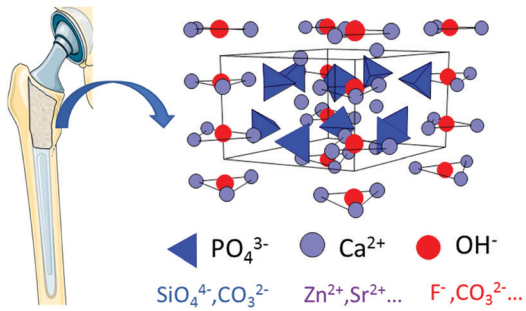
Journal of Materials Chemistry B c9tb02710f

We have presented the Graphical Abstract text and image for your article below. This brief summary of your work will appear in the contents pages of the issue in which your article appears.



Substituted hydroxyapatite coatings of bone implants

Daniel Arcos* and María Vallet-Regí*

This review is a comprehensive overview and analysis of the most important advances in the field of substituted hydroxyapatite coatings.

Please check this proof carefully. Our staff will not read it in detail after you have returned it.

Please send your corrections either as a copy of the proof PDF with electronic notes attached or as a list of corrections. **Do not edit the text within the PDF or send a revised manuscript** as we will not be able to apply your corrections. Corrections at this stage should be minor and not involve extensive changes.

Proof corrections must be returned as a single set of corrections, approved by all co-authors. No further corrections can be made after you have submitted your proof corrections as we will publish your article online as soon as possible after they are received.

Please ensure that:

- The spelling and format of all author names and affiliations are checked carefully. You can check how we have identified the authors' first and last names in the researcher information table on the next page. **Names will be indexed and cited as shown on the proof, so these must be correct.**
- Any funding bodies have been acknowledged appropriately and included both in the paper and in the funder information table on the next page.
- All of the editor's queries are answered.
- Any necessary attachments, such as updated images or ESI files, are provided.

Translation errors can occur during conversion to typesetting systems so you need to read the whole proof. In particular please check tables, equations, numerical data, figures and graphics, and references carefully.

Please return your **final** corrections, where possible within **48 hours** of receipt, by e-mail to: materialsB@rsc.org. If you require more time, please notify us by email.

Funding information

Providing accurate funding information will enable us to help you comply with your funders' reporting mandates. Clear acknowledgement of funder support is an important consideration in funding evaluation and can increase your chances of securing funding in the future.

We work closely with Crossref to make your research discoverable through the Funding Data search tool (http://search.crossref.org/funding). Funding Data provides a reliable way to track the impact of the work that funders support. Accurate funder information will also help us (i) identify articles that are mandated to be deposited in **PubMed Central (PMC)** and deposit these on your behalf, and (ii) identify articles funded as part of the **CHORUS** initiative and display the Accepted Manuscript on our web site after an embargo period of 12 months.

Further information can be found on our webpage (http://rsc.li/funding-info).

What we do with funding information

We have combined the information you gave us on submission with the information in your acknowledgements. This will help ensure the funding information is as complete as possible and matches funders listed in the Crossref Funder Registry.

If a funding organisation you included in your acknowledgements or on submission of your article is not currently listed in the registry it will not appear in the table on this page. We can only deposit data if funders are already listed in the Crossref Funder Registry, but we will pass all funding information on to Crossref so that additional funders can be included in future.

Please check your funding information

The table below contains the information we will share with Crossref so that your article can be found *via* the Funding Data search tool. **Please check that the funder names and grant numbers in the table are correct and indicate if any changes are necessary to the Acknowledgements text.**

Funder name	Funder's main country of origin	Funder ID (for RSC use only)	Award/grant number
Ministerio de Economía y Competitividad	Spain	501100003329	MAT2016-75611-R
European Research Council	European Union	501100000781	694160

Q1

Researcher information

Please check that the researcher information in the table below is correct, including the spelling and formatting of all author names, and that the authors' first, middle and last names have been correctly identified. **Names will be indexed and cited as shown on the proof, so these must be correct.**

If any authors have ORCID or ResearcherID details that are not listed below, please provide these with your proof corrections. Please ensure that the ORCID and ResearcherID details listed below have been assigned to the correct author. Authors should have their own unique ORCID iD and should not use another researcher's, as errors will delay publication.

Please also update your account on our online manuscript submission system to add your ORCID details, which will then be automatically included in all future submissions. See here for step-by-step instructions and more information on author identifiers.

First (given) and middle name(s)	Last (family) name(s)	ResearcherID	ORCID iD
Daniel	Arcos		0000-0002-5367-7272
María	Vallet-Regí	M-3378-2014	0000-0002-6104-4889

Queries for the attention of the authors

Journal: **Journal of Materials Chemistry B**

Paper: **c9tb02710f**

Title: **Substituted hydroxyapatite coatings of bone implants**

For your information: You can cite this article before you receive notification of the page numbers by using the following format: (authors), J. Mater. Chem. B, (year), DOI: 10.1039/c9tb02710f.

Editor's queries are marked on your proof like this Q1, Q2, etc. and for your convenience line numbers are indicated like this 5, 10, 15, ...

Please ensure that all queries are answered when returning your proof corrections so that publication of your article is not delayed.

Query reference	Query	Remarks
Q1	Funder details have been incorporated in the funder table using information provided in the article text. Please check that the funder information in the table is correct.	
Q2	Please confirm that the spelling and format of all author names is correct. Names will be indexed and cited as shown on the proof, so these must be correct. No late corrections can be made.	
Q3	The Graphical Abstract text has been altered for clarity. Please check that the meaning is correct.	
Q4	The sentence beginning ''Besides, the capability...'' has been altered for clarity. Please check that the meaning is correct.	
Q5	Throughout the manuscript should ''perspectives'' be changed to ''prospects''?	
Q6	The sentence beginning ''The unit cell of hydroxyapatite...'' has been altered for clarity. Please check that the meaning is correct.	
Q7	The sentence beginning ''For instance, the coating of Ti...'' has been altered for clarity. Please check that the meaning is correct.	
Q8	The meaning of the phrase ''parameters *a* and c *of*'' in the sentence beginning ''Zn^{2+} isoelectrically...'' is not clear – please provide alternative text.	
Q9	The sentence beginning ''Mg^{2+} incorporation as a soluble...'' has been altered for clarity. Please check that the meaning is correct.	
Q10	The sentence beginning ''Among the elements...'' has been altered for clarity. Please check that the meaning is correct.	
Q11	Throughout the manuscript should ''Fluor'' be changed to ''Fluoride'' or ''Fluorine''?	
Q12	Citations to Tables 2 and 3 have been added, please check that the placement of these citations is suitable. If the locations are not suitable, please indicate where in the text the citations should be inserted.	
Q13	The sentence beginning ''Certainly, these coatings...'' has been altered for clarity. Please check that the meaning is correct.	
Q14	The sentence beginning ''Besides, the Cu/Sr-Hap coating...'' has been altered for clarity. Please check that the meaning is correct.	

Q15	The sentence beginning ''For instance, samarium...'' has been altered for clarity. Please check that the meaning is correct.	
Q16	The citation to Fig. 3 in the sentence beginning ''Recently, Si-HAp coatings...'' has been changed to Fig. 2 as the text appears to discuss Fig. 2. Please check that this is correct.	
Q17	The sentence beginning ''However, microCT analysis...'' has been altered for clarity. Please check that the meaning is correct.	
Q18	The sentence beginning ''In this sense, the incorporation...'' has been altered for clarity. Please check that the meaning is correct.	
Q19	The meaning of the phrase ''called to play'' in the sentence beginning ''The incorporation of rapid...'' is not clear – please provide alternative text.	
Q20	Please note that a conflict of interest statement is required for all manuscripts. Please read our policy on Conflicts of interest (http://rsc.li/conflicts) and provide a statement with your proof corrections. If no conflicts exist, please state that ''There are no conflicts to declare''.	
Q21	Please indicate where ref. 20 should be cited in the text.	
Q22	The page numbers provided for ref. 24 appear to be incorrect. Please check and correct as necessary.	
Q23	Ref. 128: Please provide the year of publication.	
Q24	Ref. 138: Please provide the page (or article) number(s).	

Journal of Materials Chemistry B

ROYAL SOCIETY OF CHEMISTRY

REVIEW

Substituted hydroxyapatite coatings of bone implants

Cite this: DOI: 10.1039/c9tb02710f

Daniel Arcos *[ab] and María Vallet-Regí *[ab]

Surface modification of orthopedic and dental implants has been demonstrated to be an effective strategy to accelerate bone healing at early implantation times. Among the different alternatives, coating implants with a layer of hydroxyapatite (HAp) is one of the most used techniques, due to its excellent biocompatibility and osteoconductive behavior. The composition and crystalline structure of HAp allow for numerous ionic substitutions that provide added value, such as antibiotic properties or osteoinduction. In this article, we will review and critically analyze the most important advances in the field of substituted hydroxyapatite coatings. In recent years substituted HAp coatings have been deposited not only on orthopedic prostheses and dental implants, but also on macroporous scaffolds, thus expanding their applications towards bone regeneration therapies. Besides, the capability of substituted HAps to immobilize proteins and growth factors by non-covalent interactions has opened new possibilities for preparing hybrid coatings that foster bone healing processes. Finally, the most important *in vivo* outcomes will be discussed to understand the perspectives of substituted HAp coatings from a clinical point of view.

Received 29th November 2019,
Accepted 5th February 2020

DOI: 10.1039/c9tb02710f

rsc.li/materials-b

1. Introduction

Orthopedic implants improve the quality of life of millions of patients every year. Due to clinical staff training, new prosthesis designs, control of sterility and protocols for antibiotic prophylaxis, the success rate of orthopedic prostheses has significantly increased in the last decades. For instance, the success rate of hip replacements 10 years after surgery is 90–95% and 80–85%

[a] *Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria del Hospital 12 de Octubre i + 12, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain. E-mail: arcosd@ucm.es, vallet@ucm.es*

[b] *CIBER de Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Spain*

Daniel Arcos

Daniel Arcos was born in Madrid, Spain, in 1971. He received his PhD at the University Complutense of Madrid (UCM) in 2002. He is Senior Lecturer of the Department of Chemistry in Pharmaceutical Sciences at the Faculty of Pharmacy (UCM), where he carries out his research in the field of bioceramics for bone regeneration. He has published more than 100 papers in the field of bioceramics for bone tissue applications and has led several research projects on this topic. Currently he is involved in the development of substituted hydroxyapatite coatings for the treatment of bone defects under osteoporotic conditions.

María Vallet-Regí

María Vallet-Regí (Las Palmas, 1946) studied Chemistry at the Complutense University of Madrid (UCM) and received a doctorate from the same university in 1974. Currently, she is Emeritus Professor of Inorganic Chemistry and Director of the Intelligent Biomaterials Research Group of the Department of Chemistry in Pharmaceutical Sciences of the Faculty of Pharmacy of UCM. She is the author of more than 700 scientific articles and has received numerous awards and distinctions throughout her extensive scientific career.

at 20 years.[1] In the case of titanium based dental implants, the success rate is very similar.[2] Despite these clinical outcomes, thousands of prostheses and dental implants must be revised every year. The two most frequent causes for failure are insufficient bone formation around the implant immediately after implantation,[3,4] especially in osteoporotic patients,[5–8] and infection.[9]

Bone implants for load-bearing applications are generally made of metals such as titanium, Ti6Al4V alloys, stainless steel, *etc.*, which exhibit appropriate mechanical properties to be used in the clinic as dental implants and stems of hip and knee prostheses, plates, screws and other fixation devices. In the case of endosseous metal components such as dental implant roots or joint prosthesis stems, the surfaces are commonly modified by roughening and/or coating with bioactive compounds to improve the osteoconductivity. HAp is a calcium phosphate (CaP) commonly used for the fabrication of coatings, layers or thin films on the surface of prosthetic devices[10] to accelerate bone healing at early implantation times. For this aim, the coating must be biocompatible,[11] bioactive[12] and osteoconductive.[13] Besides, ASTM and ISO standards require other properties, namely the presence of a dominant crystalline phase that prevents fast coating dissolution, the presence of an amorphous phase that promotes early osteointegration without losing stability, a programmed dissolution rate of the crystalline phase (commonly HAp), an elemental composition matching the mineral phase of bone and strong implant adhesion to prevent mechanical failures under load-bearing conditions.[14,15] Currently, just a few coatings fulfil these requirements and only calcium phosphate bioceramics fabricated by plasma spraying are clinically approved for orthopedic coatings and commercially available.

Substituted HAp coatings have arisen as a very promising alternative to conventional CaP coatings. Ionic substitutions are aimed at providing additional properties to HAp such as osteoinduction or antibiotic activity. Therefore, substituted HAp coatings would be produced not only for accelerating biomechanical fixation but also for providing solutions in some pathological scenarios such as infection and osteoporosis. Currently, the field of substituted HAp coatings has become an ever-growing research field, mainly due to the variety of elements and ions with therapeutic effects discovered during the last 50 years.[16] Moreover, HAp exhibits a crystalline structure that easily allows ion incorporation by substitutive and interstitial mechanisms. These facts have opened new scenarios where coatings play an active role in the treatment of pathologies, in addition to accelerating bone healing at early implantation times. In association with the recently developed additively manufactured metallic scaffolds, substituted HAp coatings are also expanding the clinical applications of metallic implants from their conventional substitutive functions towards bone regeneration purposes. Recently, the combination of substituted HAp with nanostructures has also opened new possibilities in the field of coatings for orthopedic purposes.[17–19]

This review is devoted to the performance of substituted HAp coatings for orthopedic and dental devices. Beginning from the bioinorganic characteristics of the substituents and the most frequently applied coating methods, the *in vitro* and *in vivo* biological performance are comprehensively reviewed and analyzed.

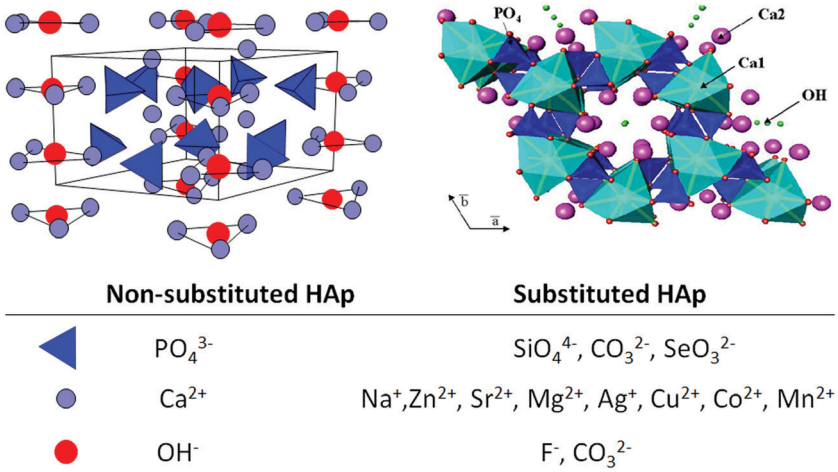
2. Crystalline structure of hydroxyapatite and ionic substitutions

Stoichiometric HAp is one of the most important bioceramics used in dentistry and orthopedic surgery. Its composition, $Ca_{10}(PO_4)_6(OH)_2$, is like the mineral component of bone tissue although there exist important crystal-chemical and microstructural differences, depending on the synthesis method. The structure of HAp can be described as a hexagonal unit cell with space group $P6_3/m$ and lattice parameters a = 9.432 Å and c = 6.881 Å, having one formula $Ca_{10}(PO_4)_6(OH)_2$ per unit cell[21] (Fig. 1). The OH^- ions and four Ca^{2+} ions at Ca(1) sites lie along columns parallel to the c axis. The OH^- are sited along the c axis and the O–H bond direction is parallel to it, without straddling the mirror planes at z = 1/4 and 3/4. The remaining six Ca^{2+} ions, positioned at Ca(2) sites, are associated with the two OH^- groups in the unit cell, where they form triangles perpendicular to the OH^-. The phosphate tetrahedrons form the remaining basic structural unit of HAp.

The bioactive behavior of stoichiometric HAp can be improved by introducing substitutions in both the cationic and anionic sublattices.[22] The cations can exhibit the same oxidation state as Ca^{2+}, such as Sr^{2+}, Pb^{2+}, Mg^{2+}, *etc.*,[23–26] and anions the same oxidation state as OH^-, such as F^- or Cl^-.[27,28] Ionic substitutions with different oxidation states are also very common[29–31] and play an important role in the chemical, structural, and microstructural properties. For example in biological apatites CO_3^{2-}, substitution for PO_4^{3-} (type B) or OH^- (type A) is a likely substitution.[32,33] In the case of B-type carbonated HAp (CHAp), single valence cations (Na^+ or K^+) are often incorporated in the Ca^{2+} positions to keep the electrical balance.[34,35]

3. Production techniques of hydroxyapatite coatings

The production techniques of HAp coatings can by classified into two main groups: physical deposition techniques and wet chemical deposition techniques.[36] Regardless of the coating technique used, the success or failure of the coating greatly depends on the previous preparation of the substrate. Surface cleaning is required to remove dirt, oils and other components coming from the machining of the prostheses. This cleaning commonly consists of an ultrasonic bath of ethanol or acetone and often includes a subsequent acid treatment (etching) and/or sand or grit-blasting to facilitate the subsequent coating attachment and stability.



