 in complete α -MEM, compared to the inorganic solution, indicated that the presence of organic compounds led to a less protective corrosion products film⁵¹. In fact, the presence of organic compounds has been reported to accelerate the degradation rate of Mg alloys by the formation of organometallic complexes with Mg^{+2} , which encourages the dissolution of the metallic substrate¹⁴⁵.

Focusing on the differences among the alloys, it is observed that the extruded Mg0.5Zn0.2Ca alloy shows the lowest total resistance compared to as-cast alloys in both solutions. The lower corrosion resistance of the extruded alloy may be associated to the lower grain size and the higher number of defects generated by the extrusion process (i.e. dislocations). However, the behavior of the as-cast alloys does not significantly differ from each other in both solution. This trend was in agreement with the data obtained from potentiodynamic curves (**Figure 34 (b)**).

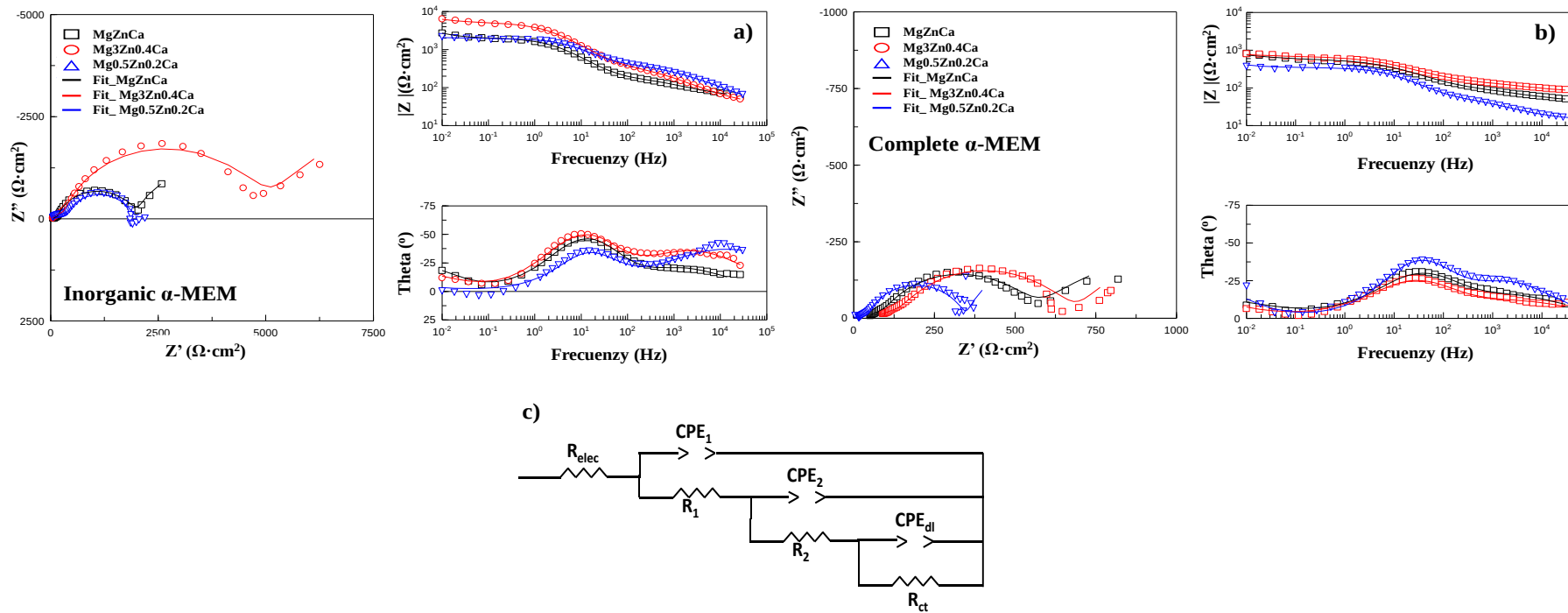


Figure 35. Nyquist and Bode plots for MgZn_xCa_y alloys in (a) inorganic α-MEM and (b) complete α-MEM solution at 37°C. (c) Electrical equivalent circuit used for the fitting.

Table 28. EIS fitted data of MgZn_xCa_y alloys in inorganic α-MEM and complete α-MEM solution at 37°C.

Solution	Alloy	R_{elec} (Ω·cm ²)	CPE_1 (μS·s ⁿ ·cm ⁻²)	n_1	R_1 (Ω·cm ²)	CPE_2 (μS·s ⁿ ·cm ⁻²)	n_2	R_2 (Ω·cm ²)	CPE_{dl} (mS·s ⁿ ·cm ⁻²)	n_3	R_{ct} (Ω·cm ²)	R_{total} (Ω·cm ²)
Inorganic α-MEM	MgZnCa	51.3	43.3	0.60	165.9	29.6	0.88	1898	10.9	0.90	2500	4564
	Mg ₃ Zn _{0.4} Ca	56.9	26.6	0.63	198.0	19.7	0.89	3014	2.1	0.51	3800	7012
	Mg _{0.5} Zn _{0.2} Ca	36.8	10.1	0.58	171.3	24.4	0.87	1489	30.6	0.56	1500	3160
Complete α-MEM	MgZnCa	41.7	71.3	0.58	81.3	77.8	0.72	452	19.2	0.56	803	1336
	Mg ₃ Zn _{0.4} Ca	71.6	84.8	0.52	107.3	83.2	0.70	520.3	46.0	0.65	750	1378
	Mg _{0.5} Zn _{0.2} Ca	14.2	64.8	0.68	49.1	88.1	0.82	286.5	81.1	0.80	500	836

4.2.2.4 Hydrogen evolution tests

4.2.2.4.1 Control of pH by daily solution replacement

Hydrogen evolution studies with periodical (every 24 h) refreshment of the medium were conducted for 120 h of immersion at 37°C in both inorganic and complete α -MEM solutions to further analyse the effect of the media (**Figure 36** (a, b)).

In general terms, the hydrogen volume collected for the three alloys was higher in complete α -MEM (up to 6 times for Mg₃Zn_{0.4}Ca and Mg_{0.5}Zn_{0.2}Ca alloys) compared to inorganic α -MEM solution. Notably, the test in inorganic α -MEM was not able to discriminate between the alloys. Besides, the higher volume of hydrogen generated in complete α -MEM was in agreement with the ranking obtained by electrochemical measurements and was related to the presence of organic compounds. In the short term, these compounds negatively affect the formation of a resistant corrosion products protective layer (as proved by EIS).

In **Figure 36** (a), the three alloys showed an increase of collected hydrogen during the first 5 h of immersion in inorganic α -MEM solution followed by a slight stabilization, probably related to the formation of a partially protective layer on the surface of the specimens³⁴⁴. This stabilization was clearer in the case of inorganic α -MEM medium, which was in agreement with the resistance values of the corrosion products measured by EIS (R_1 and R_2 , **Table 28**). Considering the average values in this medium for the three measured specimens and their standard deviations, non-significant differences were found among the studied materials (max. difference approx. 0.3 mL/cm²). These degradation rates are at least 10 times lower than those observed for Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag after 120 h of immersion in SBF under periodical (every 24 h) refreshment of the medium (**Figure 24**). The possible reason for this behavior could be the differences in the secondary phase distribution and their electrochemical activity, as previously discussed in agreement with SKPFM findings.

In the case of complete α -MEM medium (**Figure 36** (b)), the behaviour of the three materials was similar during the first 10 h of immersion. After that time, the extruded alloy started to show a higher degradation rate compared to the as-cast alloys. The H₂ volume of the Mg₃Zn_{0.4}Ca alloy increased considerably after 55 h of immersion, while for MgZnCa it began to increase after 80 h of immersion. At the end of the test,

Mg₃Zn_{0.4}Ca and Mg_{0.5}Zn_{0.2}Ca alloys exhibited similarly high H₂ volume (~1.5 mL/cm²) compared with MgZnCa alloy (0.7 mL/cm²).

Figure 36 (c, d) shows the pH evolution for MgZn_xCa_y alloys in inorganic and complete α -MEM solutions at 37°C during 24 h of immersion. The pH readings were collected periodically during the first 8 h and right before the medium replacement. All materials (regardless of the medium) showed similar trends with a pH increase from 7.2 up to 8.5-9 due to the liberation of OH⁻ during the degradation process of Mg^{345,346}. After 2 h of immersion a change of slope is observed in both media, due to the formation of a continuous corrosion products layer. In the case of the inorganic α -MEM solution, the pH slope decreases slightly after 2 h of immersion due to the protection of the corrosion products, while for complete α -MEM it increases with time, which could be related to a faster degradation due to the presence of organic compounds. However, after 24 h of immersion there are no relevant differences between the media pH.

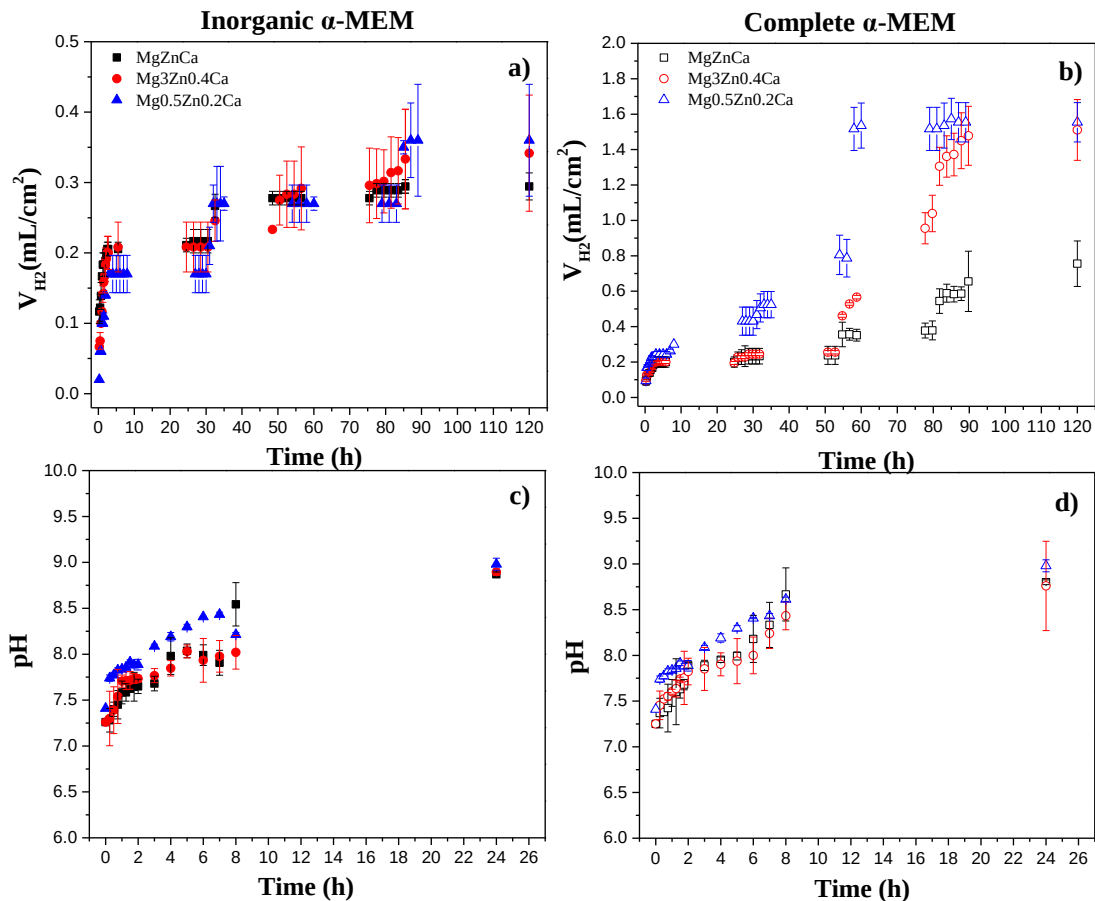


Figure 36. (a-b) Hydrogen evolution of MgZn_xCa_y alloys during 120 h of immersion at 37°C in (a) inorganic α -MEM and (b) complete α -MEM using daily solution replacement. (c, d) pH evolution of MgZn_xCa_y alloys during 24 h of immersion at 37°C in (c) inorganic α -MEM and (d) complete α -MEM solutions.

4.2.2.4.2 Control of pH under CO₂ flow

Figure 37 (a) shows hydrogen evolution results for MgZn_xCa_y alloys during 120 h of immersion in inorganic α -MEM obtained when controlling the pH at 7.4 under CO₂ flow. Both as-cast alloys present similar behavior during the short-term evaluation (up to 50 h) which correlates with the short-term electrochemical results where no significant differences between the two alloys were found (**Figure 34** and **Figure 35**). For longer immersion times (after 50 h), Mg3Zn0.4Ca started generating greater H₂ volume that indicated a higher degradation rate of this material compared to MgZnCa alloy. The extruded Mg0.5Zn0.2Ca alloy revealed much higher collected H₂ volume from the beginning of the test compared to the as-cast alloys.

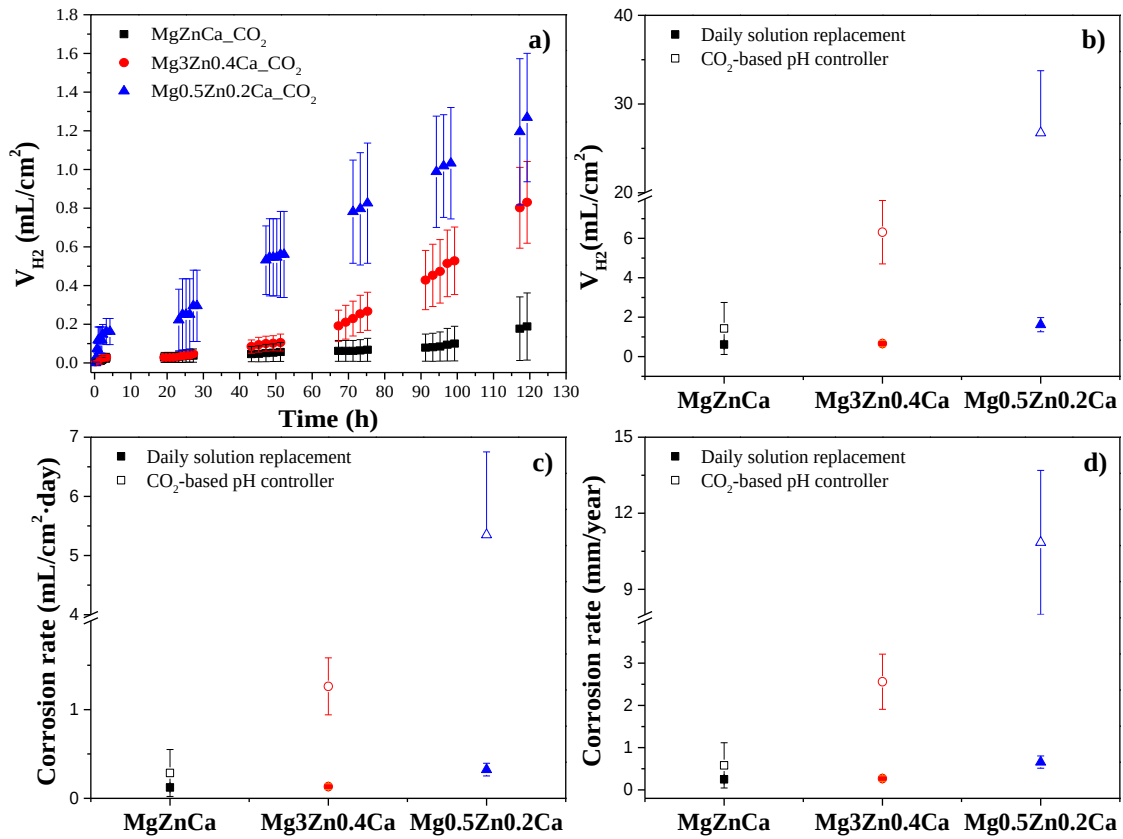


Figure 37. (a) Hydrogen evolution of MgZn_xCa_y alloys during 120 h of immersion at 37°C in inorganic α -MEM solution using CO₂-based pH controller system. Corrected values for 120 h of immersion using both pH control strategies (b) hydrogen evolution and (c, d) corrosion rate.

According to the end of the test values, the degradation performance of the studied alloys can be ranked in the following order (from the best corrosion behavior to the worst): MgZnCa < Mg3Zn0.4Ca < Mg0.5Zn0.2Ca. The higher degradation rate of the extruded material could be related to the presence of a high number of defects, typically introduced during extrusion (dislocations and twins), which is in agreement with other

reports^{347,348}. These observations highlight the fact that the processing method along with the alloying elements play an important role in the corrosion rate of the material. In the case of the as-cast alloys, Mg₃Zn_{0.4}Ca alloy showed higher degradation rate compared to MgZnCa alloy which could be associated with the higher secondary phase area fraction and more refined grain size morphology in MgZnCa acting as a barrier of corrosion.

Figure 37 (b, c) shows the comparison of the total evolved H₂ volume and the corresponding H₂ evolution rate (equivalent to degradation rate), respectively. All data have been obtained after 120 h of immersion using the two different pH control setups (**Figure 16**). The total evolved H₂ volume takes into account the amount of dissolved hydrogen limit in water (1.4 mg/mL at 37°C). It is clearly observed that the values obtained under CO₂ flow based pH control strategy were about 2-20 times higher, compared to the daily solution replacement strategy. The degradation rates of the alloys (**Figure 37** (d)) under these conditions in terms of mm/year (0.6-10.8 mm/year) indicate that the potential implants would degrade prematurely and might negatively affect the tissue regeneration process. In the case of daily media replacement, the corrosion rate was clearly underestimated, which can be ascribed to the increase of the pH during the 24 h, allowing a better stabilization of the corrosion products^{349,78}. That increase of the pH does not occur in the case of CO₂ flow based pH control strategy, and, therefore, the stabilization of the corrosion products layer is not promoted. These results demonstrated that pH plays an important role in corrosion process of Mg alloys, which brings up the concern of comparing literature results on hydrogen evolution obtained using different setups of pH control

4.2.2.4.3 Corrosion characterization

Figure 38 shows the effect of the media and pH control mode on macro-appearance and cross-sectional corrosion layer morphologies after 120 h of immersion. Specimens tested with pH control by daily replacement of the solution show a corrosion layer with a thickness below 20 µm (a-f). The corrosion layer is rich in Mg and O and the Ca/P-rich deposits⁷⁸ that form above it are better developed in complete α-MEM (d, e, f). **Table 29** shows the EDS results corresponding to the locations indicated in **Figure 38**. The presence of Ca is due to the incorporation of elements from the physiological solutions and dissolution of the substrate. P is coming only from the physiological solutions and Zn only from the substrate. The incorporation of the elements from the physiological solutions has been reported as beneficial for the overall resistance of Mg alloys¹⁴⁵.

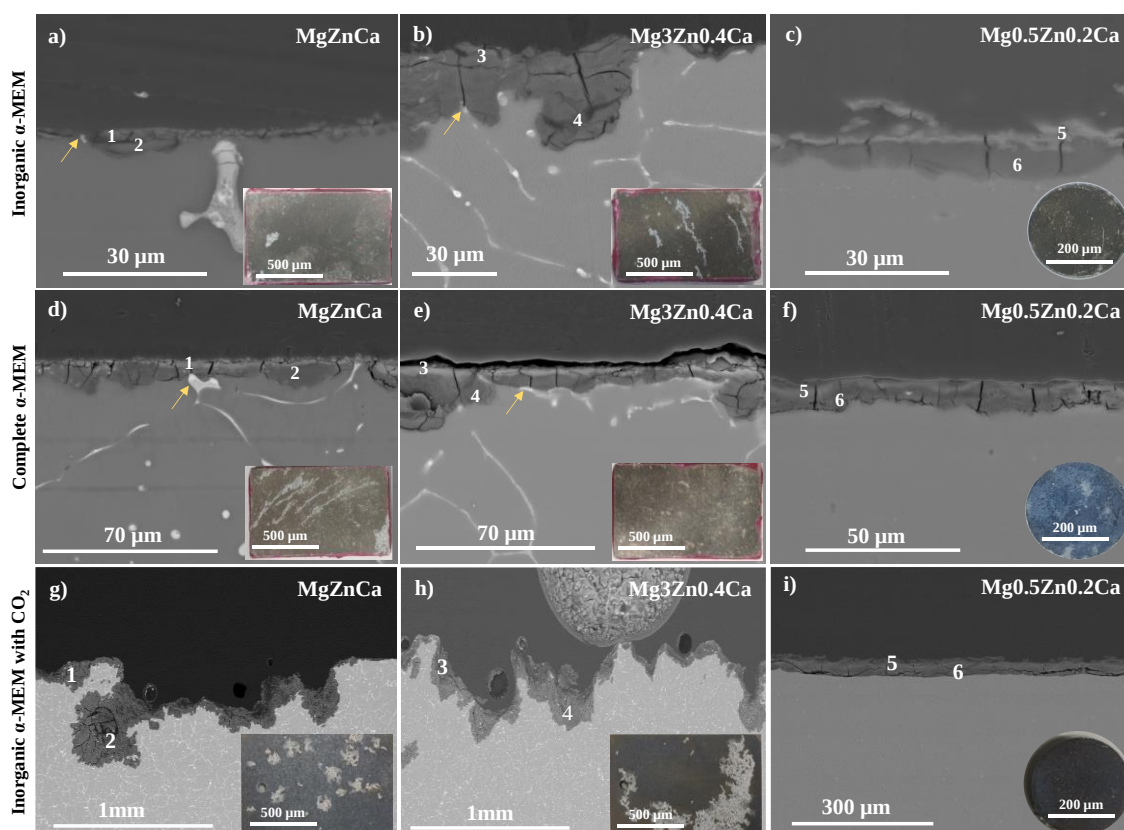


Figure 38. Backscattered electron longitudinal macro views and secondary electron cross-section of MgZn_xCa_y alloys after 120 h of immersion in (a-c) inorganic α -MEM and (d-f) complete α -MEM solutions in (a-f) daily solution replacement and (g-i) CO_2 -based pH controller systems.

Under CO_2 pH control, as-cast alloys suffer extensive localized corrosion with depths up to 160 μm , whereas the extruded alloy shows uniform degradation with a 40 μm -thick corrosion layer. The corrosion morphology of as-cast alloys is likely to be related to the constant pH conditions (pH 7.4) and the microsegregation of Ca and Zn facilitating localized corrosion mechanisms. The $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ alloy shows a greater area fraction of localized corrosion compared with the MgZnCa (~21 vs 10 %, Appendix, **Figure A1** and **Table A1**). The thickness of the corrosion products of both the localised and uniform areas presented in **Table A1** were calculated by image analysis of both the macro- and micrographs (**Figure 38**) and optical micrographs (**Figure A1**). For this purpose, the ImageJ software was employed. The pit depth and pit volume were obtained from macrographs in **Figure A1** using the software of optical focus variation profilometer. Localised corrosion in the as-cast alloys, MgZnCa and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$, is attributed to the formation of strong galvanic couples between the Zn and/or Ca-rich secondary phases and the α -Mg matrix. These second phase particles are difficult to oxidize. As a consequence of this, they remain embedded in the corrosion products as can be seen in **Figure 38** (a, b, d, e). In constant pH 7.4 conditions, where the $\text{Mg}(\text{OH})_2$ is less stable, the access of the corrosive medium to the galvanic couple

becomes easier, which causes the acceleration of the corrosion process. This is consistent with the hydrogen volume measurements.

It should be noted that as-cast alloys show a similar response when tested for short immersion times. However, after several days of immersion, the MgZnCa alloy shows better corrosion resistance than the Mg₃Zn_{0.4}Ca alloy. This is attributed here to the higher area fraction and more refined morphology of secondary phases in the MgZnCa, thus acting as a barrier for corrosion. This can be seen in **Figure 38** where corrosion mainly occurs in the α -Mg phase and the secondary phases at grain boundaries remain relatively unaffected.

According to VPD results reported in ⁴⁷, the barrier effect is expected to be provided mainly by the Ca₂Mg₆Zn₃ phase since it is more noble with respect to the α -Mg matrix, whereas the Mg₂Ca phase is more likely to dissolve due to its anodic behaviour. The results of the present study (with 0.4 Zn/Ca ratio in MgZnCa, and 4.8 Zn/Ca in Mg₃Zn_{0.4}Ca, **Table 14**) are in agreement with those of ^{346,350}, where Mg-Zn-Ca alloys with a relatively low Zn/Ca atomic ratio showed an increased formation of both Mg₂Ca and Ca₂Mg₆Zn₃ and, therefore, better corrosion resistance.

Overall, regardless of the pH control mode, hydrogen measurements demonstrate that the extruded alloy degrades much faster than as-cast alloys, despite its much lower area fraction of secondary phases and smaller grain size. This behaviour is opposite to that reported in studies ³⁵¹ and ³⁵² where the extruded versions of Mg₃Zn_{0.2}Ca and Mg_{2.5}Zn₁Ca alloys showed lower corrosion rates than the as-cast ones. In those studies, the improvement was associated with grain refinement promoting the formation of a more protective layer of corrosion products. It is important to note that in those studies the pH was not controlled during testing. Other important differences are the testing media, the alloy compositions, and the specific extrusion conditions. Whatever the case, it is evident that extruded Mg-Zn-Ca alloys reveal a different corrosion behaviour than their as-cast counterparts. Our results indicate that the higher number of defects in Mg_{0.5}Zn_{0.2}Ca alloy formed due to extrusion plays a more important role than its composition from a corrosion point of view.

Table 29. EDS analysis (at. %) of MgZn_xCa_y alloys as per **Figure 38** after 120 h of immersion in inorganic and complete α -MEM solution in the both study systems.

System	Alloy	Location	O	Na	Mg	Si	P	K	Ca	Zn	Ca/P	
Solution daily replacement	Inorganic α -MEM	MgZnCa	1	51.6	0.5	31.0	0.3	7.8	0.0	5.3	3.5	0.68
			2	66.5	0.8	25.1	0.0	3.3	0.2	2.2	2.0	0.69
		Mg3Zn0.4Ca	3	24.4	0.5	29.1	0.7	26.0	0.8	18.0	0.5	0.69
			4	44.1	0.4	41.1	0.5	9.7	0.3	3.5	0.5	0.37
		Mg0.5Zn0.2Ca	5	19.3	0.2	49.2	0.7	14.6	0.2	15.2	0.6	1.04
			6	5.5	0.3	69.5	2.6	12.3	0.2	8.7	0.9	0.70
	Complete α -MEM	MgZnCa	1	57.5	1.0	18.4	0.2	12.2	0.2	9.8	0.7	0.80
			2	58.2	0.6	30.8	0.1	4.4	0.1	3.7	2.2	0.84
		Mg3Zn0.4Ca	3	63.7	0.3	8.9	0.1	14.4	0.1	12.5	0.1	0.87
			4	66.5	0.3	30.8	0.0	1.0	-	1.2	0.2	1.28
		Mg0.5Zn0.2Ca	5	28.3	0.3	27.8	0.4	28.7	0.3	13.9	0.4	0.48
			6	59.6	0.3	38.1	0.2	1.1	0.1	0.6	0.2	0.50
CO ₂ -base pH controller	Inorganic α -MEM	MgZnCa	1	45.8	1.0	17.7	0.1	15.6	0.2	19.6	0.1	1.26
			2	57.5	0.1	42.1	0.0	-	-	0.1	0.2	-
		Mg3Zn0.4Ca	3	46.0	0.8	25.3	0.2	10.8	0.2	12.9	3.8	1.19
			4	54.2	0.2	43.0	-	0.1	0.0	0.5	2.1	4.00
		Mg0.5Zn0.2Ca	5	47.8	1.3	8.2	0.1	17.1	0.0	20.7	0.1	1.21
			6	40.8	0.7	10.8	0.4	19.6	-	26.5	0.7	1.36

4.3 Conclusions of Chapter IV

4.3.1 Alloy characterization

The microstructural and surface potential characterization evidence a significant effect of the alloying elements, the manufacturing process, and, most importantly, the presence of impurities on the formation of the microstructure, and subsequently on the corrosion mechanisms of the alloys.

Mg-Ca and Mg-Ca-Ag systems

- Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys are constituted by α -Mg grains and a discontinuous network of secondary phases distributed along the grain boundaries. The eutectic aggregates in Mg_{0.6}Ca alloy with lamellar morphologies were constituted by α -Mg/Mg₂Ca phases and polygonal, Fe, Al and Si impurities containing particles inside the grains. In Mg_{0.6}Ca₂Ag alloy, additionally contained Mg₅₄Ag₁₇ and MgAg₄ particles, contaminated with Si, Fe, Ni, Cr and Co impurities.
- SKPFM findings disclosed that Mg₂Ca phase of Mg_{0.6}Ca alloy presented anodic behavior while particles with impurities exhibited cathodic behavior compared to the matrix. In the case of Mg_{0.6}Ca₂Ag, particles with Ca/Ag ratio <1 (enriched in Ag) exhibited cathodic behavior while Ca/Ag >2 (enriched in Ca) presented anodic behavior.

Mg-Zn-Ca systems

- As-cast MgZnCa and Mg₃Zn_{0.4}Ca alloys were constituted by α -Mg grains and a discontinuous network of secondary phases along the grain boundary corresponding to Ca₂Mg₆Zn₃ and Mg₂Ca phases and Ca₂Mg₆Zn₃ particles containing impurities inside the grains. The extruded Mg_{0.5}Zn_{0.2}Ca alloy was constituted by α -Mg equiaxed grains with Ca₂Mg₆Zn₃ particles contaminated with impurities such as Fe, Si, Al, and Mg₂Ca phase distributed along the extrusion direction.
- SKPFM results revealed cathodic Volta potential values for Zn-rich (ternary) intermetallics with impurities (40-250 mV) and anodic Volta potential for Ca-rich (binary) intermetallics in the range of -40 to -100 mV. After 1 h of immersion, a decrease of VPD was observed for anodic and cathodic intermetallics, due to the formation of Ca-P compounds that contributed to the measured VPD. Corrosion occurs

in the α -Mg phase without preferential dissolution of the secondary phases at grain boundaries.

4.3.2 Corrosion evaluation

A reliable routine for screening of the corrosion resistance of the alloys and determining the realistic alloy degradation rate is proposed. To this end, EIS in α -MEM with and without organic components elucidated the role of corrosion media composition on the short-term corrosion behavior and its ability to discriminate between the corrosion resistances of different alloys. The influence of the medium pH on the alloy degradation rate was disclosed by collecting H_2 gas during immersion tests using two different strategies of pH control: a daily solution replacement and a continuous CO_2 flow.

Mg-Ca and Mg-Ca-Ag systems

- The degradation mechanism during immersion of the studied materials in SBF with daily replacement of the medium corresponded to a generalized corrosion in both alloys. The Mg_{0.6}Ca₂Ag alloy degraded at a rate of ~31 mm/year, due to the varied content of Ag and high level and variety of impurities in Mg–Ca–Ag intermetallic particles, which were mainly distributed along the grain boundaries, some of them acting as local anodes and causing the loss of the whole grain or clusters of grains as a result of a grain boundary dissolution. The same mechanism acted in Mg_{0.6}Ca alloy but to a much lesser extent, which led to a slower corrosion process at a rate of ~8 mm/year.

Mg-Zn-Ca systems

- The presence of organic components in complete α -MEM decreases the corrosion resistance of MgZn_xCa_y alloys by 2-5 times compared to α -MEM with inorganic components only. The immersion test with daily replacement of the medium underestimated the corrosion rate of the MgZn_xCa_y alloys by 2-20 times due to the stabilization of the corrosion product layer at pH>7.4. The immersion test performed at constant pH 7.4 maintained by CO_2 flow approximates the degradation conditions to those of *in-vivo*.
- Under CO_2 flow, the as-cast alloys exhibit localized corrosion, which related to the constant pH 7.4 and the microsegregation of Ca and Zn. In short term there are no significant differences in corrosion behaviour of the as-cast alloys. In long term, the

MgZnCa alloy with low Zn/Ca ratio shows better corrosion resistance than the Mg₃Zn_{0.4}Ca alloy due to the higher area fraction and more refined morphology of secondary phases acting as a barrier for corrosion.

- The extruded Mg_{0.5}Zn_{0.2}Ca alloy exhibits a uniform corrosion in all studied systems but at 2 times higher corrosion rate compared to the as-cast alloys, indicating that the higher number of defects formed during the extrusion process plays a more important role than the alloy composition.

All studied alloys exhibited high corrosion rates under CO₂ flow (0.6-10.8 mm/year) and need surface modifications as an additional strategy to control the degradation rate. A varying degree of micro-galvanic coupling in the microstructure of these alloys presents a challenge for surface coatings. The potential of PEO technology to provide protection to these alloy systems is explored in Chapter V.

5. CHAPTER V - Plasma electrolytic oxidation coatings

As it has been mentioned in the Introduction (Section 1.4.2), PEO is the most popular and effective method to improve the corrosion resistance, wear resistance and surface hardness of Mg alloys. The design of the PEO electrolyte plays a critical role in the corrosion mechanisms of coated Mg. Previous studies have demonstrated that the incorporation of Ca, P and Si into the electrolyte favors the formation of HA *in situ* during PEO and enhances the cell response *in vitro* (i.e. adhesion, proliferation, differentiation and collagen synthesis), while the presence of fluoride in the electrolyte enhances corrosion resistance but decreases the cytocompatibility with endothelial cells^{108, 111}.

Considering the above, Chapter V is focused on the design and development of bioactive ceramic coatings enriched in Ca, P, Si and/or F on cast Mg0.6Ca, Mg0.6Ca2Ag, MgZnCa and Mg3Zn0.4Ca and extruded Mg0.5Zn0.2Ca alloys in terms of corrosion behavior. The structure of the chapter is divided in two sections according to the coatings formed in: i) F-containing electrolytes (used as a corrosion performance reference) and ii) F-free electrolytes. For both types of electrolytes, chemical compositions leading to stable true solutions are explored as an alternative to compositions that form suspensions of salt precipitates. The main goals are i) to pinpoint the route for the generation of PEO coatings devoid of potential cytotoxicity problems that improve the Mg alloys corrosion performance and ii) identify a reliable corrosion screening procedure for the developed coatings. **Figure 39** shows the graphical abstract of Chapter V.

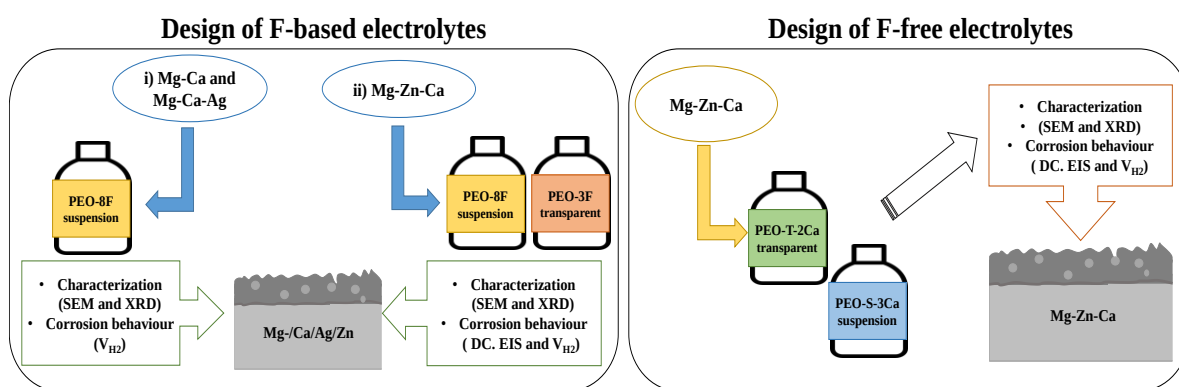


Figure 39. General scheme of Chapter V.

5.1 Fluoride-containing PEO coatings

5.1.1 Mg0.6Ca and Mg0.6Ca2Ag alloys

In order to reduce the high degradation rate of Mg0.6Ca and Mg0.6Ca2Ag alloys, ceramic coatings with high corrosion protection can be developed. In the research group, bioactive coatings with high fluorine content have been developed obtaining thick coatings with high corrosion protection. According to this, bioactive PEO coatings with high fluorine content will be developed on these alloys.

5.1.1.1 Voltage-time response and process efficiency

Figure 40 shows the voltage and current response during the anodizing of Mg0.6Ca and Mg0.6Ca2Ag alloys in suspension electrolyte with high fluorine content, further denominated as PEO-8F (**Table 17**), using a waveform signal specified in **Table 16**. The rms voltage-time curve has a marked inflection point at 60 s, which corresponds to the end of the set voltage amplitude sweep. The commencement of the plasma microdischarges was impossible to confirm visually (due to the cloudiness of the electrolyte), but it can be allocated to ~210 V and ~225 V for Mg0.6Ca2Ag and Mg0.6Ca alloys, respectively. These are points where the loss of voltage-time linearity is observed due to the dielectric breakdown of the barrier oxide layer.

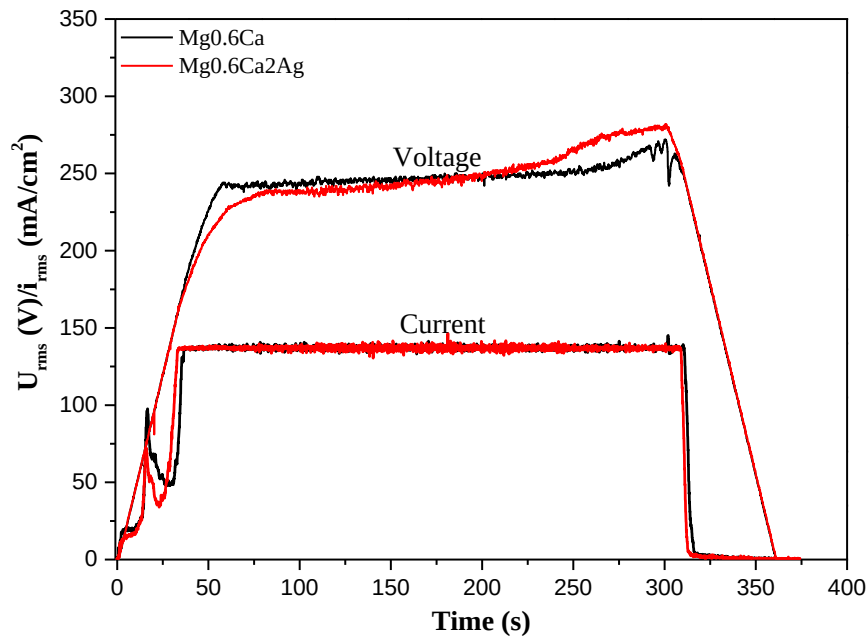


Figure 40. I_{rms} / i_{rms} versus time for both alloys with the coating formed in PEO-8F suspension electrolyte.

The voltage value oscillates around 240 V and increases at the final stage till 275 V; this fact reveals the increase of the coating thickness and impedance. With respect to the current density, it can be observed that after the initial 60 s ramp period, it reaches the previously set limit value of $138 \text{ mA} \cdot \text{cm}^{-2}$ and remains constant during the whole process. The calculated apparent specific energy consumption values were $0.032 \text{ kW} \cdot \text{h} \cdot \text{m}^{-2} \cdot \mu\text{m}^{-1}$ and $0.037 \text{ kW} \cdot \text{h} \cdot \text{m}^{-2} \cdot \mu\text{m}^{-1}$ for Mg0.6Ca and Mg0.6Ca2Ag alloys, respectively.

5.1.1.2 Coating morphologies, structure and composition

Figure 41 shows the plan view and cross-sectional coating morphologies of Mg0.6Ca/PEO-8F and Mg0.6Ca2Ag/PEO-8F coatings. In both alloys the coating surface presents a typical crater-like porous morphology associated with the sites of discharge channels, gas evolution and rapid solidification phenomena^{104,353}. In both Mg0.6Ca/PEO-8F and Mg0.6Ca2Ag/PEO-8F coatings the surface Ca/P ratio is relatively high (1.42 and 1.53, respectively) compared to the inner regions of the coating (**Table 30**), but lower than that of biological hydroxyapatite (1.67)³⁵⁴, both Ca and P contents increase towards the coating/electrolyte interface. The surface enrichment of biomaterials (e.g., of Ti alloys) in Ca and P is well known to improve the initial cell response³⁵⁵.

The cross-sectional images (**Figure 41** (c, d)) reveal relatively uniform coatings with thicknesses in the range of 32–35 μm . It can be observed that both coatings are constituted by three layers. A thin barrier layer (less than 1 μm) adjacent to the substrate is mainly composed of Mg, O and F and Ag (the latter only in the case of Mg0.6Ca2Ag, **Table 30**). An intermediate region with small pores constitutes ~40 % of the coating thickness. An outer, more compact region contains a few but relatively large pores. It is worth mentioning that EDS analysis for the Mg0.6Ca2Ag alloy did not detect the presence of Ag in the coating surface, whereas the Ag content in the inner regions did not exceed ~0.2 at. %, suggesting that a rather limiting, if any, antibacterial effect can be expected due to the presence of Ag at the initial stage of the implantation.

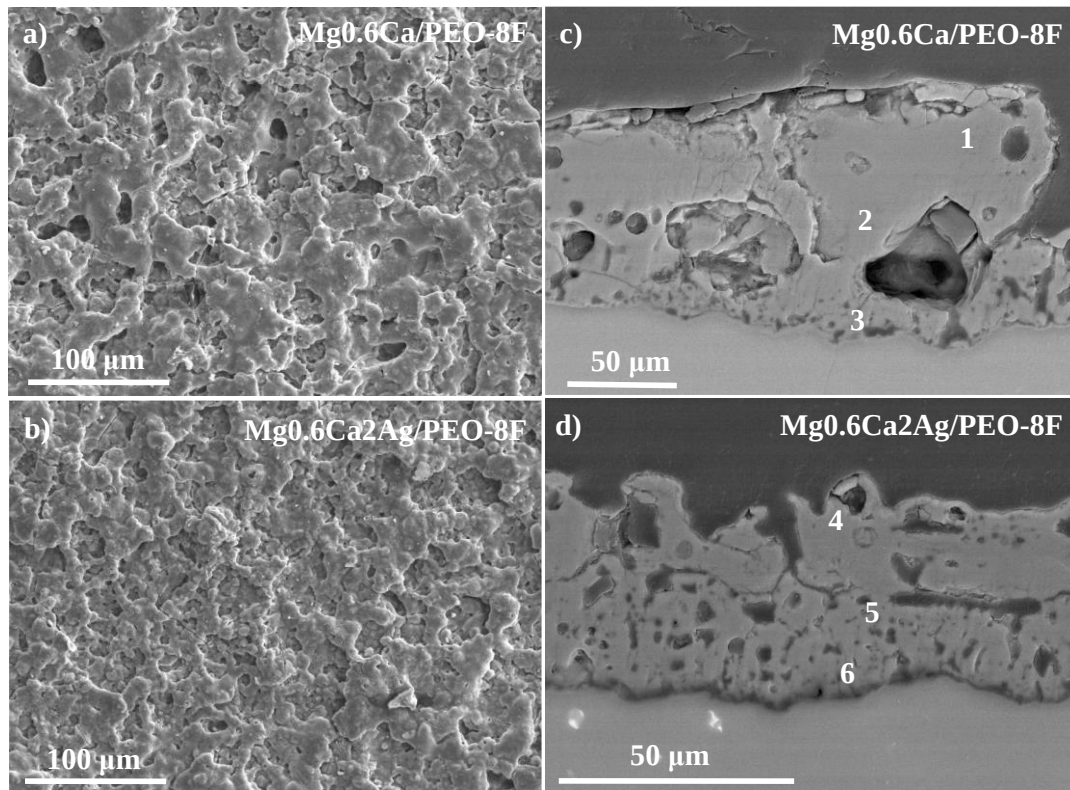


Figure 41. Backscattered electron micrographs of coating surface morphologies and cross-sections of Mg0.6Ca/PEO-8F (a, c) and Mg0.6Ca2Ag/PEO-8F (b, d).

The R_a and R_z values are the most common for the characterization of implants. The R_z value refers to the depth of the average roughness (average between the deepest valleys and highest ridges) called overlay ripple; and the R_a corresponds to the average roughness (average of the absolute values of the profile deviations). The mean value of R_a and R_z are similar for both Mg0.6Ca and Mg0.6Ca2Ag alloys as it is observed in **Table 31**, which indicates that is a suitable value for their application in biodegradable implants.

Table 30. Compositions of Mg0.6Ca/PEO-8F and Mg0.6Ca2Ag/PEO-8F by EDS (at. %).

Alloy	Location	Mg	O	F	Na	P	Ca	Ag	Ca/P
Mg0.6Ca	Plan view	22.1	44.9	16.3	4.2	5.2	7.3	–	1.42
	1	39.7	24.5	32.5	0.3	2.9	0.1	–	0.63
	2	35.2	21.2	36.7	1.9	4.4	0.6	–	0.14
	3	36.5	34.9	18.6	4.2	3.6	2.3	–	0.05
Mg0.6Ca2Ag	Plan view	27.0	42.5	16.2	3.7	4.2	6.4	–	1.53
	4	37.6	22.3	34.7	1	3.8	0.5	0.2	0.4
	5	32.4	26.3	30.1	4.3	5.1	1.5	0.2	0.3
	6	36.2	37.3	13	4.6	6.3	2.5	–	0.12

Both values are analogous, which means that the treatment by plasma electrolytic oxidation has been similar. The coating hardness values are higher compared to those for

the substrate (53 HV for Mg0.6Ca and 45 HV for Mg0.6Ca2Ag). The Mg0.6Ca alloy coating has a higher hardness than Mg0.6Ca2Ag due to the difference in porosity of both coatings, the Mg0.6Ca2Ag alloy has more pores in the outer layer. Furthermore, it can be expected that before immersion the samples will have higher values than after immersion, as the coating layer hydrates. For comparison, human bone has the following hardnesses: ~77 HV and ~24 HV for the cortical and trabecular bone of the femur, respectively ³⁵⁶.

Table 31. Surface characteristics of PEO-8F coatings formed on Mg0.6Ca and Mg0.6Ca2Ag alloys.

Parameters	Mg0.6Ca/PEO-8F	Mg0.6Ca2Ag/PEO-8F
Thickness (μm)	35	32
R _a (μm)	3.0 $\pm$ 0.4	3.4 $\pm$ 0.2
R _z (μm)	17.1 $\pm$ 0.5	15.2 $\pm$ 1.3
Hardnesses (HV)	407 $\pm$ 75	314 $\pm$ 59

*Applied: 10 g

The XRD analyses (**Figure 42**) of coated materials revealed peaks corresponding to the substrate and to crystalline phases such as MgO, MgF₂, CaF₂ and Ca₅(PO₄)₃F. The MgO is formed due to electrolytic oxidation of the substrate and the other phases are formed due to plasma-chemical reactions between the ions of the electrolyte and the substrate inside the microdischarge channels ³⁵⁷.

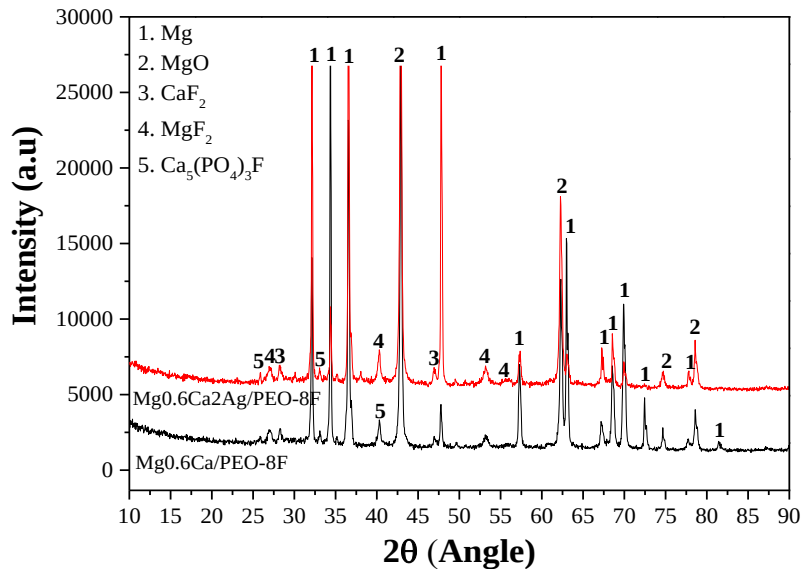


Figure 42. XRD patterns from PEO-8F coatings on Mg0.6Ca and Mg0.6Ca2Ag alloys.

5.1.1.3 Corrosion behavior of the coatings

Figure 43 (a) shows the hydrogen evolution volume and hydrogen evolution rate for Mg0.6Ca and Mg0.6Ca/PEO-8F after 60 days of immersion in m-SBF at 37°C.

Figure 43 (c) shows the volume of evolved hydrogen for Mg0.6Ca2Ag and Mg0.6Ca2Ag/PEO-8F after 4 days of immersion. As expected, the uncoated material initially exhibited a high amount of hydrogen with an evolution rate of 3.86 mL/cm²·week after 1 week of immersion, with progressive decrease to 1.02 and 1.11 mL/cm²·week after 6 and 8 weeks of immersion, respectively. The decrease of hydrogen evolution rate is due to the generation of a corrosion products layer, which acts as a partially protective coating. Mg0.6Ca/PEO-8F showed a considerably reduced hydrogen evolution rate during the first 4 weeks (~1.11 mL/cm²·week); however, after that time both non-coated and PEO-coated materials reached a similar degradation rate. Further increase of the hydrogen evolution rate was evident after 6 weeks (1.75 mL/cm²·week) due to the loss of protective properties of the inner PEO layer and increased electrochemical activities at the substrate/coating interface^{358,359,360}. The latter degradation rate corresponds to 1.9 mg/(cm²·week) of mass loss or 11 μm/week of thickness loss. Both Mg0.6Ca2Ag and Mg0.6Ca2Ag/PEO-8F exhibited an extremely high degradation rate (**Figure 43** (c)), corresponding to ~60 or ~20 mL/cm², respectively, and the experiments were stopped after 4 days of immersion.

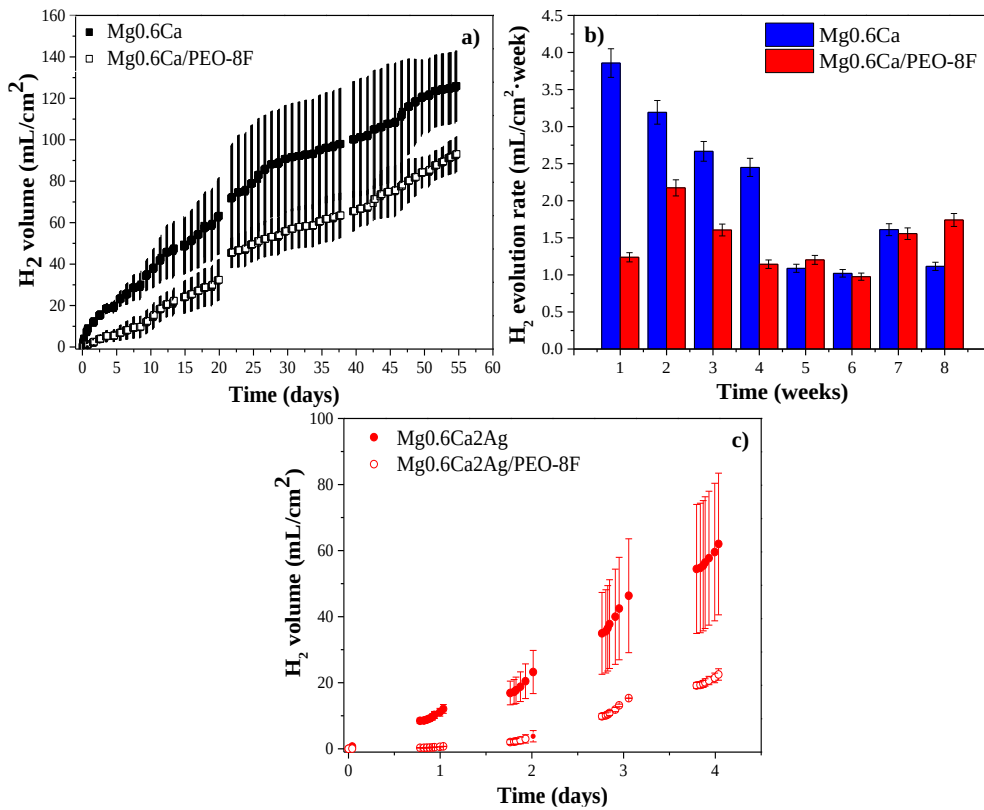


Figure 43. (a) Hydrogen volume, (b) hydrogen evolution rate of Mg0.6Ca and Mg0.6Ca/PEO-8F after 60 days. (c) Hydrogen volume of Mg0.6Ca2Ag and Mg0.6Ca2Ag/PEO-8F after 4 days of immersion in m-SBF.

Figure 44 shows the macrophotos of the degraded specimens of coated and non-coated Mg0.6Ca alloy (**Figure 44** (a, b)), both reveal a uniform corrosion with similar loss of material and dimensions. Mg0.6Ca2Ag also shows a generalized, but heavily heterogeneous corrosion morphology for the non-coated specimens (**Figure 44** (c)) and localized corrosion for the PEO-coated ones. Both coated and non-coated Mg0.6Ca2Ag specimens were found completely disintegrated after 10 days of immersion.

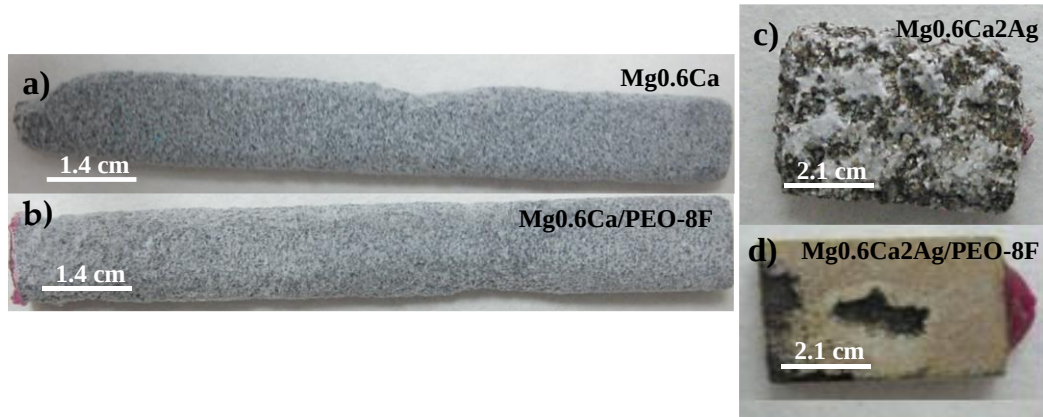


Figure 44. 3D macrophoto of (a) Mg0.6Ca, (b) Mg0.6Ca/PEO-8F after 60 days, (c) Mg0.6Ca2Ag and (d) Mg0.6Ca2Ag/PEO-8F after 4 days of immersion in m-SBF.

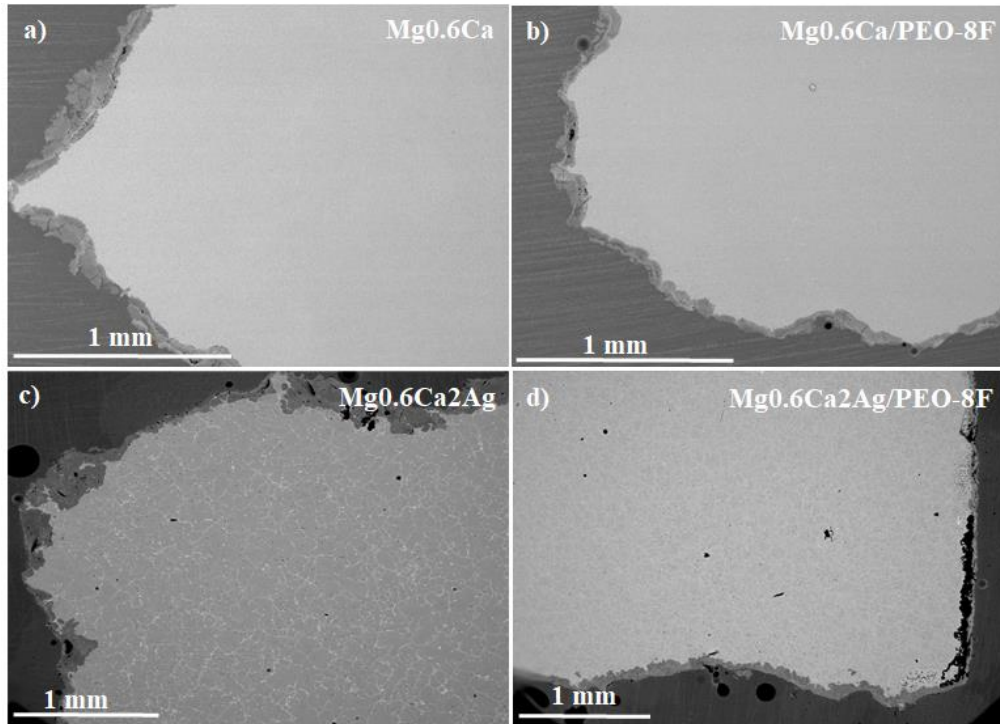


Figure 45. Cross-section before corrosion (a) Mg0.6Ca, (b) Mg0.6Ca/PEO-8F after 60 days and (c) Mg0.6Ca2Ag and (d) Mg0.6Ca2Ag/PEO-8F after 4 days of immersion in m-SBF.

SEM examination following the 60 days and 4 days immersion for Mg0.6Ca and Mg0.6Ca 2Ag alloys, respectively, revealed a heavy generalized corrosion in all cases (**Figure 45**) with no trace of attached PEO coatings left. A complete loss of adhesion is

not a typical behavior for PEO-coated Mg alloy. Typically, a 40–50 μm -thick coating would remain mostly adhered even after 8 weeks of immersion with a thick corrosion product layer developing underneath it ³¹¹.

5.1.1.4 Fluoride Release

Figure 46 shows progressive F^- ion release from $\text{Mg}_{0.6}\text{Ca}/\text{PEO-8F}$ during 24 h of immersion in 0.9 % NaCl. After 24 h of immersion, $33.757 \mu\text{g}/\text{cm}^2$ of F^- was released, which corresponds to 1.35 ppm or $71 \mu\text{M}$ of F^- for the volume of the immersion solution. According to the solubility products, K_{sp} , of fluoride containing crystalline phases in the coating, MgF_2 , CaF_2 , and $\text{Ca}_5(\text{PO}_4)_3\text{F}$ (5.16×10^{-11} , 3.58×10^{-11} , and 8.6×10^{-61} , respectively ³⁶¹), the dissolution of these compounds will produce up to a maximum of 0.88 mM of free F^- . Hence, it would be reasonable to expect a further increase of F^- with time until a solubility limit is reached. Mohedano *et al.* has shown that the solubility limit can be reached in 12 weeks ³¹¹. For comparison, average fluorine concentration in the human blood plasma is 19 ppb ³⁶². Santos-Coquillat has demonstrated that F^- tolerance limit for endothelial cells (which are more sensitive to fluoride than other types of cells) is around 1 mM ¹⁰⁵. Therefore, even in case of a maximum reached concentration of 0.88 mM F^- (the solubility limit), the material should not present cytotoxicity. However, further studies are needed in order to confirm whether this concentration would be sufficient to exhibit any antibacterial effect.

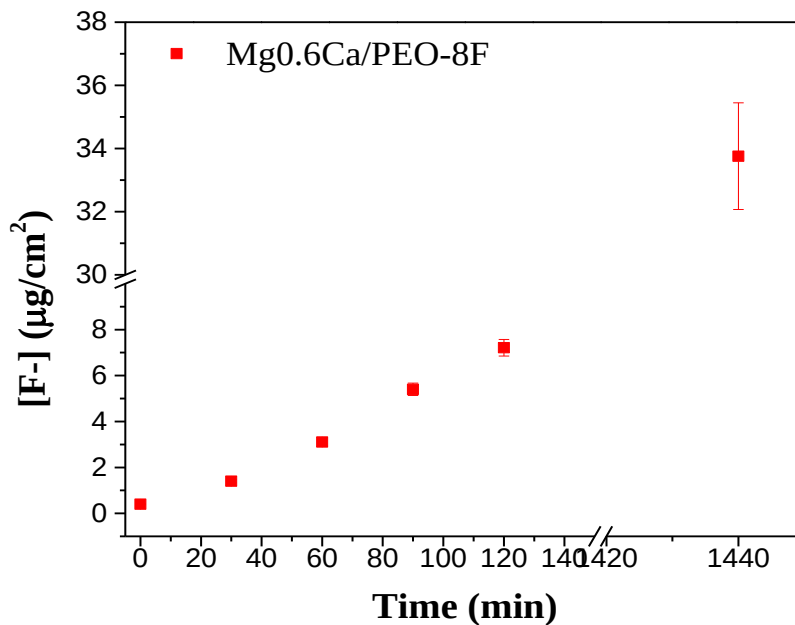


Figure 46. Fluoride ions released of $\text{Mg}_{0.6}\text{Ca}/\text{PEO-8F}$ coating for 24 h.

5.1.2 Mg-Zn-Ca alloys

Fluoride-containing PEO coatings on MgZn_xCa_y alloys were generated in two different electrolytes, with high (PEO-8F) and low (PEO-3F) fluoride content, respectively. The fluoride content in the electrolyte was modified in order to evaluate the effect on coating composition, morphology and corrosion behavior of the alloys (section 3.2.1, **Table 17**). The PEO-3F electrolyte with low fluoride content was a stable transparent solution. The optimal treatment time of 420 s was selected for PEO-8F electrolyte in accordance with the previous work performed on other alloys¹¹³. The PEO-3F electrolyte is the new, original development, therefore, the coating growth kinetics, energy consumption and coating morphologies were studied as a function of treatment time over a period of 900 s.

5.1.2.1 Voltage-time response and process efficiency

Figure 47 (a, b) shows the evolution of root mean square values of voltage and current density (U_{rms} , i_{rms} , respectively) acquired during the PEO treatment of (a) PEO-8F and (b) PEO-3F on MgZn_xCa_y alloys. In **Figure 47** (a), a linear voltage increase is observed during the 60 s ramp with a slight change of slope at ~40 s (160 V) that occurs when the limiting current density of $100 \text{ mA}\cdot\text{cm}^{-2}$ is reached. A second pronounced inflexion occurs at ~70 s, 235 V, corresponding to the initiation of plasma microdischarges due to the breakdown of oxide layer and gas, visually confirmed in PEO-3F electrolyte. Visual examination of the discharge was not possible in PEO-8F due to the opacity of the electrolyte in the presence of CaO suspended particles. At ~90 s the U_{rms} voltage attained a final constant value (~235 V) and a change of slope is observed at ~175 s after a relatively flat region for all samples. In the case of PEO-3F (**Figure 47** (b)), the current density dropped to $\sim 50 \text{ mA}\cdot\text{cm}^{-2}$ at 90 s and 240V. The drop indicates an increased coating impedance and a change in the coating growth rate due to higher resistance of the oxide material to mass and charge transfer. Microdischarges continued under further gradual decrease of the current density to $25\text{-}30 \text{ mA}\cdot\text{cm}^{-2}$ for MgZnCa and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ alloys. While for $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ alloy remains constant (~235 V) until the end of the treatment.

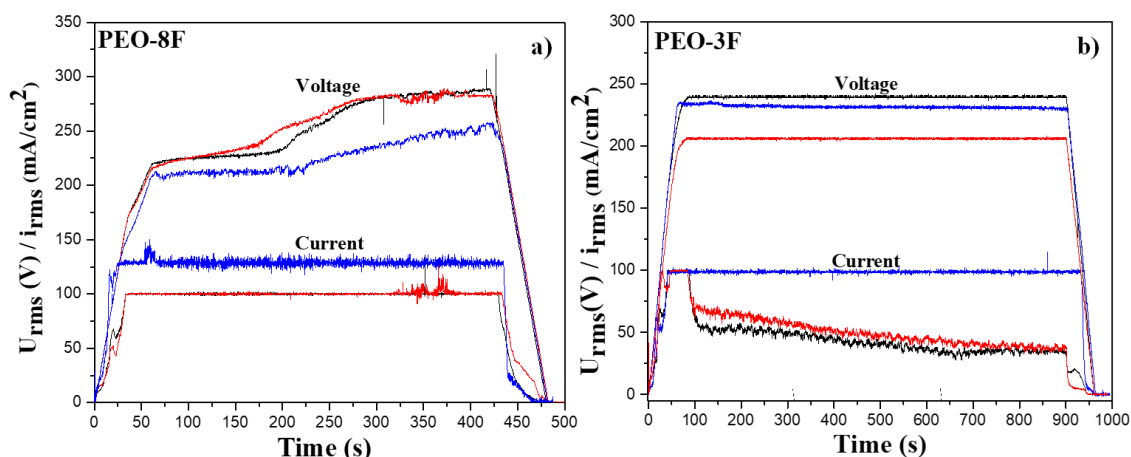


Figure 47. Current density-time curves for (a) PEO-8F and (b) PEO-3F coatings for $MgZn_xCa_y$ alloys. The colors of the curves correspond to: black-MgZnCa, red-Mg₃Zn_{0.4}Ca and blue-Mg_{0.5}Zn_{0.2}Ca for both electrolytes.

5.1.2.2 Coating morphologies, structure and composition

The evolution of the thickness and roughness values of the studied PEO coatings are disclosed in **Figure 48**. The thickness of PEO-3F coatings increases linearly with the treatment time for all alloys, being ~15-20 % greater in the case of Mg₃Zn_{0.4}Ca alloy (as it is observed in **Figure A2** (Appendix)). PEO-8F electrolyte produced the thickness in the range of 30-40 μm for all the substrates (**Figure 48** (a)), which is more than two times greater than the maximum coating thickness obtained in PEO-3F electrolyte. This behavior is in accordance with other reports where higher F content in the electrolyte improved the growth rate of the coatings^{363,364} and will be further on discussed as a function of the coating composition.

The coating thickness values were used to estimate the specific energy consumption, the energy obtained from integration of the voltage and current time transients recorded during the PEO process **Figure 48** (b)). In general terms, due to the lower coating growth kinetics, PEO-3F exhibited lower energy consumption for all substrates compared to PEO-8F. The energy consumption of PEO-3F increased with the treatment time for all substrates, the energy values for Mg_{0.5}Zn_{0.2}Ca are higher than those for as-cast alloys. In regards of the surface roughness parameters, the S_a for PEO-8F was only slightly higher compared to PEO-3F, whereas the S_{10z} was about three times higher (**Figure 48** (c)). For each respective electrolyte, the S_a values were generally similar for all substrates. For PEO-3F, the S_a and S_{10z} remained relatively constant with time for MgZnCa, decreased for Mg₃Zn_{0.4}Ca, and increased for Mg_{0.5}Zn_{0.2}Ca alloy. The roughness decrease for coatings on Mg₃Zn_{0.4}Ca alloy correlated with the decrease in their surface

pore population density (**Table 32**). Conversely, the surface pore population density increased with the processing time in the case of coatings on MgZnCa and Mg0.5Zn0.2Ca alloys, without notable effect on surface roughness.

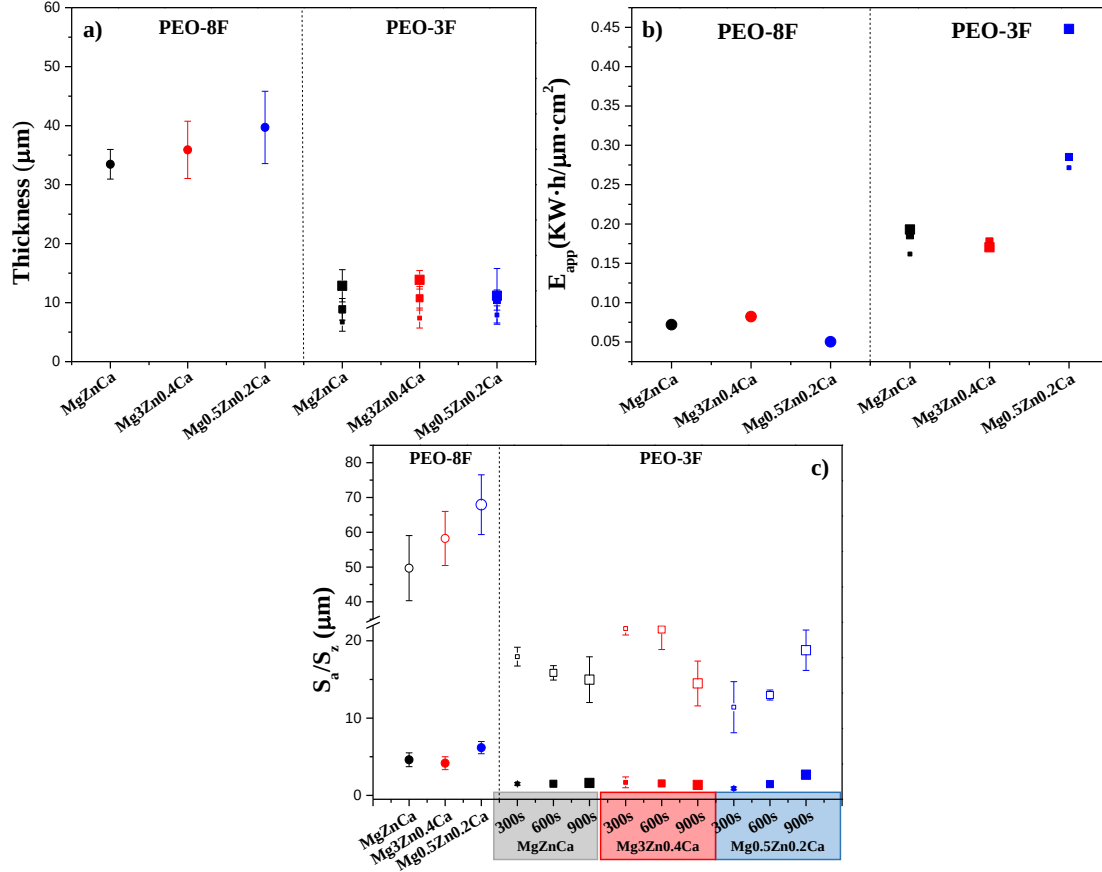


Figure 48. Evolution of (a) thickness, (b) energy consumption and (c) roughness values for PEO-8F and PEO-3F coatings on MgZn_xCa_y alloys. For PEO-3F electrolyte symbol size increases in order: 300 s, 600 s, 900 s. In (c) open symbols – S_{10z}, filled symbols – S_a.

The average pore size ranged within 0.7-1.8 μm, 0.3-1.6 μm and 1.2-1.8 μm for MgZnCa, Mg3Zn0.4Ca and Mg0.5Zn0.2Ca alloys, respectively. It is worth to mention that the average pore size was larger after 600 s of PEO-3F for the three alloys (**Figure 50** (b, e)). This is due to the transition from relatively weak discharges to more intense ones. By the end of the treatments (900 s) the more intense discharges provide sufficient energy to keep the molten material flowing for longer time and filling up the large pores.

Table 32. Surface characteristics of PEO-3F coatings.

Parameters	MgZnCa			Mg3Zn0.4Ca			Mg0.5Zn0.2Ca		
	300	600	900	300	600	900	300	600	900
Surface PPD (pore/mm²)·10⁵	(2.8 0.4)	(3.9 ± 0.5)	(10.0 ± 0.5)	(4.6 ± 0.5)	(3.6 ± 0.5)	(2.8 ± 0.4)	(0.9 ± 0.1)	(1.2 ± 0.6)	(1.0 ± 0.3)
Cross sectional pore area fraction (%)	9.3 ± 1.2	8.0 ± 1.1	6.0 ± 0.5	2.1 ± 0.8	2.7 ± 0.9	7.6 ± 0.7	4.49 ± 1.5	6.17 ± 2.2	9.19 ± 0.8
Average pore size (μm)	0.7 ± 0.2	1.8 ± 0.2	0.8 ± 0.2	0.3 ± 0.1	1.6 ± 1.1	0.5 ± 0.2	1.6 ± 0.1	1.4 ± 0.9	1.2 ± 0.3

Note: PPD - pore population density.

The surface and cross-sectional morphologies of PEO-8F and PEO-3F coatings with their respective surface area and local area EDS analysis are presented in **Figure 49- Figure 51** and **Table 33** and **Table 34**. The surface morphology of PEO-8F for all substrates (**Figure 49** (a-c)) is constituted by large surface cavities of complex shape without any defined pores, which is similar to the morphology of the coatings formed in this electrolyte on Mg0.6Ca and Mg0.6Ca2Ag alloys, as observed in Section 5.1.1.2. This type of morphology presented a problem for the estimation of surface porosity by image analysis.

The cross-sectional morphology of PEO-8F-coated samples for all alloys is constituted by two parts: i) outer layer with large sparse cavities and ii) inner part with small pores distributed close to the barrier layer. These voids are consequences of intense microdischarges and gas liberation. It is worth to mention that the morphology and roughness of PEO-8F coatings formed on MgZn_xCa_y alloys are similar to those formed on Mg0.6Ca and Mg0.6Ca2Ag alloys in the same electrolyte. The exception is the case of MgZnCa alloy, where large pores formed in the coating were arranged in a so-called pore band ^{365,366}. These pores appear to be associated with the location of the intermetallics in the grain boundaries (indicated by arrows in **Figure 49** (d)) ³⁶⁷.

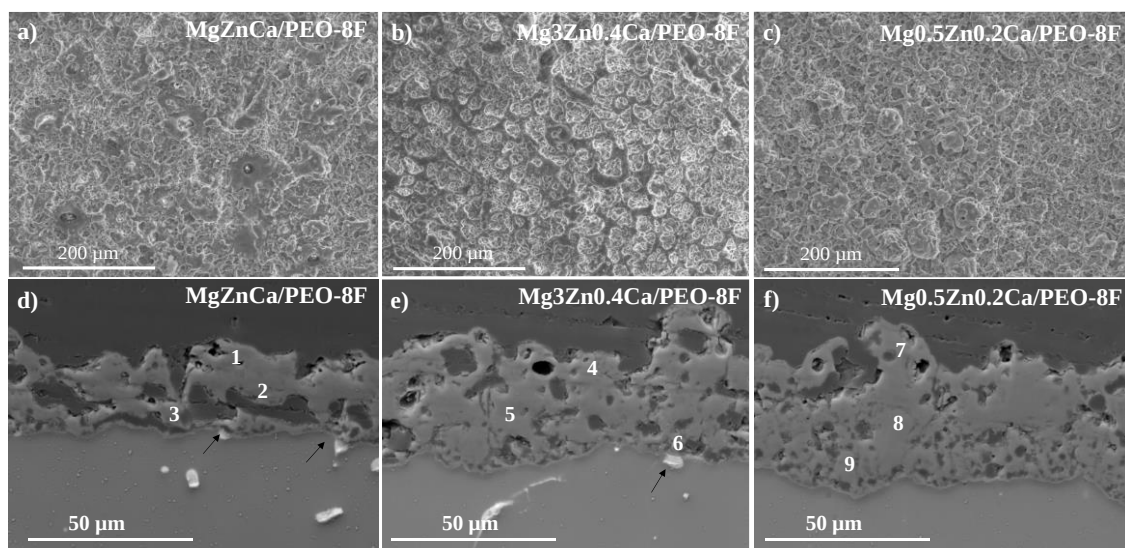


Figure 49. Secondary electron plan view micrographs and backscattered electron cross-sectional micrographs of the PEO-8F coatings. Numbers indicate where the local EDS analysis (**Table 33**) was performed.

Table 33. EDS (at. %) analysis of PEO-8F coatings on MgZn_xCa_y alloys at 420 s.

Alloy	Location	O	F	Na	Mg	P	K	Ca	Zn	Ca/P
MgZnCa	Plan view	42.3	18.9	5.4	25.5	4.2	0.2	3.3	0.3	0.79
	1	32.6	13.3	1.2	43.4	7.4	0.3	1.5	0.4	0.20
	2	20.9	33.7	1.3	37.9	5.4	0.1	0.1	0.4	0.02
	3	15.5	33.3	0.3	43.2	7.0	0.0	0.0	0.5	0.01
Mg3Zn0.4Ca	Plan view	45.2	22.5	4.2	20.6	3.1	0.3	2.6	0.1	0.86
	4	24.2	24.6	1.6	41.3	6.7	0.2	0.7	0.7	0.11
	5	21.6	29.6	0.6	40.3	7.1	0.1	-	0.7	0.01
	6	22.3	30.9	0.4	39.8	6.0	-	-	0.5	0.00
Mg0.5Zn0.2Ca	Plan view	44.3	15.6	5.8	25.9	4.7	0.3	2.8	0.2	0.6
	7	36.6	14.0	1.2	39.1	8.7	0.1	0.1	0.1	0.01
	8	28.8	20.1	1.3	42.3	7.2	0.1	-	0.2	0.01
	9	25.7	24.0	0.5	42.5	7.0	0.1	0.1	0.2	0.01

In **Figure 50**, the morphologies of PEO-3F on MgZn_xCa_y alloys disclose a pumice-like structures with pores localised in the centre of rather flat craters. This morphology is due to the gas evolution through molten coating material followed by fast solidification and gas ejection out of the discharge channel. The surface morphologies of PEO-3F at 300 s on MgZnCa and Mg3Zn0.4Ca alloys reveal heterogeneous size pores and bulging areas between the pores ((a, d, g)). However, the morphology of PEO-3F at 600 s for MgZnCa and Mg3Zn0.4Ca alloys (**Figure 50** (b, e)) comprised complex shape surface cavities with a few small pores located inside them and microcracks, the latter formed due to thermal stresses from a quick cooling process. The complex shape cavities disappear in both cast alloys at 900 s (**Figure 50** (c, f)). The surface morphology on the whole appears more homogeneous with smaller pore size (**Table 34**), which can be related to re-melting and re-generation of the surface coating material under lower intensity discharges at lower current density. In the case of extruded Mg0.5Zn0.2Ca alloy, the surface morphology remains largely the same, and only the cavity size slightly decreases with the processing time.

The cross-sectional morphology of the PEO-3F (**Figure 51**) coatings are constituted by large internal pores, some of them near the barrier layer. Similarly to the phenomena observed in PEO-8F electrolyte, the Zn-rich intermetallic particles provoke the formation of pore band region, next to the barrier layer in cast MgZnCa and Mg3Zn0.4Ca alloys at all treatment times as is indicated by arrows in **Figure 51** (a, b, d, e).

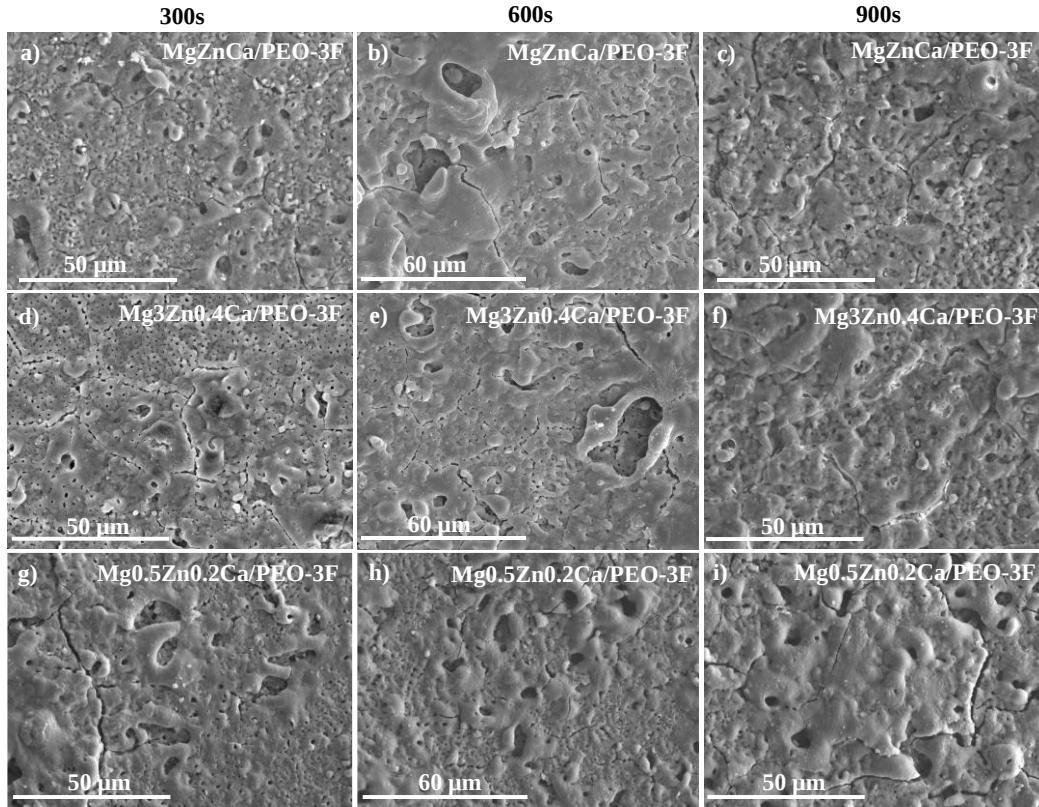


Figure 50. Secondary electron micrographs of surface morphology of the PEO-3F coatings produced on (a-c) MgZnCa, (d-f) Mg₃Zn_{0.4}Ca and (g-i) Mg_{0.5}Zn_{0.2}Ca alloys in: 300 s (a, d, g), 600 s (b, e, h) and 900 s (c, f, i).

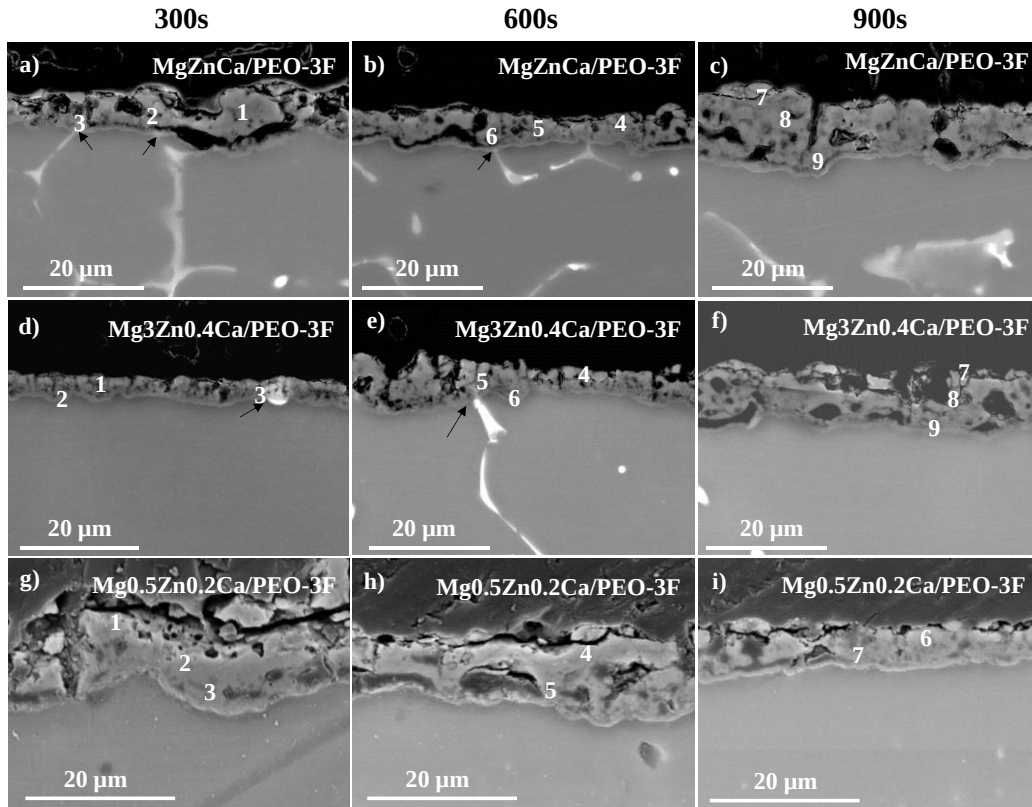


Figure 51. Backscattered electron micrographs of cross-sections of the PEO-3F coatings produced on (a-c) MgZnCa, (d-f) Mg₃Zn_{0.4}Ca and (g-i) Mg_{0.5}Zn_{0.2}Ca alloys in: 300s (a, d, g), 600 s (b, e, h) and 900 s (c, f, i).

