The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies.

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CONTENT PAGES

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ABSTRACT

Pathologies affecting the posterior segment are one of the major causes of blindness in developed countries and are becoming more prevalent due to the increase in society longevity. Sucessful therapy of diseases affecting the back of the eye requires effective concentrations of the active substance mantained during a long period of time

in the intraocular target site. Treatment of vitreoretinal diseases often include repeated intravitreous injections that are associated with adverse effects. Local administration of biodegradable microspheres offers an excellent alternative to multiple administrations, as they are able to deliver the therapeutic molecule in a controlled fashion. Furthermore, injection of microparticles is performed without the need for surgical procedures. As most of the retinal diseases are multifactorial, microspheres result especially promising because they can be loaded with more than one active substance and complemented with the inclusion of additives with pharmacological properties. Personalized therapy can be easily achieved by changing the amount of administered microspheres. Contrary to non-biodegradable devices, biodegradable PLA and PLGA microspheres disappear from the site of administration after delivering the drug. Furthermore, microspheres prepared from these mentioned biomaterials are well tolerated after periocular and intravitreal injections in animals and humans. After injection, PLA and PLGA microspheres suffer aggregation behaving like an implant. Biodegradable microspheres are potential tools in regenerative medicine for retinal repair. According to the reported results, presumably a variety of microparticulate formulations for different ophthalmic therapeutic uses will be available in the clinical practice in the near future.

1. Introduction

Pathologies affecting the posterior segment of the eye are one of the major causes of blindness in developed countries. These diseases include uveitis, diabetic retinopathy, macular edema, endophthalmitis, proliferative retinopathy, age related macular degeneration and glaucoma, among others. Generally, back of the eye diseases are chronic and degenerative.and some of them are related to elderly. Sucessful treatments of vitreoretinal diseases require effective concentrations of the active substance mantained during long periods in the target site. Statics barriers (different corneal layers, sclera, retina, blood aqueous and blood retinal barriers), dynamic barriers (tear dilution, conjunctival and choroidal blood flow, and lymphatic clearance) as well as efflux pumps, effectively limit the drug access to the posterior segment (Gaudana et al., 2010). Four routes of administration can be theoretically employed to deliver active substances to

treat retinal diseases: topical, systemic, intraocular and periocular (Herrero-Vanrell et al., 2001). The poor bioavailability of topically administered drugs limits their access to intraocular tissues. Systemic administration requires high doses to achieve adequate therapeutic levels of the drug in the eye with the risk of systemic adverse effects. Intraocular local drug administration includes injections into the anterior chamber of the eye (intracameral), in the vitreous (intravitreal) or into the periocular tissues (subconjunctival, sub-Tenon and retrobulbar). Due to the difficulty in the maintenance of therapeutic concentrations in the target site, repeated intraocular injections are required for a successful therapy causing much inconvenience to patients. Although the periocular route is getting more attention, intravitreal injections are still the most employed even being associated to non-desired effect. For example, if high doses of the therapeutic agent are administered the concentration in the retina can be toxic. Besides, successive intravitreal injections are related to adverse effects such as cataracts, retinal detachment, and haemorraghes, among others. Moreover, the risk of the non-desired effects increases with the number of injections (Herrero-Vanrell et al., 2000).

Innovative treatments as intraocular drug delivery systems have been developed to provide sustained drug concentrations of the active substance in the target site. They are constituted by a combination of drugs and biomaterials. Depending on the properties of the biomaterial (erodible or biodegradable and non-erodible or non-biodegradable), the devices can disappear from the site of administration or remain there during the lifetime of patients.

Depending on their size, devices are classified as implants (>1 mm), microparticles (1-1000 μm) and nanoparticles (1-1000 nm). Considering their physical structure they are divided into reservoir and matrix systems (Herrero-Vanrell and Cardillo 2010b).

Implants and microparticles are able to release the active substance during longer periods of time compared to nanoparticles (figure 1). Depending on their size, implantation procedure requires a surgical incision or a small perforation. Several non-biodegradable implants and one erodible device have been approved for clinical use. Non-biodegradable implants remain inside the eye or need a second surgery procedure to be removed. The non-biodegradable devices approved for clinical use are reservoir systems constituted by a nucleous of the drug surrounded by a layer of a mixture of polymers. They are loaded with antivirals (ganciclovir Vitrasert®) or anti-inflammatory drugs (fluocinolone acetonide Retisert™ and Medidur™). There is one biodegradable implant (0.45 x 6 mm) made of PLGA (Ozurdex®). The matrix device is loaded with dexamethasone (700 μg) and is approved for the treatment of retinal vein occlusion, diabetic macular edema, uveitis and post-cataract surgery (Herrero-Vanrell et al., 2011b).