Q6 Fig. 1 The unit cell of hydroxyapatite projected along the *a* axis (left) and along the *c* axis (right) showing Ca1, Ca2, tetrahedral phosphates and hydroxyl sites. Ionic substitutions with potential therapeutic effects are indicated (bottom).

3.1 Physical deposition techniques

Thermal spraying techniques. The most important physical deposition techniques for the fabrication of HAp coatings are thermal spraying techniques. They are based on processes in which the coating materials (or precursors) are heated and sprayed on the substrate. Depending on the heating method, thermal spraying includes atmospheric plasma spraying,[37] vacuum plasma spraying,[38,39] suspension plasma spraying,[40] liquid phase plasma spraying,[41] high velocity oxy-fuel spraying[42] and gas tunnel type plasma spraying.[43]

Due to the very high temperatures used in these techniques, differences between the feedstock material and the deposited coating are unavoidable. Deposited HAp and substituted HAp are dehydroxylated and partially decomposed into oxyapatite (OA) and other CaP phases. In fact, the so named HAp coatings are commonly multiphase coatings containing amorphous calcium phosphate (ACP), α or β tricalcium phosphate (α or β-TCP), OA, HAp and even CaO. In other words, the coating composition does not depend only on the feedstock material, but also on the heating and cooling processes undergone during their permanence within the plasma and cooling ratio. This multiphasic composition is decisive for the biocompatibility, since the content of more soluble phases such as ACP determines the stability of the coating. ISO rules determine that the HAp content shall be more than 95% by mass, whereas the content of α-TCP, β-TCP, TTCP and CaO shall be less than a 5% mass fraction. Regarding the coating crystallinity, the HAp phase shall have crystallinity values not less than 45% with respect to standard HAp (HAp after calcination at 1000 °C for 15 hours).[14]

Thermal spraying based techniques produce thick coatings of several tens of micrometers,[44] although the use of a feedstock as a solution or suspension can result in thinner coatings (5 to 10 μm) in comparison with thermal spraying when using dry powders. For instance, high velocity oxy-fuel spraying, a thermal spray process developed in the 90s,[45] allows the fabrication of thin films with low porosity and high adhesion, due to the velocity reached by the powder feedstock (around 800 m s^{-1}) when injected into a gas stream produced by the combustion of a fuel with oxygen.

Electrophoretic deposition (EPD). EPD is a physical coating method that consists in depositing charged colloidal HAp particles onto a conductive substrate of opposite charge.[46] The HAp particles are suspended in liquid media and subsequently deposited by the driving force of a DC electric field, the substrate being one of the electrodes. The coating thickness and morphology can be adjusted through the applied voltage, in such a way that the particle size and amount of deposited HAp increase with it. After deposition, the implant is commonly treated at 850 °C to 950 °C under high vacuum conditions.[47] EPD is a very useful technique for the fabrication of coatings onto porous structures; however, the main drawback is the shrinkage and cracking that often appear during the sintering process.

Physical vapor deposition (PVD) techniques. PVD techniques, commonly referred to as sputtering techniques, are among the most widely used methods for the preparation of HAp coatings. PVD commonly involves the use of a highly energetic beam of ions or electrons projected onto a CaP target. Ca^{2+}, PO_4^{3-} and substitution ions are pulled out from the target surface and deposited onto the substrate. Depending on the technique used to knock the ions off the target, sputtering techniques are denoted as pulsed electron deposition (PED) if the ions are pulled out from the CaP target by means of collisions with electrons;[48,49] pulsed laser deposition (PLD), or laser ablation deposition[50,51] uses a high-power laser beam to hit a CaP target resulting in a gaseous phase made of atoms, ions, molecules and clusters, which is moved towards the substrate as a plasma plume. This approach leads to thin HAp coatings of 0.05 to 5 μm thickness[52,53] and the heating of the substrate is required for crystallization. Radio-frequency magnetron sputtering (RFMS) produces coatings in large areas and with a large variety of morphologies. It is based on PVD in high vacuum conditions of CaP released into the sputtering

chamber as a gas. The particles are ionized by powerful magnets and the charged material aligns on the substrate to form the coating.[54,55] For the specific case of CaP coatings, Surmenev established the parameters that directly affect the quality of the coating, namely the discharge power, working pressure, substrate temperature, flow rate, deposition time and the subsequent thermal treatment.[56] Since Cooley *et al.* used for the first time this approach to prepare CaP coatings,[57] RFMS has been used to produce different biocompatible coatings on several substrates;[58,59] matrix-assisted pulsed laser deposition (MAPLE) was introduced as an alternative to PLD for the synthesis of thermally unstable compounds, mainly organic coatings. This technique has also shown some advantages for the fabrication of inorganic coatings as well.[60] In the case of CaP coatings, MAPLE has shown very interesting results for the fabrication of thermally unstable octacalcium phosphate coatings,[61] organic–inorganic composites[62] or even CaP coatings containing drugs.[63]

3.2 Wet chemical deposition techniques

The different chemical deposition approaches are characterized by occurring from solutions or suspensions. During the coating process, the chemical composition of the feedstock material changes by means of a chemical reaction, resulting in a different compound at the end of the coating production. Chemical deposition processes occur at moderate temperature and are mostly governed by the solution supersaturation, pH and temperature, although the addition of nucleators, inhibitors, *etc.* allows the control of the coating characteristics. In this approach, the previous conditioning of the substrate surface is compulsory. For instance, the coating of Ti implants commonly requires acid or alkaline pre-treatment to form a rich Ti–OH layer, which facilitates the subsequent calcium phosphate nucleation and growth.[64]

Chemical vapor deposition technique (CVD). CVD consists in the exposure of the substrate to volatile precursors that react or decompose on the surface. CVD has been used to prepare CaP-based coatings on metallic substrates[65] demonstrating potential for controlling the crystal phase and microstructure and providing well-adhered coatings even on complex-shaped metal substrates.[66]

Biomimetic deposition techniques. Biomimetic deposition consists in mimicking natural manufacturing methods to generate artificial bone-like HAp, which can be used to improve the osteointegration of dental and orthopedic implants. The most common process consists in the crystallization of nonstoichiometric carbonate-substituted hydroxyapatite (C-HAp) from simulated physiological solutions at low temperature conditions.[67] The biomimetic HAp crystallization takes place through the nucleation of ACP or OCP, which subsequently maturates into substituted and calcium-deficient hydroxyapatite (CDHAp). Like other chemical deposition techniques, biomimetic deposition commonly requires substrates with OH^- containing surfaces such as Si–OH, Ti–OH, Zr–OH, Nb–OH *etc.*, which can be easily obtained by acid or alkaline treatments. When these activated substrates are soaked in solutions where the ionic product overcomes the solubility constant (Kps) for HAp (for instance simulated body fluid, SBF), the hydroxyl groups promote CaP nucleation and subsequent crystallization into apatite like-phases. This technique allows for the co-deposition of other biological substances, since the coating process is carried out using mild conditions of pH and temperature.[68,69]

Sol–gel method. The sol–gel method consists in the preparation of a colloidal liquid suspension 'sol' by means of the hydrolysis and condensation of the CaP precursors (commonly phosphorous alkoxides and inorganic calcium salts).[70] Aided by the presence of an acid or basic catalyst, the sol undergoes a transition into a solid 'gel' that is deposited on the substrate. Procedures such as dip-coating have proven to be very useful for the control of the thickness, morphology and homogeneity of the coatings.[71,72] Dip-coating consists in the immersion of the substrate into a solution containing the coating precursors. In the case of HAp or substituted HAp coatings, the precursors are soluble salts of the cations (such as $Ca(NO_3)_2{\cdot}4H_2O$) and alkoxides of the anions, for instance triethyl phosphite $P(OCH_2CH_3)_3$ (TIP) and tetraethyl orthosilicate $Si(OCH_2CH_3)_4$ (TEOS) solutions in the case of silicon substituted hydroxyapatites (Si-HAp).[73] The substrate is soaked at a constant speed and the coating is deposited during the substrate withdrawal. The pulling up speed determines the coating thickness: the faster the withdrawal the thinner the coating, although several immersions can be performed in order to obtain thicker coatings.

Electrochemical deposition (ECD). The ECD technique uses a supersaturated aqueous electrolyte solution of Ca^{2+} and PO_4^{3-} ions in contact with platinum (anode) and metallic implant (cathode) electrodes connected to a current generator.[74] The electrochemical reactions occurring around the cathode lead to a pH increase and subsequent calcium phosphate nucleation and growth occurs. This approach can be carried out under ambient conditions[75] or higher temperatures in an autoclave[76] and thin coatings of less than 1 μm with uniform structures can be obtained. The main drawback is the large volume of electrolyte solution required, the hydrogen gas produced, which can lead to inhomogeneities, and the limitation to coat only conductive materials.

Micro-arc oxidation (MAO). MAO is a wet chemical technique that combines electrochemical oxidation with high voltage treatment in solutions containing calcium, phosphate and precursors of other ions as inorganic salts.[77,78] MAO has been used to prepare substituted HAp coatings on metallic implants and is a suitable approach to obtain porous and rough ceramic surfaces.[79]

Electrospray deposition (ESD). ESD involves the formation of an aerosol from a solution of calcium and phosphorous solution in a volatile organic component. This aerosol is sprayed through a nozzle over the substrate under the presence of a high voltage.[80] The coating characteristics can be tuned by controlling the precursor solution (mainly pH and concentration) and deposition parameters such as the temperature or the nozzle-to-substrate distance. In order to obtain highly

dense and homogeneous surfaces, coatings produced by ESD require a further annealing stage.

Drop on demand microdispensing (DODMD). DODMD is an additive manufacturing process which has been successfully used for fabrication of substituted hydroxyapatite coatings.[81] In this method, a suspension of a previously synthesized bioceramic is dispensed drop by drop by a micro-valve assisted by a pneumatic system. DODMD allows for depositing both organic and inorganic materials and living cells. Moreover, DODMD employs a layer-by-layer approach enabling the fabrication of multi-layered functionally graded coatings.

4. Substituted hydroxyapatite coatings

4.1 Cationic substitutions in hydroxyapatite coatings

Zinc-substituted hydroxyapatite (Zn-HAp) coatings. Zinc (Zn) is the second most abundant essential element in humans. In an average adult there are about 3 g of Zn and it is widely accepted that it is essential for all living beings. Zn enzymes catalyze the metabolic conversion (synthases, polymerases, ligases, and transferases) and the degradation (hydrolases) of proteins, nucleic acids, lipids, porphyrin precursors and other biologically important compounds. In addition to the catalytic function, Zn has a structural function in different proteins and participates in processes related to cell division, nucleic acid replication and gene transcription. Consequently, Zn is essential for growth, development and differentiation for all species, and particularly for humans. Zn deficiency leads to serious pathological effects, particularly a significant weakening of the immunological system.

Zn is only present in bone as a trace element. However, the incorporation of Zn^{2+} cations in HAp structure has attracted the interest of many researchers. Zn^{2+} isoelectrically substitutes for Ca^{2+}, resulting in the decrease of the lattice parameters *a* and c of. This is due to the difference in ionic radius between Zn^{2+} (0.074 nm) and Ca^{2+} (0.099 nm),[82] favoring the Ca(2) over the Ca(1) sites.[83] Zn^{2+} cations can incorporate in the HAp structure in a limited amount (about 20 atom%).[84]

Several authors have stated that Zn stimulates bone formation by activating proliferation and differentiation of osteoblasts.[85,86] Zn-HAp has also shown antibacterial properties against both Gram− and Gram+ bacteria. HAp doped with less than 1% of zinc ions has evidenced effective bioactivity and antibacterial properties.[87,88]

Zn-HAp coatings have been fabricated by different techniques including plasma spraying,[89] EPD,[90] sol–gel spin coating[91] and magnetron sputtering.[92] The fabrication method determines the Zn distribution within the coating. For instance, whereas the EPD method yields homogenous Zn-HAp coatings, magnetron sputtering produces Zn-HAp coatings with higher Zn concentration on the film surface.[93] Zn-HAp coatings prepared by electrochemical deposition have been also proposed for enhancing the corrosion resistance of commercially pure titanium (CP-Ti) substrates.[94] These coatings were deposited together with a calcium silicate by adding SiO_2 nanoparticles to the electrolyte solution. Certainly, the Zn-HAp/calcium silicate coating enhanced the corrosion resistance of CP-Ti, but this effect could not be attributed exclusively to the presence of Zn within the HA structure due to the presence of a secondary phase in the coating.

The optimal amount of Zn to obtain appropriate biological outcomes has been considered by different authors. Webster *et al.* showed that Zn amounts as small as 1.3% cause an increase in osteoblast responses.[95] This high response to small amounts of Zn^{2+} is very convenient, as the amount of Zn^{2+} that can be incorporated is limited, especially when high temperature methods are used. For instance, Zn-HAp coatings prepared by solution precursor plasma spraying easily decompose in the presence of Zn, resulting in the stabilization of α-TCP.[96] Zn-HAp coatings exhibit antibacterial properties in a Zn-dose dependent manner, which has been attributed to Zn^{2+} release to the local environment.

The presence of Zn^{2+} cations partially inhibits the nucleation and growth of a newly formed apatite layer, commonly occurring when HAp is in contact with SBF. This fact could be explained in terms of inhibition of the Ca^{2+} and PO_4^{3-} interactions due to the presence of Zn^{2+} on the surface.[97] Zn-HAp coatings containing 7% Zn^{2+} substitution have been prepared by the hydrothermal method.[98] Despite this decrease of *in vitro* bioactive behavior, the adhesion, proliferation and spreading studies evidenced that human osteoblast cells show better responses on Zn-HAp compared to pure HAp coatings. Since the hydrothermal method involves Zn-HAp precipitation from Zn^{2+} containing solutions, this coating is significantly thinner than pure HAp coatings (18 μm and 100 mm for Zn-HAp and HAp, respectively). However, scratch tests showed similar critical loads for both coatings, indicating that hydrothermally fabricated Zn-HAp prepared by this method presents good adhesion to titanium surfaces.

Despite the efforts for increasing the amount of Zn within HAp structure, there is not *in vivo* evidence of the positive effect of Zn-HAp coatings and the effects of Zn^{2+} cation release in bone marrow are controversial. Sogo *et al.* prepared Zn substituted β-TCP/HA composites and determined with *ex-vivo* studies an optimal Zn content of 0.316% in weight to promote bone formation.[99] Subsequently, the same group carried out *in vivo* studies evidencing that incorporation of Zn in β-TCP resulted in both favorable and unfavorable results.[100] Certainly, this material showed enhanced bone apposition to the implant surface. However, long-term studies also evidenced increased bone resorption in the medullar cavity area. These results indicate that Zn^{2+} release from CaP could be only clinically applied with small amounts of Zn^{2+} or carefully selecting implantation sites without exposure to bone marrow.

Increasing the bonding strength between HAp coatings and the substrate has been another topic of interest in the field of substituted HAp coatings. Certainly, the phase composition and the coating morphology play a fundamental role in the bone strength magnitude, and these features are strongly dependent on the coating method, especially at the substrate–coating interface. However, several studies have

evidenced that the presence of certain ionic substitutions can enhance the bonding strength for certain coating–substrate pairs. Zn substitution modifies the interfacial properties between HAp coatings and Ti substrates, mainly due to the influence of Zn^{2+} ions on the preferential HAp crystal growth along the [0 0 2] and [2 1 1] directions.[101]

Copper-Substituted hydroxyapatite (Cu-HAp) coatings. Copper is an essential trace element for most living organisms. The amount of Cu in a 70 kg adult human is about 0.15 g. Cu is a fundamental component in the catalytic site of several redox enzymes and its presence is basic for the normal development of cellular respiration, defense against free radicals, synthesis of melanin, synthesis of conjunctive tissue and iron metabolism. Cu deficiency is a serious problem especially in newborns, resulting in anemia, bone anomalies and fractures in those cases with acute deficiency. On the other hand, an excess of copper results in toxicity by accumulation in the liver and brain. In those patients with Wilson syndrome, Cu accumulation can lead to the progressive destruction of liver and nervous tissue.