Intermetallic particles are partially oxidized, note the Zn-rich regions in the barrier layer. The higher electronic conductivity of Zn-rich regions provide paths by which oxygen evolves easily and sustainably during the coating growth resulting in the formation of voids. The oxidized intermetallics are more evident in the PEO-3F coating on Mg₃Zn_{0.4}Ca alloy (**Figure 51** (d)), possibly because the alloy contains isolated intermetallic particles inside the grain. According to **Figure 51** (d), the particle size is comparable with the thickness of the coating and their Zn content is elevated (**Table 34**), hence their oxidation and disintegration is harder, especially at a reduced current density (see the current drop, (**Figure 47** (b))). The above observations are in agreement with those in the work of Martin *et al.* that pointed out the relation of segregated precipitates in Mg alloy with large discharge channels in the coatings ³⁶⁷. For Mg_{0.5}Zn_{0.2}Ca alloy, large internal pores are observed at 600 s and 900 s of treatment time as consequences of intense microdischarges and gas liberation.

The elemental composition of PEO-8F and PEO-3F was obtained by EDS analysis (**Table 33** and **Table 34**). The electrolyte-derived species from both-fluoride coatings were present throughout the entire coating thickness profiles on MgZn_xCa_y alloys. Often studies about incorporation of bioactive elements from the electrolytes neglect to disclose in detail the cross-sectional microstructure of the PEO-coated samples and the distribution of the elements along their thickness, focusing instead on the analysis of the surface ^{114,368,369}. However, the composition of the inner part of the coating, which is not in direct contact with tissue, is equally important since the elements diffuse to the coating/electrolyte interface during the corrosion process and affect the tissue cell metabolisms.

All the electrolyte species were incorporated into the coatings. Ca, P, Na, K and/or Si prevailed in the outer parts of the present coatings, whereas F dominated in the inner region, due to its well-known faster migration rate under the electric field produced during PEO process. F incorporation was found in a greater extent in the inner regions of PEO-8F and PEO-3F coatings, getting up to ~30 % and ~10 %. The concentration of F at the metal/oxide interface improves the passivation of Mg alloys due to the formation of MgF₂ ³⁷⁰. The incorporation of Zn from the substrate remained relatively independent of the electrolyte composition and the treatment time. Although, understandably, Zn content in the coatings decayed outwards and was up to 2-3 times greater for Mg₃Zn_{0.4}Ca alloy at the sites of the coatings close to the intermetallic particles (for PEO-3F: 1.4-1.6 at. %).

Ca/P ratios in the PEO-8F was found higher in the outer part of the coatings (~ 0.75) compared to the inner layer in all cases. These differences can be associated with the sources of Ca and P. Ca is readily available from the substrate and migrates outwards, whereas P migrates inwards from the electrolyte and is barely present in the barrier layer. The greatest Ca/P ratio was found for the coatings formed on Mg₃Zn_{0.4}Ca alloy (~ 0.86).

As for Ca/P ratios above 2 in the PEO-3F were observed in the inner region of the coatings despite low at. % content of both elements, whereas Ca/P ratios were below 2 in the outer layer. In the three alloys, the maximum surface incorporation of Ca, P and Si were observed at 600 s. The drop in the contents of these elements at 900 s may be related to the fact that the existing coating material is being continuously lost at the sites of microdischarges and replaced with the newly formed material ³⁷¹. As a result of current decay on MgZnCa and Mg₃Zn_{0.4}Ca alloys (**Figure 47** (a)) and, consequently, lower intensity and number of the microdischarges, the surface becomes depleted in electrolyte species. Nevertheless, for Mg_{0.5}Zn_{0.2}Ca the Ca/P ratio was much higher in the outer part (1.2 at. %) than in the inner part and increased with the treatment time from 0.4 to 1.2.

Table 34. EDS analysis (at. %) of PEO-3F coatings on MgZn_xCa_y alloys.

Alloy	Time (s)	Location	F	Na	Mg	Si	P	K	Ca	Zn	O	Ca/P
MgZnCa	300	Plan view	3.4	0.9	34.1	4	2.4	1.4	1.4	0.2	52.4	0.57
		1	2.7	0.4	35.3	4	2.5	0.9	1.1	0.1	53	0.45
		2	8	0.6	31.6	3.3	3.1	1.2	1.4	0.2	50.5	0.45
		3	0.8	0.1	47.2	0.3	0.2	-	0.5	0.3	50.5	2.36
	600	Plan view	1.3	0.6	38.3	6.6	5.3	2.9	7.3	0.4	37.4	1.11
		4	5.3	0.3	33.1	4.3	3	0.6	0.8	0	52.6	0.27
		5	8.3	0.2	34.1	3	2.7	0.7	0.5	0.1	50.3	0.17
		6	0.8	0.1	47.6	0.2	0.2	-	0.4	0.3	50.6	2
	900	Plan view	2.9	0.9	31.8	3.9	2.6	1.4	3.1	0.1	53.5	1.18
		7	2.8	0.2	34.8	4.4	2.9	0.5	0.2	0.1	54.2	0.08
		8	6.1	0.3	33.7	3	3	1.7	0.5	0.2	51.6	0.17
		9	1.8	0	47.4	0.1	0.1	-	0.1	0.2	50.2	2
Mg3Zn0.4Ca	300	Plan view	2.6	0.6	33.5	4.2	2.8	1.4	1.6	0.3	53.1	0.57
		1	4.8	0.3	35.3	3.9	3	0.4	0.8	0.1	53.9	0.27
		2	0.8	0.1	47.4	0.3	0.2	0	0.1	0.4	50.6	0.5
		3	0.7	0.3	46.4	0.2	0.1	0	0.3	1.4	50.7	3
	600	Plan view	2.4	1.1	37.7	6.1	5.2	2.9	7.4	0.6	36.7	1.42
		4	6	0.6	33	4.5	3.2	0.9	0.8	0.2	53.4	0.25
		5	5.6	0.6	34.8	2.5	2.2	1	1	1.6	50.7	0.45
		6	0.6	0.2	47.4	0.3	0.2	0	0.1	0.6	50.6	0.5
	900	Plan view	2.6	0.6	33.5	4.2	2.8	1.4	1.6	0.3	53.1	0.57
		7	4.4	0.2	37.1	3.7	2.7	0.3	0.1	0.2	53.8	0.04
		8	11.1	0.2	33.7	2.8	2.2	1.2	0.3	0.6	48	0.14
		9	0.7	0.2	47.9	0.2	0.1	0	0.2	0.5	50.4	2
Mg0.5Zn0.2Ca	300	Plan view	2.6	0.2	47.3	6.6	3.7	0.5	1.6	0.1	38.3	0.44
		1	5.3	0.3	43.3	4.7	3.7	1.7	0.4	0.1	41	0.09
		2	10.2	0.2	44.2	3.7	2.9	0.4	0.3	0.1	38.2	0.11
		3	5.2	0	42.5	5.1	4.8	0.4	0.3	0	41.7	0.14
	600	Plan view	2.4	1.1	37.7	6.1	5.2	2.9	7.4	0.6	36.7	1.22
		4	4.8	0.6	60.2	3.4	2.1	0.3	0.3	0.2	28.7	0.08
		5	4.5	0.7	59.3	3.5	2.8	0.9	0.8	0.2	28.2	0.28
	900	Plan view	2.6	0.6	33.5	4.2	2.8	1.4	1.6	0.3	53.1	1.24
		6	4.4	0.3	42.7	6.5	4.3	1.7	0.9	0	53.8	0.21
		7	11.1	0.2	61.9	3.6	2.4	0.6	0.3	0.1	48	0.13

The crystalline phases formed in the coatings were analysed by X-ray diffraction (**Figure 52**). The high intensity peaks corresponded to Mg proceeding from the base materials in the all evaluated coatings. The coating material showed MgO and amorphous material, as evidenced by the broad hump located between 2θ (25-40°). The formation of MgF_2 occurred as expected due to the presence of F in both electrolytes. MgF_2 is typically located near the barrier layer which is explained by the migration of the fluoride ions inwards³⁷⁰. In PEO-8F electrolyte, the formation of $\text{Ca}_5(\text{PO}_4)_3\text{F}$ (FA, fluorapatite), CaF_2 , $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ and $\text{Mg}_2(\text{PO}_4)\text{F}$ were promoted for all alloys, which is in accordance with previous studies of bioactive PEO coatings developed in Mg0.8Ca and AM50 alloy^{357,105}. The presence of the FA and HA suggested that the coating may promote the osseointegration between the bone and implant³⁷².

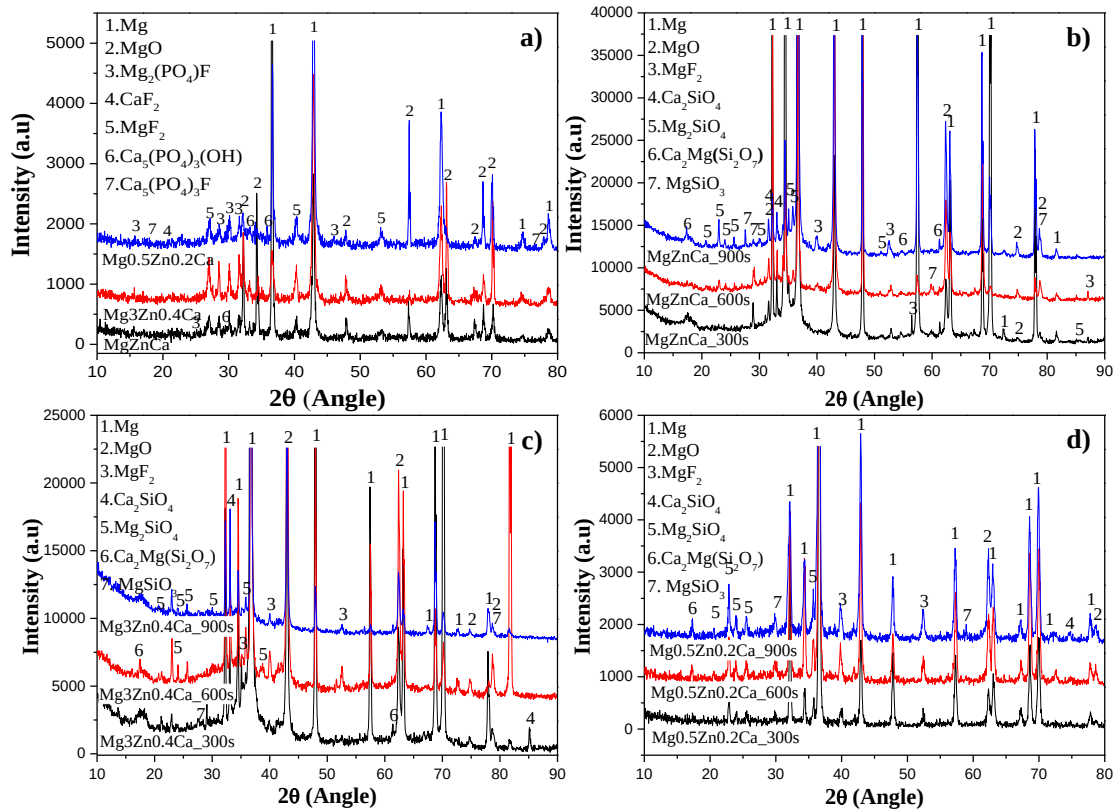


Figure 52. XRD patterns of (a) PEO-8F for MgZn_xCa_y alloys and (b-d) PEO-3F coatings on (b) MgZnCa , (c) $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}$ and (d) $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ alloys.

In PEO-3F (**Figure 52** (b-d)), the incorporation of Si into electrolyte promote the formation of magnesium and calcium silicates (MgSiO_3 , Mg_2SiO_4 , Ca_2SiO_4 , and $\text{Ca}_2\text{Mg}(\text{Si}_2\text{O}_7)$), which are known for their bioactivity as they form part of bioactive cements³⁷³. Silicon is known to facilitate more homogeneous PEO coatings as well as stabilize hydroxyapatite³⁷⁴. Si enrichment on the surface of coatings formed in silicate-based electrolytes is often associated with chemical precipitation of amorphous SiO_2 ³⁷⁵.

Nevertheless, no P-containing crystalline phases were formed, suggesting that all P is contained in amorphous material. Neither hydroxyapatite nor tricalcium phosphate was formed, since the Ca/P ratio was far below 1.67 in the electrolyte (~ 0.42 Ca/P) as well as in the outer part of the coating where the electrolyte species were mainly located (≤ 0.6 Ca/P). In comparison with PEO-8F electrolyte, the formation of MgF_2 was inhibited in PEO-3F, especially for lower treatment times; for instance, the characteristic peak at $27.3^\circ 2\theta$ was missing at all times.

5.1.2.3 Corrosion behavior of the coatings

5.1.2.3.1 Electrochemical tests

DC polarization curves (DC)

Figure 53 illustrates the potentiodynamic polarization curves obtained in inorganic α -MEM after 1 h of immersion at 37°C for bare substrates and all PEO-coated samples. The corrosion performance parameters calculated from the curves are included in **Table 35**. Polarization curves for both types of coatings show a nobler shift in the corrosion potential and a reduction in the i_{corr} , which indicates improvement of corrosion resistance compared to the substrates.

In the particular case of PEO-8F for all alloys (**Figure 53 (a)**) a reduction in i_{corr} by 2-7 times is observed in comparison with the substrate. The anodic branches of PEO-8F coatings on MgZnCa and Mg₃Zn_{0.4}Ca alloys show a steep slope due to ~ 400 mV-wide pseudo-passivity region followed by a localised corrosion. According to this, PEO-8F coatings act as barrier layer, which prevent the penetration of corrosive species towards the substrate. The coating on Mg_{0.5}Zn_{0.2}Ca shows a wider passive region (~ 700 mV) and a higher i_{corr} compared to cast alloys.

In case of PEO-3F for MgZnCa alloy, the treatments produced a noble shift of corrosion potential (by ~ 120 - 200 mV) and a reduction in i_{corr} by ~ 2 - 9 times. Above the corrosion potential, the anodic branches show a steep slope due to pseudo-passivity. This behavior, observed for ~ 150 - 200 mV, is also followed by a rapid current increase, indicating the initiation of localized corrosion. The pitting potential, E_{pit} , was similar for all three coatings and about 150 mV nobler than that of the bare substrate (**Table 35**). The coating formed in 600 s revealed the lowest i_{corr} for all three alloys.

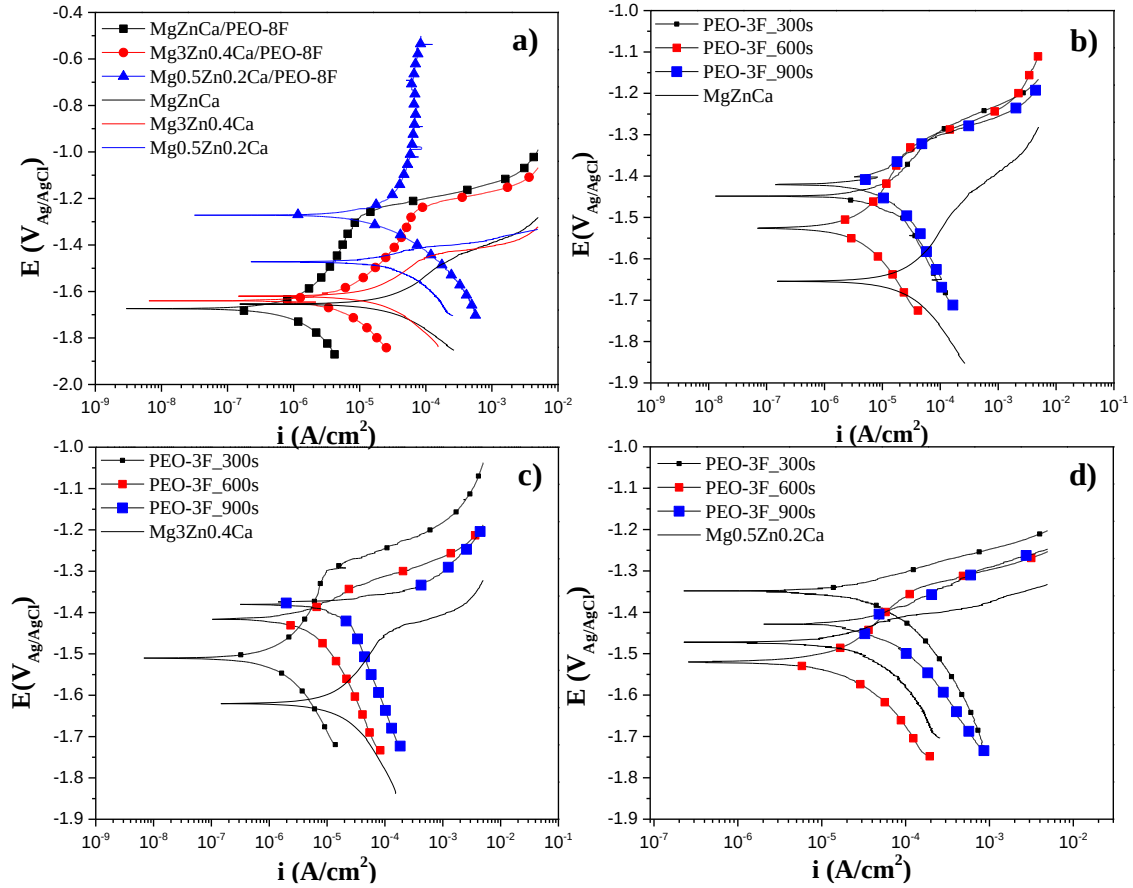


Figure 53. Polarization curves of (a) PEO-8F and (b-d) PEO-3F coatings on (b) MgZnCa, (c) Mg3Zn0.4Ca and (d) Mg0.5Zn0.2Ca alloys.

The main difference with the abovementioned behavior is observed in the PEO treatments obtained for Mg3Zn0.4Ca at longer times (600 s and 900 s) and for Mg0.5Zn0.2Ca (at 300 s and 900 s). These coated systems revealed a stronger tendency to pitting, given that E_{pit} is very close to or practically coinciding with the corrosion potential (E_{corr}) (**Table 35**). This can be associated with the previously discussed heterogeneous morphology of the coatings (**Figure 51**). In the particular case of Mg3Zn0.4Ca alloy, it may also be associated with the combination of the higher area fraction of Zn-rich intermetallics. As a result, the most homogeneous coatings of all, formed at 300 s on Mg3Zn0.4Ca alloy (**Figure 51** (d)), discloses a distinct pseudo-passive region (**Figure 53** (c)).

In general terms, PEO-3F coatings for all treatment times show more positive corrosion potentials and lower corrosion densities (by 2-3 times) compared to PEO-8F for all substrates. This behavior is in agreement with the morphologies observed in SEM images (**Figure 49** and **Figure 51**), where thinner coatings appear more compact and homogeneous with lower internal porosity.

Table 35. Corrosion characteristics from polarization curves.

Type	Time (s)	Alloy	E _{corr} (V)	i _{corr} ($\mu\text{A}\cdot\text{cm}^{-2}$)	E _{pit} (V)
Substrate	-	MgZnCa	-1.65	27.37	-1.45
	-	Mg3Zn0.4Ca	-1.62	30.25	-1.42
	-	Mg0.5Zn0.2Ca	-1.60	33.41	-1.40
PEO-3F	300	MgZnCa	-1.45	2.96	-1.30
	600	Mg3Zn0.4Ca	-1.53	1.40	-1.30
	900	Mg0.5Zn0.2Ca	-1.42	2.67	-1.32
	300	MgZnCa	-1.51	1.61	-1.28
	600	Mg3Zn0.4Ca	-1.42	1.18	-1.27
	900	Mg0.5Zn0.2Ca	-1.37	6.42	-1.35
	300	MgZnCa	-1.34	13.4	-1.32
	600	Mg3Zn0.4Ca	-1.52	6.34	-1.35
	900	Mg0.5Zn0.2Ca	-1.43	14.28	-1.37
	420	MgZnCa	-1.34	6.04	-1.28
		Mg3Zn0.4Ca	-1.34	4.55	-1.18
		Mg0.5Zn0.2Ca	-1.38	24.69	-0.70

Some PEO mechanism studies carried out on Al alloys have shown that high voltages or current may lead to thicker coatings but generate too strong microdischarges and decrease the corrosion protection of the coating³⁷⁶. For this reason, it has been deduced that the same behavior has been observed in this work for these Mg alloys.

In summary, PEO-8F and PEO-3F coatings on the three alloys revealed similar E_{pit} values (-1.23 ± 0.03 and -1.33 ± 0.05 V, respectively), regardless of the coating thickness or the alloy composition and processing route. Based on the polarization experiments, thin PEO-3F coatings with relatively low fluorine content showed lower corrosion densities compared to thicker fluorine-rich PEO-8F for all substrates due to the lower presence of internal pores. The PDP test identified three PEO-3F coatings (formed on Mg3Zn0.4Ca in 300 s and 600 s and on MgZnCa in 600 s) with three lowest and negligibly different i_{corr}, whereas only one of them (Mg3Zn0.4Ca, 300 s) also showed a clear correlation with the most homogeneous coating morphology. In other words, PDP is not sufficiently discriminating, which can be attributed to the considerable change in the surface state of Mg produced by large polarization.

Electrochemical Impedance Spectroscopy (EIS)

One of the aims of this Thesis is the development of a reliable, fast and low-maintenance coating screening process from the corrosion protection point of view in order to ensure that only the most promising candidates would pass to the *in vitro* bio-validation stage. The basic cell work experiments that determine the cytotoxicity of the biomaterial and cell viability typically take 4-5 days. Corrosion screening experiments of that duration in full cell culture media in non-sterile conditions end up in contamination almost inevitably. Therefore, it is important to elucidate the role of the composition of corrosion media on the short term corrosion behavior and on the ability of the accelerated electrochemical test to discriminate between the protective properties of different coatings. The EIS is the method of choice, since, unlike PDP, it applies a very small perturbation, close to the steady-state corrosion conditions.

For that purpose, EIS measurements of PEO-3F coatings formed on MgZnCa and Mg3Zn0.4Ca alloys in 300 s, 600 s and 900 s were carried out in both inorganic and complete α -MEM solutions at 37°C after 1 h of immersion (**Figure 55**). Complete α -MEM contains inorganic salts and amino acids, vitamins and glucose (among other organic components) that would also be present in the body fluids *in vivo* and would affect the passivation and degradation of Mg alloys.

EIS of the PEO-3F coated specimens in inorganic α -MEM and complete α -MEM (**Figure 55**), revealed the following phenomena. The coatings formed in 300 s were more protective than others in both alloys. Importantly, the results were very similar in either of the medium for each respective coating on both alloys. Although in either of the media the total impedance at low frequencies, $|Z|_{10\text{mHz}}$ did not exceed $10^4 \Omega \text{ cm}^2$, the relative improvement of the alloy corrosion resistance in the presence of the coating (up to one order of magnitude compared to $|Z|_{10\text{mHz}}$ of bare substrates (**Figure 35**, Section 4.2.2.3), was greater in complete α -MEM than in inorganic α -MEM.

In order to interpret this effect, the spectra were analysed using equivalent electrical circuits (**Figure 54**). The circuits include R_{elec} (solution resistance), R_1/CPE_1 (ascribed to the capacitive and resistance behavior of outer porous part of the coating), R_2/CPE_2 (ascribed to the capacitive and resistance behavior of the inner barrier layer), and $R_{\text{dl}}/\text{CPE}_{\text{ct}}$ related to the electrochemical activity at the substrate/electrolyte interface. Constant phase elements (CPE) are used instead of the ideal capacitors to account for the

surface roughness and heterogeneity. This equivalent circuit is in agreement with previous studies focused on the evolution of ZX11 Mg alloy in a medium similar to α -MEM⁵¹. The fitting results are presented in **Table 36**, where CPE values correspond to the admittance of the constant phase element at the radial frequency $\omega=1$ rad, in accordance with the relationship $Z=1/(CPE(j\omega)^n)$, where j is the imaginary number and $-1 \leq n \leq 1$. The main difference between the two circuits is the presence of the CPE_{dif} element (associated with the infinite Warburg diffusion through the outer porous layer) in the circuit for complete α -MEM. It suggests that a mass transfer limitation exists in the outer porous part of the PEO layer, as a result of which the access of the de-passivating amino acids to the alloy surface is blocked. The amino acids adsorbed on the coating surface are unable to reach the inner regions of the coating through the porous network of the oxide material due to the large size of their anions. This would also prevent the migration of other species.

The important implication of this finding is as follows. Whereas for bare alloys the complete α -MEM is a more aggressive medium (see Section 4.2.2.3), for PEO coated alloys the inorganic α -MEM can be considered as an inexpensive, easy maintenance and realistic medium for fast corrosion performance screening.

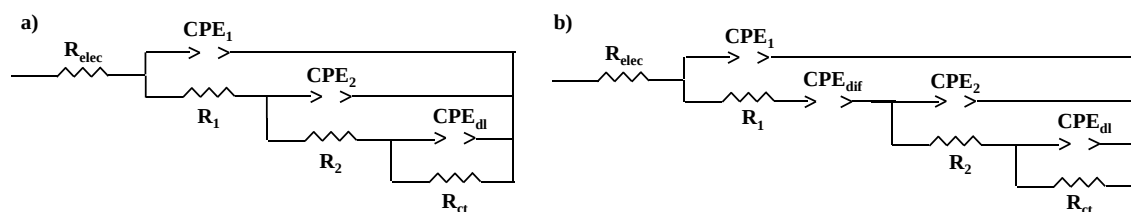


Figure 54. EIS equivalent circuits for PEO-3F coated cast $MgZn_xCa_y$ alloys after 1 h of immersion in (a) inorganic α -MEM and (b) complete α -MEM solutions at 37°C.

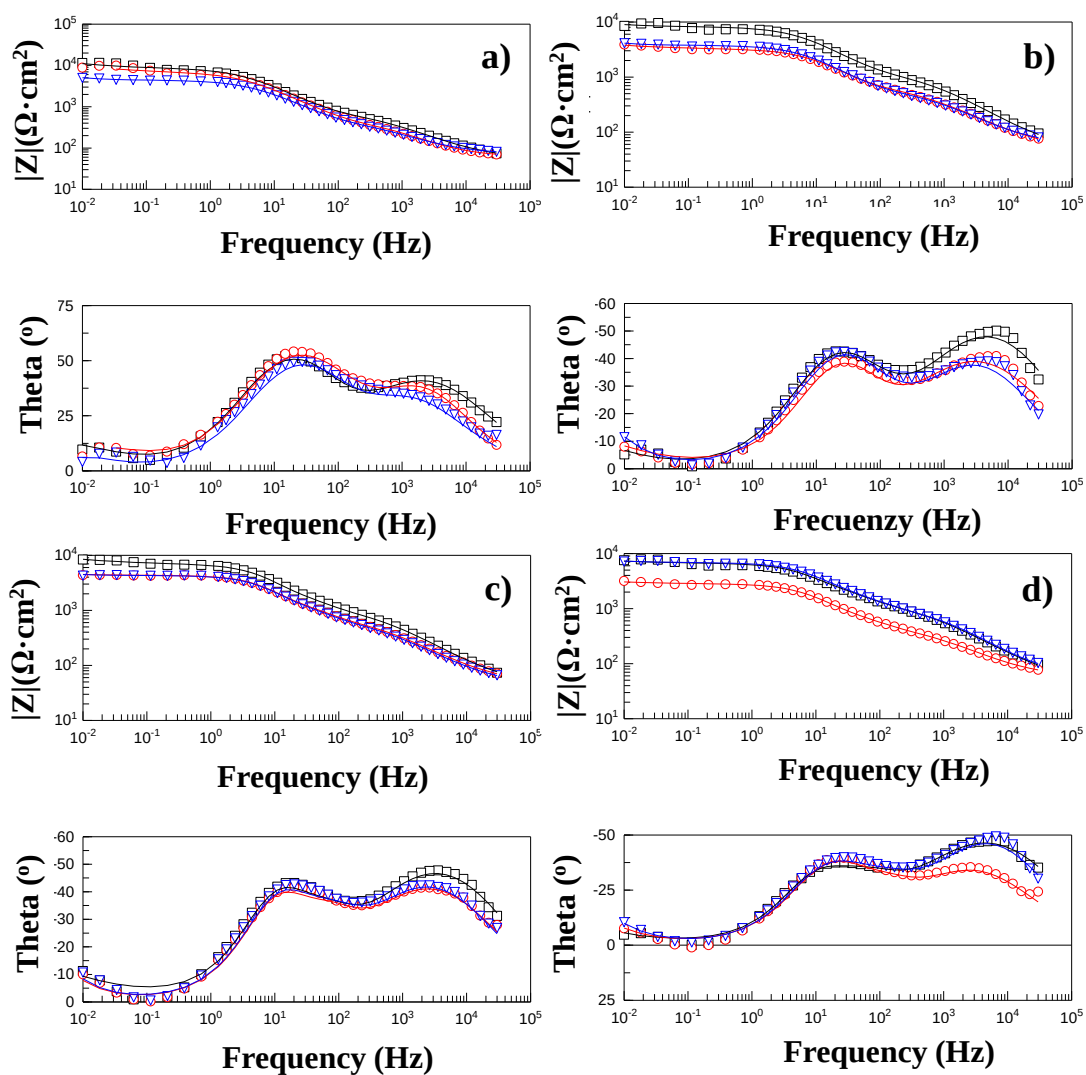


Figure 55. Bode plots of EIS spectra for PEO-3F coated MgZnCa (a, c) and Mg₃Zn_{0.4}Ca (b, d) alloys in (a, b) inorganic α -MEM and (c, d) complete α -MEM after 1 h of immersion.

Table 36. Fitted values of EIS equivalent circuits for PEO-3F on MgZnCa and Mg3Zn0.4Ca alloy in inorganic and complete α -MEM.

Medium	Alloy	Time (s)	R_{elec} ($\Omega \cdot cm^2$)	CPE_{por} ($\mu S \cdot s^n \cdot cm^{-2}$)	n_1	R_{por} ($\Omega \cdot cm^2$)	CPE_{dif} ($mS \cdot s^n \cdot cm^{-2}$)	n_{dif}	CPE_b ($\mu S \cdot s^n \cdot cm^{-2}$)	n_2	R_b ($\Omega \cdot cm^2$)	CPE_{dl} ($\mu S \cdot s^n \cdot cm^{-2}$)	n_3	R_{ct} ($\Omega \cdot cm^2$)	R_{tot} ($\Omega \cdot cm^2$)
Inorganic α -MEM	MgZnCa	300	54.76	5.72	0.71	794.1	-	-	5.99	0.86	7864	1.62	0.66	10000	18658
		600	63.14	4.02	0.79	427.2	-	-	8.79	0.81	6274	1.07	0.59	8000	14701
		900	58.39	9.37	0.72	460.5	-	-	8.50	0.84	3965	0.14	0.98	10111	14536
	Mg3Zn0.4Ca	300	48.04	2.09	0.73	1218	-	-	6.00	0.81	6956	4.86	0.67	9232	17406
		600	47.79	7.04	0.67	725.2	-	-	9.61	0.87	2501	6.19	0.57	7340	10566
		900	58.39	4.97	0.72	575.3	-	-	0.10	0.83	3178	0.134	0.90	6407	10160
Complete α -MEM	MgZnCa	300	43.46	3.66	0.71	1191	1.45	0.4	2.61	0.57	1777	5.03	0.93	3666	6634
		600	43.65	6.37	0.66	768.8	2.77	0.44	5.76	0.93	1031	13.46	0.96	1235	3035
		900	43.89	6.79	0.69	781.4	19.05	0.98	3.64	0.96	1223	6.81	0.96	2303	4307
	Mg3Zn0.4Ca	300	46.53	2.92	0.71	1320	3.76	0.47	3.63	0.89	2372	7.09	0.92	2863	6555
		600	54.65	11.18	0.65	571.1	15.01	0.69	3.74	0.99	893.8	9.63	0.98	1327	2792
		900	62.08	1.45	0.77	999.4	7.39	0.82	3.46	0.825	1878	5.44	0.85	3929	6806

Figure 56 shows the results of corrosion screening performed in inorganic α -MEM solution of the cast and extruded systems with and without coatings generated in PEO-8F and PEO-3F electrolytes at different conditions (as per **Table 17**). The diagram presents the modulus of total impedance at low frequency after 1 h of immersion at 37°C (the original EIS spectra and the respective fitting parameters can be found in **Figure A3-Figure A4, Table A2, Appendix**). High values of $|Z|_{10\text{mHz}}$ indicate low corrosion rate. For PEO-3F coatings, all alloys showed a decrease of the corrosion resistance with the increase of the treatment time. In addition, MgZnCa and Mg3Zn0.4Ca exhibited similar behavior for all treatment times while coated Mg0.5Zn0.2Ca at 300 s and 600 s showed notably higher corrosion behavior compared to the cast alloys with respective coatings.

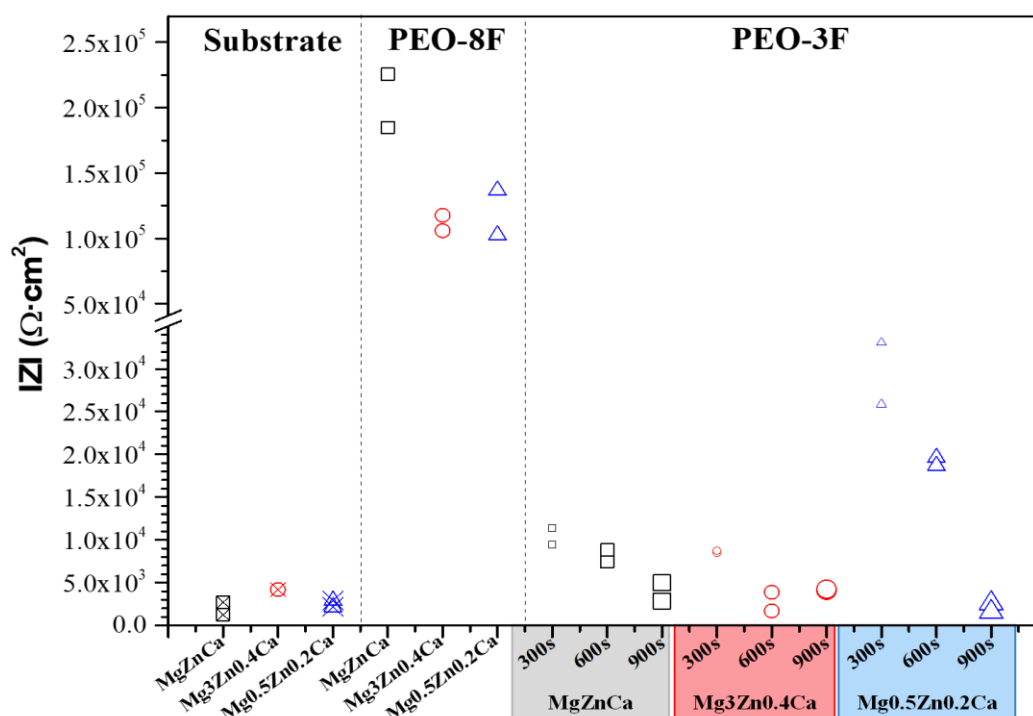


Figure 56. Scatter diagram of the impedance values obtained for PEO-8F and PEO-3F coatings in inorganic α -MEM. Symbols and colours: black squares-MgZnCa, red circles -Mg3Zn0.4Ca and blue triangles -Mg0.5Zn0.2Ca alloys. Symbol size increases in the following order of the treatment time: 300 s, 600 s and 900 s.

Comparing PEO-coated samples, PEO-8F for all alloys presented higher corrosion protection than any of the PEO-3F coatings, undoubtedly due to their greater thickness and the passivating effect of MgF_2 . In order to corroborate the ranking obtained in short-term EIS screening, 7 selected systems (including PEO-8F coating on three alloys and PEO-3F coatings formed in 300 and 600 s on cast alloys) were subjected to a 5 day-long screening by hydrogen evolution measurements in inorganic α -MEM. The selected duration of the test is equal to that of a typical *in vitro* cell proliferation test. The test will

serve to determine the degradation rate and examine the development of corrosion products during the immersion.

5.1.2.3.2 Hydrogen evolution test

Figure 57 shows the results of hydrogen evolution on bare and (a) PEO-8F and (b) PEO-3F coated samples in inorganic α -MEM with a constant pH 7.4 under flowing CO_2 .

As it can be seen in **Figure 57** (a), the slope of the curve corresponding to PEO-8F on $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}$ remains relatively constant during the test, with the degradation rate about an order of magnitude lower than that of the bare alloy. The opposite situation is observed on coated MgZnCa and $\text{Mg}_{3.5}\text{Zn}_{0.4}\text{Ca}$ alloys where negligible hydrogen volume changes were observed during ~ 70 h of immersion. After that period the degradation rate of the coated alloys accelerates and becomes approximately equal to or exceeds that of bare as-cast alloys. This behavior is highly unusual for PEO-coated Mg alloys, and is the opposite of that observed on other Mg alloys, such as $\text{Mg}_{0.8}\text{Ca}$ ³¹¹ and AZ31B³⁷⁷. Comparing the results disclosed in Section 5.1.1.3 with these, it is observed that all $\text{MgZn}_x\text{Ca}_y/\text{PEO-8F}$ systems exhibited much lower hydrogen evolution than $\text{Mg}_{0.6}\text{Ca}/\text{PEO-8F}$ and $\text{Mg}_{0.6}\text{Ca}_2\text{Ag}/\text{PEO-8F}$ (6.13 and 22.21 mL/cm²). This differences are too large to be attributed to the differences in the corrosive medium and pH control, therefore they must be defined by the substrates.

In the case of PEO-3F coatings on cast alloys, their protective effect was maintained only during the first 2-5 h, after that period the degradation rate of the coated alloys significantly accelerated (**Figure 57** (b)) and was about two times higher than that measured for PEO-8F-coated alloys. The coatings formed in 600 s demonstrated lower protective properties than those formed in 300 s, which is generally in agreement with the trend derived from EIS spectra in short-term immersion. It is worth mentioning that studies carried out by other authors on Mg-Zn-Ca alloys^{114,378,368} typically show an improved corrosion performance of PEO coated specimens. However, these studies differ from the present one in several aspects: a constant pH of the corrosive medium is not maintained, PEO coatings show high levels of fluoride or corrosion testing is done only by electrochemical methods and is limited to short immersion times.

It should be mentioned that the registered hydrogen volume corresponds to the lower limit, because part of the generated H_2 is dissolved in the medium. Once the medium is saturated in dissolved H_2 , the partial pressure becomes sufficient to displace the medium

in the burette. **Table 37** presents the corrected H_2 volume for coatings on cast alloys, according to the H_2 solubility in water at 37°C . It is evident that PEO-8F exhibits lower degradation rates than those of PEO-3F coatings, although not to a significant degree. This is related to the loss of the protective properties of the inner PEO layer and commencement of the electrochemical activities at the interface/substrate coating. The high permeability of the porous part of the PEO-3F coating is attributed to its low thickness. The low resistance of its barrier layer may be associated with low F^- content in the electrolyte and, consequently, in the coating ($\sim 4\text{--}8\%$ on average), with insufficient amount of MgF_2 formed at the coating/substrate interface. For instance, Mohedano *et al.* showed that for $\sim 40\text{ }\mu\text{m}$ -thick PEO coatings containing $\sim 20\%$ of F (also generated in an electrolyte with 8 g/L NaF) the initiation of undercoating corrosion of $Mg_{0.8}Ca$ alloy was delayed for more than two weeks³¹¹.

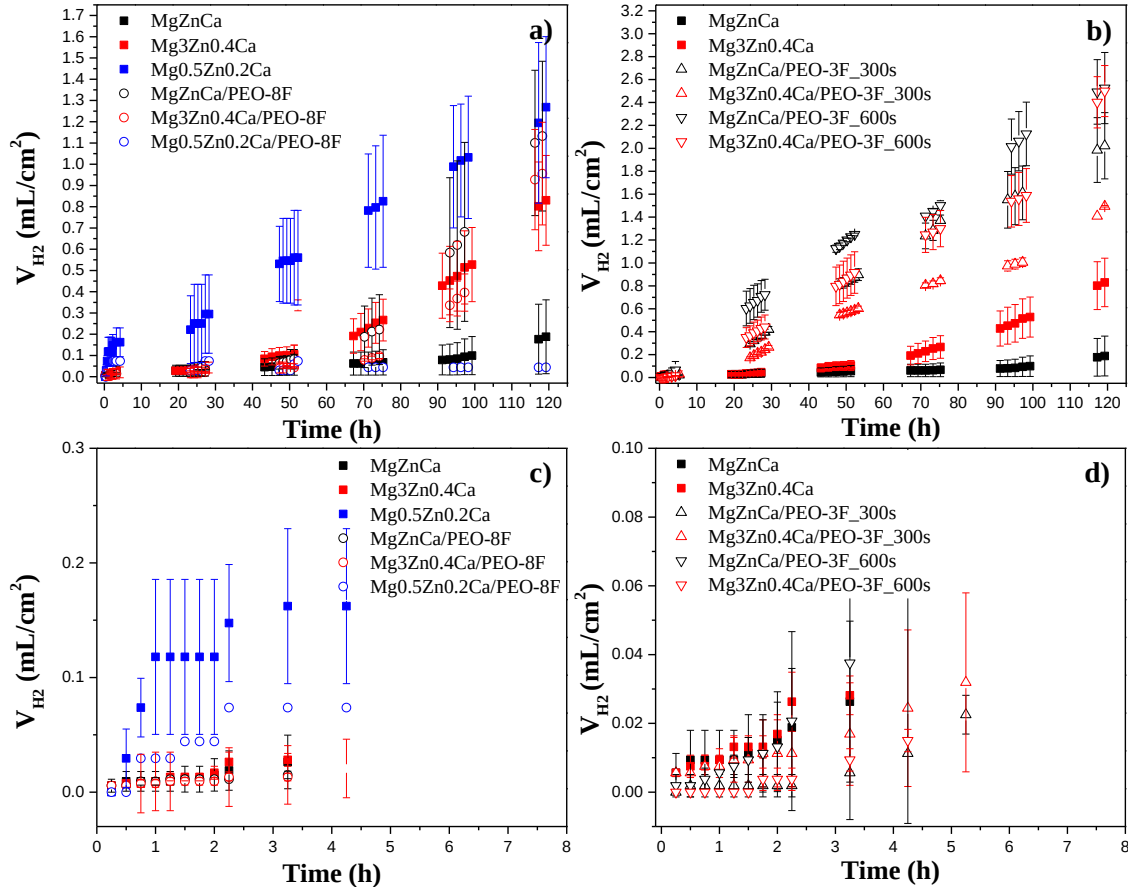


Figure 57. Hydrogen evolution of (a, c) PEO-8F coatings for all substrate and (b, d) PEO-3F coatings for $MgZnCa$ and $Mg_{3Zn0.4}Ca$ alloy after 120 h of immersion in inorganic α -MEM solutions in CO_2 flow conditions. Figures (c, d) are amplifications of the initial 8 h of immersion.

Table 37. Corrected H₂ volume for 120 h of immersion obtained from **Figure 57** (a) and (b) for as-cast MgZnCa and Mg₃Zn_{0.4}Ca alloys.

V _{H2} (mL/cm ²)	Time (s)	MgZnCa	Mg ₃ Zn _{0.4} Ca	Mg _{0.5} Zn _{0.2} Ca
PEO-8F	420	4.8 ± 1.5	4.0 ± 1.0	0.9 ± 0
PEO-3F	300	5.9 ± 0.8	4.4 ± 0	-
	600	5.9 ± 0.7	5.8 ± 0.5	-

Figure 58 illustrates the macrographs and the cross-sections of PEO-8F coatings after 120 h of H₂ evolution test (i.e. of specimens corresponding to **Figure 57** (a)). The macro appearance of the PEO-8F coatings on cast MgZnCa and Mg₃Zn_{0.4}Ca alloys revealed signs of corrosion with a clear localised attack while the coating on extruded Mg_{0.5}Zn_{0.2}Ca showed uniform corrosion. This explains the high standard deviations in collected H₂ volume for cast alloys (**Table 37**). The cross-sections of PEO-8F-coated samples disclose 8-10 µm-thick undercoating corrosion products layer with some cracks. Corrosion products layer is scalloped in cast alloys and uniform in the extruded alloy. The scalloped interface can be related to the effect of the intermetallic particles that are slow to oxidize and act as strong cathodes, as discussed in Section 4.2.2.1, Chapter IV. In all alloys the coatings appear well adhered (with the exception of the localized attack regions) even after 120 h of immersion.

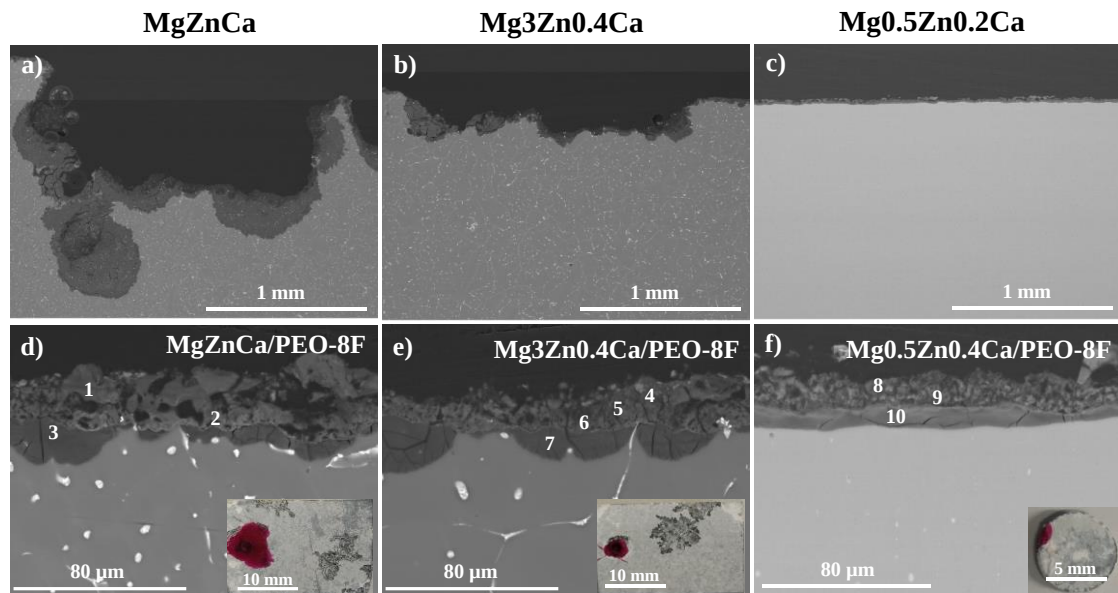
**Figure 58.** Macrographs and cross-section of PEO-8F coatings of all for MgZnCa, Mg₃Zn_{0.4}Ca and Mg_{0.5}Zn_{0.2}Ca alloys after 120 h of immersion in inorganic α-MEM.

Figure 59 (a-h) discloses the development of undercoating corrosion in PEO-3F (300 s) coated cast alloys after 18 h and 120 h of immersion. Remarkably, undercoating

corrosion progresses very quickly once the PEO barrier layer is breached, e.g. at places a ~30 μm -thick layer is formed within first 18 h of immersion (**Figure 59 (c)**).

According to the EDS analysis (**Table 38**), undercoating corrosion products layer for all PEO-8F-coated alloys mainly comprise Mg, O, P and Ca. The outer coating region locally contains more Ca than in as received state. These observations are in agreement with the formation of a protective corrosion layer on Mg in physiological solutions which is constituted by magnesium hydroxide and hydroxyapatite-like compounds^{145,379,78} that are precipitated when the near-electrode pH rises sufficiently as a result of the dissolution of $\text{Mg}(\text{OH})_2$ in the presence of Cl^- ions³⁸⁰. The elevated F content in the outer regions of the coatings may be attributed to the lixiviation of fluoride ions from the F-rich inner region.

Table 38. EDS analysis (at. %) of PEO-8F coatings for MgZn_xCa_y alloys after 120 h of immersion.

Alloy	Location	O	F	Na	Mg	P	Cl	K	Ca	Zn	Ca/P
MgZnCa	1	26.5	41.6	0.5	26.4	4.1	0.2	0.0	0.3	0.3	0.1
	2	27.3	40.3	0.7	24.7	3.6	0.1	0.0	1.9	0.9	0.8
	3	63.9	0.3	1.0	24.4	2.4	0.7	0.2	3.9	2.3	2.6
Mg3Zn0.4Ca	4	46.0	14.9	1.0	3.0	11.9	0.2	0.0	21.2	0.3	3.0
	5	34.7	33.1	0.7	22.9	5.8	0.4	0.0	1.6	0.4	0.6
	6	37.3	30.4	1.0	22.6	4.1	0.3	0.2	2.7	1.0	1.1
	7	68.3	0.8	0.6	23.6	1.7	0.1	0.1	2.3	1.7	2.0
Mg0.5Zn0.2Ca	8	20.4	44.2	0.3	29.7	4.5	0.3	0.0	0.3	0.2	0.2
	9	35.7	29.9	0.4	17.2	7.6	0.6	0.0	7.2	0.4	1.9
	10	90.2	0.2	0.6	33.8	7.0	0.4	0.2	6.4	0.6	1.8

The observed corrosion morphologies highlight several important factors in regards to the corrosion mechanism of PEO-8F and PEO-3F coated MgZn_xCa_y alloys: (i) the coatings still appear well adhered even after 120 h of immersion; (ii) fewer penetrating cracks are observed in the undercoating corrosion product layers than in bare substrates; (iii) Zn- and Ca-rich secondary phases remain embedded in the corrosion products layer under the coating (**Figure 58 (a, b)** and **Figure 59 (b, d, f, h)**). Accordingly, it may be inferred that the corrosion process of the coated samples occurs as follows (**Figure 60**): (a) corrosive species penetrate the pores and cracks of the coating; (b) noble secondary phases in the substrate form microgalvanic couples with the matrix and accelerate the

oxidation of the latter; (c) a crevice forms between the coating and the substrate, intensifying microgalvanic corrosion and, therefore, undercoating corrosion; d) the coating impedes the detachment and cracking of the corrosion products layer, resulting in entrapment therein of intermetallics that remain non-oxidized and non-dissolved.

The non-oxidized cathodic intermetallics may possess greater effective area, hence stronger microgalvanic corrosion⁵⁸. Importantly, the intermetallic cathodes are rich in Zn (**Table 33** and **Table 34**). Zn undergoes hydrolysis and produces acidification³⁸¹; as a result, the environment in the crevice under the coating is likely to be more aggressive, in terms of the pH and the gradient chloride concentration, than the bulk of the corrosive medium. This phenomena is corroborated by the higher amount of chloride in the undercoating corrosion as it is illustrated by the EDS analysis (**Table 39**). For that reason, an elevated H₂ evolution and formation of a thicker layer of corrosion products were observed on PEO coated alloys (**Figure 59** and **Figure 58**). The important role of crevice in biodegradation of Mg implants in vivo was highlighted in this report³⁸². Also, recently, Silva et al. showed that O₂ reduction can participate significantly in corrosion of Mg and its alloys⁵⁹. This may be particularly relevant to the cathodic reaction on Zn-rich intermetallics, since Zn corrodes in neutral media under oxygen depolarization. Hence, it is reasonable to expect the detrimental role of crevice in accelerated corrosion of coated Mg alloy systems like MgZn_xCa_y.

Table 39. EDS analysis (at. %) of PEO-3F_300s coatings for MgZn_xCa_y alloys after 18 h and 120 h of immersion.

Alloy	Time (h)	Location	O	F	Na	Mg	Si	P	Cl	K	Ca	Zn	Ca/P
MgZnCa	18	1	70.1	1.9	0.0	9.9	2.8	5.2	3.3	0.0	6.2	0.5	1.18
		2	56.0	0.0	0.2	22.8	0.0	5.6	1.4	0.5	12.3	1.4	2.21
Mg3Zn0.4Ca		3	56.2	2.4	1.7	11.4	1.0	10.8	1.7	0.3	12.8	2.0	1.19
		4	61.4	0	1.4	15.0	0.6	6.2	1.7	0.3	10.2	3.4	1.64
MgZnCa	120	5	57.4	0.8	0.2	24.3	13.9	1.8	0.4	0.0	0.6	0.4	0.33
		6	66.5	0.5	0.2	18.2	0.5	2.4	1.2	0.0	9.2	1.4	3.90
Mg3Zn0.4Ca		7	61.4	0.6	1.7	7.7	0.6	9.9	1.1	0.3	15.3	1.4	1.54
		8	63.5	1.2	0.7	12.6	0.0	6.6	0.5	0.5	11.9	2.6	1.82

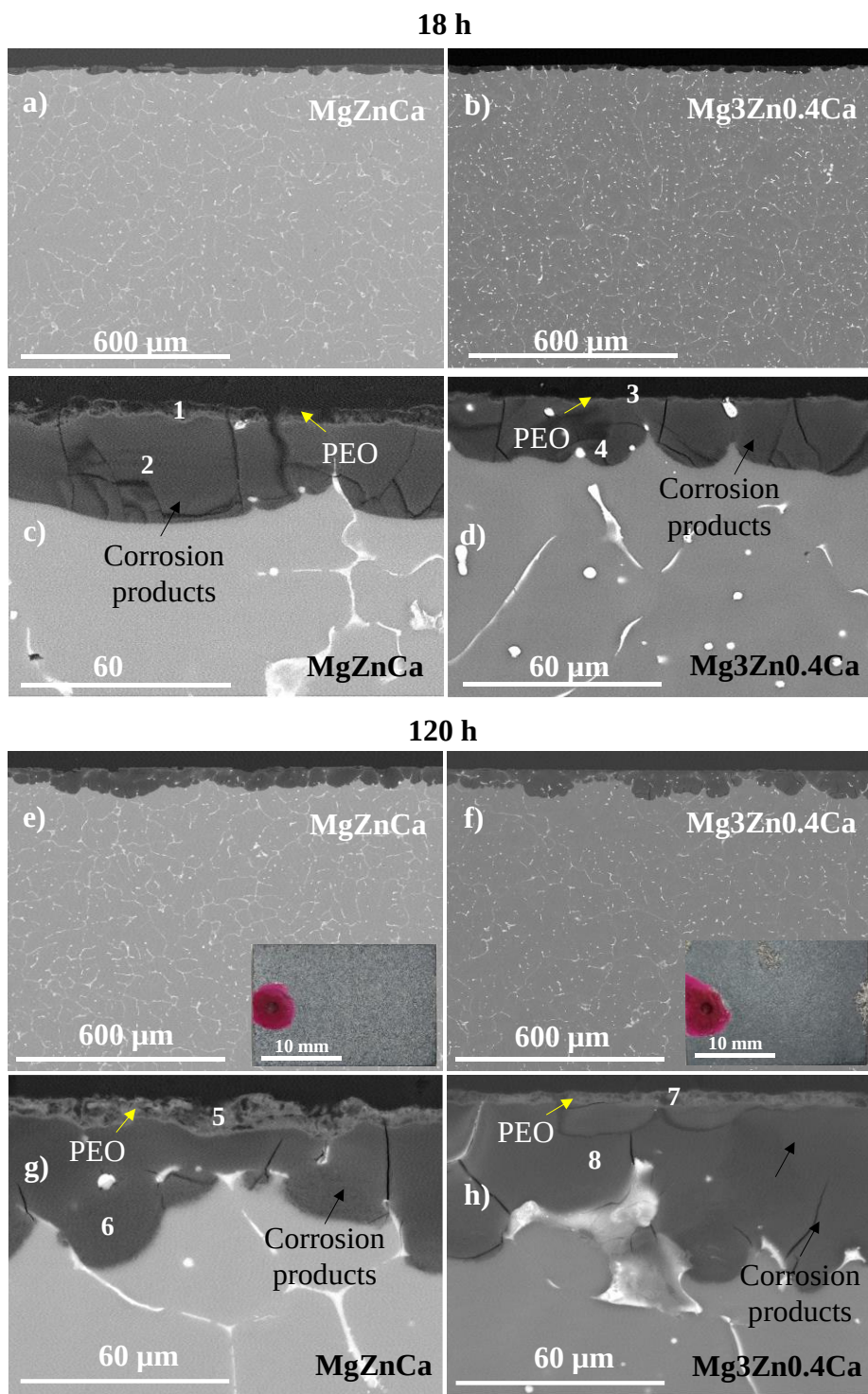


Figure 59. Cross-sectional backscattered electron micrographs after immersion in inorganic α -MEM solution under flowing CO_2 . PEO-3F (300 s): 18 h (a-d) and 120 h (e-h). Insets: macro-photographs of the specimens.

In regards to the correlation between the electrochemical and hydrogen evolution experiments, the corrosion current densities (**Table 35**) obtained from the polarization curves in inorganic α -MEM significantly underestimated the corrosion rate. According to i_{corr} of 1.48 mg cm^{-2} MgZnCa alloy would be expected to dissolve in 120 h, whereas $\sim 2.56 \text{ mg cm}^{-2}$ was actually dissolved, based on the evolved H_2 volume (**Figure 57** (b)) corrected for the amount of dissolved H_2 . The actual dissolved Mg is expected to generate about $\sim 20 \text{ }\mu\text{m}$ -thick layer of $\text{Mg}(\text{OH})_2$, which is in a good agreement with the average thickness of corrosion products layer observed in **Figure 59** (e). The difference between the i_{corr} - and H_2 - derived values is undoubtedly related to the drastic change in the corrosion rate the initial period of immersion, as can be seen in **Figure 57** (c), which must be due to the enhancement of microgalvanic couples in the presence of crevice, i.e. under the coating once the barrier layer of the latter fails. This is a common drawback of polarization tests as they provide single-time data rather than an average rate³⁸³. The abnormal polarization behavior of Mg alloys, known as negative difference effect, also complicates the interpretation³⁸⁴. Besides, hydrogen tests do not detect non-Faradaic dissolution³⁸³ and cannot reflect the contribution of oxygen reduction⁶⁰.

The EIS method can still be used for reliable qualitative corrosion screening of PEO coatings, even when the coated system is compromised by microgalvanic couples. This is demonstrated by the measurements performed on bare MgZnCa alloy and MgZnCa/PEO-3F (300 s) system after 1 h, 24 h and 120 h of immersion in inorganic α -MEM at 37°C (**Figure 61**). The results show that the initially superior corrosion resistance of the coated alloy (1 h) becomes lower than that of the bare alloy following 24 h of immersion (the original EIS spectra and the respective fitting parameters can be found in **Figure A5, Table A3**, Appendix). It further decreases after 120 h of immersion. This is consistent with the results of H_2 evolution experiments.

In summary, H_2 evolution test provides a better indication of the average corrosion rate, whereas EIS testing is more useful for direct and quick comparison of coatings. For EIS screening of different coating recipes, 24 h immersion should be considered sufficient even when a strong microgalvanic coupling is involved in the corrosion mechanism of the alloy/coating system.

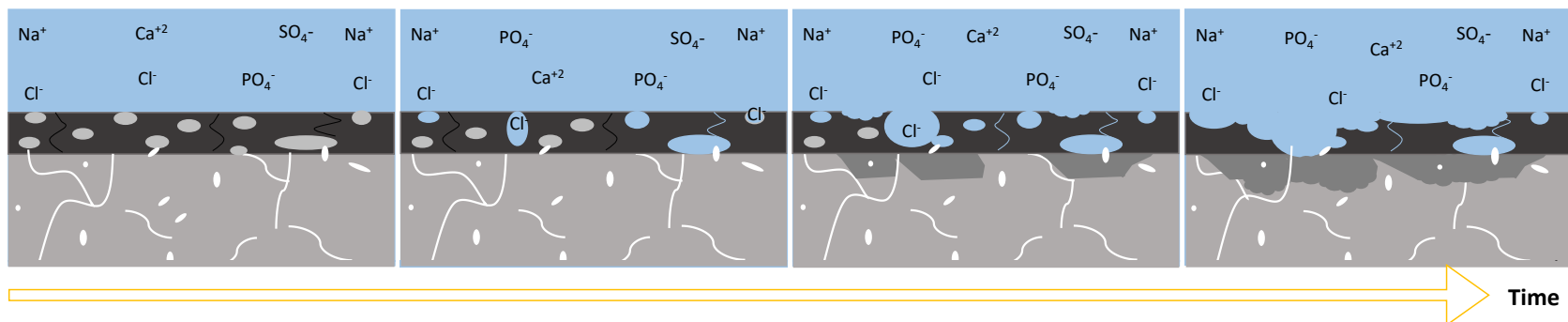


Figure 60. Schematic diagram of the corrosion mechanism of the PEO-coated samples.

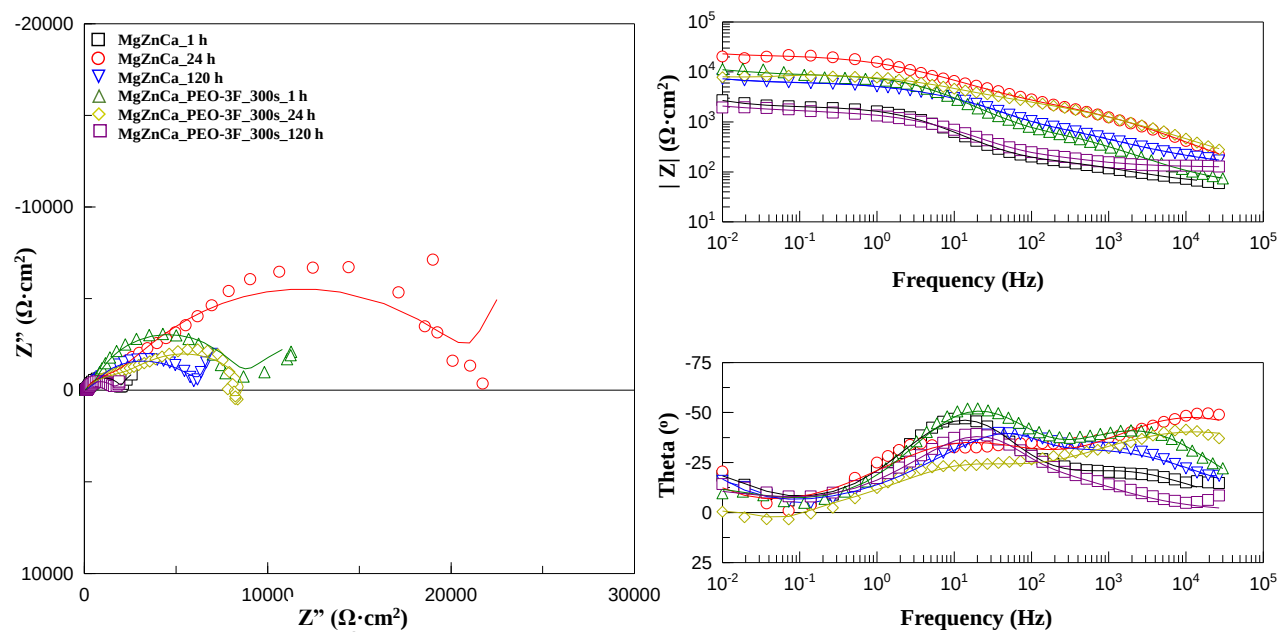


Figure 61. Nyquist and Bode diagram of MgZnCa and MgZnCa/PEO-3F (300 s) system after 1 h, 24 h and 120 h of immersion at 37°C.

5.1.2.4 Fluoride release

According to the results disclosed in the Section 5.1.1.4 and previous works on Mg0.8Ca alloy ¹⁰⁵, when coatings contain 15-20 at. % of F, the F⁻ ions release after 120 h of immersion is about 160-180 $\mu\text{g cm}^{-2}$. In the case of a reduced amount of F⁻ in the electrolyte and, as a consequence, a decreased coating thickness and F⁻ content in the coating (4-8 at. %), it would be reasonable to expect a much lower fluoride release.

Nevertheless, the maximum amount of liberated F⁻ ions (measured in 0.9 wt. % NaCl) for PEO-3F coated MgZnCa and Mg3Zn0.4Ca was 150 $\mu\text{g cm}^{-2}$ and 70 $\mu\text{g cm}^{-2}$ (**Figure 62** and **Table A4**), respectively. These values are comparable or even somewhat higher than those previously reported in Section 5.1.1.4 and in the literature for Mg0.8Ca/PEO-F systems ¹⁰⁵. The enhanced lixiviation of F⁻ may be attributed to a high permeability and hydration of the coating. This phenomena is in accordance with the similar Mg ion release from substrate and PEO-3F coatings as it is illustrated in **Figure A6** (a, c) (Appendix) due to the solubilization of corrosion products (Mg(OH)₂) and MgO. In addition, it is in agreement with the Ca, P and Si release from PEO coatings (**Figure A6** (d)). *In vitro* (e.g. for cell culture volume of 6 mL) this would generate concentrations exceeding the cytotoxicity levels (>1.0 mM) for a range of cells ¹⁰⁵. These findings imply that fluoride-free PEO electrolytes should be developed for alloy systems like MgZn_xCa_y.

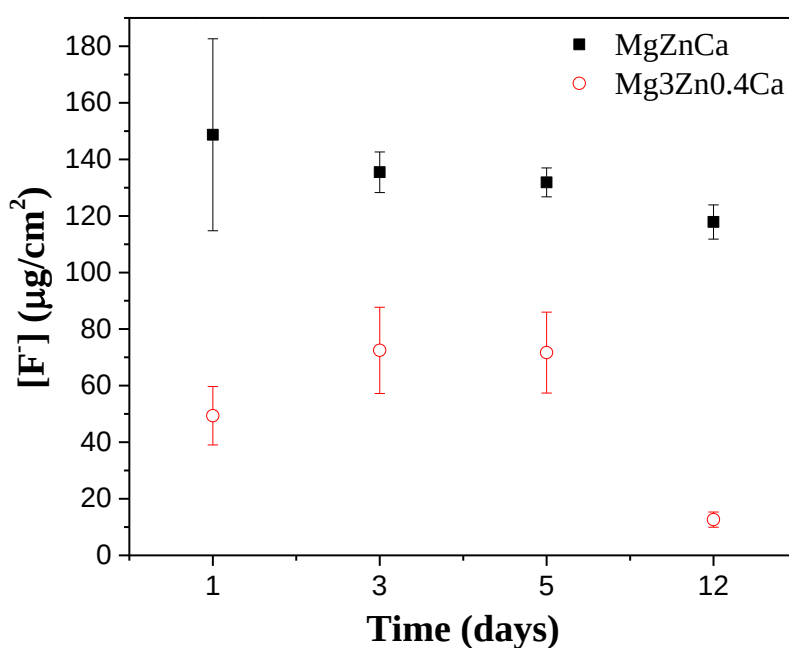


Figure 62. Fluoride release measured by ISE from PEO-3F coatings at 300 s.

5.2 Fluoride-free PEO coatings

Considering the findings from the previous section in regards of the poor corrosion resistance of Mg0.6Ca and, particularly, Mg0.6Ca2Ag system, the development and screening of fluoride-free PEO coatings were undertaken only with respect to MgZn_xCa_y alloys.

5.2.1 Voltage-time response and process efficiency

Figure 63 (a, b) shows the evolution of root mean square values of voltage and current density (U_{rms} , i_{rms}) during PEO treatment in transparent and suspension (PEO-T-2Ca and PEO-S-3Ca, respectively) electrolytes on MgZn_xCa_y alloys. The colors of the curves correspond to: black-MgZnCa, red-Mg3Zn0.4Ca and blue-Mg0.5Zn0.2Ca alloys. For both electrolytes, a linear voltage increase is observed during the 60 s ramp with a slight change of slope at ~35s (230 V) that occurs when the limiting current density of 100 mA·cm⁻² is reached. A second inflexion occurs at ~50 s corresponding to visual initiation of the plasma microdischarges indicating a change in the oxide layer growth kinetics. In PEO-T-2Ca electrolyte, the microdischarges were distributed across the surface of the sample, while in PEO-S-3Ca visual examination of the discharge was not possible because the addition of practically insoluble CaO resulted in opacity of the electrolyte. After that point, the voltage gradually reaches an approximately constant value of ~235 V in both electrolytes due to an increase in the impedance of the coatings during the process.

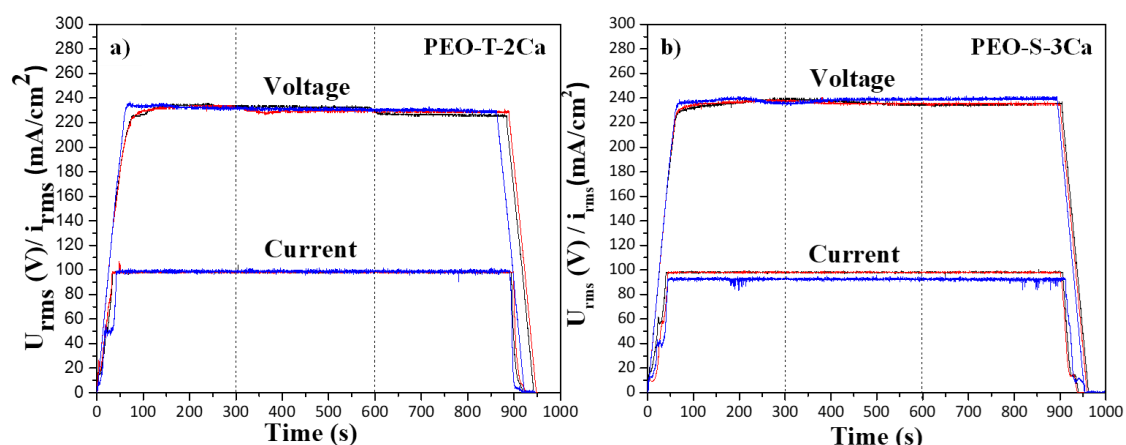


Figure 63. Current density-time curves for (a) PEO-T-2Ca and (b) PEO-S-3Ca coatings for MgZn_xCa_y alloys during 300, 600 and 900 s. The colors of the curves correspond to: black-MgZnCa, red-Mg3Zn0.4Ca and blue-Mg0.5Zn0.2Ca for both electrolytes.

5.2.2 Coating morphologies, structure and composition

Figure 64 shows the evolution of thickness values of PEO-T-2Ca and PEO-S-3Ca coatings for three alloys. The thickness of both coatings increases linearly with the

treatment time for three alloys (Appendix, **Figure A7**). Comparing the two types of electrolytes, slightly higher thicknesses (i.e. greater coating growth rate) were obtained in the suspension electrolyte for cast alloys. This could be associated with entrapment of the suspension particles from the electrolyte in the microdischarges channels and their incorporation into the coating material. In the case of the extruded alloy, no difference in coating growth kinetics was observed between the two electrolytes.

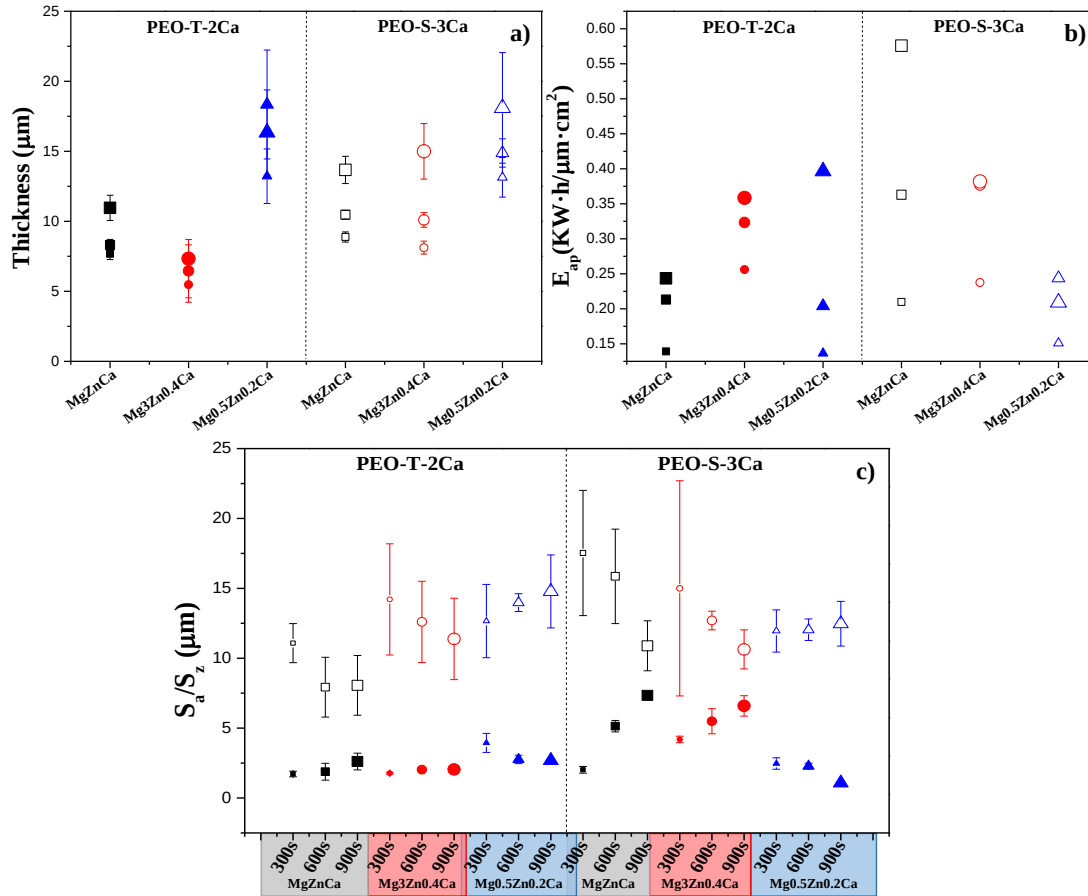


Figure 64. Evolution of (a) thickness, (b) energy consumption and (c) roughness values for PEO-T-2Ca and PEO-S-3Ca coatings on three alloys with time. Symbol size increase in order: 300 s, 600 s, 900 s. In (c) Filled symbols – S_a and open symbols – S_{10z} .

The calculated apparent specific energy consumption for all PEO-coated samples are illustrated in **Figure 64** (b). In most of the systems the results indicated an increase of energy consumption from $<0.15 \text{ kW h m}^{-2} \mu\text{m}^{-1}$ to $>0.35 \text{ kW h m}^{-2} \mu\text{m}^{-1}$ with the increase of the treatment time. Cases of a significant increase in energy for 900 s time point reflect the fact that coating growth kinetics slowed down significantly, possibly due to the development of localized intense microdischarges, typically at the edges of the specimens. Conversely cases, where the energy consumption changed the least or reduced with time (e.g. Mg3Zn0.4Ca and Mg0.5Zn0.2Ca alloys in PEO-S-3Ca electrolyte) indicating a relatively constant and increasing coating growth rate, respectively. As

expected, that happened in suspension electrolyte where the incorporation of particles from the electrolyte contributes to the formation of a new coating material.

Table 40. Surface characteristics of PEO-T-2Ca and PEO-S-3Ca coatings on MgZn_xCa_y alloys at three different treatment times.

Alloy	MgZnCa			Mg3Zn0.4Ca			Mg0.5Zn0.2Ca		
Time	300s	600s	900s	300s	600s	900s	300s	600s	900s
PEO-T-2Ca electrolyte									
Surface PPD (mm^{-2})	$1.10 \cdot 10^6$	$1.29 \cdot 10^6$	$1.60 \cdot 10^6$	$4.06 \cdot 10^6$	$4.09 \cdot 10^6$	$4.17 \cdot 10^6$	$1.09 \cdot 10^6$	$8.9 \cdot 10^5$	$7.3 \cdot 10^5$
Pore area fraction (%)	6.33	5.35	4.21	3.05	2.74	2.24	15.70	29.84	19.11
Average pore size (μm)	0.91 ± 0.28	0.77 ± 0.32	0.62 ± 0.41	0.28 ± 0.04	0.24 ± 0.03	0.25 ± 0.1	0.91 ± 0.35	2.53 ± 0.70	1.35 ± 0.23
PEO-S-3Ca electrolyte									
Surface PPD (mm^{-2})	$8.4 \cdot 10^5$	$1.32 \cdot 10^6$	$3.17 \cdot 10^6$	$3.08 \cdot 10^6$	$3.55 \cdot 10^6$	$3.99 \cdot 10^6$	$4.97 \cdot 10^6$	$4.22 \cdot 10^6$	$3.03 \cdot 10^6$
Pore area fraction (%)	19.01	9.11	3.7	4.74	4.42	1.77	4.96	2.52	1.8
Average pore size (μm)	1.19 ± 0.20	0.75 ± 0.01	0.31 ± 0.07	0.32 ± 0.12	0.28 ± 0.06	0.25 ± 0.01	0.51 ± 0.02	0.24 ± 0.07	0.45 ± 0.08

Note: PPD - pore population density.

In regards of the surface roughness, the average value of S_a of PEO-T-2Ca and PEO-S-3Ca coatings on cast MgZnCa and Mg3Zn0.4Ca alloys tend to remain constant while that of S_{10z} tends to increase with the treatment time (**Figure 64 (c)**). However, considering the standard deviation values, in most of the systems the differences within the S_a and S_{10z} cannot be viewed as statistically significant. The evolution of the surface pore population density, average pore size and pore fraction (**Table 40**) confirm that notion. The average pore size of the coatings formed in the transparent electrolyte somewhat increases with treatment time, ranging within 0.62-0.91 μm , 0.24-0.91 μm and 0.91-2.53 μm for MgZnCa, Mg3Zn0.4Ca and Mg0.5Zn0.2Ca alloys, respectively. For the suspension electrolyte the average pore size is largely similar for all alloys; the difference with the transparent electrolyte is negligible for the cast alloys. In the case of the extruded alloys, ~50-90 % pore size reduction is observed in the suspension electrolyte for all treatment times investigated.

Figure 65-Figure 67 and **Figure 66-Figure 68** show the surface and cross-sectional morphologies of PEO-T-2Ca and PEO-S-3Ca coatings for the three alloys with their respective EDS analysis (**Table 41** and **Table 42**). Treatments of cast alloys in transparent electrolyte (**Figure 65** (a-f)) result in homogeneous porosity without notable development. Extruded alloy thinner coatings (at 300 s and 600 s) revealed more heterogeneous surfaces compared to the 900 s coating, which showed more uniform pore distribution (**Figure 65** (i)).

All samples produced in suspension electrolyte showed similar uniform surface with small pores. In the extruded alloy, the coating produced at 900 s additionally features random isolated bigger open pores (**Figure 66** (i)). Thin microcracks can be observed in some cases (**Figure 65** (b) and **Figure 66** (e)); although they are unlikely to be alloy or treatment-specific. These microcracks are typical for PEO and form due to thermal stress from a quick cooling process³⁸⁵.

The cross-sectional morphology of the coatings formed on cast alloys in either of the electrolytes present small internal pores and discharge channels, some of the latter reaching the barrier layer (**Figure 67** (b, d, f) and **Figure 68** (c, e, f)). It is evident that there is no significant increment in coating thickness with time for either of the alloys or electrolytes. For respective treatment times, there are no notable morphological differences between the coatings formed on cast alloys in any of the two electrolytes. **Figure 67** (c), i.e. the microstructure of PEO-T-2Ca coating formed at 900 s, discloses an example of localized defect morphology that was commonly found in either of the cast alloys for treatment times of 600 s and 900 s. This defect features the formation of a heavily cracked, thick (~80 μm) and dense intermediate layer with scallops at the substrate interface. This morphology is typical of thermally assisted oxidation due to the formation of overheated microdischarges and has been previously observed during PEO of Mg by Arrabal *et al.*³⁸⁶. The corrosion protection afforded by coatings with this kind of cracked morphology is expected to be poor.

In case of the extruded alloy, the coatings formed in the suspension electrolyte appear more compact with less internal pores than on cast alloys; they appear to maintain their structure with the increase of the treatment time (**Figure 67** (g-i)). This is not the case in transparent electrolyte, where the coatings begin to form more internal pores when the time increases (**Figure 67** (g-h))

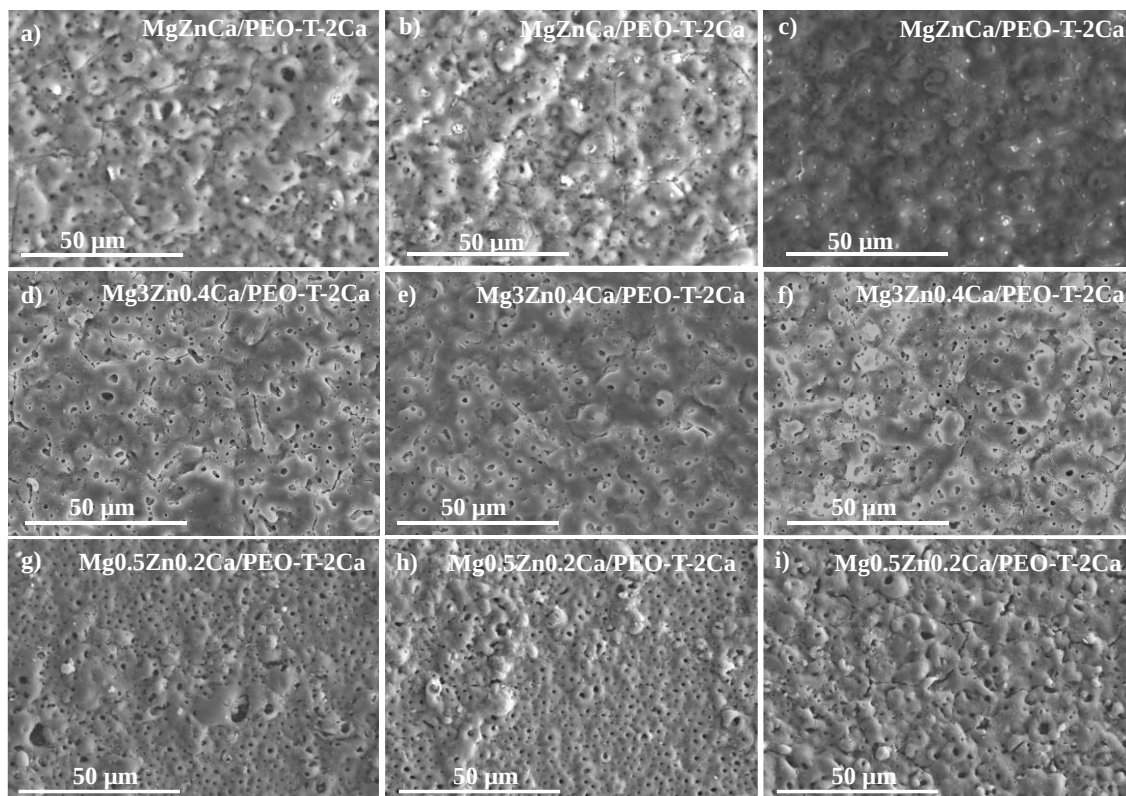


Figure 65. Secondary electron micrographs of surface morphology of the PEO-T-2Ca coatings produced on (a-c) MgZnCa, (d-f) Mg₃Zn_{0.4}Ca and (g-i) Mg_{0.5}Zn_{0.2}Ca alloys in: 300 s (a, b, c), 600 s (d, e, f) and 900 s (g, h, i).