According to their structure, microparticles receive the name of microcapsules for reservoir structures or microspheres for matrix systems (Yasukawa et al., 2004). Microcapsules are formed by a core containing the drug, which is surrounded by a layer of a polymer or a mixture of several polymers. In the microspheres, the active substance is dispersed in the polymeric network (figure 2).

Once administered, microparticles can disappear or remain in the site of administration after releasing the drug. In case of chronic posterior segment diseases biodegradable microspheres are preferred. Their use as injectable devices has become more popular over the last few decades. The main objectives of the development of biodegradable microspheres for intraocular drug delivery have been to obtain long-acting injectable drug depot formulations and specific drug targeting options.

Intraocular microparticles allow the release of the encapsulated drug, bypassing the blood–ocular barrier. The main advantage of these formulations is that they can release the drug over time with one single administration, having the same effect as multiple injections. Sustained release of active substances from microspheres reduces the need for frequent administrations and enhances patient compliance. This strategy has gained a lot of attention, especially in chronic diseases that require low concentrations of an active substance for a long period of time (Checa-Casalengua et al., 2011).

Microparticles are good candidates to be used in personalized medicine as different amounts of particles can be administered depending on patient needs. For intraocular purposes, they must be biocompatible, safe and stable, demonstrating predictable degradation kinetics. All these requierements can be achieved by the adjustement of the parameters involved in the manufacturing procedure. Furthermore, other factors such as chemical modifications of the particle surface can optimize the functionality of the system or help induce the desired response.

Over the last years, a large variety of bioactive compounds has been included in microspheres (i.e. antiproliferatives, anti-inflammatories, immunosuppressants, antibiotics and even biological therapeutic agents). For the treatment of vitreoretinal diseases, microspheres can be administered by intravitreal, periocular or suprachoroidal injection (Yasukawa et al., 2004; Herrero-Vanrell et al., 2001; Herrero-Vanrell et al., 2011a).

Biodegradable polymers such as gelatin, albumin, polyorthoesters, polyanhydrides and polyesters are preferred for the elaboration of microspheres intended for intraocular drug delivery, as they disappear from the injection site after delivering the drug (Herrero-Vanrell et al., 2013). Among them, the derivatives of poly (lactic) acid (PLA), poly (glycolic) acid (PGA) and their copolymers poly (lactic-co-glycolic) acid (PLGA) are the

most employed. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved these biopolymers for clinical use. For ophthalmic purposes, and especially for the treatment of posterior segment diseases, these erodible polymers have been employed to prepare different devices such as implants, scleral plugs, pellets, discs, films, and rods (Yasukawa et al., 2004). The degradation rate of D- or L-PLA, DL-PLA, and PGA is slower than PLGA, making it possible to select the most adequate polymer to prepare the particles. Previous experience with these polymers has shown that PLGA 50:50 (50% lactide and 50% glycolide) has short in-vivo half-life of degradation (Herrero-Vanrell et al., 2001) and degrades relatively fast to metabolic lactic and glycolic acid that are readily eliminated from the body after suffering metabolism to carbon dioxide and water mediated by Kreb's cycle (Zimmer and Kreuter, 2005).

The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies is discussed in this article.

2. **Technological aspects of microspheres for intraocular administration**

2.1. Manufacturing of microspheres

There are different methods to prepare microparticles (microcapsules and microspheres). They are based on different physico-chemical events: solvent extraction/evaporation from an emulsion, aggregation by pH adjustment or heat, coacervation (phase separation), interfacial polymerization, ionic gelation and spray drying, among others.

The most common technique for elaboration of microspheres destined to intraocular drug delivery is the solvent extraction/evaporation method from an emulsion (Freitas et al., 2005). In this technique, the polymer is first dissolved in a volatile solvent in which the drug is incorporated (inner phase). Once formed, this discontinuous phase is carefully added into a non-miscible solvent also called external phase, including a stabilizer to ensure the formation and maintenance of spherical droplets of the inner phase in the emulsion (figure 3). Then, the elimination of the dispersed phase solvent is performed by extraction/evaporation at room temperature or under vacuum, and as a result, solid polymeric microspheres are formed. Finally, the mature microspheres are recovered by filtration or centrifugation and dried (lyophillization is preferred because the high stability of the final product). Depending on the properties of the active substance, the inner phase can form different physico-chemical systems (dissolution, suspension or emulsion). In the case of biotechnological products a more sophisticated methodology is

required because of their poor stability of these products during manufacturing (i.e. proteins often have large globular structure and exhibit secondary, tertiary and, in some cases, quaternary structure that is necessary for biological activity). For these macromolecules, water-in-oil-in-water emulsion method ($W_1/O/W_2$), in which the protein is first dissolved in the inner aqueous phase (W_1), is commonly employed. Other technological approaches are currently under study for encapsulation of biotechnological products. Among them, the inclusion of stabilizers (Freitas et al., 2005) or the use of the biotechnological product in its solid state (formation of a solid-in-oil-in-water emulsion; S/O/W) (Checa-Casalengua et al., 2011) has shown effective protection of the macromolecule.