Cu^{2+} cations have been incorporated in HAp bone grafts to provide antimicrobial and bactericidal activity,[102] angiogenic potential[103] and the capability to stimulate the activity of osteoblastic cells.[104] Several authors have proposed a Cu^{2+} for Ca^{2+} substitution mechanism for the incorporation into the HAp structure.[105,106] However, recent studies have shown that an interstitial mechanism takes place during Cu^{2+} incorporation. For thermal treatment below 1100 °C, $Ca_{10}Cu_x(PO_4)_6(OH)_{2-2x}O_{2x}$ is formed with $x < 0.1$ and Cu^{2+} cations occupying the interstitial 2b Wyckoff position. Above 1100 °C, Cu rich HAp can be synthesized with the presence of Cu^{+} and Cu^{2+} also following an interstitial mechanism.[107] The potential advantages of Cu-rich HAp have been questioned due to the cytotoxicity of this cation. A high content of Cu precursors can result in the formation of CuO, which seriously compromises cell viability. There are very few studies of Cu-HAp coatings, as most of the prepared Cu-HAp have been obtained as powders or pieces. Cu-HAp coatings have been prepared by plasma spraying, but this coating did not show any advantage compared to non-substituted HAp.[108] More recently, Cu-HAp coatings have been prepared by solution precursor plasma spraying on sand-blasted stainless-steel substrates.[109] However, even after optimizing the coating parameters, impure Cu-HAp coatings were obtained mixed with CaO and CuO. No biological evaluation was carried out in this study.

In Cu-HAp coatings prepared by PLD on Ti6Al4V substrates,[110] Cu^{2+} for Ca^{2+} substitution mechanisms were described, although interstitial incorporation could not be discarded. Cu^{2+} cations were largely incorporated in the HAp structure and introduced a high degree of crystal-chemical modifications, including a reduction of the carbonate presence in the coatings. However, these differences were not reflected in the biological behavior, since Cu-HAp elicited almost identical behavior to Zn-HAp coatings with respect to pre-osteoblast proliferation and antimicrobial activity.

Silver-substituted hydroxyapatite (Ag-HAp) coatings. Silver is a metal element absent in the human body, unless accidental contamination occurs. Ag does not play any essential role for mammals since it is not found in the active site of any enzyme or exerting structural functions in cells or tissues. However, the incorporation of Ag^{+} in bioceramics has been widely studied in recent years. Ag^{+} is an effective antimicrobial agent and its inclusion in HAp coatings is used to prevent orthopedic and dental implant infections. The antimicrobial activity of Ag^{+} is related to its capability to bind to microbial DNA, preventing bacterial replication, and interacting with sulfhydryl groups of the metabolic enzymes of the bacterial electron transport chain.[111]

Ag-HAp coatings with proved antibacterial effects have been prepared by PLD,[112] co-precipitation,[113] plasma spraying,[113–115] magnetron sputtering[116] or sol–gel methods.[117] *In vivo* studies have demonstrated the antibacterial properties of thermally sprayed Ag-HAp coatings.[118] These authors proposed a subcutaneous model in rats to test the antimicrobial activity of thermally sprayed Ag-HAp coatings on titanium discs. The coating was evaluated against methicillin-resistant *S. aureus* isolated from the blood of a septic patient. Although this study could not evidence the intramedullary antibacterial effects in bone, as would be desirable for an orthopedic implant, it is one of the few *in vivo* studies developing an infection model to assess the antimicrobial activity of substituted HAp coatings. More specifically, it could be demonstrated that Ag-HAp coatings exhibit a high bactericidal effect even with a large number of bacteria inoculated (around 10^6 CFU) in the subcutaneous pocket. Plasma sprayed Ag-HAp coatings prepared on titanium substrates[108] have also evidenced that these coatings are highly hydrophilic, show low resorbability *in vitro* and exhibit excellent antibacterial effects against *S. aureus.*

Ag^{+} ions also exert a significant influence on the bonding strength. In the case of Ag-HAp on Ti substrates, the presence of Ag seems to improve the adhesion strength for electrospray deposited coatings,[119] although contradictory results were obtained by Yan *et al.*, who observed a decrease of bonding strength for Ag-HAp coatings prepared by a similar method.[120]

From a theoretical point of view, Ag^{+} (and also Zn^{2+} ions) positively influences the adhesion between HAp and α-Ti substrates. First-principles electronic structure calculations[121] predict that the work of adhesion at the interface between the (0001) planes of HA and α-Ti reaches larger values for Zn and Ag doped HAp than for stoichiometric HA/Ti interfaces. The analysis of the electronic structure of the calculated model indicates that doping with Ag or Zn increases the charge transfer between HAp and Ti slabs, reinforcing Ti–O bonds and driving the HA/Ti interface system to be more metallic.

Magnesium-substituted hydroxyapatite (Mg-HAp) coatings. Mg is an essential element with high presence in the human body. A 70 kg adult human contains about 30 g of Mg. The biological role of Mg in vertebrates is analogous to Ca in the formation of the skeleton and stabilization of cell membranes. Regarding the enzymatic activity, Mg is an essential factor for phosphate group transference reactions and in many non-oxidative nucleic acid cleavage reactions by nucleases. Mg deficiency has negative consequences on growth as well as on

mental and physical capabilities, which is a consequence of insufficient energy production due to anomalies in phosphate transference reactions.

Mg is found in natural HAp partially replacing calcium. Mg^{2+} ions inhibit HAp crystallization, avoiding the formation of large crystals and promoting the formation of more apatite nuclei. This activity is very important as nanocrystalline bone apatites are required for the appropriate bone formation–resorption turnover carried out by bone cells. Mg^{2+} deficiency affects bone growth, reduces bone density and leads to bone fragility.[122] These facts have encouraged different groups to carry out the artificial preparation of Mg-HAp in different forms, including coatings, where concentrations of Mg around 1% wt have shown optimal properties.[123] Mg-HAp coatings have been prepared by thin film deposition techniques such as magnetron sputtering, PLD, MAPLE, PED,[124] and plasma spraying,[125] as well as by electrochemical deposition methods.[126,127] Mg-HAp coatings prepared by both physical deposition and electrochemical methods show a crystallinity decrease and heterogeneous distribution of the Mg content, forming Mg-rich areas. *In vitro* cell culture tests have evidenced that osteoblasts preferentially concentrate, attach and grow in these areas, evidencing the positive effect of Mg on bone forming cells.

Electrochemical deposition of calcium orthophosphates commonly involves hydrogen gas production, which often leads to heterogeneous coatings with large pores caused by bubbles.[128] Mg^{2+} incorporation as a soluble salt leads to a decrease in both the pore size and pore volume in Mg-HAp coatings, with preferential Mg accumulation in the regions outside. Although these features could provide better corrosion resistance, Mg-HAp coatings prepared by electrochemical deposition showed similar parameters to those observed for non-substituted HAp.[127]

Strontium-substituted hydroxyapatite (Sr-HAp) coatings. Sr is considered as a non-essential element that is present in the human body. About 0.14 g of Sr is contained in an average human adult. Sr is mainly found in the mineral phase of bones, especially in those regions where bone turnover is more active.[129] The incorporation of Sr into CaPs has been motivated by its inhibitory effect on bone resorption and the improvement of bone formation in osteoporotic patients.[130] Several studies have demonstrated that Sr^{2+} for Ca^{2+} substitution in CaPs increases the activity of osteoblasts and inhibits osteoclast proliferation.[131,132]

Contrarily to Mg^{2+} and Zn^{2+}, Sr^{2+} has a larger ionic radius than Ca^{2+} (112 *vs.* 99 pm), which leads to an enlargement of the HAp unit cell and causes an increase of the cell volume. This crystalline distortion also favors the incorporation of carbonate and HPO_4^{2-} anions when Sr-HAp is prepared by low temperature synthesis methods.[133] Sr^{2+} can totally substitute for calcium in the HAp structure.[134]

Sr-HAp coatings have been fabricated on poly(etheretherketone) (PEEK) by PED.[133] Amorphous Sr doped CaP was deposited at room temperature and a subsequent annealing treatment as low as 130 °C was enough to obtain homogenous and Sr-HAp coatings with a Sr/Ca ratio very similar to the target composition. Interestingly, coatings containing high amounts of Sr^{2+} exhibit lower wettability than non-substituted HAp coatings. This fact has been also observed for Sr-HAp coatings prepared by plasma electrolytic oxidation (PEO). Teng *et al.*[135] observed that Sr-HAp coatings on Ti substrates increased the wettability for a $Sr^{2+}:Ca^{2+}$ molar ratio of 0.12, whereas $Sr^{2+}:Ca^{2+}$ of 0.25 led to a significant decrease of wettability. The low wettability of Sr-HAp with high Sr content would provide excellent protection against corrosion for metallic substrates.[136] However osteointegration strongly depends on the protein–surface interactions, which are regulated by hydrophilicity. In a recent study, Wu *et al.*[137] evidenced the positive effect of electrodeposited Sr-HAp coatings on the stability of Mg alloys, by means of protecting them from corrosion and enhancing cell proliferation. However, for those coatings with the highest Sr content, a lower expression of osteogenic markers Col-1, Runx2 and ALP was observed. These facts indicate that the Sr-HAp coating shows an optimal substitution degree that would result in the highest HAp content, hydrophilicity and cell viability. As expected, the coating procedure strongly influences the optimal Sr/Ca ratio for *in vitro* cell response. In this sense Sr-HAp coatings prepared by a pre-calcification method on an anodized titanium plate exhibited the best *in vitro* pre-osteoblast cell response for a Sr/Ca + Sr molar ratio of 0.5.[138]

Roy *et al.* prepared Sr and Mg doped HAp coatings (1 wt% in both cases) on Cp-Ti by inductively coupled radio frequency plasma spraying.[139] The coatings exhibited adhesive bond strength similar to non-doped HAp (around 17 MPa). Despite the minimal effects that Sr and Mg had on the physical properties, the presence of these substituents led to significant improvements in cell–coating interactions. Sr-HAp coatings induced better cell attachment and proliferation of human fetal osteoblasts as well as higher expression of ALP. The incorporation of Mg also resulted in biocompatible coatings, albeit it did not lead to any improvement with respect to pure HAp coatings.

Different studies carried out with highly substituted Sr-HAp point out an undeniable inhibitory effect on osteoclastogenesis, but a limited benefit to the osteoblast function, which depends on the Sr amount. A very interesting alternative has been recently proposed by Boanini *et al.* consisting in the fabrication of gradient coatings of calcium phosphates substituted with different cations.[140] By means of Combinatorial Matrix Assisted Pulsed Laser Evaporation (C-MAPLE), Sr-HA/Zinc β-TCP coatings were prepared with a homogeneous distribution of the two phosphates. This strategy consists in combinatorial coating fabrication aimed to obtain synergies from the osteogenic properties of Zn and the osteoclasts inhibitory effects of Sr. These authors could demonstrate that the cell response can be modulated as a function of compositional intermixing of both substituted calcium phosphates. The osteoblast activity improved as a function of the Zn β-TCP content, whereas a higher presence of Sr-HAp led to the inhibition of osteoclastogenesis without affecting the osteoblast biocompatibility.

Other substituted hydroxyapatite coatings (Co^{2+}, Na^{+}, Mn^{2+}, and Ce^{3+}). In addition to the above described examples, there

are some other substituted HAp coatings that are mentioned in just a few papers, so that a detailed description and discussion are not always possible. However, there are some examples including substitution with essential cations or rare earths which have shown interesting results that deserve to be mentioned.

Cobalt is an essential trace element with a very specific function in humans. Among the elements of the first transition series, Co is the least abundant in the Earth's crust and sea water. In fact, vitamin B_{12} and its derivatives are the only Co containing compounds with biological activity. A 70 kg adult human contains about 0.003 g of Co, being the second least abundant essential element after molybdenum. However, several studies involving Co-doped bioceramics[141–143] point out an angiogenic effect of Co^{2+}, which could favor the neovascularization of newly formed bone by inducing hypoxia conditions. These studies have been commonly done on bulk and powder materials, but studies on Co-HAp coatings are very scarce. Co-HAp coatings have been recently prepared by electrodeposition on Ti22Nb6Zr alloy.[144] Although no biological effects were evaluated, this article evidences the improvement of corrosion resistance with the incorporation of Co^{2+}. This effect is explained in terms of the higher particle aggregation undergone by HAp particles during electrodeposition in the presence of Co^{2+}. Consequently, the electrodeposited Co-HAp coatings are denser and less porous than the undoped HAp coating, thus providing corrosion protection to the metal alloy.

Enhanced corrosion protection has been also obtained with sodium substituted hydroxyapatite (Na-HAp) coatings prepared by electrophoretic deposition.[145] In this case, the coating is made of a composite containing Na-HAp and chitosan that is deposited on 316L stainless steel previously coated with poly(*O*-phenylenediamine). This coating showed *in vitro* bioactive behavior and high corrosion resistance in SBF. However, no conclusion could be obtained regarding the presence of Na^+ in the ceramic component, since the required comparison with non-substituted HA was not carried out.

Manganese is an essential trace element that shows the most potent capacity for binding to integrins.[146] Mn occupies the active site of several metalloenzymes and acts as a Lewis acid (as Mn^{2+}) catalyzing hydrolytic reactions and as a redox catalyzer when Mn exhibits high oxidation states. A 70 kg adult human contains about 0.02 g of Mn. Several studies have endeavored to use Mn to enhance the osteoconductivity of Ti substrates by means of its potent cell adhesion-promoting effect.[147–149] These coatings supported a better cell response with increased viability, proliferation and ALP activity in osteoblastic cells compared with bare Ti or untreated β-TCP film. More recently, Mn-HAp coatings have been produced by electrodeposition on ZnO coated stainless steel.[150] This bilayer coating improved the corrosion resistance, mechanical properties, and metal ion leach-out performance as well as the *in vitro* bioactivity and biocompatibility. However, Mn^{2+} does not always provide beneficial effects when it is associated with other coatings different from calcium phosphates. Park *et al.* studied the effects of Mn incorporation into a titanium oxide coating on titanium.[151] These authors demonstrated that Mn incorporation, instead of providing biological benefits, impaired cell behavior by decreasing cellular attachment, spreading, proliferation, ALP activity, and osteoblast phenotype gene expression compared with the bare Ti surface. This apparently contradictory observation could be explained in terms of the different kinetic release of Mn ions from the CaP coating and Ti oxide layers, which would differ because of the differences in biodegradation rate.

Yttrium substituted HAp (Y-HAp) coatings have been proposed to improve osteoblast (or bone-forming cell) function over undoped HA.[152] Based on the enhancement of the mechanical properties and conductivity in different ceramics, Y was incorporated in HAp powders and deposited on titanium substrates. Greater amounts of calcium deposition by osteoblasts cultured on Y-HAp coatings were observed compared with undoped plasma-sprayed HA coatings.

HAp doped with rare earths has gained interest in recent years. For instance, Ce^{3+} or Ce^{4+} substitutions in HAp have shown antimicrobial activity.[153] Recently, cerium-doped hydroxyapatite (Ce-HAp)/collagen coatings have been prepared by means of a biomimetic process on Ti substrates.[154] In this case, Ce^{4+} was added as $Ce(SO_4)_2{\cdot}4H_2O$ with a final Ce^{4+} concentration as low as 0.5% in a supersaturated solution of $CaCl_2$ and NaH_2PO_4, thus maintaining a cerium concentration within the therapeutic range.[155] The Ce-HAp coatings so obtained showed 92.61 and 73.59% bactericidal ratios for *E. coli* and *S. aureus*, respectively, indicating a higher efficiency against Gram-negative bacterial strains. No cytotoxicity studies were carried out so the information about their potential application in biological systems is very restricted. Table 1 summarizes the most relevant studies carried out on cation-substituted HAp coatings and the enhanced biological function observed *in vitro*.