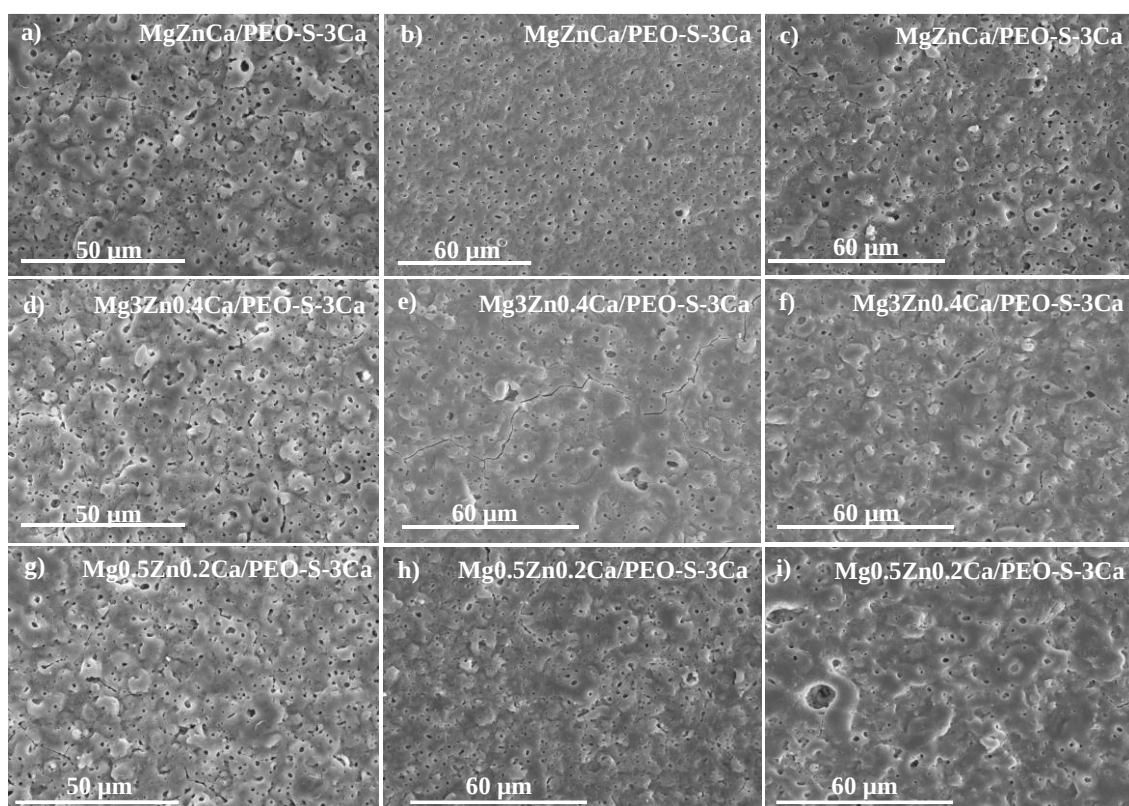


Figure 66. Secondary electron micrographs of surface morphology of the PEO-S-3Ca coatings produced on (a-c) MgZnCa, (d-f) Mg₃Zn_{0.4}Ca and (g-i) Mg_{0.5}Zn_{0.2}Ca alloys in: 300 s (a, b, c), 600 s (d, e, f) and 900 s (g, h, i).

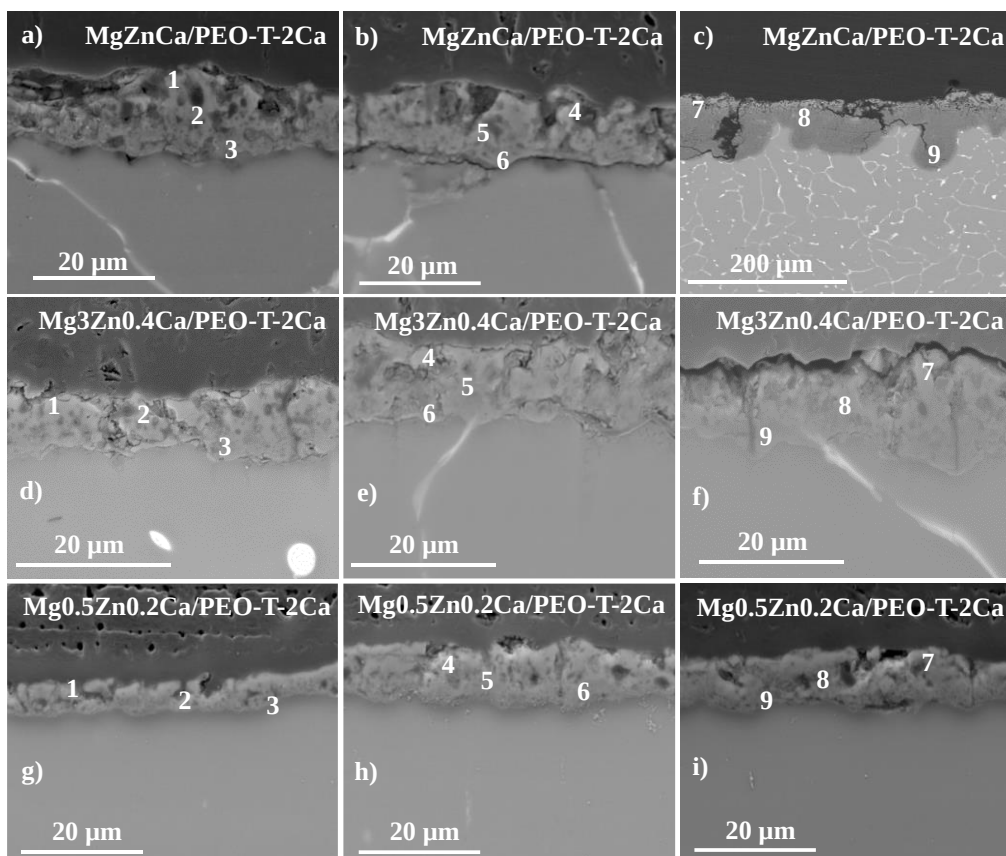


Figure 67. Backscattered electron micrographs of cross-sections of the PEO-T-2Ca coatings produced on (a-c) MgZnCa, (d-f) Mg3Zn0.4Ca and (g-i) Mg0.5Zn0.2Ca alloys in: 300 s (a, b, c), 600 s (d, e, f) and 900 s (g, h, i).

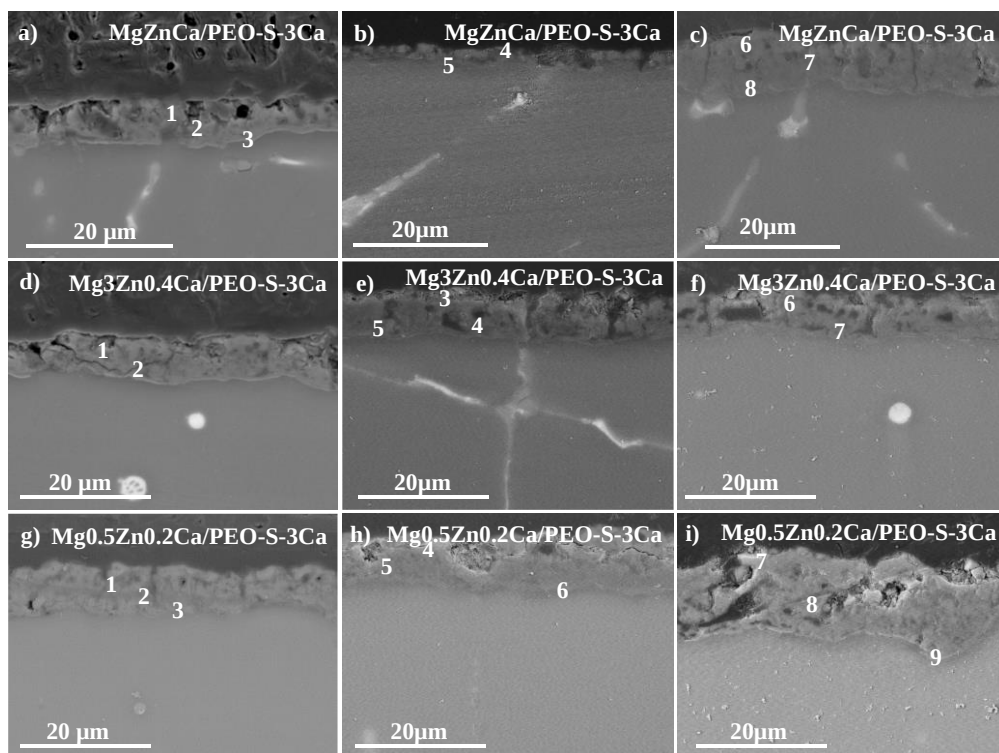


Figure 68. Backscattered electron micrographs of cross-sections of PEO-S-3Ca coatings produced on (a-c) MgZnCa, (d-f) Mg3Zn0.4Ca and (g-i) Mg0.5Zn0.2Ca alloys in: 300 s (a, b, c), 600 s (d, e, f) and 900 s (g, h, i).

A semiquantitative analysis of the elemental composition of the coating was performed by EDS (**Table 41** and **Table 42**). The composition of the coatings studied was determined by the composition of the electrolyte. For instance, Si, Ca, P, and K were mainly incorporated in the outer part of the coating while Zn incorporation is higher in the inner part of all PEO-T-2Ca coatings. That occurs because Si and P migrate from the electrolyte inwards, while Zn migrate from the substrate outwards. Ca incorporation mainly occurs from the electrolyte.

Similar behavior was observed for PEO-S-3Ca, but the incorporation of Ca from this electrolyte significantly decreased with the increase of the treatment time for all alloys. For instance, the surface Ca/P ratios in coatings formed in PEO-S-3Ca electrolyte decreased with the treatment time from ~1.2 to ~0.2 for all alloys. This could be explained by the development of greater intensity (i.e. longer lasting) microdischarges in the suspension electrolyte with time. As a result, these discharges cause a destructive effect, which is associated with the loss of the previously formed material at the site of microdischarges and the formation of new material ⁴³. In comparison, Ca/P ratios in the surface of the coatings formed in PEO-T-2Ca electrolyte were two times lower for all alloys, but were maintained relatively constant with the increase of the treatment time. This is an indication of milder character of microdischarges that took place in this electrolyte.

Table 41. EDS analysis of PEO-T-2Ca coatings in at. % as per **Figure 65** and **Figure 67**.

Alloy	Time (s)	Location	O	Na	Mg	Si	P	K	Ca	Zn	Ca/P
MgZnCa	300	Plan view	56.1	2.1	27.0	7.3	3.9	1.7	1.8	0.1	0.46
		1	54.9	1.9	27.9	8.3	4.1	2.2	0.8	0.1	0.20
		2	52.5	0.6	35.6	5.7	4.1	1.1	0.3	0.2	0.07
		3	51.0	0.4	41.2	4.4	2.0	0.7	0.1	0.3	0.05
	600	Plan view	57.5	3.2	25.1	6.9	3.4	2.0	1.7	0.1	0.50
		4	53.2	1.3	27.7	9.6	4.7	1.6	1.8	0.1	0.38
		5	52.0	1.1	32.3	7.2	4.7	1.9	0.7	0.1	0.15
		6	40.1	0.2	53.5	3.5	1.9	0.4	0.2	0.2	0.11
	900	Plan view	57.5	2.7	25.0	7.6	3.2	2.2	1.7	0.1	0.53
		7	51.5	1.9	31.0	8.4	4.0	1.6	1.5	0.2	0.38
		8	42.3	0.9	42.5	8.2	4.6	1.4	1.5	0.7	0.33
		9	43.4	0.3	53.0	2.4	1.3	0.2	0.1	0.5	0.08
Mg3Zn0.4Ca	300	Plan view	55.5	2.6	26.7	7.7	3.4	2.2	1.7	0.1	0.50
		1	53.4	1.2	31.0	9.1	4.6	0.7	1.1	0.1	0.24
		2	48.3	0.3	44.8	3.8	1.9	0.9	0.1	0.3	0.05
		3	33.1	0.2	62.0	2.8	1.3	0.3	0.0	0.5	0.00
	600	Plan view	57.4	1.6	27.1	7.8	3.6	1.0	1.3	0.2	0.36
		4	50.2	1.6	31.9	8.7	4.4	1.4	1.8	0.1	0.41
		5	41.7	0.5	45.9	5.7	3.8	0.7	1.0	0.6	0.26
		6	48.6	0.4	47.9	1.9	0.7	0.3	0.1	0.2	0.14
	900	Plan view	55.6	2.6	26.5	7.6	3.5	2.2	1.7	0.4	0.49
		7	52.9	2.2	30.1	8.2	3.5	1.8	1.1	0.2	0.31
		8	41.2	1.3	37.2	10.4	5.2	2.0	2.0	0.7	0.38
		9	37.1	0.3	56.9	2.9	1.8	0.1	0.1	0.8	0.06
Mg0.5Zn0.2Ca	300	Plan view	45.9	1.0	38.1	9.9	4.9	0.0	1.2	0.0	0.24
		1	51.5	1.1	31.5	10.6	4.4	0.2	0.8	0.1	0.18
		2	48.8	0.8	36.9	7.7	4.7	0.5	0.5	0.1	0.11
		3	14.8	0.2	80.5	1.8	1.1	0.1	1.1	0.4	1.00
	600	Plan view	42.5	0.4	40.0	11.7	5.0	0.5	0.8	0.0	0.16
		4	49.4	0.5	36.0	9.3	4.0	0.4	0.3	0.1	0.08
		5	48.9	1.0	38.7	6.2	3.4	1.3	0.3	0.1	0.09
		6	40.5	0.2	51.3	5.1	2.4	0.3	0.1	0.2	0.04
	900	Plan view	45.6	0.9	41.9	9.0	2.9	1.3	0.7	0.1	0.24
		7	49.1	1.1	35.0	8.9	3.9	1.1	0.8	0.1	0.21
		8	49.8	0.3	37.8	7.6	3.9	0.4	0.2	0.1	0.05
		9	39.5	0.3	53.2	4.7	1.8	0.2	0.1	0.2	0.06

Table 42. EDS analysis of PEO-S-3Ca coatings in at. % as per **Figure 66** and **Figure 68**.

Alloy	Time (s)	Location	O	Na	Mg	Si	P	K	Ca	Zn	Ca/P
MgZnCa	300	Plan view	55.5	2.8	27.4	8.0	2.6	0.4	3.2	0.2	1.23
		1	36.6	2.2	35.4	14.7	4.0	0.9	6.0	0.3	1.50
		2	48.8	1.6	33.8	10.5	2.7	0.2	2.2	0.1	0.81
		3	45.3	0.9	44.1	7.8	1.2	0.1	0.5	0.2	0.42
	600	Plan view	42.1	0.5	38.3	14.1	4.1	0.0	0.9	0.2	0.22
		4	48.4	1.7	34.2	9.4	2.7	0.7	2.6	0.4	0.96
		5	37.6	0.3	55.7	4.8	0.6	0.0	0.2	0.8	0.33
		6	43.3	2.1	35.7	12.9	3.6	0.7	0.4	0.6	0.11
	900	Plan view	40.7	0.6	39.9	14.5	3.9	0.3	0.0	0.2	0.00
		7	37.1	2.3	42.5	12.4	3.9	1.0	0.6	0.2	0.15
		8	31.2	0.6	60.3	6.3	1.4	0.0	0.1	0.1	0.07
		9	54.6	3.5	26.9	7.6	2.8	0.6	3.8	0.3	1.36
Mg3Zn0.4Ca	300	Plan view	48.4	1.7	34.2	9.4	2.7	0.7	2.6	0.4	0.96
		1	37.6	0.3	55.7	4.8	0.6	0.0	0.2	0.8	0.33
		2	42.7	2.2	37.0	12.9	2.8	0.9	1.3	0.2	0.46
	600	Plan view	34.9	0.9	49.3	11.3	2.9	0.3	0.6	0.0	0.21
		3	27.6	0.7	64.5	5.6	1.6	0.0	0.2	0.0	0.13
		4	41.9	1.9	37.4	12.0	2.8	1.0	1.0	0.4	0.36
		5	34.5	0.7	44.7	14.7	4.5	0.2	0.3	0.8	0.07
	900	Plan view	12.4	0.5	80.2	4.7	1.6	0.0	0.0	0.9	0.00
		6	53.7	3.9	26.6	7.8	3.4	0.8	3.8	0.0	1.12
		7	45.8	2.2	34.2	14.1	2.9	0.6	0.1	0.1	0.03
Mg0.5Zn0.2Ca	300	Plan view	44.1	0.3	50.6	4.0	0.8	0.1	0.1	0.1	0.13
		1	44.4	2.6	37.5	14.6	3.0	1.0	0.1	0.3	0.03
		2	37.6	1.3	41.6	14.1	4.5	0.5	0.3	0.4	0.07
		3	36.0	0.9	50.2	8.8	3.7	0.0	0.4	0.1	0.11
	600	Plan view	37.2	1.6	52.7	8.0	2.1	0.5	0.0	0.0	0.00
		4	38.7	2.1	37.1	12.9	6.8	1.0	1.3	0.1	0.19
		5	36.5	0.9	40.6	13.1	8.2	0.6	0.2	0.1	0.02
		6	36.0	0.5	50.9	7.5	5.3	0.0	0.1	0.3	0.02
	900	Plan view	4.7	0.1	92.2	1.5	1.0	0.1	0.1	0.4	0.10
		7	55.5	2.8	27.4	8.0	2.6	0.4	3.2	0.2	1.23
		8	36.6	2.2	35.4	14.7	4.0	0.9	6.0	0.3	1.50
		9	48.8	1.6	33.8	10.5	2.7	0.2	2.2	0.1	0.81

The X-Ray diffraction patterns of the all the studied coatings are displayed in **Figure 69**. All coatings reveal high intensity peaks of Mg (from the substrate), MgO and the presence of amorphous material corresponding to a peak broadening between 25-40° (2 θ). In addition, Mg₂SiO₄ (forsterite) were formed on all substrates for both electrolytes.

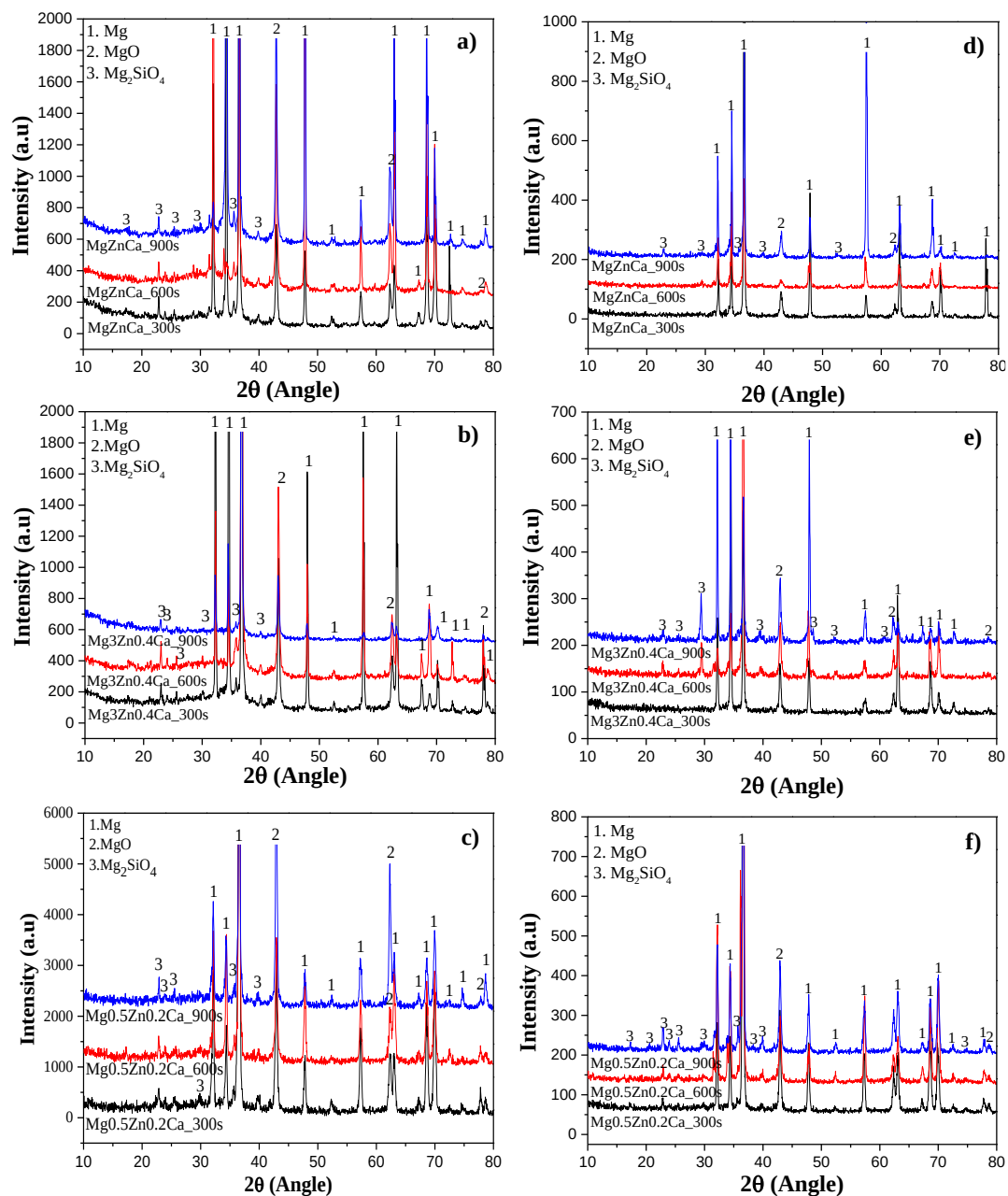


Figure 69. XRD patterns of (a-c) PEO-T-2Ca and (d-f) PEO-S-3Ca coatings on (a, d) MgZnCa, (b, e) Mg₃Zn_{0.4}Ca and (c, f) Mg_{0.5}Zn_{0.2}Ca alloys.

The silicate species from the electrolyte precipitated as amorphous SiO₂, giving rise to Si-rich top layers, as seen in **Table 41** and **Table 42**. During the microdischarges, SiO₂ reaction with MgO forms forsterite at the range of 1100-1400°C. The presence of this compound enhances the compactness of the coatings and improves the mechanical

properties and the corrosion resistance of the coatings^{387,388}. The absence of Ca- and P-containing crystalline phases in PEO-T-2Ca and PEO-S-3Ca coatings suggests that these species present in amorphous state.

5.2.3 Corrosion behavior of the coatings

5.2.3.1 Electrochemical test

DC polarization curves

Figure 70 shows the polarization curves of PEO-T-2Ca and PEO-S-3Ca coatings on the three studied alloys after 1 h of immersion in inorganic part of α -MEM at 37°C. The electrochemical parameters calculated from the curves are presented in **Table 43**.

PEO-T-2Ca coatings produced a noble shift in E_{corr} of MgZnCa and Mg3Zn0.4Ca alloys by 100-200 mV and a reduction of corrosion current densities by 9-10 times. After the E_{corr} , a ~200 mV-wide pseudo-passivity region is observed in the anodic branches of the curves. The E_{pit} is similar (about -1.30 V) in both alloys and is about 300 mV nobler than that of the substrate. The main difference is in the pseudo-passivity of the 900 s coating on MgZnCa alloy, where it is only ~100 mV-wide. This coating reveals a stronger tendency to pitting, which can be associated with local heterogeneities in the coating due to thermally-assisted oxidation (as shown in **Figure 70** (c)). The coatings formed at 600 s on MgZnCa and 300 s on Mg3Zn0.4Ca alloy revealed the lowest i_{corr} out of the three coatings.

The PEO-T-2Ca-coated samples of the extruded alloy present significant differences compared to the coated cast alloys, revealing a nobler E_{corr} (by ~150 mV) and no pseudo-passivation range. It should be noted that the E_{corr} of Mg0.5Zn0.2Ca alloy substrate is also about ~150 mV nobler than that of the cast alloys. The i_{corr} , on the other hand, was higher by 2-3 times compared to that of the substrate. It should be noted that the anodic branches of coated extruded alloy systems disclose neither a well-defined pseudo-passivity region nor a marked pitting potential, especially for longer treatment times (**Figure 70** (c)). The latter may be reasonably called pseudo-Tafel behavior, i.e. the anodic behavior of these systems resembles active dissolution. In this case, a uniform corrosion development should be reasonably expected under the coating.

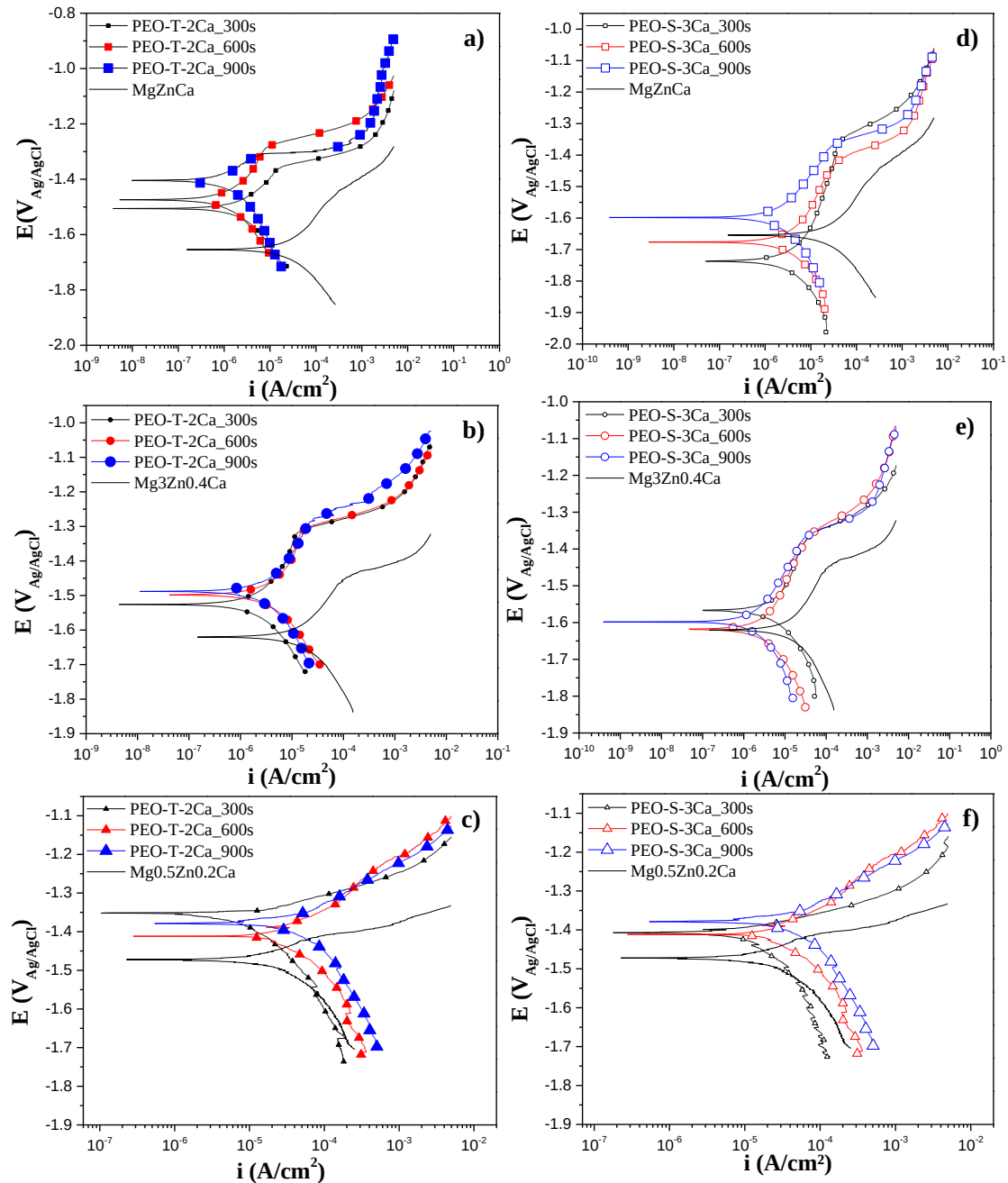


Figure 70. Polarization curves of (a-c) PEO-T-2Ca and (d-f) PEO-S-3Ca coatings on (a, d) MgZnCa, (b, e) Mg3Zn0.4Ca and (c, f) Mg0.5Zn0.2Ca alloys. Filled symbols- PEO-T-2Ca and open symbols- PEO-S-3Ca coatings. Symbol shape: square-MgZnCa, circle-Mg3Zn0.4Ca and triangle-Mg0.5Zn0.2Ca alloys. Symbol size increases in the following order of the treatment time: 300 s, 600 s and 900 s.

The PEO-S-3Ca systems show a behavior very similar to that of PEO-T-2Ca coatings on the respective alloys, in regards to the shifting of E_{corr} to higher values. The i_{corr} decreased with the increase of the treatment time for cast alloys and increased for the extruded alloy. Comparing all the coating/alloy systems, the lowest i_{corr} values were observed for PEO-S-3Ca coatings on Mg3Zn0.4Ca alloy, within $0.84\text{--}1.97 \mu\text{A}\cdot\text{cm}^{-2}$ range. The i_{corr} for the extruded alloys systems were 10-30 times higher than that of cast alloys (**Table 43**). In comparison to the corrosion current data available in the literature

for PEO-coated Mg-Zn-Ca systems with alloy compositions similar to the ones of this study, the i_{corr} values obtained in the present work are lower by two times. For instance, the PEO process developed on Mg3Zn0.4Ca and Mg1.78Zn0.51Ca alloys yielded i_{corr} of 2.38-7.28 and 5.25 mA/cm², respectively ^{114,389}. This highlights the important influence of the composition of the electrolyte and the parameters of the PEO process on the corrosion performances of the PEO/MgZn_xCa_y systems.

Table 43. Corrosion characteristics from polarization curves of PEO-T-2Ca and PEO-S-3Ca coatings.

Electrolyte	Alloy	Time (s)	E_{corr} (V)	i_{corr} ($\mu\text{A}/\text{cm}^2$)	E_{pit} (V)
PEO-T-2Ca	MgZnCa		-1.65	27.37	-1.45
	Mg3Zn0.4Ca		-1.62	30.25	-1.42
	Mg0.5Zn0.2Ca		-1.47	33.41	-1.40
	MgZnCa	300	-1.51	2.32	-1.35
		600	-1.47	1.33	-1.27
		900	-1.4	1.43	-1.31
	Mg3Zn0.4Ca	300	-1.53	2.5	-1.31
		600	-1.5	3.76	-1.3
		900	-1.49	3.02	-1.28
	Mg0.5Zn0.2Ca	300	-1.35	11.02	-
		600	-1.41	55.4	-
		900	-1.38	60.79	-
	MgZnCa	300	-1.74	6.33	-1.35
		600	-1.68	5.66	-1.4
		900	-1.6	3.85	-1.36
	Mg3Zn0.4Ca	300	-1.57	1.97	-1.36
		600	-1.62	0.99	-1.35
		900	-1.6	0.84	-1.34
PEO-S-3Ca	Mg0.5Zn0.2Ca	300	-1.41	20.2	-
		600	-1.41	47.32	-
		900	-1.38	60.32	-

In summary, all coatings on MgZnCa and Mg3Zn0.4Ca alloy exhibited a positive shift (200-400 mV) in E_{corr} and a reduction of corrosion density compared to the respective substrates. The coatings on extruded Mg0.5Zn0.2Ca exhibited a stronger tendency to uniform corrosion for all treatment times. MgZnCa alloy in PEO-T-2Ca coatings presented lower corrosion density while the opposite trend is observed on Mg3Zn0.4Ca and Mg0.5Zn0.2Ca alloys.

Electrochemical Impedance Spectroscopy

The screening of PEO-T-2Ca and PEO-S-3Ca coatings on MgZnCa, Mg₃Zn_{0.4}Ca and Mg_{0.5}Zn_{0.2}Ca alloys is illustrated in **Figure 71** according to their modulus of total impedance at low frequency ($|Z|_{10\text{mHz}}$) after 1 h of immersion in inorganic α -MEM at 37°C.

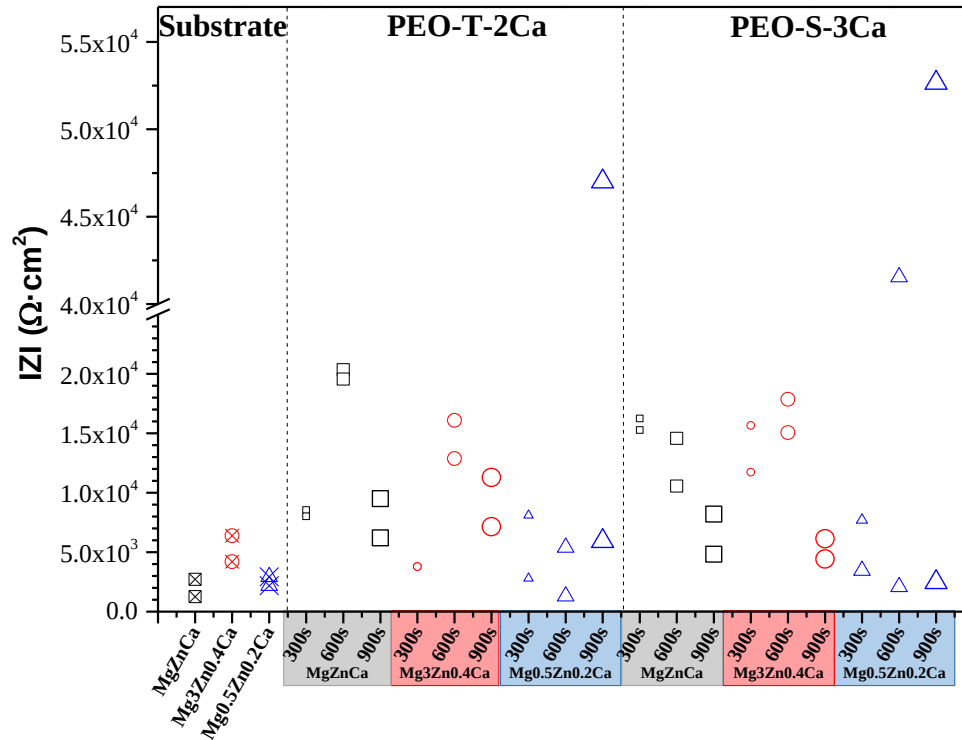


Figure 71. Scatter diagram of the impedance values for studied PEO-T-2Ca and PEO-S-3Ca coatings. Colours indicate: black-MgZnCa, red-Mg₃Zn_{0.4}Ca and blue-Mg_{0.5}Zn_{0.2}Ca alloys. Symbol size: small-300 s, medium-600 s and large-900 s of treatment time.

Several tendencies can be identified:

- i) The coatings produced in the transparent electrolyte at 600 s show greater corrosion protection for cast MgZnCa and Mg₃Zn_{0.4}Ca alloys than those formed at 300 and 900 s treatment times. For Mg₃Zn_{0.4}Ca alloy the latter treatments revealed insignificant improvement compared to bare substrate.
- ii) The coatings produced in the suspension electrolyte at 300 s provided considerably higher corrosion protection for both cast alloys, in comparison with transparent electrolyte. Overall, the corrosion resistance tended to decrease with PEO treatment time for cast alloys coated in suspension electrolyte.
- iii) In the case of extruded Mg_{0.5}Zn_{0.2}Ca alloy, the coatings showed low repeatability in corrosion resistance for longer PEO treatment times. For the thinnest coatings (300 s), the repeatability was adequate for both electrolytes.

iv) Overall, the two electrolytes were comparable in terms of relatively similar modulus of total impedance obtained for the respective treatment times for all the alloys. MgZnCa coated in PEO-T-2Ca at 600 s made an exception, as its $|Z|_{10\text{mHz}}$ was two times greater compared to its PEO-S-3Ca counterpart.

The EIS screening identified PEO-T-2Ca (600 s) and PEO-S-3Ca (300 s) coatings as the most protective ones for MgZnCa alloy, according to the highest $|Z|_{10\text{mHz}}$ with greatest repeatability. For Mg₃Zn_{0.4}Ca alloy, the 600 s treatments in either of the electrolytes were most protective. The coatings formed on Mg_{0.5}Zn_{0.2}Ca in either of the electrolytes were inferior, suffering from low repeatability at 600 and 900 s treatment times.

In order to understand the contribution of various electrochemical processes that take place in the coating/substrate systems into their corrosion resistance, a detailed analysis of the EIS spectra of the three alloys was performed for selected treatment times in each PEO electrolyte. **Figure 72** illustrates the Nyquist and Bode plots of the EIS spectra for 600 s coatings produced in PEO-T-2Ca electrolyte and 300 s coatings produced in PEO-S-3Ca electrolyte. The respective equivalent circuits and the fitting parameters are presented in **Figure 73** and **Table 44**.

The equivalent circuit (**Figure 73**) used to interpret the EIS results of PEO-T-2Ca and PEO-S-3Ca coatings for cast alloys includes R_{el} (solution resistance), R_1/CPE_1 (resistive and capacitive response of the outer-intermediate part of the coatings), R_2/CPE_2 (behavior of inner barrier layer), and R_{ct}/CPE_{dl} (electrochemical activity at the substrate/electrolyte interface). However, the circuit for the coated Mg_{0.5}Zn_{0.2}Ca alloy includes only R_1/CPE_1 and R_{ct}/CPE_{dl} corresponding to the resistive/capacitive response of the coating and of the substrate/electrolyte interface, respectively. In the equivalent circuits, the impedance of CPE is calculated by the following equation: $Z=1/(CPE(j\omega)^n)$; where j is the imaginary number and $-1 \leq n \leq 1$.

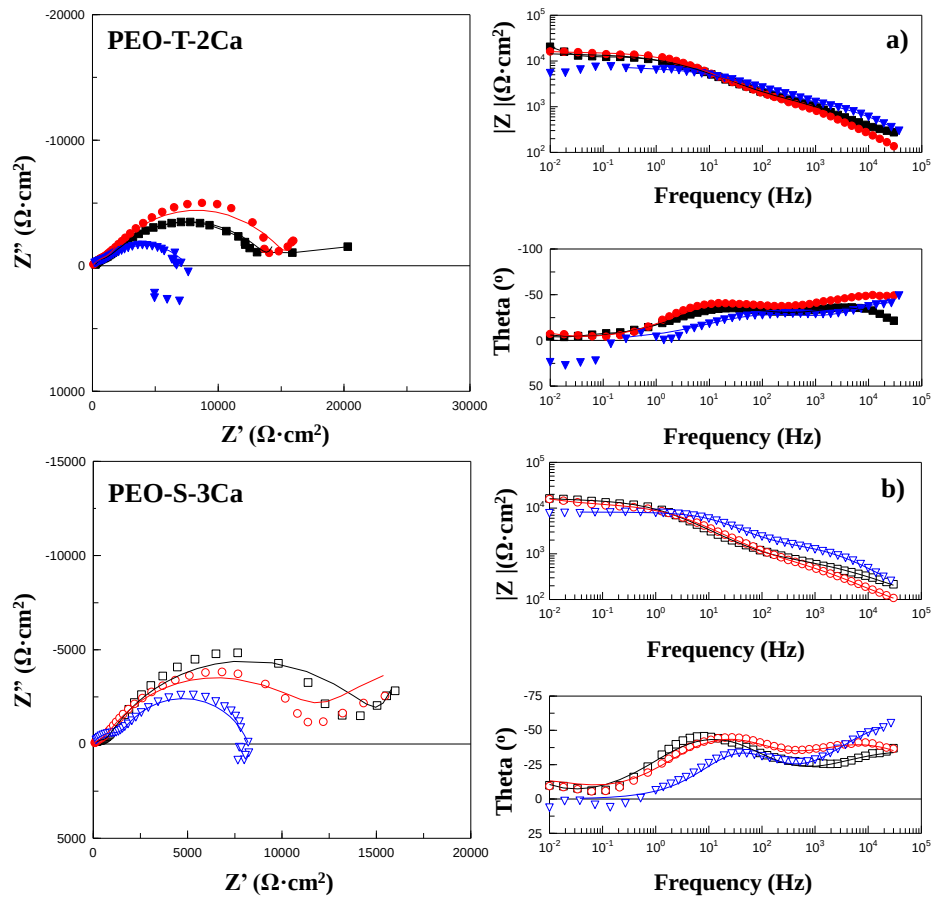


Figure 72. Nyquist and Bode plots of (a) PEO-T-2Ca and (b) PEO-S-3Ca coatings for MgZnCa, Mg3Zn0.4Ca and Mg0.5Zn0.2Ca alloys after 1 h of immersion in inorganic α -MEM solution at 37°C. Colours: black-MgZnCa, red-Mg3Zn0.4Ca and blue-Mg0.5Zn0.2Ca alloys. Filled symbols- PEO-T-2Ca and open symbols-PEO-S-3Ca.

The fitted data indicate that in all coating systems, the barrier layer is the main factor responsible for the corrosion protection of the alloy (R_1 is lower than R_2). This behavior correlates with the higher values of CPE_2 compared to CPE_1 by 2-10 times indicating the poor corrosion resistance of the porous layer. According to the differences among the alloys, R_{ct} values of cast MgZnCa and Mg3Zn0.4Ca alloys are about two times greater compared to those for the extruded Mg0.5Zn0.2Ca alloy, suggesting a greater electrochemical activity and dissolution processes at the substrate/coating interface in the latter.

In regards to the differences between alloys, the coated cast alloys systems exhibited similar corrosion behavior, with about four times higher R_{total} compared to the coated extruded alloy ($R_{total} \sim 26-33 \text{ k}\Omega \cdot \text{cm}^2$ vs. $\sim 7.5-8.2 \text{ k}\Omega \cdot \text{cm}^2$). On cast alloys, the resistance of the porous part (R_1) was about 10 times lower than the resistance of the barrier layer (R_2), indicating easy permeability of the outer part by corrosive species from the medium.

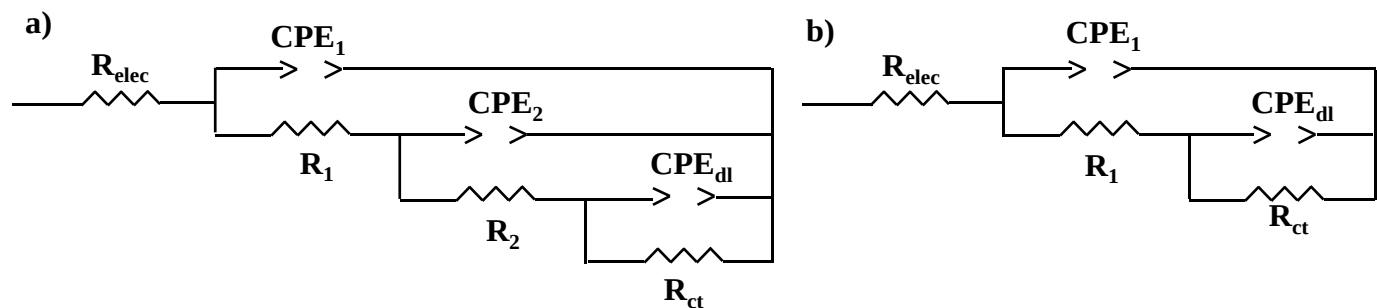


Figure 73. EIS equivalent circuits for (a) PEO-T-2Ca and PEO-S-3Ca-coated samples on MgZnCa and Mg3Zn0.4Ca alloys, (b) PEO-T-2Ca and PEO-S-3Ca coated samples on Mg0.5Zn0.2Ca alloy. Both cases were carried out after 1 h of immersion in inorganic α -MEM.

Table 44. Fitted electrical parameters of EIS spectra of PEO-T-2Ca at 600 s and PEO-S-3Ca at 300 s after 1 h of immersion in inorganic α -MEM solution.

Electrolyte	Alloy	R_{elec}	CPE_1	n_1	R_1	CPE_2	n_2	R_2	CPE_{dl}	n_3	R_{ct}	R_{total}
		($\Omega \cdot cm^2$)	($\mu S \cdot s^n \cdot cm^{-2}$)		($\Omega \cdot cm^2$)	($\mu S \cdot s^n \cdot cm^{-2}$)		($\Omega \cdot cm^2$)	($mS \cdot s^n \cdot cm^{-2}$)		($\Omega \cdot cm^2$)	($\Omega \cdot cm^2$)
PEO-T-2Ca	MgZnCa	178	1.49	0.7	1422	11.66	0.62	12053	1.72	0.44	16305	29780
	Mg3Zn0.4Ca	19.17	3.6	0.63	2013	6.52	0.71	13705	6.22	0.93	16800	32518
	Mg0.5Zn0.2Ca	58.34	0.41	0.72	1090	-	-	-	10.6	0.57	6400	7490
PEO-S-3Ca	MgZnCa	31.99	7.29	0.53	883.4	121.2	0.71	15101	5.73	0.97	16957	32941
	Mg3Zn0.4Ca	38.54	6.92	0.61	1005	8.74	0.71	11305	1.04	0.67	14062	26372
	Mg0.5Zn0.2Ca	14.54	0.69	0.71	1711	-	-	-	4.3	0.79	6500	8211

The PEO-S-3Ca coatings in particular showed lower values of the outer layer resistance (R_1) by two times in both alloys in comparison with the PEO-T-2Ca coatings, possibly due to the greater pore area fraction and slightly bigger pore size (**Table 40**). The opposite situation was observed for the extruded alloy, where R_1 of PEO-S-3Ca exhibited higher value than for the PEO-T-2Ca, which, again, correlated with the differences in the surface porosity of these coatings. Overall, the fact that the coatings on extruded alloy presented only one time constant (R_1/CPE_1), suggest that they get easily and uniformly hydrated and permeated and their barrier layer resistance is in discriminable from the resistance of the rest of the coating.

PEO-T-2Ca and PEO-S-3Ca coatings formed at 600 s and 300 s, respectively, on each alloys were further evaluated by hydrogen evolution measurements in inorganic α -MEM in order to determine the degradation rate and to examine the development of corrosion products during the period of immersion comparable to that of a standard *in vitro* cell proliferation test.

5.2.3.2 Hydrogen evolution test

Figure 74 shows the hydrogen evolution for the three coated $MgZn_xCa_y$ alloys during 120 h of immersion in inorganic α -MEM solution at 37°C under flow of CO_2 controlling the pH at 7.4. The curves for bare alloys are included as reference.

According to **Figure 74** (a), all PEO-T-2Ca specimens showed a quick acceleration of corrosion rate after 5 h of immersion as observed in the Figure (c, d) which is related to the loss of the protective properties of the coatings. After that time, the degradation rate of $MgZnCa$ - and $Mg_3Zn_{0.4}Ca$ -coated specimens remained relatively constant (no marked change of the slopes) until the end of the test. However, for the coated $Mg_{0.5}Zn_{0.2}Ca$, the H_2 evolution increased much faster since the beginning. Taking into account the total hydrogen volume after 120 h for PEO-T-2Ca-coated specimens, $Mg_{0.5}Zn_{0.2}Ca$ present higher value (10 mL/cm²) compared to the cast alloys (~2 mL/cm²). This behavior confirms the trend revealed in both DC and AC electrochemical experiments and may be attributed to the lack of barrier layer resistance in extruded alloy system in accordance with the predictions derived from EIS spectra fittings (**Figure 72**, **Table 44**). Therefore, coated specimens exhibited 2-10 times higher corrosion rate compared to bare substrates which is opposite to what is typically found in studies of PEO-treated Mg alloys.

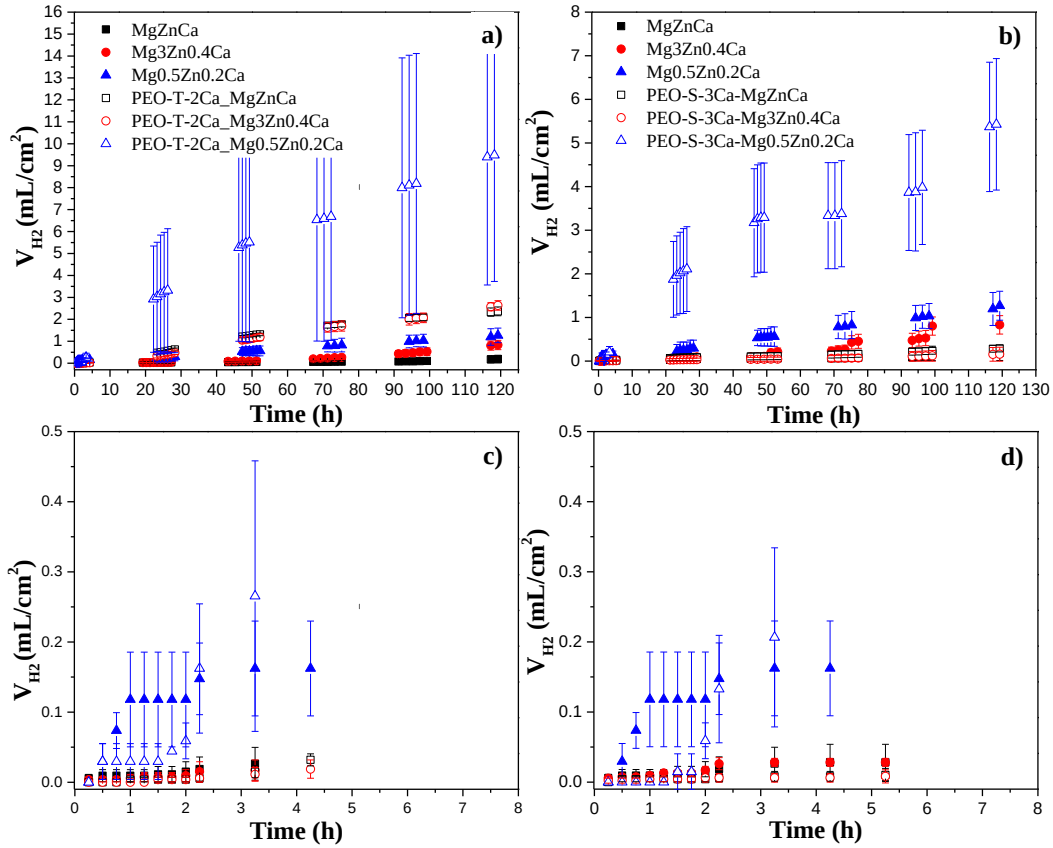


Figure 74. Hydrogen evolution for (a) PEO-T-2Ca and (b) PEO-S-3Ca coatings for all alloys after 120 h of immersion in inorganic α -MEM solution at 37°C and constant pH 7.4. Figures (c, d) are amplified views of the initial immersion period in (a, b).

The cast and extruded systems with coatings obtained in suspension electrolyte disclosed differing behavior (**Figure 74** (b)). The slopes of H₂ evolution curves for MgZnCa and Mg3Zn0.4Ca-coated specimens remained shallow and relatively constant, indicating that coatings maintained their protective effect during the test. PEO-S-3Ca-coated on MgZnCa and Mg3Zn0.4Ca alloys showed, respectively, similar and lower H₂ volume after 120 h of immersion (0.29 and 0.12 mL/cm²) compared to bare substrates (0.18 and 0.88 mL/cm²). However, the extruded alloy/coating system exhibited the same accelerated corrosion tendency with respect to the bare substrate as observed for the Mg0.5Zn0.2Ca coated in transparent electrolyte: 5.4 mL/cm² of collected H₂ vs. 1.26 mL/cm² for the bare substrate.

The experimentally collected total hydrogen volume was corrected for all systems in order to take into account the amount of the dissolved gas. The newly calculated values permitted a comparison of the true degradation rates of the coated systems (**Table 45**).

Table 45. Corrected H₂ volume obtained from **Figure 74** (a) and (b) for three alloys in 120 h of immersion.

V _{H2} corrected (mL/cm ²)	Time (s)	MgZnCa	Mg3Zn0.4Ca	Mg0.5Zn0.2Ca
PEO-T-2Ca	600	5.8 ± 0.5	6.4 ± 3.7	26.7 ± 2.3
PEO-S-3Ca	300	4.7 ± 5.0	2.52 ± 1.0	15.1 ± 4.2

Comparing the two coating types, it is evident that the coatings produced in suspension electrolyte offered more protection for all studied alloys. Further, greater corrosion resistance was exhibited by Mg3Zn0.4Ca alloy with PEO-S-3Ca coating then by MgZnCa alloy with the same coating, whereas no significant differences were observed between these alloys when coated in transparent electrolyte. Clearly, the immersion test results allowed for greater discrimination between the coated systems than the EIS measurements after 1 h of immersion (**Figure 72, Table 44**). This may be associated with the initiation and extent of localized corrosion that can only be revealed at longer immersion times. In case of the coated extruded alloy, the degradation rate was 3-5 times greater than that of the coated cast alloys, in agreement with the tendency revealed during electrochemical testing.

The obtained results were compared, where possible, with those published in the literature. **Table 46** presents a survey of reports focused on the development of bioactive PEO coatings on a variety of Mg-Zn-Ca alloys in order to improve the corrosion resistance of Mg alloys in physiological media. It should be mentioned, that all of these works are focused on studying cast Mg-Zn-Ca alloys, and none on the extruded alloys. The majority of these reports used Ca-P, Ca-P-Si or Si-based suspension electrolytes forming coatings with a thickness in the range of 10-40 µm. The corrosion performance of these systems was evaluated by electrochemical or immersion tests revealing that PEO coatings improved on the corrosion performance of the substrates.

In the present Thesis, only electrochemical studies (PDP and EIS) disclosed a superior performance of the coated systems, compared with bare substrates, while 120 h-long immersion tests revealed greater degradation of the most of the coating/substrate systems. Direct comparison of the long-term protective capacity of the coatings reported in the literature with those studied in the present work was difficult due to several differing aspects: i) in the immersion tests cited in **Table 46**, the volume of released hydrogen was not measured and a constant pH of the corrosive medium was not maintained during the

test; ii) only plan view examination of the corrosion products was carried out after the tests, which is not insightful for determination of the corrosion mechanism.

In order to further understand the reasons for the corrosion behaviour of the coated systems investigated in the present study, macro- and micro-morphological examination of the specimens after 120 h of immersion was carried out (**Figure 75**). In regards to cast alloys, the coatings formed in suspension electrolyte do not show any sign of localised corrosion (**Figure 75** (b, d)), whereas for the coatings formed in transparent electrolyte the localised process have already started (**Figure 75** (a, c)). The macro-appearance of the extruded Mg0.5Zn0.2Ca alloy with either type of coating suggested a uniform corrosion process (**Figure 75** (e, f)). The detailed cross-sectional examination of the better performing PEO-S-3Ca coatings for the three alloys (**Figure 75** (g-i)) disclosed a thick corrosion products layer in the range of 10-50 μm with the presence of cracks. Remarkably, in cast alloys the hydrated coating was still present over the corrosion products. These findings are similar to those regarding the F-containing coatings earlier discussed in this Chapter (Section (5.1.2.3)). It is evident that the second phase network in the grain boundaries of cast alloys was halting the corrosion progress (**Figure 75** (g, h)).

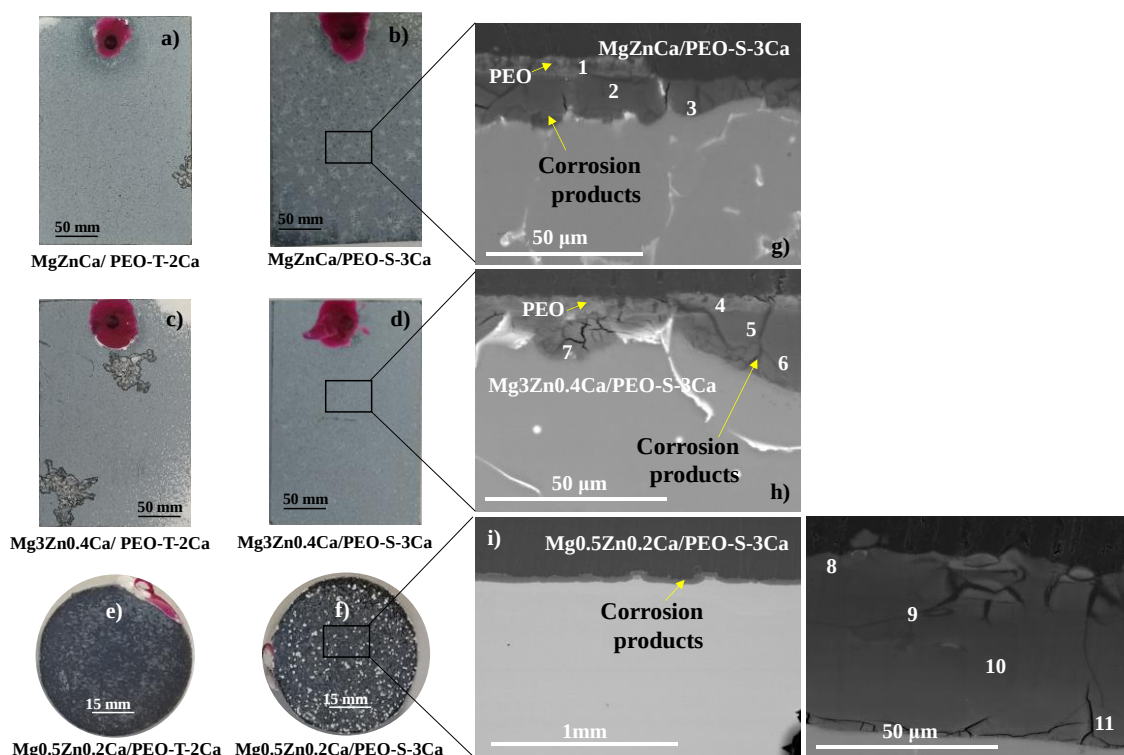


Figure 75. Macrographs of (a, c, e) PEO-T-2Ca and (b, d, f) PEO-S-3Ca coatings on (a, b) MgZnCa, (c, d) Mg3Zn0.4Ca and (e, f) Mg0.5Zn0.2Ca alloys and respective cross-sectional backscattered electron micrographs (g, h, i) of PEO-S-3Ca coatings after 120 h of H_2 evolution test.

Table 46. Survey on corrosion behavior of PEO coated Mg-Zn-Ca alloys.

Alloy		PEO coating			Corrosion behavior				Ref.
Composition	Manufacture	Composition	Thickness (μm)	Treatment time	EIS*	PDP*	Immersion test	Characterization post-corrosion	
Mg4.71Zn0.6Ca	Cast	20 g/L Na ₂ SiO ₃ , 4 g/L NaOH, and 5 g/L HA (grain size of 100 nm)	5 μm	-	Hanks' solution	-	36 days in Hanks' solution Replacing every two days	Macrophotos and XRD	390
Mg1.74Zn0.55Ca		i) 15.0 g/L Na ₂ SiO ₃ ·9H ₂ O ii) 7.5 g/L C ₃ H ₇ O ₆ PCa (calcium glycerophosphate, 7.5 g/L(NaPO ₃) ₆ and 5 g/L KOH	i) 35 μm ii) 40 μm	-	1, 12, 24 and 48 h SBF	SBF	21 days in SBF No solution replacement, pH measured during the test	Longitudinal SEM images and XRD	391
Mg2.53Zn0.51Ca		10 g/L NaOH, 15 g/L Na ₂ SiO ₃ and 10 g/L Ca(H ₂ PO ₄) ₂	10 μm	-	-	-	2, 4, 8 and 12 weeks <i>in vivo</i>	Macrophotos	392
Mg1.78Zn0.51Ca		i) 15.0 g/L Na ₂ SiO ₃ ·9H ₂ O ii) 7.5 g/L C ₃ H ₇ O ₆ PCa (calcium glycerophosphate, 7.5 g/L(NaPO ₃) ₆ and 5 g/L KOH	i) 35 μm ii) 40 μm	5 min	SBF	-	18 days in SBF Replacing every two days pH measured during the test	Longitudinal SEM images and XRD	
Mg1.74Zn0.55Ca		2.85 g/L (C ₆ H ₅ O ₇) ₂ Ca ₃ ·4H ₂ O, 1.90 g/L Na ₃ PO ₄ , 5 g/L KOH, 7 g/L NH ₄ HF ₂ , 5 ml/L C ₃ H ₈ O ₃ , 5 ml/L N(CH ₂ CH ₂ OH) ₃ and 7.5 ml/L H ₂ O ₂	-	10 min	SBF	-	-	Longitudinal SEM images	378
Mg2Zn0.24Ca		0.1 mol/L Na ₃ PO ₄ ·12H ₂ O, 0.05 mol NaOH, and 0.01 mol/L Na ₂ SiO ₃ ·9H ₂ O	-	-	SBF	-	5 days in SBF pH measured during the test	Longitudinal SEM images and XRD	394

*EIS: Electrochemical Impedance Spectroscopy and PDP: Potentiodynamic Polarization

In the extruded alloy the coating was mostly detached, the corrosion layer was at least twice thicker and advanced unimpeded, given the uniform microstructure of the alloy (**Figure 75 (i)**). In comparison with the results of other published works, the onset of corrosion in the present systems is rather early. For instance, on PEO-coated Mg_{0.8}Ca the initiation of the undercoating corrosion product layer was reported after 2 weeks of immersion³¹¹, but the coatings in question were 3-4 times thicker and contained a high level of fluorine.

The EDS analysis of the corrosion products formed as a result of degradation of PEO-coated alloys is presented in **Table 47**. Overall, the corrosion product layer in all cases contain elevated levels of Ca and P (up to ~5 at. %). Importantly, these are far below the Ca and P content (~10-12 at. % and ~5-6 at.%, respectively) in the undercoating corrosion products for fluoride-based PEO-3F systems (**Figure 59, Table 39**), suggesting that PEO-S-3Ca coating system is much less permeable and possesses better barrier properties. The still attached coatings also contain far greater amount of these species (~11-13 at. %) than the as-received coatings (~2-4 at. %, see Section 5.2.2). This is undoubtedly the result of precipitation of calcium phosphates from the inorganic α -MEM solution. Several works have reported that the main corrosion products formed on Mg-Zn-Ca alloys are constituted by Mg(OH)₂, Ca₁₀(PO₄)₆(OH)₂, (Ca,Mg)₃(PO₄)₂, Mg(HCO₃)(OH)·2H₂O or MgCO₃·3H₂O^{390,395,392}.

Additionally, Zn was found both in inner and outer part of the corrosion products (e.g. points 2, 4, 5, 6, **Table 41** and **Table 42**) due to the diffusion of Zn from the bulk material. It may participate in formation of amorphous ZnPO₄, ZnCO₃ and/or Zn(OH)₂^{29,30}. Ca/P ratio in the attached parts of the coatings and the corrosion products was elevated and ranged from 0.86 to 4.00, and were mostly non-stoichiometric with respect to 1.5 or 1.67 ratio of apatite and hydroxyapatite. Elevated content and lixiviation of Ca and P are expected to contribute to the growth of the bone, improving the biocompatibility of the implant in the human body^{396,397}.

Several important factors corresponding to the corrosion mechanisms were illustrated by post-corrosion characterization: a) the coatings appeared well adhered after 120 h of immersion for the cast alloys while those on extruded alloy were completely gone; b) the presence of cracks is evident in the corrosion product layer and c) a few Zn-rich intermetallics are embedded into the corrosion products on cast alloys (**Figure 75 (g, h)**).

Table 47. Composition (at. %) of the PEO-S-3Ca coatings on all MgZn_xCa_y alloys 120 h of immersion at locations as per **Figure 75**.

Alloy	Point	O	Na	Mg	Si	P	Cl	K	Ca	Zn	Ca/P
MgZnCa	1	55.5	0.4	12.8	4.1	12.1	0.5	0.2	13.5	0.4	1.12
	2	54.3	0.7	30.6	0.3	5.2	1.0	0.2	5.6	1.4	1.08
	3	47.8	0.3	48.0	0.0	0.3	0.3	0.0	1.2	0.4	4.00
Mg3Zn0.4Ca	4	56.5	1.1	11.5	1.9	13.4	0.4	0.0	11.5	2.6	0.86
	5	71.1	0.7	22.0	0.0	1.7	0.3	0.1	2.1	1.4	1.24
	6	57.8	0.7	30.0	0.3	3.5	0.7	0.2	3.5	2.6	1.00
	7	51.6	0.3	46.0	0.0	0.3	0.1	0.0	0.3	0.7	1.00
Mg0.5Zn0.2Ca	8	60.2	0.9	12.0	0.0	9.7	0.7	0.0	13.8	0.7	1.42
	9	56.0	0.7	17.5	0.0	10.0	1.2	0.0	12.1	0.9	1.21
	10	48.5	1.1	17.7	0.2	11.2	1.1	0.4	17.7	1.1	1.58
	11	70.1	0.8	19.2	0.0	2.5	0.3	0.0	5.1	0.5	2.04

According to this, the degradation of all PEO-coated MgZn_xCa_y alloys in inorganic α -MEM proceeds in accordance with the following mechanism: i) the corrosive species from the media penetrate through the microdefects in the outer porous layer, leading to progressive hydration of the coating and degradation of the inner barrier layer; ii) the corrosion products react with the species from the media leading to the precipitation of Ca-P based compounds which inhibit the degradation of MgZn_xCa_y alloys; iii) the Zn- and impurity-rich intermetallics that oxidize very slowly form galvanic couples with the matrix and accelerate the corrosion process of the adjacent matrix grains, as discussed in Chapter IV; iv) the coating prevents the detachment of the corrosion product layer providing an additional protection; v) the cracked undercoating corrosion products layer is a crevice between the adhered PEO coating and the substrate and accelerates the corrosion rate, as discussed in Section 5.1.2.3.2 of the present Chapter.

Figure 76 illustrates the comparison of the corrected hydrogen volume for all studied PEO coatings and alloys systems after 120 h of immersion in inorganic α -MEM. Comparing the different PEO electrolytes, it can be seen that PEO-8F/Mg0.5Zn0.2Ca system presents the lowest corrected hydrogen volume compared to the rest of the coatings and alloys. This phenomenon may be due to the fact that the coating is a) thick enough to protect against corrosion; b) forms a barrier layer uncompromised by inclusion of large cathodic intermetallics due to a uniform microstructure of the alloy. However, although this coating provides greater corrosion protection, its high fluoride content may present premature cell death during the first days of culture. Fluoride-free coatings on the

extruded Mg_{0.5}Zn_{0.2}Ca alloy presented 3-5 times lower protection compared with all the other systems, which is related to the lack of barrier layer resistance, as revealed by EIS.

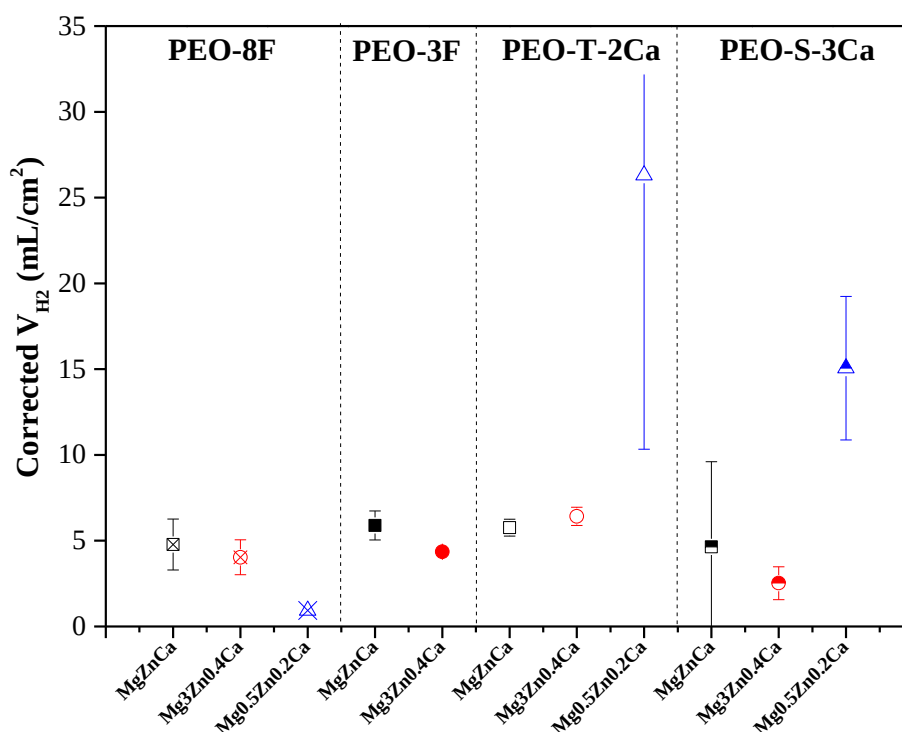


Figure 76. Comparative of corrected hydrogen volume for all substrates obtained following 120 h of immersion in inorganic α -MEM at 37°C under CO₂ flow control. PEO treatment times were: PEO-8F – 420 s, PEO-3F – 300 s, PEO-T-2Ca – 600 s, PEO-S-3Ca – 300 s.

All systems based on fluoride-free coatings and cast alloys presented a behavior comparable to that of fluoride-based systems, with the exception of PEO-S-3Ca/Mg₃Zn_{0.4}Ca, whose degradation rate, 2.09 ± 1.02 mm/year, according to the corrected H₂ volume, was about two times lower which is attributed to its lower permeability and superior barrier properties. Nevertheless, these results suggest that stand-alone PEO coatings formed on these alloys do not have a sufficient protective effect due to the high impurity content in the secondary phases of the alloys, which lead to the acceleration of the corrosion process at the coating/substrate interface incrustated with intermetallics. For this reason, in order to improve the corrosion protection of these PEO systems, further studies will focus on the design and development of hybrid hierarchical systems consisting of ceramic and polymeric layers. Considering the corrosion screening findings of this Chapter, the PEO-S-3Ca coating formed at 300 s on Mg₃Zn_{0.4}Ca alloy was selected for the design and development of hybrid systems and their subsequent functionalisation.

5.3 Conclusions of Chapter V

I. Fluoride-containing PEO coatings

Cast Mg0.6Ca and Mg0.6Ca2Ag alloys

- PEO-8F coatings of 32-35 μm thicknesses, containing bioactive calcium fluoride, magnesium fluoride and fluorapatite phases, were generated on Mg0.6Ca and Mg0.6Ca2Ag alloys for biomedical implant applications.
- The hydrogen evolution rate for PEO-8F coatings on Mg0.6Ca 3D prototypes evaluated during long term immersion tests (up to 60 days) was 3.86 mL/cm² week during the first month of immersion in SBF and 1.75 mL/cm² week during the second month of immersion. The uncoated alloy generated two times more hydrogen in the first month of the test.
- The PEO coatings liberate fluoride ions during immersion in 0.9 % NaCl. The fluoride release is increased throughout 24 h of immersion time for Mg0.6Ca/PEO-8F. The fluoride reserves of the coating have not been completely depleted in the course of 24 h immersion.

Cast MgZnCa and Mg3Zn0.4Ca and extruded Mg0.5Zn0.2Ca alloys

- PEO coatings containing Ca, P and Si with high (PEO-8F) and low (PEO-3F) F containing electrolytes were developed on MgZn_xCa_y alloys. PEO-8F coatings exhibited thickness of 30-40 μm while PEO-3F coatings showed a linear coating growth kinetics yielding ~14-15 μm -thick coatings in 900 s of treatment.
- All coatings are comprised by magnesium oxide, magnesium fluoride and fluorapatite phases. In the case of PEO-3F, calcium and magnesium silicates are formed and phosphorous is incorporated into the amorphous phase.
- PEO-8F and PEO-3F morphologies on cast alloys are strongly influenced by Zn-rich intermetallic particles that are embedded in the coatings in a partially oxidized state and cause the formation of large voids nearby the Zn-rich sites.
- The presence of amino acids and other organic components of complete α -MEM has no influence on Mg alloy/PEO system corrosion resistance, due to their adsorption on the coating surface and inability to migrate towards the coating/substrate interface. Therefore, the inorganic α -MEM can be regarded as an inexpensive, reliable and easy maintenance corrosion screening medium for Mg alloy/PEO systems. A 24 h

immersion is recommended for EIS screening of PEO coatings on alloys with a strong micro-galvanic coupling.

- PEO-8F coatings present insignificantly lower degradation rate than PEO-3F coatings on cast alloys, which is associated with localized corrosion attack.
- The easy permeability of the PEO-3F coatings and insufficient stability of the barrier layer (deficiency of MgF_2) formed in low-fluoride electrolyte allows the early initiation of undercoating corrosion.
- A more aggressive corrosive environment and enhanced micro-galvanic couple effect between the Zn-rich intermetallic particles at the coating/substrate interface and the Mg matrix take place in the undercoating crevice, which leads to faster degradation, often in form of localized corrosion of the coated alloys, compared to non-coated substrates. High permeability and hydration of the coatings results in the enhanced lixiviation of F^- ($\sim 70\text{-}150\ \mu\text{g}/\text{cm}^2$) from the coatings that can reach the cytotoxicity levels for a variety of cells.

II. Fluoride-free PEO coatings

Cast MgZnCa and Mg₃Zn_{0.4}Ca and extruded Mg_{0.5}Zn_{0.2}Ca alloys

- Ca-P containing coatings formed in transparent and suspension electrolytes were developed on MgZn_xCa_y alloys. The thickness and roughness of PEO-T-2Ca and PEO-S-3Ca coatings increase linearly with the treatment time in the range of 5-18 μm for all studied alloys.
- PEO-T-2Ca and PEO-S-3Ca coatings formed at all treatment times were mainly constituted by MgO and Mg_2SiO_4 phases. Additionally, PEO-T-2Ca and PEO-S-3Ca coatings presented an amorphous state corresponding to Ca and P species.
- Two-step corrosion screening using EIS measurements after 1 h of immersion and 120 h-long H_2 evolution test confirmed that coatings developed in fluoride-free electrolytes can offer corrosion protection for cast alloys equal to that provided by the fluoride-containing coatings, thus eliminating the risk of fluoride-related cytotoxicity.
- The developed thin stand-alone PEO coatings on MgZn_xCa_y alloys are susceptible to easy permeability by the electrolyte and subsequent breach of the barrier layer, which leads to the formation of crevice between the PEO layer and substrate filled by undercoating corrosion products.
- The presence of Zn-rich impurities containing noble intermetallics at the coating/substrate interface form microgalvanic couples and are responsible for driving

the undercoating corrosion at a rate that may exceed the degradation rate of the bare substrate, particularly in a medium where pH is controlled to remain constant at 7.4. This behavior takes at least 24 h of immersion to reveal itself in H₂ evolution test.

- The system identified as the most corrosion resistant, Mg₃Zn_{0.4}Ca/PEO-S-3Ca-300 s, exhibits lesser permeability than the other studied systems due to superior barrier properties. It does not provide a sufficiently long delay before the onset of corrosion and generates $2.52 \pm 1.0 \text{ mL/cm}^2$ ($2.09 \pm 1.02 \text{ mm/year}$) of H₂ in 120 h, which may lead to the development of a hostile environment for cells.
- PEO-coated cast or extruded MgZn_xCa_y alloys require an additional protection strategy in order to reduce the degradation rate of the system.

6. CHAPTER VI – Hybrid PEO/Polymer coatings

As stated in the Introduction (Section 1.6), the infiltration of a PEO layer with a polymer can improve the corrosion resistance of a PEO/Mg alloy system. In addition, there must be a proper control of the surface structure of the polymer top-layer at the micrometre scale along with the chemical composition, as these play a critical role in the bioactivity of the material¹⁰⁰. However, only few reports on hybrid PEO/polymer coating systems for Mg exist, and those focus on Mg-Al alloys and only planar polymer top-coats 206,207,209,210.

On that basis, Chapter VI aims to design and develop hierarchical porous polymer top-coats (PCL or PLA) over Mg3Zn0.4Ca/PEO-S-3Ca system selected in the previous section. In particular, the optimization of the polymer top-coats using BF and dip coating techniques and the analysis of the corrosion behavior of the full hybrid hierarchical coating (HHC) system are carried out. The structure of this chapter is divided in three main sections: (i) the optimization of the experimental parameters to obtain controlled homogeneous polymer film thickness, pore size and surface porosity. (ii) Detailed characterization of the top-coat layer morphology, roughness and wettability. (iii) The corrosion behaviour evaluation of the hybrid hierarchical coating system by hydrogen evolution test. **Figure 77** shows the graphical abstract of Chapter VI.

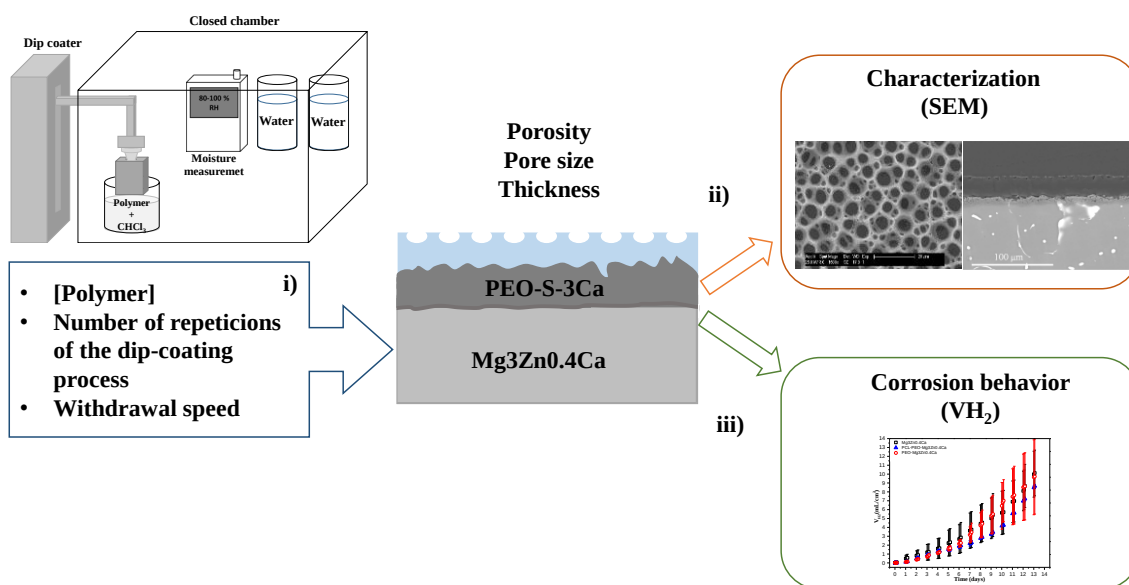


Figure 77. General scheme of Chapter VI.

6.1 Strategy for the preparation of polymer-coated PEO treated Mg alloys: towards hybrid hierarchical metal/ceramic/polymer biomaterials

The preparation of the hybrid polymeric-ceramic materials was carried out following a procedure based on two consecutive steps. First, the Mg₃Zn_{0.4}Ca alloy specimens (10 × 10 × 3 mm) were treated by PEO in order to obtain ceramic porous surface layer with controlled thickness. After this step, the PEO-treated sample was coated using BF method in order to form a porous polymer top-coat layer. The deposition of the PLA and PCL layer was carried out using dip coating technique by the immersion of the sample into polymer solution (using a volatile solvent) and upon taking out of the solution, the specimen was allowed to dry in a moist atmosphere. The schematic of dip coating process is shown in **Figure 78**. Interestingly, during the evaporation process, the liquid-air interface cools down to temperatures below 0°C. In these conditions, the water vapour start to condense on the evaporating surface. This condensation occurs during the evaporation process, thus, finally forming micrometre size water droplets. The complete evaporation of the solvent and the water droplets finally leads to the formation of a porous surface. The experimental conditions are critical to modulate the surface porosity. In this study, the temperature was maintained around 25°C and the relative humidity (RH) was 98-99 %. However, other parameters such as the polymer concentration or the number of repetitions of the dip coating process will be explored in order to optimize the pore formation.

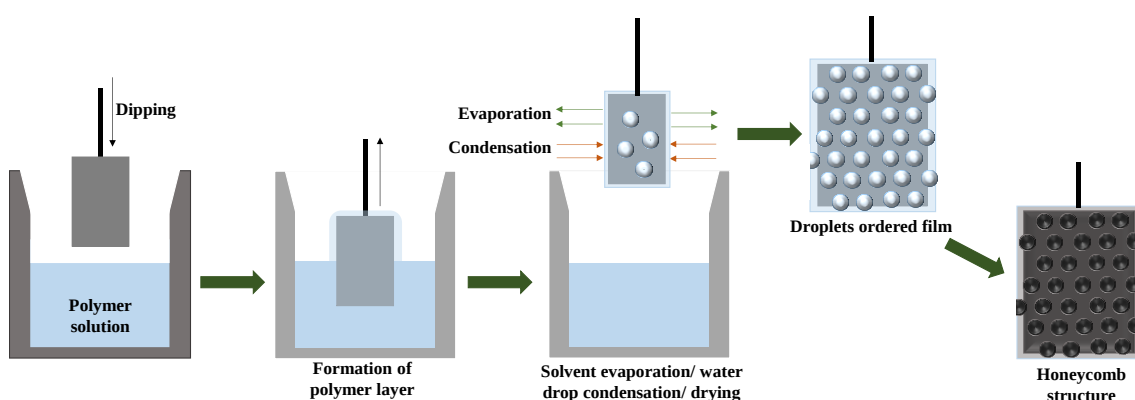


Figure 78. Scheme of combination of dip coating technology and breath figures method adapted from ^{398,399}.

6.2 Polymer Breath Figures: analysis of the pore formation and coating thickness

In order to optimize the generation of a homogeneous polymer coating with a surface porous structure, the experimental parameters of the dip coating and BF technique were varied. In particular, the polymer concentration, withdrawal speed and number of repetitions of the dip coating process in terms of porosity, pore size and thickness were the experimental parameters selected for the optimization.