2.2. Technological properties of microspheres

Once obtained, microspheres are morphologically characterized by using different techniques such as optic microscopy and Scanning Electron Microscopy (SEM), among others (figure 4). Particle size analysis is carried out by laser diffraction techniques. Infrared (IR) spectroscopy helps characterize the polymer and the encapsulated drug. Differential scanning calorimetry (DSC) analysis allows establishing the impact of the microencapsulation procedure and sterilization on the characteristics of the microspheres and possible interactions between the polymer and the encapsulated drug (Brittner et al., 1999). One interesting aspect is to evaluate the difference between amorphous and crystalline state of the microparticle components performed by X-Ray diffraction. Gel permeation chromatography (GPC) is employed to determine the molecular weight of formulation components, mainly the polymeric substance. (Herrero-Vanrell and Refojo, 2001; Martinez-Sancho et al., 2004a; Checa-Casalengua et al., 2011; Herrero-Vanrell et al., 2013).

Encapsulation efficiency is a critical parameter and gives the rate between the theoretical amount of active substance in the microspheres and the actual amount after the microencapsulation procedure. The drug loading in the microspheres is generally expressed as micrograms of drug per milligram of microspheres. Both parameters are determined using the following mathematical equations:

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Experimental drug loading}}{\text{Theoretical drug loading}} \times 100$$

$$\text{Drug Loading}(\%) = \frac{\text{Amount of drug}}{\text{Amount of microparticles}} \times 100$$

The ability of the microparticulate systems to deliver the drug in a controlled fashion is studied by means of in-vitro release experiments. Assays must simulate the in-vivo conditions. To do this, particles are suspended in an aqueous solvent (usually PBS, pH 7.4) in sink conditions. Then, the samples are placed in a shaker bath with constant agitation. At fixed time intervals, the supernatant is removed, measuring the drug concentration (Herrero-Vanrell et al., 2007). The same volume of fresh medium is replaced to continue the release study. Drug release studies can also be assessed by dyalisis. This method is especially useful for the release study of poor soluble drugs (He et al., 2006). Additives can be employed to modulate the release rate of the active substance from the particles. The use of additives in the microparticulate technology will be more detailed below.

2.3. Sterilization of microspheres

Microspheres destined to intraocular administration must comply with the sterility assurance requirements described in Pharmacopoieas. A final sterilization is preferred over preparation of microspheres under aseptic conditions. A sterility assurance level (SAL) of 10^{-6} (statistical probability of finding 1 contaminated unit is 1 million) is generally acepted for pharmacopoeial sterilization procedures (Yaman, 2001).

Among the final available sterilization methods, ethylene oxide, gamma irradiation and autoclaving are the most employed. However, their use is limited due to instability of the materials (drug and/or polymer) or the production of toxic residues during the process. Some of the biopolymers commonly used to prepare microparticles for ophthalmic drug delivery, such as PLA and PLGA cannot undergo terminal sterilization by steam in a standard autoclave. In the case of thermally sensitive biomaterials, gamma irradiation is one of the preferred options because of its high capacity of penetration. The gamma irradiation dose required to assure sterilization of a pharmaceutical product is 25 kGy (Herrero-Vanrell et al., 2001). However, it is well known that γ-radiation can induce structural changes in both the polymer (Sintzel et al., 1998) and in the encapsulated drug, especially if the active molecule is a protein (Montanari et al., 1998; Jain et al., 2011). It has been described that irradiation can induce non-desired events such as dose-dependent chain scission as well as molecular weight reduction of the polymer, affecting the behaviour of the final product (Nijsen et al., 2002). Moreover, in the case of proteins, special care must be put on denaturation and degradation processes because

they affect the integrity and bioactivity of the therapeutic agent (Jain et al., 2011). Several strategies have been reported to overcome the risk associated to ionizing radiation of intraocular microparticulate formulations. The use of low temperatures (dry ice) during the sterilization of microspheres loaded with low molecular weight drugs (i.e. acyclovir, ganciclovir and celcoxib) has demonstrated to maintain the properties of the formulation after gamma irradiation exposure (Herrero-Vanrell et al., 2000; Martinez-Sancho et al., 2004b; Amrite et al., 2006). However, in the case of microspheres loaded with biological products, the use of low temperatures during irradiation does not provide complete protection against undesirable reactions (i.e. protein carbonylation and hydroperoxide generation) (Sintzel et al., 1998). The use of antioxidants combined with the inclusion of the active agent in its solid form has been proposed as a succesful technological strategy to promote protein stability during sterilization (Mohanan et al., 2012; Checa-Casalengua et al., 2012).