4.2 Anionic substitutions in hydroxyapatite coatings

Silicon-substituted hydroxyapatite (Si-HAp) coatings. Silicon is an essential trace element for most living organisms. A human adult of 70 kg contains about 1.4 g of silicon. The unique soluble Si form in physiological conditions is silicic acid, $Si(OH)_4$, which can be dissolved up to 2 mM at pH = 7. The essentiality of Si for animal life was established by Carlisle[157] by means of the determination of deficiency symptoms in rats and chickens fed with silicon depleted diets,[156] demonstrating that Si deficiency led to serious effects on bone growth and defective formation of connective tissue. Silicon or silicates substitute for phosphorus, or phosphates, with subsequent charge imbalance.[158] Although the amount of Si that can be incorporated into HAp ranges between 0.1 and 0.5 wt%,[159–162] Si-HAps have evidenced higher bioactive behavior compared to non-substituted apatites.[163–167]

Si-HAp coatings have been prepared by magnetron sputtering.[168] Since the Si substitution is very limited, HAp and Si were sputtered from different targets instead of a single Si-HAp one, yielding a layer of Si-HAp of 0.7 μm in thickness and with a Si content of approximately 0.8 wt%. *In vitro* cell

Table 1 Cation-substituted HAp coatings and the enhanced biological function observed with *in vitro* studies

Coating	Function	Substrate	Fabrication method	Ref.
Zn-HAp	Osteoblast function	Titanium, Ti6Al4V	Plasma spraying	89 and 96
	Corrosion resistance	Ti-Coated silicone	Electrophoretic deposition	90
	Bactericidal	Silicon	Sol–gel spin coating	91
		Titanium; silicon	Magnetron sputtering	92 and 93
		Titanium	Electrochemical deposition	94
		Titanium	Hydrothermal coating	98
		Ti6Al4V	Pulsed laser deposition	110
Cu-HAp	Bactericidal	Titanium	Plasma spraying	108
	Angiogenesis	Stainless-steel	Plasma spraying	109
	Osteoblast function	Ti6Al4V	Pulsed laser deposition	110
Ag-HAp	Bactericidal	Titanium, Ti6Al4V	Plasma spraying	108 and 113–115
	Adhesion strength	Titanium	Electrospraying	119
Mg-Hap	Osteoblast function	Titanium	Plasma spraying	125
		Ti6Al4V	Electrochemical deposition	126
Sr-HAp	Osteoblast function	PEEK	Pulse electron deposition	133
	Osteoclast inhibition	Titanium	Plasma electrolytic oxidation	135
	Corrosion resistance	Magnesium alloy	Hydrothermal, electrodeposition	136 and 137
		Titanium	Radio frequency plasma spraying	139
Co-HAp	Angiogenesis	Ti22Nb6Zr	Electrodeposition	144
	Corrosion resistance			
Na-HAp	Corrosion resistance	316L stainless steel	Electrophoretic deposition	145
Mn-HAp	Mechanical properties	Titanium	Pulsed laser deposition	147–149
	Corrosion resistance	Stainless steel	Electrodeposition	150
Y-HAp	Osteoblast function	Titanium	IonTite™	152
Ce-HAp	Bactericidal	Titanium	Biomimetic	154

culture studies showed that Si-HAp thin coatings exhibited high bioactivity and biofunctionality. Attachment and growth of human osteoblast-like (HOB) cells was observed during the culture period, with more formation of extracellular matrix compared to the uncoated titanium substrate, although no comparison was established with pure HAp coatings. The same research group have carried out the preparation of Si-HAp coatings comparing the influence of the Si content: 0.8 wt%, 2.2 wt%, and 4.9 wt% on Ti to evaluate the long-term *in vitro* biocompatibility effects of these coatings *in vitro*.[169,170] HOB cells showed improved adhesion on the coated surfaces with increasing Si content and developed mature cytoskeletons with well-defined actin stress fibers in the cell membranes. However, the reactivity provided by Si in the Si-HAp with the highest substitution resulted in fast dissolution that hindered the initial cell attachment, concluding that a Si content of 2.2 wt% would be the best substitution degree to improve the bioactive behavior of HAp thin films.

Surmeneva *et al.* have widely studied the microstructural characteristics of Si-HAp prepared by magnetron sputtering.[171–173] The microstructure and coating composition can be controlled by changing the bias voltage from 0 V to −50 and −100 V.[172] For instance, the coating thickness decreases with the magnitude of negative bias, whereas the Ca/P and Ca/P + Si ratios increase. Anyway, all the coatings exhibited good biocompatibility with respect to MG-63 osteoblast cells. Silicon incorporation also exerts an influence on the mechanical properties of magnetron-sputtered Si-HAp.[173] The nanohardness and elastic modulus decrease as a function of Si content due to microstructural modifications derived from silicon incorporation. In addition, the adhesion behavior is also influenced by Si substitution. Whereas coating failure occurred due to low cohesion in non-substituted HAp coatings, Si-HAp with 1.2 at% deformed plastically without crack formation and a mixed elastic–plastic behavior was observed for the Si-HAp coating with 4.6 atom% of Si.

Si-HAp coatings have been also successfully prepared by PLD,[174] sol–gel chemistry followed by dip coating,[175] spin coating,[176] electrochemical deposition using electrolytes containing Na_2SiO_3 as a silicon source[177,178] and by means of hydrothermal treatment with metasilicic acid, H_2SiO_3, of a previously pure HA coating prepared by CVD methods,[179] being one of the most widely investigated substituted HAp coatings.

Fluor-substituted hydroxyapatite (F-HAp) coatings. Fluor is an essential trace element with a presence of 2.6 g in a 70 kg adult human. Although essential, there is not much knowledge about the biological functions of F^- and its biochemical activity is not well defined. Most of the F^- content is in the skeleton and teeth where it isoelectronically substitutes for OH^- in HAp. F^- anions are also found in extracellular fluids at very low concentration (micromolar levels) and even lower in the intracellular compartment.

The incorporation of F^- into the HAp structure reduces the dissolution rate and increases its hardness.[180] Certainly, partial substitution of F^- for OH^- ions, which results in $Ca_{10}(PO_4)_6(OH)_{2-x}F_x$, is a well-known strategy for enhancing the structural stability and resistance to dissolution with respect to non-substituted HAp. The lower solubility of F-HAp allows the preparation of very thin coatings (below 1 micrometer) that otherwise would be very unstable under *in vivo* conditions, and would result in implant failure by early degradation. F-HAp thin films have been recently prepared by PLD[181] producing coatings of 1 μm in thickness, with higher resistance to dissolution and better cell attachment of human mesenchymal stem cells (HMSCs) compared to HAp coatings.

In vitro studies indicate that the F^- for OH^- substitution enhances cell proliferation and reduces bacterial activity.[182] Antibacterial properties of F-HAp coatings on stainless steel substrates have been proposed for the prevention and treatment of peri-implantitis in dental implants.[183] The antibacterial property against different pathogens was dependent on the coating crystallinity, which could be controlled with a post-hydrothermal treatment. In this sense both low crystalline and highly ordered crystalline F-HAp coatings reduced the bacterial viability, although the ordered one also reduced the bacterial adhesion. This study concludes that F^- content, rather than release, is the most influential variable, although the surface characteristics also have a significant impact on the bacterial adhesion, as it has been recently demonstrated for nanopatterned Ti coatings on orthopedic devices.[184]

F-HAps coatings have been prepared by different methods including sol–gel[185] or slip coating methods.[186] There is some controversy regarding the positive or negative biological activity of fluoride with respect to osteoblast cells. Some studies have shown that osteoblast cells exhibit lower proliferation rates on fluoridated coatings compared with cells cultured on non-substituted HAp coatings, pointing out the potential toxicity of F^- anions.[187] However, the beneficial or detrimental effects of F^- in HAp coatings seem to be dosage-dependent. For instance, electrodeposition can be used for preparing these coatings by adding NaF into the electrolyte.[188] This method is appropriate to obtain coatings with a thickness of a few microns (around 5 micrometers) and F/Ca ratios as high as 0.125, *i.e* a nominal composition of $Ca_{10}(PO_4)_6(OH)_{0.75}F_{1.25}$. However, the best bonding strength on titanium substrates, lower dissolution rate and most appropriate biological activity were obtained for those coatings with moderate F contents, with substitution for OH^- in the range $Ca_{10}(PO_4)_6(OH)_{0.75-1}F_{1.25-1}$.

Carbonate-substituted hydroxyapatite (C-HAp) coatings. C-HAp has been synthesized as powders, grains, pieces and coatings for bone grafting applications. The interest in C-HAp arises from the chemical composition of biological apatites which are non-stoichiometric, Ca-deficient and carbonated. This ionic substitution contributes to the higher solubility of biological apatites, compared with the stoichiometric and highly crystalline synthetic ones. The presence of CO_3^{2-} in the HAp structure helps to keep constant bone regeneration through dissolution–crystallization cycles.

C-HAp coatings have been prepared by RF-magnetron sputtering,[189,190] electrophoretic deposition[191–193] biomimetic deposition,[194,195] electrochemical deposition,[196] pulsed laser deposition,[197] hydrothermal crystallization[198] and even by a hybrid process of plasma spraying and hydrothermal synthesis.[199] Since C-HAp coatings are more soluble than HAp, they are likely to provide a better osteogenic response, although the coating stability can be seriously compromised. Different strategies have been used to overcome this potential drawback. For instance, C-HAp coatings deposited on Ti substrates by RF-magnetron sputtering[200,201] have been obtained as dense and well adhered films with controlled elemental composition. B-type C-HAp coatings have been fabricated by this method with a rough and homogeneous microstructure that facilitates the development of hMSC, differentiated cells and bone explanted osteoblasts. The coating adherence was improved by introducing a buffer layer of $CHA_{1-x}Ti_x$ (x = 0–1) with a chemical gradient between the Ti substrate and the $Ca_{10-2x/3}(PO_4)_{6-x}(CO_3)_x(OH)_{2-x/3}$ coating. Another strategy for enhancing the stability of C-HAp coatings is the formation of composites with biocompatible polymers. Tang *et al.* prepared chitosan/C-HAp on Ti6Al4V substrates by electrophoretic deposition of $CaCO_3$ and subsequent treatment in a phosphate buffer solution until transformation into C-HAp.[192] However, the coating exhibited numerous cracks and macropores between C-HAp particles. This drawback was resolved by soaking the sample in a chitosan solution, which filled the cracks (linking the C-HAp particles) while keeping the pores, which could positively contribute to osteointegration after implantation.

The biomimetic method is one of the easiest alternatives for the preparation of C-HAp coatings. By soaking preconditioned substrates in highly saturated SBF, C-HAp spontaneously nucleates and grows on the surface, with disregard of the substrate morphology, as biomimetic deposition is not a line-of-sight technique. Moreover, the coating roughness can be easily controlled by the solution concentration and time exposure of the substrate to the biomimetic solution. Costa *et al.*[194] prepared C-HAp coatings biomimetically deposited on polycaprolactone discs from SBFx7 and SBFx10 during periods of 24 and 48 hours. Whereas SBFx7 led to almost smooth C-HAp coatings, SBFx10 led to micro-rough topographies. All the coatings were biocompatible with respect to osteoblasts but, interestingly, different topographies elicited different responses with respect to osteoclast cells: smooth coatings allowed high osteoclast resorptive activity whereas micro-rough coatings partially hindered the formation of actine rings. In this way, the biomimetic technique would produce C-HAp coatings that improve osteoconductivity while minimizing osteoclastic resorption.

Other anion-substituted hydroxyapatite coatings. Since the HAp structure allows for many different substitutions, some groups have explored other possibilities with less conventional anions than those reviewed in previous sections. Boron is considered an essential element for plants, but not for animals. Although some studies point out several benefits for osteogenic differentiation[202] further research is required before B is

accepted as an essential nutrient for humans. B is incorporated as borate ions by substitution of phosphate. B-HAp coatings have been fabricated on chitosan scaffolds[203] and MC3T3-E1 cell cultures evidenced enhanced proliferation associated with B release, indicating the potential of these coatings for *in vitro* bone tissue engineering applications.

Selenium is a trace essential element for humans. Around 15 mg of Se is found in an average human. Selenium-doped hydroxyapatite (Se-HAp) coatings have been prepared by several methods such as PLD[204] or biomimetic techniques including selenite ions in SBF.[200] Rodríguez-Valencia *et al.*[205] have hypothesized on the osteogenic, antitumoral and antibiotic activity of Se incorporated as SeO_3^{2-} anions into the HAp structure. Coatings with 2.7 at% of Se resulted in significant osteogenic activity of MC3T3-E1 pre-osteoblasts, a significant antiproliferative effect on cancerous osteoblasts (MG63), and antibiofilm properties against *S. epidermidis* and *S. aureus* bacterial strains (Table 2).

4.3 Co-substituted hydroxyapatite coatings

Co-substitution is a very interesting strategy to optimize the biological performance of Hap coatings. Taking advantage of the different ions available for substitution and the variety of biological effects, the presence of two or more substituents can lead to synergistic, complementary or compensatory effects that provide added value to Hap coatings. Combination of cations with antimicrobial and osteogenic properties is one of the most attractive options. The antimicrobial efficiency of Ag^+ is explained in terms of inhibition of the bacterial replication process against both Gram-negative and Gram-positive bacteria.[206] However, Ag^+ cations also exhibit toxicity to human cells above a certain concentration, even if they are incorporated in Hap coatings.[207] This toxicity seems to be related to the affinity of Ag^+ to the ALP high affinity metal site and the incorporation of a second cation able to alleviate the potential negative effect of Ag^+ has been considered. Geng *et al.* evaluated the antibacterial effect and biocompatibility of Ag/Sr-Hap hydrothermally coated on Ti substrates.[208,209] The addition of Sr^{2+} significantly decreases the Ag^+ toxicity, in such a way that co-substituted coatings keep the antibacterial effect with low silver substitution, while maintaining the proliferation capability of pre-osteoblast cells. Sr^{2+} ions seem to counteract the silver toxicity by reducing the quantity of Ag^+ that gets into the cells by competing for binding sites of specific cellular function and promoting cell differentiation.

The antibacterial effects of Cu^{2+} can be also limited due to the potential toxicity of this cation. Due to the small amount of Cu^{2+} that can be incorporated and the risk of segregation of cytotoxic phases,[210] more attention has been paid to Cu containing co-substituted Hap coatings. For instance, co-substitution with Zn^{2+} provides much better results than single Cu^{2+} incorporation into Hap coatings. Cu/Zn-Hap coatings prepared by electrodeposition on pure titanium substrates exhibit antimicrobial activity associated with Cu^{2+} cations, whereas Zn^{2+} makes up for the cytotoxicity of Cu^{2+}.[211] Zn and Cu incorporation also led to an increase of corrosion resistance compared to non-substituted Hap coatings. This could be associated with the reduced grain size of Cu/Zn-Hap coatings, which plays an important role in elevating the electron activity at the grain boundaries and consequently improving the corrosion protection. Certainly, these coatings exhibited antimicrobial activity against *E. coli*, but the study could not clearly assign this effect to Cu^{2+} or to the concomitant effect of Zn^{2+} release.

Sr has been also proposed as a secondary substituent to alleviate the potential toxicity of Cu-Hap coatings.[212] Cu/Sr-Hap

Table 2 Anion-substituted HAp coatings and the enhanced biological functions observed with *in vitro* studies

Coating	Function	Substrate	Fabrication method	Ref.
Si-HAp	Osteoblast function	Titanium	Magnetron sputtering	168–173
	Osteoinduction	Titanium	Pulsed laser deposition	174
	Mechanical properties	Ti6Al4V	Dip coating	175
	Bonding strength	Zirconia	Spin coating	176
		Mg5Zn0.3Ca, SiC	Electrochemical deposition	177 and 178
		C/C composite	CLVD/hydrothermal	179
F-HAp	Structural stability	Titanium	Pulsed laser deposition	181
	Osteoblast attachment	Stainless steel	Hydrothermal method	183
		Stainless steel	Sol–gel	185
		Zirconia	Slip coating	186
		Titanium	Electrochemical deposition	188
C-HAp	Osteoblast function	Titanium	RF-Magnetron sputtering	189, 190, 200 and 201
		Ti6Al4V	Electrophoretic deposition	191–193
		Polycaprolactone, Ti	Biomimetic deposition	194 and 195
		TiO_2 nanotubes	Electrochemical deposition	196
		Titanium	Hydrothermal method	198
		Ti6Al4V	Plasma spraying/hydrothermal	199
B-HAp	Osteoblast function	Chitosan	Microwave assisted precipitation	203
Se-Hap	Osteoblast function	Titanium	Pulsed laser deposition	204
	Antitumoral	Ti6Al4V	Biomimetic	205
	Bactericidal			

coatings have been prepared by electrodepositon on CP-Ti and the antibacterial activity and cytocompatibility have been evaluated. The lattice parameters of Cu/Sr-Hap were enlarged with respect to pure Hap, evidencing that Sr^{2+}, with a larger ionic radius than Ca^{2+}, plays a major role in the crystal changes occurring in Hap. The coatings exhibited a bactericidal effect against *E. coli* (near to 91% inactivation), although the antimicrobial ratio did not reach the 99% inactivation required to be considered as antibacterial. Besides, the Cu/Sr-Hap coating Q14 increases the number of viable preosteoblast seeds compared to CP-Ti and stimulated the differentiation towards the osteoblast phenotype.