First of all, the withdrawal speed during the dip coating process required to form a homogeneous coating was explored. According to this, different withdrawal speeds were employed varying from 0.2 to 2 mm/s. In this first series of experiments, 3D printed PLA pieces (40 x 5 x 2 mm) were used as a support substrate. The polymer concentration was set to 25 mg/mL and a single repetition of the dip coating process was carried out. **Figure 79** illustrates the SEM images of the generated surfaces, the analysis of pore fraction area and the average of pores size. Pores are formed at 1 mm/s suggesting that a high withdrawal speed leads to a higher meniscus in comparison to 0.2 mm/s. This together with a rapid evaporation of the solvent leads to the formation of pores on the PLA surface. An increase of the withdrawal speed leads to the formation of microporous surfaces and the formation of multilayers of pores. This phenomena is due to the combination of 3D ordering in BF and rapid solidification during withdrawal speed as it is observed in **Figure 80**. The condensed water droplets sink from the top layer, which is coated by new nucleated water droplets. However, the films are solidified faster than by the direct evaporation method; the pores produced in the top layer are less susceptible to sink before the films solidify completely⁴⁰⁰. Excellent results are obtained for 1.5 and 2 mm/s being similar in both cases. For this reason, 2 mm/s is selected for the next step.

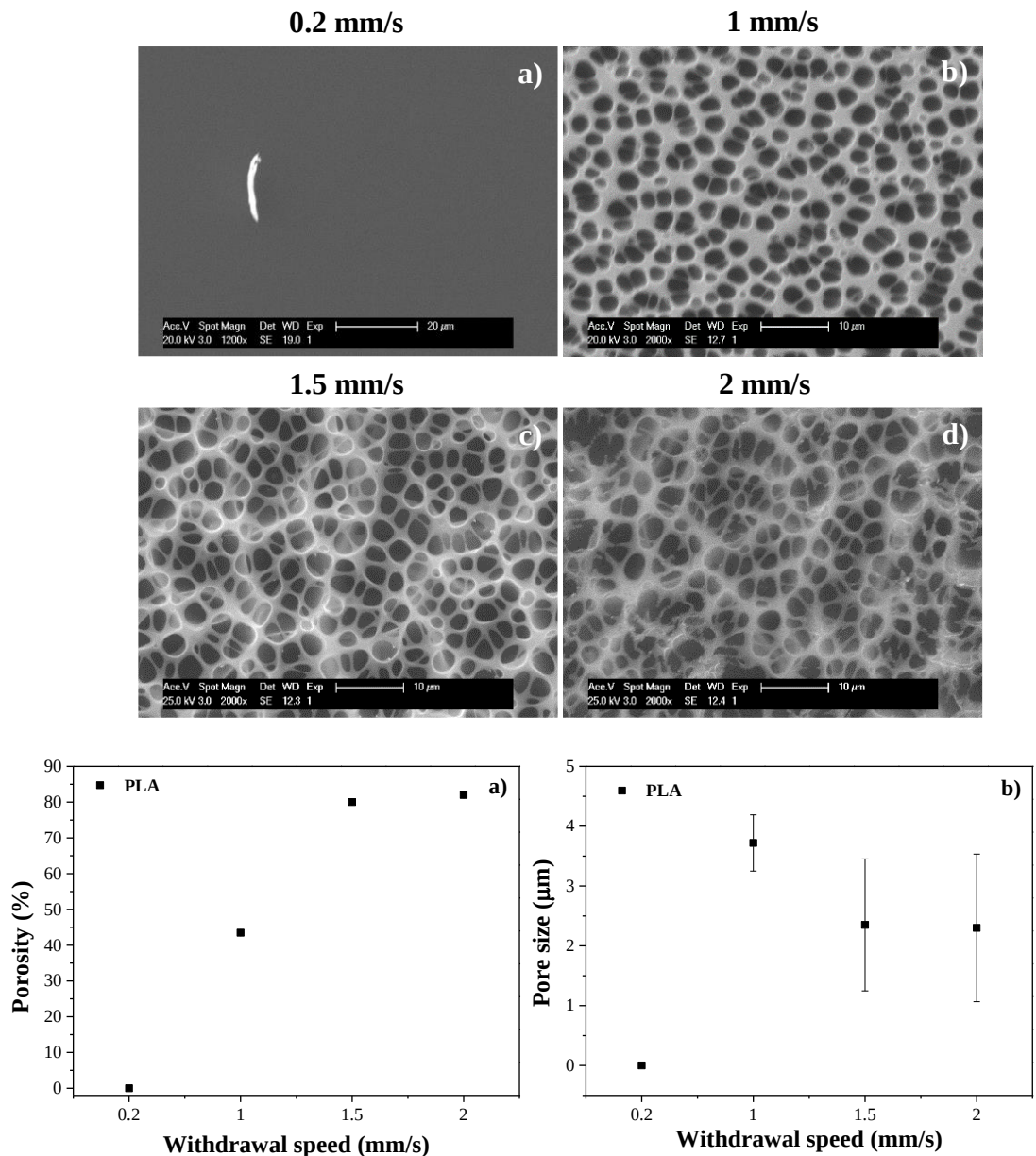


Figure 79. Effect of the withdrawal speed of dip coating in 3D PLA printed pieces on (a-d) PLA film morphology (e) porosity and (f) pore size.

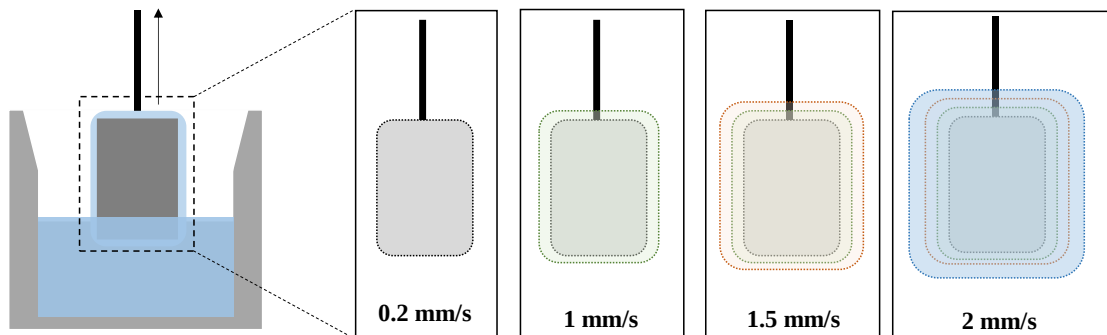


Figure 80. Scheme of the formation of PLA layer with increasing polymer thickness layer when the withdrawal speed is increased.

This preliminary set of experiments served to confirm that the combination of dip coating and BFs can be used to form porous surfaces and to establish the employed optimum withdrawal speed range for the polymer concentration.

The second series of tests were carried out on Mg alloys, more precisely, we employed Mg₃Zn_{0.4}Ca/PEO-S-3Ca specimen as substrate. For these series of experiments, both PLA and PCL polymers are employed. **Figure 81** shows the top view images of PLA and PCL, the analysis of the pore fraction area and the average of pores size at different concentrations (25, 50, 75 and 100 mg/mL) using a withdrawal speed of 2 mm/s and 1 cycle of dip coating process.

The polymer coatings prepared at lower concentrations (25 and 50 mg/mL) formed planar and heterogeneous surfaces at locations where the polymer layer did not cover all the pores produced in the PEO treatment. In contrast, the use of higher polymer concentrations for the dip coating process (75 and 100 mg/mL) covered all the PEO coating pores and formed a homogeneous polymer layer. Particularly interesting are the cases (c) and (d) presented in **Figure 81**, in these two cases porous polymer surfaces were obtained. PLA coating prepared using polymer concentrations of 100 mg/mL formed a homogeneous microporous surface with average pore size of $1.5 \pm 0.3 \mu\text{m}$, while PCL at 75 mg/mL formed a heterogeneous morphology with a pore size of $10.9 \pm 0.2 \mu\text{m}$. It is worth mentioning that higher polymer concentration ($\geq 100 \text{ mg/L}$) leads to a lower ability to form pores on the surface of the sample. The low amount of solvent to be evaporated does not allow for condensation of enough amount of water vapour to form the micropores.

In addition to the top-views, in order to investigate the formation of micropores at the polymer surface, cross-sections were analysed to address the layer thickness of the polymer layer (bottom panel in **Figure 81** and graphs enclosed in **Figure 83**). As expected, the thickness of the PLA layer formed in the range of the explored concentrations gradually increased with the polymer concentration from $2.9 \pm 0.5 \mu\text{m}$ for 25 mg/mL to $36.8 \pm 1.1 \mu\text{m}$ for 100 mg/mL (**Figure 83**).

In the case of PCL, the polymer coatings prepared using 25 mg/mL polymer solutions did not cover the PEO treated surface homogeneously, while above 50 mg/mL a homogeneous polymer layer is formed with a $12.6 \pm 2.3 \mu\text{m}$ for 75 mg/mL and $28.4 \pm 1.3 \mu\text{m}$ for 100 mg/mL. In both cases, the thickness of the polymer coating gradually

increased with the polymer concentration (**Figure 82**), being slightly higher for PLA than for PCL coatings in all cases. Based on these findings, the polymer concentration of 75 mg/mL was selected as the optimal concentration in order to evaluate the role of the number of the cycles of dip coating process on the surface morphology.

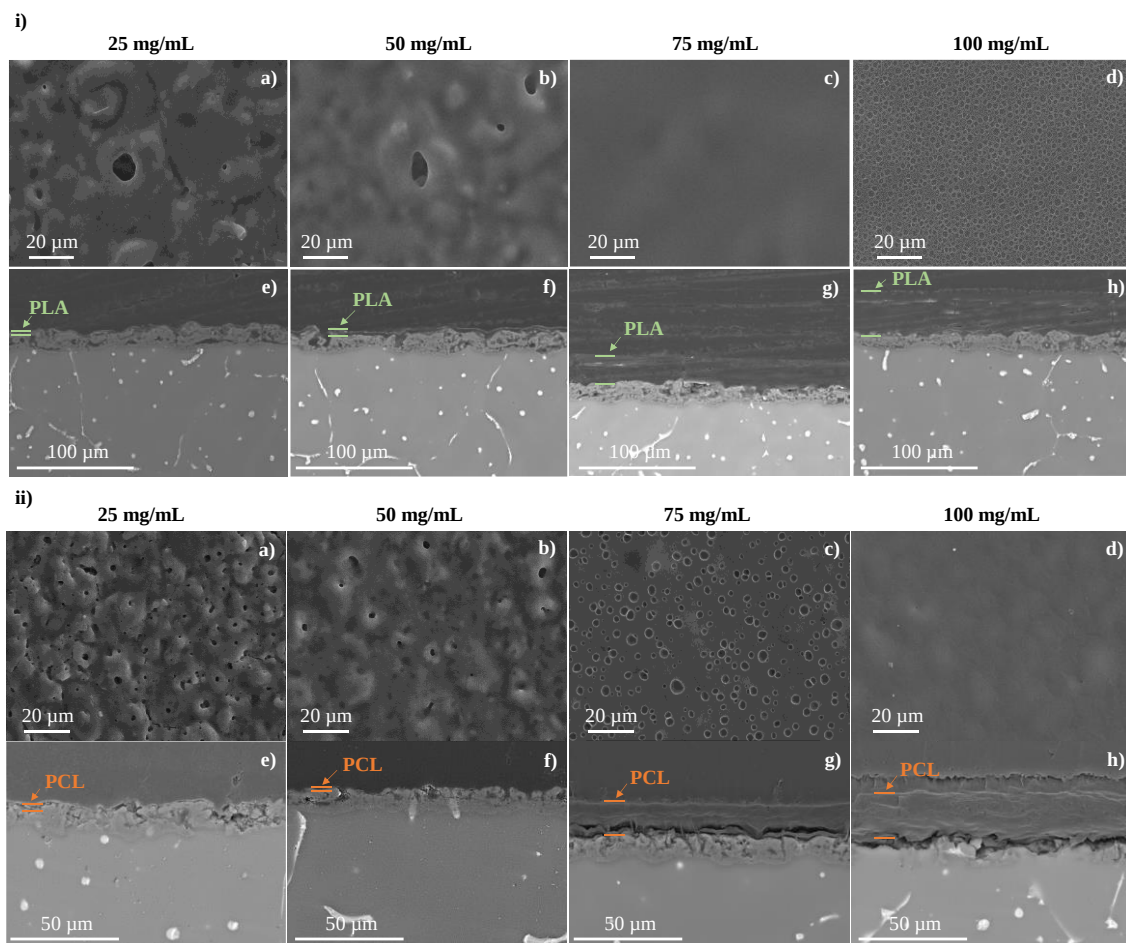


Figure 81. (a-d) Secondary electron top view image and (e-h) backscattered electron secondary electron cross-section images of (i) PLA and (ii) PCL on PEO-coated samples at 25, 50, 75 and 100 mg/mL.

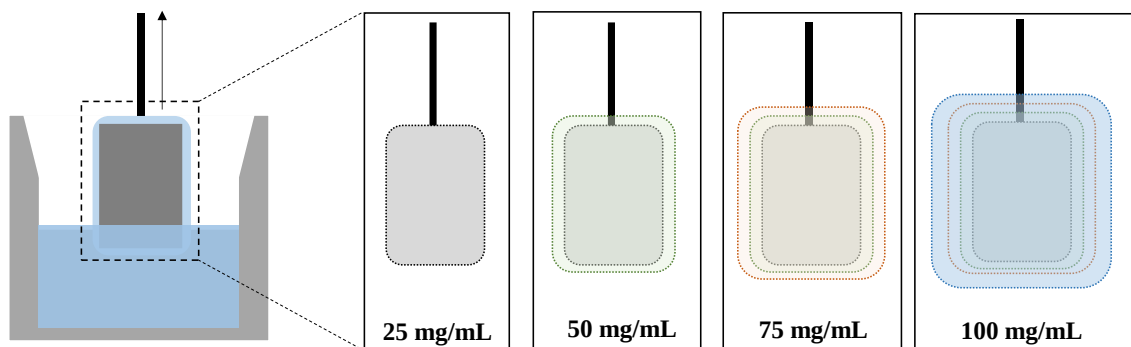


Figure 82. Scheme of the formation of polymer layer with variable thickness by increasing the polymer concentration.

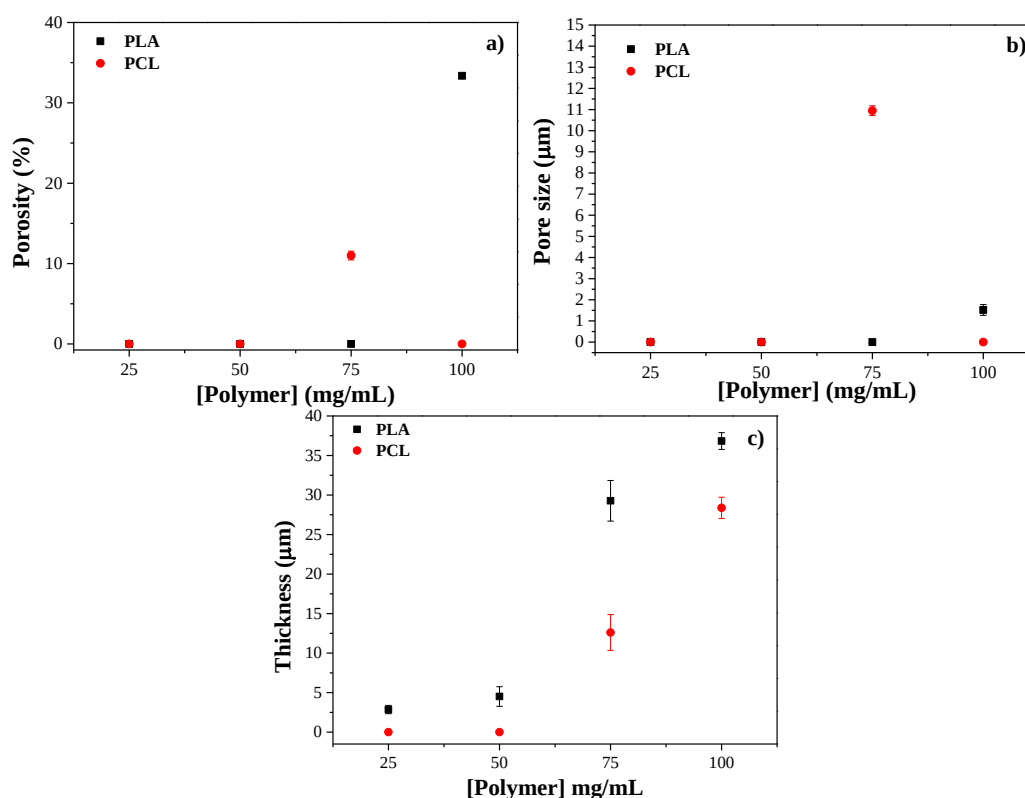


Figure 83. Variation of (a) pore fraction area, (b) average pores size and (c) thickness at different concentrations of PLA and PCL.

Figure 84 shows the SEM images of the polymer coatings prepared using either PLA or PCL but varying the number of dipping cycles (1, 2 and 3). The polymer concentrations (using 75 mg/mL) and the withdrawal speed (2 mm/s) were maintained constant for all the experiments. As evidenced in **Figure 84** for the coatings prepared using PLA, 2 and even 3 cycles lead to rather homogeneous surfaces with small pore sizes in the range from 1.8 ± 0.5 to 2.8 ± 1.0 μm. Both routes create a multi-layered porous structure due to the sinking of condensed water droplets while a planar surface (without any pore) is formed when a single dip coating process is carried out. In the case of the coatings prepared using PCL polymeric solutions, heterogeneous morphologies are observed in the first dip coating step. However, homogeneous porous polymer coatings were formed upon repetition of the dip coating process 2 and 3 times. Interestingly, an increase of the number of cycles produced surfaces with larger average pore size in the range of 1.4 ± 0.6 μm for 1 cycle and 11.1 ± 2.2 μm for 3 cycles (**Figure 85**).

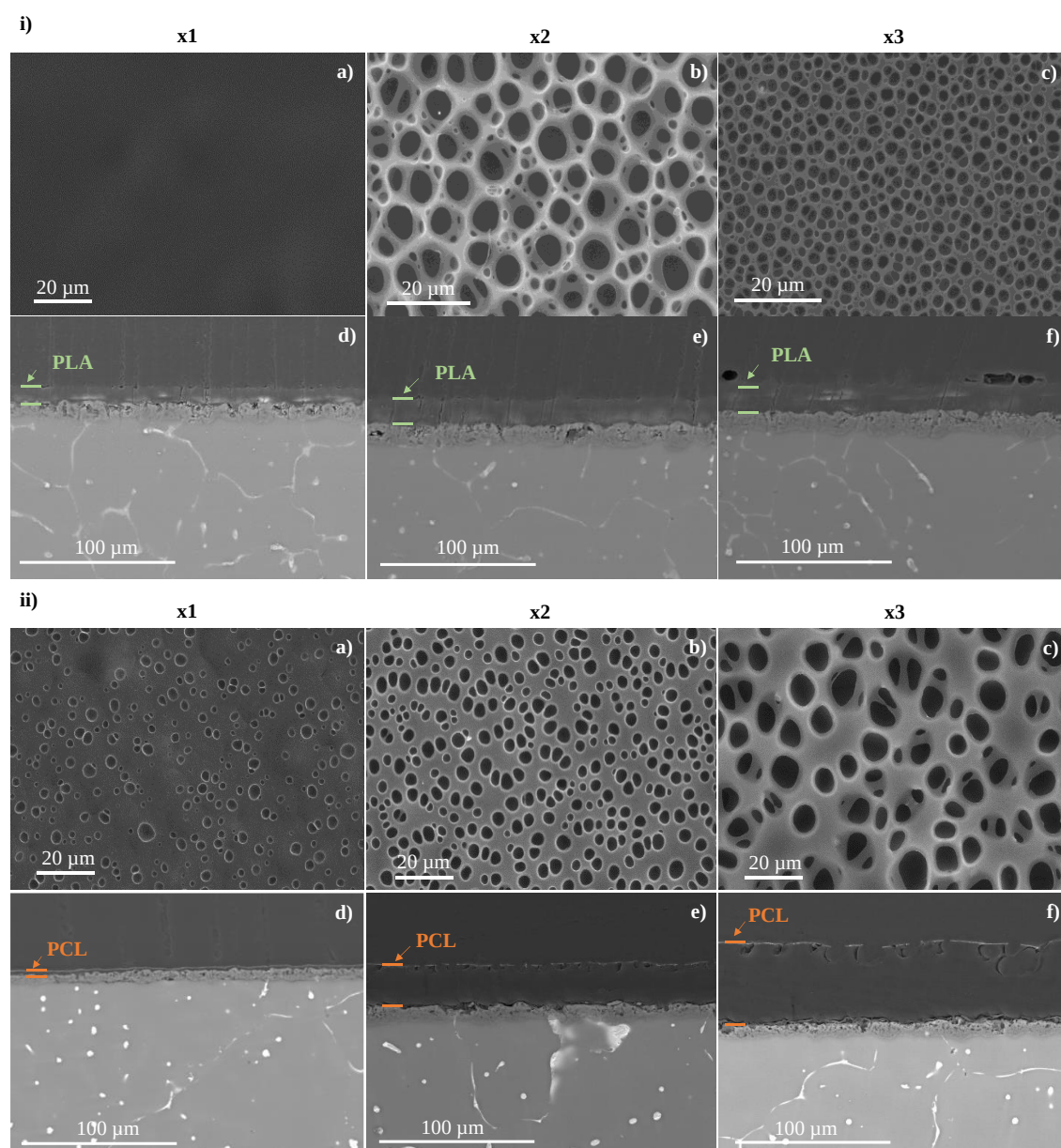


Figure 84. (a-c) Secondary electron top view and (d-f) backscattered electron cross-section images of (i) PLA and (ii) PCL on PEO-coated samples at 1, 2 and 3 cycles at 75 mg/mL of polymer concentration and 2 mm/s.

As a result, the pore size of both PLA-based and PCL-based polymer coatings increased with the number of cycles and, thus, the pore size could be, at least to some extent, modulated. This phenomenon is due to the fact that condensed water droplets sink from the upper layer, and this upper layer is covered by new nucleated water droplets. In addition, the films solidify slowly, so it is more likely that the droplets formed in the top layer do not sink before the films solidify completely, forming larger pores. In the case of the three cycles for PCL, there is a 3D structure because of the combination of 3D BF sorting and rapid solidification during the retreat speed rate, as it is observed in **Figure 86**.

According to the cross-sectional profiles examined to measure the thickness of the polymer layer, a homogeneous polymer coating was formed for both polymers for all the cases independently of the number of the cycles. The thickness increased linearly (from $29.3 \pm 2.6 \mu\text{m}$ for 1 cycle to $57.8 \pm 0.3 \mu\text{m}$ for 3 cycles and 12.6 ± 0.2 for 1 cycle to $53.3 \pm 2.2 \mu\text{m}$ for 3 cycles for PLA and PCL, respectively) with the increase of the number of cycles (**Figure 85**). The difference between the two can be associated to slight viscosity differences between the polymeric solutions, being higher for PLA. In view of the results observed in **Figure 85**, 2 dip coating cycles were selected for the rest of the experiments focussed on the optimization of the withdrawal speed during the dip coating process.

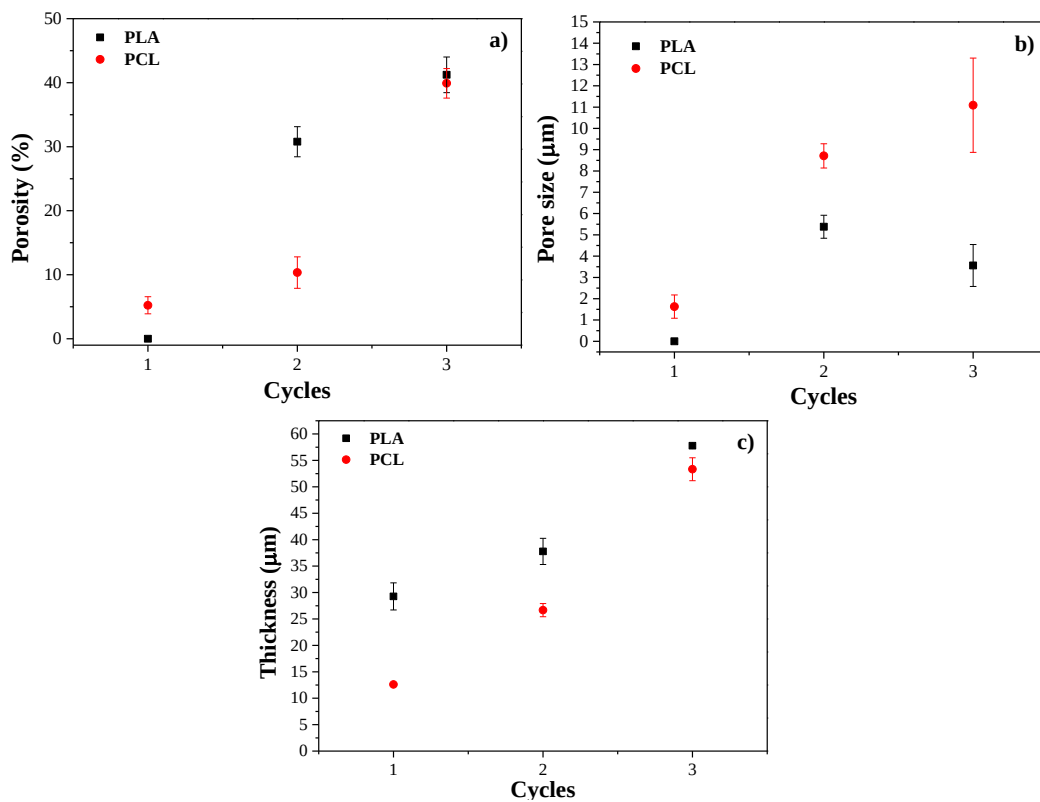


Figure 85. Variation of (a) pore fraction area, (b) average pore size and (c) thickness at different number of cycles.

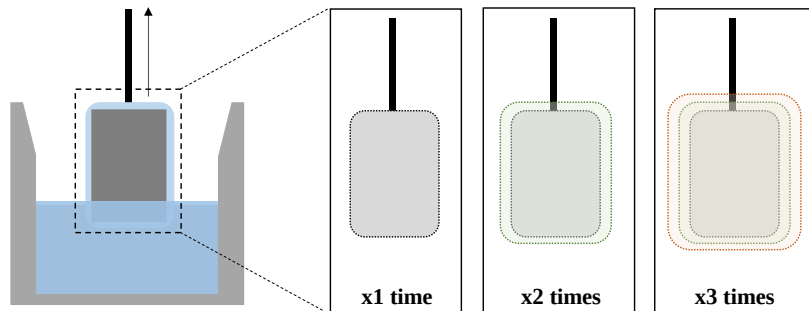


Figure 86. Scheme of the formation of polymer layer when it is varied the number of dip coating cycles repeated up to 3 times.

In **Figure 87** the top view and the cross-sectional profiles of the polymer coatings obtained by varying the dip coating withdrawal speed (0.5, 1 and 2 mm/s) are depicted. For all the cases, 75 mg/mL concentration and 2 cycles were used. It can be appreciated that the increase of the withdrawal speed for PLA specimens leads to the formation of homogeneous surfaces with small pore size (in the range of $1.9 \pm 0.8 \mu\text{m}$ for 1 mm/s and $4.4 \pm 1.0 \mu\text{m}$ for 2 mm/s), while a slightly planar surface is formed for the lowest withdrawal speed (**Figure 87**). For 2 mm/s, the creation of multilayer pore structure by the sinking of condensed water droplets is observed. On the contrary, a homogeneous distribution surface is formed for PCL specimens at 1 and 2 mm/s, while a planar surface is formed for 0.5 mm/s. In general terms, the increase of the withdrawal speed leads to the formation of microporous and multilayer surfaces for both PLA- and PCL- based polymer coating. An increase in the withdrawal speed leads to faster solidification by the rapid evaporation of the solvent which avoids the sinking of water droplets, and thus their growth. Higher speeds result in smaller pores, especially in the shallower layers, as the deeper layers have already solidified as it is illustrated in **Figure 89**.

As expected, the thickness of PLA and PCL top-coats increase with the increase of the withdrawal speed from $2.6 \pm 0.3 \mu\text{m}$ for PCL at 0.5 mm/s up to $37.8 \pm 2.5 \mu\text{m}$ for PLA at 2 mm/s (**Figure 89**). A homogeneous thin layer is formed for PLA and PCL at 0.5 and 1 mm/s while a homogeneous thicker polymer film is generated at 2 mm/s, being thicker for PLA in all cases. Higher withdrawal speeds (as schematically shown in **Figure 88**) increased the amount of polymer solution deposited onto the surface of the substrate and thus produced thicker films. For this reason, 2 mm/s is selected as optimal parameter of the process.

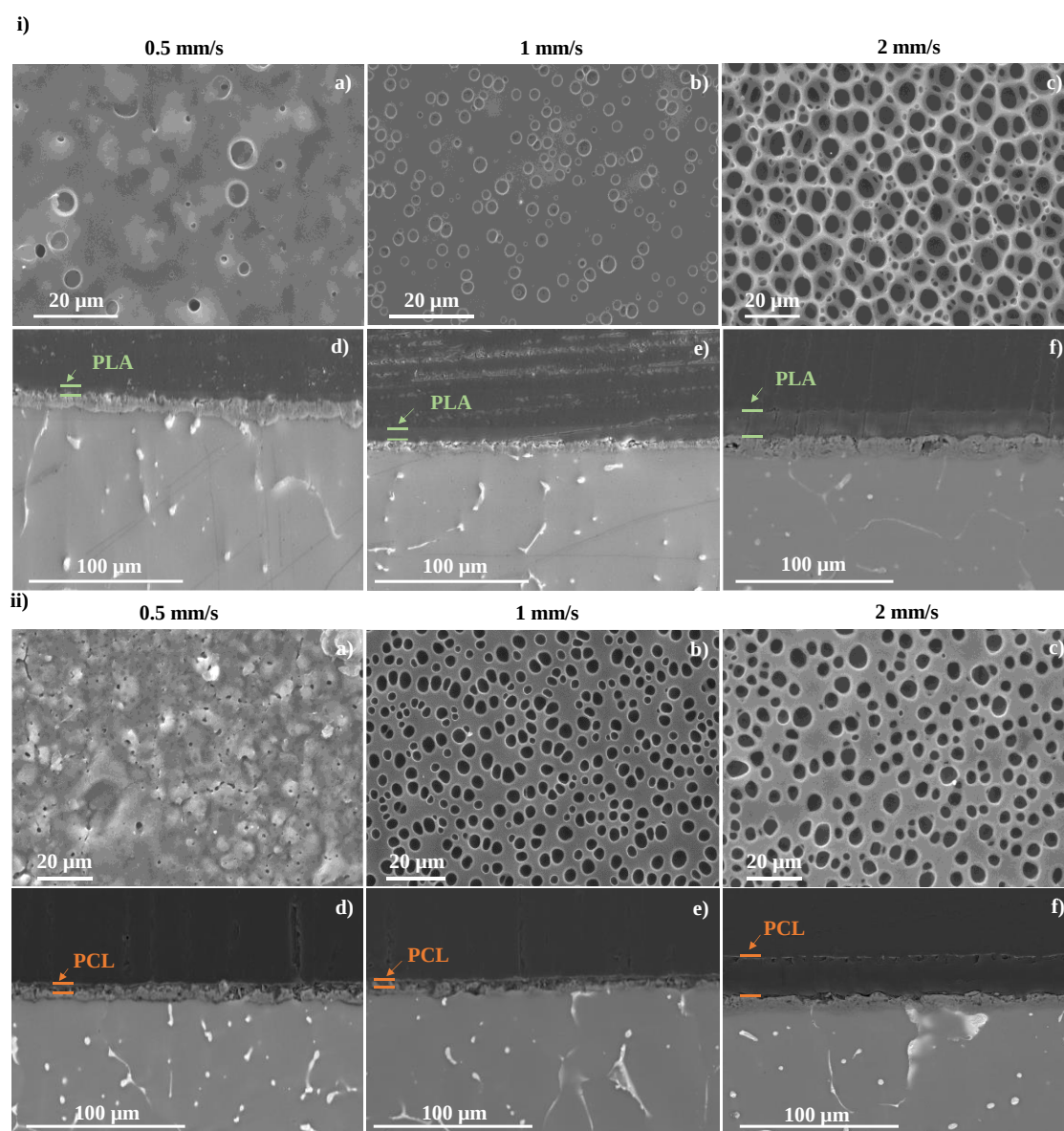


Figure 87. (a-c) Secondary electron top view and (d-f) backscattered electron cross-section images of (i) PLA and (ii) PCL on PEO-coated samples at withdrawal speed of 0.5, 1 and 2 mm/s at 75 mg/mL of polymer concentration and 2 cycles.

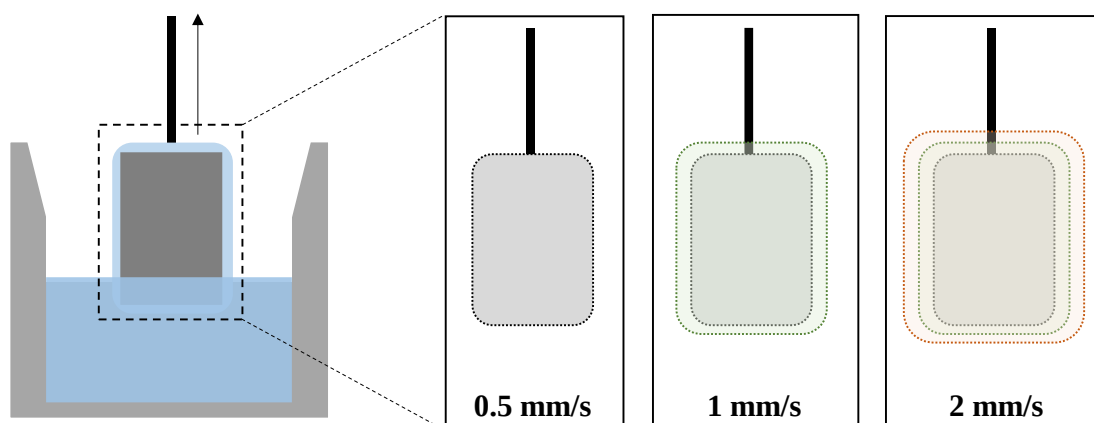


Figure 88. Scheme of the formation of polymer layer with increasing thickness by varying the withdrawal speed.

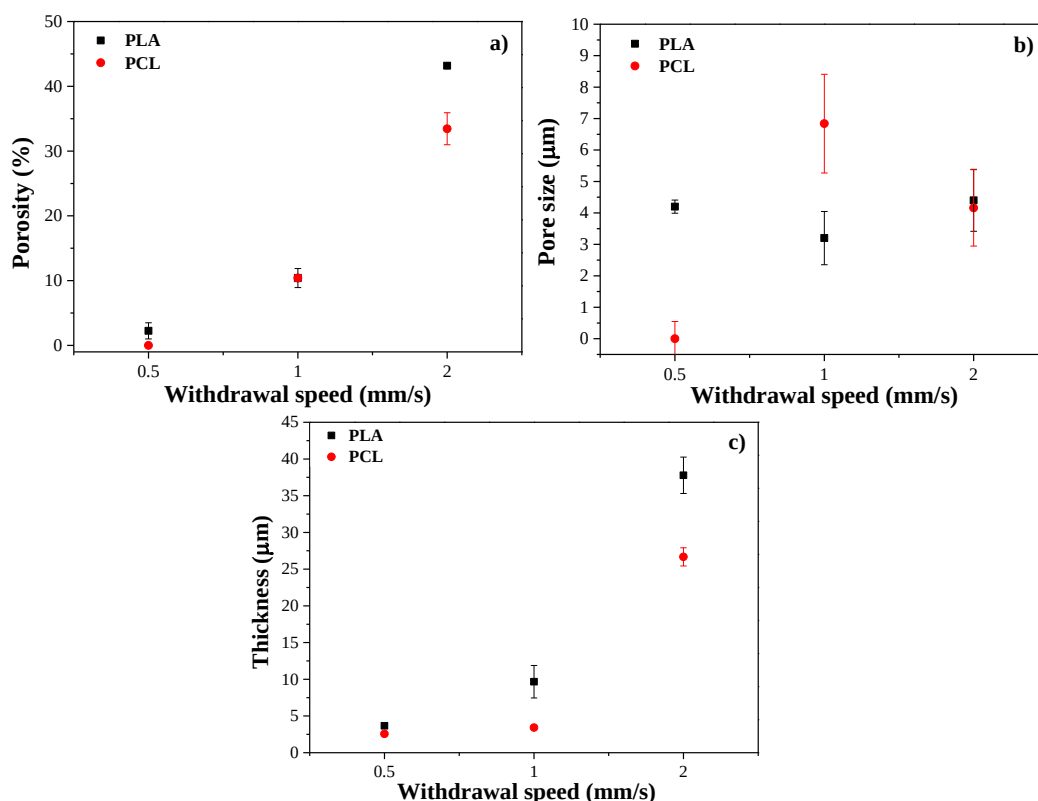


Figure 89. Variation of (a) pore fraction area, (b) average pore size and (c) thickness at different withdrawal speed.

In summary, as a result of the optimization process, samples prepared using 75 mg/mL, 2 cycles and 2 mm/s withdrawal speed were selected in order to evaluate the resultant corrosion behavior for both PLA and PCL-coated samples.

6.3 Evaluation of the corrosion properties of the hybrid hierarchical PEO/polymer coatings

Prior to the evaluation of the corrosion performance of the hybrid materials, the plan view and cross-sectional coating morphologies, the roughness (S_a) and the surface wettability of PLA/PEO-S-3Ca and PCL/PEO-S-3Ca-coated on Mg3Zn0.4Ca alloy were characterized (**Table 48**).

The cross-section images (**Figure 90**) of PLA-based and PCL-based hybrid coatings indicate the formation of homogeneous coatings with 28 ± 0.4 and 36 ± 1.2 μm thicknesses. These coatings present two layers structure: the first layer, in contact with the alloy substrate is constituted by a PEO coating which provides protection against the corrosion for the whole system. The second, polymer outer part presents a microporous structure. This porous structure, as it has been reported before, allows for an excellent interaction with the cells. For instance, Santos-Coquillat *et al.* developed a

microstructured PCL top-coat with interconnected porosity over a Ca-P-Si based PEO coating on Mg0.8Ca alloy using a BF method without dip coating technique. Interestingly, the PCL top-coat formed an open, fully permeable porous (2-30 μm pore size) scaffold that allowed excellent cell proliferation after 96 h where part of the cells were trapped within the pores ²⁰⁵.

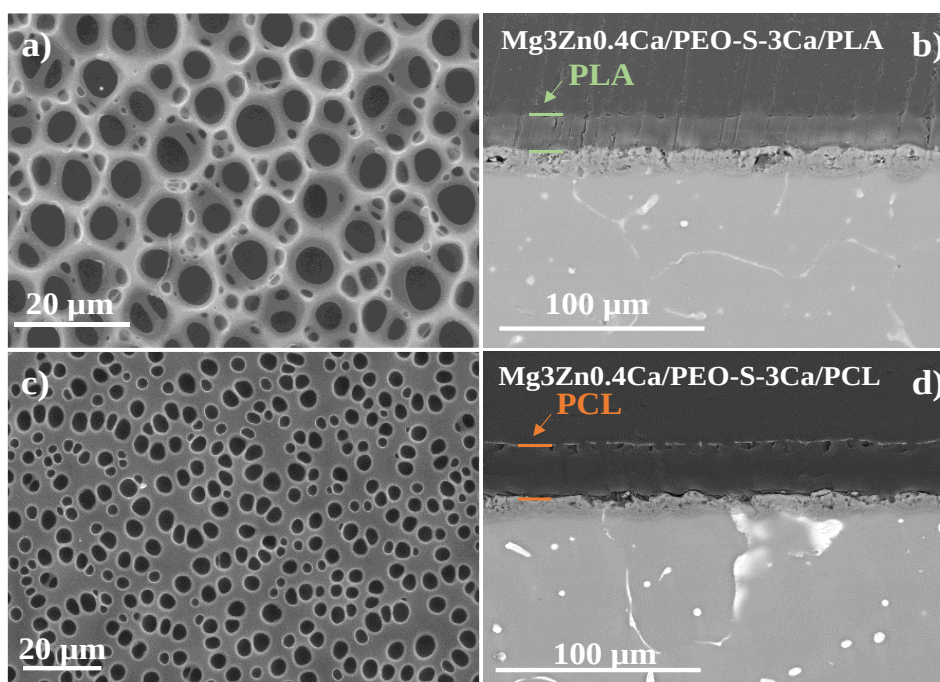


Figure 90. (a, c) SE plan view micrographs and (b, d) BSE cross-section of SEM images for (a, b) PLA/PEO-coated Mg3Zn0.4Ca and (c, d) PCL/PEO-coated Mg3Zn0.4Ca.

The variation of the surface roughness values (S_a , average height of selected area and S_z , maximum height of selected area) was analysed by using optical profilometry (**Table 48**). PEO-coated sample presented a higher S_a value due to the high surface porosity with lower average pore size generated by the PEO treatment. In contrast, PLA and PCL surface S_a values are significantly lower due to the larger pores (with average pore sizes in the range of 7-10 μm). As a result of the relatively large thickness of the polymer top-coats, the waviness of the PEO ceramic layer is reduced, which reduced the S_a values from 4.2 μm (stand-alone PEO layer) down to 2.5 μm and 3.1 μm for PLA and PCL top-coats of the hybrid coating system, respectively.

The wettability of the surface significantly changes upon deposition of the polymer layer. PEO is a highly hydrophilic surface with contact angles of $\sim 23.6^\circ$. However, when it is coated with PLA or PCL, there is an increase of the contact angle up to $\sim 95^\circ$. This means that PLA and PCL are mildly hydrophobic: as the surface becomes porous, the trapped air stays inside the pores consequently increasing its contact angle. For

comparison, the contact angle of PCL and PLA BF films formed on glass substrate by drop deposition are $65.3 \pm 1.1^\circ$ and $106.5 \pm 0.8^\circ$, respectively. This means that BF technique is sensitive to the topography of the underlying substrate as well as to the laminar flow of the polymer solution during dip coating at the contact angle, both of which affect the formation of the pores, and consequently, the wettability.

Table 48. Surface characteristics (thickness, contact angle, roughness measurements) of the studied coatings.

Sample	Thickness (μm)	Average pore size (μm)	Contact Angle ($^\circ$)	S _a (μm)	S _{10z} (μm)
Mg3Zn0.4Ca/PEO-S-3Ca	7.8 ± 0.8	0.3 ± 0.1	23.6 ± 0.8	4.2 ± 0.23	21.0 ± 7.7
Mg3Zn0.4Ca/PEO-S-3Ca/PLA	36.0 ± 1.2	10 ± 1.1	95.0 ± 1.1	3.1 ± 0.2	18.1 ± 7.0
Mg3Zn0.4Ca/PEO-S-3Ca/PCL	28.0 ± 0.4	7 ± 1.9	94.4 ± 2.3	2.5 ± 0.5	17.7 ± 6.5

The preliminary assessment of the effect of the PCL polymer top-coat on corrosion performance of the PEO-coated Mg3Zn0.4Ca alloy was carried out in pseudo-physiological immersion test. This test used a H₂ collection set-up first developed by Daavari et al.²²³, where it was denominated as "quasi-in vivo" referring to the regulation of the medium pH as in human body conditions. Although, it must be recognized that the medium used in the test is inorganic one, whereas in vivo, the proteins and amino acids play an additional complicated role in corrosion mechanism. **Figure 91** shows the hydrogen evolution after 13 days of immersion for Mg3Zn0.4Ca, PEO-coated and PCL/PEO-coated specimens in inorganic α -MEM solution at 37°C with a constant pH of 7.4 under CO₂ flow. At the initial stage of immersion, a slightly lower hydrogen volume is observed for PEO and PEO/PCL specimens, compared to non-coated substrate. After 8 days of immersion, PEO treated surface and PEO/PCL-coated specimens showed an exponential increase of hydrogen volume. At the end of the test, substrate and PEO treated surface present the same hydrogen volume ($\sim 10 \text{ mL/cm}^2$), while PEO/PCL system exhibited slightly lower hydrogen volume ($\sim 8.5 \text{ mL/cm}^2$). Surprisingly, the protective effect of PCL top-coat was overall insignificant, its main contribution consisting in better repeatability between (lower standard deviation, **Figure 91**).

The macrographs of PEO and PEO/PCL specimens (**Figure 91**) disclosed a filiform corrosion morphology, which suggested that both coating systems could be easily permeated by the corrosion species from the media during the first hours of immersion.

According to the literature, the corrosion behavior of hybrid PLA/PEO coating on Mg-Al alloy obtained using dip coating over 14 days of immersion in SBF was 6-10 times superior to that of a stand-alone PEO system, which was attributed to the sealing of the pores and cracks of the PEO coatings^{207,206}. However, the opposite situation is observed, PCL/PEO-coated specimens showed a remarkable lack of protection, especially during the first 5 days of immersion. In order to understand this behavior, the cross-sections of Mg3Zn0.4Ca/PEO-S-3Ca and PCL/Mg3Zn0.4Ca/PEO-S-3Ca specimens were analysed in more detail (**Figure 92**) after 4 and 8 h of “quasi-in vivo” immersion.

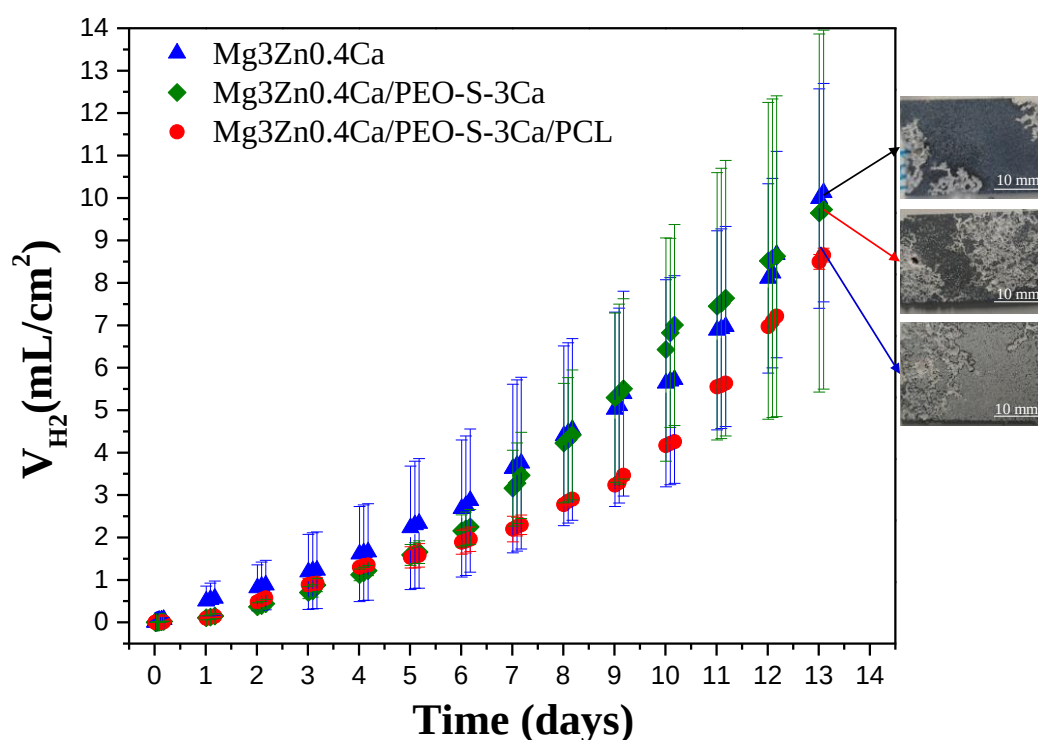


Figure 91. Hydrogen evolution of Mg3Zn0.4Ca, PEO and PCL-coated specimens after 13 days of immersion in inorganic part of α -MEM at 37°C at constant pH 7.4.

At neither point PEO specimens show corrosion products under the PEO coating, while PCL/PEO-S-3Ca specimen reveals the presence of crevice at PCL/PEO interface, in some regions of the sample (**Figure 92** (d)). Also, a 3-5 μm -thick layer of undercoating corrosion products is formed under PEO layer (**Figure 92** (c)) after 4 h of immersion (**Figure 92** (f)). The low adhesion between PCL and PEO and subsequent crevice formation may be associated with the moisture entrapped between the layers during the dip coating, carried out in high humidity environment of a closed chamber. The moisture is absorbed by the ceramic layer, leaving a gap, where corrosive medium enters.

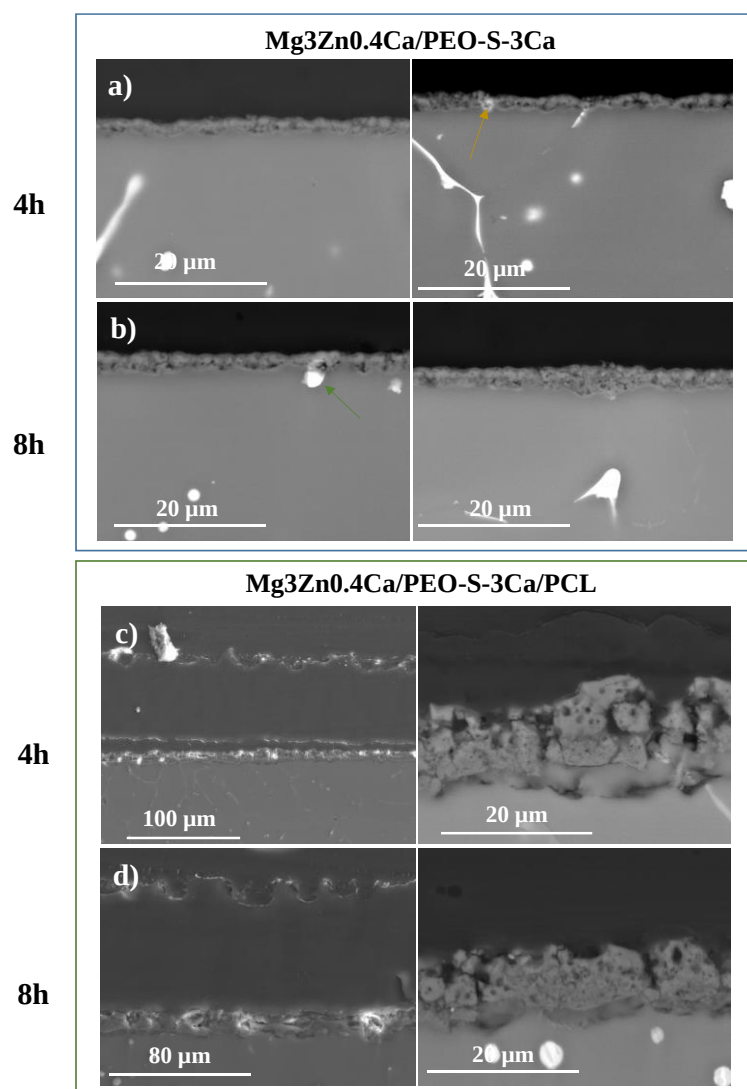


Figure 92. Backscattered electron cross-section images of (a-c) PEO treated surface and (d-f) PCL-coated samples after (a, c) 4 h and (b, d) 8 h of immersion in inorganic α -MEM at 37°C.

As described in Section 5.2.3.2, the corrosion protection of the PEO coating depends on the corrosion resistance of its barrier layer. TEM analysis of the PEO-S-3Ca coating (**Figure 93** (a)) shows a barrier layer of about $\sim 0.6 \mu\text{m}$ thickness with a complex stratified morphology comprising an upper porous region ($\sim 400 \text{ nm}$), separated from the rest of the PEO coating material by a submicron-size pore band, and an inner region ($\sim 200 \text{ nm}$) adjacent to the substrate. It is worth mentioning that several finer nanovoid bands are located inside the inner region of the barrier layer (**Figure 93** (c, d)), delimiting the $<100 \text{ nm}$ -thick strata of the barrier layer.

A curvature of the barrier layer/substrate interface is also seen below the pores, which means that the pores represent the bottom of the discharge channels. **Figure 93** (b, c) shows discharge channels cutting across almost the entire thickness of the barrier layer. This phenomena is consistent with previous findings of Arrabal and Daavari in PEO

treated surface in AZ91-D and AZ31 alloys^{386,223}. It is unclear whether the stratification of the barrier layer is exclusive to AC PEO regime, as no detailed TEM studies of PEO coatings formed in DC mode have been reported so far.

The low protection of the PEO/PCL- and PEO-coated specimens may therefore be attributed to the presence of these thin strata and penetrating discharge channels in the inner region of the barrier layer. This provides an easy access for corrosive species from the medium to the substrate, initiating the early undercoating corrosion.

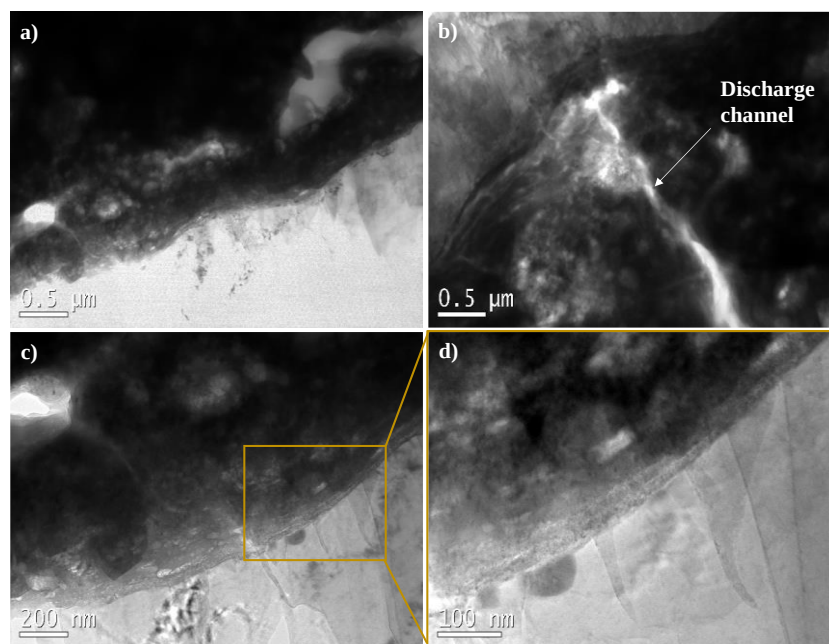


Figure 93. TEM micrographs of PEO-S-3Ca coating acquired at 200 kV from different regions of the barrier layer.

The findings suggest that the initiation of the corrosion mechanism of PCL/PEO-S-3Ca system involves the following steps: i) the presence of crevice between PCL/PEO-S-3Ca interface causes the easy penetration of the corrosive species through the pores and the discharge channels of the PEO coating; ii) the presence of noble partially oxidized secondary phases in the PEO layer (indicated by arrows in **Figure 92** (a)) allows the formation of galvanic couples with the matrix accelerating the corrosion process⁴⁰¹ (see Section 4.2.2.1).

6.4 Optimization of the corrosion resistance of the hybrid hierarchical PEO/polymer coatings

As result of the above mentioned findings, a need for further optimization of the dip coating process became evident. The strategy to avoid the presence of moisture at the polymer/PEO interface consists of the preparation of a solid (i.e. pore-free) polymer layer in conditions of low relative humidity (<40 %). More precisely, in order to avoid the

presence of a crevice at polymer/PEO interface, a thin sealing PCL layer was applied onto PEO in normal humidity conditions, using a 0.3 mm/s withdrawal speed, followed by an application of a thick and porous polymer top-coat inside the closed chamber using the previously established conditions (2 mm/s, 2 cycles, 75 mg/mL). The scheme of this process that results in the generation of a HHC system is illustrated in **Figure 94**.

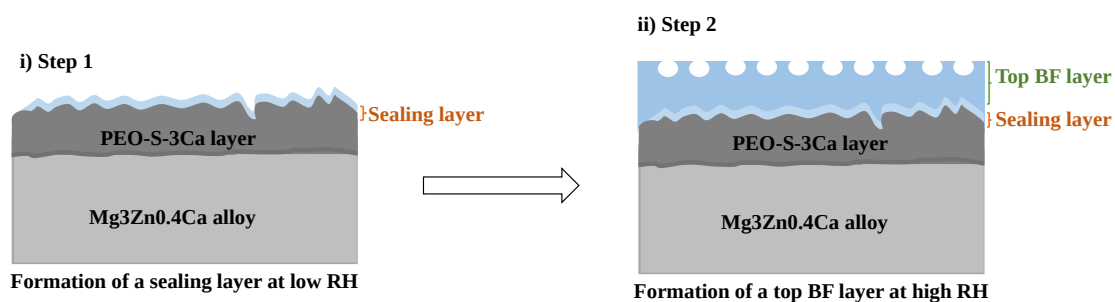


Figure 94. Scheme of steps followed in order to improve the adhesion between PEO treated surface and the polymer layer.

Figure 95 shows the micrographs of top views and cross-sections of PLA and PCL-coated samples obtained following the described new strategy. PLA_{BF} and PCL_{BF} surfaces disclosed an average of pore size of 6.4 ± 1.0 and 8.2 ± 0.6 μm , respectively. Both polymer layers are constituted by two sublayers. A thin sealing sublayer exhibits a good adhesion between polymer and ceramic coatings due to the penetration of the polymer into the pores and cracks of the PEO (**Figure 95** (c, f)).

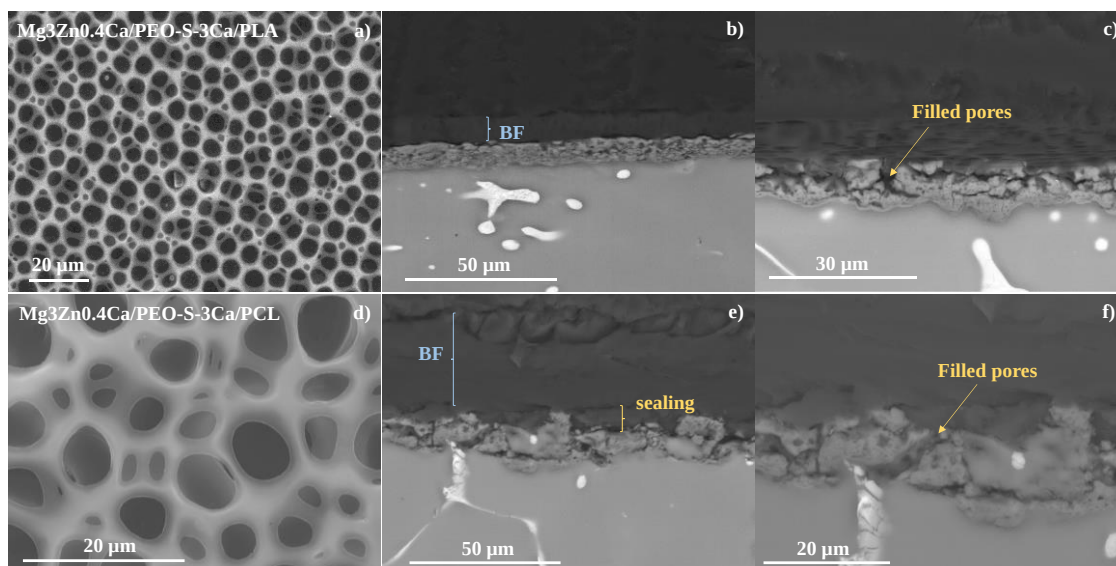


Figure 95. (a, d) Secondary electron top view images and (b-f) backscattered electron cross-section images of (a-c) PLA and (d-f) PCL-coated samples.

The hydrogen evolution results for Mg3Zn0.4Ca, PEO-coated and PLA and PCL-based HHC systems after 17 days of immersion in inorganic α -MEM at 37°C using a constant pH (7.4) under CO₂ flow are illustrated in **Figure 96**. As can be seen, the slope

of the curve corresponding to the PLA- and PCL-coated samples remains relatively constant during the testing period and the evolved hydrogen volume is 0.5 mL/cm^2 . This means that the HHC system could provide a long-term corrosion protection for a Mg alloy. Different studies have demonstrated that the short-term (1 h) corrosion behavior of hybrid coatings on Mg alloys comprising a planar polymer top-coat is improved by 2-3 times compared to a PEO coating ²⁰⁶. However, the HHC strategy developed in the present Thesis offers a 10-times reduction of the degradation rate of a Mg-Zn-Ca alloy system down to 1.6 and 1.9 mm/year based on corrected H_2 volume, PCL and PLA (respectively).

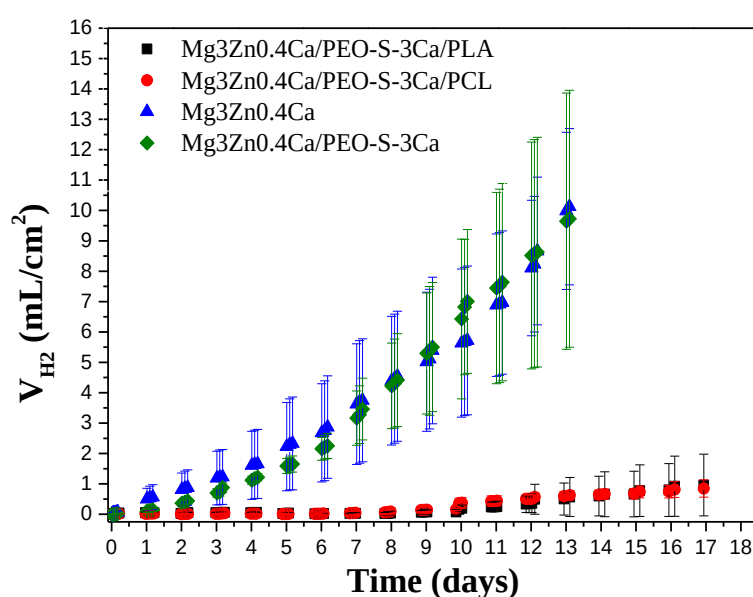


Figure 96. Hydrogen evolution of bulk material, PEO, PLA and PCL-coated samples after 17 days in inorganic α -MEM of immersion at 37°C .

Figure 97 illustrates the cross-sections of PLA and PCL-based HHC after 17 days of immersion in inorganic α -MEM solution at 37°C . Both specimens show regions with localized (**Figure 97** (a, e)) and uniform corrosion under the PEO coating (**Figure 97** (b, f)). It is worth mentioning that the polymer layer is detached from PEO layer in pitting corrosion areas, but is intact in the uniform corrosion regions. In addition, **Figure 97** (c, g) shows the formation of polymer bubbles, which are formed by the accumulation of H_2 gas at the PEO/substrate interface during the Mg degradation process. It is important to note that the corrosion inside of the bubble is very intense, possibly due to higher concentration of Cl^- and the reduction of pH caused by massive formation of $\text{Mg}(\text{OH})_2$ (**Figure 97** (g, h)). Further, the accumulation of high H_2 gas causes the bubble to stretch and grow until a brittle rupture ⁴⁰² as occurs in PLA as it can be seen in **Figure 97** (c, d). However, the PCL bubbles can undergo high mechanical strain at PCL/PEO interface due

to the high flexibility of this polymer⁴⁰² (**Figure 97** (g, h)). When bubbles finally rupture, the accumulated H₂ gas is released and collected in the burette, which is in accordance with the slight increase of hydrogen volume observed after 10 days of immersion (**Figure 96**).

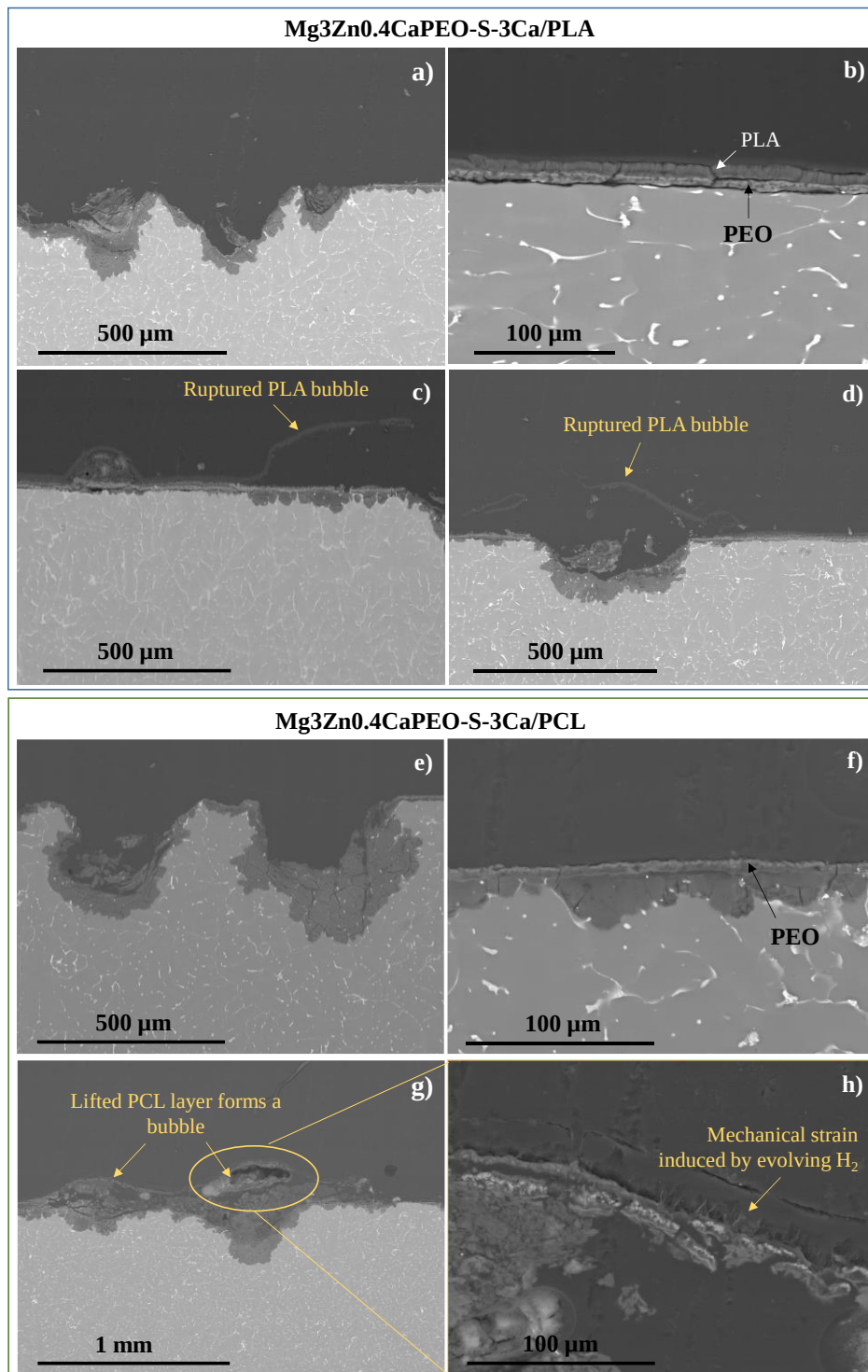


Figure 97. Backscattered electron cross-section image of PLA (a-d) and PCL (e-h)/PEO-S-3Ca-Mg3Zn0.4Ca alloy after 17 days of immersion in inorganic α -MEM solution.

The corrosion of Mg with the developed polymer-ceramic HHCs occurs according to the following mechanism: i) the penetration of the corrosive species from the media through the defects in polymer layer, ii) the hydration of PEO coating by the penetration of the media through pores and cracks in PEO; iii) PEO barrier layer failure with subsequent formation of undercoating corrosion products; iv) formation of microgalvanic couples between nobler intermetallic inclusions at the PEO/Mg interface and the Mg matrix, accelerating the corrosion process (explained in more detail in Section 5.2.3.2); v) formation of a crevice at PCL_{sealing}/PCL_{BF} interface and at PEO/substrate interface. As a consequence of the latter the formation of stretched polymer bubbles at the coating/substrate interface causes a more aggressive Cl⁻-concentrated environment inside, activating the pitting corrosion mechanism. The accumulation of more H₂ gas allows the bubble to grow causing mechanical stress and bursting or delamination of the polymer layer which facilitates further ingress of corrosive medium towards the coating/substrate interface.

In order to verify the corrosion mechanism of the developed hybrid materials *in vitro*, the degradation of 3D PCL/PEO-S-3Ca/Mg3Zn0.4Ca specimens have been analysed in more detail after 4 days of immersion in complete α -MEM (**Figure 98**). It should be noted that the polymer layer shows discontinuities at the edges of the sample that act as stress-raisers during solidification and shrinkage of the polymer. These defects induce localised formation of thick corrosion products (**Figure 98** (a, b)). Failed edges facilitate propagation of the crevice at the PCL/PEO interface (**Figure 98** (c)). In addition, the presence of crevice at PCL_{sealing}/PCL_{BF} interface is observed **Figure 98** (d). The phenomenon suggests that the high humidity of the chamber and the hydrophobic behavior of PCL allows the water droplets to be accumulated on the surface of the sample compromising the adhesion between polymer layers.

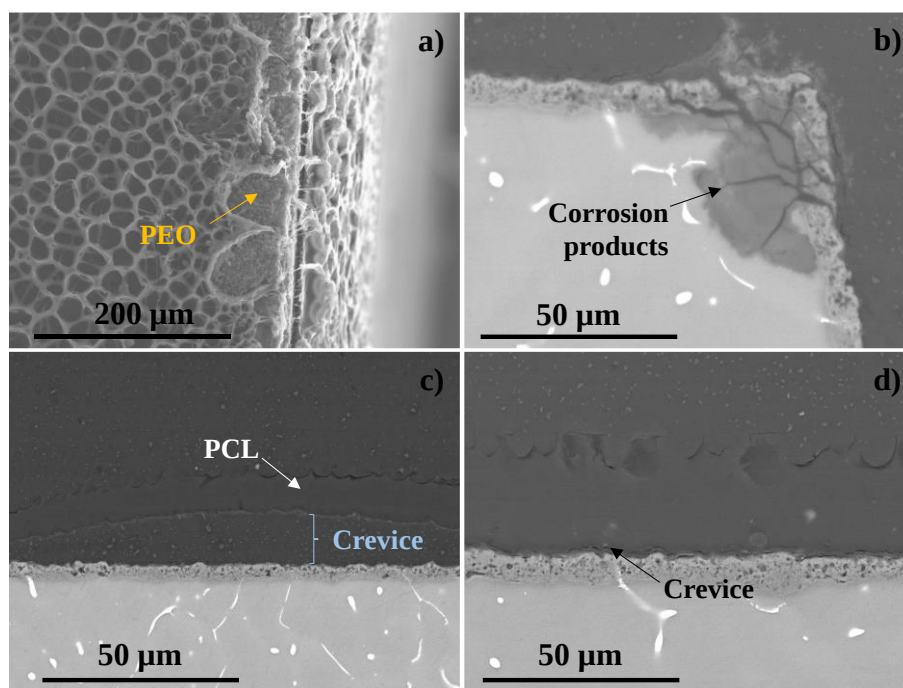


Figure 98. (a) Secondary electron top view image and (b-d) backscattered electron cross-section images of Mg3Zn0.4Ca/PEO-S-3Ca/PCL specimen after 4 days of immersion in complete α -MEM.

Summarizing the presented results, porous PLA and/or PCL layers can be prepared on PEO/Mg alloy system via relatively simple methods, such as dip coating and BF. The optimization of the process parameters such as polymer concentration, withdrawal speed and number of dipping cycles allowed the design and development of hybrid hierarchical coating systems with adequate pore size, and thicknesses in order to establish an equilibrium between corrosion protection and biocompatibility. The corrosion behavior of hybrid coating systems can be compromised by the lack of adhesion at the BF polymer/PEO interface due to the high humidity of the BF chamber. A sealing layer formed outside of the high humidity chamber corrects the problem and demonstrates an enhanced penetration of polymer into the PEO pores. Hierarchical polymer top-coat comprising a sealing layer formed at low RH and a porous BF layer formed at high RH considerably enhances the corrosion resistance of Mg3Zn0.4Ca alloy.

The combination of PEO ceramic layer and porous hierarchical polymer top-coat allows to control the degradation rate of Mg alloy-based systems, which is demonstrated in the present study. This approach signals the potential of a hybrid strategy to create a hybrid 3D implant material with hierarchical structure for biomedical applications that can be employed as a drug carrier to prevent infection and inflammatory effect in the implant vicinity area.

6.5 Conclusions of Chapter VI

Optimization of the coating process parameters

- BF and dip coating techniques were evaluated in terms of porosity, pore size and thickness of microstructured polymer films in order to optimize the parameters of the process using different polymer concentration, withdrawal speed and dipping cycles.
- Low polymer concentrations (25 and 50 mg/mL) do not form a homogeneous polymer layer and do not cover completely PEO pores. While at high concentrations, 100 mg/mL of PLA and 75 mg/mL of PCL, a homogeneous layer covering all PEO pores is formed with pore size in the range of 2-12 μm , being higher for PCL. For both polymers the BF film thickness increases up to 35 μm with the increase of the polymer concentration.
- The increase of dipping cycles allows the increase of the pore size, porosity and thickness of PLA and PCL. The porosity and thickness (from 10 to 60 μm) are higher for PLA compared to PCL.
- The porosity and pore size increase with the increase of the withdrawal speed for PLA and PCL, being slightly higher for PCL. The thickness increases from 5 to 40 μm for both polymers, being higher for PLA.

Corrosion behavior

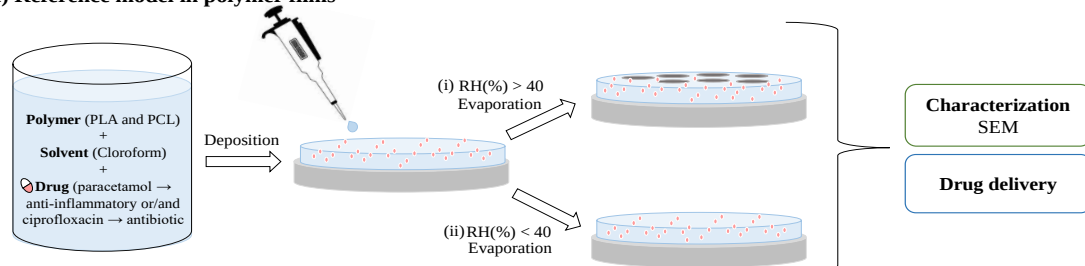
- Mg3Zn0.4Ca/PEO-S-3Ca/PCL_{BF} hybrid system showed negligibly higher corrosion resistance compared to stand-alone PEO-coated system and the substrate due to low adhesion between the polymer and the PEO layer caused by adsorbed moisture in a high humidity chamber.
- A sealing layer applied in low humidity conditions followed by a porous and thicker BF polymer layer form a hierarchical polymer top-coat that ensures a good adhesion at the ceramic/polymer interface.
- Hybrid hierarchical coating systems based on PLA or PCL improve the corrosion protection of stand-alone PEO-coated systems by 10-12 times, ensuring the degradation rate of Mg alloy of 1.6 and 1.9 mm/year, respectively.
- Although the corrosion behavior is improved, there are still limiting factors that need to be further studied in order to avoid the formation of edge defects in the polymeric layer when coating a real 3D implant prototypes.

7. CHAPTER VII- Drug release by hybrid coatings

As mentioned in the Introduction (Section 1.7), the incorporation of an implant into human body for fixation of open fractured bones and joint revision surgery may leads, in some cases, implant rejection, inflammation or microbial infection ⁴⁰³. In order to avoid these problems, functionalised hybrid hierarchical systems comprising of porous polymer top-coat are a promising strategy to control the drug release during the first days of implantation ²⁴⁷. However, the effect of the drug on the degradation rate of the hybrid material is important because, in case the drug prescribed for clinical reasons is a corrosion accelerator, the hybrid coating that releases will have to provide a sufficient level of corrosion protection. Up to date, only two work evaluated the effect of drug release (paracetamol and amoxicillin) on the Mg-Al alloys ^{148,125}.

According to the above, Chapter VII is focused on the design and development of HHC incorporating anti-inflammatory and antibiotic agents in order to reduce the potential problems associated with the implantation and enhance corrosion resistance of Mg-based implant. The structure of this chapter is divided in two sections: (i) evaluation of different parameters such as drug concentration, polymer film composition and roughness deposited on glass substrates on drug delivery capacity. (ii) Evaluation of corrosion inhibition effect of drug loaded HHC by electrochemical measurements, hydrogen evolution test and localised measurements techniques. The graphical abstract of this chapter is displayed in **Figure 99**.

i) Reference model in polymer films



ii) Real model in 3D hybrid hierarchical system

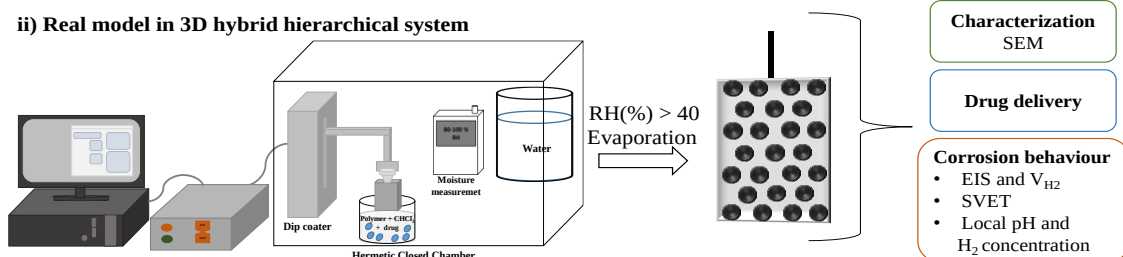


Figure 99. General scheme of Chapter VII.

7.1 UV-vis spectra calibration for the anti-inflammatory and antibiotics agents release

The first experiments were conducted to select the anti-inflammatory and antibiotic agents with the aim to incorporate the drugs into a polymer layer that may help to avoid the problems associated to the post-surgery period. For this purpose, three anti-inflammatory agents and three antibiotics were evaluated by UV-visible spectroscopy in the range of 200-400 nm. The calibration curve of acetylsalicylic acid (aspirin) is observed in **Figure 100**. The rest of the calibration experiments for paracetamol, naproxen, gentamicin, streptomycin and ciprofloxacin are illustrated in Appendix (**Figure A8** and **Figure A9**). The experiment is carried out at different drug concentrations in inorganic α -MEM at 25°C and evidences a gradual increase of the absorbance as a function of the agent concentration.

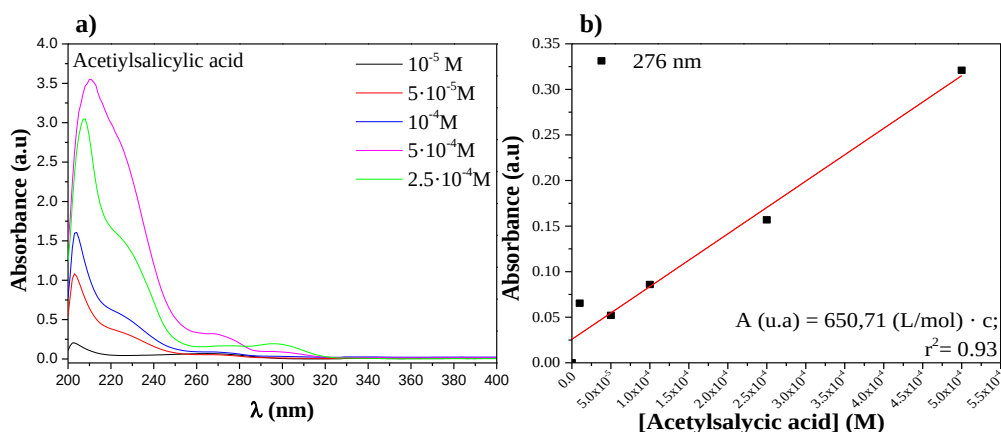


Figure 100. (a) Absorption spectra and (b) standard curve of acetylsalicylic acid at different concentration in inorganic α -MEM at 25°C.

In order to select an adequate model drug, anti-inflammatory agents (paracetamol, naproxen and aspirin) and antibiotic agents (streptomycin, gentamicin and ciprofloxacin), were investigated in terms of their effect on the corrosion behavior of Mg alloys. The experiment was carried out evaluating the hydrogen evolution during immersion of MgZnCa alloy sample (1 cm^2) in inorganic α -MEM solution with 0.01M a dissolved of pharmaceutical agent at room temperature. **Figure 101** reveals that in the presence of streptomycin and ciprofloxacin in physiological medium entails a higher increase the degradation rates of Mg, while in the case of paracetamol, naproxen, aspirin and gentamicin, lower degradation rates were observed in comparison to the substrate in drug-free medium after 26 h of immersion. It is worth mentioning that ciprofloxacin and paracetamol agents present high solubility in inorganic α -MEM compared to the rest of pharmaceutical agents, which makes their detection easier using UV-visible spectroscopy

technique. For this reason, paracetamol and ciprofloxacin, as an example of corrosion inhibitor and accelerator, respectively, were selected for the optimization of the experimental parameters of the encapsulation/delivery process.

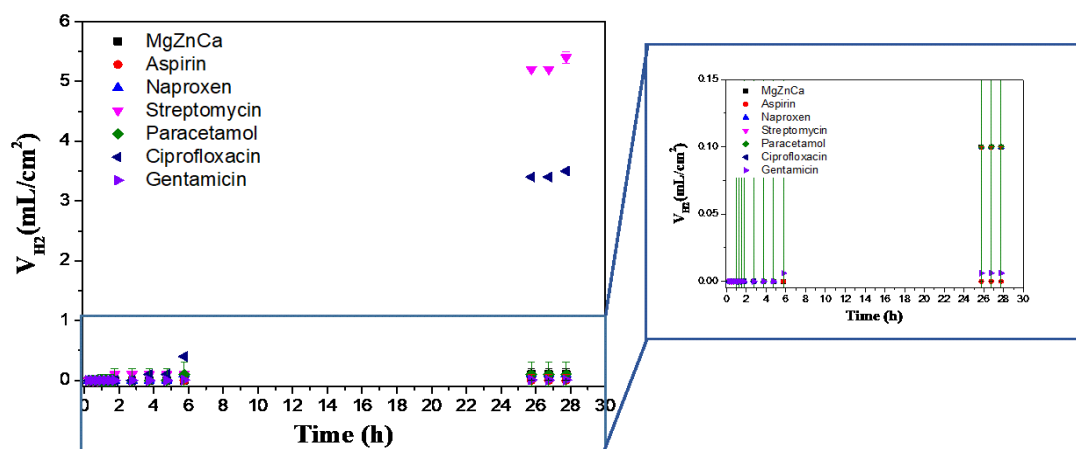


Figure 101. Hydrogen evolution of anti-inflammatory and antibiotic agents after 26 h of immersion in inorganic α -MEM at 25°C on MgZnCa alloy dissolving 0.01 M of drug in the medium.