3. **Technological strategies to optimize the drug release from microspheres**

As therapy must be directed to personalized medicine, the characteristics of the formulation must cover the particular requirements of the patient. With this idea, several technological approaches have been employed to optimize intraocular microparticulate formulations. The inclusion of small molecules or biotechnological products as solids in the particles has proven to maintain their stability (Herrero-Vanrell et al., 2000; Checa-Casalengua et al., 2011). As most of the pathologies affecting the posterior segment are multifactorial, the co-encapsulation of two or more active substances results especially attractive (Herrero-Vanrell et al., 2009). Several excipients are useful to adjust the release of the drug to the therapeutic requirements. They can remain inside the particles or be eliminated after manufacturing. Special attention has to be paid in the first case, as the substances must be well tolerated. Recently, the use of additives with pharmacological properties is one of the most promising approaches to optimize intraocular microparticle formulations.

3.1. Hydrophilic additives in microspheres

Hydrophilic additives have been employed to increase the encapsulation efficiency. Lysozime as a model protein was encapsulated in biodegradable microspheres and the effect of different additives (amphiphilic stabilizer, basic salt and lyoprotectant) was evaluated. The highest encapsulation efficiency was observed by using $NaHCO_3$ (15-94%) although the in-vitro release characteristics were worsened. Cooperative effects in

terms of encapsulation of lysozime were described with the use of rat serum albumin (RSA), sucrose and $NaHCO_3$ as additives during the microencapsulation procedure (Srinivasan et al., 2005).

Adjuvants such as polyethylene glycol (PEG 1000), pluronic F68 or gelatin increased the drug release rate from PLGA microspheres destined to intravitreal administration. Addition of PEG 1000 (30%) or pluronic F68 (3%) accelerated the release of cyclosporine (CyS) from PLGA microspheres (Mw 15,000 g/mol; 75:25) prepared by a extraction/solvent evaporation method. In this study, pluronic F68 increased the release of CyS more significantly and maintained the structural integrity of particles after 2 months of the release experiment (He et al., 2006). Martinez Sancho et al. (2004a) incorporated gelatin in the aqueous phase of the O/W emulsion during the preparation of acyclovir loaded PLGA microspheres to produce a higher drug release rate. The optimizacion of the formulation was performed applying a two-factor level experimental design. The analyzed variables were the amount of the drug included in the formulation and the gelatin added to the continuous phase of the emulsion. The best formulation according to the results was prepared with a drug:polymer ratio of 2:10 and adding gelatin to the aqueous phase (final concentration 0.08%). Microspheres released acyclovir at a constant rate for 63 days (1.73 ± 0.08 µg acyclovir/day/mg microspheres). Thanks to the addition of gelatin, the optimized formulation reduced by 40% the theoretical dosage of microspheres to be administered with respect to initial studies.

3.2. Lipophilic additives in microspheres

Oily compounds have been included as additives in the microspheres to modulate the drug release rate. If microparticles are prepared by the solvent extraction/evaporation emulsion technique, the lipophilic substance is added to the organic phase of the emulsion, remaining inside the particles after maduration. In the past decades, our research group has been evaluating the inclusion of oily additives not only to control drug release but also to improve the technological properties of microspheres. They are able to promote an increase of drug encapsulation efficiency, extend the release of the active substances and even protect a biological product from degradation (Herrero-Vanrell et al., 1998; Martinez-Sancho et al., 2003; Barcia et al., 2005; Checa-Casalengua et al., 2011; Checa-Casalengua et al., 2012).

3.3. Additives with pharmacological properties

An additional advantage of using adjuvants is that some of them own

pharmacological properties that make them interesting in the ophthalmic therapy. This is the case of retinoic acid (vitamin A) and the vitamin E with antiproliferative and antioxidant properties that have already been included in the microspheres. Inclusion of Vitamin A in acyclovir PLGA 50:50 microspheres resulted in a more prolonged release of the drug (Martinez-Sancho et al., 2006). In the case of biological products, vitamin E was used in combination with the Glial Cell-line Derived Neurotrophic Factor (GDNF) (Checa-Casalengua et al., 2011) for glaucoma treatment. Under the technological point of view, the addition of vitamin E increased the encapsulation efficiency of GDNF, protected the biotechnological product from degradation and extended the release of the active substance.