Mg/Sr-Hap coatings have been prepared by plasma spraying.[213] This coating exhibited high bonding strength after subsequent thermal treatment at 500 °C and induced MC3T3-E1 preosteblast proliferation. Whereas Mg/Sr-Hap coatings prepared by plasma spraying do not show microstructural differences with respect to undoped HA, other attempts to prepare Mg/Sr-HAP by deposition methods yielded different outcomes. Mg^{2+} cations influence the microstructure of hydroxyapatite coatings, when the fabrication method involves nucleation and crystallization from liquid solutions. For instance, Mg/Sr-Hap coatings prepared by electrochemical deposition indicate that the presence of Mg as a co-dopant led to less crystalline and irregular coatings, in agreement with the inhibitory effect of Hap crystallization attributed to Mg^{2+}.[214] This fact would question the convenience of incorporating Mg^{2+} in Hap when using wet route deposition-based techniques.

Substitution of Hap with rare earth elements has been a field of interest in recent decades, mainly due to the potential capability of Hap to retain radionuclides and be used for storage of nuclear waste.[215] Recently, the use of substituted Hap with rare earths for biomedical purposes has been investigated and has opened new alternatives in the field of bone implants. For instance, samarium (Sm) substitution in Hap has demonstrated antibacterial activity, improvement of the osteo- Q15 blast performance and also activity as a radiotherapeutic agent in preventing caries.[216,217] On the other hand, certain rare earth cations such as Gd^{3+} improve the mechanical properties and corrosion resistance in metal alloys.[218] Considering these antecedents, Sm/Gd-Hap coatings have been prepared by electrodeposition on stainless steel substrates.[219] Different Sm/Gd ratios were incorporated in the Hap structure. Sm/Gd-Hap coatings containing a 1 : 1 Sm/Gd ratio exhibited an interconnected granular structure with uniform coverage, whereas 2 : 1 and 1 : 2 Sm/Gd ratios resulted in heterogeneous coatings made of agglomerated particles. The main consequence is that Sm/Gd-Hap coatings with similar contents of rare earths showed much better protection against corrosion. Besides, Sm provided antibacterial properties against *E. coli* and *S. aureus* together with high cytocompatibility with respect to MC3T3-E1 preosteoblastic cells.

Co-substituted Hap coatings have been also prepared by incorporating cations and anions simultaneously. These coatings are mainly prepared by introducing metal cations like Co^{2+},[220] Zn^{2+}[221] or Ag^{+}[222] in F-Hap, because F^{-} ions exert a dose-dependent effect on osteoblast proliferation and osteogenic differentiation[223] that compensates the toxic effects of transition metal cations. Birgani *et al.*[220] incorporated F^{-} and Co^{2+} ions in CaPs prepared by a biomimetic method on the surface of culture well plates. Although the crystalline phase formed seemed to correspond to OCP, the incorporation of F^{-} rendered this CaP more apatitic. The incorporation of Co^{2+} into the CaP coating upregulates the expression of VEGF and CD31 angiogenic markers of hMSCs compared to CaP without Co^{2+}. However, Co^{2+} suppresses the ALP activity and decreases the expression of bone sialoprotein (BSP), which results in reduced mineralization of hMSC. The incorporation of fluoride compensated the adverse effects of Co^{2+}, favoring osteogenesis while keeping the angiogenic effect.

Zn/F-Hap coatings have been successfully fabricated by ED on cp-Ti, obtaining totally crack-free and dense layers, which led to a decrease in the corrosion current densities of Ti-cp in physiological solutions.[221] Since ED is a low temperature process it allowed for obtaining nanostructured coatings. The nanopatterned surface together with the continuous Zn^{2+} release results in good osteoblast proliferation, and promoted ALP expression in MC3T3-E1 preosteoblast cells.

The incorporation of bactericidal species into F-Hap also provides interesting cationic–anionic combinations. Ag^{+} has been incorporated into fluorapatite to fabricate hybrid coatings with TiO_2 nanotubes on Ti substrates.[222] The Ag/F-Hap coatings form rod shaped nanoparticles that get into the voids of TiO_2 nanotubes, thus increasing the adhesion strength of the coatings. Fluoride incorporation also increases the dissolution resistance whereas the presence of Ag^{+} killed all viable *S. aureus* in the antibacterial tests. In addition, the hybrid coating increased the corrosion resistance by two orders of magnitude and showed high cell viability when MC3T3-E1 preosteoblasts were cultured on it.

Co-substitution can only occur to a limited extent as it causes crystal-chemical disorders that result in apatite decomposition and segregation of other phases. Research interest has been focused on preparing multimaterial coatings made of singly substituted Haps. Using production techniques that provide accurate control of the deposition pattern such as drop on demand microdispensing, the distribution of singly substituted HAPs can be designed in a homogeneous or controlled manner.[224] Multimaterial coatings have been prepared by Lim *et al.*[225] by means of depositing Si-Hap and Ag-Hap on glass substrates previously coated with a Hap layer. Interestingly the homogeneous distribution of Ag-Hap at every alternate position in the Si-Hap/Ag-Hap coating prevented *S. aureus* adhesion to the same extent as the pure Ag-Hap coating, even though 50% of Ag-Hap was replaced by Si-Hap. This result evidences the importance of the homogeneity and pattern deposition of Ag^{+} in the coating, although the total elimination of bacteria was not achieved in any case. On the other hand, the osteogenic response of the Si-Hap/Ag-Hap coating was significantly lower than the pure Si-Hap coating, indicating that the stimulatory effect of silicon is mainly dose-dependent (Table 3).

5. *In vivo* studies of substituted hydroxyapatite coatings

In vivo animal models have been used to determine fundamental features of substituted HAp coatings such as bone regeneration at the peri-implant site, bone adhesion, angiogenesis, *etc.*, which allows for determining the potential advantages provided by the ions incorporated in HAp. Table 4 shows the different ionic substitutions incorporated into HAp coatings, together with the expected biological outcomes and the animal models that they have been tested with. Zhao *et al.* studied the effects of Mg in Mg-HAp coatings on the osseointegration of dental implants.[226] These authors observed enhanced *in vitro* cell proliferation, higher ALP activity and more osteocalcin production compared to pure HAp. However, only temporary effects could be identified *in vivo*. A slightly higher bone implant contact (BIC) for the Mg-HAp coatings was observed 2 weeks after implantation, whereas no significant differences were observed with respect to BIC or the amount of bone between threads after 4 and 8 weeks.

Ke *et al.* incorporated MgO and SiO_2 within plasma sprayed hydroxyapatite coatings on titanium implants.[227] The MgO/SiO_2-HAp coatings exhibited increased osteogenesis, osteointegration and bone mineralization compared with non-substituted HAp after implantation in rats' femurs. More interestingly, the pushout tests evidenced that the MgO/SiO_2-HAp coatings exhibited a shear modulus much higher than uncoated and HAp coated implants (96% and 56.4%, respectively), demonstrating the better quality of the bone–implant interface for these coatings in a quantitative way. More recently, the same group coated Ti and Ti6Al4V implants with a ternary dopant system within HAp.[228] This system was aimed at inducing osteogenesis, angiogenesis and infection control by means of ZnO, SiO_2 and Ag_2O incorporation, respectively. For this study, these authors used the same rat femur model, evidencing that the Zn/Si/Ag-HAp coating led to higher bone formation at the early stage as well as more mineralization compared with non-substituted HAp coatings. The Zn/Si/Ag-HAp coatings resulted in better osteointegration with higher shear modulus during the push out tests, although no evidence of angiogenesis or antibacterial properties was demonstrated *in vivo*.

Early degradation of substituted HAp coatings prepared by low temperature methods is one of the most serious concerns for transferring these devices to clinical applications. This is the case of C-HAp coatings prepared by biomimetic methods. Measuring the coating degradation within bone involves certain difficulties that can be partially overcome using ectopic models. For instance, Barrère *et al.*[229] implanted Ti6Al4V pieces biomimetically coated with C-HAp, using a subcutaneous rat model to determine the coating degradation and the biological behavior derived from the solubility of the coatings. Interestingly a dissolution–precipitation mechanism took place under *in vitro* conditions with immersion time in α-MEM. However, *in vivo* studies showed that no dissolution occurred of the C-HAp coating. On the contrary, coating calcification could be observed on these implants.

F-HAp coatings can be considered as an alternative to HAp for avoiding early coating degradation. For instance, plasma sprayed F-HAp coatings on Ti6Al4V do not degrade after 12 and 25 weeks of being implanted into the femora and humeri of adult goats, whereas HAp coatings showed extensive degradation for the same period.[230] Other studies confirmed these observations by evidencing an almost equal degree of bone apposition for plasma sprayed HAp and F-HAp coatings, but less F-HAp coating dissolution within the first 3 months of implantation.[231,232] Studies on a goat maxilla model obtained similar results, as no significant differences were found in the histomorphometrical analysis between F-HAp and HAp coated implants, although higher coating thickness reduction was observed for pure HAp coatings.[233]

Osteoporotic animal models are very convienient for the evaluation of substituted HAp coatings aimed at treating patients having bones of low quality. Ovariectomized animal models are often used for these studies since they mimic the osteoporosis conditions in humans. Sr-HAp coatings have been proposed for application in osteoporotic conditions and implanted in the femur of ovariectomized rats.[234] Different amounts of Sr were incorporated in HAp, but the Sr-HAp coatings with the highest Sr content led to the highest bone formation. Biomechanical tests demonstrated the beneficial effects of coatings with 20 mol% of Sr substitution on implant fixation with respect to 5%, 10% and 0% Sr-HAp coatings, evidencing the positive *in vivo* effect of this element under osteoporotic conditions.

Table 3 Co-substituted Hap coatings

Coating	Function	Substrate	Fabrication method	Ref.
Ag/Sr-HAp	Antibacterial/osteoblast function	Titanium	Hydrothermal method	208 and 209
Cu/Zn-HAp	Antibacterial/biocompatibility/corrosion resistance	Titanium	Electrodeposition	211
Cu/Sr-HAp	Antibacterial/osteoblast function	Titanium	Electrodeposition	212
Mg/Sr- HAp	Mechanical properties/osteoblast function	Ti6Al4V	Plasma spraying	213
		Ti40Nb	Electrochemical deposition	214
Sm/Gd-HAp	Antibacterial/corrosion resistance	Stainless steal	Electrodeposition	219
Co/F-HAp	Angiogenesis/osteogenesis	Culture plates	Biomimetic method	220
Zn/F-HAp	Corrosion resistance/osteogenesis	Titanium	Electrodeposition	221
Ag/F-HAp	Coating stability/adhesion strength/antibacterial	Titanium	Electrodeposition	222
Si-HAp/Ag-HAp	Osteogenesis/antibacterial	Glass	Drop on demand microdispensing	225

Table 4 *In vivo* studies carried out with substituted HAp coatings

Coating	Substrate	Fabrication method	*In vivo* animal model	Ref.
Ag-HAp	Titanium	Thermal spraying	Rat, subcutaneous	117
Mg-HAp	Ti, Ti6Al4V	Electrochemical deposition	Rabbit, bone	226
	Ti6Al4V	Pulsed laser deposition	Rabbit, bone	245
	Titanium	Plasma spraying	Rat, bone	227
Sr-HAp	Titanium	Electrochemical deposition	Rat, bone	234
	Titanium (oxidized)	Biomimetic method		235
Si-HAp	Titanium (oxidized)	Biomimetic method	Rat, bone	235
	Porous titanium	Biomimetic method	Rabbit, bone	242
	Porous titanium	Precipitation method	Rabbit, bone	243
	Porous Ti6l4V	Dip coating	Sheep, bone	244
F-HAp	Ti6Al4V	Plasma spraying	Goat, bone	230 and 231
	Ti6Al4V	Plasma spraying	Rabbit, bone	232
	Titanium	Plasma spraying	Goat, bone	233
C-HAp	Ti6Al4V	Biomimetic method	Rat, subcutaneous	229
Sr/Si·HAp	Porous titanium	Biomimetic method	Rat, bone	236
Zn/Si/Ag-HAp	Ti, Ti6Al4V	Plasma spraying	Rat, bone	228

Sr-HAp and Si-HAp coatings have been also deposited by using biomimetic methods on screw-shaped implants and placed in the tibia metaphysis of rats.[235] The association of small amounts of Si and Sr with these coatings improves the bioactive behavior, especially at the very early stages after implantation. Si seems to stimulate bone apposition, whereas Sr fosters bone formation in the area within the threads. These positive effects of Sr and Si cosubstitution in CaP coatings have been recently confirmed by Bose *et al.*[236] This group prepared Si–Sr CaP coatings on Ti cylinders previously coated with TiO_2 nanotubes by electrochemical anodization, a technique previously developed by the same group.[237] In agreement with previous work, these researchers could demonstrate that Sr and Si substitution in CaP coatings led to an increase of osteoid formation around the implant in the early stages (4 weeks) after implantation in the distal femur of rats.

6. *In vivo* studies of scaffolds coated with substituted-HAp

Traditionally, HAp based coatings have been aimed at improving the bone adhesion of solid implantable devices (prosthesis stems, dental implants, fixation screws, *etc.*) designed for bone substitution or fixation applications. An alternative to the current substitutive strategies in the treatment of bone defects is the concept of functionalized metallic macroporous scaffolds.[238] These scaffolds must facilitate osteogenesis and new blood vessel formation within their macroporous structure, while exhibiting optimal mechanical behavior. Both aspects are mandatory for bone regeneration of critical defects, particularly in osteoporotic bones, where the implant integration with the hosting bone is seriously affected due to the low bone formation rate in the peri-implant region.[239] The surface functionalization of these metal structures with a highly bioactive bioceramic has emerged as a very interesting alternative.[240,241]

The preparation of Si-HAp coatings by low temperature methods and their *in vivo* evaluation have been carried out by several research groups.[242,243] For this aim Ti or Ti alloy macroporous scaffolds have been often chosen as substrates. Zhang *et al.*[242] proposed Ti scaffolds prepared by fiber sintering coated with Si-HAp by a biomimetic method. The scaffolds exhibited a porosity of 67% with a pore size of 150–600 μm. This study compared uncoated Ti scaffolds with pure HAp coated Ti and Si-HAp coated scaffolds when implanted in New Zealand rabbits. Both the HAp and Si-HAp coatings led to a significant increase of the bone ingrowth rate compared to uncoated Ti, evidencing that both coatings enhanced the osteoconductive properties. Moreover, the Si-HAp coated scaffolds exhibited significantly higher bone ingrowth than the HAp coated scaffolds. After four weeks, 90% of the pore area was covered by new bone tissue, evidencing the positive role of Si substitution in this kind of coatings.

Recently, Si-HAp coatings prepared by a sol–gel route on Ti6Al4V macroporous scaffolds have demonstrated a synergistic effect when they are associated with vascular endothelial growth factor (VEGF) in osteoporotic sheep[244] (Fig. 2). Ti6Al4V macroporous structures were fabricated by electron beam melting to obtain customized highly macroporous structures, which were coated with Si-HAp by the dip coating method. Subsequently, VEGF was immobilized on the coatings by soaking the specimens in a solution of VEGF in a phosphate buffered solution. *In vitro* studies demonstrated that the SiHAp coatings stimulated the proliferation of MC3T3-E1 pre-osteoblastic cells, whereas the adsorption of VEGF stimulates the proliferation of EC_2 mature endothelial cells. When these scaffolds were implanted in osteoporotic sheep, only the simultaneous

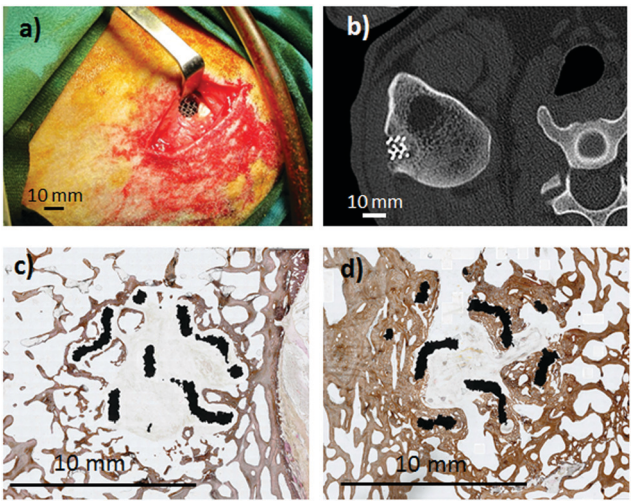


Fig. 2 Implantation of a macroporous Ti6Al4V scaffold coated with Si-HAp/VEGF in an osteoporotic sheep model (a). Computed tomography scan image of the implant within the bone defect (b). Histological overview of bones implanted with uncoated Ti6Al4V microporous implants (c) and SiHAp/VEGF coated implants (d).

presence of the SiHAp coating and VEGF led to a significant increase of new tissue formation in osteoporotic bone, demonstrating that, under osteoporotic conditions, both osteogenesis and vascularization must be strengthened to reach bone regeneration within these metallic scaffolds.