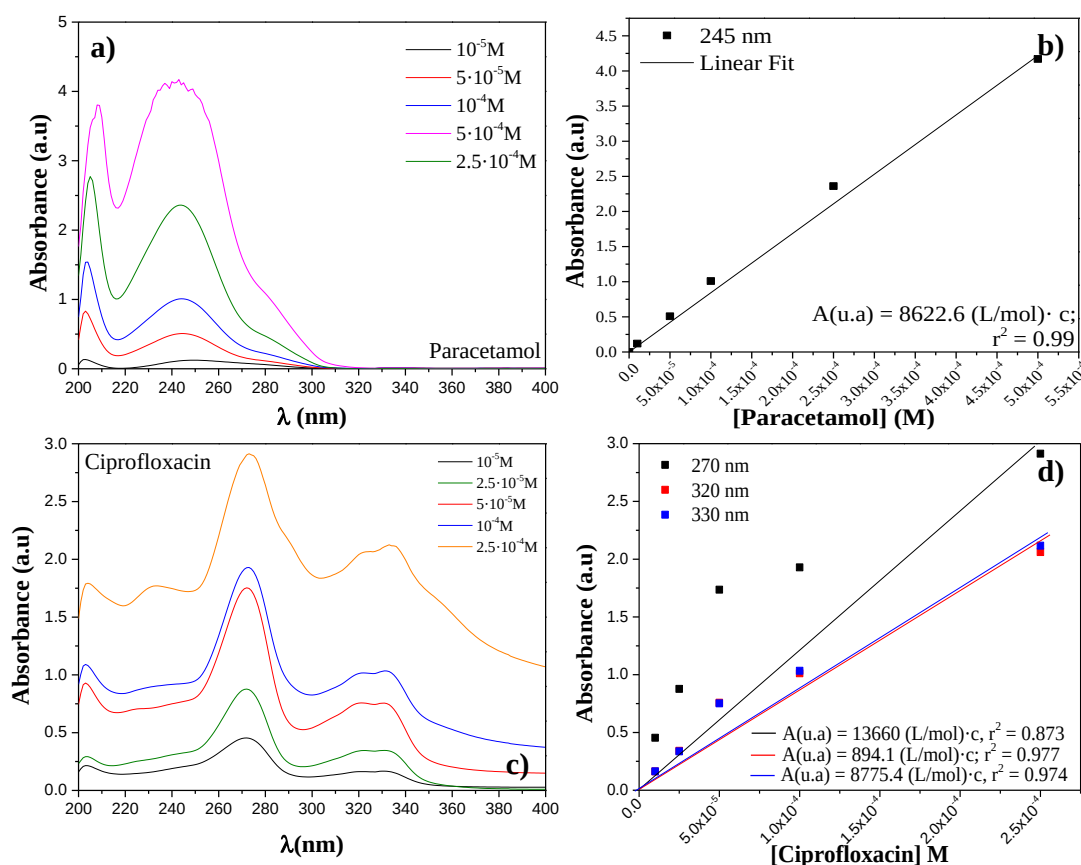


Figure 102. (a, c) Absorption spectra and (b, d) standard curve of paracetamol (PAR) and ciprofloxacin (CIP) in inorganic α -MEM at 25°C.

Taking into account these results, it is observed that paracetamol (PAR) and ciprofloxacin (CIP) present a maximum in absorption at $\lambda_{\max} = 245$ nm for PAR and at $\lambda_{\max} = 272, 320$ and 330 nm for CIP (**Figure 102** (a, c)). The calibration curves of PAR

suggested that the increase of PAR concentration acquires a good linearity while CIP deviates from linearity for 270 nm when the concentration increases, as indicated by R^2 values. However, the opposite situation is observed for 320 and 330 nm with linearity correlation coefficients of (R^2) 0.9314 and 0.9379, respectively. For this reason, the signal at 330 nm was selected to evaluate the CIP release.

7.1 Methodology to fabricate polymer coatings under controlled environmental humidity

7.1.1 Polymer coatings on glass wafers loaded with the drug (model system).

A critical aspect in the success of an implant is related to their ability to adapt during the first days of implantation. An interesting approach to improve this initial response to metal device implantation involves the use of biocompatible polymer coatings. However, it is necessary to select a biodegradable polymer with a low degradation rate and appropriate mechanical properties. Taking into account these requirements, PCL and PLA have been extensively studied. However, there are important differences between the two. PLA presents excellent mechanical properties but high degradation rates. Therefore, in view of their use for the fabrication of orthopaedic implants, a high degradation rate may lead to premature implant failure. Whereas PCL presents longer degradation times but lower mechanical properties. Herein, we will explore the use of these two materials separately but also will combine them and prepare polymer blends with properties that benefit from the best of the two materials.

As mentioned before, an important factor that helps to avoid or at least significantly reduce an implant rejection during the first days of implantation, is functionalisation of the polymer layer by incorporating drugs and modulating their release. Two of the factors affecting the drug release are the degradation rate of the polymer layer and its T_g (glass transition temperature). As PLA has a T_g around 50-60°C that would result in a slow release at room temperature, while PCL has a lower T_g and it would result in an increased drug release rate. Therefore, it is interesting to explore how a control over the drug release can be also achieved producing polymer coatings by combination of both polymers (PCL and PLA) at different compositions.

In order to understand the pore formation and the ability of the polymer to encapsulate and release the selected drugs, it was prepared a planar and porous polymer coatings on glass wafers instead of using Mg substrates. This system serves as a reference model to

understand and optimize the drug release from the polymeric coating reducing the cost and the number of Mg substrate samples required. **Figure 103** shows the strategy for the preparation of this system model which has been inspired by other previous studies ⁴⁰⁴. The polymer solution (PCL and/or PLA as blend and paracetamol and/or ciprofloxacin as a drug are dissolved in chloroform) was placed in a cylindrical glass and allowed to evaporate in a closed vessel chamber in which the relative humidity could be varied.

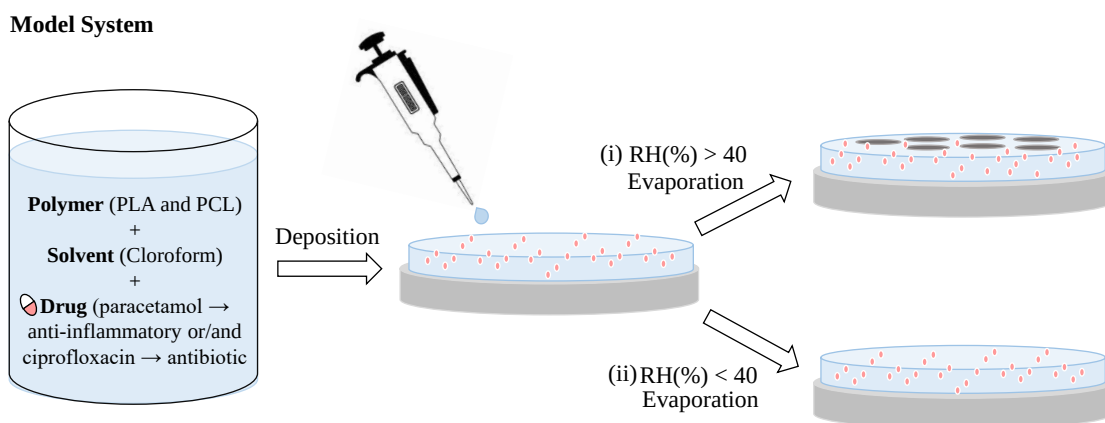


Figure 103. Deposition of polymer solution dissolving pharmaceutical agents on glass disc.

More precisely, the different solutions were deposited and allowed to evaporate in air with low relative humidity (below 40 %) or in an environment with high humidity (above 90 %) using a closed chamber with water reservoirs. The polymer solution concentration and the amount of drug embedded within the polymer film were also explored to study the release process.

First of all, the formation of microporous polymer surfaces was explored varying the polymer solution volume and the polymer concentration. For this purpose, different deposition volumes (150, 100, 90 and 50 μL) were employed at three different polymer concentration (30, 50 and 75 mg/mL) of PLA and PCL to prepare the films. As has been already mentioned, PCL and PLA are selected for these experiments due to the different degradation times and because they have been successfully used for the fabrication of porous interfaces. These experiments were carried by the deposition of these volumes into a glass disk (12 mm diameter) as described in Section 3.6.1.

Secondly, the incorporation of the drug into the polymer coating was explored. The drug concentration was evaluated in planar PCL and PLA films prepared at a constant low relative humidity (RH < 40%) until complete evaporation of the chloroform at room temperature. 1, 2, 5, 10 and 20 wt. % of PAR and 2 and 5 wt. % of CIP were incorporated

in the polymer solution and then deposited and allow to evaporate into circular glass as illustrated in **Figure 103** (ii).

Thirdly, the effect of the surface structuring on the drug release was explored. The drug release was studied in both porous and planar films incorporating 2 and 5 wt. % of PAR and CIP in the polymer solution. Porous films were obtained using BF method with a constant relative humidity of >90 % in a hermetic closed chamber at room temperature (**Figure 103** (i)) while planar films were developed using the experimental conditions mentioned before (**Figure 103** (ii)). In addition, polymer films were prepared using different polymer compositions of PLA/PCL (100/0, 25/75, 50/50, 75/25 and 0/100) in the blend incorporating 2 and 5 wt. % of PAR or CIP. The objective of this analysis is to understand the role of the polymer properties on the drug release process and explore the possibility to modulate the release by simply changing the composition of the polymer film.

7.1.2 Formation of microporous polymer films on glass substrate (Model system).

7.1.1.1 Formation of planar PCL and PLA films: evaporation under low relative humidity (RH) conditions.

Planar polymer surfaces were prepared onto glass wafer substrates (**Figure 103** (ii)) using PCL and PLA and incorporating 5 wt. % of PAR and CIP at a constant low relative humidity (below 40 %).

Figure 104 shows illustrative SEM images of the polymer surfaces obtained using 5 wt. % of (a, b) PAR and (c, d) CIP on (a, c) PLA and (b, d) PCL films. These images evidenced that the incorporation of PAR and CIP to the film plays an important role in the resultant surface morphology of the films. It is worth mentioning that PAR is homogeneously distributed in both PCL and PLA polymer layer which may be associated with a great solubility of PAR in chloroform. The incorporation of CIP in the planar films is evidenced by the presence of CIP needle-like structures trapped and uniformly distributed inside of PCL and PLA films. Most probably, the poor solubility of CIP in chloroform do not allow for a homogeneous surface morphology.

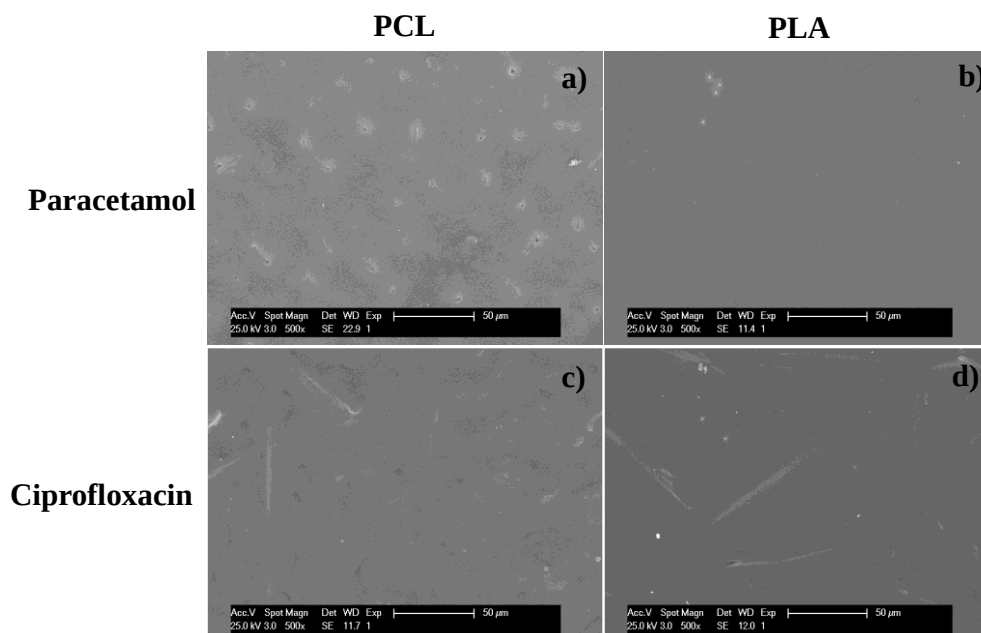


Figure 104. SEM images of (a, c) PCL and (b, d) PLA at 5 wt. % of PAR and CIP films obtained at low relative humidity conditions (below 40 %) and polymer concentration of 30 mg/mL for PLA and PCL.

The wettability of these films is evaluated in order to determinate the influence of the incorporation of these pharmaceutical agents. The contact angle of PAR specimens corresponds to $81.2 \pm 1.2^\circ$ and $87.2 \pm 0.5^\circ$ for PCL and PLA, respectively, while for CIP they are $79.4 \pm 3.2^\circ$ and $77.2 \pm 2.4^\circ$. The contact angle of this films increases compared with those for non-loaded planar films (PCL: $76.6 \pm 1.1^\circ$ and PLA: $75.5 \pm 0.8^\circ$). This suggests that the incorporation of either of agents influences on the contact angle of PCL and PLA decreases the wettability of the films, although they remain hydrophilic⁴⁰⁵.

7.1.1.2 Formation of porous films from polymer blends: evaporation under high RH condition

In order to explore the pore formation, the contact angle and the drug release, two different series of experiments were carried out. The incorporation of PAR and CIP in PLA, PCL and their blends was carried out using a constant amount of PAR and CIP of 2 and 5 wt. % at high relative humidity (>90 %). More precisely, the blend compositions investigated were: 100/0, 25/75, 50/50, 75/25 and 0/100 wt. % of PLA/PCL. On the other hand, it is investigated the role of the amount of incorporated PAR and CIP (2 and 5 wt. %) in the films in the formation of microporous structures. The surface images obtained by SEM of these systems are illustrated in **Figure 105** and **Figure 106**. The evaluation of pore size and the contact angle are displayed in **Figure 107**.

a) Incorporation of PAR in PLA, PCL and their blends

According to **Figure 105**, pores are formed in all studied conditions. In the case of pure PLA and 25/75 (PLA/PCL) blend, the incorporation of PAR at 2 and 5 wt. % leads to the formation of small pores forming regular porous surfaces. Similar behavior is observed for the blends with high PCL content (50/50 and 25/75) and pure PCL forming large pores with regular porous surfaces. It is worth mentioning that the increase of PCL in the blends slightly reduces the pore size of the blend from 24 to 8 μm for 2 wt. % of PAR, while remains constant around $\sim 25 \mu\text{m}$ for 5 wt. % PAR (**Figure 107 (a)**).

The increase of PAR content from 2 to 5 wt. % leads to the increase of the average pore size while maintaining a regular distribution. The increase of PAR from 2 wt. % to 5 wt. % in PCL improves the homogeneity of pore distribution and reduces the coagulation of the droplets as it is observed in PCL at 2 wt. % (**Figure 105 (j)**). According to these results, it appears that the incorporation of PAR plays a key role on the pore formation and distribution leading to the formation of regular microporous surfaces. Most probably, this phenomenon may be attributed to the high solubility of PAR in chloroform preventing or at least significantly reducing the coagulation of the droplets.

In order to explore the influence of the incorporation of PAR in the films on the surface wettability, the static contact angle measurements were carried out (**Figure 107 (b)**). i) The contact angle decreases for non-loaded samples with the increase of PCL content in the blend. The incorporation of PAR showed two different trends: blends with low PCL content exhibited a lower contact angle and blends with high PCL content exhibited higher contact angles comparing with non-loaded blends.

Moreover, the contact angle remains constant for loaded blends with low PCL content (75/25, 50/50 pure PLA) while an increase in the contact angle was observed for specimens with higher PCL content in the blend (25/75 and pure PCL). However, non-significant differences were found in the increase of PAR concentration from 2 to 5 wt. % for all blends. According to these results, the incorporation of PAR and the PCL content in the blend have an influence in the wettability of the film.

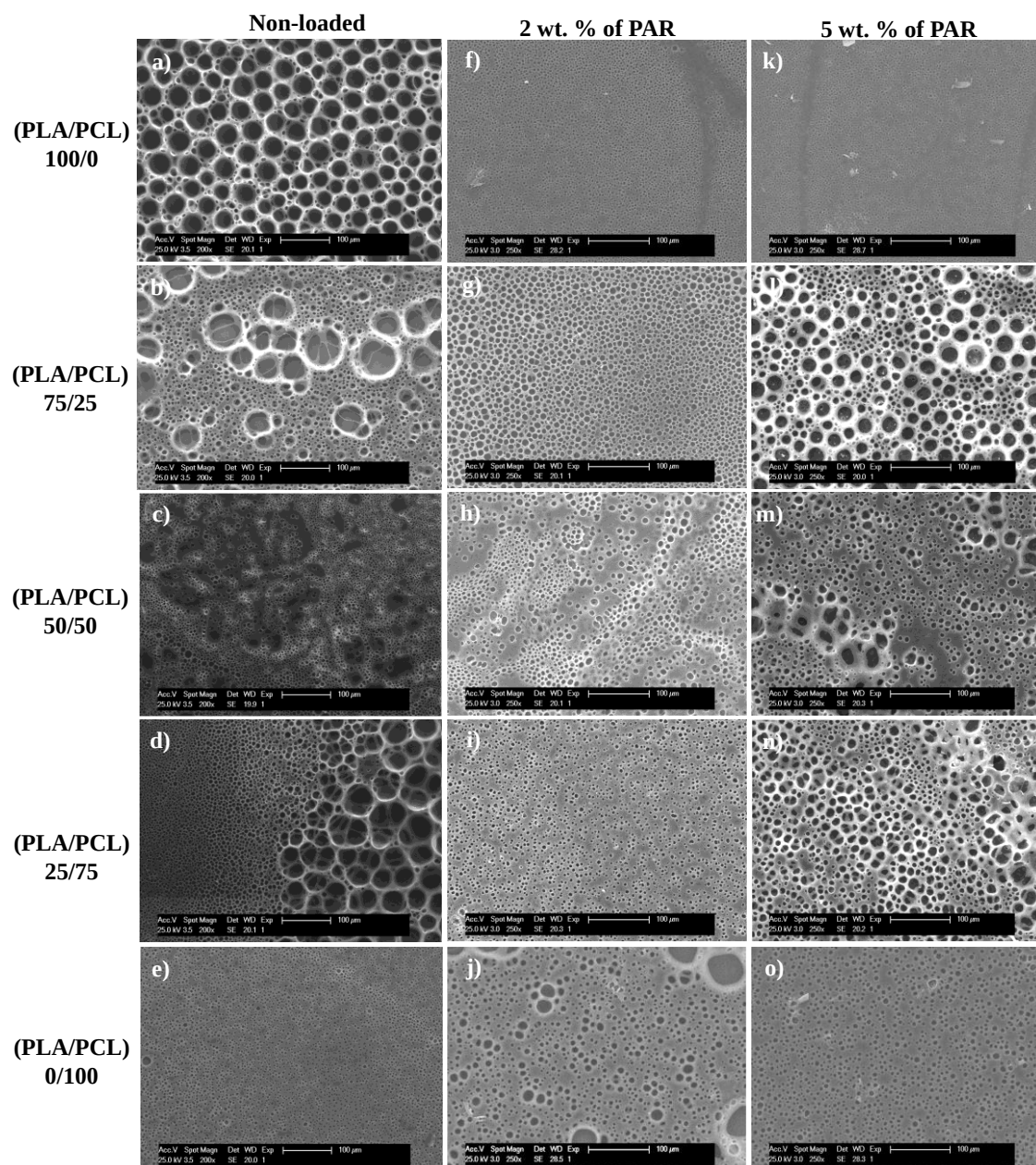


Figure 105. SEM images of the porous films prepared using different composition of PLA/PCL (100/0, 25/75, 50/50, 75/25 and 0/100) (a-e) non-loaded and loaded specimens with (f-j) 2 and (k-o) 5 wt. % of PAR obtained at high relative humidity conditions (>90 %) and polymer concentration of 30 mg/mL PLA and PCL.

b) Incorporation of CIP in PLA, PCL and their blends

In contrast to the irregular surfaces observed above, the porous surfaces prepared using 2 and 5 wt. % of CIP (**Figure 106**) exhibit regular microporous morphology with hexagonally packed arrays of pores in some cases. For pure PLA and for all blends prepared, the incorporation of CIP at both concentrations, 2 and 5 wt. %, leads to the formation of small pores and regular porous surfaces forming hexagonally packed structures. However, in the case of pure PCL incorporating CIP leads to larger and irregular porous surfaces. This phenomenon is attributed to the coagulation of the

condensed water droplets. In addition, the increase of the PCL in the blend leads to the increase of the pore size from 8 μm to 20 μm for 2 wt. % and 24 to 58 μm for 5 wt. % (Figure 107 (c)) of CIP.

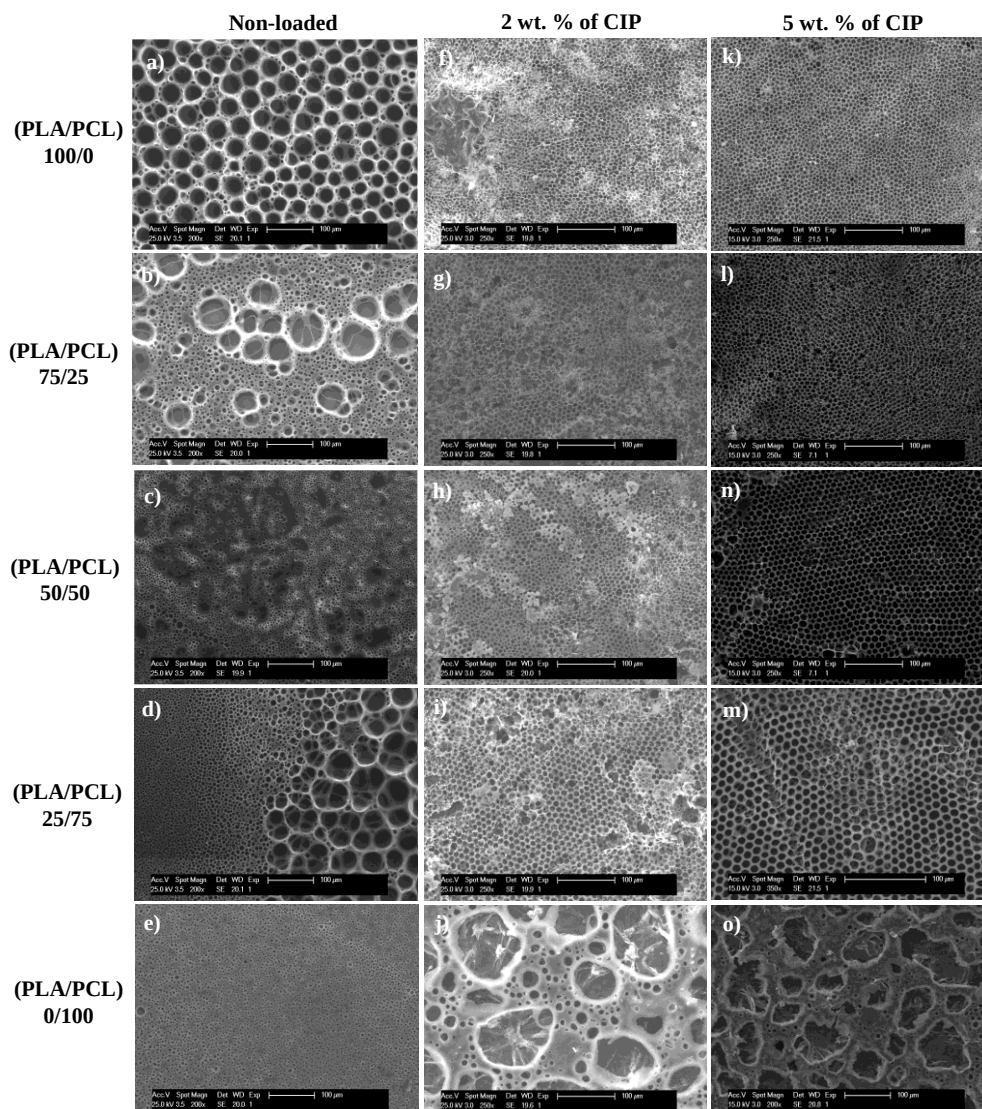


Figure 106. SEM images of the porous films at different composition of PLA/PCL (100/0, 25/75, 50/50, 75/25 and 0/100) (a-e) non-loaded and loaded specimens with (f-j) 2 and (k-o) 5 wt. % of CIP obtained at high relative humidity conditions (>90 %) and polymer concentration of 30 mg/mL of PLA and PCL.

The increase of CIP content from 2 to 5 wt. % in the blends produced microstructured films with increased average pore size from 8 μm to 58 μm as displayed in Figure 107 (c). According to the results, the incorporation of CIP has an influence on the pore formation and distribution, forming regular hexagonal structures, which may be associated with the low solubility of CIP in chloroform avoiding the coagulation of the droplets.

In order to explore the influence of the incorporation of CIP in the surface wettability the contact angle values were measured and are depicted in **Figure 107** (d). The incorporation of CIP leads to a slightly decrease in the contact angle of blends with lower PCL content (75/25 and pure PLA) compared to non-loaded blends. However, an slightly increase of the contact angle was observed for blends with higher PCL content (50/50, 25/75 and pure PCL) compared to non-loaded samples. Overall, the contact angle of loaded blends remains within the range of 90-105° with the increase of PCL content. Similar trend was observed for the increase of CIP concentration from 2 to 5 wt. % For this reason, it is concluded that the incorporation of CIP in the blend do not have a significant effect on the surface wettability of the films, which remain mildly hydrophobic.

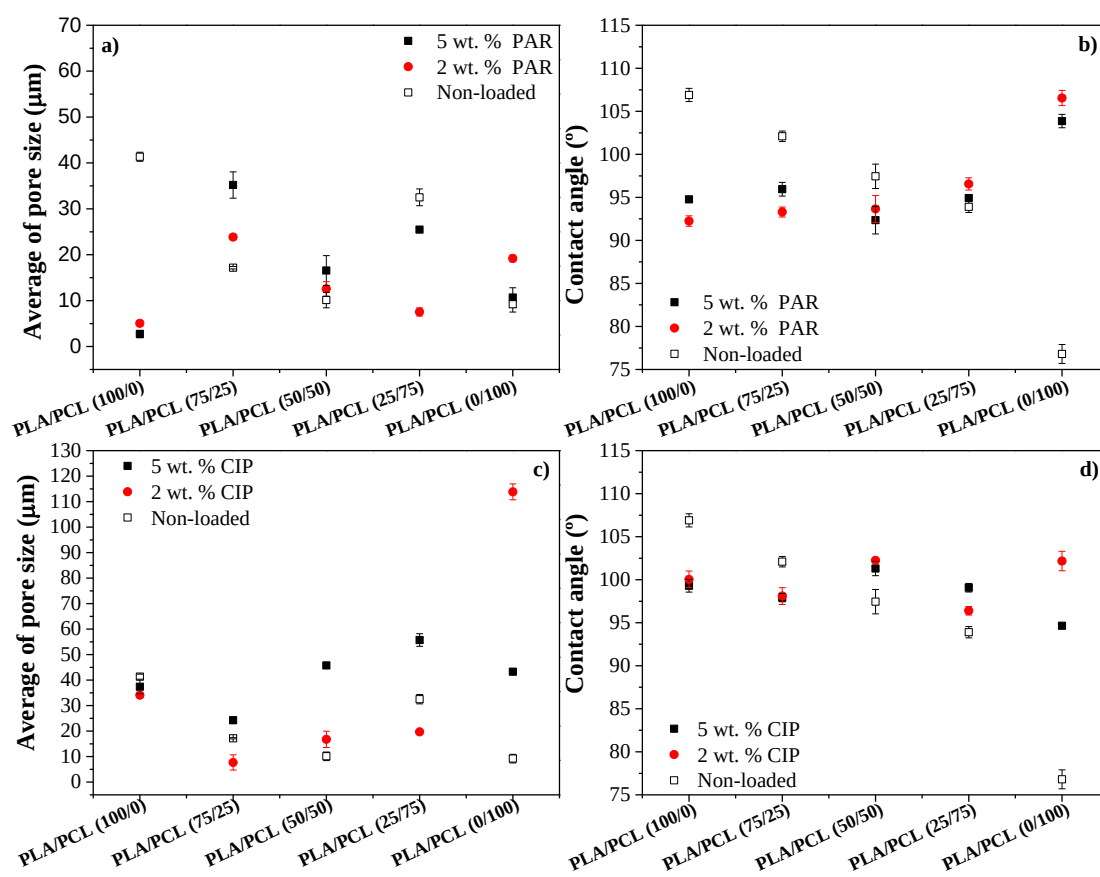


Figure 107. Variation of (a, c) pore size and (b, d) contact angle of porous films from **Figure 105** and **Figure 106**.

c) Multifunctional polymer coatings: prepared by simultaneous incorporation of PAR and CIP

In order to explore the influence of the incorporation of both (PAR and CIP) agents in the pore size and distribution, contact angle and drug release have been investigated at different composition of the blends (100/0, 25/75, 50/50, 75/25 and 0/100 wt. % of PLA/PCL). For this purpose, 5 wt. % of PAR and 5 wt. % of CIP were dissolved in the polymer solution. The plan view of these specimens are illustrated in **Figure 108** and the evaluation of pore size and contact angle are shown in **Figure 109**.

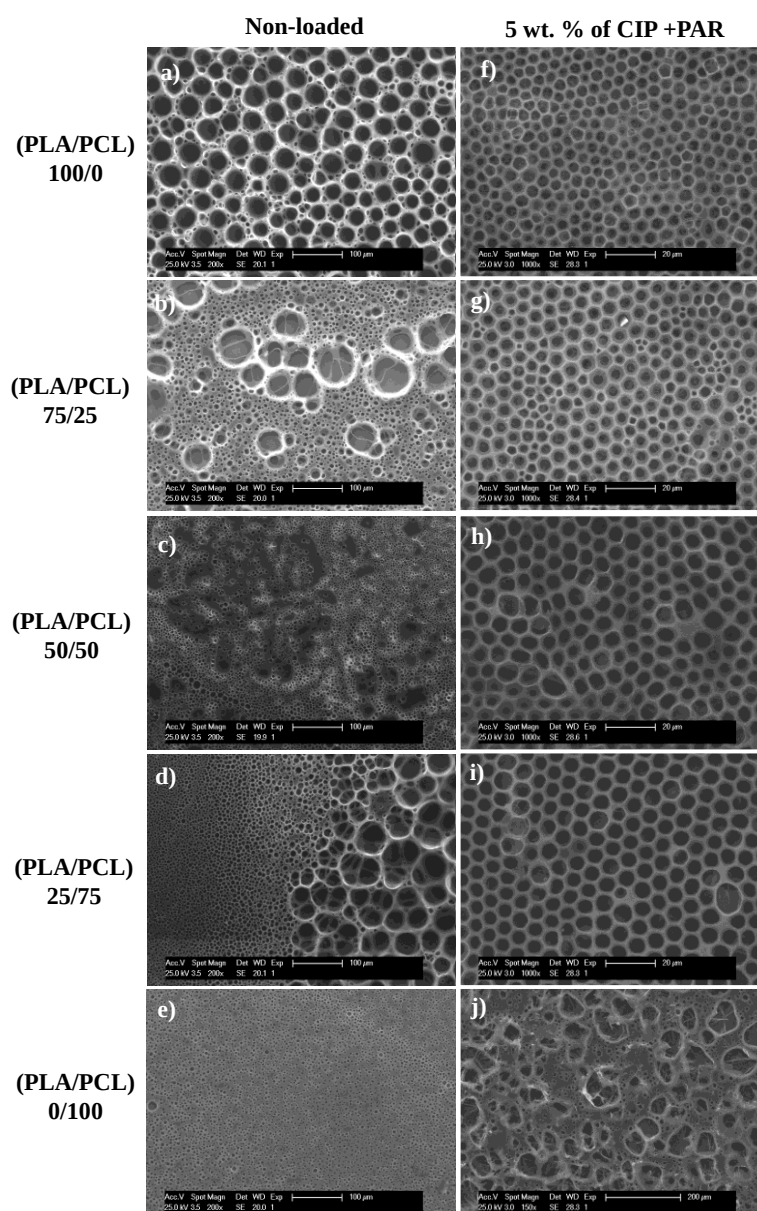


Figure 108. Plan view of (a-e) non-loaded and loaded specimens with (f-j) 5 wt. % of PAR and 5 wt. % of CIP incorporated to different composition of PLA/PCL (25/75, 50/50, 75/25, 100/0 and 0/100) obtained at high relative humidity conditions (>90 %) and polymer concentration of 30 mg/mL for PLA and PCL.

As shown in **Figure 108**, the first important conclusion is that microporous surface were formed in all the cases independently of the blend composition. In the case of PLA, the incorporation of PAR and CIP mixture leads to the decrease of the pores size while keeping the hexagonal packed structure. For all polymer blends, the incorporation of the drug mixture produced regular hexagonal packed micropores with slightly higher pore sizes in comparison to the pristine polymer blends. However, the opposite situation is observed for pure PCL blend where larger and irregular porous surface was generated. This type of hexagonal structures and irregular surfaces, for PCL film, are similar to those in **Figure 106** (k-o), which suggests that the formation of this structure is due the incorporation of CIP in the film that favours the condensation of the condensed microdroplets. It is worth mentioning that the increase of PCL content in the blends (**Figure 108** (f-j)) leads to the increase of the pore size from 5 μm to 9 μm (**Figure 109** (a)). This suggests that the combination of both CIP and PAR agents has an influence on the pore formation, distribution and size.

The influence of PAR and CIP incorporation on the surface hydrophilicity was investigated by static contact angle measurements (**Figure 109** (b)). The incorporation of PAR and CIP agents in the blends leads to a decrease in the contact angle for pure PLA film. However, the opposite situation was observed for pure PCL film, showing higher contact angle compared to PCL non-loaded specimen. In the case of the blends, 4° lower contact angle was observed compared to non-loaded specimens. The increase of PCL content in the blend leads to decrease of the contact angle. Thus, the combination of both CIP and PAR agents has an influence on the wettability of the films due to the hydrophilic nature of both compounds.

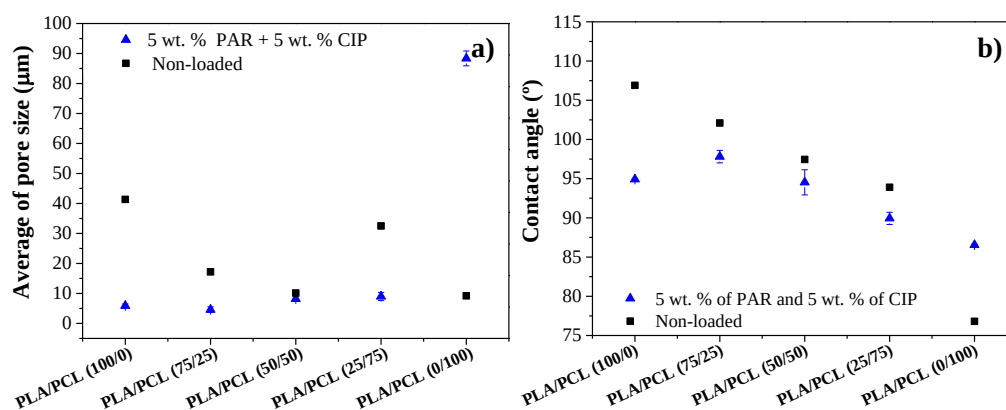


Figure 109. Evaluation of (a) pore size and (b) contact angle of porous films from **Figure 108**.

According to these results, the blend composition and the incorporation of drugs have an important effect on the pore size and its distribution. PAR films form regular porous

surfaces with small pores size while CIP films form regular pores surface with hexagonal packed structures.

In the case of variations in surface wettability, PAR incorporation shows two different trends: blends with low PCL content exhibited lower contact angle, while the opposite situation was observed for high PCL content in the blend. Furthermore, the increase of PCL in the blends leads to an increase of the contact angle of the loaded specimens. Similar trend was observed for CIP incorporation. However, in CIP loaded blends the contact angle remains relatively constant with the increase of PCL content. Non-significant differences were observed with the increase of PAR or CIP (2 and 5 wt. %) concentration for all blends studied.

The incorporation of a mixture of both (5 wt. %) CIP and (5 wt. %) PAR agents leads to the formation of hexagonally packed arrays with lower pore size compared to the individual agents. Regarding wettability, the incorporation of the drug mixture in the blends leads to the decrease of the contact angle for pure PLA film and an increase of contact angle for pure PCL film. In the case of the blends, lower contact angle was observed compared to non-loaded specimens. The increase of PCL content in the blend leads to the decrease of the contact angle.

7.2 Drug release from reference model system

7.2.1 Effect of drug concentration in polymer films on the drug release kinetics in planar PCL and PLA films

In this section, the drug release from the reference model system is investigated. Drug loaded polymer films were placed onto glass substrate (as it has been discussed in Section 7.2) in order to evaluate the effect of the different studied parameters on the drug delivery. For this reason, three different experiments were carried out to understand the effect of (i) drug concentration in planar films, (ii) roughness in porous and planar films, and (iii) multi-loaded porous films. These experiments are carried out by analysing the drug release into 4 mL of inorganic α -MEM solution using UV-Vis spectrophotometer in the range of 200-400 nm.

First of all, it is investigated the effect of drug concentration. The release profiles of PAR and CIP from planar PCL and PLA films are displayed in **Figure 110** after 240 h of immersion in inorganic α -MEM solution at 37°C.

As can be observed in **Figure 110 (a)**, an increase of the drug concentration in the PCL film leads to the increase of the drug release rate. A similar conclusion can be obtained for those films containing PAR in PLA films. The drug release from PCL films incorporating 2 and 5 wt. % of PAR started with a burst release during the first hours of immersion followed by slow and steady release. Similar trend is observed in other report, published by Volokhova *et al.* where PAR is released from electrospun PCL scaffolds⁴⁰⁶. They evidenced that the burst release depends on the drug state (molecular dispersion, particles, crystals etc.) and distribution across the fibre from amorphous regions in the polymer. However, at 10 and 20 wt. % concentration of PAR exhibits a fast release from the beginning which is probably due to the rapid solubilisation of the aggregates trapped in the surface of the film^{407,408}.

In the case of PAR, the release from PLA is prolonged over time followed by a stabilisation of the curve after 160 h of immersion (**Figure 110 (b)**) which is in agreement with this report⁴⁰⁹. It is worth mentioning that PCL exhibits higher PAR delivery in comparison to PLA for all the explored concentrations (1, 2 and 5 wt. %) of PAR. The probable reason for this is the to low glass transition and melting temperature ($T_g = -60^\circ\text{C}$ and $T_m = 60^\circ\text{C}$)⁴¹⁰ of PCL compared to PLA ($T_g = 55^\circ\text{C}$ and $T_m = 165^\circ\text{C}$)⁴¹¹ which allows PCL to be softer at 37°C . This could favour the diffusion of the PAR from the PCL polymer matrix leading to the higher release during the first hour of immersion. In the case of PLA, the high stiffness of the material reduced the diffusion rate of the PAR in the polymer matrix thus leading to longer diffusion times.

The CIP release from PCL and PLA films increases with the amount of CIP within the film. This effect was also observed in the case of PAR in both PCL and PLA films. The release profile in PCL and PLA exhibits an initial burst release, followed by prolonged release over time and stabilisation of the curve after 160 h of immersion until the end of the test (**Figure 110 (c, d)**). This behavior has been also observed in others reports where the CIP was released from polymer microparticles and nanoparticles^{412,413}.

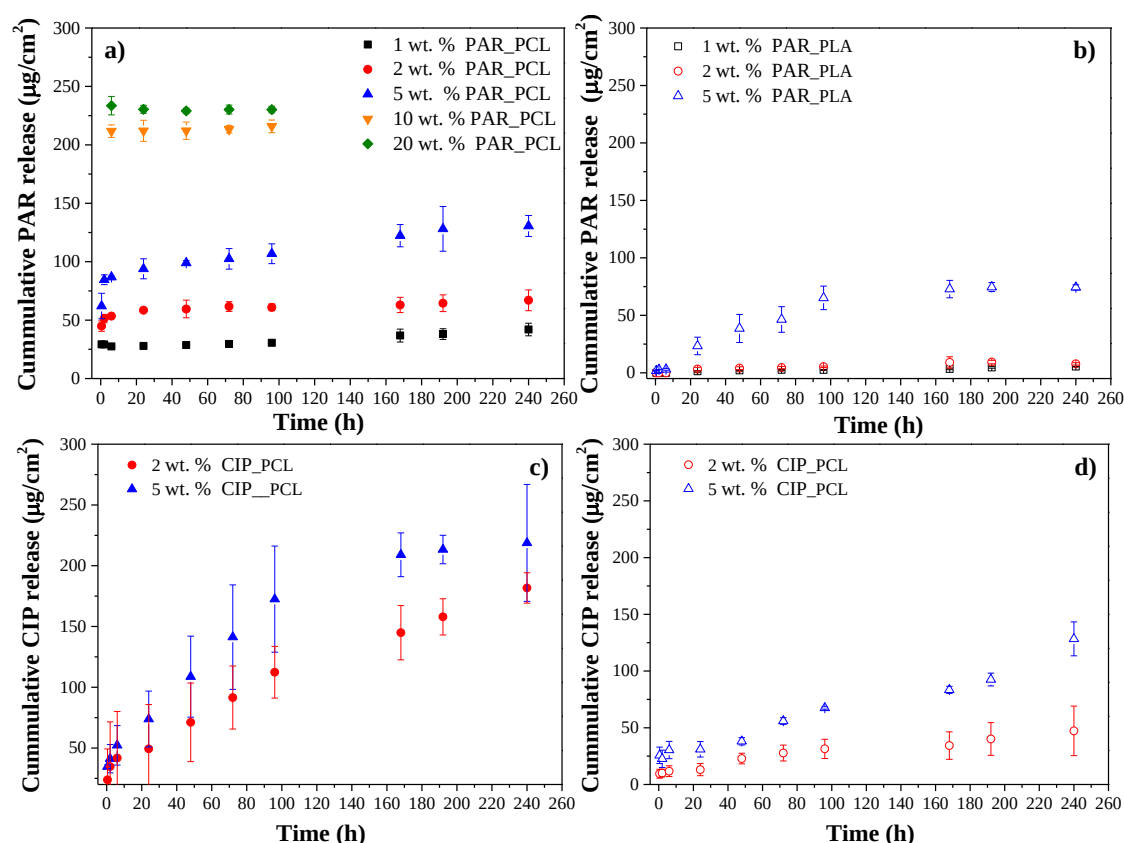


Figure 110. (a, b) PAR and (c, d) CIP release from (a, c) PCL and (b, d) PLA planar films at different drug concentration (1-20 wt. %) during 240 h of immersion in inorganic α-MEM solution at 37°C.

According to these reports the initial burst release can be attributed to the loosely bound drug molecules at the surface of the particle, while the subsequent sustained release reflects the progressive erosion of the polymer and diffusion of the encapsulated drug from deeper layers of the polymer matrix. It is worth mentioning that CIP presented higher release rates from PCL specimens in comparison to PLA at both concentration (2 and 5 wt. %) of CIP. As it was discussed before, this can be associated to the lower T_g of PCL leading to greater diffusion of the CIP within the polymer matrix ⁴¹⁴.

Comparing PAR and CIP at 2 and 5 wt. % in PCL and PLA films, it is observed that CIP exhibit slightly higher release rates in comparison to PAR in both polymers films. This effect may be associated with the solubility differences of PAR and CIP in inorganic α-MEM solution. According to the literature, the solubility in water at 25°C of CIP is 30 mg/mL and of PAR is 14.3 mg/mL ^{415,416}. The CIP release from PCL and PLA films increases with the amount of CIP within the film. This effect was also observed in the case of PAR in both PCL and PLA films.

The incorporation of 1 wt. % of PAR in the films leads to lower drug release rates while the incorporation of 10 and 20 wt. % leads to fast delivery from the beginning of

the experiment. For this reason, in order to reduce the initial burst produced using higher CIP and PAR concentrations the following studies will be carried out with 2 and 5 wt. % of PAR and CIP.

7.2.2 Effect of the surface topography on drug release kinetics: PAR and CIP release from microporous polymer (PLA and PCL) surfaces

In order to evaluate the effect of the topography of the film on PAR and CIP release, porous surfaces (obtained via the BFs method) and planar surfaces were prepared using different blends and different % of both drugs. More precisely, the polymer composition of PLA/PCL was varied: 100/0, 25/75, 50/50, 75/25 and 0/100, and 2 and 5 wt. % PAR and CIP were incorporated in these blends. **Figure 112** presents the 2 wt. % of (a, b) PAR and (c, d) CIP release profiles from (a, c) porous and (b, d) planar surfaces film recorded up to 240 h of immersion in inorganic α -MEM at 37°C.

As it is observed in **Figure 112** (a, b), all cases showed an initial burst PAR release during the first 6 h of immersion followed by a slow and steady release. This burst release may be associated with the fraction of PAR that is on the surface of the sample, while the rest of the release may be associated with diffusion of the drug out of the polymer matrix.

On the one hand, PAR release from porous blends (**Figure 112** (a)) increases with the increase of PCL content in the blends. This may be associated with the low T_g of PCL, that allows a faster diffusion of PAR through the polymer matrix, as it was discussed in the previous Section (7.3.1) and previous report ⁴¹⁷. Similar trend is observed for planar films (**Figure 112** (b)), where an increase of PCL content in the blend leads to increase of the PAR release. Comparing porous and planar films, it is observed that porous films, for all blends, exhibited higher PAR release by 2-4 times compared to planar films. This suggests that porous films have a higher exposed area to the medium compared to planar films. This could be described by Wenzel model, which assumes that the liquid droplet has a complete contact with the rough surface ⁴¹⁸.

On the other hand, for CIP release from porous films (**Figure 112** (c)) an initial burst release during 6 h of immersion followed by a slower release over a longer period of time is observed. This burst release can be associated with the fraction of drug on the surface, while the subsequent sustained release reflects the diffusion of the encapsulated drug from the deep layers of the polymer matrix. Furthermore, non-significant differences are found

in all porous films except for PLA/PCL (25/75), that showed lower CIP release during the first 100 h of immersion.

According to the literature, Maxime *et al.*⁴¹⁹ evaluated the CIP release from PCL/Poly(DL-lactide) (PDLLA) blends after 23 days of immersion in PBS. They demonstrated that the CIP release is largely dependent on the polymer blend composition, an increase of PCL in the blends leads to an increase of CIP release due to the higher diffusion of CIP in the polymer matrix. However, our results differed and this tendency was not observed. Taking this into account, the observed behavior may be associated with the combination of two factors: (i) pore size, where all blends showed quite similar pore size and therefore similar exposed area to the medium, and (ii) the binding affinity⁴²⁰ of the CIP to PCL and PLA, being similar in both cases.

Planar films (**Figure 112** (d)) presented a linear increase of CIP release from pure PCL during the first 120 h of immersion followed by slow release over longer periods of time. In general terms, the increasing PCL content in the film leads to an increase of CIP release, being higher for pure PCL and lower for pure PLA. This phenomenon suggests that the increase of PCL content allows an easier diffusion of the CIP in the polymer matrix due to the low T_g of PCL, as it was mention in the previous Section 7.3.1.

Comparing planar and porous surfaces, it can be concluded that planar films exhibited higher CIP release rates compared to porous films. The possible reason for this is that in a porous films, during the first hours of immersion there is a quick release rate of CIP due to the drug being trapped on the surface of the sample (**Figure 111** (a)). However, the diffusion of the rest of CIP is limited over time because the drug is trapped deep in the inner dense region of the film, and its diffusion outwards takes time, as it is illustrated in **Figure 111** (b).

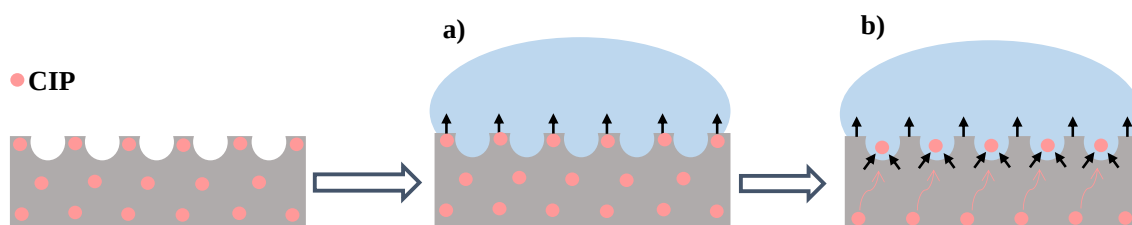


Figure 111. Scheme of CIP release from porous films. (a) First 6 hours of immersion and (b) over time.

In general terms, PAR and CIP profiles release from porous films are similar, lying in the range of 60-160 $\mu\text{g}/\text{cm}^2$ and 100-160 $\mu\text{g}/\text{cm}^2$, respectively over 260 h. This suggests, that the solubility of CIP in water is not a limiting factor for drug release, as it was proposed before Section 7.3.1. Therefore, the drug binding affinity to PCL and PLA could

also play a key role. For planar surfaces it is evidenced that blends incorporating CIP show a higher drug release compared to blends incorporating PAR. Accordingly, this may be associated to the binding affinity of the drug to the polymers (PLA and PCL) and/or the solubility of the drug in the medium (being higher for CIP).

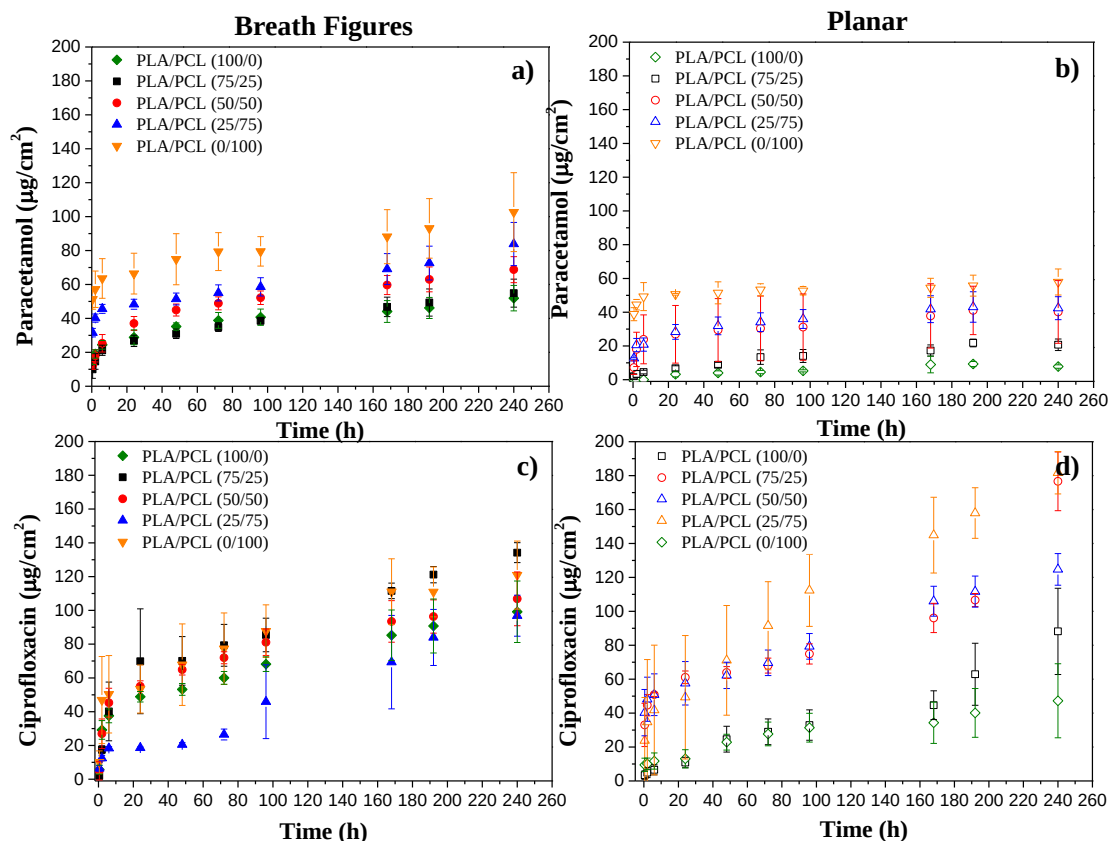


Figure 112. Drug release of (a, b) PAR and (c, d) CIP at 2 wt. % from different composition of PLA/PCL 100/0, 25/75, 50/50, 75/25 and 0/100) and from (a, c) porous and (b, d) planar films.

In **Figure 113** (a), it can be observed that in all cases with greater initial load (at 5 wt. %) an initial burst of PAR release occurs during the first 6 h of immersion followed by a slow and steady release. Non-significant differences of PAR release are observed for pure PCL and those blends with higher PCL content (25/75 and 50/50 of PLA/PCL). Similar trends were observed for pure PLA and the 75/25 of PLA/PCL blend. These tendencies suggest two different behaviors depending on the amount of PCL in the blend. Those prepared with PCL >50 % present a release profile similar to the one observed for pure PCL, however, those with PCL <50 % in the PLA/PCL blends will have the same drug release behaviors as for pure PLA.

The blends 25/75 and 50/50 (PLA/PCL) and pure PCL present a slightly higher PAR release in comparison to the release measured for films prepared using either the 75/25 (PLA/PCL) blend and/or pure PLA. To the best of our knowledge, this may be attributed

to the combination of two main factors: the diffusion of PAR in the polymer matrix and the presence of pores and their size. The increase of PCL content allows for an easier diffusion of the PAR in the polymer matrix due to the low T_g of PCL.

A similar trend to that of porous surfaces is observed for planar films (**Figure 113 (b)**), where higher release is obtained for blends with high PCL content because of the faster diffusion of the PAR in the PCL regions ⁴¹⁷.

Finally, comparing planar and porous surfaces, both types of films present similar behavior of PAR release except for pure PLA film and PLA/PCL(75/25) blend, that showed slightly higher PAR release in porous films. The possible reason for the obtained similarity in PAR release trends may be attributed to the fact that the pore size and distribution in PAR-loaded blends is quite heterogeneous (**Figure 104 (k-o)**) do not allow for a further exhaustive analysis). Although pore formation is observed for all samples, the pore sizes change from one sample to another, this introduces a rather large dispersion in measured released values. According to the obtained results, it may be concluded that, due to this variability, the PAR release from porous and planar films is practically independent of the surface topography.

According to **Figure 113 (c)**, the CIP release profile, measured for the porous films and different blends compositions, present a burst release during the first 3 h followed by a slower release over longer periods of time. This burst release can be associated with loose bounding of the drug to the surface, while the subsequent sustained release reflects the diffusion of the encapsulated drug from deeper layers of the polymeric matrix. As a result, the release profile can be divided into two different regions, i.e. (i) initial release at short periods of time and (ii) subsequent release at long periods of time. On the one hand, a short period of time (from 10 to 120 h) shows that pure PCL and pure PLA films exhibit a higher CIP release than PLA/PCL blends. PLA/PCL blends present almost the same quantitative CIP release. On the other hand, at long period of time (from 160 to 240 h) a plateau is observed and non-significant differences of CIP release are observed for all cases, pure polymers and blends.

Planar films (**Figure 113 (d)**) presented a linear increase of CIP release during the first 120 h of immersion followed by slow release over longer period of time. In general terms, the increase of PCL content in the film leads to the increase of CIP release, the latter being higher for pure PCL and lower for pure PLA. This phenomenon suggests that the increase

of PCL content allows for an easier diffusion of the CIP in the polymer matrix due to the low T_g of PCL.

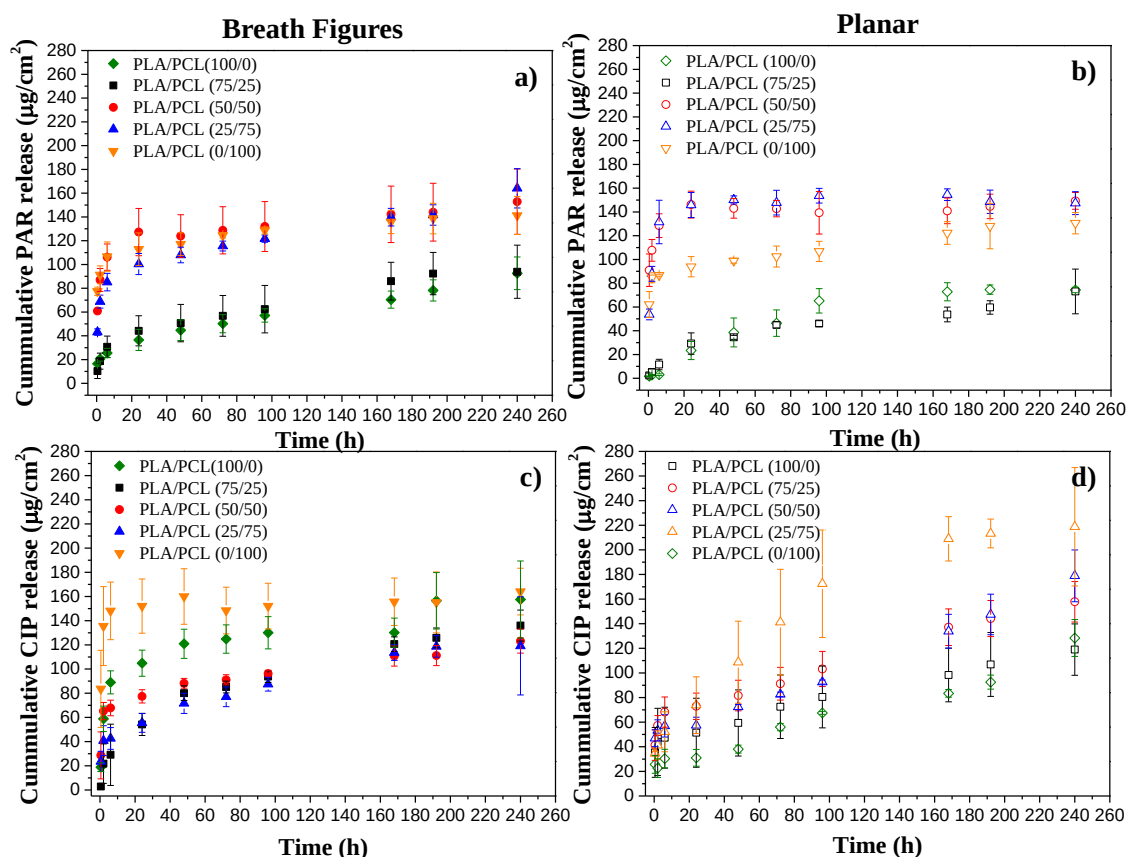


Figure 113. Drug release of (a, b) PAR and (c, d) CIP at 5 wt. % from different composition of PLA/PCL 100/0, 25/75, 50/50, 75/25 and 0/100 and from (a, c) porous and (b, d) during 240 h of immersion in inorganic α -MEM solution at 37°C.

Comparing the planar and porous films, it can be noted that the planar films shows a slightly higher CIP release than porous films, same as in the case of samples loaded with 2 wt. % of CIP. The possible explanation is that for porous films in the first hours of immersion there is a high rate of CIP release because the drug is on the surface of the sample (**Figure 111** (a)). Whereas the diffusion of CIP is limited over time because the drug is trapped inside the pores, preventing its diffusion out of the sample, as it is displayed in **Figure 111** (b).

The comparison between PAR and CIP release from porous films is largely similar for both drugs. However, planar surfaces incorporating CIP showed a higher drug release compared to PAR-loaded samples, this may be associated to the combination of two factors. i) binding affinity of the drug to the PLA and PCL polymers and ii) the solubility of the drug in the medium. This trend is similar to the results of **Figure 112** which suggests that the PAR and CIP release behavior is independent of the drug concentration.

In regards to the effect of the drug concentration, in general terms for all PAR-loaded samples, porous and planar films showed a higher PAR release at 5 wt. % compared to those at 2 wt. %. This behavior is in agreement with the results obtained in the previous Section 7.3.1. However, non-significant differences were found between 2 and 5 wt. % for CIP blends suggesting that the incorporation of higher amount of drug does not affect the drug release trend. The possible reason for this behavior may be associated with the distribution of the CIP needles in the polymer layer due to the low solubility of CIP in chloroform. The needles in contact with the medium will be dissolved and released, while those that are trapped within the matrix will take longer to come out due to the poor diffusion of the needles within the polymer matrix. For this reason, it is possible that if the test were performed for longer periods of time for the samples with 5 wt. % of CIP, higher cumulative CIP release could be observed over time.

According to these results, it is possible to modulate the pore size via blends of polymers in order to obtain an enhanced control over the drug release along the first hours of implantation. Therefore, porous surfaces are selected for the following studies of the release of a mixed load of the two drugs, each at 5 wt. %.

7.2.3 Multi-drug loaded porous polymer films: effect of the simultaneous incorporation of both PAR and CIP within the films on the drug release

In order to explore the influence of the combination of both (PAR and CIP) agents on the drug release, porous films were prepared using PLA/PCL blends incorporating 5 wt. % of PAR and 5 wt. % of CIP. The measurements of PAR and CIP release from multi-loaded system were carried out separately. **Figure 114** discloses the release profiles of (a) PAR and (b) CIP loaded at different PLA/PCL blends (100/0, 25/75, 50/50, 75/25 and 0/100 wt. %) after 240 h of immersion in inorganic α -MEM at 37°C.

A burst release of each drug is observed at the 1st hour followed by a sustained delivery over at least 240 h of immersion. The increase of PCL in blends leads to the slight increase of PAR and CIP release except for 75/25 (PLA/PCL) that is slightly higher compared to the rest of the blends. This may be attributed to the competing effect of two factors: (i) the PCL content in the film and (ii) pore size. The higher PCL content allows the easier diffusion of the PAR and CIP in the matrix due to the lower T_g of PCL, as it was discussed before. The increase of pore size leads to lower exposed area to medium and then lower drug release, which is in accordance with the higher drug release of 75/25 (PLA/PCL) by the small pore size (**Figure 107 (b)**).

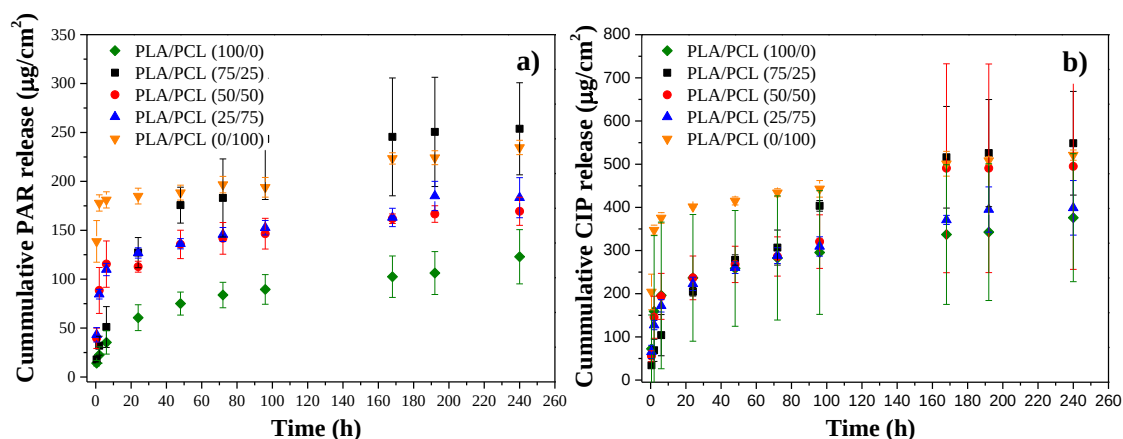


Figure 114. (a) PAR and (b) CIP release from porous films incorporating a mix of 5 wt. % of PAR and 5 wt. % of CIP during 240 h of immersion in inorganic α -MEM solution at 37°C.

Comparing these results those for mono-drug loaded systems (**Figure 113** (a, c)) it is observed that the combination of both drugs in the porous films leads to the increase of each drug release by 2 and 7 times for PAR and CIP, respectively. possible synergetic interactions of CIP with PAR leading to an accelerated release of each. This is consistent with other reports where the influence of orally administered CIP and PAR on the human body was evaluated^{421,422}.

Taking into account these result, the following can be summarized:

- *Kinetic study for the effect of PAR load in the polymer planar films.* The increase of drug load (studied from 1 to 20 wt. % of PAR) leads to an increase of PAR release. PLA films present lower drug release compared to PCL as PLA presents a higher T_g reducing the diffusion of the drug in the polymer matrix.
- *Effect of the surface topography on PAR release at 2 and 5 wt. %.* Porous blends showed an increase of PAR release with the increase of PCL content in the film. Similar behavior was observed for planar surfaces, which is attributed to the lower T_g of PCL. For 2 wt. % loaded samples, porous surfaces showed higher PAR release compared to planar surfaces, while for 5 wt. %, porous and planar surfaces showed similar behavior of drug release due to the higher exposed area to the medium in porous surfaces. The increase of PAR concentration from 2 to 5 wt. % leads to the increase of PAR release for all blends, porous and planar surfaces, due to the more uniform distribution of PAR in the polymer layer controlling the drug release
- *Effect of the surface topography on the CIP release at 2 wt. %.* Non-significant differences of CIP release are found for all porous blends as the CIP release depends on the pore size and the biding affinity between drug and polymer. Two different tendencies are observed at short and long release periods of time for 5 wt. % of CIP.

For short periods of time pure PCL and pure PLA films exhibited higher CIP release than PLA/PCL blends, while non-significant differences of CIP release are observed for all cases at long periods of time.

The same CIP release behavior was observed for both CIP concentrations (2 and 5 wt. %). i) For planar surfaces, the increase of PCL content in the blends increased the CIP release, which is associated to the lower T_g of PCL. ii) For porous and planar films, similar final CIP release was observed, the main difference is the release period, as CIP is trapped in the porous over the time preventing its diffusion out of the samples, then its diffusion depends on the distribution of the needles in the polymer matrix.

- *Multi-drug loaded systems.* The combination of PAR and CIP in the blends leads to a synergistically increased drug release in comparison to the films prepared with mono-drug load.

In order to probe the level of corrosion protection provided by a hybrid hierarchical coating system to a Mg alloy, the degradation rate of drug-loaded systems will be evaluated in more detail in the next section. Due to the high complexity of drug release from the blends with different composition of PCL/PLA and due to the insufficient knowledge of the degradation process of the blends, mono-drug loaded pure PCL, has been selected for investigation. PCL is a polymer with a low degradation rate although it presents poor mechanical properties. PCL films with 5 wt. % of PAR or CIP are selected in order to evaluate the corrosion inhibiting and accelerating effects of the drugs during - short- and long-term degradation of 3D hybrid hierarchical Mg₃Zn_{0.4}Ca alloy-based systems by EIS, hydrogen evolution test and local electrochemical measurements.

7.3 Functionalization of hybrid hierarchical coating on Mg₃Zn_{0.4}Ca alloy

7.3.1 Generation of hierarchical polymer top-coat.

Using the optimized conditions, identified in Chapter VI and Section 7.3, a two-step procedure for the preparation of the loaded hierarchical polymeric top-coat on ceramic PEO layer is proposed as follows, using dip coating technique and BF method. A 75 mg/mL PCL concentration is used in each one of the steps (using conditions optimized in the previous Chapter). The first step involves the formation of a sealing polymer layer on Mg₃Zn_{0.4}Ca/PEO-S-3Ca specimen at 0.3 mm/s of withdrawal speed and a single dip coating cycle at a constant low relative humidity of <40 %. This layer is carried out in

order to increase the adhesion between ceramic and polymer layer by infiltrating the polymer into the cracks and pores of the ceramic layer, improving the corrosion protection of the system. In the second step, a loaded porous polymer top-coat layer is developed by BF method using 2 dip coating cycles at 2 mm/s at a high relative humidity of 98-99 % in an hermetic closed chamber (**Figure 115**). The PAR and CIP agents were incorporated into the polymer solution using 5 wt. % of drug only in the second step.

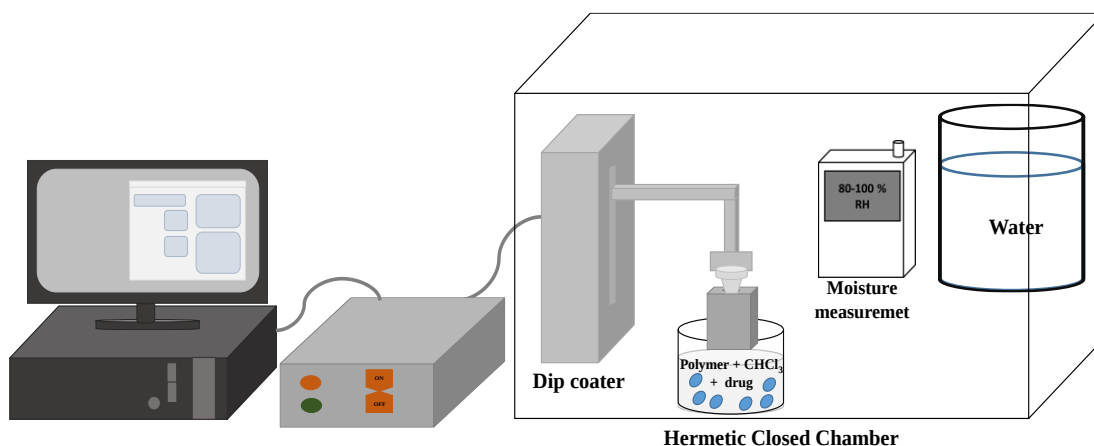


Figure 115. Preparation of loaded porous hybrid ceramic-polymeric materials.

In order to evaluate the drug release from these systems, specimens were immersed during 240 h in 4 mL of inorganic α -MEM solution at 37°C. At regular time intervals, an aliquot of 2 mL was removed from the solution and then added back in order to maintain the sink condition constant. The collected aliquots are analysed using UV/VIS spectrometer at $\lambda_{\text{max}} = 245$ nm for PAR and 320 nm for CIP in triplicate.

The experiments described above allowed us to determine the drug release behavior of PAR and CIP in the polymers employing a glass substrate as model. This section is devoted to taking advantage of the knowledge acquired previously (see Section 7.3.1), by exploring the drug release process in hybrid hierarchical polymer-ceramic coatings.

7.3.2 Characterization of loaded hybrid hierarchical coatings

In order to explore the pore formation, the water contact angle and the drug release from functionalized hybrid hierarchical coating (HHC) were evaluated. The results of the study of these parameters are illustrated in **Figure 116** and **Table 49**.

Figure 116 shows the SEM images of loaded porous surfaces of HHCs where a homogeneous pore distribution can be seen for both PAR and CIP specimens. The pores in CIP specimen exhibit a larger size, around of 12.2 ± 1.1 μm , while for PAR specimen

smaller pores, around $4.5 \pm 0.8 \mu\text{m}$, are formed in comparison with non-loaded PCL specimen ($8.5 \pm 0.3 \mu\text{m}$), as illustrated in **Table 49**. Therefore, it can be concluded that the incorporation of active principles in the polymer layer has an influence on the pores formation, size and distribution. This is in agreement with the observations made in Section 7.2. It is worth mentioning that PAR polygonal particles with different size are incrustated or deposited on the surface of PCL layer (**Figure 116** (a, b)). However, the incorporation of CIP occurs in form of needles penetrating the porous PCL layer (**Figure 116** (c, d)) which is in accordance, with results obtained with stand-alone polymer BF films on glass (**Figure 104** (c)). This may be associated with the solubility of the drugs in the polymer PCL solution, which is lower for CIP compared to PAR.

In terms of wettability, PCL is a practically hydrophobic surface with a contact angle of $88.9 \pm 5.4^\circ$. This is explained by the Wenzel model that assumes that the groove under the droplet is filled with gas⁴²³. Further, if the PCL layer is loaded with CIP and PAR, there is a tendency to slight increase the average contact angle up to $92.3 \pm 7.6^\circ$ for PAR and $96.1 \pm 3.0^\circ$ for CIP. However, these differences cannot be considered statistically significant. According to the literature, PAR and CIP are hydrophilic compounds^{424,425}, although, clearly, hydrophobicity of the PCL_{BF} surface is maintained in their presence.

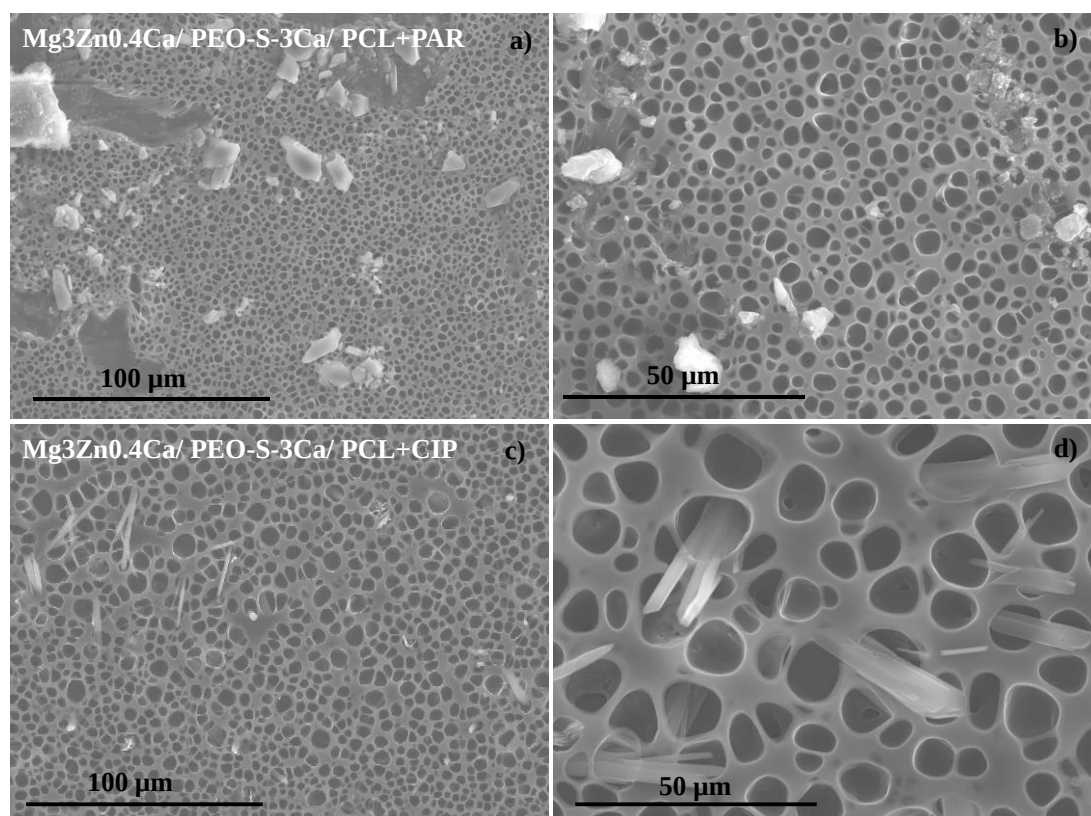


Figure 116. BSE plan view images of (a, b) PCL+PAR and (c, d) PCL+CIP-coated specimens.

Table 49. Evaluation of some parameters of loaded HHC.

Specimens	Pore size (μm)	Porosity (%)	S_a (μm)	Contact angle ($^\circ$)
Mg3Zn0.4Ca/PEO-S-3Ca/PCL	8.5 ± 2.8	40.05	0.60 ± 0.2	88.9 ± 5.4
Mg3Zn0.4Ca/PEO-S-3Ca/PCL+PAR	4.8 ± 0.8	44.75	0.25 ± 0.02	92.3 ± 7.6
Mg3Zn0.4Ca/PEO-S-3Ca/PCL+CIP	12.2 ± 1.1	45.51	0.5 ± 0.1	96.1 ± 3.0

This can be explained by the fact that the roughness and porosity of the surface are not affected by the drug incorporation in a significant way (**Table 49**). The pore size and porosity were evaluated based on **Figure 116** micrographs and the S_a were derived from **Figure A10** (Appendix) analysing the whole area of each 2D micrographs. The incorporation of PAR and CIP revealed a slight tendency to increase the porosity while maintaining the pore size within the same order compared to non-loaded samples. Therefore, the Wenzel model conditions remained practically unchanged, producing the same order of slight hydrophobicity.

7.3.3 Controlled drug release from loaded hybrid hierarchical coating

The next step is the estimation of PAR and CIP delivery from Mg3Zn0.4Ca/PEO-S-3Ca/PCL specimen at different time points. **Figure 117** shows the PAR and CIP release from Mg3Zn0.4Ca/PEO-S-3Ca/PCL specimen during 240 h of immersion in inorganic α -MEM solution at 37°C.

The release profiles of PAR and CIP from Mg3Zn0.4Ca/PEO-S-3Ca/PCL reveal a slow increase of the drug release during the first 120 h of immersion followed by a slower release over the remaining time. The final PAR and CIP release corresponds to 64 and 155 $\mu\text{g/mL}$, respectively, after 240 h of immersion (**Figure 117** (a)). As expected, CIP specimens present higher released fraction of the total load (61 %) compared to PAR (34 %) as it is observed in **Figure 117** (b). The possible reason for the higher CIP release is possibly due to the fact that part of the needles is in contact with the medium. Their solubilisation forms a hole in the polymer layer allowing an easy penetration of the medium into the polymer layer. The limit of PAR and CIP in the human blood plasma for adults correspond to 10-20 mg/mL and 5 mg/mL, respectively, hence the detected release appears to be safely within these guidelines without a risk for liver damage.

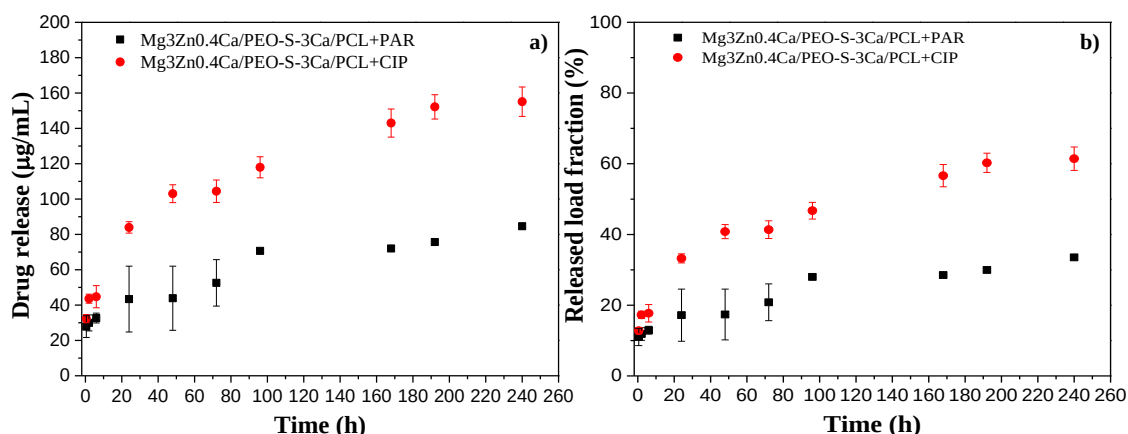


Figure 117. (a) PAR and CIP release and (b) release loaded fraction from $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}/\text{PCL+PAR}$ and $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}/\text{PCL+CIP}$ specimens during 240 h of immersion in inorganic α -MEM solution at 37°C .

Reports of drug eluting from hybrid ceramic-polymeric systems carried out up to date mostly refer to Ti and its alloys^{426,427,428,429}. Only a few works are developed on Mg alloys, which are focused on the design of hybrid material using monolithic top-coat dispersing microspheres/nanoparticles or polymer nanoparticle drug-carriers^{305,304}. The main studied active principles correspond to anti-inflammatory, antibiotics and immunosuppressants agents with the aim to prevent the infection and restenosis avoiding the premature failure of implants^{166,302,430}. According to previous reports, a burst release during the first hour of immersion followed by long-term slow release is observed.

A comparison between the model system (porous PCL film on glass) and real system (HHC on Mg alloy) is illustrated in **Figure 118**. Differences between the models are found, where higher PAR and CIP release from porous PCL films of model system are observed. In addition, these loaded PCL films reveal a burst release of $\sim 50\%$ of the load during the first 2 h of immersion followed by the release of the remaining load (up to 80-97 % of the total) in 48 h. However, in case of $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}/\text{PCL}$, a gradual release over time is observed for PAR and CIP, releasing only 34 and 61 % of the load, respectively, over the period of 8 d (190 h). The possible reason of this may be associated to the fact that Mg^{+2} and Ca^{+2} are chelating with PAR^0 and CIP^0 (Zwitterionic) leading to the formation of insoluble chelates in the physiological medium⁴³¹ reducing the CIP and PAR release. This is in good agreement with other reports where the complexation of different cations with CIP and PAR structures were shown, reducing the bioavailability of the drugs^{432,433}.

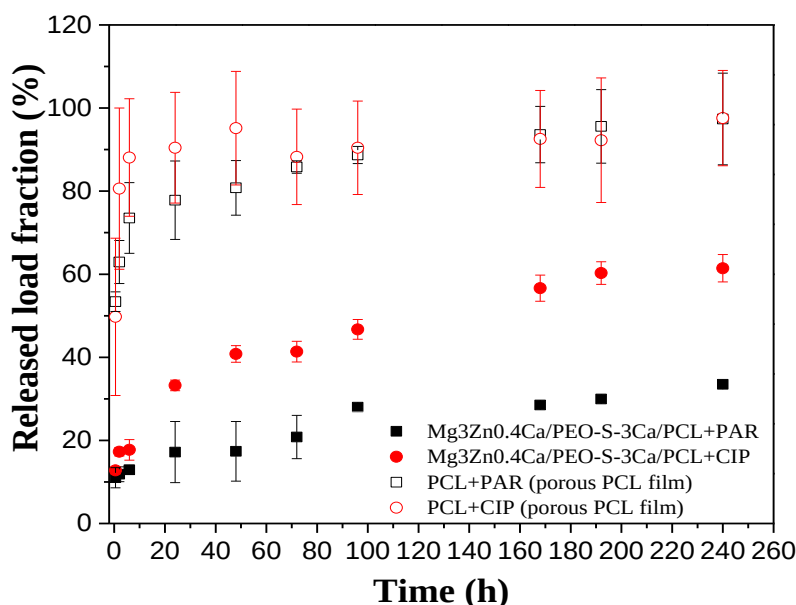


Figure 118. Comparison released load fraction from porous PCL films and loaded hybrid systems.

In addition to the release kinetics, another interesting effect as a possibility for achieving a certain level of protection against corrosion of the hybrid coating when the drug is released. The following section aims to evaluate the inhibitory effect of PAR and CIP in terms of corrosion behavior. For this purpose, the evaluation will be carried out in the short and long term by electrochemical measurements and hydrogen evolution tests.

7.3.4 Corrosion behavior and inhibitor effect of load hybrid ceramic-polymeric materials

7.3.4.1 Electrochemical Impedance Spectroscopy

Figure 119 (a) illustrates the evaluation of the total impedance modulus at low frequencies (0.01 Hz) of Mg3Zn0.4Ca/PEO-S-3Ca/PCL, Mg3Zn0.4Ca/PEO-S-3Ca/PCL+PAR and Mg3Zn0.4Ca/PEO-S-3Ca/PCL+CIP specimens. The test was carried out at over 48 h of immersion (starting from 30 min) in inorganic α -MEM at 37°C maintaining constant agitation. The pH evolution was measured during the test (**Figure 119** (b)). One order of magnitude drop of the impedance value (at low frequencies) due to the characteristic delamination of the PCL layer at the edge of the sample, as described in previous Chapter VI (Section 6.4). These areas appear as the localised corrosion initiation sites (inset macrographs in **Figure 119** (a)). The presence of defects at the edges of the specimens allows an easy access for corrosive species from the medium leading to premature failure of the specimens.