4. Syringeability and injectability of microspheres

Syringeability and injectability are key-product performance parameters of intraocular dosage forms. The former refers to the ability of an injectable therapeutic to pass easily through a hypodermic needle or transfer from a vial prior to an injection, while the latter is related to the performance of the intraocular formulation during injection. Properly syringeability and injectability assures to administer the prescribed dose of the active substance. Microspheres are injected as a conventional suspension so special care must be taken in the preparation of homogenous particle dispersion in the clinical practice. Injectability studies of microsphere suspensions are directed to select the optimal diameter and length of needles, by calculating the force recorded to inject the selected concentration of particles. The application of a maximum ejection force of 12 Nw over 10 seconds is considered suitable for a proper injection. The vehicles used to resuspend microspheres can influence the time of particle precipitation. BSS or isotonic phosphate buffer solutions pH 7.4 are mainly used as vehicles. Solutions composed of viscosity enhancers can also be employed to delay the clumping of particles. If microspheres clogging occurs, biopolymers such as hydroxypropylmethylcellulose or hyaluronic acid added in the aqueous vehicle help improve both syringeability and injectability (Herrero-Vanrell and Refojo, 2001; Gomes do Santos et al., 2006). These pseudoplastic polymers are commonly used as surgical aids in ophthalmology. Moreover, these polymeric solutions are transparent and biocompatible, being rapidly diluted in the intraocular fluids and eliminated from the eye (Chan et al., 1984; Herrero-Vanrell et al., 2001).

Rojas et al. (2013) analyzed the influence of the time elapsed between the preparation of the microsphere suspension and the injection on the clumpling of polyesteramide derivative particles for intraocular administration. With this objective,

suspension of particles were released through different needles gauges (25G, 27G, 30G and 32G) and quantified in a Nebauer chamber cell counting. According to authors, the clumping of the particles had a clear relation with the time, being greater at 30 minutes after suspension preparation. No significant differences were observed in the amount of microspheres released through the different needle gauges. Martinez- Sancho et al. (2004a) evaluated the injectability of sterilized acyclovir PLGA 50:50 microparticles (15,000 g/mol) (20-40 μm) by injecting them through different gauge needles (27G, 25G, and 21G) employed in the clinical practice. The results of syringeability indicated neither partial nor complete blockage of the suspension flow. Authors concluded that these microspheres were suitable for intraocular injection through a 27-gauge needle. Suprachoroidal administration of microspheres requires more sophisticated technology. Patel et al. (2011), have evaluated the use of microneedles for suprachoriodal injection of nanoparticles (20-1000 nm) and microparticles (10 μm). These devices penetrate only a few hundred micrometers into the sclera (Patel et al., 2012). Parameters such as microneedle length, pressure and particle size are critical parameters to render optimal delivery into the suprachoroidal space.

5. **Determination of the amount of microspheres for injection**

The amount of microspheres to inject for the treatment of vitreoretinal diseases depends on the therapeutic window of the drug, its intravitreal pharmacokinetic as well as on the drug payload in the particles and its release kinetic (Barcia et al., 2009). Theoretically, the amount of microspheres (A) to be intravitreally administered can be calculated through the following pharmacokinetic equation:

$A \times K_0 = Css \times Cl$

Where Css is the steady state concentration of the drug in the vitreous that must be achieved and maintained for therapeutic efficacy. Cl is the drug clearance in the vitreous. Cl is a product of Ke (drug elimination constant in the vitreous) and Vd (vitreous distribution volume). K_0 is the theoretical zero-order drug release rate per mg of microspheres.

If microspheres are administered by another route different than the direct deposit of the formulation in the vitreous cavity, the amount of microspheres for injection can be calculated by using pharmacokinetic modelling. In this case, different ocular tissues (sclera, choroid, retinal pigment epithelium and vitreous) must be introduced in the equation, also taking into account the clearance of the active substance via choroidal circulation (Ranta and Urtti, 2006).

6. **Tolerance of microparticles for intraocular administration**

Tolerance studies for microspheres can be performed in-vitro and in-vivo (both in animals and humans).

6.1. In-vitro tolerance studies

In-vitro tolerance studies give preliminary information about the response of cells to formulations and avoid the use of experimental animals in the first stages of a pharmaceutical product development. Immortalized retinal pigment epithelial cell lines and primary cultures of retinal cells are the most employed in formulations destined to intravitreal administration (Szurman et al., 2009). Other cells lines with high sensitivity are also used to test tolerance of ophthalmic formulations. This is the case of peritoneal macrophages used to study the in-vitro tolerance of polymers and microspheres for intraocular drug delivery (Andrés-Guerrero et al., 2013). Recently, ex-vivo models of retinal diseases have been used to test tolerance of polymers and particles (Arango-Gonzalez et al., 2013).

6.2. Tolerance studies in animals

Local administration of microparticles involves mainly intravitreal and periocular (sub-Tenon, subconjuntival and posterior juxtaescleral) injections (Herrero-Vanrell et al., 2001; Kompella et al., 2003; Amrite and Kompella, 2005; Ranta and Urtti, 2006; Barcia et al., 2009, Checa-Casalengua et al., 2011). Suprachoroidal administration using microneedles has been also performed (Patel et al., 2011; 2012) (figure 5).

The injection volumes usually reported are between 50-100 µL (Herrero-Vanrell et al., 2013), although higher quantities can be accommodated in periocular spaces. The toxicity and biocompatibility of the PLGA drug delivery systems have been evaluated after injection. In-vivo ocular tolerance of drug delivery systems depends mainly on their composition and site of administration.