Mg-HAp coatings on porous implants also enhance both early and long-term osteogenesis. Mróz *et al.*[245] have prepared Mg-HAp and Mg-substituted octacalcium phosphate coatings by PLD on porous titanium-based implants. When these porous scaffolds were implanted in a rabbit femoral model for 6 months, the histopathological analysis revealed that all the implants, the coated ones and the uncoated reference, were biocompatible, exhibiting bone ingrowth within the pores. However, microCT analysis evidenced a significantly higher bone volume for implants coated with Mg-HAp compared to the uncoated and Mg-OCP coatings. Unfortunately, this work did not include a group of pure HAp coated implants to establish the positive role of Mg at long-term implantation stages.

7. Summary and future perspectives

Substituted HAp coatings provide a very interesting alternative to improve the performance of dental and orthopedic implants. The different ionic substitutions allowed by the HAp structure, together with the variety of fabrication methods, provide new characteristics to the commercially available plasma sprayed CaP coatings. For instance, Si-HAp coatings deposited on porous metallic implants have shown excellent bone regeneration capabilities through the synergy of osteoconductive HAp with the osteoinductive behavior of the soluble silica species released from the coatings. Other ionic substituents like Sr^{2+}, Mg^{2+} or Zn^{2+} stimulate bone healing and are expected to provide relevant advances for the treatment of osteoporotic fractures.

Infection is also a serious cause of orthopedic implant failure. Thousands of metallic orthopedic prostheses are revised every year because of infections caused by *S. aureus* and *S. epidermidis*. In this sense, the incorporation of ions with antimicrobial properties to lower the risk of infection has become a priority research field for the development of new coatings. Substitutions with Ag^{+} and Cu^{2+} have evidenced positive *in vitro* results with bacterial strains. However, *in vivo* studies are very scarce, perhaps because of the difficulty in designing appropriate *in vivo* infection models in bone. The development of these models will be mandatory for the further development of these devices. Other biological activities such as angiogenesis or antitumoral properties can be obtained by the incorporation of Co^{2+} or SeO_3^{2-}, although much more *in vitro* and *in vivo* evidence is required before being translated into clinical applications.

The association of drugs and osteogenic macromolecules with substituted HAp coatings has opened new possibilities for the treatment of bone pathologies. Some attempts have been carried out involving the antiosteoporotic activity of Sr^{2+} in combination with zoledronate acid[246] or providing complementary therapeutic effects with the antibiotic activity of vancomycin.[247] Substituted HAp coatings combined with biological entities provide great potential to improve new bone formation at the peri-implant site or bone regeneration in macroporous scaffolds. The combination of Mg substituted β-TCP and C-HAp with recombinant human bone morphogenetic protein-2 (rhBMP-2) has shown excellent behavior with respect to rhBMP-2 delivery, which resulted in superior bone formation within scaffolds.[248] The increasing knowledge about the synergies of substituted HAp with proteins,[249] growth factors,[244] or microRNAs[250] will eventually result in customized coatings for each specific clinical challenge.

The incorporation of rapid prototyping methods as a new strategy for coating fabrication is called to play a fundamental role in this field. Techniques such as drop on demand microdispensing allow coating customization, distributing at the micrometer scale different substituted HAp. These techniques also provide new tools for incorporation and controlled release of drugs from the coating to the local environment. However, all these possibilities will be carried out successfully only if appropriate *in vivo* animal models (osteoporotic, infection, tumoral, *etc.*) are previously developed.

Transferring new biomedical devices from the lab to clinical applications is a difficult challenge. Coatings are not an exception. As an estimative approximation, one technology project out of ten results in a biomedical device that goes to the clinical trial phase.[251] Innovation in the biomaterials industry is commonly technology driven but always to satisfy unmet clinical needs. In this sense, the large amount of scientific literature evidences that research on new coatings is widely supported by technology development. There are many fabrication methods that allow for manufacturing substituted hydroxyapatite coatings, which have evidenced satisfactory *in vitro* behavior.

Certainly, improving coating adhesion and reducing the costs related to industrial upscaling can be considered as the critical issues for spreading the application of novel coatings.[252,253] But accelerating early bone–implant integration and the treatment and/or prevention of prothesis infection remain as unmet clinical needs that must be also satisfied. The variety of production techniques and compositions should play a relevant role in transferring new substituted HAp coatings to clinical practice. However, the current scenario largely differs from this. Only a few CaP bioceramics fabricated by plasma spraying are commercially available. The causes of this lack of translation can be found in different aspects. Firstly, *in vivo* studies concerning substituted HAp coatings are very scarce, which is partially caused by the lack of appropriate animal models. One of the most relevant clinical needs is to avoid prosthesis infection and different cationic substitutions have been introduced in HAp coatings. However, there are not any *in vivo* studies including a bone infection model to test these devices, and only some subcutaneous models have been proposed.

The second hurdle is the regulatory processes toward market approval. In this sense new substituted HAp coatings fabricated with alternative technologies to plasma spraying are considered as devices that do not have any equivalent on the market. In these cases, pre-market approval (PMAs) is compulsory, which increases the financial and time costs compared to products having equivalent ones in the market, which just require 510k notification. This fact would make novel coatings less attractive to investors, especially because the biggest hurdle can come at the very end of the process with the clinical trials, when a large part of the investment has been already made.

In conclusion, commercial translation of substituted HAp coatings is not exclusively driven by the available technology. Evidence-based definition of the unmet clinical need and preclinical studies with appropriate animal models are mandatory to reduce the failure risk during clinical trials. In addition, regulatory filing and an effective marketing strategy would be required to allow novel HAp products to enter the market.

Conflicts of interest

Q20

Acknowledgements

D. A. would like to thank the Ministerio de Economía y Competitividad for financial support (project MAT2016-75611-R AEI/FEDER, UE). M. V.-R. acknowledges funding from the European Research Council (Advanced Grant VERDI); ERC-2015-AdG Proposal 694160.

References

1 R. W. Crawford and D. W. Murray, *Ann. Rheum. Dis.*, 1997, **56**, 455.
2 A. D. Pye, D. E. A. Lockhart, M. P. Dawson, C. A. Murray and A. J. Smith, *J. Hosp. Infect.*, 2009, **72**, 104.
3 A. P. Tomsia, M. E. Launey, J. S. Lee, M. H. Mankani, U. G. K. Wegst and E. Saiz, *Int. J. Oral Maxillofac. Implants*, 2011, **26**, 25.
4 E. M. Christenson, K. S. Anseth, L. J. J. P. van der Beucken, C. K. Chan, B. Ercn and J. A. Jansen, *et al.*, *J. Orthop. Res.*, 2007, **25**, 11.
5 D. Arcos, A. R. Boccaccini, M. Bohner, A. Díez-Pérez, M. Epple, E. Gómez-Barrena, A. Herrera, J. A. Planell, L. Rodríguez-Mañas and M. Vallet-Regí, *Acta Biomater.*, 2014, **10**, 1793.
6 M. Fini, G. Giavaresi, P. Torricelli, A. Krajewski, A. Ravaglioli, M. M. Belmonte, G. Biagini and R. Giardino, *J. Bone Jt. Surg.*, 2001, **83**, 139.
7 M. Fini, G. Giavaresi, P. Torricelli, V. Borsari, R. Giardino, A. Nicolini and A. Carpi, *Biomed. Pharmacother.*, 2004, **58**, 487.
8 M. Franchi, M. Fini, G. Giavaresi and V. Ottani, *Micron*, 2005, **36**, 630.
9 D. Campoccia, L. Montanaro and C. R. Ariola, *Biomaterials*, 2006, **27**, 2331.
10 M. Vallet-Regí and D. Arcos, *Nanoceramics in clinical use. From materials to applications. RSC Nanoscience and Nanotechnology*, RSC, Cambridge, 2016.
11 D. F. Williams, *The Williams dictionary of Biomaterials*, Liverpool University Press, Liverpool, 1999.
12 L. L. Hench, *J. Am. Ceram. Soc.*, 1991, **74**, 1487.
13 J. H. M. Goosen, A. J. Kums, B. J. Kolln and C. C. P. M. Verheyen, *Arch. Orthop. Trauma. Surg.*, 2009, **129**, 1165.
14 ISO 13779-3:2008. Implants for surgery-hydroxyapatite-Part 3: Chemical analysis and characterization of crystallinity and phase purity.
15 ISO 13779-2:2008. Implants for surgery-hydroxypatite- Part 2: Coatings of hydroxyapatite.
16 P. Chellan and P. J. Sadler, *Philos. Trans. R. Soc., A*, 2015, **373**, 20140182.
17 H. Qiao, G. Song, Y. Huang, H. Yang, S. Han, X. Zhang, Z. Wang, J. Ma, X. Bu and L. Fu, *RSC Adv.*, 2019, **9**, 13348.
18 H. Qiao, Q. Zou, C. Yuan, X. Zhang, S. Han, Z. Wang, X. Bu., H. Tang and Y. Huang, *Ceram. Int.*, 2018, **44**, 16632.
19 Y. Huang, W. Wang, X. Zhang, X. Liu, Z. Xu, S. Han, Z. Su, H. Liu, Y. Gao and H. Yang, *Ceram. Int.*, 2018, **44**, 5528.
20 J. C. Elliott, *Structure and Chemistry of the Apatites and Other Calcium orthophosphates, Studies in Inorganic Chemistry 18*, Elsevier, Amsterdam, 1999. Q21
21 M. I. Kay, R. A. Young and A. S. Posner, *Nature*, 1964, **204**, 1050.
22 J. T. B. Ratnayake, M. Mucalo and G. J. Dias, *J. Biomed. Mater. Res., Part B*, 2017, **105**, 1285–1299.
23 A. Bigi, A. Ripamonti, S. Brückner, M. Gazzano, N. Roveri and S. A. Thomas, *Acta Crystallogr., Sect. B: Struct. Sci.*, 1989, **45**, 247.
24 A. Bigi, E. Falini, M. Foresti, M. Gazzano, A. Ripamonti and N. Roveri, *Acta Crystallogr., Sect. B: Struct. Sci.*, 1996, **52**, 879–812. Q22

25 C. Ergun, T. J. Webster, R. Bizios and R. H. Doremus, *J. Biomed. Mater. Res.*, 2002, **59**, 305.
26 T. J. Webster, C. Ergun, R. H. Doremus and R. Bizios, *J. Biomed. Mater. Res.*, 2002, **5**, 312.
27 R. Zapanta LeGeros, *Arch. Oral Biol.*, 1974, **20**, 6313.
28 P. E. Mackie, J. C. Elliott and R. A. Young, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1972, **28**, 1840.
29 J. C. Elliott, G. Bonel and J. C. Trombe, *J. Appl. Crystallogr.*, 1980, **13**, 618.
30 B. G. DeBoer, *Acta Crystallogr., Sect. B: Struct. Sci.*, 1991, **47**, 683.
31 A. Serret, M. V. Cabañas and M. Vallet-Regí, *Chem. Mater.*, 2000, **12**, 3836.
32 R. A. Young and P. E. Mackie, *Mater. Res. Bull.*, 1980, **15**, 17.
33 R. M. Wilson, J. C. Elliott and S. E. P. Dowker, *Am. Mineral.*, 1999, **84**, 1406.
34 E. A. P. De Maeyer, R. M. H. Verbeeck and D. E. Naessens, *Inorg. Chem.*, 1993, **32**, 5709.
35 R. M. H. Verbeeck, E. A. P. De Maeyer and F. C. M. Driessens, *Inorg. Chem.*, 1995, **34**, 2084.
36 R. A. Surmenev, M. A. Surmeneva and A. A. Ivanova, *Acta Biomater.*, 2014, **10**, 557.
37 R. B. Heiman, *Surf. Coat. Technol.*, 2006, **201**, 2012.
38 H. C. Gledhill, I. G. Turner and C. Doyle, *Biomaterials*, 1999, **20**, 315.
39 H. C. Gledhill, I. G. Turner and C. Doyle, *Biomaterials*, 2001, **22**, 1233.
40 H. Podlesak, L. Pawlowski, R. D'Haese, J. Laurenys, T. Lampke and S. Bellayer, *J. Therm. Spray Technol.*, 2010, **19**, 657.
41 Y. Huang, L. Song, X. liu, Y. Xiao, Y. Wu, J. Chen, F. Wu and Z. Gu, *Biofabrication*, 2010, **2**, 045003.
42 S. Hasan and J. Stokes, *J. Therm. Spray Technol.*, 2011, **20**, 186.
43 M. F. Morks and A. Kobayashi, *Appl. Surf. Sci.*, 2007, **253**, 7136.
44 S. Dorozhkin, *Prog. Biomater.*, 2012, **1**, 1.
45 H. Oguchi, K. Ishikawa, S. Ojima, Y. Hirayaa, K. Seto and G. Eguchi, *Biomaterials*, 1992, **13**, 471.
46 A. R. Boccaccini, S. Keim, R. Ma, Y. Li and I. Zhitomirsky, *J. R. Soc., Interface*, 2010, **7**, S581.
47 L. Besra and M. Liu, *Prog. Mater. Sci.*, 2007, **52**, 1.
48 J. M. Choi, H. E. Kim and I. S. Lee, *Biomaterials*, 2000, **21**, 469.
49 P. G. Coelho and J. E. Lemons, *J. Biomed. Mater. Res.*, 2009, **90**, 351.
50 P. Rajesh, C. V. Muraleedharan, M. Komath and H. Varma, *J. Mater. Sci.: Mater. Med.*, 2011, **22**, 497.
51 M. Jelinek, M. Weiserova, T. Kocourek, M. Zezulova and J. Strnad, *Laser Phys.*, 2011, **21**, 1265.
52 L. Q. Tri and D. H. C. Chua, *Appl. Surf. Sci.*, 2009, **256**, 76.
53 M. Sygnatowich and A. Tiwari, *Mater. Sci. Eng., C*, 2009, **29**, 1071.
54 J. G. C. Wolke, K. van Dijk, H. G. Schaeken, K. de Groot and J. A. Jansen, *J. Biomed. Mater. Res.*, 1994, **28**, 1477.
55 K. van Dijk, H. G. Schaeken, J. C. G. Wolke and J. A. Jansen, *Biomaterials*, 1996, **17**, 405.
56 R. A. A. Surmenev, *Surf. Coat. Technol.*, 2012, **206**, 2035.
57 D. R. Cooley, A. F. van Dellen, J. O. Burgess and S. Windeler, *J. Prosthet. Dent.*, 1992, **67**, 93.
58 R. Snysers, E. Bousser, D. Music, J. Jensen, S. Hocquet and J. M. Schneider, *Plasma Processes Polym.*, 2008, **5**, 168.
59 V. M. Ievlev, E. P. Domashevskaya, V. I. Putlaiev, Y. D. Tretyakov, S. M. Barinov, E. K. Belonogov, A. V. Kostyuchenko, M. I. Petrzhik and F. V. Kiryukhantsev-Korneev, *Glass Phys. Chem.*, 2008, **34**, 608.
60 A. Bigi, E. Boanini, C. Capuccini, M. Fini, I. N. Mihailescu, C. Ristoscu, F. Sima and P. Torricelli, *Biomaterials*, 2009, **30**, 6168.
61 E. Boanini, P. Torricelli, M. Fini, F. Sima, F. Servan, I. N. Mihailescu and A. Bigi, *J. Inorg. Biochem.*, 2012, **107**, 65.
62 G. Negroiu, R. M. Piticescu, G. C. Chitanu, I. N. Mihailescu, L. Zdrentu and M. Miroiu, *J. Mater. Sci.: Mater. Med.*, 2008, **18**, 1537.
63 A. Bigi, E. Boanini, C. Capuccini, M. Fini, I. N. Mihailescu, C. Ristoscu, F. Sima and P. Torricelli, *Biomaterials*, 2009, **30**, 6168.
64 X. X. Wang, S. Hayakawa, K. Tsuru and A. Osaka, *J. Biomed. Mater. Res.*, 2000, **52**, 172.
65 M. V. Cabañas and M. Vallet-Regí, *J. Mater. Chem.*, 2003, **3**, 1104.
66 T. Goto and H. Katsui, Chemical Vapor Deposition of Ca–P–O Film Coating, in *Interface Oral Health Science 2014*, ed. K. Sasaki, O. Suzuki and N. Takahashi, Springer, Tokyo, 2015.
67 T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, *J. Biomed. Mater. Res.*, 1990, **24**, 721.
68 A. L. A. Escada, J. P. B. Machado, S. G. Shneider, M. C. R. Alves Rezende and A. P. R. Alves Claro, *J. Mater. Sci.: Mater. Med.*, 2011, **22**, 2457.
69 A. H. Aparecida, M. V. L. Fook and A. C. Guastaldi, *J. Mater. Sci.: Mater. Med.*, 2009, **20**, 1215.
70 L. L. Hench and J. K. West, *Chem. Rev.*, 1990, **90**, 33.
71 I. Izquierdo-Barba, M. Vallet-Regí, J. M. Rojo, E. Blanco and L. Esquivias, *J. Sol-Gel Sci. Technol.*, 2003, **26**, 1179.
72 I. Izquierdo-Barba, A. Asenjo, L. Esquivias and M. Vallet-Regí, *Eur. J. Inorg. Chem.*, 2003, 1608.
73 N. Hijón, M. V. Cabañas, J. Peña and M. Vallet-Regí, *Acta Biomater.*, 2006, **2**, 567.
74 D. Qiu, L. Yang, Y. Yin and A. Wang, *Surf. Coat. Technol.*, 2011, **205**, 3280.
75 S. Lin, R. Z. Legeros and J. P. Legeros, *J. Biomed. Mater. Res., Part A*, 2003, **66**, 819.
76 S. Ban and S. Maruno, *J. Biomed. Mater. Res.*, 1998, **42**, 387.
77 H. Cimenoglu, M. Gunyuz, G. T. Kose, M. Baydogan, F. Ugurlu and C. Sener, *Mater. Charact.*, 2011, **62**, 304.
78 R. Luo, Z. Liu, F. Yan, Y. Kong and Y. Zhang, *Appl. Surf. Sci.*, 2013, **266**, 57.
79 F. Liu, Y. Song, F. Wang, T. Shimizu, K. Igarashi and L. Zhao, *J. Biosci. Bioeng.*, 2005, **100**, 100.