Interestingly, both PAR- and CIP-loaded systems, despite the initially 2-3 times lower $|Z|_{100\text{mHz}}$ values compared to the drug-free system, demonstrate a recovery of the impedance value after 6 h of immersion. After that, Mg3Zn0.4Ca/PEO-S-3Ca/PCL+PAR and Mg3Zn0.4Ca/PEO-S-3Ca/PCL+CIP maintain higher corrosion resistance compared to non-loaded coated samples. This suggests that both agents present an inhibiting effect on the corrosion behavior, which is similar to the observation made in regards to CIP for Ti alloy⁴²⁹. In the present HHC system on Mg alloy, this effect could be attributed to the formation of insoluble chelates such as Mg/Ca-CIP⁴³¹ or Mg/Ca-PAR⁴³³ (Appendix (**Figure A11**)) that could precipitate in the cracks and pores of the PEO coating blocking the access of the corrosive species from the medium. The complexation of these compounds is predicted to be thermodynamically favourable at neutral pH as it was reported in works of pharmacokinetics of fluoroquinolone group to which CIP belongs^{432,434}.

Figure 119 (b) displays the evolution of pH during the test. Similar trend is observed for all specimens with a linear increase during the first 6 h of immersion from 7.18 to 8.6. After 24 h, all of them exhibited a stabilisation of the pH around 8.8-9.0 due to the formation of corrosion products layer. This may be due to the fact that at the beginning the defects at the edges of the sample allow the easy penetration of the corrosive species from the medium causing the acceleration of degradation rate of Mg3Zn0.4Ca alloy. Furthermore, the increase of pH favours the precipitation of Ca-P compounds reducing the degradation rate of Mg, as it was illustrated in **Figure 119** (a).

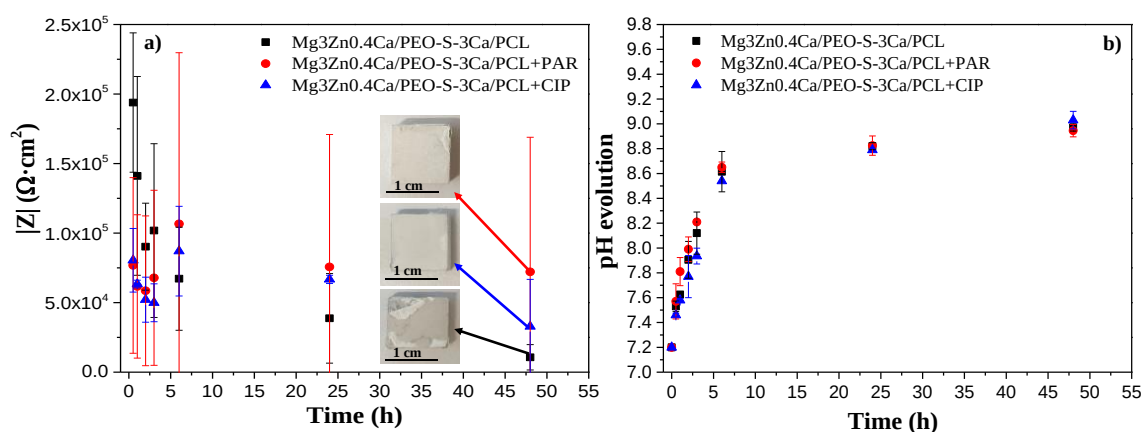


Figure 119. Evaluation of (a) total impedance modulus at 10 mHz and (b) pH evolution of Mg3Zn0.4Ca/PEO/PCL, Mg3Zn0.4Ca/PEO/PCL+PAR and Mg3Zn0.4Ca/PEO/PCL+CIP specimens at 30 min, 1 h, 2 h, 3 h, 6 h, 24 h and 48 h of immersion in inorganic α -MEM at 37°C.

7.3.4.2 Hydrogen evolution test

In order to evaluate the corrosion behavior of these specimens during a long-term period, hydrogen evolution tests were carried out for Mg₃Zn_{0.4}Ca/PEO/PCL, Mg₃Zn_{0.4}Ca/PEO/PCL+PAR and Mg₃Zn_{0.4}Ca/PEO/PCL+CIP over 11 days of immersion in inorganic α -MEM at 37°C under flow of CO₂ at constant pH of 7.4 (**Figure 120**).

As can be seen in **Figure 120**, the slope of the curves for all three systems increase rapidly during the first 3 h of immersion which is attributed to the penetration of the corrosive species through the defects of edges as shown in previous Section (7.4.4.1). Then, the slope remains relatively constant during 120 h of immersion for all specimens due to the formation of corrosive semi-protective layer reducing the degradation rate of Mg. The protective effect of Mg₃Zn_{0.4}Ca/PEO/PCL+PAR and Mg₃Zn_{0.4}Ca/PEO/PCL was maintained only the first 6 days, after that period the degradation rate of these specimens was significantly accelerated by 3-10 times compared to Mg₃Zn_{0.4}Ca/PEO/PCL+CIP. For the latter, the hydrogen volume remained relatively constant during the whole test, this behavior being in agreement with the trend observed during the electrochemical measurements (**Figure 119 (a)**).

Although both agents may form these insoluble chelates with cations, CIP and PAR exhibited different corrosion behavior after 6 days of immersion. The possible reason for this fact may be attributed to the $pK_a = 9.38$ of PAR, which means that at $pH \geq 7.4$, the phenol group of PAR becomes increasingly deprotonated and can facilitate the formation of insoluble chelates with divalent cations^{434,435,436}. It is worth mentioning that the corrosion resistance of PAR-loaded system following 24 h of immersion and above (**Figure 119**) is higher compared to CIP, which can be attributed to the formation of these insoluble chelates of PAR, with the increase of the pH to 9.0.

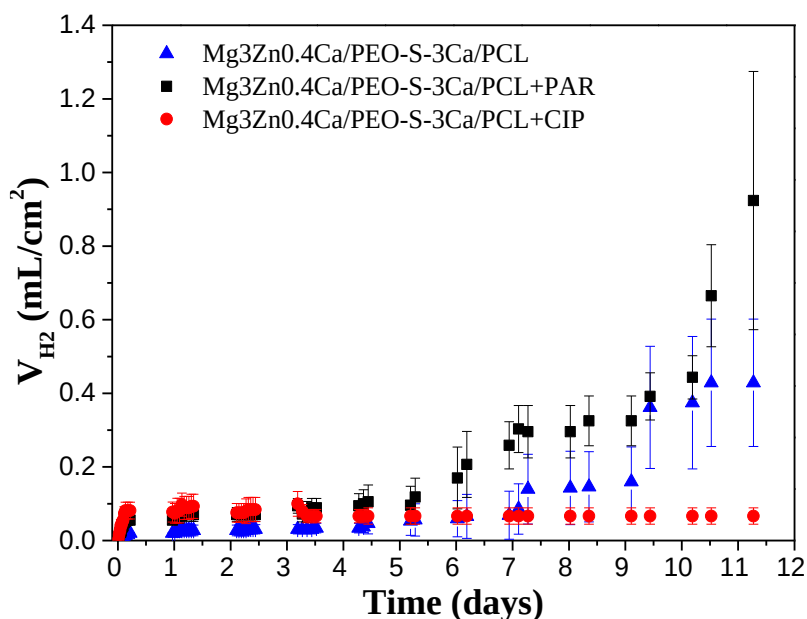


Figure 120. Hydrogen evolution of Mg3Zn0.4Ca/PEO/PCL, Mg3Zn0.4Ca/PEO/PCL+PAR and Mg3Zn0.4Ca/PEO/PCL+CIP specimens after 11 days of immersion in inorganic α -MEM at 37°C under flow of CO₂.

In “quasi-in vivo” H₂ collection system, where the pH is forcibly controlled at 7.4, this deprotonation cannot take place and weaker PAR/Me(II) chelates are formed, hence the increased degradation rate after 6 d of immersion, compared with CIP-loaded system. Also, pH at 7.4 does not favour the stability of Mg(OH)₂, therefore, initially both complex-forming drugs seen to slightly accelerate the degradation rate of the coated systems.

The cross-sectional examination of Mg3Zn0.4Ca/PEO/PCL+PAR and Mg3Zn0.4Ca/PEO/PCL+CIP systems after 24 h of immersion (**Figure 121**) showed the presence of crevice between PCL_{sealing}/PEO layer in both cases. Furthermore, it can be seen that the PEO coating is hydrated (as indicated by arrows in **Figure 121** (b, e)) and corrosion products have formed below PEO coating that contain Cl⁻ (**Table 51**). This suggests that during the first hours of immersion, the PCL_{BF} layer defects present at the edges of the sample allow an easy access of the corrosive species through the polymer delamination crevice to the cracks and pores of the outer PEO layer and the discharge channels in the barrier PEO layer (as seen in Chapter VI, Section 6.4) to the substrate, thus initiating the degradation of the latter.

The inhibition efficiency of PAR and CIP has been calculated using the measured hydrogen evolution rate (RV_H) obtained from the expression ($RV_H = (V_t - V_i) / (T_t - T_i)$), where V_t and V_i are volumes of hydrogen evolved at times T_t and T_i, respectively. The

inhibition efficiency ($\eta\%$) is calculated using the expression $\eta\% = \frac{RV_H - RV_{H(\text{blank})}}{RV_{H(\text{blank})}}$ where $RV_{H(\text{blank})}$ and $RV_{H(\text{inh})}$ are the rates of hydrogen evolution in the absence and presence of pharmaceutical agents⁴³⁷. The results (**Table 50**) indicate that CIP presents an inhibiting efficiency of 75 % while PAR (-252 %) is a severe corrosion accelerator for Mg3Zn0.4Ca/PEO/PCL system. To date, the effect of CIP was only investigated on corrosion of mild steel and bronze, where it exhibited a 86 % and 57 % inhibiting effect, respectively, in HCl at 200 ppm^{151,150} (vs. 160 ppm of CIP in the present Thesis). PAR, on the other hand, was reported to have 80-96 % inhibition efficiency for AZ91 in 30 % of ethylene glycol at 0.01-1 mM of drug concentration¹⁴⁸.

Table 50. Evaluation of the inhibiting efficiency of paracetamol and ciprofloxacin in different materials.

Sample	RV _H	η (%)
CIP	0.01	74.1
PAR	0.09	-252.5
PCL	0.03	-

It is worth mentioning that **Figure 121** discloses more affected areas in the PAR specimen compared to CIP, suggesting the acceleration of degradation rate of Mg alloy by high solubility of Mg/Ca-PAR complexes at neutral pH. However, this observation is not reflected in the hydrogen volume values for PAR-system at 24 hours of immersion, i.e. these could not be discriminated from those for CIP-system. It was suggested that the H₂ dissolved at low corrosion rate⁴³⁸ may be considered the typical cause of error in H₂ evolution test⁵⁸.

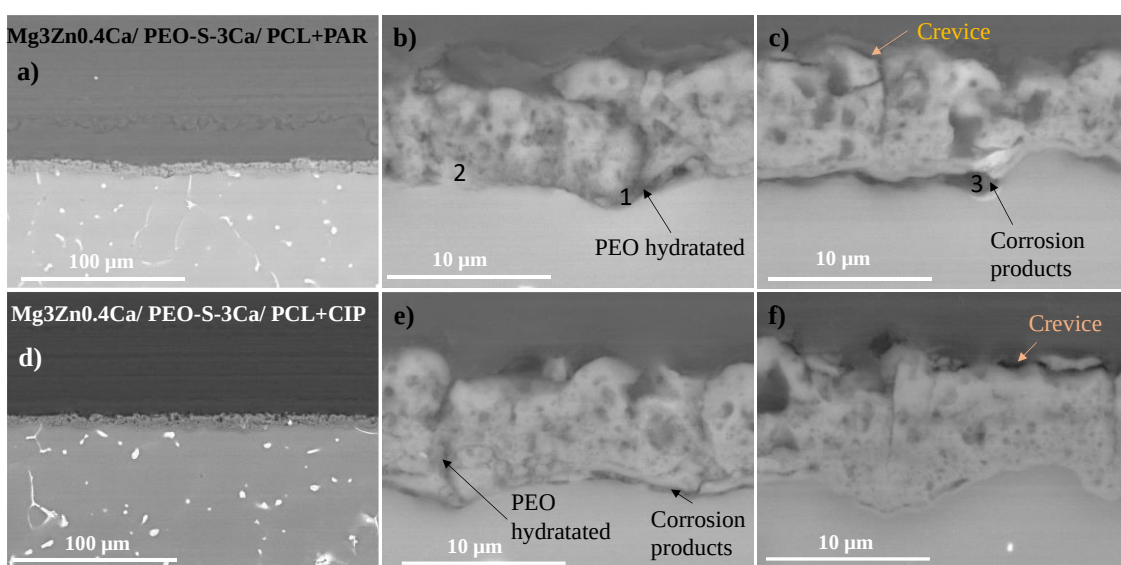


Figure 121. Secondary-electron cross-section images of (a-c) Mg3Zn0.4Ca/PEO-S-3Ca/PCL+PAR and (d-f) Mg3Zn0.4Ca/PEO-S-3Ca/PCL+CIP after 24 h of immersion in inorganic α -MEM at 37°C.

Table 51. EDS analysis (at. %) of corrosion products and PEO layer after 24 h of immersion in Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL+PAR specimen.

Location	O	Na	Mg	Si	P	Cl	K	Ca	Zn	Ca/P
1	40.6	0.4	50.4	5.4	0.5	-	0.1	0.1	2.6	0.25
2	51.0	0.6	40.5	6.1	1.1	0.1	0.1	0.1	0.6	0.07
3	17.2	0.9	69.2	10.1	0.6	0.3	0.2	0.4	1.2	0.73

Therefore, for cases that are outside the resolution capacity of H₂ evolution test, local current density, local pH and dissolved hydrogen monitoring are considered more appropriate for probing the degradation mechanism and inhibiting effect. Considering the results obtained in this section, the Mg₃Zn_{0.4}Ca/PEO/PCL+PAR system can be ruled out from further investigation by local electrochemical methods. Furthermore, this drug has no use in orthopaedic and cardiovascular implants, as its anti-inflammatory effect is not local, it has to be administered orally as the brain regulates the anti-inflammatory function. Nevertheless, it was useful in the present study as a clear illustration of a possibility of a severe corrosion accelerating effect of a drug contained in a HHC, which may lead to a premature implant failure within a few weeks of implantation. Localised measurements on CIP-loaded and CIP-free HHC systems will be carried out in the following section in order to determine the corrosion inhibiting mechanism of the drug.

7.3.4.3 Localised measurements (SVET, local pH and H₂ concentration)

Scanning Vibrating Electrode Technique (SVET), local pH and dissolved hydrogen concentration monitoring are powerful methods to provide information on the local electrochemical activity on the surface of Mg. **Figure 122** illustrates the visual appearances of the specimens and local current density of non-loaded and CIP-loaded specimens after 1, 12 and 24 h of immersion in inorganic α -MEM at 37°C.

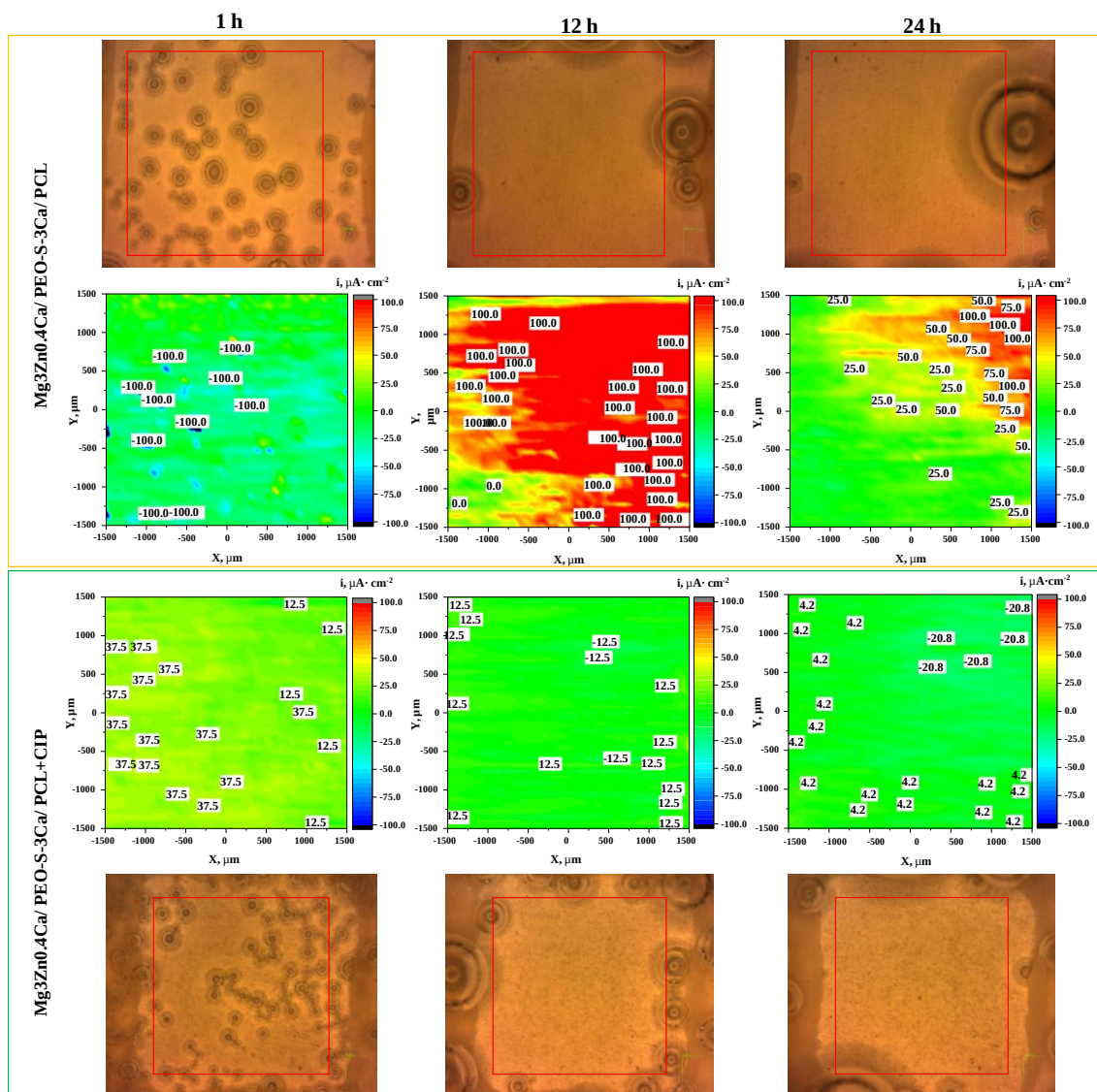


Figure 122. Optical micrographs and SVET current density distribution on non-loaded and CIP loaded Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL samples after 1, 12 and 24 h of immersion in α -MEM at 37°C.

Localized cathodic activity is developed on the surface of PCL while predominantly anodic activity is observed in CIP surface after 1 h of immersion. After 12 h, the current density becomes intensely and overwhelmingly anodic for PCL ($100 \mu\text{A}/\text{cm}^2$), while CIP demonstrates uniformly low current densities that are maintained up to 24 h. The corrosion inhibiting effect of CIP is about an order of magnitude. In PCL specimen the electrochemical activity gradually localises with time. The formation of more cathodic regions is possibly attributed to the increase of pH by the migration of hydroxide ions that occurs at the early stages of corrosion.

Taking into account the concept of H_2 evolution test, the anodic magnesium oxidation ($\text{Mg} \rightarrow \text{Mg}^{2+} + 2\text{e}^-$) and the cathodic hydrogen reduction ($2\text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{OH}^- + \text{H}_2$) occurs on Mg alloys surface. For this reason, the local concentration of dissolved H_2 and

the local pH can be used as indicators of the cathodic reaction. The solubility of H_2 in water is 1.4 mg/mL at 37°C and its local concentration in the corrosive solution is affected by the chemical/electrochemical reactions occurring on the surface of Mg during the immersion.

The visual appearance, the variation of local pH and dissolved H_2 concentration over a period of 24 h for CIP-free and CIP-loaded systems are displayed in **Figure 123** and **Figure 124**, respectively. According to **Figure 123** (b, e, h), the pH in CIP-free system within 1 h of immersion quickly increases from 7.2 (pH of inorganic α -MEM as received) up to 7.95 and then is stabilised at 8.33 after 24 h of immersion, demonstrating a uniform electrochemical activity of the sample surface. This corresponds to the increasing trend of dissolved H_2 concentration, from 1.88 $\mu\text{mol/L}$ at 1 h to 2.17 $\mu\text{mol/L}$ after 24 h of immersion. The dissolved H_2 maps are mostly uniform.

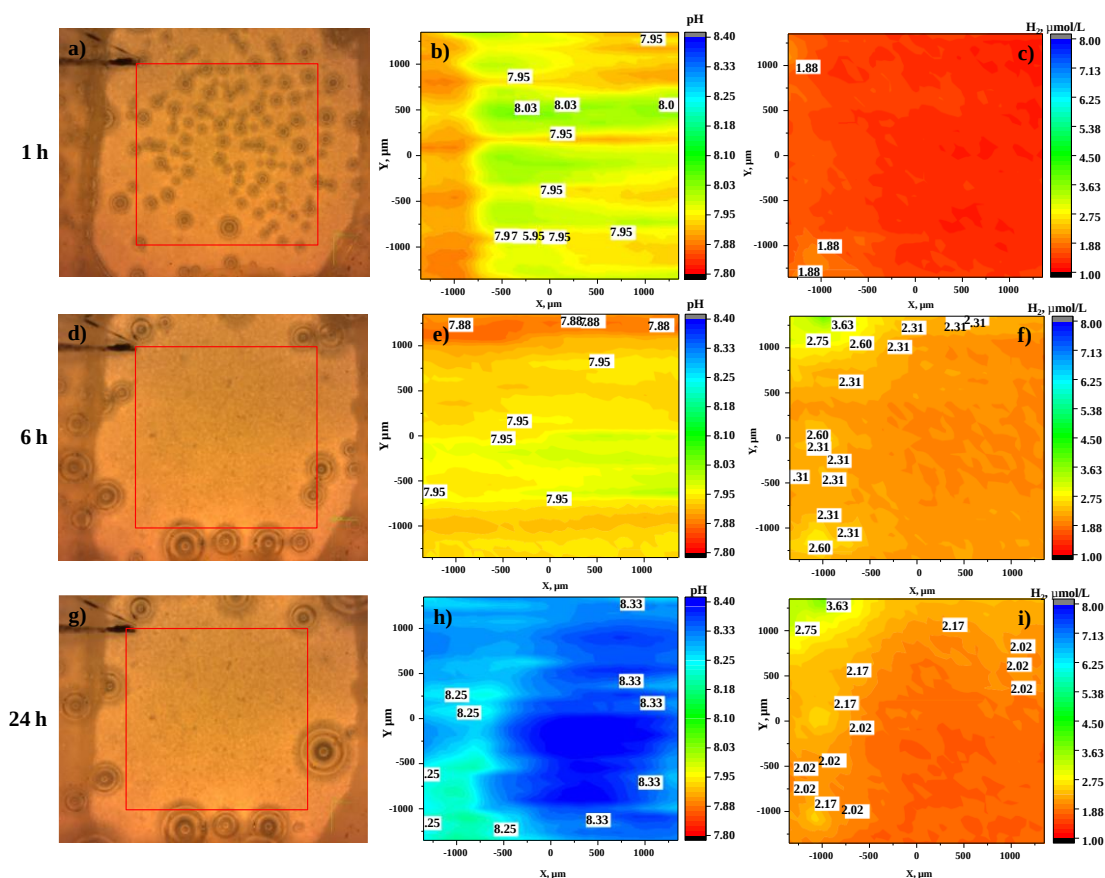


Figure 123. (a, d, g) The visual appearances, (b, c, h) distribution of pH and (c, f, i) H_2 concentration of $Mg_3Zn_{0.4}Ca/PEO-S-3Ca/PCL$ during 1 h, 6 h and 24 h in inorganic α -MEM at 37°C.

In the case of CIP-loaded system, the local pH map reveals a drastic alkalization within 1 h of immersion up to pH 8.33, **Figure 124** (b). However, the pH decreases with time and stabilises around 7.95 after 24 h of immersion (**Figure 124** (e, h)). This acidification

is in discord with the evolution of dissolved H_2 concentration, which increases progressively from 3.19 $\mu\text{mol/L}$ within 1 h of immersion to 6.02 $\mu\text{mol/L}$ after 24 h of immersion (**Figure 124** (c, d, i)). Notably, localised areas with a high amount of dissolved H_2 (green and yellow areas, **Figure 124** (f, i)) appear after 6 h, which suggest an increased corrosion activity, but do not lead to a pH increase.

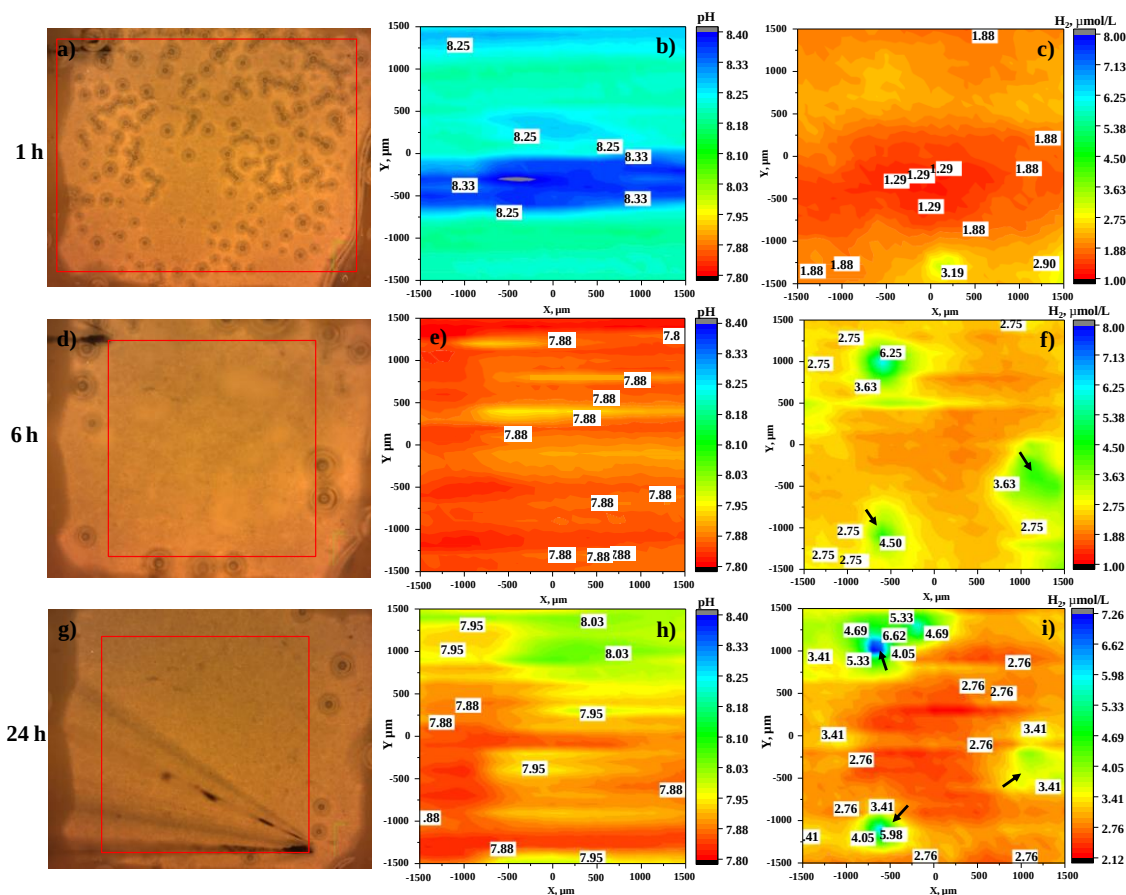


Figure 124. (a, d, g) The visual appearances, (b, c, h) distributions of local pH and (c, f, i) H_2 concentration of $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}/\text{PCL+CIP}$ in inorganic α -MEM at 37°C after 1, 6 and 24 h.

This apparent disagreement between pH maps and dissolved H_2 maps may be explained by the continuous CIP release (**Figure 124**) into the medium. Considering that CIP exhibits two pK_a values of 5.9 and 8.89⁴³⁹, at pH 7.4 it exists as a $\sim 100\%$ zwitterion. When corrosion of Mg shifts the local pH to 8.3, the equilibrium CIP^0 (Zwitterion) $\leftrightarrow$ CIP^- (Anion) is still shifted to the left (**Figure 125**). As CIP is being released from the coating, the increase of the zwitterion CIP^0 fraction reduces the pH⁴⁴⁰. The high release of zwitterionic specie and low pH generates more aggressive conditions, where $\text{Mg}(\text{OH})_2$ is less stable. This tendency should be accelerating the corrosion process, but the opposite happens due to the formation of insoluble Me(II)-CIP chelates ($\text{Me} = \text{Mg}, \text{Ca}$ released from the coating) that precipitate on the surface of PEO coating.

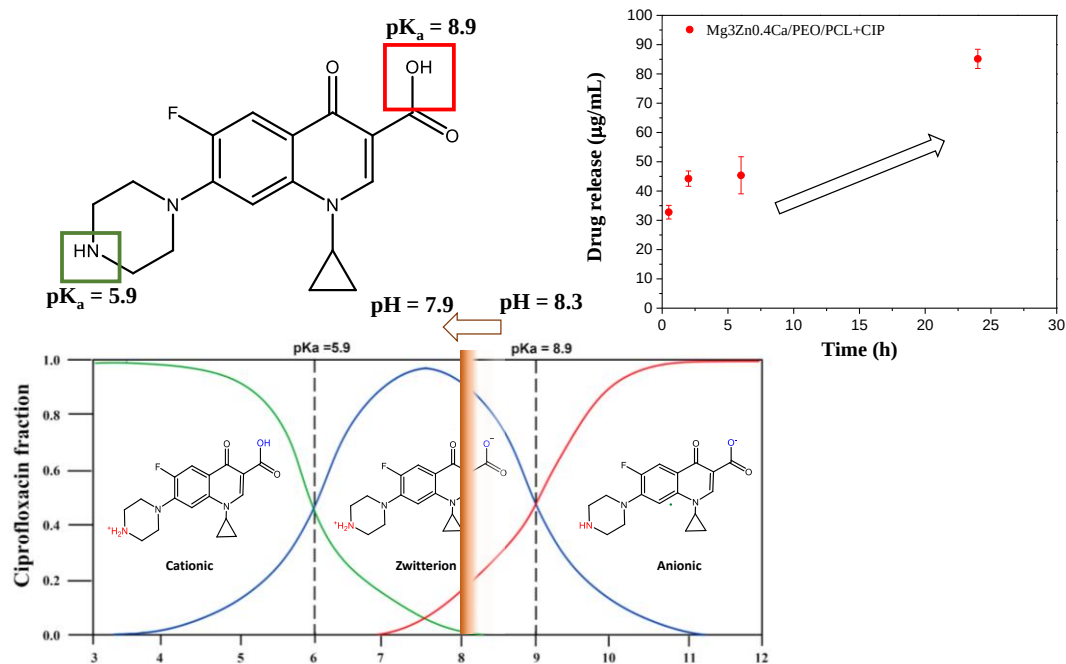


Figure 125. Regulation of pH by CIP zwitterion release.

Therefore, the initial acceleration of Mg corrosion due to the complexing of the released Mg^{2+} ions is followed by the inhibiting action of the precipitated insoluble chelates, which explains the observed trends in local pH, dissolved H_2 and current density activity in SVET maps over 24 h (**Figure 122**).

The cross-sectional SEM examination of Mg3Zn0.4Ca/PEO/PCL and Mg3Zn0.4Ca/PEO/PCL+CIP after 24 h of local pH and dissolved H_2 concentration measurements (**Figure 126**) reveals the formation of crevice at the PCL_{sealing}/PCL_{BF} and PCL_{sealing}/PEO interface in both systems. These crevices lead to the easy penetration of the corrosion species from the electrolyte causing the hydration of PEO layer (**Figure 126** (a-c)) followed by the formation of corrosion products layer under the PEO coating (**Figure 126** (c)). In addition, the presence of these crevices accelerate the corrosion process due to high ingress of chloride ions (**Table 51**, point 2 and 3) as it was discussed in Chapter V (Section 5.2.3). This is in agreement with the results of collected hydrogen volume (**Figure 120**) and local electrochemical measurements (**Figure 122** and **Figure 123**). In the case of Mg3Zn0.4Ca/PEO/PCL+CIP specimen the damage at the PEO/substrate interface is negligible compared with CIP-free specimen, and the coating itself bears lesser signs of hydration.

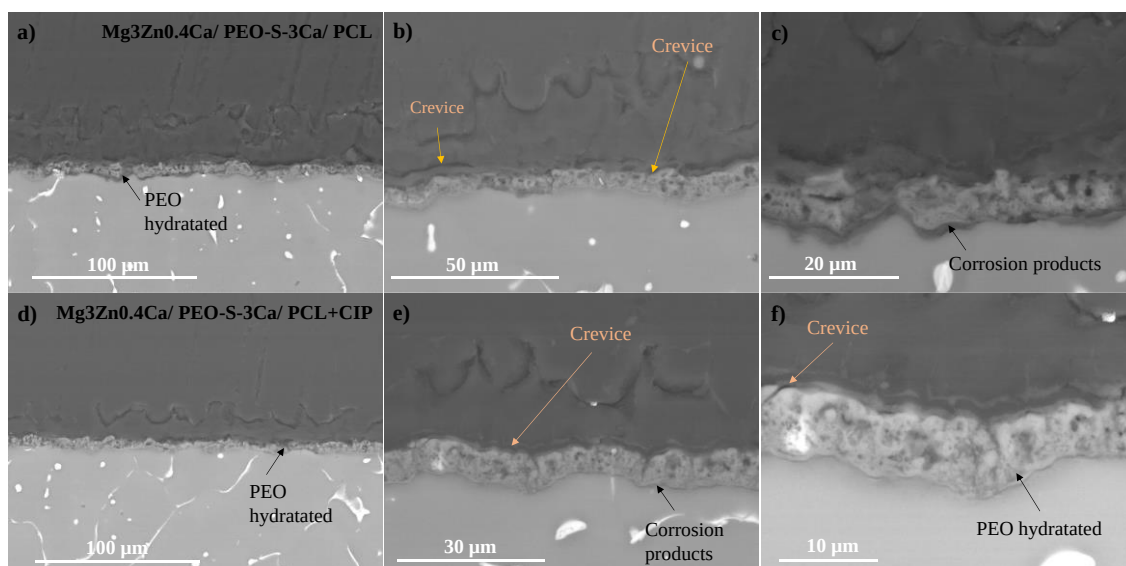


Figure 126. Secondary electron cross-section images of (a-c) Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL and (d-f) Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL+CIP after local pH and local H₂ concentration measurements in inorganic α -MEM at 37°C.

This evidence corroborates the obtained electrochemical and hydrogen evolution results (**Figures 121–127**) and supports the idea of the formation of insoluble chelates whose precipitation helps to seal the cracks and pores of the PEO coating reducing the degradation rate of Mg. Similar conclusions in regards to inhibiting effect of 8-HQ inhibitor loaded into a PEO coating on Mg alloy were reached by Vaghefinazari *et al.*⁴⁴¹. This is not always the case, as the same authors have shown in⁴⁴²: the corrosion inhibiting or accelerating effect of the organic species loaded into PEO coatings will entirely depend on the solubility of the resultant complex compounds. Remarkably, CIP is a corrosion accelerator with respect to bare Mg substrate, undoubtedly due to its complexing ability. However, the Mg(II)-CIP complex precipitates apparently are unable to form a well-adhered protective film on non-surface modified substrate. The porous nature of PEO coatings, on the other hand, has the capacity to trap and lock the precipitates, resulting in an inhibiting effect.

In order to understand the contribution of the electrochemical processes occurring in different parts of the hybrid coatings on their corrosion resistance, a detailed analysis of the previously presented EIS spectra of drug-loaded and drug-free specimens (**Figure 119** (a)) was performed for selected times (1 and 24 h). **Figure 127** displays the resistances of different parts of CIP-loaded and non-loaded hybrid coatings: PCL layer (R_2), outer PEO (R_3) and barrier PEO layer (R_4) resistance in inorganic α -MEM at 37°C. The Nyquist and Bode plots of the EIS spectra and the equivalent circuit for loaded and non-loaded

hybrid coatings are illustrated in **Figure A12** (Appendix). The fitting parameters are presented in **Table A5** of Appendix.

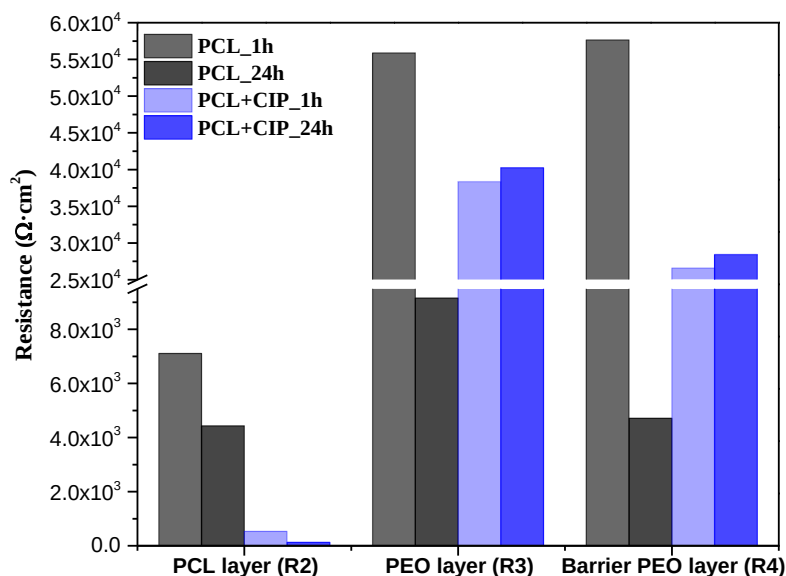
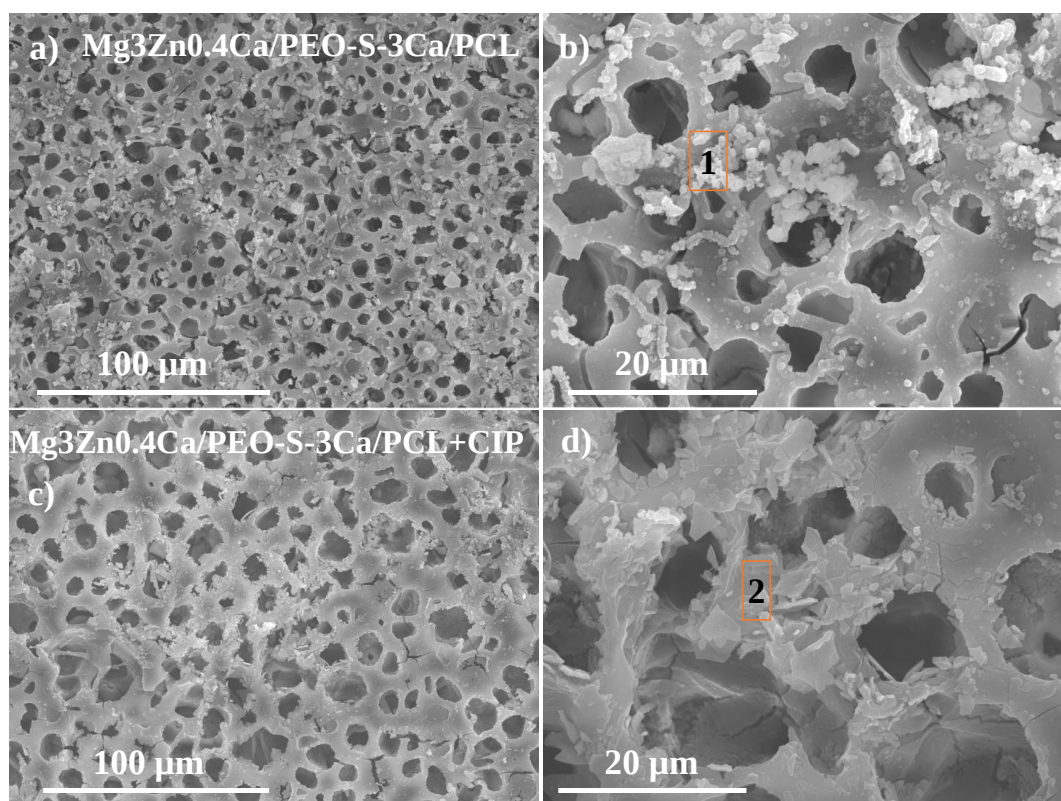


Figure 127. The evolution of resistance of different parts of hybrid coatings: PCL layer (R_2), outer PEO (R_3) and barrier PEO layer (R_4) during 1 and 24 h of immersion in inorganic α -MEM at 37°C for CIP-loaded and CIP-free specimens.

According to **Figure 127**, R_2 , R_3 , R_4 are higher for non-loaded specimen at 1 h of immersion compared to CIP-loaded specimen. R_3 and R_4 decrease for drug-free system after 24 h due to the penetration of corrosive species through the edges defects and the cracks in PCL layer (**Figure 128** (a, b)) leading to the hydration of PEO layer and subsequent degradation of the substrate. The presence of discharge channels in the barrier PEO layer provide additional pathways for corrosive species, as it was discussed in previous Chapter VI (Section 6.4), causing an acceleration of Mg which is in accordance with low R_4 value of drug-free system after 24 h (**Figure 127**). In addition, this is in agreement with higher values of local pH and dissolved H_2 concentration for this system (**Figure 124** (h, i)).

However, the opposite situation is observed for CIP-loaded specimen that retains 3 times higher R_3 and R_4 values after 24 h of immersion. The possible reason for these high values can be found in the penetration of the corrosive species from the medium that allows for the formation of insoluble chelates such as $Me(II)$ -CIP ($Me(II) = Mg^{+2}$ and/or Ca^+ , easily lixiviated from the PEO layer ¹⁰⁵).



e)

Point/ EDS	C	O	Na	Mg	Si	P	Cl	K	Ca	N
(at. %)										
1	59.6	33.3	0.1	0.6	2.7	0.0	0.0	3.3	0.4	-
2	52.5	32.4	0.1	0.5	-	2.5	0.0	0.0	3.1	8.6

Figure 128. Backscattered SEM images of (a, b) Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL and (c, d) Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL specimen after EIS measures (48 h). EDS analysis (at. %) of (b, d) images.

The insoluble chelates are precipitated in the cracks and pores of PEO coatings blocking the passage of the corrosive species from the medium which is corroborated with higher values of R_3 and R_4 compared to non-loaded sample. This is corroborated by morphological and compositional examination of the specimen surface (**Figure 128**). The precipitates formed on the CIP-free specimen surface are discrete Si-rich particles (amorphous SiO₂ tends to form and precipitate at neutral pH as a result of lixiviation of Si from PEO coating)¹⁰⁵. An irregular Ca/P containing plate-like crystals with high N content (EDS analysis of point 2 (**Figure 128** (d, e)) are evident on the surface of CIP-loaded specimen, nitrogen being the characteristic marker of CIP. These complex precipitates also seem to ensure a lesser cracking of the PCL_{BF} layer.

It can be concluded that it is possible to develop drug-functionalised hybrid hierarchical ceramic-polymer coatings with high protective capacity. They could be used

for a multitude of applications, such as orthopaedic implants or cardiovascular stents. This type of coatings may be optimised for immunosuppressive and antiproliferative drugs (e.g. sirolimus, paclitaxel) for stents and other antibiotics (e.g. vancomycin, amoxicillin etc.) for orthopaedic implants. However, it appears imperative to carry out a corrosion screening of the extended range of relevant pharmaceutical agents in order to ensure the adequate durability of the implant while being able to adapt the characteristics of the implant to a specific patient and application by selecting the drug for each individual. The use of biodegradable hierarchical coatings in combination with corrosion screening approach could lead to improved therapeutics in many clinical applications.

7.4 Conclusions of Chapter VII

Evaluation of parameters of the process by reference model system

- The incorporation of PAR in a porous polymer film affects the pore formation. Regular porous surfaces with small pores were formed for pure PLA and 75/25 (PLA/PCL) blend and larger pores for pure PCL, 50/50 and 25/75 (PLA/PCL) blends. The increase of PCL fraction decreases the pore size for 2 wt. % of PAR, while the pore size remained nearly constant for 5 wt. % of PAR. Additionally, for any blend, the increase of PAR content from 2 to 5 wt. % tended to increase the pore size.
- The incorporation of CIP in porous polymer films has an influence on the pore formation and distribution. A regular hexagonal packed structure with small pores was formed in the presence of CIP in all PLA/PCL blends, except for pure PCL that promotes larger pores and irregular porous surfaces. The increase of PCL increases the pore size in the presence of CIP. The increase of the CIP from 2 to 5 wt. % increases the pore size in all the blends.
- By incorporating both drugs (5 wt. % of CIP + 5 wt. % of PAR) in the films, small pores with regular hexagonal packed structure are formed except for PCL film, that formed rather large pores with irregular distribution. This type of structure is associated to the incorporation of CIP, that significantly influenced the pore formation and distribution. The increase of PCL in the blend leads to the formation of hexagonal structures with similar pore size.
- The kinetic study of PAR (from 1 to 20 wt. %) showed that an increase of drug concentration leads to higher drug release. PLA films presents lower drug release compared to PCL as PLA presents a higher T_g .

- Effect of the surface topography on PAR release at 2 and 5 wt. % was revealed. Porous blends showed an increase of PAR release with the increase of PCL content in the film. Similar behavior was observed for planar surfaces. For 2 wt. % loaded samples, porous surfaces showed higher PAR release compared to planar surfaces, while for 5 wt. %, porous and planar surfaces showed similar behavior of drug release. The increase of PAR concentration from 2 to 5 wt. % leads to the increase of PAR release for all blends, porous and planar surfaces.
- Effect of the surface topography on the CIP release was revealed. Non-significant differences of CIP release are found for all porous blends with 2 wt.% CIP load. Two different tendencies are observed at short and long periods of time for 5 wt. % of CIP load. For short periods of time pure PCL and pure PLA films exhibited higher CIP release than PLA/PCL blends, while non-significant differences of CIP release are observed for all cases at long periods of time. The same CIP release behavior was observed for both CIP concentrations (2 and 5 wt. %): i) for planar surfaces, the increase of PCL content in the blends increased the CIP release. ii) Porous and planar films showed similar final CIP release.
- In a mixed load of both PAR and CIP pharmaceutical agents in the blends the CIP release dominates over PAR, which may be attributed to an interaction between PAR and CIP. Additionally, both PAR and CIP present higher drug release compared with the release of a mono-drug load, due to a possible synergistic interaction between the agents.

Evaluation of corrosion inhibiting effect of functionalized hybrid hierarchical coating system on Mg₃Zn_{0.4}Ca alloy

- The pores formation on the top-coat PCL_{BF} layer is influenced by the presence of PAR and CIP in the polymer/chloroform solution. A homogeneous distribution of pores is achieved in both cases, but small pores with a size of 4 µm were formed for PAR-loaded system, while large pores with 12 µm size were formed for CIP-loaded one.
- The PAR and CIP release from Mg₃Zn_{0.4}Ca/PEO-S-3Ca/PCL increases slowly over the time followed by a stabilization of the curve. After 10 days of immersion, CIP specimen showed higher fraction of the released load by two times (67 %) compared to PAR (34 %) due to the easy access of the water into the inner structure of PCL_{BF} layer when CIP needles partially buried in the layer are solubilized.

- The short-term (48 h) corrosion resistance for all samples decreased with time. The drug-free HHC exhibited higher corrosion protection than PAR- or CIP-loaded HCC only during first 3 h of immersion. The drug-loaded system presented overall higher and more stable corrosion protection with time than the drug-free system due to the formation of insoluble chelates that block the passage of corrosive species from the medium through PEO layer reducing the degradation rate of Mg.
- Local current density distribution maps revealed an order of magnitude corrosion inhibiting effect of CIP on PEO/PCL-coated Mg₃Zn_{0.4}Ca alloy. Local pH, dissolved H₂ concentration and EIS measurements together with the drug-release profile and analysis of acid-base equilibrium of CIP disclosed the corrosion inhibition mechanism. 74 % of inhibition efficiency in “quasi-in vivo” conditions is achieved due to the formation of insoluble chelate complexes of CIP with Mg and Ca cations released from coated Mg, that seal the coating system defects reducing the degradation rate of Mg₃Zn_{0.4}Ca.

In conclusion, these results are highly relevant to understanding the relation between the influence of drug loading and release on corrosion behavior of Mg alloys with functionalized hybrid hierarchical coating systems, which is the first step towards the development of customized corrosion protection systems for implants in orthopaedic or cardiovascular applications.

8. CONCLUSIONS

The design and development of biodegradable materials is one of the greatest challenges for applications in temporary orthopaedic and cardiovascular implants. In this Thesis, a new hybrid hierarchical material based on magnesium alloys is investigated combining two different levels of functionalization: electrochemical modification by plasma electrolytic oxidation and deposition of a biodegradable polymer layer using a *Breath Figures* and Dip coating methods. In Chapters IV and V, different magnesium alloys and their surface modification produced by plasma electrolytic oxidation have been characterized and tested in terms of corrosion behavior. In Chapters VI and VII, hybrid hierarchical coating systems and their drug-loaded versions have been investigated for Mg₃Zn_{0.4}Ca alloy in terms of corrosion behavior and drug delivering capacity. The most relevant findings of this work are summarized as followed:

❖ Chapter IV

As-cast Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloys consisted of α -Mg grains and a discontinuous network of secondary phases distributed along the grain boundaries. Eutectic aggregates of α -Mg/Mg₂Ca and polygonal particles with impurity phases were located inside the grains in Mg_{0.6}Ca alloy, while the Mg_{0.6}Ca₂Ag alloy presented binary Mg-Ag particles with impurities. Surface potential maps obtained by SKPFM for Mg_{0.6}Ca alloy revealed anodic behavior for Mg₂Ca phases (-50 mV) while particles with impurities exhibited cathodic behavior (20 mV). For Mg_{0.6}Ca₂Ag, particles with Ca/Ag ratio <1 (enriched in Ag) exhibited cathodic behavior (20 mV); Ca/Ag >2 (enriched in Ca) led to anodic behavior (- 27 mV).

The corrosion mechanism of Mg_{0.6}Ca and Mg_{0.6}Ca₂Ag alloy corresponds to generalized corrosion. Mg_{0.6}Ca₂Ag showed higher degradation rate because particles at the grain boundaries acted as local anodes causing the loss of the whole grain or clusters of grains by the grain boundary dissolution. The same corrosion mechanism happened in Mg_{0.6}Ca alloy but to a lesser extent.

The microstructure of as-cast MgZnCa and Mg₃Zn_{0.4}Ca alloys consisted of a discontinuous network of secondary phases distributed along the grain boundaries with Ca₂Mg₆Zn₃ particles inside the grains. The extruded Mg_{0.5}Zn_{0.2}Ca alloy consisted of α -Mg equiaxed grains and second phase particles with impurities aligned in the extrusion

Conclusions

direction. According to SKPFM results, Zn-rich (ternary) intermetallics with impurities exhibited cathodic behavior (40-250 mV) while Ca-rich (binary) intermetallics showed anodic behavior (-40 to -100 mV). During short-term immersion, Zn-rich and Ca-rich intermetallics experience a decrease of VPD due to the formation of surface Ca-P compounds that contribute to the measured VPD. Corrosion occurs in the α -Mg phase without preferential dissolution of the secondary phases at grain boundaries.

A reliable short-term screening by EIS was proposed in order to discriminate between the corrosion resistance of different alloys. The corrosion resistance of MgZn_xCa_y alloys was lower by 2-5 times in complete α -MEM compared to inorganic α -MEM solution.

Realistic long-term degradation rate testing under flowing CO_2 concluded that as-cast alloys presented higher corrosion resistance by 2 times compared to the extruded alloy. The as-cast alloys exhibited extensive localized corrosion due to the constant pH conditions and the microsegregation of Ca and Zn. In short term, there are no significant differences in corrosion behaviour of the as-cast alloys. The MgZnCa alloy showed a lower extent of localized corrosion than $\text{Mg}_{0.5}\text{Zn}_{0.4}\text{Ca}_{0.1}$ alloy due to the higher area fraction and more refined morphology of secondary phases acting as a corrosion barrier. The extruded $\text{Mg}_{0.5}\text{Zn}_{0.2}\text{Ca}_{0.3}$ alloy presented uniform corrosion. Its two times greater corrosion rate compared to the as-cast alloys is attributed to high number of defects formed during the extrusion process that play a more important role during corrosion than the alloy composition.

The pH control in the medium significantly influences the degradation rate of Mg alloys over long-term periods. If the pH is controlled at 7.4 with CO_2 flow, the degradation rate of the alloys is 2-20 times higher than in periodic solution replacement set-up because the former does not allow for the stabilisation of Ca-P-based compounds with semi-protective capacity.

❖ Chapter V

Bioactive plasma electrolytic oxidation (PEO) coatings were successfully developed on Mg-Ca, Mg-Ca-Ag and Mg-Zn-Ca alloys in two different types of electrolytes, fluoride- and fluoride-free ones. While coating compositions and morphologies were mostly controlled by the electrolyte composition and the parameters of the process, the coating protective capacity was heavily influenced by the substrate.

Fluoride-containing PEO coatings with minimal energy consumption and 30-35 μm -thickness have been formed on Mg0.6Ca and Mg0.6Ca2Ag alloys. It was found that at similar coating morphologies and similarly high levels of fluoride in the coatings, only the coated Mg0.6Ca alloy was able to sustain a long-term degradation period at 1.75 mL/cm² per week rate, whereas the coated Mg0.6Ca2Ag alloy disintegrated in a matter of 10 days. Fluoride release into 0.9 wt. % NaCl was found to be approximately ~ 33.76 $\mu\text{g}/\text{cm}^2$ after 24 h for the coating formed on Mg0.6Ca alloy, which is to be expected that the material not present cytotoxicity.

For Mg-Zn-Ca alloys, the high level of fluoride in the electrolyte (PEO-8F) led to the formation of thick coatings in the range of 30-45 μm , while F⁻-lean electrolytes developed thinner (14-15 μm) coatings. In both types of the electrolytes, the PEO morphology formed on cast alloys was strongly influenced by the presence of Zn-rich particles at the coating/substrate interface causing the formation of large voids inside the coatings. The corrosion performance of PEO-8F coatings was slightly higher compared to PEO-3F for all three alloys. The findings were attributed to the easy permeability of PEO-3F caused by the deficiency of MgF₂ in the barrier layer, the presence of crevice, i.e. the undercoating corrosion products layer, at the coating/substrate interface and the strong micro-galvanic couple formed between Zn-rich particles and Mg matrix.

Fluoride-free PEO coatings promoted the development of thinner coatings, up to 18 μm , for all studied alloys, mainly constituted by magnesium oxide and magnesium silicates. No substantial differences in either the chemical composition of the coatings or the phase composition were observed for the alloys. Screening of fluoride-free PEO coatings on Mg-Zn-Ca alloys revealed that they offered corrosion protection of cast alloys similar to that provided by fluorine-containing coatings. Nevertheless, the degradation in general followed the same mechanism, involving easy permeation of the coating by corrosive medium, subsequent failure of the barrier layer and development of a crevice in the form of undercoating corrosion product layer. The coating on the Mg3Zn0.4Ca alloy formed in suspension F⁻-free electrolyte (PEO-S-3Ca) showed the highest corrosion protection compared to the other coating/alloy systems due to greater barrier properties. The results strongly suggested that the PEO treatments studied in the present Thesis required an additional protection strategy to reduce the degradation rate of the system.

❖ *Chapter VI*

New biodegradable system, denominated hybrid hierarchical coatings (HHC) constituted by ceramic (PEO) and biodegradable polymeric (PLA and PCL) layers, have been investigated on Mg₃Zn_{0.4}Ca alloy. After the optimization of the dip coating process parameters evaluating the porosity, pore size and thickness of polymer coating, it is concluded that low concentrations, low withdrawal speed and one dipping cycle formed heterogeneous porous surfaces with low porosity, small pore size and thin layers unevenly covering the PEO pores. Conversely, high polymer concentration, high withdrawal speed and various cycles of the dip-coating process led to formation of thick multi-layered porous structures.

HHC with a polymer top-coat formed at 75 mg/mL (PLA and PCL), 2 mm/s and 2 dipping cycles showed slightly higher corrosion protection compared to stand-alone PEO coating but it was not sufficient to protect the alloy from corrosion over long period of time. A new strategy was developed and successfully implemented in order to enhance the adhesion at the polymer/PEO interface. A thin sealing polymer layer generated in the absence of moisture followed by a thick porous polymer top-coat layer yielded a 2-3 times enhancement of corrosion performance and was considered as suitable candidate for further functionalization as drug carrier.

❖ *Chapter VII*

The design of new biodegradable biomaterials requires the introduction of different features such as the incorporation of pharmacological agents in order to provide a multifunctional effect as an anti-inflammatory and/or antibacterial effect and to offer extra protection against corrosion if the drug has a corrosion-accelerating effect.

According to this philosophy, the tuning of the drug delivery system via variation of drug concentration and polymer blend composition, using a model system of a polymer film deposited on a glass (PLA, PCL and/or PCL/PLA), was carried out, evaluating the porosity, pore size and drug release. After the optimization of the functionalization process parameters to obtain porous surfaces with homogeneous pore sizes, it is concluded that the incorporation of PAR leads to the formation of small pores with regular porous surface for higher PLA content in the blend and larger pores for high PCL content. The incorporation of CIP leads to the formation of hexagonal packed structures independent of the blend composition. Similar hexagonal structure was formed by incorporating a

mixture of 5 wt. % PAR and 5 wt. % CIP. For both agents, the increase of drug concentration from 2 to 5 wt. % formed larger pores.

The effect of the surface topography on PAR release at 2 and 5 wt. % showed that porous and planar film blends presented an increase of PAR release with the increase of PCL content. For 2 wt. % drug load, porous surfaces showed higher PAR release compared to planar surfaces, while for 5 wt. %, porous and planar surfaces showed similar drug release behavior. The increase of PAR concentration from 2 to 5 wt. % leads to the increase of PAR release for all blends. In the case of CIP release, non-significant differences were found for all porous blends at 2 and 5 wt. % at long periods of time. Same CIP release behavior was observed for both CIP concentrations (2 and 5 wt. %) in planar surfaces, the increase of PCL content in the blends increased the CIP release. Porous and planar films showed similar final CIP release. In a mixed load of both PAR (5 wt. %) and CIP (5 wt. %) agents in the blends, the CIP release dominates over PAR.

For functionalized $\text{Mg}_3\text{Zn}_{0.4}\text{Ca}/\text{PEO-S-3Ca}/\text{PCL}_{\text{sealing}}/\text{PCL}_{\text{BF}}$ HHC system, a gradual release of PAR and CIP over time was achieved liberating 34 and 61 % of the load over 240 h, as opposed to burst release and complete liberation of the load observed for stand-alone PCL film. The enhanced control over drug liberation is related to its reduced bioavailability associated with the chelation of Mg^{+2} and Ca^{+2} cations with PAR^0 and CIP^0 zwitterions.

The system loaded with CIP and PAR showed enhanced short-term corrosion protection by the formation of insoluble chelate complexes of PAR and CIP. While in the long term, the CIP-loaded system showed a higher corrosion protection by 3-10 times than the non-loaded and PAR-loaded systems due to the chelated complexes with PAR are formed at pH 9. The inhibition efficiency of CIP was 75 % while that of PAR was -252 %, showing a corrosion acceleration effect of hybrid system for PAR. Local current density distribution maps showed a corrosion inhibitory effect of CIP by an order of magnitude compared to the non-loaded system. Local pH, dissolved H_2 concentration and EIS measurements, combined with the drug release profile and acid-base equilibrium analysis of CIP, showed the corrosion inhibition effect as a results of the formation of insoluble chelate complexes of CIP with Mg and Ca cations released from the Mg-coated sealing the defects in the coating system.

Conclusions

The results of this Thesis reveal that it is possible to develop multifunctional biodegradable systems based on magnesium alloys that could become an alternative to the state-of-the-art in orthopaedic and cardiovascular implant applications. The results of **Chapters IV** and **V** demonstrate that tailored stand-alone bioactive PEO surface treatments for biocompatible Mg alloys present low corrosion protection in “quasi-in vivo” physiological conditions. Chapters **VI** and **VII** prove that it is possible to obtain protective hybrid hierarchical coatings comprising a ceramic layer and a homogeneously porous polymer top-coat. These HHC can be functionalized with pharmaceutical agents and provide a controlled drug release over an extended period of time and a corrosion inhibiting effect.

9. APPENDIX

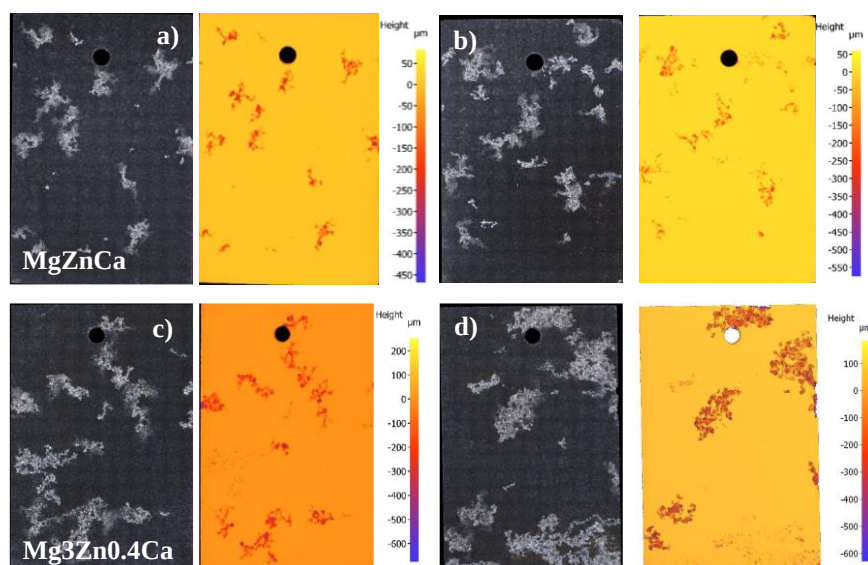


Figure A1. Optical micrographs and variation of surface topography rendering for the two sides of (a, b) MgZnCa and (c, d) Mg₃Zn_{0.4}Ca alloy specimens after 120 h of immersion in inorganic α -MEM with CO₂-based pH controller.

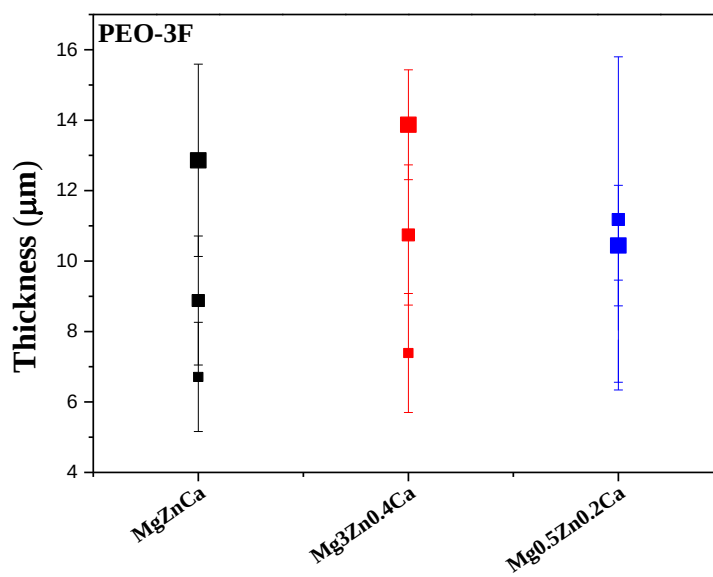


Figure A2. Evolution of PEO-3F coating thickness for MgZnCa, Mg₃Zn_{0.4}Ca and Mg_{0.5}Zn_{0.2}Ca alloys after 300 s, 600 s and 900 s of treatment time. Symbol size increases in order: 300 s, 600 s, 900 s. Note that for Mg_{0.5}Zn_{0.2}Ca alloy the 300 s data point overlaps with that of 900 s.

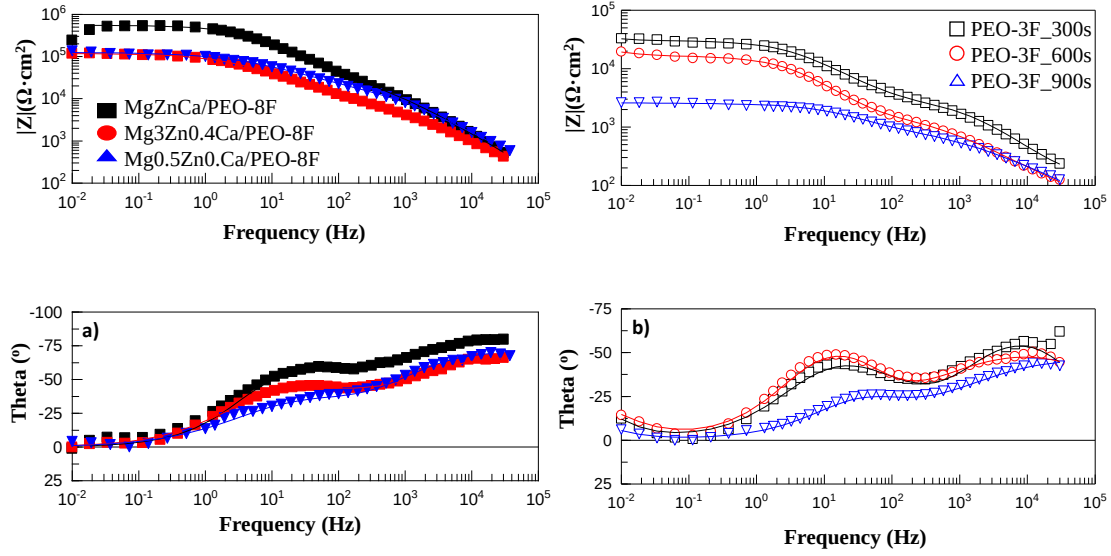


Figure A3. Bode plots of (a) PEO-8F coatings for all substrates and (b) PEO-3F for Mg0.5Zn0.2Ca alloy after 1 h of immersion in inorganic α -MEM solutions at 37°C.

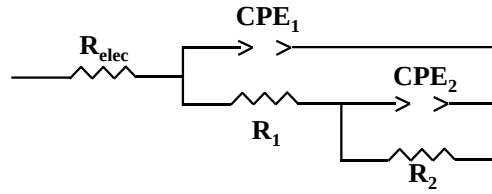


Figure A4. Equivalent circuit of Mg0.5Zn0.2Ca/PEO-8F after 1 h of immersion in inorganic α -MEM solution at 37°C as per Figure 55.

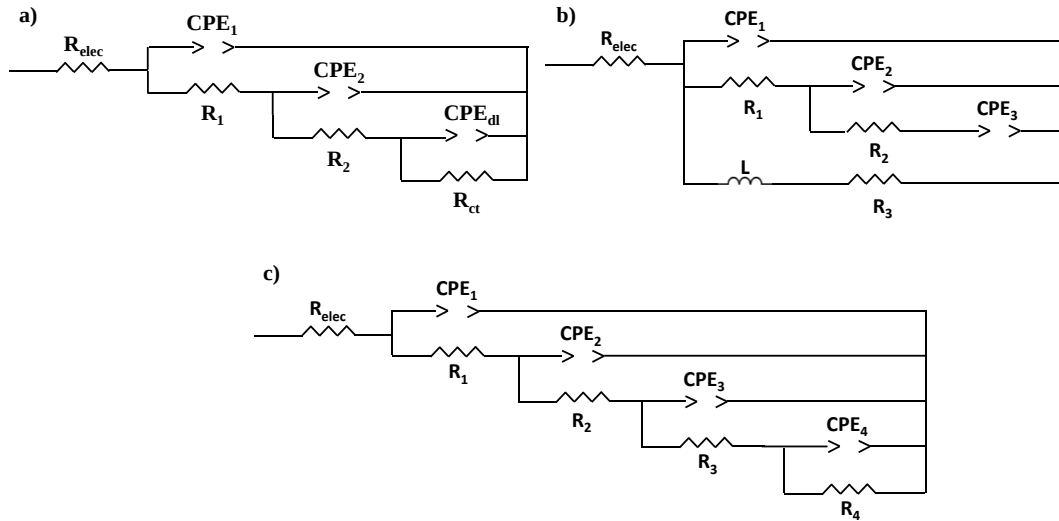


Figure A5. Equivalent circuits for MgZnCa and MgZnCa/PEO-3F system after 1 h, 24 h and 120 h of immersion in inorganic α -MEM solution as per Figure 61.

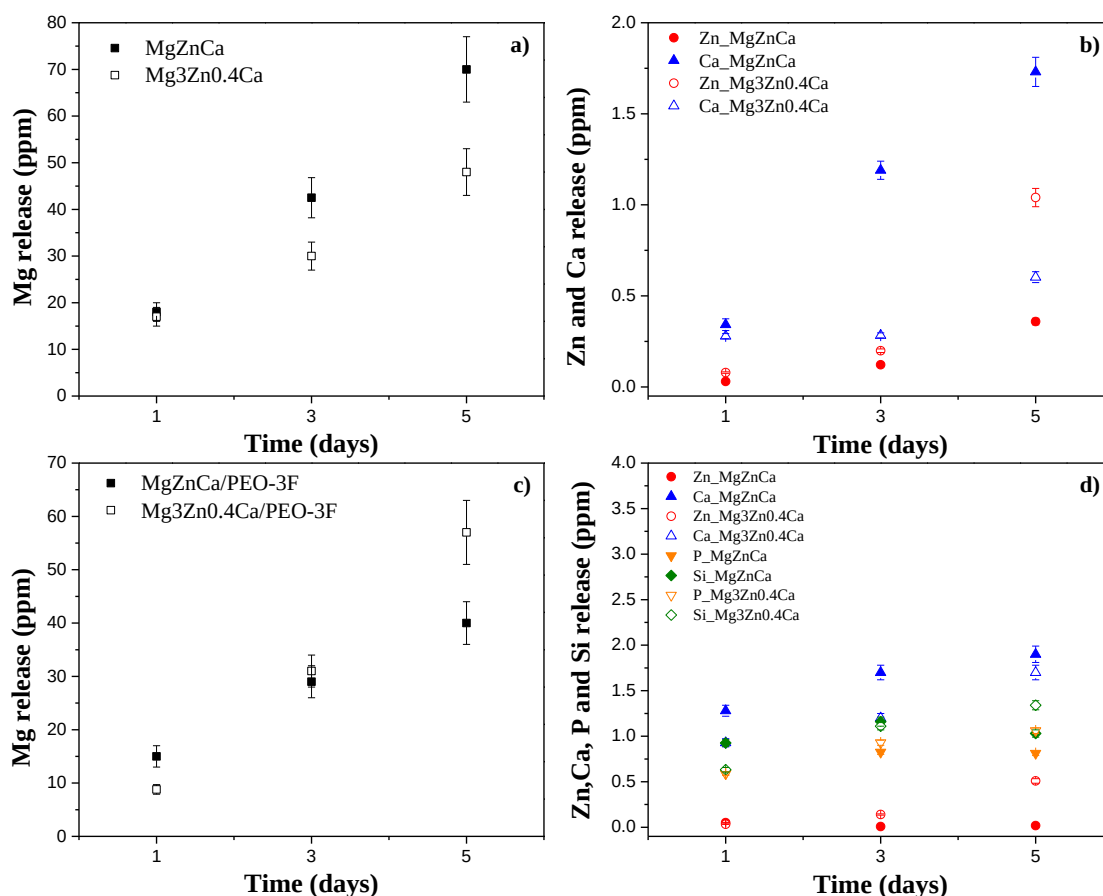


Figure A6. Ion release measured by ICP-OES following the immersion of (a, b) MgZnCa and Mg3Zn0.4Ca substrates and (c, d) PEO-3F coatings formed in 300 s in 0.9 wt.% NaCl.

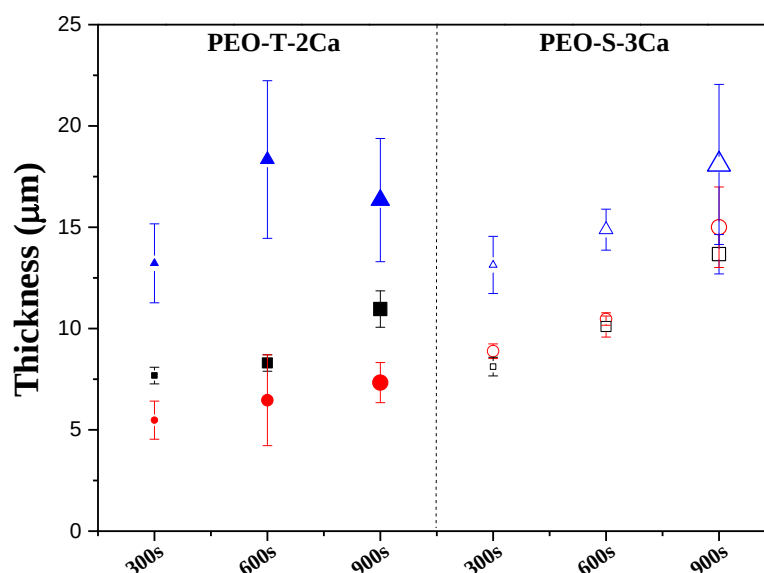


Figure A7. Evolution of thickness of PEO-T-2Ca and PEO-S-3Ca for MgZnCa (black symbols), Mg3Zn0.4Ca (red symbols) and Mg0.5Zn0.2Ca (blue symbols) alloys at three treatment time. Symbol size increases in order: 300 s, 600 s, 900 s. Filled symbols – PEO-T-2Ca and open symbols – PEO-S-3Ca.

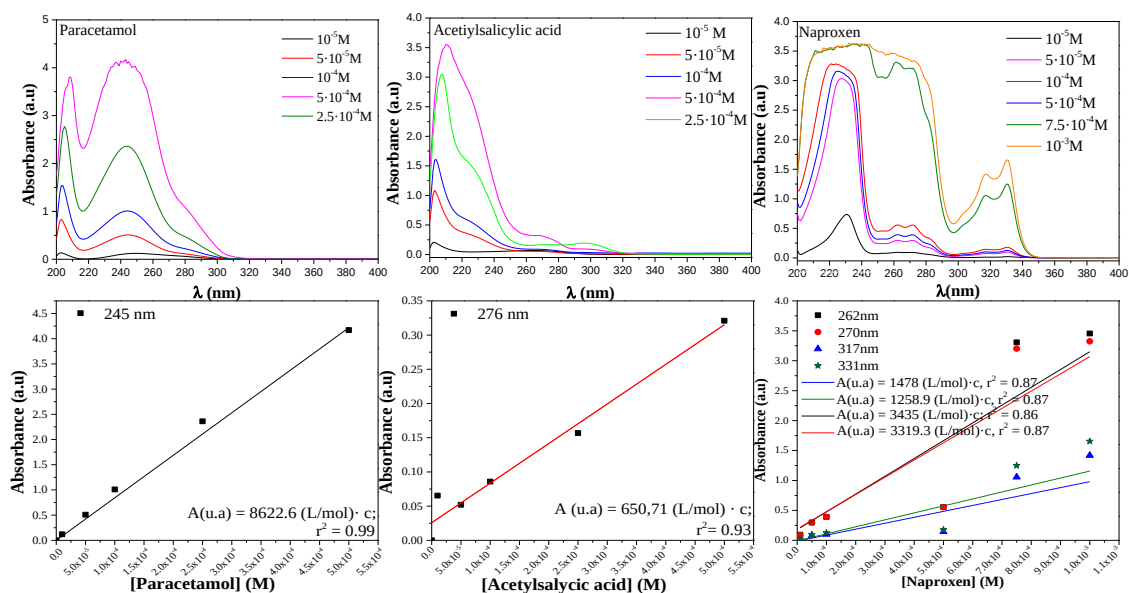


Figure A8. Absorption spectra and standard curves of anti-inflammatory agents at different concentrations in inorganic α -MEM solution at 25°C.

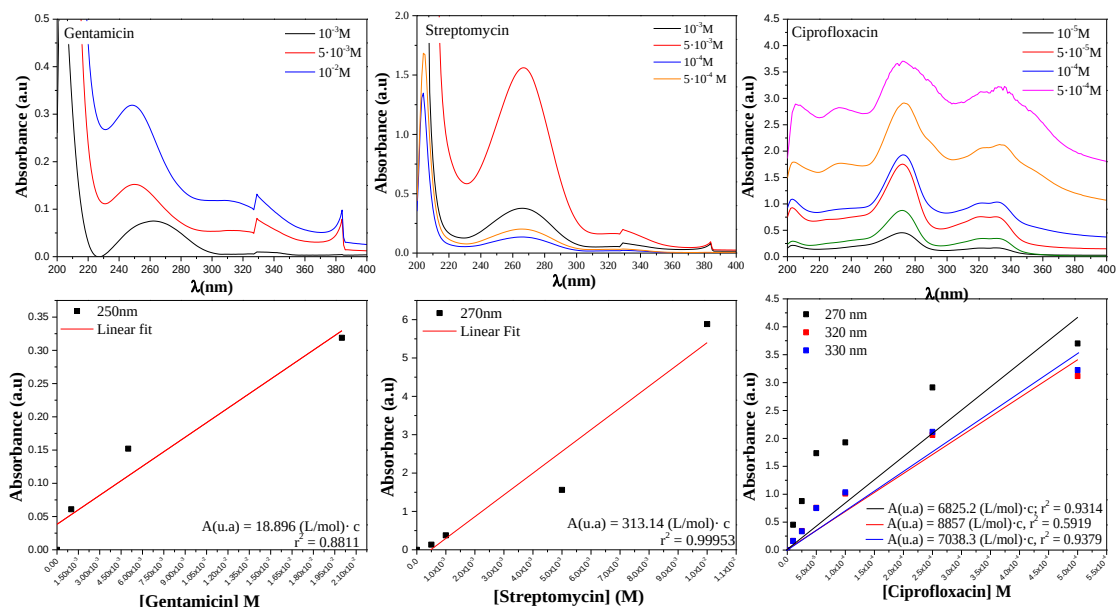


Figure A9. Absorption spectra and standard curves of different antibiotic agents at different concentrations in inorganic α -MEM solution at 25°C.

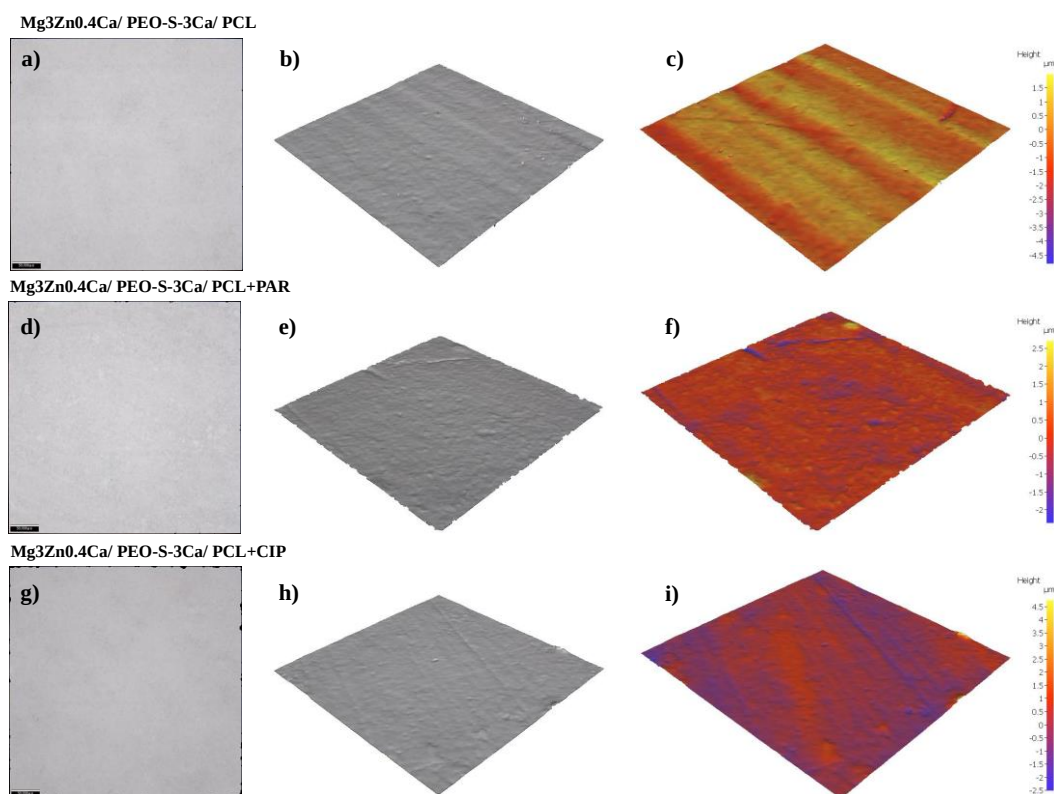


Figure A10. (a, d, g) 2D optical micrographs, (b, e, h) 3D rendering and (c, f, i) variation of surface topography in 3D of (a-c) PCL, (d-f) PCL+PAR and (g-i) PCL+CIP specimens. 2D micrographs scale bar: 50 μm .

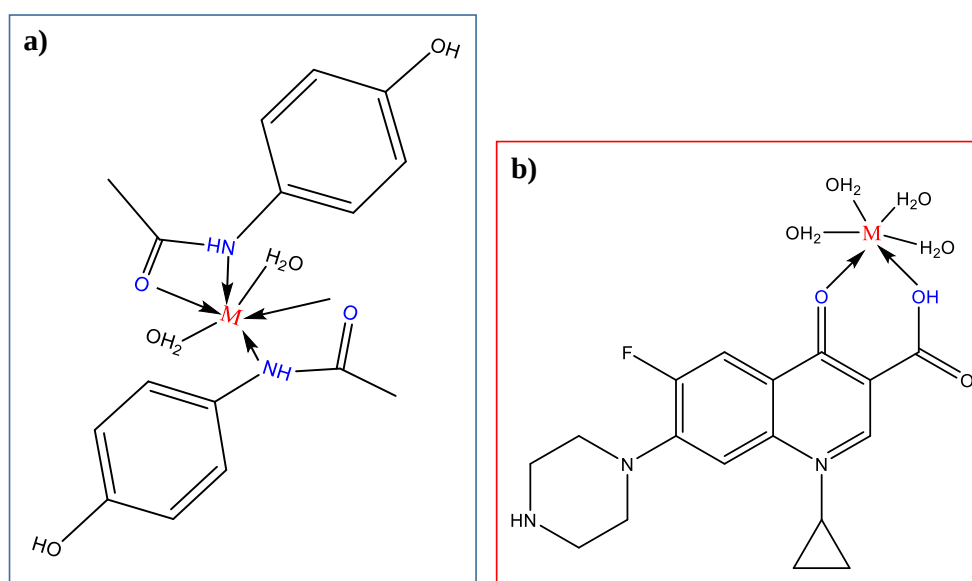


Figure A11. Molecular structure of (a) paracetamol and (b) ciprofloxacin complexes, where (M = Mg(II), Ca(II)).