6.2.1. Intravitreal administration

Several reactions have been described after intraocular administration of PLGA microspheres. The most frequent is the tendency of particles to aggregate by forming a whitish depot (Khooebi et al., 1991). The permanence of the aggregate is variable as the

polymer takes different times to degrade. By histological studies, a mild localized foreign body reaction was described after the administration of ganciclovir-loaded microparticles in rabbits. In the same study, histopathologic analysis at 4 and 8 weeks post-injection showed mononuclear cells and multinucleated giant cells surrounding the particles, with no involvement of the retina or other ocular structures (Veloso et al., 1997). The studies showed minimal focal disruption of the retinal architecture in eyes receiving both ganciclovir-loaded and blank microspheres. In general, the foreign body response is associated with the type of polymer and decreased with time. Particles remained at the implantation site and, according to some authors, twelve weeks after injection in rabbit eyes only pieces of microparticles could be recognized remaining at the injection site (Visscher et al., 1985; Moritera et al., 1992).

Among the clinical signs, inflammation is the most frequently described after intravitreal injection of PLGA microparticles in rabbits. This reaction is similar to the one reported for sutures made of PLGA and after intramuscular injection of particles in rabbits (Visscher et al., 1985). Inflammation signs were associated to early stages after injection and disappeared 2-4 weeks after administration. In any case, no retinal and choroidal damage were observed 35 days after administration (Giordano et al., 1995).

6.2.2. Periocular administration

No inflammatory reaction in the retina nor surrounding tissues has been associated to PLGA microspheres after periocular injection. Mild conjunctival congestion is the most frequent clinical sign reported. Periocular route allows higher amounts of particles in the injection site than intraviteral administration without noticeable adverse signs. Periocular injection in pigs of 100 mg of blank microspheres (42.5 μm) or microspheres containing 25% or 50% of the kinase inhibitor PKC412 (67.7 μm) caused mild conjunctival reaction that was similar among the three groups. There were no discernible signs of inflammation or irritation. Ten days after injection the microspheres appeared as bulges beneath the conjunctiva (Saishin et al., 2003). Similarly, administration of PLGA microspheres loaded with celecoxib did not produce signs of inflammation 60 days after administration. Furthermore, no significant changes were observed in the thickness of retinal layers between untreated rats and animals receiving the celecoxib microparticles. Visual inspection of the site of action (periocular tissue) did not reveal the presence of inflammation, including redness and edema (Amrite et. al, 2006). In case of rabbits, no adverse signs were observed after juxtaescleral injection of 5 mg of PLGA particles (blank and loaded with dexamethasone) suspended in BSS. Authors reported only

conjunctival congestion at the administration site (24 h and 2 weeks post-injection for unloaded microspheres and 24 h and 1 week for dexamethasone-loaded microspheres) and concluded that PLGA microparticles are suitable for juxtascleral injection in rabbits with no adverse affects (Herrero-Vanrell et al., 2010a).

In terms of intraocular tolerance, the nature of the polymer is critical. Furthermore, the biological response to a biomaterial depends on the physiological nature of the tissue. Rincon et al. (2005) evaluated the response to microparticles prepared from an elastin derivative poly (valine-proline-alanine-valine-guanine) (VPAVG) in different tissues. The authors reported no inflammatory response after subcutaneous injection of different amounts of particles (1.5 mg and 2.5 mg) in the hind-paw of the rat. Similar results regarding to the absence of inflammation were reported after intravitreal injection of 2.5 mg of poly (VPAVG) microparticles in which a few rabbits of the experimental group presented inflammation signs (2/11). However, at the end of the study (28 days after injection) 45% (5/11) of the animals showed tractional retinal detachment. This adverse effect was related to certain fibroblastic activity induced by the polymer. These results confirm the importance of testing tolerance in the specific ocular site.

6.2.3 Suprachoroidal administration

Nanoparticles (20 nm and 500 nm) and microparticles (1 μm and 10 μm) of fluospheres were administered into the space located between the sclera and choroids (Patel et al., 2012). The fundus of the injected rabbit eyes appeared normal with no inflammation or abnormalities as compared with uninjected eyes.

6.3. Tolerance studies in humans

6.3.1. Intravitreal administration

Cardillo et al. in 2006 reported a preliminary study of the potential use of PLGA microspheres for the treatment of macular edema. Intravitreal sustained-release triamcinolone microspheres system (RETAAC) was injected in human eyes. In this study, authors reported a good tolerance for the particles after their intravitreal injection. Although special concern is related to the risk of visual-impairment after intravitreous injection of microparticles, this preliminary investigation in humans has shown the opposite. The tendency of the microspheres to aggregate and condensate at the site of the injection leaving a free visual axis was reported after clinical evaluation in patients receiving the treatment. No inflammation signs were reported although it should be taken into account that, in this case, the anti-inflammatory substance could attenuate the inflammatory reaction.