80 R. Bosco, R. E. U. Edreira, J. G. C. Wolke, S. C. G. Leeuwenburgh and J. J. J. P. van den Beucken, *Surf. Coat. Technol.*, 2013, **233**, 91.

81 E. S. Thian, L. Chang, P. N. Lim, B. Gurucharan, J. Sun and J. Y. H. Fuh, *Surf. Coat. Technol.*, 2012, **231**, 29.

82 F. Miyaji, Y. Kono and Y. Suyama, *Mater. Res. Bull.*, 2005, **40**, 209.

83 Y. Tang, H. F. Chappell, M. T. Dove, R. J. Reeder and Y. J. Lee, *Biomaterials*, 2009, **30**, 2864.

84 F. Ren, R. Xin, X. Ge and Y. Leng, *Acta Biomater.*, 2009, **5**, 3141.

85 M. Yamaguchi, H. Oishi and Y. Suketa, *Biochem. Pharmacol.*, 1987, **36**, 4007.

86 M. Yamaguchi, *J. Trace Elem. Exp. Med.*, 1998, **11**, 119.

87 V. Stanić, S. Dimitrijević, J. Antíc-Stankovíc, M. Mitríc, B. Jokíc, I. B. Plecas and S. Raicevic, *Appl. Surf. Sci.*, 2010, **256**, 6083.

88 E. S. Thian, T. Konishi, Y. Kawanobe, P. N. Lim, C. Choong, B. Ho and M. Aizawa, *J. Mater. Sci.: Mater. Med.*, 2013, **24**, 437.

89 A. V. Lyasnikova, O. A. Dudareva, V. N. Lyasnikov, O. A. Markelova and I. P. Grishina, *Glass Ceram.*, 2018, **75**, 163.

90 T. G. Peñaflor Galindo, T. Kataoka, S. Fujii, M. Okuda and M. Tagaya, *Colloid Interface Sci. Commun.*, 2016, **10–11**, 15.

91 D. Predoi, S. L. Iconaru, M. V. Predoi, N. Buton and M. Motelica-Heino, *Coatings*, 2019, **9**, 256.

92 K. A. Prosolov, O. A. Belyavskaya, J. Linders, K. Loza, O. Prymak, C. Mayer, J. V. Rau, M. Epple and Y. P. Sharkeev, *Coatings*, 2019, **9**, 220.

93 E. O. López, A. L. Rossi, P. L. Bernardo, R. O. Freitas, A. Mello and A. M. Rossi, *Ceram. Int.*, 2019, **45**, 793.

94 Y. Huang, H. Zhang, H. Qiao, X. Nian, X. Zhang, W. Wang, X. Zhang, X. Chang, S. Han and X. Pang, *Appl. Surf. Sci.*, 2015, **357**, 1776.

95 T. J. Webster, E. A. Massa-Schlueter, J. L. Smith and E. B. Slamovich, *Biomaterials*, 2004, **25**, 2111.

96 R. Sergi, D. Bellucci, R. T. Candidato Jr, L. Lusvarghi, G. Bolelli, L. Pawlowski, G. Candiani, L. Atomare, L. De Nardo and V. Cannillo, *Surf. Coat. Technol.*, 2018, **352**, 84.

97 R. T. Candidato, C. Thouzellier and L. Pawlowski, *J. Biomed. Mater. Res., Part B*, 2018, **106**, 2101.

98 I. Y. Ortiz, A. R. dos Santos, A. M. Costa, E. Mavropoulos, M. N. Tanaka, M. H. Prado da Silva and S. de Souza Camargo Jr., *Ceram. Int.*, 2016, **42**, 15502.

99 Y. Sogo, T. Sakurai, K. Onuma and A. Ito, *J. Biomed. Mater. Res., Part A*, 2002, **62**, 457.

100 H. Kawamura, A. Ito, T. Muramatsu, S. Miyakawa, N. Ochiai and T. Tateishi, *J. Biomed. Mater. Res., Part A*, 2003, **65**, 468.

101 J. Ma and J. Qin, *Cryst. Growth Des.*, 2015, **15**, 1273.

102 G. Borkow and J. Gabbay, *Curr. Med. Chem.*, 2005, **12**, 2163.

103 C. Wu, Y. Zhou, M. Xu, P. Han, L. Chen, J. Chang and Y. Xiao, *Biomaterials*, 2013, **34**, 422.

104 A. Ewald, C. Kappel, E. Vorndran, C. Moseke, M. Gelinsky and U. Gbureck, *J. Biomed. Mater. Res., Part A*, 2012, **100**, 2392.

105 M. Pujari and P. N. Patel, *J. Solid State Chem.*, 1989, **83**, 100.

106 S. Shanmucham and B. Gopal, *Ceram. Int.*, 2014, **40**, 15655.

107 S. Gomes, C. Vichery, S. Descamps, H. Martinez, A. Kaur, A. Jacobs, J.-M. Nedelec and G. Renaudin, *Acta Biomater.*, 2018, **65**, 462.

108 A. V. Lyasnikova, O. A. Markelova, O. A. Dudareva, V. N. Lyasnikov, A. P. Barabash and S. P. Shpinyak, *Powder Metall. Met. Ceram.*, 2016, **55**, 97.

109 R. B. Unabia, S. Bonebeau, R. T. Candidato Jr and L. Pawlowski, *Surf. Coat. Technol.*, 2018, **353**, 370.

110 B. M. Hidalgo-Robatto, M. López. Álvarez, A. S. Azevedo, J. Dorado, J. Serra and N. F. Azevedo, *Surf. Coat. Technol.*, 2018, **333**, 168.

111 R. O. Darouiche, *Clin. Infect. Dis.*, 1999, **29**, 137.

112 M. Jelinek, T. Kocourek, K. Jurek, J. Remsa, J. Miksovsky and M. Weisasrová, *Appl. Phys. A: Mater. Sci. Process.*, 2010, **101**, 615.

113 Y. K. Chen, X. B. Zheng, Y. T. Xie, H. Ji, C. X. Ding and H. W. Li, *et al.*, *Surf. Coat. Technol.*, 2010, **205**, 1892.

114 Y. Chen, Z. Zheng, Y. Xie, C. Ding, H. Ruan and C. Fan, *J. Mater. Sci.: Mater. Med.*, 2008, **19**, 3603.

115 G. A. Fielding, M. Roy, A. Bandyopadhyay and S. Bose, *Acta Biomater.*, 2012, **8**, 3144.

116 W. Chen, Y. Liu, H. S. Courtney, M. Bettenga, C. M. Agrawal and J. D. Bumgardner, *et al.*, *Biomaterials*, 2006, **27**, 5512.

117 W. Chen, S. Oh, A. P. Ong, N. Oh, Y. Liu, H. S. Courtney, M. Appleford and J. L. Ong, *J. Biomed. Mater. Res., Part A*, 2007, **82**, 899.

118 T. Shimazaki, H. Miyamoto, Y. Ando, I. Noda, Y. Yonekura and S. Kawano, *J. Biomed. Mater. Res., Part B*, 2010, **92**, 386.

119 O. Gokcekaya, T. J. Webster, K. Ueda, T. Narushima and C. Ergun, *Mater. Sci. Eng., C*, 2017, **77**, 555.

120 Y. Yan, X. Zhang, Y. Huang, Q. Ding and X. Pang, *Appl. Surf. Sci.*, 2014, **314**, 348.

121 J. P. Sun and Y. Song, *ACS Appl. Nano Mater.*, 2018, **9**, 4940.

122 R. K. Rude, F. R. Singer and H. E. Gruber, *J. Am. Coll. Nutr.*, 2009, **28**, 131.

123 A. Banyopadhyay, S. Bernard, W. Xue and S. Bose, *J. Am. Ceram. Soc.*, 2006, **89**, 2675.

124 G. Graziani, M. Boi and M. Bianchi, *Coatings*, 2018, **8**, 269.

125 S. Bose, A. Vu, K. Emshadi and A. Bandyopadhyay, *Mater. Sci. Eng., C*, 2018, **88**, 166.

126 J. Min and H.-C. Choe, *Appl. Surf. Sci.*, 2018, **432**, 294.

127 B. Bakin, T. Koc Delice, U. Tiric, I. Birlik and A. Ak Azem, *Surf. Coat. Technol.*, 2016, **301**, 29.

128 P. Layrolle. Calcium phosphate coatings, in *Comprehensive Biomaterials*, ed. P. Ducheyne, K. Healy, D. W. Hutmacher, D. W. Grainger and C. J. Kirkpatric, Elsevier, Amsterdam, Netherlands, vol. 1, pp. 223–229. Q23

129 S. G. Dahl, P. Allain, P. J. Marie, Y. Mauras, G. Boivin, P. Ammann, Y. Tsouderos, P. D. Delmas and C. Christiansen, *Bone*, 2001, **28**, 446.

130 P. J. Marie, *Curr. Opin. Pharmacol.*, 2005, **5**, 633.

131 N. D. Ravi, R. Balu and T. S. Sampath Kumar, *J. Am. Ceram. Soc.*, 2012, **95**, 2700.

132 C. Capuccini, P. Torricelli, E. Boanini, M. Gazzano, R. Giardino and A. Bigi, *J. Biomed. Mater. Res., Part A*, 2009, **89**, 594.

133 M. Bianchi, L. Degli Esposti, A. Ballardini, F. Liscio, M. Berni, A. Gambardella, S. C. G. Leeuwenburgh, S. Sprio, A. Tampieri and M. Iafisco, *Surf. Coat. Technol.*, 2017, **319**, 191.

134 A. Bigi, E. Boanini, C. Capuccini and M. Gazzano, *Inorg. Chim. Acta*, 2007, **360**, 1009.

135 H.-P. Teng, H.-Y. Lin, Y.-H. Huang and F.-H. Lu, *Surf. Coat. Technol.*, 2018, **350**, 1112.

136 Y. Li, S. Shen, L. Zhu, S. Cai, Y. Jiang, R. Ling, S. Jiang, Y. Lin, S. Hua and G. Xu, *J. Ceram. Soc. Jpn.*, 2019, **127**, 158.

137 X. Wu, W. Lin, D. Li, H. Guo, P. Li and Y. Fan, *RSC Adv.*, 2019, **9**, 15013.

138 T.-D. T. Nguyen, Y.-S. Jang, M.-H. Lee and T.-S. Bae, *J. Appl. Biomater. Funct. Mater.*, 2019, **17**.

139 M. Roy, A. Bandyopadhyay and S. Bose, *J. Biomed. Mater. Res., Part B*, 2011, **99**, 258.

140 E. Boanini, P. Torricelli, F. Sima, E. Axente, M. Fini, I. N. Mihailescu and A. Bigi, *J. Inorg. Biochem.*, 2018, **183**, 1.

141 Z. T. Birgani, E. Fennema, M. J. Gijbels, J. de Boer, C. A. van Blitterswijk and P. Habibovic, *Acta Biomater.*, 2016, **36**, 267.

142 Z. T. Birgani, N. Gharraee, A. Malhotra, C. A. van Blitterswijk and P. Habibovic, *Biomed. Mater.*, 2016, 015020.

143 C. Wu, Y. Zhou, W. Fan, P. Han, J. Chang, J. Yuen, M. Ahang and Y. Xiao, *Biomaterials*, 2012, **33**, 2076.

144 R. Drevet, Y. Zhukova, S. Dubinskiy, A. Kazakbiev, V. Naumenko, M. Abakumov, J. Fauré, H. Benhayoune and S. Prokoshkin, *J. Alloys Compd.*, 2019, **793**, 576.

145 K. Prem Ananth, J. Sun and J. Bai, *Mater. Today Chem.*, 2018, **10**, 153.

146 A. P. Mould, S. K. Akiyama and M. J. Humphries, *J. Biol. Chem.*, 1995, **270**, 26270.

147 E. Gyorgy, P. Toricelli, G. Socol, M. Iliescu, I. Mayer, I. N. Mihailescu, A. Bigi and J. Werckman, *J. Biomed. Mater. Res., Part A*, 2004, **71**, 353.

148 A. Bigi, B. Bracci, F. Cuisinier, R. Elkaim, M. Fini, I. Mayer, I. N. Mihailescu, G. Socol, L. Sturba and P. Torricelli, *Biomaterials*, 2005, **26**, 2381.

149 F. Sima, G. Socol, E. Axente, I. N. Mihailescu, L. Zdrentu, S. M. Petrescu and I. Mayer, *Appl. Surf. Sci.*, 2007, **254**, 1155.

150 K. Prem Ananth, J. Sun and J. Bai, *Int. J. Mol. Sci.*, 2018, **19**, 2340.

151 J. W. Park, Y. J. Kim and J. H. Jang, *Appl. Surf. Sci.*, 2011, **258**, 977.

152 M. Sato, M. A. Sambito, A. Aslani, N. M. Kalkhoran, E. B. Slamovich and T. J. Webster, *Biomaterials*, 2006, **27**, 2358.

153 Y. Lin, Z. Yang and J. Cheng, *J. Rare Earths*, 2007, **25**, 452.

154 G. Ciobani and M. Harja, *Ceram. Int.*, 2019, **45**, 2852.

155 M. A. Jakupec, P. Unfried and B. K. Keppler, *Rev. Physiol., Biochem. Pharmacol.*, 2005, **153**, 101.

156 E. M. Carlisle, *Science*, 1970, **167**, 179.

157 E. M. Carlisle, *Calcif. Tissue Int.*, 1981, **33**, 27.

158 I. R. Gibson, S. M. Best and W. Bonfield, *J. Biomed. Mater. Res.*, 1999, **44**, 422.

159 I. R. Gibson, S. M. Best and W. Bonfield, *J. Am. Ceram. Soc.*, 2002, **85**, 2771.

160 D. Arcos, J. Rodríguez-Carvajal and M. Vallet-Regí, *Chem. Mater.*, 2004, **16**, 2300.

161 D. Arcos, J. Rodríguez-Carvajal and M. Vallet-Regí, *Solid State Sci.*, 2004, **6**, 987.

162 D. Arcos, S. Sánchez-Salcedo, I. Izquierdo-Barba, L. Ruiz, J. González-Calbet and M. Vallet-Regí, *J. Biomed. Mater. Res.*, 2006, **78**, 762.

163 M. C. Matesanz, J. Linares, M. Oñaderra, M. J. Feito, F. J. Martínez-Vázquez, S. Sánchez-Salcedo, D. Arcos, M. Teresa Portolés and M. Vallet-Regí, *Colloids Surf., B*, 2015, **133**, 304.

164 M. C. Matesanz, J. Linares, I. Lilue, S. Sánchez-Salcedo, M. J. Feito, D. Arcos, M. Vallet-Regí and M. T. Portolés, *J. Mater. Chem. B*, 2014, **2**, 2910.

165 M. Vallet-Regí and D. Arcos, *J. Mater. Chem.*, 2005, **15**, 1509.

166 N. Patel, S. M. Best, W. Bonfield, I. R. Gibson, K. A. Hing, E. Damien and P. A. Revell, *J. Mater. Sci.: Mater. Med.*, 2002, **13**, 1199.