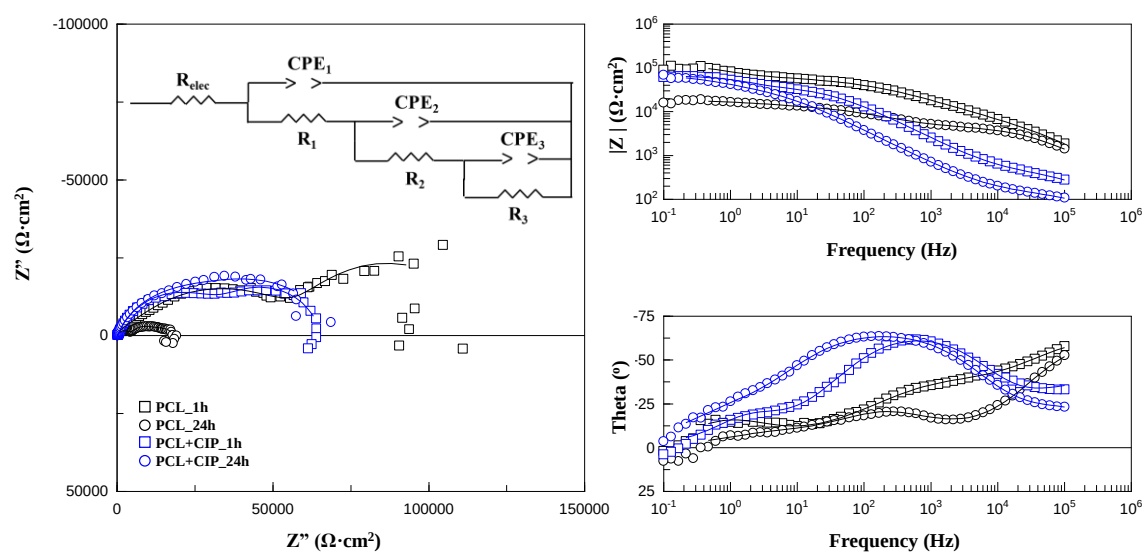


Figure A12. Nyquist and Bode diagrams of non-loaded and CIP-loaded specimens after 1 h and 24 h of immersion in inorganic α -MEM solution at 37°C.

Table A1. Corrosion morphology characteristics obtained by image analysis as per **Figure A1** and macroimages/SEM images of **Figure 38**.

Parameters	MgZnCa	Mg3Zn0.4Ca	Mg0.5Zn0.2Ca	MgZnCa	Mg3Zn0.4Ca	Mg0.5Zn0.2Ca
	Daily replacement of solution			CO ₂ -based pH control		
Image analysis of micro- and macro-graphs						
Localised corrosion area fraction (%)	2.01	10.18	-	10.37	20.76	-
Uniform layer corrosion product thickness (µm)	13.38	16.70	4.39	17.74	6.48	44.73
Localized corrosion depth* (µm)	55.44	128.35	-	163.11	147.94	-
Optical profilometry						
Pit depth (µm)	-	-	-	76.36	43.45	-
Pit volume (mm ³)	-	-	-	0.29	1.33	-

Table A2. Fitted electrical parameters of EIS spectra after 1 h of immersion in inorganic α -MEM solution as per **Figure A3**.

Alloy	Electrolyte	Time (s)	R_{elec} ($\Omega \cdot cm^2$)	CPE_1 ($\mu S \cdot s^n \cdot cm^{-2}$)	n_1	R_1 ($\Omega \cdot cm^2$)	CPE_2 ($\mu S \cdot s^n \cdot cm^{-2}$)	n_2	R_2 ($\Omega \cdot cm^2$)	CPE_{dl} ($mS \cdot s^n \cdot cm^{-2}$)	n_3	R_{ct} ($\Omega \cdot cm^2$)
Mg0.5Zn0.2Ca	PEO-3F	300	108.6	0.25	0.83	2593	3.47	0.73	27935	2.26	0.99	33142
		600	24.6	4.16	0.64	1931	3.42	0.87	14824	2.13	0.90	75611
		900	5.22	9.04	0.56	1114	12.7	0.80	1368	13.64	0.5	2600
MgZnCa	PEO-8F	420	74.93	0.17	0.92	12155	1.147	0.66	115480	0.35	0.82	62500
Mg3Zn0.4Ca			61.95	0.22	0.91	6645	1.47	0.64	71777	0.38	0.6	5163
Mg0.5Zn0.2Ca			21.65	0.0074	0.81	16366	-	-	-	1.21	0.59	110380

Table A3. Fitted electrical parameters of EIS spectra of MgZnCa and MgZnCa/PEO-3F at 300 s after 1 h, 24 h and 120 h in inorganic α -MEM solution as per **Figure 61**.

Sample	R_{elec} ($\Omega \cdot cm^2$)	CPE_1 ($\mu S \cdot s^n \cdot cm^{-2}$)	n_1	R_1 ($\Omega \cdot cm^2$)	CPE_2 ($\mu S \cdot s^n \cdot cm^{-2}$)	n_2	R_2 ($\Omega \cdot cm^2$)	CPE_{dl} ($mS \cdot s^n \cdot cm^{-2}$)	n_3	R_{ic} ($\Omega \cdot cm^2$)	L	CPE_4 ($mS \cdot s^n \cdot cm^{-2}$)	n_4	R_4 ($\Omega \cdot cm^2$)
MgZnCa_1 h	51.35	43.3	0.60	165.9	29.6	0.88	1898	10.9	0.90	2500	-	-	-	-
MgZnCa_24 h	50	97.99	0.71	1966	3.58	0.74	6876	$5.92 \cdot 10^6$	0.98	11617	-	2.2	0.9	20000
MgZnCa_120 h	117.6	0.23	0.53	1878	1.16	0.99	4621	5.60	0.91	13133	-	-	-	-
MgZnCa_300s_1 h	42.98	3.73	0.71	1231	3.07	0.94	2172	4.08	0.98	3395	-	-	-	-
MgZnCa_300s_24 h	50.27	4.25	0.56	3060	15.88	0.65	6117	0.32	0.95	8229	25485	-	-	-
MgZnCa_300s_120 h	123.9	0.76	0.64	303.8	6.58	0.99	1242	7.65	0.59	1900	-	-	-	-

Table A4. Fluoride release measured by ISE as per **Figure 62**.

Time (days)	[F ⁻] (μg/cm ²)	
	MgZnCa	Mg3Zn0.4Ca
1	148.70 ± 33.9	49.4 ± 10.3
3	135.5 ± 7.2	72.5 ± 15.2
5	131.9 ± 5.1	71.7 ± 14.3
12	117.9 ± 6.0	12.7 ± 2.7

Table A5. Fitted electrical parameters of EIS spectra of non-loaded and CIP-loaded specimens after 1 h and 24 h of immersion in inorganic α-MEM solution at 37°C as per **Figure A12**.

R_{elec} (Ω·cm ²)	CPE1 (μS·s ⁿ ·cm ⁻²)	n₁	R1 (Ω·cm ²)	CPE2 (μS·s ⁿ ·cm ⁻²)	n₂	R2 (Ω·cm ²)	CPE_{dl} (mS·s ⁿ ·cm ⁻²)	n₃	R_{tc} (Ω·cm ²)	R_{elec} (Ω·cm ²)
PCL_1 h	61.63	0.02	0.78	7105	0.36	0.56	55877	5.53	0.8	57642
PCL_24 h	61.63	0.02	0.78	4426	1.34	0.69	9154	28.48	0.69	4706
PCL+C_1 h	61.63	0.21	0.71	528.6	0.24	0.81	38366	5.00	0.90	26579
PCL+C_24 h	61.63	0.361	0.78	120	1.44	0.77	40246	12.20	0.76	28420

10. REFERENCES

- (1) Amini, A. R.; Wallace, J. S.; Nukavarapu, S. P. Short-Term and Long-Term Effects of Orthopedic Biodegradable Implants. *J. Long. Term Eff. Med. Implants* **2011**, *21* (2), 93–122. <https://doi.org/10.1615/JLongTermEffMedImplants.v21.i2.10>.
- (2) Manivasagam, G.; Suwas, S. Biodegradable Mg and Mg Based Alloys for Biomedical Implants. *Mater. Sci. Technol.* **2014**, *30* (5), 515–520. <https://doi.org/10.1179/1743284713Y.00000000500>.
- (3) Zeng, R.; Dietzel, W.; Witte, F.; Hort, N.; Blawert, C. Progress and Challenge for Magnesium Alloys as Biomaterials. *Adv. Eng. Mater.* **2008**, *10* (8), B3–B14. <https://doi.org/10.1002/adem.200800035>.
- (4) Eddy Jai Poinern, G.; Brundavanam, S.; Fawcett, D. Biomedical Magnesium Alloys: A Review of Material Properties, Surface Modifications and Potential as a Biodegradable Orthopaedic Implant. *Am. J. Biomed. Eng.* **2013**, *2* (6), 218–240. <https://doi.org/10.5923/j.ajbe.20120206.02>.
- (5) Prakasam, M.; Locs, J.; Salma-Ancane, K.; Loca, D.; Largeteau, A.; Berzina-Cimdina, L. Biodegradable Materials and Metallic Implants—A Review. *J. Funct. Biomater.* **2017**, *8* (4), 44. <https://doi.org/10.3390/jfb8040044>.
- (6) Godavitarne, C.; Robertson, A.; Peters, J.; Rogers, B. Biodegradable Materials. *Orthop. Trauma* **2017**, *31* (5), 316–320. <https://doi.org/10.1016/j.mporth.2017.07.011>.
- (7) Chakraborty Banerjee, P.; Al-Saadi, S.; Choudhary, L.; Harandi, S. E.; Singh, R. Magnesium Implants: Prospects and Challenges. *Materials* **2019**, *12* (1), 136. <https://doi.org/10.3390/ma12010136>.
- (8) Staiger, M. P.; Pietak, A. M.; Huadmai, J.; Dias, G. Magnesium and Its Alloys as Orthopedic Biomaterials: A Review. *Biomaterials* **2006**, *27* (9), 1728–1734. <https://doi.org/10.1016/j.biomaterials.2005.10.003>.
- (9) Yusop, A. H.; Bakir, A. A.; Shaharom, N. A.; Abdul Kadir, M. R.; Hermawan, H. Porous Biodegradable Metals for Hard Tissue Scaffolds: A Review. *Int. J. Biomater.* **2012**, *2012*, 641430. <https://doi.org/10.1155/2012/641430>.
- (10) Walker, J.; Shadanbaz, S.; Woodfield, T. B. F.; Staiger, M. P.; Dias, G. J. Magnesium Biomaterials for Orthopedic Application: A Review from a Biological Perspective: Magnesium Biomaterials for Orthopedic Application. *J. Biomed. Mater. Res. B Appl. Biomater.* **2014**, *102* (6), 1316–1331. <https://doi.org/10.1002/jbm.b.33113>.
- (11) Antoniac, I.; Miculescu, M.; (Păltânea), V.; Stere, A.; Quan, P.; Paltanea, G.; Alina, R.; Earar, K. Magnesium-Based Alloys Used in Orthopedic Surgery. *Materials* **2022**, *15*, 1148. <https://doi.org/10.3390/ma15031148>.
- (12) Heublein, B. Biocorrosion of Magnesium Alloys: A New Principle in Cardiovascular Implant Technology? *Heart* **2003**, *89* (6), 651–656. <https://doi.org/10.1136/heart.89.6.651>.
- (13) Rapetto, C.; Leoncini, M. Magmaris: A New Generation Metallic Sirolimus-Eluting Fully Bioresorbable Scaffold: Present Status and Future Perspectives. *J. Thorac. Dis.* **2017**, *9* (S9), S903–S913. <https://doi.org/10.21037/jtd.2017.06.34>.
- (14) Luthringer, B. J. C.; Feyerabend, F.; Willumeit-Römer, R. Magnesium-Based Implants: A Mini-Review. *Magnes. Res.* **2014**, *27* (4), 142–154. <https://doi.org/10.1684/mrh.2015.0375>.
- (15) Wittchow, E.; Adden, N.; Riedmüller, J.; Savard, C.; Waksman, R.; Braune, M. Bioresorbable Drug-Eluting Magnesium-Alloy Scaffold: Design and Feasibility in

- a Porcine Coronary Model. *EuroIntervention* **2013**, 8 (12), 1441–1450. <https://doi.org/10.4244/EIJV8I12A218>.
- (16) Haude, M.; Erbel, R.; Erne, P.; Verheye, S.; Degen, H.; Böse, D.; Vermeersch, P.; Wijnbergen, I.; Weissman, N.; Prati, F.; Waksman, R.; Koolen, J. Safety and Performance of the Drug-Eluting Absorbable Metal Scaffold (DREAMS) in Patients with de-Novo Coronary Lesions: 12 Month Results of the Prospective, Multicentre, First-in-Man BIOSOLVE-I Trial. *The Lancet* **2013**, 381 (9869), 836–844. [https://doi.org/10.1016/S0140-6736\(12\)61765-6](https://doi.org/10.1016/S0140-6736(12)61765-6).
- (17) Waksman, R.; Prati, F.; Bruining, N.; Haude, M.; Böse, D.; Kitabata, H.; Erne, P.; Verheye, S.; Degen, H.; Vermeersch, P.; Di Vito, L.; Koolen, J.; Erbel, R. Serial Observation of Drug-Eluting Absorbable Metal Scaffold: Multi-Imaging Modality Assessment. *Circ. Cardiovasc. Interv.* **2013**, 6 (6), 644–653. <https://doi.org/10.1161/CIRCINTERVENTIONS.113.000693>.
- (18) Haude, M.; Ince, H.; Abizaid, A.; Toelg, R.; Lemos, P. A.; von Birgelen, C.; Christiansen, E. H.; Wijns, W.; Neumann, F.-J.; Kaiser, C.; Eeckhout, E.; Lim, S. T.; Escaned, J.; Garcia-Garcia, H. M.; Waksman, R. Safety and Performance of the Second-Generation Drug-Eluting Absorbable Metal Scaffold in Patients with de-Novo Coronary Artery Lesions (BIOSOLVE-II): 6 Month Results of a Prospective, Multicentre, Non-Randomised, First-in-Man Trial. *The Lancet* **2016**, 387 (10013), 31–39. [https://doi.org/10.1016/S0140-6736\(15\)00447-X](https://doi.org/10.1016/S0140-6736(15)00447-X).
- (19) Seitz, J.-M.; Lucas, A.; Kirschner, M. Magnesium-Based Compression Screws: A Novelty in the Clinical Use of Implants. *JOM* **2016**, 68 (4), 1177–1182. <https://doi.org/10.1007/s11837-015-1773-1>.
- (20) Biber, R.; Pauser, J.; Geßlein, M.; Bail, H. J. Magnesium-Based Absorbable Metal Screws for Intra-Articular Fracture Fixation. *Case Rep. Orthop.* **2016**, 2016, 1–4. <https://doi.org/10.1155/2016/9673174>.
- (21) Ding, W. Opportunities and Challenges for the Biodegradable Magnesium Alloys as Next-Generation Biomaterials. *Regen. Biomater.* **2016**, 3 (2), 79–86. <https://doi.org/10.1093/rb/rbw003>.
- (22) Witte, F.; Kaese, V.; Haferkamp, H.; Switzer, E.; Meyer-Lindenberg, A.; Wirth, C. J.; Windhagen, H. In Vivo Corrosion of Four Magnesium Alloys and the Associated Bone Response. *Biomaterials* **2005**, 26 (17), 3557–3563. <https://doi.org/10.1016/j.biomaterials.2004.09.049>.
- (23) Li, G.; Zhang, L.; Wang, L.; Yuan, G.; Dai, K.; Pei, J.; Hao, Y. Dual Modulation of Bone Formation and Resorption with Zoledronic Acid-Loaded Biodegradable Magnesium Alloy Implants Improves Osteoporotic Fracture Healing: An in Vitro and in Vivo Study. *Acta Biomater.* **2018**, 65, 486–500. <https://doi.org/10.1016/j.actbio.2017.10.033>.
- (24) Mao, L.; Shen, L.; Niu, J.; Zhang, J.; Ding, W.; Wu, Y.; Fan, R.; Yuan, G. Nanophasic Biodegradation Enhances the Durability and Biocompatibility of Magnesium Alloys for the Next-Generation Vascular Stents. *Nanoscale* **2013**, 5 (20), 9517. <https://doi.org/10.1039/c3nr02912c>.
- (25) Thomann, M.; Krause, Ch.; Bormann, D.; von der Höh, N.; Windhagen, H.; Meyer-Lindenberg, A. Comparison of the Resorbable Magnesium . Alloys LAE442 Und MgCa0.8 Concerning Their Mechanical Properties, Their Progress of Degradation and the Bone-Implant-Contact after 12 Months Implantation Duration in a Rabbit Model. *Mater. Werkst.* **2009**, 40 (1–2), 82–87. <https://doi.org/10.1002/mawe.200800412>.

- (26) Gusieva, K.; Davies, C. H. J.; Scully, J. R.; Birbilis, N. Corrosion of Magnesium Alloys: The Role of Alloying. *Int. Mater. Rev.* **2015**, *60* (3), 169–194. <https://doi.org/10.1179/1743280414Y.00000000046>.
- (27) Xin, Y.; Hu, T.; Chu, P. K. In Vitro Studies of Biomedical Magnesium Alloys in a Simulated Physiological Environment: A Review. *Acta Biomater.* **2011**, *7* (4), 1452–1459. <https://doi.org/10.1016/j.actbio.2010.12.004>.
- (28) Radha, R.; Sreekanth, D. Insight of Magnesium Alloys and Composites for Orthopedic Implant Applications – a Review. *J. Magnes. Alloys* **2017**, *5* (3), 286–312. <https://doi.org/10.1016/j.jma.2017.08.003>.
- (29) Wang, H.; Shi, Z. In Vitro Biodegradation Behavior of Magnesium and Magnesium Alloy. *J. Biomed. Mater. Res. B Appl. Biomater.* **2011**, *98B* (2), 203–209. <https://doi.org/10.1002/jbm.b.31769>.
- (30) Chen, Y. J.; Li, Y. J.; Walmsley, J. C.; Dumoulin, S.; Skaret, P. C.; Roven, H. J. Microstructure Evolution of Commercial Pure Titanium during Equal Channel Angular Pressing. *Mater. Sci. Eng. A* **2010**, *527* (3), 789–796. <https://doi.org/10.1016/j.msea.2009.09.005>.
- (31) Nakamura, Y.; Tsumura, Y.; Tonogai, Y.; Shibata, T.; Ito, Y. Differences in Behavior among the Chlorides of Seven Rare Earth Elements Administered Intravenously to Rats. *Fundam. Appl. Toxicol.* **1997**, *37* (2), 106–116. <https://doi.org/10.1006/faat.1997.2322>.
- (32) Chen, Y.; Xu, Z.; Smith, C.; Sankar, J. Recent Advances on the Development of Magnesium Alloys for Biodegradable Implants. *Acta Biomater.* **2014**, *10* (11), 4561–4573. <https://doi.org/10.1016/j.actbio.2014.07.005>.
- (33) Kirkland, N. T.; Birbilis, N.; Walker, J.; Woodfield, T.; Dias, G. J.; Staiger, M. P. In-Vitro Dissolution of Magnesium-Calcium Binary Alloys: Clarifying the Unique Role of Calcium Additions in Bioresorbable Magnesium Implant Alloys. *J. Biomed. Mater. Res. B Appl. Biomater.* **2010**, *95B* (1), 91–100. <https://doi.org/10.1002/jbm.b.31687>.
- (34) Li, Z.; Gu, X.; Lou, S.; Zheng, Y. The Development of Binary Mg–Ca Alloys for Use as Biodegradable Materials within Bone. *Biomaterials* **2008**, *29* (10), 1329–1344. <https://doi.org/10.1016/j.biomaterials.2007.12.021>.
- (35) Kim, W.-C.; Kim, J.-G.; Lee, J.-Y.; Seok, H.-K. Influence of Ca on the Corrosion Properties of Magnesium for Biomaterials. *Mater. Lett.* **2008**, *62* (25), 4146–4148. <https://doi.org/10.1016/j.matlet.2008.06.028>.
- (36) Jeong, Y. S.; Kim, W. J. Enhancement of Mechanical Properties and Corrosion Resistance of Mg–Ca Alloys through Microstructural Refinement by Indirect Extrusion. *Corros. Sci.* **2014**, *82*, 392–403. <https://doi.org/10.1016/j.corsci.2014.01.041>.
- (37) Rad, H. R. B.; Idris, M. H.; Kadir, M. R. A.; Farahany, S. Microstructure Analysis and Corrosion Behavior of Biodegradable Mg–Ca Implant Alloys. *Mater. Des.* **2012**, *33*, 88–97. <https://doi.org/10.1016/j.matdes.2011.06.057>.
- (38) Song, Y.; Han, E.-H.; Shan, D.; Yim, C. D.; You, B. S. The Effect of Zn Concentration on the Corrosion Behavior of Mg–XZn Alloys. *Corros. Sci.* **2012**, *65*, 322–330. <https://doi.org/10.1016/j.corsci.2012.08.037>.
- (39) Gu, X.; Zheng, Y.; Cheng, Y.; Zhong, S.; Xi, T. In Vitro Corrosion and Biocompatibility of Binary Magnesium Alloys. *Biomaterials* **2009**, *30* (4), 484–498. <https://doi.org/10.1016/j.biomaterials.2008.10.021>.
- (40) He, Y.; Tao, H.; Zhang, Y.; Jiang, Y.; Zhang, S.; Zhao, C.; Li, J.; Zhang, B.; Song, Y.; Zhang, X. Biocompatibility of Bio-Mg-Zn Alloy within Bone with Heart,

- Liver, Kidney and Spleen. *Sci. Bull.* **2009**, 54 (3), 484–491. <https://doi.org/10.1007/s11434-009-0080-z>.
- (41) Riaz, U.; Shabib, I.; Haider, W. The Current Trends of Mg Alloys in Biomedical Applications—A Review. *J. Biomed. Mater. Res. B Appl. Biomater.* **2019**, 107 (6), 1970–1996. <https://doi.org/10.1002/jbm.b.34290>.
- (42) Gopi, D.; Shinyjoy, E.; Kavitha, L. Synthesis and Spectral Characterization of Silver/Magnesium Co-Substituted Hydroxyapatite for Biomedical Applications. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **2014**, 127, 286–291. <https://doi.org/10.1016/j.saa.2014.02.057>.
- (43) Gu, X.-N.; Zheng, Y.-F. A Review on Magnesium Alloys as Biodegradable Materials. *Front. Mater. Sci. China* **2010**, 4 (2), 111–115. <https://doi.org/10.1007/s11706-010-0024-1>.
- (44) Rosalbino, F.; De Negri, S.; Saccone, A.; Angelini, E.; Delfino, S. Bio-Corrosion Characterization of Mg–Zn–X (X = Ca, Mn, Si) Alloys for Biomedical Applications. *J. Mater. Sci. Mater. Med.* **2010**, 21 (4), 1091–1098. <https://doi.org/10.1007/s10856-009-3956-1>.
- (45) Bakhsheshi Rad, H. R.; Idris, M. H.; Kadir, M. R. A.; Farahany, S.; Fereidouni, A.; Yahya, M. Y. Characterization and Corrosion Behavior of Biodegradable Mg–Ca and Mg–Ca–Zn Implant Alloys. *Appl. Mech. Mater.* **2011**, 121–126, 568–572. <https://doi.org/10.4028/www.scientific.net/AMM.121-126.568>.
- (46) Zander, D.; Zumdick, N. A. Influence of Ca and Zn on the Microstructure and Corrosion of Biodegradable Mg–Ca–Zn Alloys. *Corros. Sci.* **2015**, 93, 222–233. <https://doi.org/10.1016/j.corsci.2015.01.027>.
- (47) Bakhsheshi-Rad, H. R.; Abdul-Kadir, M. R.; Idris, M. H.; Farahany, S. Relationship between the Corrosion Behavior and the Thermal Characteristics and Microstructure of Mg–0.5Ca–XZn Alloys. *Corros. Sci.* **2012**, 64, 184–197. <https://doi.org/10.1016/j.corsci.2012.07.015>.
- (48) Yin, P.; Li, N. F.; Lei, T.; Liu, L.; Ouyang, C. Effects of Ca on Microstructure, Mechanical and Corrosion Properties and Biocompatibility of Mg–Zn–Ca Alloys. *J. Mater. Sci. Mater. Med.* **2013**, 24 (6), 1365–1373. <https://doi.org/10.1007/s10856-013-4856-y>.
- (49) Wong, C.-C.; Wong, P.-C.; Tsai, P.-H.; Jang, J. S.-C.; Cheng, C.-K.; Chen, H.-H.; Chen, C.-H. Biocompatibility and Osteogenic Capacity of Mg–Zn–Ca Bulk Metallic Glass for Rabbit Tendon–Bone Interference Fixation. *Int. J. Mol. Sci.* **2019**, 20 (9), 2191. <https://doi.org/10.3390/ijms20092191>.
- (50) Wang, C.; Fang, H.; Qi, X.; Hang, C.; Sun, Y.; Peng, Z.; Wei, W.; Wang, Y. Silk Fibroin Film-Coated MgZnCa Alloy with Enhanced in Vitro and in Vivo Performance Prepared Using Surface Activation. *Acta Biomater.* **2019**, 91, 99–111. <https://doi.org/10.1016/j.actbio.2019.04.048>.
- (51) Hou, R.; Victoria-Hernandez, J.; Jiang, P.; Willumeit-Römer, R.; Luthringer-Feyerabend, B.; Yi, S.; Letzig, D.; Feyerabend, F. In Vitro Evaluation of the ZX11 Magnesium Alloy as Potential Bone Plate: Degradability and Mechanical Integrity. *Acta Biomater.* **2019**, 97, 608–622. <https://doi.org/10.1016/j.actbio.2019.07.053>.
- (52) Hradilova, M.; Vojtech, D. Mechanical Properties of As-Cast and Extruded Mg–4Zn and Mg–4Zn–0.4Ca Alloys. *Kov. Mater.* **2014**, 52, 361–370. https://doi.org/10.4149/km_2014_6_361.
- (53) Moradnezhad, S.; Razaghian, A.; Taghiabadi, R.; Abedi, H. R.; Salandari-Rabori, A.; Emamy, M. Effect of Ca Additions on Evolved Microstructures and Subsequent Mechanical Properties of a Cast and Hot-Extruded Mg–Zn–Zr

- Magnesium Alloy. *Int. J. Adv. Manuf. Technol.* **2019**, *104* (9), 4265–4275. <https://doi.org/10.1007/s00170-019-04260-6>.
- (54) Du, Y.; Liu, D.; Ge, Y. Effects of Ce Addition on Mechanical and Corrosion Properties of the As-Extruded Mg–Zn–Ca Alloy. *J. Mater. Eng. Perform.* **2021**, *30* (1), 488–496. <https://doi.org/10.1007/s11665-020-05318-3>.
- (55) Sezer, N.; Evis, Z.; Kayhan, S. M.; Tahmasebifar, A.; Koç, M. Review of Magnesium-Based Biomaterials and Their Applications. *J. Magnes. Alloys* **2018**, *6* (1), 23–43. <https://doi.org/10.1016/j.jma.2018.02.003>.
- (56) Wang, Y.; Fu, P.; Wang, N.; Peng, L.; Kang, B.; Zeng, H.; Yuan, G.; Ding, W. Challenges and Solutions for the Additive Manufacturing of Biodegradable Magnesium Implants. *Engineering* **2020**, *6* (11), 1267–1275. <https://doi.org/10.1016/j.eng.2020.02.015>.
- (57) Gonzalez, J.; Hou, R. Q.; Nidadavolu, E. P. S.; Willumeit-Römer, R.; Feyerabend, F. Magnesium Degradation under Physiological Conditions – Best Practice. *Bioact. Mater.* **2018**, *3* (2), 174–185. <https://doi.org/10.1016/j.bioactmat.2018.01.003>.
- (58) Esmaily, M.; Svensson, J. E.; Fajardo, S.; Birbilis, N.; Frankel, G. S.; Virtanen, S.; Arrabal, R.; Thomas, S.; Johansson, L. G. Fundamentals and Advances in Magnesium Alloy Corrosion. *Prog. Mater. Sci.* **2017**, *89*, 92–193. <https://doi.org/10.1016/j.pmatsci.2017.04.011>.
- (59) Silva, E. L.; Lamaka, S. V.; Mei, D.; Zheludkevich, M. L. The Reduction of Dissolved Oxygen During Magnesium Corrosion. *ChemistryOpen* **2018**, *7* (8), 664–668. <https://doi.org/10.1002/open.201800076>.
- (60) Mei, D.; Wang, C.; Nienaber, M.; Pacheco, M.; Barros, A.; Neves, S.; Reis, R.; Zhu, S.; Bohlen, J.; Letzig, D.; Guan, S.; Zheludkevich, M. L.; Lamaka, S. V. Corrosion Behavior of Mg Wires for Ureteral Stent in Artificial Urine Solution. *Corros. Sci.* **2021**, *189*, 109567. <https://doi.org/10.1016/j.corsci.2021.109567>.
- (61) Feliu, S.; Veleza, L.; García-Galvan, F. Effect of Temperature on the Corrosion Behavior of Biodegradable AZ31B Magnesium Alloy in Ringer’s Physiological Solution. *Metals* **2019**, *9* (5), 591. <https://doi.org/10.3390/met9050591>.
- (62) Zainal Abidin, N. I.; Martin, D.; Atrens, A. Corrosion of High Purity Mg, AZ91, ZE41 and Mg₂Zn_{0.2}Mn in Hank’s Solution at Room Temperature. *Corros. Sci.* **2011**, *53* (3), 862–872. <https://doi.org/10.1016/j.corsci.2010.10.008>.
- (63) Kirkland, N. T.; Birbilis, N. Influence of Environmental Variables on In Vitro Performance. In *Magnesium Biomaterials: Design, Testing, and Best Practice*; Kirkland, N. T., Birbilis, N., Eds.; SpringerBriefs in Materials; Springer International Publishing: Cham, 2014; pp 39–72. https://doi.org/10.1007/978-3-319-02123-2_3.
- (64) Burstein, G. T.; Liu, C.; Souto, R. M. The Effect of Temperature on the Nucleation of Corrosion Pits on Titanium in Ringer’s Physiological Solution. *Biomaterials* **2005**, *26* (3), 245–256. <https://doi.org/10.1016/j.biomaterials.2004.02.023>.
- (65) Liu, M.; Wang, J.; Zhu, S.; Zhang, Y.; Sun, Y.; Wang, L.; Guan, S. Corrosion Fatigue of the Extruded Mg–Zn–Y–Nd Alloy in Simulated Body Fluid. *J. Magnes. Alloys* **2020**, *8* (1), 231–240. <https://doi.org/10.1016/j.jma.2019.09.009>.
- (66) Ghali, E.; Dietzel, W.; Kainer, K.-U. General and Localized Corrosion of Magnesium Alloys: A Critical Review. *J. Mater. Eng. Perform.* **2004**, *13* (1), 7–23. <https://doi.org/10.1361/10599490417533>.
- (67) Singh Raman, R. K.; Jafari, S.; Harandi, S. E. Corrosion Fatigue Fracture of Magnesium Alloys in Bioimplant Applications: A Review. *Eng. Fract. Mech.* **2015**, *137*, 97–108. <https://doi.org/10.1016/j.engfracmech.2014.08.009>.

- (68) Peron, M.; Torgersen, J.; Berto, F. Mg and Its Alloys for Biomedical Applications: Exploring Corrosion and Its Interplay with Mechanical Failure. *Metals* **2017**, *7* (7), 252. <https://doi.org/10.3390/met7070252>.
- (69) Walker, J.; Shadanbaz, S.; Kirkland, N. T.; Stace, E.; Woodfield, T.; Staiger, M. P.; Dias, G. J. Magnesium Alloys: Predicting in Vivo Corrosion with in Vitro Immersion Testing. *J. Biomed. Mater. Res. B Appl. Biomater.* **2012**, *100B* (4), 1134–1141. <https://doi.org/10.1002/jbm.b.32680>.
- (70) Pogorielov, M.; Husak, E.; Solodivnik, A.; Zhdanov, S. Magnesium-Based Biodegradable Alloys: Degradation, Application, and Alloying Elements. *Interv. Med. Appl. Sci.* **2017**, *9* (1), 27–38. <https://doi.org/10.1556/1646.9.2017.1.04>.
- (71) Chen, K.; Xie, X.; Tang, H.; Sun, H.; Qin, L.; Zheng, Y.; Gu, X.; Fan, Y. In Vitro and in Vivo Degradation Behavior of Mg–2Sr–Ca and Mg–2Sr–Zn Alloys. *Bioact. Mater.* **2020**, *5* (2), 275–285. <https://doi.org/10.1016/j.bioactmat.2020.02.014>.
- (72) Xi, Z.; Wu, Y.; Xiang, S.; Sun, C.; Wang, Y.; Yu, H.; Fu, Y.; Wang, X.; Yan, J.; Zhao, D.; Wang, Y.; Zhang, N. Corrosion Resistance and Biocompatibility Assessment of a Biodegradable Hydrothermal-Coated Mg–Zn–Ca Alloy: An in Vitro and in Vivo Study. *ACS Omega* **2020**, *5* (9), 4548–4557. <https://doi.org/10.1021/acsomega.9b03889>.
- (73) Xin, Y.; Hu, T.; Chu, P. K. Influence of Test Solutions on In Vitro Studies of Biomedical Magnesium Alloys. *J. Electrochem. Soc.* **2010**, *157* (7), C238. <https://doi.org/10.1149/1.3421651>.
- (74) Sanchez, A. H. M.; Luthringer, B. J. C.; Feyerabend, F.; Willumeit, R. Mg and Mg Alloys: How Comparable Are in Vitro and in Vivo Corrosion Rates? A Review. *Acta Biomater.* **2015**, *13*, 16–31. <https://doi.org/10.1016/j.actbio.2014.11.048>.
- (75) Willbold, E.; Weizbauer, A.; Loos, A.; Seitz, J.-M.; Angrisani, N.; Windhagen, H.; Reifenrath, J. Magnesium Alloys: A Stony Pathway from Intensive Research to Clinical Reality. Different Test Methods and Approval-Related Considerations: MAGNESIUM ALLOYS: A STONY PATHWAY FROM INTENSIVE RESEARCH TO CLINICAL REALITY. *J. Biomed. Mater. Res. A* **2017**, *105* (1), 329–347. <https://doi.org/10.1002/jbm.a.35893>.
- (76) Kokubo, T.; Takadama, H. How Useful Is SBF in Predicting in Vivo Bone Bioactivity? *Biomaterials* **2006**, *27* (15), 2907–2915. <https://doi.org/10.1016/j.biomaterials.2006.01.017>.
- (77) Schille, Ch.; Braun, M.; Wendel, H. P.; Scheideler, L.; Hort, N.; Reichel, H.-P.; Schweizer, E.; Geis-Gerstorfer, J. Corrosion of Experimental Magnesium Alloys in Blood and PBS: A Gravimetric and Microscopic Evaluation. *Mater. Sci. Eng. B* **2011**, *176* (20), 1797–1801. <https://doi.org/10.1016/j.mseb.2011.04.007>.
- (78) Mei, D.; Lamaka, S. V.; Lu, X.; Zheludkevich, M. L. Selecting Medium for Corrosion Testing of Bioabsorbable Magnesium and Other Metals – A Critical Review. *Corros. Sci.* **2020**, *171*, 108722. <https://doi.org/10.1016/j.corsci.2020.108722>.
- (79) Hiromoto, S.; Yamamoto, A.; Maruyama, N.; Somekawa, H.; Mukai, T. Influence of PH and Flow on the Polarisation Behaviour of Pure Magnesium in Borate Buffer Solutions. *Corros. Sci.* **2008**, *50* (12), 3561–3568. <https://doi.org/10.1016/j.corsci.2008.09.026>.
- (80) Li, N.; Zheng, Y. Novel Magnesium Alloys Developed for Biomedical Application: A Review. *J. Mater. Sci. Technol.* **2013**, *29* (6), 489–502. <https://doi.org/10.1016/j.jmst.2013.02.005>.
- (81) Lévesque, J.; Hermawan, H.; Dubé, D.; Mantovani, D. Design of a Pseudo-Physiological Test Bench Specific to the Development of Biodegradable Metallic

- Biomaterials. *Acta Biomater.* **2008**, *4* (2), 284–295. <https://doi.org/10.1016/j.actbio.2007.09.012>.
- (82) Törnqvist, E.; Annas, A.; Granath, B.; Jalkestén, E.; Cotgreave, I.; Öberg, M. Strategic Focus on 3R Principles Reveals Major Reductions in the Use of Animals in Pharmaceutical Toxicity Testing. *PLoS ONE* **2014**, *9* (7). <https://doi.org/10.1371/journal.pone.0101638>.
- (83) Loos, A.; Rohde, R.; Haverich, A.; Barlach, S. In Vitro and In Vivo Biocompatibility Testing of Absorbable Metal Stents. *Macromol. Symp.* **2007**, *253* (1), 103–108. <https://doi.org/10.1002/masy.200750715>.
- (84) Burmester, A.; Willumeit-Römer, R.; Feyerabend, F. Behavior of Bone Cells in Contact with Magnesium Implant Material: Bone Cells and Magnesium Implant Material Interaction. *J. Biomed. Mater. Res. B Appl. Biomater.* **2017**, *105* (1), 165–179. <https://doi.org/10.1002/jbm.b.33542>.
- (85) Fischer, J.; Prüfrock, D.; Hort, N.; Willumeit, R.; Feyerabend, F. Improved Cytotoxicity Testing of Magnesium Materials. *Mater. Sci. Eng. B* **2011**, *176* (11), 830–834. <https://doi.org/10.1016/j.mseb.2011.04.008>.
- (86) Wang, Y.; Zhang, S.; Zeng, X.; Ma, L. L.; Weng, W.; Yan, W.; Qian, M. Osteoblastic Cell Response on Fluoridated Hydroxyapatite Coatings. *Acta Biomater.* **2007**, *3* (2), 191–197. <https://doi.org/10.1016/j.actbio.2006.10.002>.
- (87) Okawachi, H.; Ayukawa, Y.; Atsuta, I.; Furuhashi, A.; Sakaguchi, M.; Yamane, K.; Koyano, K. Effect of Titanium Surface Calcium and Magnesium on Adhesive Activity of Epithelial-Like Cells and Fibroblasts. *Biointerphases* **2012**, *7* (1), 27. <https://doi.org/10.1007/s13758-012-0027-9>.
- (88) Zhang, B.; Hou, Y.; Wang, X.; Wang, Y.; Geng, L. Mechanical Properties, Degradation Performance and Cytotoxicity of Mg–Zn–Ca Biomedical Alloys with Different Compositions. *Mater. Sci. Eng. C* **2011**, *31* (8), 1667–1673. <https://doi.org/10.1016/j.msec.2011.07.015>.
- (89) Ulum, M. F.; Caesarendra, W.; Alavi, R.; Hermawan, H. In-Vivo Corrosion Characterization and Assessment of Absorbable Metal Implants. *Coatings* **2019**, *9* (5), 282. <https://doi.org/10.3390/coatings9050282>.
- (90) Lin, Y.; Liu, J.; Bian, D.; Huang, Z.; Lin, Z.; Wang, M.; Chu, X.; Li, M.; Zhang, Y.; Zheng, Y. In Vivo Studies on Mg–1Sc Alloy for Orthopedic Application: A 5-Months Evaluation in Rabbits. *Mater. Lett.* **2020**, *262*, 127130. <https://doi.org/10.1016/j.matlet.2019.127130>.
- (91) Mao, L.; Yuan, G.; Wang, S.; Niu, J.; Wu, G.; Ding, W. A Novel Biodegradable Mg–Nd–Zn–Zr Alloy with Uniform Corrosion Behavior in Artificial Plasma. *Mater. Lett.* **2012**, *88*, 1–4. <https://doi.org/10.1016/j.matlet.2012.08.012>.
- (92) Mousa, H. M.; Chan, H. P.; Kim, C. S. Surface Modification of Magnesium and Its Alloys Using Anodization for Orthopedic Implant Application. In *Magnesium Alloys*; Aliofkhazraei, M., Ed.; InTech, 2017; pp 219–239. <https://doi.org/10.5772/66341>.
- (93) Franz, S.; Rammelt, S.; Scharnweber, D.; Simon, J. C. Immune Responses to Implants – A Review of the Implications for the Design of Immunomodulatory Biomaterials. *Biomaterials* **2011**, *32* (28), 6692–6709. <https://doi.org/10.1016/j.biomaterials.2011.05.078>.
- (94) Wang, J.; Tang, J.; Zhang, P.; Li, Y.; Wang, J.; Lai, Y.; Qin, L. Surface Modification of Magnesium Alloys Developed for Bioabsorbable Orthopedic Implants: A General Review. *J. Biomed. Mater. Res. B Appl. Biomater.* **2012**, *100B* (6), 1691–1701. <https://doi.org/10.1002/jbm.b.32707>.

- (95) Dong, H. *Surface Engineering of Light Alloys: Aluminium, Magnesium and Titanium Alloys*; Elsevier, 2010.
- (96) Salman, S. A.; Okido, M. Anodization of Magnesium (Mg) Alloys to Improve Corrosion Resistance. In *Corrosion Prevention of Magnesium Alloys*; Elsevier, 2013; pp 197–231. <https://doi.org/10.1533/9780857098962.2.197>.
- (97) Blawert, C.; Bala Srinivasan, P. Plasma Electrolytic Oxidation Treatment of Magnesium Alloys. In *Surface Engineering of Light Alloys*; Elsevier, 2010; pp 155–183. <https://doi.org/10.1533/9781845699451.2.155>.
- (98) Jiang, B. L.; Wang, Y. M. 5 - Plasma Electrolytic Oxidation Treatment of Aluminium and Titanium Alloys. In *Surface Engineering of Light Alloys*; Dong, H., Ed.; Woodhead Publishing, 2010; pp 110–154. <https://doi.org/10.1533/9781845699451.2.110>.
- (99) Barati Darband, Gh.; Aliofkhazraei, M.; Hamghalam, P.; Valizade, N. Plasma Electrolytic Oxidation of Magnesium and Its Alloys: Mechanism, Properties and Applications. *J. Magnes. Alloys* **2017**, *5* (1), 74–132. <https://doi.org/10.1016/j.jma.2017.02.004>.
- (100) Sankara Narayanan, T. S. N.; Park, I. S.; Lee, M. H. Strategies to Improve the Corrosion Resistance of Microarc Oxidation (MAO) Coated Magnesium Alloys for Degradable Implants: Prospects and Challenges. *Prog. Mater. Sci.* **2014**, *60*, 1–71. <https://doi.org/10.1016/j.pmatsci.2013.08.002>.
- (101) Yerokhin, A. L.; Snizhko, L. O.; Gurevina, N. L.; Leyland, A.; Pilkington, A.; Matthews, A. Discharge Characterization in Plasma Electrolytic Oxidation of Aluminium. *J. Phys. Appl. Phys.* **2003**, *36* (17), 2110–2120. <https://doi.org/10.1088/0022-3727/36/17/314>.
- (102) Xue, W.; Deng, Z.; Chen, R.; Zhang, T. Growth Regularity of Ceramic Coatings Formed by Microarc Oxidation on Al–Cu–Mg Alloy. *Thin Solid Films* **2000**, *372* (1), 114–117. [https://doi.org/10.1016/S0040-6090\(00\)01026-9](https://doi.org/10.1016/S0040-6090(00)01026-9).
- (103) Chang, L. Growth Regularity of Ceramic Coating on Magnesium Alloy by Plasma Electrolytic Oxidation. *J. Alloys Compd.* **2009**, *468* (1–2), 462–465. <https://doi.org/10.1016/j.jallcom.2008.01.069>.
- (104) Mohedano, M.; Lu, X.; Matykina, E.; Blawert, C.; Arrabal, R.; Zheludkevich, M. L. Plasma Electrolytic Oxidation (PEO) of Metals and Alloys. In *Encyclopedia of Interfacial Chemistry*; Elsevier, 2018; pp 423–438. <https://doi.org/10.1016/B978-0-12-409547-2.13398-0>.
- (105) Santos-Coquillat, A.; Esteban-Lucia, M.; Martinez-Campos, E.; Mohedano, M.; Arrabal, R.; Blawert, C.; Zheludkevich, M. L.; Matykina, E. PEO Coatings Design for Mg-Ca Alloy for Cardiovascular Stent and Bone Regeneration Applications. *Mater. Sci. Eng. C* **2019**, *105*, 110026. <https://doi.org/10.1016/j.msec.2019.110026>.
- (106) Zhang, T.; Wu, X.; Huang, H.; Zhang, Y.; Li, M.; Lan, G.; Xia, H.; Yin, Q. The Beneficial Influence of Microarc Oxidation-Coated Magnesium Alloy on the Adhesion, Proliferation and Osteogenic Differentiation of Bone Marrow Stromal Cells. *Mater. Lett.* **2014**, *137*, 362–365. <https://doi.org/10.1016/j.matlet.2014.09.002>.
- (107) Matykina, E.; Arrabal, R.; Mohedano, M.; Pardo, A.; Merino, M. C.; Rivero, E. Stability of Plasma Electrolytic Oxidation Coating on Titanium in Artificial Saliva. *J. Mater. Sci. Mater. Med.* **2013**, *24* (1), 37–51. <https://doi.org/10.1007/s10856-012-4787-z>.
- (108) Jia, Z. J.; Li, M.; Liu, Q.; Xu, X. C.; Cheng, Y.; Zheng, Y. F.; Xi, T. F.; Wei, S. C. Micro-Arc Oxidization of a Novel Mg–1Ca Alloy in Three Alkaline KF

- Electrolytes: Corrosion Resistance and Cytotoxicity. *Appl. Surf. Sci.* **2014**, 292, 1030–1039. <https://doi.org/10.1016/j.apsusc.2013.11.038>.
- (109) Tian, P.; Peng, F.; Wang, D.; Liu, X. Corrosion Behavior and Cytocompatibility of Fluoride-Incorporated Plasma Electrolytic Oxidation Coating on Biodegradable AZ31 Alloy. *Regen. Biomater.* **2017**, 4 (1), 1–10. <https://doi.org/10.1093/rb/rbw036>.
- (110) Yao, Z. P.; Liu, X. R.; Li, L. L.; Qi, S. F.; Jiang, Z. H. Effects of Sodium Fluoride on Structure and Corrosion Resistance of Plasma Electrolytic Oxidation Ceramic Coatings on Magnesium Alloys. *CORROSION* **2010**, 66 (8), 085002–085002–085006. <https://doi.org/10.5006/1.3479952>.
- (111) Gu, X. N.; Li, N.; Zhou, W. R.; Zheng, Y. F.; Zhao, X.; Cai, Q. Z.; Ruan, L. Corrosion Resistance and Surface Biocompatibility of a Microarc Oxidation Coating on a Mg–Ca Alloy. *Acta Biomater.* **2011**, 7 (4), 1880–1889. <https://doi.org/10.1016/j.actbio.2010.11.034>.
- (112) Shahri, Z.; Allahkaram, S. R.; Soltani, R.; Jafari, H. Study on Corrosion Behavior of Nano-Structured Coatings Developed on Biodegradable as Cast Mg–Zn–Ca Alloy by Plasma Electrolyte Oxidation. *Surf. Coat. Technol.* **2018**, 347, 225–234. <https://doi.org/10.1016/j.surfcoat.2018.04.087>.
- (113) Moreno, L.; Mohedano, M.; Mingo, B.; Arrabal, R.; Matykina, E. Degradation Behaviour of Mg0.6Ca and Mg0.6Ca2Ag Alloys with Bioactive Plasma Electrolytic Oxidation Coatings. *Coatings* **2019**, 9 (6), 383. <https://doi.org/10.3390/coatings9060383>.
- (114) Wang, Y.; Chen, M.; Zhao, Y. Preparation and Corrosion Resistance of Microarc Oxidation-Coated Biomedical Mg–Zn–Ca Alloy in the Silicon–Phosphorus-Mixed Electrolyte. *ACS Omega* **2019**, 4 (25), 20937–20947. <https://doi.org/10.1021/acsomega.9b01998>.
- (115) Ibrahim, H.; Dehghanghadikolaie, A.; Advincula, R.; Dean, D.; Luo, A.; Elahinia, M. Ceramic Coating for Delayed Degradation of Mg-1.2Zn-0.5Ca-0.5Mn Bone Fixation and Instrumentation. *Thin Solid Films* **2019**, 687, 137456. <https://doi.org/10.1016/j.tsf.2019.137456>.
- (116) Jiang, H. B.; Wu, G.; Lee, S.-B.; Kim, K.-M. Achieving Controllable Degradation of a Biomedical Magnesium Alloy by Anodizing in Molten Ammonium Bifluoride. *Surf. Coat. Technol.* **2017**, 313, 282–287. <https://doi.org/10.1016/j.surfcoat.2017.01.090>.
- (117) Xiong, Y.; Yang, Z.; Hu, X.; Song, R. Bioceramic Coating Produced on AZ80 Magnesium Alloy by One-Step Microarc Oxidation Process. *J. Mater. Eng. Perform.* **2019**, 28 (3), 1719–1727. <https://doi.org/10.1007/s11665-019-03925-3>.
- (118) Robinson, H. J.; Markaki, A. E.; Collier, C. A.; Clyne, T. W. Cell Adhesion to Plasma Electrolytic Oxidation (PEO) Titania Coatings, Assessed Using a Centrifuging Technique. *J. Mech. Behav. Biomed. Mater.* **2011**, 4 (8), 2103–2112. <https://doi.org/10.1016/j.jmbbm.2011.07.009>.
- (119) Zhou, W.; Shen, T.; Aung, N. N. Effect of Heat Treatment on Corrosion Behaviour of Magnesium Alloy AZ91D in Simulated Body Fluid. *Corros. Sci.* **2010**, 52 (3), 1035–1041. <https://doi.org/10.1016/j.corsci.2009.11.030>.
- (120) Whiteside, P.; Matykina, E.; Gough, J. E.; Skeldon, P.; Thompson, G. E. In Vitro Evaluation of Cell Proliferation and Collagen Synthesis on Titanium Following Plasma Electrolytic Oxidation. *J. Biomed. Mater. Res. A* **2010**, 94A (1), 38–46. <https://doi.org/10.1002/jbm.a.32664>.

- (121) Sul, Y.-T.; Johansson, C.; Albrektsson, T. Oxidized Titanium Screws Coated with Calcium Ions and Their Performance in Rabbit Bone. *Int. J. Oral Maxillofac. Implants* **2002**, *17*, 625–634.
- (122) Rao, X.; Chu, C. L.; Sun, Q.; Zheng, Y. Y. Fabrication and Apatite Inducing Ability of Different Porous Titania Structures by PEO Treatment. *Mater. Sci. Eng. C* **2016**, *66*, 297–305. <https://doi.org/10.1016/j.msec.2016.04.038>.
- (123) Martínez Campos, E.; Santos-Coquillat, A.; Mingo, B.; Arrabal, R.; Mohedano, M.; Pardo, A.; Ramos, V.; López Lacomba, J.-L.; Matykina, E. Albumin Loaded PEO Coatings on Ti — Potential as Drug Eluting Systems. *Surf. Coat. Technol.* **2015**, *283*, 44–51. <https://doi.org/10.1016/j.surfcoat.2015.10.045>.
- (124) Zhao, Y.; Chen, X.; Li, S.; Zeng, R.; Zhang, F.; Wang, Z.; Guan, S. Corrosion Resistance and Drug Release Profile of Gentamicin-Loaded Polyelectrolyte Multilayers on Magnesium Alloys: Effects of Heat Treatment. *J. Colloid Interface Sci.* **2019**, *547*, 309–317. <https://doi.org/10.1016/j.jcis.2019.04.017>.
- (125) Heakal, F. E.-T.; Bakry, A. M. Role of Amoxicillin in Enhancing AZ31 Alloy Degradation Resistance and Its Monitoring Using Nano-Pd Electrochemical Sensor. *Mater. Chem. Phys.* **2019**, *234*, 224–236. <https://doi.org/10.1016/j.matchemphys.2019.06.004>.
- (126) Ji, X.-J.; Gao, L.; Liu, J.-C.; Jiang, R.-Z.; Sun, F.-Y.; Cui, L.-Y.; Li, S.-Q.; Zhi, K.-Q.; Zeng, R.-C.; Wang, Z.-L. Corrosion Resistance and Antibacterial Activity of Hydroxyapatite Coating Induced by Ciprofloxacin-Loaded Polymeric Multilayers on Magnesium Alloy. *Prog. Org. Coat.* **2019**, *135*, 465–474. <https://doi.org/10.1016/j.porgcoat.2019.06.048>.
- (127) Bordbar-Khiabani, A.; Yarmand, B.; Sharifi-Asl, S.; Mozafari, M. Improved Corrosion Performance of Biodegradable Magnesium in Simulated Inflammatory Condition via Drug-Loaded Plasma Electrolytic Oxidation Coatings. *Mater. Chem. Phys.* **2020**, *239*, 122003. <https://doi.org/10.1016/j.matchemphys.2019.122003>.
- (128) Toorani, M.; Aliofkhazraei, M.; Golabadi, M.; Rouhaghdam, A. S. Effect of Lanthanum Nitrate on the Microstructure and Electrochemical Behavior of PEO Coatings on AZ31 Mg Alloy. *J. Alloys Compd.* **2017**, *719*, 242–255. <https://doi.org/10.1016/j.jallcom.2017.05.169>.
- (129) Phuong, N. V.; Fazal, B. R.; Moon, S. Cerium- and Phosphate-Based Sealing Treatments of PEO Coated AZ31 Mg Alloy. *Surf. Coat. Technol.* **2017**, *309*, 86–95. <https://doi.org/10.1016/j.surfcoat.2016.11.055>.
- (130) Kartsonakis, I. A.; Stanciu, S. G.; Matei, A. A.; Karaxi, E. K.; Hristu, R.; Karantonis, A.; Charitidis, C. A. Evaluation of the Protective Ability of Typical Corrosion Inhibitors for Magnesium Alloys towards the Mg ZK30 Variant. *Corros. Sci.* **2015**, *100*, 194–208. <https://doi.org/10.1016/j.corsci.2015.07.028>.
- (131) Dinodi, N.; Shetty, A. N. Alkyl Carboxylates as Efficient and Green Inhibitors of Magnesium Alloy ZE41 Corrosion in Aqueous Salt Solution. *Corros. Sci.* **2014**, *85*, 411–427. <https://doi.org/10.1016/j.corsci.2014.04.052>.
- (132) Huang, D.; Hu, J.; Song, G.-L.; Guo, X. Inhibition Effect of Inorganic and Organic Inhibitors on the Corrosion of Mg–10Gd–3Y–0.5Zr Alloy in an Ethylene Glycol Solution at Ambient and Elevated Temperatures. *Electrochimica Acta* **2011**, *56* (27), 10166–10178. <https://doi.org/10.1016/j.electacta.2011.09.002>.
- (133) Wierzbicka, E.; Vaghefinazari, B.; Lamaka, S. V.; Zheludkevich, M. L.; Mohedano, M.; Moreno, L.; Visser, P.; Rodriguez, A.; Velasco, J.; Arrabal, R.; Matykina, E. Flash-PEO as an Alternative to Chromate Conversion Coatings for Corrosion Protection of Mg Alloy. *Corros. Sci.* **2021**, *180*, 109189. <https://doi.org/10.1016/j.corsci.2020.109189>.

- (134) Brusciotti, F.; Snihirova, D. V.; Xue, H.; Montemor, M. F.; Lamaka, S. V.; Ferreira, M. G. S. Hybrid Epoxy–Silane Coatings for Improved Corrosion Protection of Mg Alloy. *Corros. Sci.* **2013**, *67*, 82–90. <https://doi.org/10.1016/j.corsci.2012.10.013>.
- (135) Hu, R.-G.; Zhang, S.; Bu, J.-F.; Lin, C.-J.; Song, G.-L. Recent Progress in Corrosion Protection of Magnesium Alloys by Organic Coatings. *Prog. Org. Coat.* **2012**, *73* (2), 129–141. <https://doi.org/10.1016/j.porgcoat.2011.10.011>.
- (136) Yang, W.; Li, Q.; Xiao, Q.; Liang, J. Improvement of Corrosion Protective Performance of Organic Coating on Low Carbon Steel by PEO Pretreatment. *Prog. Org. Coat.* **2015**, *89*, 260–266. <https://doi.org/10.1016/j.porgcoat.2015.09.003>.
- (137) Lamaka, S. V.; Xue, H. B.; Meis, N. N. A. H.; Esteves, A. C. C.; Ferreira, M. G. S. Fault-Tolerant Hybrid Epoxy-Silane Coating for Corrosion Protection of Magnesium Alloy AZ31. *Prog. Org. Coat.* **2015**, *80*, 98–105. <https://doi.org/10.1016/j.porgcoat.2014.11.024>.
- (138) Lamaka, S. V.; Knörschild, G.; Snihirova, D. V.; Taryba, M. G.; Zheludkevich, M. L.; Ferreira, M. G. S. Complex Anticorrosion Coating for ZK30 Magnesium Alloy. *Electrochimica Acta* **2009**, *55* (1), 131–141. <https://doi.org/10.1016/j.electacta.2009.08.018>.
- (139) Gnedenkov, A. S.; Sinebryukhov, S. L.; Mashtalyar, D. V.; Gnedenkov, S. V. Protective Properties of Inhibitor-Containing Composite Coatings on a Mg Alloy. *Corros. Sci.* **2016**, *102*, 348–354. <https://doi.org/10.1016/j.corsci.2015.10.026>.
- (140) Gnedenkov, A. S.; Sinebryukhov, S. L.; Mashtalyar, D. V.; Gnedenkov, S. V. Localized Corrosion of the Mg Alloys with Inhibitor-Containing Coatings: SVET and SIET Studies. *Corros. Sci.* **2016**, *102*, 269–278. <https://doi.org/10.1016/j.corsci.2015.10.015>.
- (141) Frignani, A.; Grassi, V.; Zanutto, F.; Zucchi, F. Inhibition of AZ31 Mg Alloy Corrosion by Anionic Surfactants. *Corros. Sci.* **2012**, *63*, 29–39. <https://doi.org/10.1016/j.corsci.2012.05.012>.
- (142) Fayomi, O. S. I.; Akande, I. G.; Nsikak, U. An Overview of Corrosion Inhibition Using Green and Drug Inhibitors. *J. Phys. Conf. Ser.* **2019**, *1378*, 022022. <https://doi.org/10.1088/1742-6596/1378/2/022022>.
- (143) Shamnamol, G. K.; Sreelakshmi, K. P.; Ajith, G.; Jacob, J. M. Effective Utilization of Drugs as Green Corrosion Inhibitor – A Review. *AIP Conf. Proc.* **2020**, *2225* (1), 070006. <https://doi.org/10.1063/5.0005931>.
- (144) Verma, C. Drugs as Environmentally Benign Corrosion Inhibitors for Ferrous and Nonferrous Materials in Acid Environment: An Overview. *J. Mater. Environ. Sci.* **2017**, *8* (11), 4040–4051.
- (145) Lamaka, S. V.; Vaghefinazari, B.; Mei, D.; Petrauskas, R. P.; Höche, D.; Zheludkevich, M. L. Comprehensive Screening of Mg Corrosion Inhibitors. *Corros. Sci.* **2017**, *128*, 224–240. <https://doi.org/10.1016/j.corsci.2017.07.011>.
- (146) Anadebe, V. C.; Onukwuli, O. D.; Abeng, F. E.; Okafor, N. A.; Ezeugo, J. O.; Okoye, C. C. Electrochemical-Kinetics, MD-Simulation and Multi-Input Single-Output (MISO) Modeling Using Adaptive Neuro-Fuzzy Inference System (ANFIS) Prediction for Dexamethasone Drug as Eco-Friendly Corrosion Inhibitor for Mild Steel in 2 M HCl Electrolyte. *J. Taiwan Inst. Chem. Eng.* **2020**, *115*, 251–265. <https://doi.org/10.1016/j.jtice.2020.10.004>.
- (147) Raghavendra, N. Expired Naproxen Drug as a Robust Corrosion Inhibitor of Al in 3 M Hydrochloric Acid System. **2020**, *42* (4), 917–922.

- (148) Fekry, A. M.; Fatayerji, M. Z. Electrochemical Corrosion Behavior of AZ91D Alloy in Ethylene Glycol. *Electrochimica Acta* **2009**, *54* (26), 6522–6528. <https://doi.org/10.1016/j.electacta.2009.06.025>.
- (149) Sathasivam, K.; Jeeva, P. A.; Dinakaran, R.; Narayanan, S. The Inhibitive Action of Eco-Friendly Compound on the Corrosion of Mild Steel in Brine Water. **2017**, *20*, 1–12.
- (150) Srinivasulu, A.; Kasthuri, P. Study of Inhibition and Adsorption Properties of Mild Steel Corrosion by Expired Pharmaceutical Gentamicin Drug in Hydrochloric Acid Media. *Orient. J. Chem.* **2017**, *33*, 2616–2624. <https://doi.org/10.13005/ojc/330559>.
- (151) Akpan, I. A.; Offiong, N.-A. O. Inhibition of Mild Steel Corrosion in Hydrochloric Acid Solution by Ciprofloxacin Drug. *Int. J. Corros.* **2013**, *2013*, e301689. <https://doi.org/10.1155/2013/301689>.
- (152) Oyekunle, D. T.; Agboola, O.; Ayeni, A. O. Corrosion Inhibitors as Building Evidence for Mild Steel: A Review. *J. Phys. Conf. Ser.* **2019**, *1378* (3), 032046. <https://doi.org/10.1088/1742-6596/1378/3/032046>.
- (153) Shukla, S. K.; Quraishi, M. A. The Effects of Pharmaceutically Active Compound Doxycycline on the Corrosion of Mild Steel in Hydrochloric Acid Solution. *Corros. Sci.* **2010**, *52* (2), 314–321. <https://doi.org/10.1016/j.corsci.2009.09.017>.
- (154) Wong, H. M.; Yeung, K. W. K.; Lam, K. O.; Tam, V.; Chu, P. K.; Luk, K. D. K.; Cheung, K. M. C. A Biodegradable Polymer-Based Coating to Control the Performance of Magnesium Alloy Orthopaedic Implants. *Biomaterials* **2010**, *31* (8), 2084–2096. <https://doi.org/10.1016/j.biomaterials.2009.11.111>.
- (155) Gray, J. E.; Luan, B. Protective Coatings on Magnesium and Its Alloys — a Critical Review. *J. Alloys Compd.* **2002**, *336* (1), 88–113. [https://doi.org/10.1016/S0925-8388\(01\)01899-0](https://doi.org/10.1016/S0925-8388(01)01899-0).
- (156) Blacklock, J.; Sievers, T. K.; Handa, H.; You, Y.-Z.; Oupický, D.; Mao, G.; Möhwald, H. Cross-Linked Bio-reducible Layer-by-Layer Films for Increased Cell Adhesion and Transgene Expression. *J. Phys. Chem. B* **2010**, *114* (16), 5283–5291. <https://doi.org/10.1021/jp100486h>.
- (157) Rezwan, K.; Chen, Q. Z.; Blaker, J. J.; Boccaccini, A. R. Biodegradable and Bioactive Porous Polymer/Inorganic Composite Scaffolds for Bone Tissue Engineering. *Biomaterials* **2006**, *27* (18), 3413–3431. <https://doi.org/10.1016/j.biomaterials.2006.01.039>.
- (158) Agrawal, C. M. Biodegradable Polymers for Orthopaedic Applications. In *Polymer Based Systems on Tissue Engineering, Replacement and Regeneration*; Reis, R. L., Cohn, D., Eds.; NATO Science Series; Springer Netherlands: Dordrecht, 2002; pp 25–36. https://doi.org/10.1007/978-94-010-0305-6_3.
- (159) Li, L.-Y.; Cui, L.-Y.; Zeng, R.-C.; Li, S.-Q.; Chen, X.-B.; Zheng, Y.; Kannan, M. B. Advances in Functionalized Polymer Coatings on Biodegradable Magnesium Alloys – A Review. *Acta Biomater.* **2018**, *79*, 23–36. <https://doi.org/10.1016/j.actbio.2018.08.030>.
- (160) Chan, W. Y.; Chian, K. S.; Tan, M. J. In Vitro Metal Ion Release and Biocompatibility of Amorphous Mg₆₇Zn₂₈Ca₅ Alloy with/without Gelatin Coating. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2013**, *33* (8), 5019–5027. <https://doi.org/10.1016/j.msec.2013.08.025>.
- (161) Ravi Kumar, M. N. V. A Review of Chitin and Chitosan Applications. *React. Funct. Polym.* **2000**, *46* (1), 1–27. [https://doi.org/10.1016/S1381-5148\(00\)00038-9](https://doi.org/10.1016/S1381-5148(00)00038-9).

- (162) Heise, S.; Virtanen, S.; Boccaccini, A. R. Tackling Mg Alloy Corrosion by Natural Polymer Coatings—A Review. *J. Biomed. Mater. Res. A* **2016**, *104* (10), 2628–2641. <https://doi.org/10.1002/jbm.a.35776>.
- (163) Scharnagl, N.; Blawert, C. 14 - Polymer-Based Degradable Coatings for Metallic Biomaterials. In *Surface Coating and Modification of Metallic Biomaterials*; Wen, C., Ed.; Woodhead Publishing, 2015; pp 393–422. <https://doi.org/10.1016/B978-1-78242-303-4.00014-4>.
- (164) Prabhu, D. B.; Gopalakrishnan, P.; Ravi, K. R. Coatings on Implants: Study on Similarities and Differences between the PCL Coatings for Mg Based Lab Coupons and Final Components. *Mater. Des.* **2017**, *135*, 397–410. <https://doi.org/10.1016/j.matdes.2017.09.043>.
- (165) Tang, J.; Wang, J.; Xie, X.; Zhang, P.; Lai, Y.; Li, Y.; Qin, L. Surface Coating Reduces Degradation Rate of Magnesium Alloy Developed for Orthopaedic Applications. *J. Orthop. Transl.* **2013**, *1* (1), 41–48. <https://doi.org/10.1016/j.jot.2013.06.003>.
- (166) Santos-Coquillat, A.; Martínez-Campos, E.; Mora Sánchez, H.; Moreno, L.; Arrabal, R.; Mohedano, M.; Gallardo, A.; Rodríguez-Hernández, J.; Matykina, E. Hybrid Functionalized Coatings on Metallic Biomaterials for Tissue Engineering. *Surf. Coat. Technol.* **2021**, *422*, 127508. <https://doi.org/10.1016/j.surfcoat.2021.127508>.
- (167) Zhou, H.; Lawrence, J. G.; Bhaduri, S. B. Fabrication Aspects of PLA-CaP/PLGA-CaP Composites for Orthopedic Applications: A Review. *Acta Biomater.* **2012**, *8* (6), 1999–2016. <https://doi.org/10.1016/j.actbio.2012.01.031>.
- (168) Liu, S.; Qin, S.; He, M.; Zhou, D.; Qin, Q.; Wang, H. Current Applications of Poly(Lactic Acid) Composites in Tissue Engineering and Drug Delivery. *Compos. Part B Eng.* **2020**, *199*, 108238. <https://doi.org/10.1016/j.compositesb.2020.108238>.
- (169) Zimmermann, T.; Ferrandez-Montero, A.; Lieblich, M.; Ferrari, B.; González-Carrasco, J. L.; Müller, W.-D.; Schwitalla, A. D. In Vitro Degradation of a Biodegradable Polylactic Acid/Magnesium Composite as Potential Bone Augmentation Material in the Presence of Titanium and PEEK Dental Implants. *Dent. Mater.* **2018**, *34* (10), 1492–1500. <https://doi.org/10.1016/j.dental.2018.06.009>.
- (170) Zhao, D.; Zhu, T.; Li, J.; Cui, L.; Zhang, Z.; Zhuang, X.; Ding, J. Poly(Lactic-Co-Glycolic Acid)-Based Composite Bone-Substitute Materials. *Bioact. Mater.* **2021**, *6* (2), 346–360. <https://doi.org/10.1016/j.bioactmat.2020.08.016>.
- (171) Lai, Y.; Li, Y.; Cao, H.; Long, J.; Wang, X.; Li, L.; Li, C.; Jia, Q.; Teng, B.; Tang, T.; Peng, J.; Eglin, D.; Alini, M.; Grijpma, D. W.; Richards, G.; Qin, L. Osteogenic Magnesium Incorporated into PLGA/TCP Porous Scaffold by 3D Printing for Repairing Challenging Bone Defect. *Biomaterials* **2019**, *197*, 207–219. <https://doi.org/10.1016/j.biomaterials.2019.01.013>.
- (172) Chen, J.-Y.; Chen, X.-B.; Li, J.-L.; Tang, B.; Birbilis, N.; Wang, X. Electrospayed PLGA Smart Containers for Active Anti-Corrosion Coating on Magnesium Alloy AMLite. *J. Mater. Chem. A* **2014**, *2* (16), 5738. <https://doi.org/10.1039/c3ta14999d>.
- (173) Mamaghani, M. Y.; Pishvaei, M.; Kaffashi, B. Synthesis of Latex Based Antibacterial Acrylate Polymer/Nanosilver via in Situ Miniemulsion Polymerization. *Macromol. Res.* **2011**, *19* (3), 243–249. <https://doi.org/10.1007/s13233-011-0307-0>.
- (174) Yazdimamaghani, M.; Pourvala, T.; Motamedi, E.; Fathi, B.; Vashaei, D.; Tayebi, L. Synthesis and Characterization of Encapsulated Nanosilica Particles with an

- Acrylic Copolymer by in Situ Emulsion Polymerization Using Thermoresponsive Nonionic Surfactant. *Materials* **2013**, *6* (9), 3727–3741. <https://doi.org/10.3390/ma6093727>.
- (175) Yazdimamaghani, M.; Razavi, M.; Vashae, D.; Tayebi, L. Development and Degradation Behavior of Magnesium Scaffolds Coated with Polycaprolactone for Bone Tissue Engineering. *Mater. Lett.* **2014**, *132*, 106–110. <https://doi.org/10.1016/j.matlet.2014.06.036>.
- (176) Kim, Y.-K.; Lee, K.-B.; Kim, S.-Y.; Jang, Y.-S.; Kim, J. H.; Lee, M.-H. Improvement of Osteogenesis by a Uniform PCL Coating on a Magnesium Screw for Biodegradable Applications. *Sci. Rep.* **2018**, *8* (1), 13264. <https://doi.org/10.1038/s41598-018-31359-9>.
- (177) Zomorodian, A.; Ribeiro, I. A.; Fernandes, J. C. S.; Matos, A. C.; Santos, C.; Bettencourt, A. F.; Montemor, M. F. Biopolymeric Coatings for Delivery of Antibiotic and Controlled Degradation of Bioresorbable Mg AZ31 Alloys. *Int. J. Polym. Mater.* **2017**, *66* (11), 533–543. <https://doi.org/10.1080/00914037.2016.1252347>.
- (178) Lynge, M. E.; Westen, R. van der; Postma, A.; Städler, B. Polydopamine—a Nature-Inspired Polymer Coating for Biomedical Science. *Nanoscale* **2011**, *3* (12), 4916–4928. <https://doi.org/10.1039/C1NR10969C>.
- (179) Liu, Y.; Ai, K.; Lu, L. Polydopamine and Its Derivative Materials: Synthesis and Promising Applications in Energy, Environmental, and Biomedical Fields. *Chem. Rev.* **2014**, *114* (9), 5057–5115. <https://doi.org/10.1021/cr400407a>.
- (180) Guo, Y.; Jia, S.; Qiao, L.; Su, Y.; Gu, R.; Li, G.; Lian, J. Enhanced Corrosion Resistance and Biocompatibility of Polydopamine/Dicalcium Phosphate Dihydrate/Collagen Composite Coating on Magnesium Alloy for Orthopedic Applications. *J. Alloys Compd.* **2020**, *817*, 152782. <https://doi.org/10.1016/j.jallcom.2019.152782>.
- (181) Singer, F.; Schlesak, M.; Mebert, C.; Höhn, S.; Virtanen, S. Corrosion Properties of Polydopamine Coatings Formed in One-Step Immersion Process on Magnesium. *ACS Appl. Mater. Interfaces* **2015**, *7* (48), 26758–26766. <https://doi.org/10.1021/acsami.5b08760>.
- (182) Sinha, V. R.; Singla, A. K.; Wadhawan, S.; Kaushik, R.; Kumria, R.; Bansal, K.; Dhawan, S. Chitosan Microspheres as a Potential Carrier for Drugs. *Int. J. Pharm.* **2004**, *274* (1), 1–33. <https://doi.org/10.1016/j.ijpharm.2003.12.026>.
- (183) Ordikhani, F.; Tamjid, E.; Simchi, A. Characterization and Antibacterial Performance of Electrodeposited Chitosan–Vancomycin Composite Coatings for Prevention of Implant-Associated Infections. *Mater. Sci. Eng. C* **2014**, *41*, 240–248. <https://doi.org/10.1016/j.msec.2014.04.036>.
- (184) Adhikari, U.; Rijal, N. P.; Khanal, S.; Pai, D.; Sankar, J.; Bhattarai, N. Magnesium Incorporated Chitosan Based Scaffolds for Tissue Engineering Applications. *Bioact. Mater.* **2016**, *1* (2), 132–139. <https://doi.org/10.1016/j.bioactmat.2016.11.003>.
- (185) Abinaya, B.; Prasith, T. P.; Ashwin, B.; Chandran, S. V.; Selvamurugan, N. Chitosan in Surface Modification for Bone Tissue Engineering Applications. *Biotechnol. J.* **2019**, *14* (12), 1900171. <https://doi.org/10.1002/biot.201900171>.
- (186) Barani, B.; Lakshminarayanan, A. K.; Subashini, R. Microstructural Characteristics of Chitosan Deposited Az91 Magnesium Alloy. *Mater. Today Proc.* **2019**, *16*, 456–462. <https://doi.org/10.1016/j.matpr.2019.05.115>.
- (187) Wagermaier, W.; Fratzl, P. *Collagen*; Elsevier, 2012; pp 35–55. <https://doi.org/10.1016/B978-0-444-53349-4.00247-8>.

- (188) Davidenko, N.; Hamaia, S.; Bax, D. V.; Malcor, J.-D.; Schuster, C. F.; Gullberg, D.; Farndale, R. W.; Best, S. M.; Cameron, R. E. Selecting the Correct Cellular Model for Assessing of the Biological Response of Collagen-Based Biomaterials. *Acta Biomater.* **2018**, *65*, 88–101. <https://doi.org/10.1016/j.actbio.2017.10.035>.
- (189) Zhao, N.; Zhu, D. Collagen Self-Assembly on Orthopedic Magnesium Biomaterials Surface and Subsequent Bone Cell Attachment. *PLOS ONE* **2014**, *9* (10), e110420. <https://doi.org/10.1371/journal.pone.0110420>.
- (190) Mushahary, D.; Wen, C.; Kumar, J. M.; Lin, J.; Harishankar, N.; Hodgson, P.; Pande, G.; Li, Y. Collagen Type-I Leads to in Vivo Matrix Mineralization and Secondary Stabilization of Mg–Zr–Ca Alloy Implants. *Colloids Surf. B Biointerfaces* **2014**, *122*, 719–728. <https://doi.org/10.1016/j.colsurfb.2014.08.005>.
- (191) Barbeck, M.; Kühnel, L.; Witte, F.; Pissarek, J.; Precht, C.; Xiong, X.; Krastev, R.; Wegner, N.; Walther, F.; Jung, O. Degradation, Bone Regeneration and Tissue Response of an Innovative Volume Stable Magnesium-Supported GBR/GTR Barrier Membrane. *Int. J. Mol. Sci.* **2020**, *21* (9), 3098. <https://doi.org/10.3390/ijms21093098>.
- (192) Burkersroda, F. von; Schedl, L.; Göpferich, A. Why Degradable Polymers Undergo Surface Erosion or Bulk Erosion. *Biomaterials* **2002**, *23* (21), 4221–4231. [https://doi.org/10.1016/S0142-9612\(02\)00170-9](https://doi.org/10.1016/S0142-9612(02)00170-9).
- (193) Zberg, B.; Uggowitzer, P. J.; Löffler, J. F. MgZnCa Glasses without Clinically Observable Hydrogen Evolution for Biodegradable Implants. *Nat. Mater.* **2009**, *8* (11), 887–891. <https://doi.org/10.1038/nmat2542>.
- (194) Xu, L.; Yamamoto, A. In Vitro Degradation of Biodegradable Polymer-Coated Magnesium under Cell Culture Condition. *Appl. Surf. Sci.* **2012**, *258* (17), 6353–6358. <https://doi.org/10.1016/j.apsusc.2012.03.036>.
- (195) Xu, L.; Yamamoto, A. Characteristics and Cytocompatibility of Biodegradable Polymer Film on Magnesium by Spin Coating. *Colloids Surf. B Biointerfaces* **2012**, *93*, 67–74. <https://doi.org/10.1016/j.colsurfb.2011.12.009>.
- (196) Makkar, P.; Kang, H. J.; Padalhin, A. R.; Park, I.; Moon, B.-G.; Lee, B. T. Development and Properties of Duplex MgF2/PCL Coatings on Biodegradable Magnesium Alloy for Biomedical Applications. *PLoS ONE* **2018**, *13* (4). <https://doi.org/10.1371/journal.pone.0193927>.
- (197) Hornberger, H.; Virtanen, S.; Boccaccini, A. R. Biomedical Coatings on Magnesium Alloys – A Review. *Acta Biomater.* **2012**, *8* (7), 2442–2455. <https://doi.org/10.1016/j.actbio.2012.04.012>.
- (198) Sharma, D.; Rawat, M.; Sharma, J.; Ahuja, S. K.; Chandra, A.; Barman, S.; Arya, R. K. Polymer Coatings Methods. In *Polymer Coatings*; CRC Press, 2020; pp 11–31.
- (199) Wang, C.; Yi, Z.; Sheng, Y.; Tian, L.; Qin, L.; Ngai, T.; Lin, W. Development of a Novel Biodegradable and Anti-Bacterial Polyurethane Coating for Biomedical Magnesium Rods. *Mater. Sci. Eng. C* **2019**, *99*, 344–356. <https://doi.org/10.1016/j.msec.2019.01.119>.
- (200) Ahmadi Lakalayeh, G.; Rahvar, M.; Haririan, E.; Karimi, R.; Ghanbari, H. Comparative Study of Different Polymeric Coatings for the Next-Generation Magnesium-Based Biodegradable Stents. *Artif. Cells Nanomedicine Biotechnol.* **2018**, *46* (7), 1380–1389. <https://doi.org/10.1080/21691401.2017.1369424>.
- (201) Abdal-hay, A.; Barakat, N. A. M.; Lim, J. K. Influence of Electrospinning and Dip-Coating Techniques on the Degradation and Cytocompatibility of Mg-Based Alloy. *Colloids Surf. Physicochem. Eng. Asp.* **2013**, *420*, 37–45. <https://doi.org/10.1016/j.colsurfa.2012.12.009>.

- (202) Abdal-hay, A.; Dewidar, M.; Lim, J. K. Biocorrosion Behavior and Cell Viability of Adhesive Polymer Coated Magnesium Based Alloys for Medical Implants. *Appl. Surf. Sci.* **2012**, *261*, 536–546. <https://doi.org/10.1016/j.apsusc.2012.08.051>.
- (203) Bautista, A. Filiform Corrosion in Polymer-Coated Metals. *Prog. Org. Coat.* **1996**, *28* (1), 49–58. [https://doi.org/10.1016/0300-9440\(95\)00555-2](https://doi.org/10.1016/0300-9440(95)00555-2).
- (204) Bierwagen, G. P. Reflections on Corrosion Control by Organic Coatings. *Prog. Org. Coat.* **1996**, *28* (1), 43–48. [https://doi.org/10.1016/0300-9440\(95\)00588-9](https://doi.org/10.1016/0300-9440(95)00588-9).
- (205) Santos-Coquillat, A.; Martínez-Campos, E.; Vargas-Alfredo, N.; Arrabal, R.; Rodríguez-Hernández, J.; Matykina, E. Hierarchical Functionalized Polymeric-Ceramic Coatings on Mg-Ca Alloys for Biodegradable Implant Applications. *Macromol. Biosci.* **2019**, *19* (10), 1900179. <https://doi.org/10.1002/mabi.201900179>.
- (206) Muñoz, M.; Torres, B.; Mohedano, M.; Matykina, E.; Arrabal, R.; López, A. J.; Rams, J. PLA Deposition on Surface Treated Magnesium Alloy: Adhesion, Toughness and Corrosion Behaviour. *Surf. Coat. Technol.* **2020**, *388*, 125593. <https://doi.org/10.1016/j.surfcoat.2020.125593>.
- (207) Butt, M. S.; Maqbool, A.; Umer, M. A.; Saleem, M.; Malik, R. A.; Alarifi, I. M.; Alrobei, H. Enhanced Mechanical Properties of Surface Treated AZ31 Reinforced Polymer Composites. *Crystals* **2020**, *10* (5), 381. <https://doi.org/10.3390/cryst10050381>.
- (208) Soleymani, F.; Emadi, R.; Sadeghzade, S.; Tavangarian, F. Bioactivity Behavior Evaluation of PCL-Chitosan-Nanobaghdadite Coating on AZ91 Magnesium Alloy in Simulated Body Fluid. *Coatings* **2020**, *10* (3), 231. <https://doi.org/10.3390/coatings10030231>.
- (209) Hiromoto, S.; Doi, K. Self-Healing Behavior of Sodium Polyacrylate-Hydroxyapatite Coatings on Biodegradable Magnesium Alloy. *CORROSION* **2017**, *73* (12), 1461–1477. <https://doi.org/10.5006/2509>.
- (210) Butt, M. S.; Bai, J.; Wan, X.; Chu, C.; Xue, F.; Ding, H.; Zhou, G. Mechanical and Degradation Properties of Biodegradable Mg Strengthened Poly-Lactic Acid Composite through Plastic Injection Molding. *Mater. Sci. Eng. C* **2017**, *70*, 141–147. <https://doi.org/10.1016/j.msec.2016.08.051>.
- (211) Gnedenkov, S. V.; Sinebryukhov, S. L.; Mashtalyar, D. V.; Egorkin, V. S.; Sidorova, M. V.; Gnedenkov, A. S. Composite Polymer-Containing Protective Coatings on Magnesium Alloy MA8. *Corros. Sci.* **2014**, *85*, 52–59. <https://doi.org/10.1016/j.corsci.2014.03.035>.
- (212) Li, L.-H.; Sankara Narayanan, T. S. N.; Kim, Y. K.; Kong, Y.-M.; Park, I. S.; Bae, T. S.; Lee, M. H. Deposition of Microarc Oxidation–Polycaprolactone Duplex Coating to Improve the Corrosion Resistance of Magnesium for Biodegradable Implants. *Thin Solid Films* **2014**, *562*, 561–567. <https://doi.org/10.1016/j.tsf.2014.04.004>.
- (213) Hubbell, J. A. *In Situ* Material Transformations in Tissue Engineering. *MRS Bull.* **1996**, *21* (11), 33–35. <https://doi.org/10.1557/S0883769400031821>.
- (214) Lotfi, M.; Nejib, M.; Naceur, M. Cell Adhesion to Biomaterials: Concept of Biocompatibility. *Adv. Biomater. Sci. Biomed. Appl.* **2013**. <https://doi.org/10.5772/53542>.
- (215) Eisenbarth, E.; Meyle, J.; Nachtigall, W.; Breme, J. Influence of the Surface Structure of Titanium Materials on the Adhesion of Fibroblasts. *Biomaterials* **1996**, *17* (14), 1399–1403. [https://doi.org/10.1016/0142-9612\(96\)87281-4](https://doi.org/10.1016/0142-9612(96)87281-4).
- (216) Murphy, W.; Black, J.; Hastings, G. *Handbook of Biomaterial Properties*; Springer, 2016.