6.3.2. Periocular administration

Paganelly et al. (2009) injected periocularly 2 mg of PLGA (50:50) microspheres (mean size 1.07 ± 0.35 µm) loaded with cyprofloxacin (0.99 mg) in a combination of a solution of 25 mg of triamcinolone acetonide in humans. The combined treatment was compared with the topical administration of prednisolone (1%) and ciprofloxacin (3%) eye drops during 4 weeks. The safety of both treatments was evaluated (intraocular pressure, biomicroscopy, and ophthalmoscopic findings) resulting in the same ocular tolerance for both pharmacological therapies after cataract surgery.

7. Movement of particles after injection

The movement of particles after intravitreal and periocular injection has been studied in different animal models and humans.

7.1. Intravitreal administration

PLGA microparticles are not expected to move as they have the tendency to aggregate several days after their intravitreal injection, which has been previously observed in animals and humans (Giordano et al., 1995; Herrero-Vanrell et al., 2001., Cardillo et al., 2006; Barcia et al.,2009) (figure 6). In the case of intravitreal injection, the influence of the presence and absence of the lens on the movement of microspheres has been described in rabbits. Intravitreous injected microparticles (7-10 micrometer size) were retained in the vitreous cavity in phakic eyes while some particles moved to the anterior chamber in aphakic eyes (Algvere and Bill 1979).

7.2. Periocular administration

In the case of periocular injection, the size of particles affects their ocular distribution. After subconjunctival injection in rats, fluorescent polystyrene particles in the nano- size (20 and 200 nm) and micro-range (2 µm) behaved differently depending on their size. While particles higher than 200 nm were retained in the site of injection up to 60 days, lower sizes were able to move across the sclera and were rapidly cleared by the systemic and lymphatic circulation (Amrite and Kompella, 2005).

8. **Degradation of PLA and PLGA microspheres**

PLGA is amorphous and, in general terms, its degradation rate is faster than the one observed for the more crystalline PLA polymer. For example, the 50:50 PLGA has shorter half-life than the 75:25 PLGA, and this one degrades faster than PLA (Li, 1999). The degradation of these polymers takes place by hydrolysis of its ester linkages in the presence of water (Giordano et al., 1995). Among the polymers with the same composition, the lower molecular weight of the polymers and copolymers the faster the degradation rate (Herrero-Vanrell and Refojo, 2001).

8.1. In-vitro degradation of microspheres

Degradation of microspheres depends on the polymer properties, the possible interaction among components and on the characteristics of the microparticles. Moritera et al. (1991) demonstrated the influence of polymer composition and molecular weight of PLA and PLGA polymers on the release rate (in-vitro and in-vivo) of microspheres (50 μm) loaded with 5-fluorouracil (5-FU). Not surprinsingly, PLGA (3,300 g/mol) microspheres showed a faster in-vitro release (98% of the encapsulated drug) in only 2 days, while the 3,400 g/mol and 4,700 g/mol PLA particles took almost 7 days to release 85% and 70% of the 5-FU, respectively. It is interesting to note that the presence of the drug may affect degradation time of the particles (Visscher et al., 1985; Maulding et al., 1991). The influence of the acidic or basic properties of the active substance encapsulated in the microparticles in the enhancement of the hydrolytic degradation of the polymers has been reported (Delgado et al., 1996; Li 1999). Morphology change studies in microspheres have been performed during the in-vitro release assays. Checa-Casalengua et al. (2011) observed no changes in the morphology of GDNF loaded microspheres after 2 weeks of the release study. However, surface erosion of the particles appeared at 4 weeks. The erosion resulted more evident and particles began to aggregate after 6 weeks of in-vitro incubation, being completed after 8 weeks of the assay (figure 7).

8.2. In-vivo degradation of microspheres

Degradation of erodible microparticle systems after injection depends on their characteristics (i.e. size, structure, drug loading) as well as on the polymer properties. The amount and the size (total surface area) of the microspheres also govern the degradation rate. Smaller size microparticles degrade faster than larger sizes (Herrero-Vanrell et al., 2001; Grizzi et al., 1995). As mentioned previously, experience has

demonstrated that the PLA and PLGA polymers suffer biodegradation. The rate of polymer biodegradation (in-vitro and in-vivo) depends on PLA:PGA ratio and molecular weight of the polymer (higher molecular weights degrade slower than low molecular weight polymers) (Miller et al., 1977). Furthermore, surgery procedures have shown to accelerate microparticle clearance. As an example, Moritera et al. (1991) studied the influence of vitrectomy in rabbits. Clearance from the vitreous cavity was accelerated in animals that underwent vitrectomy. According to authors, particles gradually reduced their size faster in vitrectomized eyes. On the other hand, when Giordano et al. (1995) evaluated the biodegradation and clearance time of unloaded microspheres of a relatively low molecular weight polymer (inherent viscosity 0.2 dL/g) from the vitreous cavity in rabbits after gas vitrectomy, they found evidence of the microparticles up to 24 weeks postinjection.