167 A. E. Porter, C. M. Botelho, M. A. Lopes, J. D. Santos, S. M. Best and W. Bonfield, *J. Biomed. Mater. Res., Part A*, 2004, **69**, 670.

168 E. S. Thian, J. Huang, S. M. Best, Z. H. Barber and W. Bonfield, *Biomaterials*, 2005, **26**, 2947.

169 E. S. Thian, J. Huang, S. M. Best, Z. H. Barber, R. A. Brooks, N. Rushton and W. Bonfield, *Biomaterials*, 2006, **27**, 2692.

170 E. S. Thian, J. Huang, M. E. Vickers, S. M. Best, Z. H. Barber and W. Bonfield, *J. Mater. Sci.*, 2006, **41**, 709.

171 M. A. Surmeneva, M. V. Chaikina, V. I. Zaikovskiy, V. F. Pichugin, O. Prymak and M. Epple., *et al.*, *Surf. Coat. Technol.*, 2013, **218**, 39.

172 M. A. Surmeneva, A. Kovtum, A. Peetsch, S. N. Goroja, A. Sharonova and V. F. Picugin., *et al.*, *RSC Adv.*, 2013, **3**, 11240.

173 M. A. Surmeneva, T. M. Mukhametkaliyev, A. I. Tyurin, A. D. Teresov, N. N. Koval, T. S. Pirozhkova, I. A. Shuvarin, A. V. Shuklinov, A. O. Zhigachev, C. Oehr and R. A. Surmenev, *Surf. Coat. Technol.*, 2015, **275**, 176.

174 J. V. Rau, I. Cacciotti, S. Laureti, M. Fosca, G. Varvaro and A. Latini, *J. Biomed. Mater. Res., Part B*, 2015, **103**, 1621.

175 N. Hijon, M. V. Cabañas, J. Peña and M. Vallet-Regí, *Acta Biomater.*, 2006, **2**, 567.

176 J.-Y. Cha, C.-H. Lim and Y.-J. Kim, *J. Ceram. Soc. Jpn.*, 2018, **126**, 940.

177 C. Dehghanian, N. Aboudzadeh and M. A. Shokrgozr, *Mater. Chem. Phys.*, 2018, **203**, 27.

178 L. Kezhi, G. Qian, Z. Leilei, Z. Yulei, L. Shoujie, G. Kebing and L. Shaoxian, *Ceram. Int.*, 2017, **43**, 1410.

179 X. Xin-Bo, N. Xin-Ye, L. Ya-Yun, C. Cen-Cen, Z. Ji-Zhao and Z. Xie-Rong, *Sci. Rep.*, 2016, **6**, 31309.

180 E. O. López, A. L. Rossi, B. S. Archanjo, R. O. Ospina, A. Mello and A. M. Rossi, *Surf. Coat. Technol.*, 2015, **264**, 163.

181 Y. Hashimoti, M. Ueda, Y. Kohiga, K. Imura and S. Hontsu, *Dent. Mater. J.*, 2018, **37**, 408.
182 R. E. Marquis, *Can. J. Microbiol.*, 1995, **41**, 955.
183 A. Alhilou, T. Do, L. Mizban, B. H. Clarkson, D. J. Wood and M. G. Katsikogianni, *ACS Omega*, 2016, **1**, 264.
184 R. Alvarez, S. Muñoz-Piña, M. U. González, I. Izquierdo-Barba, I. Fernández-Martínez, V. Rico, D. Arcos, A. García-Valenzuela, A. Palmero, M. Vallet-Regí, A. R. González-Elipe and J. M. García-Martín, *Nanomaterials*, 2019, **9**, 1217.
185 M. Caligari Conti, G. Xerri, F. Peyrouzet, P. Schembri Wismayer, E. Sionagra, D. Matovani, D. Vella and J. Buhagiar, *Surf. Eng.*, 2019, **35**, 255.
186 B. León, M. Albano, L. Garrido, E. Ferraz, A. Rosa and P. T. Oliveira, *Int. J. Appl. Ceram. Technol.*, 2018, **15**, 1415.
187 E. J. Lee, S. H. Lee, H. W. Kim, Y. M. Kong and H. E. Kim, *Biomaterials*, 2005, **26**, 3843.
188 J. Wang, Y. Chao, Q. Wan, Z. Zhu. and H. Yu, *Acta Biomater.*, 2009, **5**, 1798.
189 L. E. Sima, G. E. Stan, C. O. Morosanu, A. Melinescu and R. Melinte, *et al.*, *J. Biomed. Mater. Res.*, 2010, **95**, 1203.
190 L. Winning, L. Robinson, A. R. Boyd, I. A. El Karim, F. T. Lundy and B. J. Meenan, *J. Biomed. Mater. Res., Part A*, 2017, **105**, 1692.
191 Y.-P. Guo, Y.-B. Yao, C.-Q. Ning, L.-F. Chu and Y.-J. Guo, *Mater. Lett.*, 2011, **65**, 1007.
192 S. Tang, B. Tian, Y.-J. Guo, Z.-A. Zhu and Y.-P. Guo, *Surf. Coat. Technol.*, 2014, **251**, 210.
193 S. Tang, B. Tian, Q.-F. Ke, Z.-A. Zhu and Y.-P. Guo, *RSC Adv.*, 2014, **4**, 41500.
194 D. O. Costa, P. D. H. Prowse, T. Chrones, S. M. Sims, D. W. Hamilton, A. S. Rizkalla and S. J. Dixon, *Biomaterials*, 2013, **34**, 7215.
195 M. Stigter, J. Bezemer, K. de Groot and P. Layrolle, *J. Controlled Release*, 2004, **99**, 127.
196 F. S. Utku, E. Seckin, G. Goller, C. Tamerler and M. Urgen, *Ceram. Int.*, 2014, **40**, 15479.
197 J. V. Rau, A. Generosi, S. Laureti, V. S. Komlev, D. Ferro, S. Nunziante Cesaro, B. Paci, V. Rossi Albertini, E. Agostinelli and S. M. Barinov, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1813.
198 X. Wei, C. Fu, K. Savino and M. Z. Yates, *Cryst. Growth Des.*, 2012, **12**, 3474.
199 Y. Han, K. Xu, G. Montay, T. Fu and J. Lu, *J. Biomed. Mater. Res.*, 2002, **60**, 511.
200 L. E. Sima, G. E. Stan, C. O. Morosanu, A. Melinescu and R. Melinte, *et al.*, *J. Biomed. Mater. Res.*, 2010, **95**, 1203.
201 L. Winning, L. Robinson, A. R. Boyd, I. A. El Karim, F. T. Lundy and B. J. Meenan, *J. Biomed. Mater. Res., Part A*, 2017, **105**, 1692.
202 M. Gumusderelioglu, E. O. Tuncay, G. Kaynak, T. T. Demirtas, R. S. Aydin Tigli and S. S. Hakki, *J. Trace Elem. Med. Biol.*, 2015, **31**, 120.
203 E. Ö. Tunçay, T. T. Demirtaş and M. Gümüşderelioğlu, *J. Trace Elem. Med. Biol.*, 2017, **40**, 72.
204 C. Rodríguez-Valencia, P. Freixeiro, J. Serra, C. M. Ferreirós, P. González and M. López-Álvarez, *Biomed. Mater.*, 2017, **12**, 015028.
205 B. Yilmaz, Z. Evis, A. Tezcaner and S. Banerjee, *Int. J. Appl. Ceram. Technol.*, 2016, **13**, 1059.
206 Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim and J. O. Kim, *J. Biomed. Mater. Res.*, 2000, **52**, 662.
207 M. Roy, G. A. Fielding, H. Beyenal, A. Bandyopadhyay and S. Bose, *ACS Appl. Mater. Interfaces*, 2012, **4**, 1341.
208 Z. Geng, Z. Cui, Z. Li, S. Zhu, Y. Liang, Y. Liu, X. Li, X. He, X. Yu, R. Wang and X. Yang, *Mater. Sci. Eng., C*, 2016, **58**, 467.
209 Z. Geng, R. Wang, X. Zhuo, Z. Li, Y. Huang, L. Ma, Z. Cui, S. Zhu, Y. Liang, Y. Liu, H. Bao, X. Li, Q. Huo, Z. Liu and X. Yang, *Mater. Sci. Eng., C*, 2017, **71**, 852.
210 L. Yang, S. Perez-Amodio, F. Y. F. Barrere-de Groot, V. Everts, C. A. van Blitterswijk and P. Habibovic, *Biomaterials*, 2006, **31**, 2976.
211 Y. Huang, X. Zhang, H. Mao, T. Li, R. Zhao, Y. Yan and X. Pang, *RSC Adv.*, 2015, **5**, 17076.
212 Y. Huang, M. Hao, X. Nian, H. Qiao, X. Zhang, X. Zhan, G. Song, J. Guo, X. Pang and H. Zhang, *Ceram. Int.*, 2016, **42**, 11876.
213 L. Cao, I. Ullah, N. Li, S. Niu, R. Sun, D. Xia, R. Yang and X. Zhang, *J. Mater. Sci. Technol.*, 2019, **35**, 719.
214 L. Morejón-Alonso, C. Mochales, L. Nascimento and W.-D. Müller, *Mater. Lett.*, 2019, **248**, 65.
215 D. Arcos, J. Rodríguez-Carvajal and M. Vallet-Regí, *Chem. Mater.*, 2005, **17**, 57.
216 S. D. Morais, J. Coelho, M. P. Ferraz, P. S. Gomes, M. H. Fernandes, N. S. Hussain, J. D. Santos and M. A. Lopes, *J. Mater. Chem. B*, 2014, **2**, 5872.
217 C. S. Ciobanu, C. L. Popa and D. Predoi, *J. Nanomater.*, 2014, 780686.
218 M. Yang, C. Qin, F. Pan and T. Zhou, *J. Rare Earths*, 2011, **29**, 550.
219 S. Sathiskumar, K. Louis, E. Shinyjoy and D. Gopi, *Ind. Eng. Chem. Res.*, 2016, **55**, 6331.
220 Z. T. Birgani, N. Gharraee, A. Malhotra, C. A. van Blitterswijk and P. Habibovic, *Biomed. Mater.*, 2016, **11**, 015020.
221 Y. Huang, X. Zhang, H. Qiao., M. Hao, H. Zhang, Z. Xua, X. Zhang, X. Pang and H. Lin, *Ceram. Int.*, 2016, **42**, 1903.
222 Y. Huang, G. Song, X. Chang, Z. Wang, X. Zhang, S. Han, Z. Su, H. Yang, D. Yang and X. Zhang, *Int. J. Nanomed.*, 2018, **13**, 2665.
223 W. Qu, D. Zhong, P. Wu, J. Wang and B. Han, *J. Bone Miner. Metab.*, 2008, **26**, 328.
224 X. Qiu, P. N. Lim, S. Y. Tong and E. S. Thian, *Mater. Technol.*, 2018, **33**, 406.
225 P. N. Lim, Z. Wang, L. Cahng, T. Konishi, C. Choong, B. Ho and E. S. Thian, *J. Mater. Sci.: Mater. Med.*, 2017, **28**, 3.
226 S.-F. Zhao, Q.-H. Jiang, S. Peel, X.-X. Wang and F.-M. He, *Clin. Oral Implants Res.*, 2013, **24**, 34.
227 D. Ke, S. F. Robertson, W. S. Dernell, A. Bandyopadhyay and S. Bose, *ACS Appl. Mater. Interfaces*, 2017, **9**, 25731.

228 A. A. Vu, S. F. Robertson, D. Ke, A. Bandyopadhyay and S. Bose, *Acta Biomater.*, 2019, **92**, 325.

229 F. Barrère, C. M. van der Valk, R. A. J. Dalmeijer, C. A. van Blitterswijk, K. de Groot and P. Layrolle, *J. Biomed. Mater. Res., Part A*, 2003, **64**, 378.

230 W. J. Dhert, C. P. A. T. Klein, J. G. Wolke, E. A. Van der Velde, K. de Groot and P. M. Rozing, *J. Biomed. Mater. Res.*, 1991, **25**, 1183.

231 W. J. Dhert, C. P. A. T. Klein, J. A. Jansen, E. A. van der Velde, R. C. Vrieste, P. M. Rozing and K. de Groot, *J. Biomed. Mater. Res.*, 1993, **27**, 127.

232 W. J. Dhert, P. Thompson, C. P. A. T. Klein, K. de Groot, P. M. Rozing and L. E. Ericson, *J. Mater. Sci.: Mater. Med.*, 1994, **5**, 59.

233 H. Caulier, S. Vercaigne, I. Naert, J. P. C. M. van der Waerden, J. G. C. Wolke, W. Kalk and J. A. Jansen, *J. Biomed. Mater. Res.*, 1997, **34**, 121.

234 Z. S. Tao, B.-L. Bai, X. W. He, W. Liu, H. Li, Q. Zhou, T. Sun, Z.-L. Huang, K.-K. Tu, Y.-X. Lv, W. Cui and L. Yang, *Med. Biol. Eng. Comput.*, 2016, **54**, 1959.

235 A. M. Ballo, W. Xia, A. Palmquist, C. Lindahl, L. Emanuelsson, J. Lausma, H. Engqvist and P. Thompsen, *J. R. Soc., Interface*, 2012, **9**, 1615.

236 S. Bose, D. Banerjee, A. Shivaram, S. Tarafder and A. Bandyopadhyay, *Mater. Des.*, 2018, **151**, 102.

237 K. Das, S. Bose and A. Bandyopadhyay, *J. Biomed. Mater. Res., Part A*, 2009, **90**, 225.

238 P. Heinl, L. Muller, C. Korner, R. F. Singer and F. A. Muller, *Acta Biomater.*, 2008, **4**, 1536.

239 M. Franchi, M. Fini, G. Giavaresi and V. Ottani, *Micron*, 2005, **36**, 630.

240 T. J. Webster, C. Ergun, R. H. Doremus, R. W. Siegel and R. Bizios, *Biomaterials*, 2000, **21**, 1803.

241 I. Izquierdo-Barba, A. Asenjo, L. Esquivias and M. Vallet-Regí, *Eur. J. Inorg. Chem.*, 2003, 1608.

242 E. Zhang and C. Zou, *Acta Biomater.*, 2009, **5**, 1732.

243 A. Ilea, O.-G. Vrabie, A.-M. Babtan, V. Miclaus, F. Ruxanda, M. Sarkozi, L. Barbu-Tudoran, V. Mager, C. Berce, B. A. Bosca, N. B. Petrescu, O. Cadar, R. S. Campian and R. Barabás, *J. Mater. Sci.: Mater. Med.*, 2019, **30**, 26.

244 I. Izquierdo-Barba, L. Santos-Ruiz, J. Becerra, M. J. Feito, D. Fernández-Villa, M. C. Serrano, I. Díaz-Güemes, B. Fernández-Tomé, S. Enciso, F. M. Sánchez-Margallo, D. Monopoli, H. Afonso, M. T. Portolés, D. Arcos and M. Vallet-Regí, *Acta Biomater.*, 2019, **83**, 456.

245 W. Mróz, B. Budner, R. Syroka, G. Golansky Niedzielski, A. Slósarczyk, D. Schwarze and T. E. L. Douglas, *J. Biomed. Mater. Res., Part B*, 2015, **103**, 151.

246 E. Boanini, P. Torricelli, F. Sima, E. Axente, M. Fini, I. N. Mihailescu and A. Bigi, *J. Colloid Interface Sci.*, 2015, **448**, 1.

247 D. Nancy and N. Rajendran, *Int. J. Biol. Macromol.*, 2018, **110**, 197.

248 M. Dadsetan, T. Guda, M. B. Runge, D. Mijares, R. Z. LeGeros, J. P. LeGeros, D. T. Silliman, L. Lu, J. C. wenke, P. R. Brown Baer and M. J. Yaszemski, *Acta Biomater.*, 2015, **18**, 9.

249 B. Huang, Y. Yuan, T. Li, S. Ding, W. Zhang, Y. Gu and C. Liu, *Sci. Rep.*, 2016, **6**, 24323.

250 Z. Geng, X. Wang, J. Zhao, Z. Li, L. Ma, S. Zhu, Y. Liang, Z. Cui, H. He and X. Yang, *Biomater. Sci.*, 2018, **6**, 2654.

251 Y. Bayon, M. Bohner, D. Eglin, P. Procter, R. G. Richards, J. Weber and D. I. Zeugolis, *J. Mater. Sci.: Mater. Med.*, 2016, **27**, 144.

252 R. Bosco, J. Van Den Beucken, S. Leeuwenburgh and J. Jansen, *Coatings*, 2012, **2**, 95.

253 R. A. Surmenev, *Surf. Coat. Technol.*, 2012, **206**, 2035.