- (217) Marmur, A. Equilibrium Contact Angles: Theory and Measurement. *Colloids Surf. Physicochem. Eng. Asp.* **1996**, 116 (1), 55–61. [https://doi.org/10.1016/0927-7757\(96\)03585-6](https://doi.org/10.1016/0927-7757(96)03585-6).
- (218) Morra, M.; Occhiello, E.; Garbassi, F. Knowledge about Polymer Surfaces from Contact Angle Measurements. *Adv. Colloid Interface Sci.* **1990**, 32 (1), 79–116. [https://doi.org/10.1016/0001-8686\(90\)80012-O](https://doi.org/10.1016/0001-8686(90)80012-O).
- (219) Amani, H.; Arzaghi, H.; Bayandori, M.; Dezfuli, A. S.; Pazoki-Toroudi, H.; Shafiee, A.; Moradi, L. Controlling Cell Behavior through the Design of Biomaterial Surfaces: A Focus on Surface Modification Techniques. *Adv. Mater. Interfaces* **2019**, 6 (13), 1900572. <https://doi.org/10.1002/admi.201900572>.
- (220) Liu, P.; Pan, X.; Yang, W.; Cai, K.; Chen, Y. Improved Anticorrosion of Magnesium Alloy via Layer-by-Layer Self-Assembly Technique Combined with Micro-Arc Oxidation. *Mater. Lett.* **2012**, 75, 118–121. <https://doi.org/10.1016/j.matlet.2012.02.016>.
- (221) Guo, M.; Cao, L.; Lu, P.; Liu, Y.; Xu, X. Anticorrosion and Cytocompatibility Behavior of MAO/PLLA Modified Magnesium Alloy WE42. *J. Mater. Sci. Mater. Med.* **2011**, 22 (7), 1735–1740. <https://doi.org/10.1007/s10856-011-4354-z>.
- (222) Gao, J. H.; Guan, S. K.; Chen, J.; Wang, L. G.; Zhu, S. J.; Hu, J. H.; Ren, Z. W. Fabrication and Characterization of Rod-like Nano-Hydroxyapatite on MAO Coating Supported on Mg–Zn–Ca Alloy. *Appl. Surf. Sci.* **2011**, 257 (6), 2231–2237. <https://doi.org/10.1016/j.apsusc.2010.09.080>.
- (223) Daavari, M.; Atapour, M.; Mohedano, M.; Sánchez, H. M.; Rodríguez-Hernández, J.; Matykina, E.; Arrabal, R.; Taherizadeh, A. Quasi-in Vivo Corrosion Behavior of AZ31B Mg Alloy with Hybrid MWCNTs-PEO/PCL Based Coatings. *J. Magnes. Alloys* **2021**. <https://doi.org/10.1016/j.jma.2021.09.010>.
- (224) Hu, J.; Hardy, C.; Chen, C.-M.; Yang, S.; Voloshin, A. S.; Liu, Y. Enhanced Cell Adhesion and Alignment on Micro-Wavy Patterned Surfaces. *PLOS ONE* **2014**, 9 (8), e104502. <https://doi.org/10.1371/journal.pone.0104502>.
- (225) Martínez, E.; Engel, E.; Planell, J. A.; Samitier, J. Effects of Artificial Micro- and Nano-Structured Surfaces on Cell Behaviour. *Ann. Anat. - Anat. Anz.* **2009**, 191 (1), 126–135. <https://doi.org/10.1016/j.aanat.2008.05.006>.
- (226) Norman, J. J.; Desai, T. A. Methods for Fabrication of Nanoscale Topography for Tissue Engineering Scaffolds. *Ann. Biomed. Eng.* **2006**, 34 (1), 89–101. <https://doi.org/10.1007/s10439-005-9005-4>.
- (227) González-Henríquez, C. M.; Sarabia Vallejos, M. A.; Rodríguez-Hernández, J. Introduction to Surface Instabilities and Wrinkle Formation. In *Wrinkled Polymer Surfaces: Strategies, Methods and Applications*; González-Henríquez, C. M., Rodríguez-Hernández, J., Eds.; Springer International Publishing: Cham, 2019; pp 3–18. https://doi.org/10.1007/978-3-030-05123-5_1.
- (228) Widawski, G.; Rawiso, M.; François, B. Self-Organized Honeycomb Morphology of Star-Polymer Polystyrene Films. *Nature* **1994**, 369 (6479), 387–389. <https://doi.org/10.1038/369387a0>.
- (229) Calejo, M. T.; Ilmarinen, T.; Skottman, H.; Kellomäki, M. Breath Figures in Tissue Engineering and Drug Delivery: State-of-the-Art and Future Perspectives. *Acta Biomater.* **2018**, 66, 44–66. <https://doi.org/10.1016/j.actbio.2017.11.043>.
- (230) Zhu, L.-W.; Wan, L.-S.; Jin, J.; Xu, Z.-K. Honeycomb Porous Films Prepared from Porphyrin-Cored Star Polymers: Submicrometer Pores Induced by Transition of Monolayer into Multilayer Structures. *J. Phys. Chem. C* **2013**, 117 (12), 6185–6194. <https://doi.org/10.1021/jp311439p>.

- (231) Stenzel, M. H.; Barner-Kowollik, C.; Davis, T. P. Formation of Honeycomb-Structured, Porous Films via Breath Figures with Different Polymer Architectures. *J. Polym. Sci. Part Polym. Chem.* **2006**, *44* (8), 2363–2375. <https://doi.org/10.1002/pola.21334>.
- (232) Muñoz-Bonilla, A.; Fernández-García, M.; Rodríguez-Hernández, J. Towards Hierarchically Ordered Functional Porous Polymeric Surfaces Prepared by the Breath Figures Approach. *Prog. Polym. Sci.* **2014**, *39* (3), 510–554. <https://doi.org/10.1016/j.progpolymsci.2013.08.006>.
- (233) Zhang, A.; Bai, H.; Li, L. Breath Figure: A Nature-Inspired Preparation Method for Ordered Porous Films. *Chem. Rev.* **2015**, *115* (18), 9801–9868. <https://doi.org/10.1021/acs.chemrev.5b00069>.
- (234) Tian, Y.; Jiao, Q.; Ding, H.; Shi, Y.; Liu, B. The Formation of Honeycomb Structure in Polyphenylene Oxide Films. *Polymer* **2006**, *47* (11), 3866–3873. <https://doi.org/10.1016/j.polymer.2006.03.081>.
- (235) Peng, J.; Han, Y.; Yang, Y.; Li, B. The Influencing Factors on the Macroporous Formation in Polymer Films by Water Droplet Templating. *Polymer* **2004**, *45* (2), 447–452. <https://doi.org/10.1016/j.polymer.2003.11.019>.
- (236) Wu, X.; Jones, M. D.; Davidson, M. G.; Chaudhuri, J. B.; Ellis, M. J. Surfactant-Free Poly(Lactide-Co-Glycolide) Honeycomb Films for Tissue Engineering: Relating Solvent, Monomer Ratio and Humidity to Scaffold Structure. *Biotechnol. Lett.* **2011**, *33* (2), 423–430. <https://doi.org/10.1007/s10529-010-0438-y>.
- (237) Gong, J.; Xu, B.; Tao, X.; Li, L. Honeycomb Microstructured Silicon Oxycarbide Sheets from Silicon-Containing Graft Copolymer Films. *Plasma Process. Polym.* **2014**, *11* (11), 1001–1009. <https://doi.org/10.1002/ppap.201400060>.
- (238) Du, M.; Zhu, P.; Yan, X.; Su, Y.; Song, W.; Li, J. Honeycomb Self-Assembled Peptide Scaffolds by the Breath Figure Method. *Chem. – Eur. J.* **2011**, *17* (15), 4238–4245. <https://doi.org/10.1002/chem.201003021>.
- (239) Zhang, R.; Wang, J.; Wang, M.; He, Y. Fabrication of Honeycomb Polyvinyl Butyral Film under Humidity Provided by Super Saturated Salt Solutions. *J. Appl. Polym. Sci.* **2012**, *124* (1), 495–500. <https://doi.org/10.1002/app.34795>.
- (240) Pilati, F.; Montecchi, M.; Fabbri, P.; Synytska, A.; Messori, M.; Toselli, M.; Grundke, K.; Pospiech, D. Design of Surface Properties of PET Films: Effect of Fluorinated Block Copolymers. *J. Colloid Interface Sci.* **2007**, *315* (1), 210–222. <https://doi.org/10.1016/j.jcis.2007.06.046>.
- (241) Muñoz-Bonilla, A.; Ibarboure, E.; Papon, E.; Rodriguez-Hernandez, J. Engineering Polymer Surfaces with Variable Chemistry and Topography. *J. Polym. Sci. Part Polym. Chem.* **2009**, *47* (9), 2262–2271. <https://doi.org/10.1002/pola.23305>.
- (242) Kasai, W.; Kondo, T. Fabrication of Honeycomb-Patterned Cellulose Films. *Macromol. Biosci.* **2004**, *4* (1), 17–21. <https://doi.org/10.1002/mabi.200300054>.
- (243) Wang, Y.; Liu, Z.; Huang, Y.; Han, B.; Yang, G. Micropatterned Polymer Surfaces Induced by Nonsolvent. *Langmuir* **2006**, *22* (4), 1928–1931. <https://doi.org/10.1021/la051646d>.
- (244) Connal, L. A.; Gurr, P. A.; Qiao, G. G.; Solomon, D. H. From Well Defined Star-Microgels to Highly Ordered Honeycomb Films. *J. Mater. Chem.* **2005**, *15* (12), 1286–1292. <https://doi.org/10.1039/B414421J>.
- (245) Cheng, C. X.; Tian, Y.; Shi, Y. Q.; Tang, R. P.; Xi, F. Porous Polymer Films and Honeycomb Structures Based on Amphiphilic Dendronized Block Copolymers. *Langmuir* **2005**, *21* (14), 6576–6581. <https://doi.org/10.1021/la050187d>.

- (246) Velayudhan, S.; Kumar, P. R. A.; Nair, P. D. A Novel, Single Step, Highly Sensitive In-Vitro Cell-Based Metabolic Assay Using Honeycomb Microporous Polymer Membranes. *J. Biomed. Nanotechnol.* **2015**, *11* (4), 590–599. <https://doi.org/10.1166/jbn.2015.2066>.
- (247) Ponnusamy, T.; Lawson, L. B.; Freytag, L. C.; Blake, D. A.; Ayyala, R. S.; John, V. T. In Vitro Degradation and Release Characteristics of Spin Coated Thin Films of PLGA with a “Breath Figure” Morphology. *Biomater* **2012**, *2* (2), 77–86. <https://doi.org/10.4161/biom.20390>.
- (248) Imming, P.; Buss, T.; Dailey, L. A.; Meyer, A.; Morck, H.; Ramadan, M.; Rogosch, T. A Classification of Drug Substances According to Their Mechanism of Action. *Pharm.* **2004**, *59* (8), 579–589.
- (249) Bose, S.; Tarafder, S. Calcium Phosphate Ceramic Systems in Growth Factor and Drug Delivery for Bone Tissue Engineering: A Review. *Acta Biomater.* **2012**, *8* (4), 1401–1421. <https://doi.org/10.1016/j.actbio.2011.11.017>.
- (250) Castiglioni, S.; Cazzaniga, A.; Albisetti, W.; Maier, J. A. M. Magnesium and Osteoporosis: Current State of Knowledge and Future Research Directions. *Nutrients* **2013**, *5* (8), 3022–3033. <https://doi.org/10.3390/nu5083022>.
- (251) Borhani, S.; Hassanajili, S.; Ahmadi Tafti, S. H.; Rabbani, S. Cardiovascular Stents: Overview, Evolution, and next Generation. *Prog. Biomater.* **2018**, *7*, 175–205. <https://doi.org/10.1007/s40204-018-0097-y>.
- (252) Kipshidze, N.; Dangas, G.; Tsapenko, M.; Moses, J.; Leon, M. B.; Kutryk, M.; Serruys, P. Role of the Endothelium in Modulating Neointimal Formation: Vasculoprotective Approaches to Attenuate Restenosis after Percutaneous Coronary Interventions. *J. Am. Coll. Cardiol.* **2004**, *44* (4), 733–739. <https://doi.org/10.1016/j.jacc.2004.04.048>.
- (253) Ferraz, M. P.; Mateus, A. Y.; Sousa, J. C.; Monteiro, F. J. Nanohydroxyapatite Microspheres as Delivery System for Antibiotics: Release Kinetics, Antimicrobial Activity, and Interaction with Osteoblasts. *J. Biomed. Mater. Res. A* **2007**, *81A* (4), 994–1004. <https://doi.org/10.1002/jbm.a.31151>.
- (254) Teupe, C.; Meffert, R.; Winckler, S.; Ritzerfeld, W.; Törmälä, P.; Brug, E. Ciprofloxacin-Impregnated Poly-L-Lactic Acid Drug Carrier. *Arch. Orthop. Trauma Surg.* **1992**, *112* (1), 33–35. <https://doi.org/10.1007/BF00431041>.
- (255) Wei, G.; Kotoura, Y.; Oka, M.; Yamamuro, T.; Wada, R.; Hyon, S.-H.; Ikada, Y. A Bioabsorbable Delivery System for Antibiotic Treatment of Osteomyelitis. The Use of Lactic Acid Oligomer as a Carrier. *J. Bone Joint Surg. Br.* **1991**, *73*, 246–252. <https://doi.org/10.1302/0301-620X.73B2.2005148>.
- (256) Simchi, A.; Tamjid, E.; Pishbin, F.; Boccaccini, A. R. Recent Progress in Inorganic and Composite Coatings with Bactericidal Capability for Orthopaedic Applications. *Nanomedicine Nanotechnol. Biol. Med.* **2011**, *7* (1), 22–39. <https://doi.org/10.1016/j.nano.2010.10.005>.
- (257) Avés, E. P.; Estévez, G. F.; Sader, M. S.; Sierra, J. C. G.; Yurell, J. C. L.; Bastos, I. N.; Soares, G. D. A. Hydroxyapatite Coating by Sol–Gel on Ti–6Al–4V Alloy as Drug Carrier. *J. Mater. Sci. Mater. Med.* **2009**, *20* (2), 543–547. <https://doi.org/10.1007/s10856-008-3609-9>.
- (258) Stigter, M.; Bezemer, J.; de Groot, K.; Layrolle, P. Incorporation of Different Antibiotics into Carbonated Hydroxyapatite Coatings on Titanium Implants, Release and Antibiotic Efficacy. *J. Controlled Release* **2004**, *99* (1), 127–137. <https://doi.org/10.1016/j.jconrel.2004.06.011>.

- (259) Antoci, V. J.; Adams, C. S.; Hickok, N. J.; Shapiro, I. M.; Parvizi, J. Antibiotics for Local Delivery Systems Cause Skeletal Cell Toxicity In Vitro. *Clin. Orthop. Relat. Res.* **2007**, *462*, 200–206. <https://doi.org/10.1097/BLO.0b013e31811ff866>.
- (260) Sanpo, N.; Ang, S. M.; Cheang, P.; Khor, K. A. Antibacterial Property of Cold Sprayed Chitosan-Cu/Al Coating. *J. Therm. Spray Technol.* **2009**, *18* (4), 600. <https://doi.org/10.1007/s11666-009-9391-5>.
- (261) Marambio-Jones, C.; Hoek, E. M. V. A Review of the Antibacterial Effects of Silver Nanomaterials and Potential Implications for Human Health and the Environment. *J. Nanoparticle Res.* **2010**, *12* (5), 1531–1551. <https://doi.org/10.1007/s11051-010-9900-y>.
- (262) Roy, M.; Bandyopadhyay, A.; Bose, S. In Vitro Antimicrobial and Biological Properties of Laser Assisted Tricalcium Phosphate Coating on Titanium for Load Bearing Implant. *Mater. Sci. Eng. C* **2009**, *29* (6), 1965–1968. <https://doi.org/10.1016/j.msec.2009.03.009>.
- (263) Anderson, J. M.; Rodriguez, A.; Chang, D. T. Foreign Body Reaction to Biomaterials. *Semin. Immunol.* **2008**, *20* (2), 86–100. <https://doi.org/10.1016/j.smim.2007.11.004>.
- (264) Rahmati, M.; Mobasheri, A.; Mozafari, M. Inflammatory Mediators in Osteoarthritis: A Critical Review of the State-of-the-Art, Current Prospects, and Future Challenges. *Bone* **2016**, *85*, 81–90. <https://doi.org/10.1016/j.bone.2016.01.019>.
- (265) Brooks, E. K.; Der, S.; Ehrensberger, M. T. Corrosion and Mechanical Performance of AZ91 Exposed to Simulated Inflammatory Conditions. *Mater. Sci. Eng. C* **2016**, *60*, 427–436. <https://doi.org/10.1016/j.msec.2015.11.059>.
- (266) Udipi, K.; Ornberg, R. L.; Thurmond, K. B.; Settle, S. L.; Forster, D.; Riley, D. Modification of Inflammatory Response to Implanted Biomedical Materials in Vivo by Surface Bound Superoxide Dismutase Mimics. *J. Biomed. Mater. Res.* **2000**, *51* (4), 549–560. [https://doi.org/10.1002/1097-4636\(20000915\)51:4<549::AID-JBM2>3.0.CO;2-Z](https://doi.org/10.1002/1097-4636(20000915)51:4<549::AID-JBM2>3.0.CO;2-Z).
- (267) Vishwakarma, A.; Bhise, N. S.; Evangelista, M. B.; Rouwkema, J.; Dokmeci, M. R.; Ghaemmaghami, A. M.; Vrana, N. E.; Khademhosseini, A. Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response. *Trends Biotechnol.* **2016**, *34* (6), 470–482. <https://doi.org/10.1016/j.tibtech.2016.03.009>.
- (268) Hume, P. S.; He, J.; Haskins, K.; Anseth, K. S. Strategies to Reduce Dendritic Cell Activation through Functional Biomaterial Design. *Biomaterials* **2012**, *33* (14), 3615–3625. <https://doi.org/10.1016/j.biomaterials.2012.02.009>.
- (269) Zhong, Y.; Bellamkonda, R. V. Dexamethasone-Coated Neural Probes Elicit Attenuated Inflammatory Response and Neuronal Loss Compared to Uncoated Neural Probes. *Brain Res.* **2007**, *1148*, 15–27. <https://doi.org/10.1016/j.brainres.2007.02.024>.
- (270) Peter, B.; Pioletti, D. P.; Laib, S.; Bujoli, B.; Pilet, P.; Janvier, P.; Guicheux, J.; Zambelli, P.-Y.; Bouler, J.-M.; Gauthier, O. Calcium Phosphate Drug Delivery System: Influence of Local Zoledronate Release on Bone Implant Osteointegration. *Bone* **2005**, *36* (1), 52–60. <https://doi.org/10.1016/j.bone.2004.10.004>.
- (271) Garbuz, D. S.; Hu, Y.; Kim, W. Y.; Duan, K.; Masri, B. A.; Oxland, T. R.; Burt, H.; Wang, R.; Duncan, C. P. Enhanced Gap Filling and Osteoconduction Associated with Alendronate-Calcium Phosphate-Coated Porous Tantalum. *JBJS* **2008**, *90* (5), 1090–1100. <https://doi.org/10.2106/JBJS.G.00415>.

- (272) Trajkovski, B.; Petersen, A.; Strube, P.; Mehta, M.; Duda, G. N. Intra-Operatively Customized Implant Coating Strategies for Local and Controlled Drug Delivery to Bone. *Adv. Drug Deliv. Rev.* **2012**, *64* (12), 1142–1151. <https://doi.org/10.1016/j.addr.2012.05.016>.
- (273) Tsiridis, E.; Upadhyay, N.; Giannoudis, P. Molecular Aspects of Fracture Healing: Which Are the Important Molecules? *Injury* **2007**, *38* (1, Supplement), S11–S25. <https://doi.org/10.1016/j.injury.2007.02.006>.
- (274) Ginebra, M. P.; Traykova, T.; Planell, J. A. Calcium Phosphate Cements as Bone Drug Delivery Systems: A Review. *J. Controlled Release* **2006**, *113* (2), 102–110. <https://doi.org/10.1016/j.jconrel.2006.04.007>.
- (275) Sachse, A.; Wagner, A.; Keller, M.; Wagner, O.; Wetzel, W.-D.; Layher, F.; Venbrocks, R.-A.; Hortschansky, P.; Pietraszczyk, M.; Wiederanders, B.; Hempel, H. J.; Bossert, J.; Horn, J.; Schmuck, K.; Mollenhauer, J. Osteointegration of Hydroxyapatite-Titanium Implants Coated with Nonglycosylated Recombinant Human Bone Morphogenetic Protein-2 (BMP-2) in Aged Sheep. *Bone* **2005**, *37* (5), 699–710. <https://doi.org/10.1016/j.bone.2005.06.011>.
- (276) Liu, Y.; de Groot, K.; Hunziker, E. B. BMP-2 Liberated from Biomimetic Implant Coatings Induces and Sustains Direct Ossification in an Ectopic Rat Model. *Bone* **2005**, *36* (5), 745–757. <https://doi.org/10.1016/j.bone.2005.02.005>.
- (277) Liu, Y.; Enggist, L.; Kuffer, A. F.; Buser, D.; Hunziker, E. B. The Influence of BMP-2 and Its Mode of Delivery on the Osteoconductivity of Implant Surfaces during the Early Phase of Osseointegration. *Biomaterials* **2007**, *28* (16), 2677–2686. <https://doi.org/10.1016/j.biomaterials.2007.02.003>.
- (278) Henslee, A. M.; Spicer, P. P.; Yoon, D. M.; Nair, M. B.; Meretoja, V. V.; Witherel, K. E.; Jansen, J. A.; Mikos, A. G.; Kasper, F. K. Biodegradable Composite Scaffolds Incorporating an Intramedullary Rod and Delivering Bone Morphogenetic Protein-2 for Stabilization and Bone Regeneration in Segmental Long Bone Defects. *Acta Biomater.* **2011**, *7* (10), 3627–3637. <https://doi.org/10.1016/j.actbio.2011.06.043>.
- (279) Zilberman, M.; Kraitzer, A.; Grinberg, O.; Elsner, J. J. Drug-Eluting Medical Implants. *Handb. Exp. Pharmacol.* **2010**, No. 197, 299–341. https://doi.org/10.1007/978-3-642-00477-3_11.
- (280) Babapulle, M. N.; Eisenberg, M. J. Coated Stents for the Prevention of Restenosis: Part II. *Circulation* **2002**, *106* (22), 2859–2866. <https://doi.org/10.1161/01.CIR.0000038984.30279.89>.
- (281) Ormiston, J. A.; Webster, M. W. I.; Armstrong, G. First-in-Human Implantation of a Fully Bioabsorbable Drug-Eluting Stent: The BVS Poly-L-Lactic Acid Everolimus-Eluting Coronary Stent. *Catheter. Cardiovasc. Interv.* **2007**, *69* (1), 128–131. <https://doi.org/10.1002/ccd.20895>.
- (282) Vasconcelos, D. P.; Águas, A. P.; Barbosa, M. A.; Pelegrín, P.; Barbosa, J. N. The Inflammasome in Host Response to Biomaterials: Bridging Inflammation and Tissue Regeneration. *Acta Biomater.* **2019**, *83*, 1–12. <https://doi.org/10.1016/j.actbio.2018.09.056>.
- (283) Raphel, J.; Holodniy, M.; Goodman, S. B.; Heilshorn, S. C. Multifunctional Coatings to Simultaneously Promote Osseointegration and Prevent Infection of Orthopaedic Implants. *Biomaterials* **2016**, *84*, 301–314. <https://doi.org/10.1016/j.biomaterials.2016.01.016>.
- (284) Bagherifard, S. Mediating Bone Regeneration by Means of Drug Eluting Implants: From Passive to Smart Strategies. *Mater. Sci. Eng. C* **2017**, *71*, 1241–1252. <https://doi.org/10.1016/j.msec.2016.11.011>.

- (285) Radin, S.; Campbell, J. T.; Ducheyne, P.; Cuckler, J. M. Calcium Phosphate Ceramic Coatings as Carriers of Vancomycin. *Biomaterials* **1997**, *18* (11), 777–782. [https://doi.org/10.1016/S0142-9612\(96\)00190-1](https://doi.org/10.1016/S0142-9612(96)00190-1).
- (286) Kofron, M.; Laurencin, C. Bone Tissue Engineering by Gene Delivery☆. *Adv. Drug Deliv. Rev.* **2006**, *58* (4), 555–576. <https://doi.org/10.1016/j.addr.2006.03.008>.
- (287) Tang, S.; Tian, B.; Ke, Q.-F.; Zhu, Z.-A.; Guo, Y.-P. Gentamicin-Loaded Carbonated Hydroxyapatite Coatings with Hierarchically Porous Structures: Drug Delivery Properties, Bactericidal Properties and Biocompatibility. *RSC Adv* **2014**, *4* (78), 41500–41509. <https://doi.org/10.1039/C4RA05493H>.
- (288) Nyan, M.; Hao, J.; Miyahara, T.; Noritake, K.; Rodriguez, R.; Kasugai, S. Accelerated and Enhanced Bone Formation on Novel Simvastatin-Loaded Porous Titanium Oxide Surfaces. *Clin. Implant Dent. Relat. Res.* **2014**, *16* (5), 675–683. <https://doi.org/10.1111/cid.12045>.
- (289) Teng, F.-Y.; Tai, I.-C.; Ho, M.-L.; Wang, J.-W.; Weng, L. W.; Wang, Y. J.; Wang, M.-W.; Tseng, C.-C. Controlled Release of BMP-2 from Titanium with Electrodeposition Modification Enhancing Critical Size Bone Formation. *Mater. Sci. Eng. C* **2019**, *105*, 109879. <https://doi.org/10.1016/j.msec.2019.109879>.
- (290) Bigham, A.; Saudi, A.; Rafienia, M.; Rahmati, S.; Bakhtiyari, H.; Salahshouri, F.; Sattary, M.; Hassanzadeh-Tabrizi, S. A. Electrophoretically Deposited Mesoporous Magnesium Silicate with Ordered Nanopores as an Antibiotic-Loaded Coating on Surface-Modified Titanium. *Mater. Sci. Eng. C* **2019**, *96*, 765–775. <https://doi.org/10.1016/j.msec.2018.12.013>.
- (291) Sultana, N.; Bora, P.; Sarma, B. Chapter 10 - Nanocarriers in Drug Delivery System: Eminence and Confront. In *Smart Nanocontainers*; Nguyen-Tri, P., Do, T.-O., Nguyen, T. A., Eds.; Micro and Nano Technologies; Elsevier, 2020; pp 159–178. <https://doi.org/10.1016/B978-0-12-816770-0.00010-1>.
- (292) Hoang Thi, T. T.; Cao, V. D.; Nguyen, T. N. Q.; Hoang, D. T.; Ngo, V. C.; Nguyen, D. H. Functionalized Mesoporous Silica Nanoparticles and Biomedical Applications. *Mater. Sci. Eng. C* **2019**, *99*, 631–656. <https://doi.org/10.1016/j.msec.2019.01.129>.
- (293) Alsehli, M. Polymeric Nanocarriers as Stimuli-Responsive Systems for Targeted Tumor (Cancer) Therapy: Recent Advances in Drug Delivery. *Saudi Pharm. J.* **2020**, *28* (3), 255–265. <https://doi.org/10.1016/j.jsps.2020.01.004>.
- (294) Mura, S.; Nicolas, J.; Couvreur, P. Stimuli-Responsive Nanocarriers for Drug Delivery. *Nat. Mater.* **2013**, *12* (11), 991–1003. <https://doi.org/10.1038/nmat3776>.
- (295) Wang, Y.; Shim, M. S.; Levinson, N. S.; Sung, H.-W.; Xia, Y. Stimuli-Responsive Materials for Controlled Release of Theranostic Agents. *Adv. Funct. Mater.* **2014**, *24* (27), 4206–4220. <https://doi.org/10.1002/adfm.201400279>.
- (296) Cho, I.-H.; Shim, M. K.; Jung, B.; Jang, E. H.; Park, M.-J.; Kang, H. C.; Kim, J.-H. Heat Shock Responsive Drug Delivery System Based on Mesoporous Silica Nanoparticles Coated with Temperature Sensitive Gatekeeper. *Microporous Mesoporous Mater.* **2017**, *253*, 96–101. <https://doi.org/10.1016/j.micromeso.2017.06.042>.
- (297) Etmianfar, M. R.; Sheykhholeslami, S. O. R.; Khalili, V.; Mahdavi, S. Biocompatibility and Drug Delivery Efficiency of PEG-b-PCL/Hydroxyapatite Bilayer Coatings on Nitinol Superelastic Alloy. *Ceram. Int.* **2020**, *46* (8, Part B), 12711–12717. <https://doi.org/10.1016/j.ceramint.2020.02.038>.
- (298) Jaworska, J.; Jelonek, K.; Kajzer, W.; Szewczenko, J.; Kaczmarczyk, B.; Marcinkowski, A.; Janeczek, H.; Pastusiak, M.; Basiaga, M.; Kasperczyk, J.

- Comparison of Biodegradable Poly(Glycolide- ϵ -Caprolactone) and Poly(Glycolide- ϵ -Caprolactone-d,l-Lactide) Coatings Enriched with Ciprofloxacin Formed on Ti6Al4V Alloy. *Polym. Degrad. Stab.* **2018**, *155*, 136–144. <https://doi.org/10.1016/j.polymdegradstab.2018.07.013>.
- (299) Calori, I. R.; Braga, G.; de Jesus, P. da C. C.; Bi, H.; Tedesco, A. C. Polymer Scaffolds as Drug Delivery Systems. *Eur. Polym. J.* **2020**, *129*, 109621. <https://doi.org/10.1016/j.eurpolymj.2020.109621>.
- (300) Harrison, K. Introduction to Polymeric Drug Delivery Systems. In *Biomedical Polymers*; Elsevier, 2007; pp 33–56. <https://doi.org/10.1533/9781845693640.33>.
- (301) Marjanović-Balaban, Ž.; Jelić, D. Polymeric Biomaterials in Clinical Practice. In *Biomaterials in Clinical Practice : Advances in Clinical Research and Medical Devices*; Zivic, F., Affatato, S., Trajanovic, M., Schnabelrauch, M., Grujovic, N., Choy, K. L., Eds.; Springer International Publishing: Cham, 2018; pp 101–117. https://doi.org/10.1007/978-3-319-68025-5_4.
- (302) Ji, X.-J.; Gao, L.; Liu, J.-C.; Wang, J.; Cheng, Q.; Li, J.-P.; Li, S.-Q.; Zhi, K.-Q.; Zeng, R.-C.; Wang, Z.-L. Corrosion Resistance and Antibacterial Properties of Hydroxyapatite Coating Induced by Gentamicin-Loaded Polymeric Multilayers on Magnesium Alloys. *Colloids Surf. B Biointerfaces* **2019**, *179*, 429–436. <https://doi.org/10.1016/j.colsurfb.2019.04.029>.
- (303) Lu, P.; Fan, H.; Liu, Y.; Cao, L.; Wu, X.; Xu, X. Controllable Biodegradability, Drug Release Behavior and Hemocompatibility of PTX-Eluting Magnesium Stents. *Colloids Surf. B Biointerfaces* **2011**, *83* (1), 23–28. <https://doi.org/10.1016/j.colsurfb.2010.10.038>.
- (304) Xu, X.; Lu, P.; Guo, M.; Fang, M. Cross-Linked Gelatin/Nanoparticles Composite Coating on Micro-Arc Oxidation Film for Corrosion and Drug Release. *Appl. Surf. Sci.* **2010**, *256* (8), 2367–2371. <https://doi.org/10.1016/j.apsusc.2009.10.069>.
- (305) Lu, P.; Liu, Y.; Guo, M.; Fang, H.; Xu, X. Corrosion and Drug Release Properties of EN-Plating/PLGA Composite Coating on MAO Film. *Mater. Sci. Eng. C* **2011**, *31* (7), 1285–1289. <https://doi.org/10.1016/j.msec.2011.04.005>.
- (306) Rohwerder, M.; Turcu, F. High-Resolution Kelvin Probe Microscopy in Corrosion Science: Scanning Kelvin Probe Force Microscopy (SKPFM) versus Classical Scanning Kelvin Probe (SKP). *Electrochimica Acta* **2007**, *53* (2), 290–299. <https://doi.org/10.1016/j.electacta.2007.03.016>.
- (307) Jacobs, H. O.; Leuchtmann, P.; Homan, O. J.; Stemmer, A. Resolution and Contrast in Kelvin Probe Force Microscopy. *J. Appl. Phys.* **1998**, *84* (3), 1168–1173. <https://doi.org/10.1063/1.368181>.
- (308) Schmutz, P.; Frankel, G. S. Characterization of AA2024-T3 by Scanning Kelvin Probe Force Microscopy. *J. Electrochem. Soc.* **1998**, *145* (7), 2285. <https://doi.org/10.1149/1.1838633>.
- (309) Tonna, C.; Wang, C.; Mei, D.; Lamaka, S. V.; Zheludkevich, M. L.; Buhagiar, J. Biodegradation Behaviour of Fe-Based Alloys in Hanks' Balanced Salt Solutions: Part I. Material Characterisation and Corrosion Testing. *Bioact. Mater.* **2022**, *7*, 426–440. <https://doi.org/10.1016/j.bioactmat.2021.05.048>.
- (310) Mingo, B.; Mohedano, M.; Blawert, C.; del Olmo, R.; Hort, N.; Arrabal, R. Role of Ca on the Corrosion Resistance of Mg–9Al and Mg–9Al–0.5Mn Alloys. *J. Alloys Compd.* **2019**, *811*, 151992. <https://doi.org/10.1016/j.jallcom.2019.151992>.
- (311) Mohedano, M.; Luthringer, B. J. C.; Mingo, B.; Feyerabend, F.; Arrabal, R.; Sanchez-Egido, P. J.; Blawert, C.; Willumeit-Römer, R.; Zheludkevich, M. L.; Matykina, E. Bioactive Plasma Electrolytic Oxidation Coatings on Mg-Ca Alloy

- to Control Degradation Behaviour. *Surf. Coat. Technol.* **2017**, *315*, 454–467. <https://doi.org/10.1016/j.surfcoat.2017.02.050>.
- (312) Bitá, A.; Antoniac, A.; Cotrut, M. C.; Vasile, E.; Ion, C.; Niculescu, M.; Antoniac, I. In Vitro Degradation and Corrosion Evaluation of Mg-Ca Alloys for Biomedical Applications. *J. Optoelectron. Adv. Mater.* **2016**, *18*, 394–398.
- (313) Deng, M.; Höche, D.; Lamaka, S. V.; Snihirova, D.; Zheludkevich, M. L. Mg-Ca Binary Alloys as Anodes for Primary Mg-Air Batteries. *J. Power Sources* **2018**, *396*, 109–118. <https://doi.org/10.1016/j.jpowsour.2018.05.090>.
- (314) Yang, L.; Shi, Y.; Shen, L.; Zhang, E.; Qin, G.; Lu, X.; Zhou, X. Effect of Ag on Cathodic Activation and Corrosion Behaviour of Mg-Mn-Ag Alloys. *Corros. Sci.* **2021**, *185*, 109408. <https://doi.org/10.1016/j.corsci.2021.109408>.
- (315) Zhang, X.; Zhang, Y.; Lv, Y.; Dong, Z.; Yang, L.; Zhang, E.; Hashimoto, T.; Zhou, X. PEO Coating on Mg-Ag Alloy: The Incorporation and Release of Ag Species. *J. Magnes. Alloys* **2022**. <https://doi.org/10.1016/j.jma.2021.12.006>.
- (316) Du, J.; Zhang, A.; Yang, M.; Li, M.; Xiong, S. Atomic Cluster Structures, Phase Stability and Physicochemical Properties of Binary Mg-X (X= Ag, Al, Ba, Ca, Gd, Sn, Y and Zn) Alloys from Ab-Initio Calculations. *Intermetallics* **2018**, *95*. <https://doi.org/10.1016/j.intermet.2018.02.005>.
- (317) Laws, K. J.; Shamlaye, K. F.; Granata, D.; Koloadin, L. S.; Löffler, J. F. Electron-Band Theory Inspired Design of Magnesium–Precious Metal Bulk Metallic Glasses with High Thermal Stability and Extended Ductility. *Sci. Rep.* **2017**, *7* (1), 3400. <https://doi.org/10.1038/s41598-017-03643-7>.
- (318) Ben-Hamu, G.; Eliezer, D.; Kaya, A.; Na, Y. G.; Shin, K. S. Microstructure and Corrosion Behavior of Mg–Zn–Ag Alloys. *Mater. Sci. Eng. A* **2006**, *435–436*, 579–587. <https://doi.org/10.1016/j.msea.2006.07.109>.
- (319) Tie, D.; Feyerabend, F.; Mueller, W.-D.; Schade, R.; Liefelth, K.; Kainer, K.; Willumeit, R. Antibacterial Biodegradable Mg-Ag Alloys. *Eur. Cell. Mater.* **2013**, *25*, 284–298. <https://doi.org/10.22203/eCM.v025a20>.
- (320) Mathieu, S.; Rapin, C.; Steinmetz, J.; Steinmetz, P. A Corrosion Study of the Main Constituent Phases of AZ91 Magnesium Alloys. *Corros. Sci.* **2003**, *45* (12), 2741–2755. [https://doi.org/10.1016/S0010-938X\(03\)00109-4](https://doi.org/10.1016/S0010-938X(03)00109-4).
- (321) Ballerini, G.; Bardi, U.; Bignucolo, R.; Ceraolo, G. About Some Corrosion Mechanisms of AZ91D Magnesium Alloy. *Corros. Sci.* **2005**, *47*, 2173–2184. <https://doi.org/10.1016/j.corsci.2004.09.018>.
- (322) Zhao, T.; Hu, Y.; Pan, F.; He, B.; Guan, M.; Yuan, Y.; Tang, A. Effect of Zn Content on the Microstructure and Mechanical Properties of Mg–Al–Sn–Mn Alloys. *Materials* **2019**, *12* (19), 3102. <https://doi.org/10.3390/ma12193102>.
- (323) Pulido-González, N.; Torres, B.; García-Rodríguez, S.; Rodrigo, P.; Bonache, V.; Hidalgo-Manrique, P.; Mohedano, M.; Rams, J. Mg–1Zn–1Ca Alloy for Biomedical Applications. Influence of the Secondary Phases on the Mechanical and Corrosion Behaviour. *J. Alloys Compd.* **2020**, *831*, 154735. <https://doi.org/10.1016/j.jallcom.2020.154735>.
- (324) Pulido-González, N.; Torres, B.; Rodrigo, P.; Hort, N.; Rams, J. Microstructural, Mechanical and Corrosion Characterization of an as-Cast Mg–3Zn–0.4Ca Alloy for Biomedical Applications. *J. Magnes. Alloys* **2020**, *8* (2), 510–522. <https://doi.org/10.1016/j.jma.2020.02.007>.
- (325) Orlov, D.; Ralston, K. D.; Birbilis, N.; Estrin, Y. Enhanced Corrosion Resistance of Mg Alloy ZK60 after Processing by Integrated Extrusion and Equal Channel Angular Pressing. *Acta Mater.* **2011**, *59* (15), 6176–6186. <https://doi.org/10.1016/j.actamat.2011.06.033>.

- (326) Jiang, P.; Blawert, C.; Hou, R.; Bohlen, J.; Konchakova, N.; Zheludkevich, M. L. A Comprehensive Comparison of the Corrosion Performance, Fatigue Behavior and Mechanical Properties of Micro-Alloyed MgZnCa and MgZnGe Alloys. *Mater. Des.* **2020**, *185*, 108285. <https://doi.org/10.1016/j.matdes.2019.108285>.
- (327) Lu, X.; Zhao, G.; Zhou, J.; Zhang, C.; Yu, J. Microstructure and Mechanical Properties of the As-Cast and As-Homogenized Mg-Zn-Sn-Mn-Ca Alloy Fabricated by Semicontinuous Casting. *Materials* **2018**, *11* (5). <https://doi.org/10.3390/ma11050703>.
- (328) Chun, J. S.; Byrne, J. G. Precipitate Strengthening Mechanisms in Magnesium Zinc Alloy Single Crystals. *J. Mater. Sci.* **1969**, *4* (10), 861–872. <https://doi.org/10.1007/BF00549777>.
- (329) Zhao, C.; Chen, X.; Pan, F.; Gao, S.; Zhao, D.; Liu, X. Effect of Sn Content on Strain Hardening Behavior of As-Extruded Mg-Sn Alloys. *Mater. Sci. Eng. A* **2018**, *713*, 244–252. <https://doi.org/10.1016/j.msea.2017.12.074>.
- (330) Gao, J.; Guan, S.; Ren, Z. W.; Sun, Y. F.; Zhu, S. J.; Wang, B. Homogeneous Corrosion of High Pressure Torsion Treated Mg–Zn–Ca Alloy in Simulated Body Fluid. *Mater. Lett. - MATER LETT* **2011**, *65*, 691–693. <https://doi.org/10.1016/j.matlet.2010.11.015>.
- (331) Wei, L.; Li, J.; Zhang, Y.; Lai, H. Effects of Zn Content on Microstructure, Mechanical and Degradation Behaviors of Mg-XZn-0.2Ca-0.1Mn Alloys. *Mater. Chem. Phys.* **2020**, *241*, 122441. <https://doi.org/10.1016/j.matchemphys.2019.122441>.
- (332) Zhang, Y.; Feng, X.; Huang, Q.; Li, Y.; Yang, Y. Anisotropic Mechanical and Corrosion Properties of Directionally Solidified Mg-3Zn-0.2Ca Alloy. *J. Alloys Compd.* **2022**, *895*, 162687. <https://doi.org/10.1016/j.jallcom.2021.162687>.
- (333) Bahmani, A.; Arthanari, S.; Shin, K. S. Corrosion Behavior of Mg–Mn–Ca Alloy: Influences of Al, Sn and Zn. *J. Magnes. Alloys* **2019**, *7* (1), 38–46. <https://doi.org/10.1016/j.jma.2018.11.004>.
- (334) Gnedenkov, A. S.; Sinebryukhov, S. L.; Filonina, V. S.; Egorkin, V. S.; Ustinov, A. Yu.; Sergienko, V. I.; Gnedenkov, S. V. The Detailed Corrosion Performance of Bioresorbable Mg-0.8Ca Alloy in Physiological Solutions. *J. Magnes. Alloys* **2021**. <https://doi.org/10.1016/j.jma.2021.11.027>.
- (335) Mingo, B.; Arrabal, R.; Mohedano, M.; Mendis, C. L.; del Olmo, R.; Matykina, E.; Hort, N.; Merino, M. C.; Pardo, A. Corrosion of Mg-9Al Alloy with Minor Alloying Elements (Mn, Nd, Ca, Y and Sn). *Mater. Des.* **2017**, *130*, 48–58. <https://doi.org/10.1016/j.matdes.2017.05.048>.
- (336) Acharya, M. G.; Shetty, A. N. The Corrosion Behavior of AZ31 Alloy in Chloride and Sulfate Media – A Comparative Study through Electrochemical Investigations. *J. Magnes. Alloys* **2019**, *7* (1), 98–112. <https://doi.org/10.1016/j.jma.2018.09.003>.
- (337) Xin, Y.; Liu, C.; Zhang, X.; Tang, G.; Tian, X.; Chu, P. K. Corrosion Behavior of Biomedical AZ91 Magnesium Alloy in Simulated Body Fluids. *J. Mater. Res.* **2007**, *22* (7), 2004–2011. <https://doi.org/10.1557/jmr.2007.0233>.
- (338) N. Dezfouli, S.; Huan, Z. G.; Mol, J. M. C.; Leeftang, M.; Chang, J.; Zhou, J. Influence of HEPES Buffer on the Local PH and Formation of Surface Layer during in Vitro Degradation Tests of Magnesium in DMEM. *Prog. Nat. Sci.* **2014**, *24*. <https://doi.org/10.1016/j.pnsc.2014.08.009>.
- (339) Zhang, T.; Shao, Y.; Meng, G.; Cui, Z.; Wang, F. Corrosion of Hot Extrusion AZ91 Magnesium Alloy: I-Relation between the Microstructure and Corrosion Behavior. *Corros. Sci.* **2011**, *53* (5), 1960–1968. <https://doi.org/10.1016/j.corsci.2011.02.015>.

- (340) Aung, N. N.; Zhou, W. Effect of Grain Size and Twins on Corrosion Behaviour of AZ31B Magnesium Alloy. *Corros. Sci.* **2010**, *52* (2), 589–594. <https://doi.org/10.1016/j.corsci.2009.10.018>.
- (341) Millán-Ramos, B.; Morquecho-Marín, D.; Silva-Bermudez, P.; Ramírez-Ortega, D.; Depablos-Rivera, O.; García-López, J.; Fernández-Lizárraga, M.; Victoria-Hernández, J.; Letzig, D.; Almaguer-Flores, A.; Rodil, S. E. Biocompatibility and Electrochemical Evaluation of ZrO₂ Thin Films Deposited by Reactive Magnetron Sputtering on MgZnCa Alloy. *J. Magnes. Alloys* **2021**. <https://doi.org/10.1016/j.jma.2021.07.010>.
- (342) Fang, H.; Wang, C.; Zhou, S.; Zheng, Z.; Lu, T.; Li, G.; Tian, Y.; Suga, T. Enhanced Adhesion and Anticorrosion of Silk Fibroin Coated Biodegradable Mg–Zn–Ca Alloy via a Two-Step Plasma Activation. *Corros. Sci.* **2020**, *168*, 108466. <https://doi.org/10.1016/j.corsci.2020.108466>.
- (343) Bakhsheshi-Rad, H. R.; Hamzah, E.; Fereidouni-Lotfabadi, A.; Daroonparvar, M.; Yajid, M. a. M.; Mezbahul-Islam, M.; Kasiri-Asgarani, M.; Medraj, M. Microstructure and Bio-Corrosion Behavior of Mg–Zn and Mg–Zn–Ca Alloys for Biomedical Applications. *Mater. Corros.* **2014**, *65* (12), 1178–1187. <https://doi.org/10.1002/maco.201307588>.
- (344) Ibrahim, H.; Klarner, A. D.; Poorganji, B.; Dean, D.; Luo, A. A.; Elahinia, M. Microstructural, Mechanical and Corrosion Characteristics of Heat-Treated Mg–1.2Zn–0.5Ca (Wt%) Alloy for Use as Resorbable Bone Fixation Material. *J. Mech. Behav. Biomed. Mater.* **2017**, *69*, 203–212. <https://doi.org/10.1016/j.jmbbm.2017.01.005>.
- (345) Chu, P.-W.; Le Mire, E.; Marquis, E. A. Microstructure of Localized Corrosion Front on Mg Alloys and the Relationship with Hydrogen Evolution. *Corros. Sci.* **2017**, *128*, 253–264. <https://doi.org/10.1016/j.corsci.2017.09.022>.
- (346) Abdel-Gawad, S. A.; Shoeib, M. A. Corrosion Studies and Microstructure of Mg–Zn–Ca Alloys for Biomedical Applications. *Surf. Interfaces* **2019**, *14*, 108–116. <https://doi.org/10.1016/j.surf.2018.11.011>.
- (347) Neil, W. C.; Forsyth, M.; Howlett, P. C.; Hutchinson, C. R.; Hinton, B. R. W. Corrosion of Heat Treated Magnesium Alloy ZE41. *Corros. Sci.* **2011**, *53* (10), 3299–3308. <https://doi.org/10.1016/j.corsci.2011.06.005>.
- (348) Song, G.-L.; Xu, Z. Crystal Orientation and Electrochemical Corrosion of Polycrystalline Mg. *Corros. Sci.* **2012**, *63*, 100–112. <https://doi.org/10.1016/j.corsci.2012.05.019>.
- (349) Johnston, S.; Shi, Z.; Atrens, A. The Influence of PH on the Corrosion Rate of High-Purity Mg, AZ91 and ZE41 in Bicarbonate Buffered Hanks' Solution. *Corros. Sci.* **2015**, *101*, 182–192. <https://doi.org/10.1016/j.corsci.2015.09.018>.
- (350) Larionova, T. V.; Park, W.-W.; You, B.-S. A Ternary Phase Observed in Rapidly Solidified Mg–Ca–Zn Alloys. *Scr. Mater.* **2001**, *45* (1), 7–12. [https://doi.org/10.1016/S1359-6462\(01\)00982-4](https://doi.org/10.1016/S1359-6462(01)00982-4).
- (351) Liu, D.; Guo, C.; Chai, L.; Sherman, V. R.; Qin, X.; Ding, Y.; Meyers, M. A. Mechanical Properties and Corrosion Resistance of Hot Extruded Mg–2.5Zn–1Ca Alloy. *Mater. Sci. Eng. B* **2015**, *195*, 50–58. <https://doi.org/10.1016/j.mseb.2015.02.001>.
- (352) Min, Y.; Debaio, L.; Runfang, Z.; Minfang, C. Microstructure and Properties of Mg–3Zn–0.2Ca Alloy for Biomedical Application. *Rare Met. Mater. Eng.* **2018**, *47* (1), 93–98. [https://doi.org/10.1016/S1875-5372\(18\)30078-X](https://doi.org/10.1016/S1875-5372(18)30078-X).

- (353) Yerokhin, A. L.; Nie, X.; Leyland, A.; Matthews, A.; Dowey, S. J. Plasma Electrolysis for Surface Engineering. *Surf. Coat. Technol.* **1999**, 122 (2), 73–93. [https://doi.org/10.1016/S0257-8972\(99\)00441-7](https://doi.org/10.1016/S0257-8972(99)00441-7).
- (354) Scapin, M. A.; Guilhen, S. N.; Cotrim, M. E. B.; Pires, M. A. F. DETERMINATION OF Ca/P MOLAR RATIO IN HYDROXYAPATITE (HA) BY X-RAY FLUORESCENCE TECHNIQUE. *São Paulo* **2015**, 7.
- (355) Mohedano, M.; Matykina, E.; Arrabal, R.; Pardo, A.; Merino, M. C. Metal Release from Ceramic Coatings for Dental Implants. *Dent. Mater.* **2014**, 30 (3), e28–e40. <https://doi.org/10.1016/j.dental.2013.12.011>.
- (356) Pan, Y.; Chen, C.; Feng, R.; Cui, H.; Gong, B.; Zheng, T.; Ji, Y. Effect of Calcium on the Microstructure and Corrosion Behavior of Microarc Oxidized Mg-XCa Alloys. *Biointerphases* **2018**, 13 (1), 011003. <https://doi.org/10.1116/1.5003320>.
- (357) Matykina, E.; Garcia, I.; Arrabal, R.; Mohedano, M.; Mingo, B.; Sancho, J.; Merino, M. C.; Pardo, A. Role of PEO Coatings in Long-Term Biodegradation of a Mg Alloy. *Appl. Surf. Sci.* **2016**, 389, 810–823. <https://doi.org/10.1016/j.apsusc.2016.08.005>.
- (358) Gao, Y.; Yerokhin, A.; Matthews, A. DC Plasma Electrolytic Oxidation of Biodegradable Cp-Mg: In-Vitro Corrosion Studies. *Surf. Coat. Technol.* **2013**, 234, 132–142. <https://doi.org/10.1016/j.surfcoat.2012.11.035>.
- (359) Arrabal, R.; Matykina, E.; Viejo, F.; Skeldon, P.; Thompson, G. E. Corrosion Resistance of WE43 and AZ91D Magnesium Alloys with Phosphate PEO Coatings. *Corros. Sci.* **2008**, 50 (6), 1744–1752. <https://doi.org/10.1016/j.corsci.2008.03.002>.
- (360) Gao, Y.; Yerokhin, A.; Matthews, A. Deposition and Evaluation of Duplex Hydroxyapatite and Plasma Electrolytic Oxidation Coatings on Magnesium. *Surf. Coat. Technol.* **2015**, 269, 170–182. <https://doi.org/10.1016/j.surfcoat.2015.01.030>.
- (361) McCann, H. G. The Solubility of Fluorapatite and Its Relationship to That of Calcium Fluoride. *Arch. Oral Biol.* **1968**, 13 (8), 987–1001. [https://doi.org/10.1016/0003-9969\(68\)90014-9](https://doi.org/10.1016/0003-9969(68)90014-9).
- (362) Agalakova, N. I.; Gusev, G. P. Molecular Mechanisms of Cytotoxicity and Apoptosis Induced by Inorganic Fluoride. *ISRN Cell Biol.* **2012**, 2012, e403835. <https://doi.org/10.5402/2012/403835>.
- (363) Liu, F.; Yu, J.; Song, Y.; Shan, D.; Han, E.-H. Effect of Potassium Fluoride on the In-Situ Sealing Pores of Plasma Electrolytic Oxidation Film on AM50 Mg Alloy. *Mater. Chem. Phys.* **2015**, 162, 452–460. <https://doi.org/10.1016/j.matchemphys.2015.06.014>.
- (364) Kazanski, B.; Kossenko, A.; Zinigrad, M.; Lugovskoy, A. Fluoride Ions as Modifiers of the Oxide Layer Produced by Plasma Electrolytic Oxidation on AZ91D Magnesium Alloy. *Appl. Surf. Sci.* **2013**, 287, 461–466. <https://doi.org/10.1016/j.apsusc.2013.09.180>.
- (365) Yang, J.; Lu, X.; Blawert, C.; Di, S.; Zheludkevich, M. L. Microstructure and Corrosion Behavior of Ca/P Coatings Prepared on Magnesium by Plasma Electrolytic Oxidation. *Surf. Coat. Technol.* **2017**, 319, 359–369. <https://doi.org/10.1016/j.surfcoat.2017.04.001>.
- (366) Lu, X.; Blawert, C.; Mohedano, M.; Scharnagl, N.; Zheludkevich, M. L.; Kainer, K. U. Influence of Electrical Parameters on Particle Uptake during Plasma Electrolytic Oxidation Processing of AM50 Mg Alloy. *Surf. Coat. Technol.* **2016**, 289, 179–185. <https://doi.org/10.1016/j.surfcoat.2016.02.006>.

- (367) Martin, J.; Nominé, A. V.; Stef, J.; Nominé, A.; Zou, J. X.; Henrion, G.; Grosdidier, T. The Influence of Metallurgical State of Substrate on the Efficiency of Plasma Electrolytic Oxidation (PEO) Process on Magnesium Alloy. *Mater. Des.* **2019**, *178*, 107859. <https://doi.org/10.1016/j.matdes.2019.107859>.
- (368) Pan, Y.; He, S.; Wang, D.; Huang, D.; Zheng, T.; Wang, S.; Dong, P.; Chen, C. In Vitro Degradation and Electrochemical Corrosion Evaluations of Microarc Oxidized Pure Mg, Mg–Ca and Mg–Ca–Zn Alloys for Biomedical Applications. *Mater. Sci. Eng. C* **2015**, *47*, 85–96. <https://doi.org/10.1016/j.msec.2014.11.048>.
- (369) Lim, S.-G.; Choe, H.-C. Corrosion Phenomena of PEO-Treated Films Formed in Solution Containing Mn, Mg, and Si Ions. *Appl. Surf. Sci.* **2019**, *477*, 50–59. <https://doi.org/10.1016/j.apsusc.2017.12.209>.
- (370) Carboneras, M.; García-Alonso, M. C.; Escudero, M. L. Biodegradation Kinetics of Modified Magnesium-Based Materials in Cell Culture Medium. *Corros. Sci.* **2011**, *53* (4), 1433–1439. <https://doi.org/10.1016/j.corsci.2011.01.014>.
- (371) Matykina, E.; Doucet, G.; Monfort, F.; Berkani, A.; Skeldon, P.; Thompson, G. E. Destruction of Coating Material during Spark Anodizing of Titanium. *Electrochimica Acta* **2006**, *22* (51), 4709–4715. <https://doi.org/10.1016/j.electacta.2006.01.021>.
- (372) Dorozhkin, S. V. Bioceramics of Calcium Orthophosphates. *Biomaterials* **2010**, *31* (7), 1465–1485. <https://doi.org/10.1016/j.biomaterials.2009.11.050>.
- (373) Prati, C.; Gandolfi, M. G. Calcium Silicate Bioactive Cements: Biological Perspectives and Clinical Applications. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **2015**, *31* (4), 351–370. <https://doi.org/10.1016/j.dental.2015.01.004>.
- (374) Khan, A. F.; Saleem, M.; Afzal, A.; Ali, A.; Khan, A.; Khan, A. R. Bioactive Behavior of Silicon Substituted Calcium Phosphate Based Bioceramics for Bone Regeneration. *Mater. Sci. Eng. C* **2014**, *35*, 245–252. <https://doi.org/10.1016/j.msec.2013.11.013>.
- (375) Monfort, F.; Berkani, A.; Matykina, E.; Skeldon, P.; Thompson, G. E.; Habazaki, H.; Shimizu, K. Development of Anodic Coatings on Aluminium under Sparking Conditions in Silicate Electrolyte. *Corros. Sci.* **2007**, *49* (2), 672–693. <https://doi.org/10.1016/j.corsci.2006.05.046>.
- (376) Mécuson, F.; Czerwicz, T.; Belmonte, T.; Dujardin, L.; Viola, A.; Henrion, G. Diagnostics of an Electrolytic Microarc Process for Aluminium Alloy Oxidation. *Surf. Coat. Technol.* **2005**, *200* (1), 804–808. <https://doi.org/10.1016/j.surfcoat.2005.01.076>.
- (377) Wierzbicka, E.; Pillado, B.; Mohedano, M.; Arrabal, R.; Matykina, E. Calcium Doped Flash-PEO Coatings for Corrosion Protection of Mg Alloy. *Metals* **2020**, *10* (7), 916. <https://doi.org/10.3390/met10070916>.
- (378) Pan, Y.; Chen, C.; Wang, D.; Huang, D. Dissolution and Precipitation Behaviors of Silicon-Containing Ceramic Coating on Mg–Zn–Ca Alloy in Simulated Body Fluid. *Colloids Surf. B Biointerfaces* **2014**, *122*, 746–751. <https://doi.org/10.1016/j.colsurfb.2014.08.012>.
- (379) Zhang, S.; Li, J.; Song, Y.; Zhao, C.; Zhang, X.; Xie, C.; Zhang, Y.; Tao, H.; He, Y.; Jiang, Y.; Bian, Y. In Vitro Degradation, Hemolysis and MC3T3-E1 Cell Adhesion of Biodegradable Mg–Zn Alloy. *Mater. Sci. Eng. C* **2009**, *29* (6), 1907–1912. <https://doi.org/10.1016/j.msec.2009.03.001>.
- (380) Tie, D.; Feyerabend, F.; Hort, N.; Willumeit, R.; Hoeche, D. XPS Studies of Magnesium Surfaces after Exposure to Dulbecco’s Modified Eagle Medium, Hank’s Buffered Salt Solution, and Simulated Body Fluid. *Adv. Eng. Mater.* **2010**, *12* (12), B699–B704. <https://doi.org/10.1002/adem.201080070>.

- (381) Zhu, M.; Pan, G. Quantum Chemical Studies of Mononuclear Zinc Species of Hydration and Hydrolysis. *J. Phys. Chem. A* **2005**, *109* (33), 7648–7652. <https://doi.org/10.1021/jp045560p>.
- (382) Wu, H.; Zhang, C.; Lou, T.; Chen, B.; Yi, R.; Wang, W.; Zhang, R.; Zuo, M.; Xu, H.; Han, P.; Zhang, S.; Ni, J.; Zhang, X. Crevice Corrosion – A Newly Observed Mechanism of Degradation in Biomedical Magnesium. *Acta Biomater.* **2019**, *98*, 152–159. <https://doi.org/10.1016/j.actbio.2019.06.013>.
- (383) Kirkland, N. T.; Birbilis, N.; Staiger, M. P. Assessing the Corrosion of Biodegradable Magnesium Implants: A Critical Review of Current Methodologies and Their Limitations. *Acta Biomater.* **2012**, *8* (3), 925–936. <https://doi.org/10.1016/j.actbio.2011.11.014>.
- (384) Song, G.; Atrens, A.; StJohn, D. An Hydrogen Evolution Method for the Estimation of the Corrosion Rate of Magnesium Alloys. In *Essential Readings in Magnesium Technology*; Mathaudhu, S. N., Luo, A. A., Neelameggham, N. R., Nyberg, E. A., Sillekens, W. H., Eds.; Springer International Publishing: Cham, 2016; pp 565–572. https://doi.org/10.1007/978-3-319-48099-2_90.
- (385) Chaharmahali, R.; Fattah-alhosseini, A.; Babaei, K. Surface Characterization and Corrosion Behavior of Calcium Phosphate (Ca-P) Base Composite Layer on Mg and Its Alloys Using Plasma Electrolytic Oxidation (PEO): A Review. *J. Magnes. Alloys* **2020**. <https://doi.org/10.1016/j.jma.2020.07.004>.
- (386) Arrabal, R.; Matykina, E.; Hashimoto, T.; Skeldon, P.; Thompson, G. E. Characterization of AC PEO Coatings on Magnesium Alloys. *Surf. Coat. Technol.* **2009**, *203* (16), 2207–2220. <https://doi.org/10.1016/j.surfcoat.2009.02.011>.
- (387) Brindley, G. W.; Hayami, R. Kinetics and Mechanism of Formation of Forsterite (Mg_2SiO_4) by Solid State Reaction of MgO and SiO_2 . *Philos. Mag. J. Theor. Exp. Appl. Phys.* **1965**, *12* (117), 505–514. <https://doi.org/10.1080/14786436508218896>.
- (388) Liu, J.; Lu, Y.; Jing, X.; Yuan, Y.; Zhang, M. Characterization of Plasma Electrolytic Oxidation Coatings Formed on Mg–Li Alloy in an Alkaline Silicate Electrolyte Containing Silica Sol. *Mater. Corros.* **2009**, *60* (11), 865–870. <https://doi.org/10.1002/maco.200805204>.
- (389) Dou, J.; Wang, J.; Lu, Y.; Chen, C.; Yu, H.; Ma, R. L.-W. Bioactive MAO/CS Composite Coatings on Mg–Zn–Ca Alloy for Orthopedic Applications. *Prog. Org. Coat.* **2021**, *152*, 106112. <https://doi.org/10.1016/j.porgcoat.2020.106112>.
- (390) Ly, X.; Yang, S.; Nguyen, T. Effect of Equal Channel Angular Pressing as the Pretreatment on Microstructure and Corrosion Behavior of Micro-Arc Oxidation (MAO) Composite Coating on Biodegradable Mg–Zn–Ca Alloy. *Surf. Coat. Technol.* **2020**, *395*, 125923. <https://doi.org/10.1016/j.surfcoat.2020.125923>.
- (391) Dou, J.; Gu, G.; Chen, C.; Pan, Y. Characterization and Biodegradation Behavior of Micro-Arc Oxidation Coatings Formed on Mg–Zn–Ca Alloys in Two Different Electrolytes. *RSC Adv.* **2016**, *6* (106), 104808–104818. <https://doi.org/10.1039/C6RA22666C>.
- (392) Zhang, N.; Zhao, D.; Liu, N.; Wu, Y.; Yang, J.; Wang, Y.; Xie, H.; Ji, Y.; Zhou, C.; Zhuang, J.; Wang, Y.; Yan, J. Assessment of the Degradation Rates and Effectiveness of Different Coated Mg–Zn–Ca Alloy Scaffolds for in Vivo Repair of Critical-Size Bone Defects. *J. Mater. Sci. Mater. Med.* **2018**, *29* (9), 138. <https://doi.org/10.1007/s10856-018-6145-2>.
- (393) Dou, J.; Chen, Y.; Chi, Y.; Li, H.; Gu, G.; Chen, C. Preparation and Characterization of a Calcium–Phosphate–Silicon Coating on a Mg–Zn–Ca Alloy

- via Two-Step Micro-Arc Oxidation. *Phys. Chem. Chem. Phys.* **2017**, *19* (23), 15110–15119. <https://doi.org/10.1039/C7CP02672B>.
- (394) Gao, J. H.; Shi, X. Y.; Yang, B.; Hou, S. S.; Meng, E. C.; Guan, F. X.; Guan, S. K. Fabrication and Characterization of Bioactive Composite Coatings on Mg-Zn-Ca Alloy by MAO/Sol-Gel. *J. Mater. Sci. Mater. Med.* **2011**, *22* (7), 1681–1687. <https://doi.org/10.1007/s10856-011-4349-9>.
- (395) Dou, J.; Zhao, Y.; Lu, L.; Gu, G.; Yu, H.; Chen, C. Effect of the Second-Step Voltages on the Structural and Corrosion Properties of Silicon–Calcium–Phosphate (Si–CaP) Coatings on Mg–Zn–Ca Alloy. *R. Soc. Open Sci.* **2018**, *5* (10), 172410. <https://doi.org/10.1098/rsos.172410>.
- (396) Virtanen, S. Biodegradable Mg and Mg Alloys: Corrosion and Biocompatibility. *Mater. Sci. Eng. B* **2011**, *176* (20), 1600–1608. <https://doi.org/10.1016/j.mseb.2011.05.028>.
- (397) Gnedenkov, A. S.; Lamaka, S. V.; Sinebryukhov, S. L.; Mashtalyar, D. V.; Egorkin, V. S.; Imshinetskiy, I. M.; Zheludkevich, M. L.; Gnedenkov, S. V. Control of the Mg Alloy Biodegradation via PEO and Polymer-Containing Coatings. *Corros. Sci.* **2021**, *182*, 109254. <https://doi.org/10.1016/j.corsci.2021.109254>.
- (398) Dong, R.; Wang, F.; Li, Z.; Chen, Z.; Zhang, H.; Jin, C. Honeycombed TiO₂ Films Prepared by Combining Breath Figure and Sol Gel Methods. *Surf. Coat. Technol.* **2015**, *276*, 391–398. <https://doi.org/10.1016/j.surfcoat.2015.06.040>.
- (399) Mansouri, J.; Yapit, E.; Chen, V. Polysulfone Filtration Membranes with Isoporous Structures Prepared by a Combination of Dip-Coating and Breath Figure Approach. *J. Membr. Sci.* **2013**, *444*, 237–251. <https://doi.org/10.1016/j.memsci.2013.05.022>.
- (400) Park, M. S.; Kim, J. K. Breath Figure Patterns Prepared by Spin Coating in a Dry Environment. *Langmuir* **2004**, *20* (13), 5347–5352. <https://doi.org/10.1021/la035915g>.
- (401) Moreno, L.; Mohedano, M.; Arrabal, R.; Matykina, E. Development and Screening of (Ca-P-Si-F)-PEO Coatings for Biodegradability Control of Mg-Zn-Ca Alloys. *J. Magnes. Alloys* **2022**. <https://doi.org/10.1016/j.jma.2021.12.011>.
- (402) Jeong, H.; Rho, J.; Shin, J.-Y.; Lee, D. Y.; Hwang, T.; Kim, K. J. Mechanical Properties and Cytotoxicity of PLA/PCL Films. *Biomed. Eng. Lett.* **2018**, *8* (3), 267–272. <https://doi.org/10.1007/s13534-018-0065-4>.
- (403) Drews, J. Drug Discovery: A Historical Perspective. *Science* **2000**, *287* (5460), 1960–1964. <https://doi.org/10.1126/science.287.5460.1960>.
- (404) Akoumeh, R.; Elzein, T.; Martínez-Campos, E.; Reviriego, F.; Rodríguez-Hernández, J. Fabrication of Porous Films from Immiscible Polymer Blends: Role of the Surface Structure on the Cell Adhesion. *Polym. Test.* **2020**, *91*, 106797. <https://doi.org/10.1016/j.polymertesting.2020.106797>.
- (405) Poelma, F. G.; Breäs, R.; Tukker, J. J. Intestinal Absorption of Drugs. III. The Influence of Taurocholate on the Disappearance Kinetics of Hydrophilic and Lipophilic Drugs from the Small Intestine of the Rat. *Pharm. Res.* **1990**, *7* (4), 392–397. <https://doi.org/10.1023/a:1015827624296>.
- (406) Volokhova, A. A.; Kudryavtseva, V. L.; Spiridonova, T. I.; Kolesnik, I.; Goreninskii, S. I.; Sazonov, R. V.; Remnev, G. E.; Tverdokhlebov, S. I. Controlled Drug Release from Electrospun PCL Non-Woven Scaffolds via Multi-Layering and e-Beam Treatment. *Mater. Today Commun.* **2021**, *26*, 102134. <https://doi.org/10.1016/j.mtcomm.2021.102134>.

- (407) Ajmal, G.; Bonde, G. V.; Mittal, P.; Khan, G.; Pandey, V. K.; Bakade, B. V.; Mishra, B. Biomimetic PCL-Gelatin Based Nanofibers Loaded with Ciprofloxacin Hydrochloride and Quercetin: A Potential Antibacterial and Anti-Oxidant Dressing Material for Accelerated Healing of a Full Thickness Wound. *Int. J. Pharm.* **2019**, *567*, 118480. <https://doi.org/10.1016/j.ijpharm.2019.118480>.
- (408) Campbell, K.; Qi, S.; Craig, D. Q. M.; McNally, T. Paracetamol-Loaded Poly(ϵ -Caprolactone) Layered Silicate Nanocomposites Prepared Using Hot-Melt Extrusion. *J. Pharm. Sci.* **2009**, *98* (12), 4831–4843. <https://doi.org/10.1002/jps.21787>.
- (409) Kalani, M.; Yunus, R. Effect of Supercritical Fluid Density on Nanoencapsulated Drug Particle Size Using the Supercritical Antisolvent Method. *Int. J. Nanomedicine* **2012**, *7*, 2165–2172. <https://doi.org/10.2147/IJN.S29805>.
- (410) Nair, N. R.; Sekhar, V. C.; Nampoothiri, K. M.; Pandey, A. 32 - Biodegradation of Biopolymers. In *Current Developments in Biotechnology and Bioengineering*; Pandey, A., Negi, S., Soccol, C. R., Eds.; Elsevier, 2017; pp 739–755. <https://doi.org/10.1016/B978-0-444-63662-1.00032-4>.
- (411) Farah, S.; Anderson, D. G.; Langer, R. Physical and Mechanical Properties of PLA, and Their Functions in Widespread Applications — A Comprehensive Review. *Adv. Drug Deliv. Rev.* **2016**, *107*, 367–392. <https://doi.org/10.1016/j.addr.2016.06.012>.
- (412) Silva, D. M.; Liu, R.; Gonçalves, A. F.; da Costa, A.; Castro Gomes, A.; Machado, R.; Vongsvivut, J.; J. Tobin, M.; Sencadas, V. Design of Polymeric Core-Shell Carriers for Combination Therapies. *J. Colloid Interface Sci.* **2021**, *587*, 499–509. <https://doi.org/10.1016/j.jcis.2020.12.001>.
- (413) Thomas, N.; Thorn, C.; Richter, K.; Thierry, B.; Prestidge, C. Efficacy of Poly-Lactic-Co-Glycolic Acid Micro- and Nanoparticles of Ciprofloxacin Against Bacterial Biofilms. *J. Pharm. Sci.* **2016**, *105* (10), 3115–3122. <https://doi.org/10.1016/j.xphs.2016.06.022>.
- (414) Ebrahimifar, M.; Taherimehr, M. Evaluation of In-Vitro Drug Release of Polyvinylcyclohexane Carbonate as a CO₂-Derived Degradable Polymer Blended with PLA and PCL as Drug Carriers. *J. Drug Deliv. Sci. Technol.* **2021**, *63*, 102491. <https://doi.org/10.1016/j.jddst.2021.102491>.
- (415) PubChem. *Hazardous Substances Data Bank (HSDB)*: 6987. <https://pubchem.ncbi.nlm.nih.gov/source/hsdb/6987> (accessed 2022-07-28).
- (416) Jouyban, A.; Azarmir, O.; Mirzaei, S.; Hassanzadeh, D.; Ghafourian, T.; Acree, W. E.; Nokhodchi, A. Solubility Prediction of Paracetamol in Water-Ethanol-Propylene Glycol Mixtures at 25 and 30 Degrees C Using Practical Approaches. *Chem. Pharm. Bull. (Tokyo)* **2008**, *56* (4), 602–606. <https://doi.org/10.1248/cpb.56.602>.
- (417) Zahedi, P.; Karami, Z.; Rezaeian, I.; Jafari, S. H.; Mahdaviani, P.; Abdolghaffari, A. H.; Abdollahi, M. Preparation and Performance Evaluation of Tetracycline Hydrochloride Loaded Wound Dressing Mats Based on Electrospun Nanofibrous Poly(Lactic Acid)/Poly(ϵ -Caprolactone) Blends. *J. Appl. Polym. Sci.* **2012**, *124*. <https://doi.org/10.1002/app.35372>.
- (418) Nagayama, G.; Zhang, D. Intermediate Wetting State at Nano/Microstructured Surfaces. *Soft Matter* **2020**, *16* (14), 3514–3521. <https://doi.org/10.1039/C9SM02513H>.
- (419) Dzikowski, M.; Castanié, N.; Guedon, A.; Verrier, B.; Primard, C.; Sohier, J. Antibiotic Incorporation in Jet-Sprayed Nanofibrillar Biodegradable Scaffolds for

- Wound Healing. *Int. J. Pharm.* **2017**, *532* (2), 802–812. <https://doi.org/10.1016/j.ijpharm.2017.08.117>.
- (420) Zupančič, Š.; Sinha-Ray, S.; Sinha-Ray, S.; Kristl, J.; Yarin, A. L. Long-Term Sustained Ciprofloxacin Release from PMMA and Hydrophilic Polymer Blended Nanofibers. *Mol. Pharm.* **2016**, *13* (1), 295–305. <https://doi.org/10.1021/acs.molpharmaceut.5b00804>.
- (421) Van Kolfsochten, A. A.; Olling, M.; Van Noordwijk, J. Pharmacokinetic Interactions between Indomethacin and Paracetamol in the Rat. *Pharm. Weekbl. Sci.* **1985**, *7* (1), 15–19. <https://doi.org/10.1007/BF01962864>.
- (422) Issa, M. M.; Nejem, R. M.; El-Abadla, N. S. Oral Ciprofloxacin Affects the Pharmacokinetics of Paracetamol in Saliva. *Clin. Drug Investig.* **2006**, *26* (4), 223–226. <https://doi.org/10.2165/00044011-200626040-00007>.
- (423) Han, S.; Yang, R.; Li, C.; Yang, L. The Wettability and Numerical Model of Different Silicon Microstructural Surfaces. *Appl. Sci.* **2019**, *9* (3), 566. <https://doi.org/10.3390/app9030566>.
- (424) Nam, S.-W.; Choi, D.-J.; Kim, S.-K.; Her, N.; Zoh, K.-D. Adsorption Characteristics of Selected Hydrophilic and Hydrophobic Micropollutants in Water Using Activated Carbon. *J. Hazard. Mater.* **2014**, *270*, 144–152. <https://doi.org/10.1016/j.jhazmat.2014.01.037>.
- (425) Kłosińska-Szmurło, E.; Pluciński, F. A.; Grudzień, M.; Betlejewska-Kielak, K.; Biernacka, J.; Mazurek, A. P. Experimental and Theoretical Studies on the Molecular Properties of Ciprofloxacin, Norfloxacin, Pefloxacin, Sparfloxacin, and Gatifloxacin in Determining Bioavailability. *J. Biol. Phys.* **2014**, *40* (4), 335–345. <https://doi.org/10.1007/s10867-014-9354-z>.
- (426) Wang, L.; Zheng, H.; Du, C.; Shi, Z.; Ren, L.; Wang, Y. Construct Scaffold-like Delivery System with Poly (Lactic-Co-Glycolic) Microspheres on Micro-Arc Oxidation Titanium. *Appl. Surf. Sci.* **2013**, *266*, 81–88. <https://doi.org/10.1016/j.apsusc.2012.11.095>.
- (427) Dawes, G. J. S.; Fratila-Apachitei, L. E.; Necula, B. S.; Apachitei, I.; Witkamp, G. J.; Duszczek, J. Release of PLGA–Encapsulated Dexamethasone from Microsphere Loaded Porous Surfaces. *J. Mater. Sci. Mater. Med.* **2010**, *21* (1), 215–221. <https://doi.org/10.1007/s10856-009-3846-6>.
- (428) Thomas, M. B.; Metoki, N.; Mandler, D.; Eliaz, N. In Situ Potentiostatic Deposition of Calcium Phosphate with Gentamicin-Loaded Chitosan Nanoparticles on Titanium Alloy Surfaces. *Electrochimica Acta* **2016**, *222*, 355–360. <https://doi.org/10.1016/j.electacta.2016.10.186>.
- (429) Sasireka, A.; Rajendran, R.; Priya, P.; Raj, V. Ciprofloxacin-Loaded Ceramic/Polymer Composite Coatings on Ti with Improved Antibacterial and Corrosion Resistance Properties for Orthopedic Applications. *ChemistrySelect* **2019**, *4* (4), 1166–1175. <https://doi.org/10.1002/slct.201803769>.
- (430) Xu, W.; Yagoshi, K.; Asakura, T.; Sasaki, M.; Niidome, T. Silk Fibroin as a Coating Polymer for Sirolimus-Eluting Magnesium Alloy Stents. *ACS Appl. Bio Mater.* **2020**, *3* (1), 531–538. <https://doi.org/10.1021/acsabm.9b00957>.
- (431) Walden, D. M.; Khotimchenko, M.; Hou, H.; Chakravarty, K.; Varshney, J. Effects of Magnesium, Calcium, and Aluminum Chelation on Fluoroquinolone Absorption Rate and Bioavailability: A Computational Study. *Pharmaceutics* **2021**, *13* (5), 594. <https://doi.org/10.3390/pharmaceutics13050594>.
- (432) Bridle, M. J.; Janesko, B. G. Computational Study of Fluoroquinolone Binding to and Its Applicability to Future Drug Design. *Int. J. Quantum Chem.* **2017**, *117* (20), e25428. <https://doi.org/10.1002/qua.25428>.

- (433) Refat, M. S.; Mohamed, G. G.; El-Sayed, M. Y.; Killa, H. M. A.; Fetooh, H. Spectroscopic and Thermal Degradation Behavior of Mg(II), Ca(II), Ba(II) and Sr(II) Complexes with Paracetamol Drug. *Arab. J. Chem.* **2017**, *10*, S2376–S2387. <https://doi.org/10.1016/j.arabjc.2013.08.017>.
- (434) Chandrathilaka, A.; Ileperuma, O.; Hettiarachchi, C. Spectrophotometric and PH-Metric Studies on Pb(II), Cd(II), Al(III) and Cu(II) Complexes of Paracetamol and Ascorbic Acid. *J. Natl. Sci. Found. Sri Lanka* **2013**, *41*, 337–344. <https://doi.org/10.4038/jnsfsr.v41i4.6253>.
- (435) Sultan, M. A.; Karim, A.; Kandory, A.; Al-metwali, A. Synthesis and Characterization of Al(III) Complex with Paracetamol. **2020**. <https://doi.org/10.25258/IJDDT.V10I1.23>.
- (436) Prabhath, M. R. R.; Williams, L.; Bhat, S. V.; Sharma, P. Recent Advances in Cyanamide Chemistry: Synthesis and Applications. *Molecules* **2017**, *22* (4), 615. <https://doi.org/10.3390/molecules22040615>.
- (437) Eduok, U. M.; Umoren, S.; Udoh, A. Synergistic Inhibition Effects between Leaves and Stem Extracts of *Sida Acuta* and Iodide Ion for Mild Steel Corrosion in 1 M H₂SO₄ Solutions. *Arab. J. Chem.* **2012**, *5*. <https://doi.org/10.1016/j.arabjc.2010.09.006>.
- (438) Deng, M.; Wang, L.; Höche, D.; V. Lamaka, S.; Wang, C.; Snihirova, D.; Jin, Y.; Zhang, Y.; L. Zheludkevich, M. Approaching “Stainless Magnesium” by Ca Micro-Alloying. *Mater. Horiz.* **2021**, *8* (2), 589–596. <https://doi.org/10.1039/D0MH01380C>.
- (439) Palacio, D. A.; Rivas, B. L.; Urbano, B. F. Ultrafiltration Membranes with Three Water-Soluble Polyelectrolyte Copolymers to Remove Ciprofloxacin from Aqueous Systems. *Chem. Eng. J.* **2018**, *351*, 85–93. <https://doi.org/10.1016/j.cej.2018.06.099>.
- (440) Yang, L.; Qin, X.; Jiang, X.; Gong, M.; Yin, D.; Zhang, Y.; Zhao, B. SERS Investigation of Ciprofloxacin Drug Molecules on TiO₂ Nanoparticles. *Phys. Chem. Chem. Phys.* **2015**, *17* (27), 17809–17815. <https://doi.org/10.1039/C5CP02666K>.
- (441) Vaghefinazari, B.; Lamaka, S. V.; Blawert, C.; Serdechnova, M.; Scharnagl, N.; Karlova, P.; Wieland, D. C. F.; Zheludkevich, M. L. Exploring the Corrosion Inhibition Mechanism of 8-Hydroxyquinoline for a PEO-Coated Magnesium Alloy. *Corros. Sci.* **2022**, *203*, 110344. <https://doi.org/10.1016/j.corsci.2022.110344>.
- (442) Vaghefinazari, B.; Wang, C.; Mercier, D.; Mei, D.; Seyeux, A.; Marcus, P.; Blawert, C.; Lamaka, S. V.; Zheludkevich, M. L. Adverse Effect of 2,5PDC Corrosion Inhibitor on PEO Coated Magnesium. *Corros. Sci.* **2021**, *192*, 109830. <https://doi.org/10.1016/j.corsci.2021.109830>.