9. Microspheres as therapeutical tools for the treatment of vitreoretinal diseases

Microspheres intended for the treatment of posterior segment diseases have been injected by periocular or intravitreal route. Although several studies have been conducted employing the topical route, there is no evidence yet of effective drug concentrations in the vitreous after this administration route.

Depending on their potential use, PLGA microparticles have been loaded with different drugs. Microspheres destined to the treatment of proliferative retinopathy have been prepared with adriamycin and retinoic acid, anti-inflammatory and immunosuppressants (dexamethasone and cyclosporine) were tested for uveitis, budesonide and celecoxib for diabetic retinopathy, triamcionolone acetonide for macular edema, acyclovir for herpes infection, ganciclovir for cytomegalovirus retinitis, neurotrophic factors such as GDNF for neuroprotection in glaucoma, anti-endothelial growth factor agent (anti-VEGF) for age-related macular degeneration (AMD), tauroursodeoxycholic acid (TUDCA) for photoreceptor rescue in retinitis pigmentosa, the protein kinase C (PKC412) inhibitor for choroidal neovascularization (CNV), a combination of steroids (triamcinolone) and antibiotic agents (cyprofloxacin) to prevent ocular inflammation and infection after cataract surgery and antisense TGF-β2 phosphorothioate oligonucleotides to prevent post-surgical fibrosis.

9.1. Proliferative Vitreoretinopathy (PVR)

Microparticles intended for the treatment of PVR have been loaded with different

drugs with antiproliferative activity (Moritera et al. 1991; 1992).

Low molecular weight PLA (3,400 g/mol) microspheres (50 µm size) loaded with adryamicine (1%) were intravitreally administered in a rabbit model of PVR and in healthy animals. The study tried to compare the administration of the active substance in solution with a long-term release of the drug (Moritera et al., 1991). Suspensions of microspheres (10 mg and 3 mg) were injected. A decrease of retinal detachment (RD) from 50% to 10% in PVR was observed in rabbits 4 weeks after administration of 10 mg of adryamicine-PLGA microspheres. On the contrary, 3 mg of PLA microspheres containing 3 µg of adryamicine was not able to decrease the rate of retinal detachment. Authors found a significant decrease in retinal toxicity of the injection of microspheres in comparison with the administration of the same amount of drug in solution with neither histological abnormalities nor electrophysiological changes in the treated eyes. The same authors observed a faster clearance of the drug and the microspheres in pathologic eyes compared with healthy eyes.

Giordano et al. (1993) studied the intravitreous release of retinoic acid (RA) in a rabbit model of PVR caused by a lipopolysaccharide (LPS) injection. Microspheres released the drug in-vitro for 40 days. In-vivo studies showed that the incidence of tractional detachment 2 months after the administration of 5 mg of RA-loaded microspheres with a dose of 110 µg of RA was effectively reduced when compared to blank microspheres.

9.2. Uveitis

The term “uveitis” is used to denote any intraocular inflammatory condition without reference to the underlying cause (Rodriguez et al., 1996). Corticosteroids have proven efficient anti-inflammatory activity for the treatment of acute ocular inflammations such as uveitis. Intravitreal injections of steroids provide therapeutic drug levels (Gaudio, 2004) but only for short periods of time. Due to the short half-life of corticosteroids the maintenance of effective intravitreal concentrations in the target site is difficult to attain (Kwak and D´Amico, 1992).

Barcia et al. (2009) developed dexamethasone-PLGA microspheres (53 µm) for intravitreal administration to prevent intraocular inflammation in an animal model of uveitis. To this, 10 mg of PLGA 50:50 (0.2 dL/g) microspheres containing 141 µg of dexamethasone/mg microspheres were suspended in 0.1 mL of isotonized PBS and injected in an animal model of inflammation. One week before the administration of particles a lipopolysaccharide (LPS) injection was performed. At one day and one week after microsphere injection, the intraocular inflammation caused by LPS injection

resulted significantly lower in treated animals compared to the group receiving blank particles. In the same study, a second injection of LPS was performed to simulate secondary uveitis (30 days after microspheres injection). No inflammation signs were observed in the animals treated with PLGA microspheres loaded with the anti-inflammatory agent after a second LPS injection, demonstrating that an effective concentration of the drug was still present. He et al. (2006) prepared cyclosporine (CyS) PLGA (75:25) of low molecular weight (15,000 g/mol) microparticles (50 μm) intended to treat uveitis and other intraocular immune disorders. Microparticle formulations maintained sustained therapeutic concentrations of CyS for at least 65 days in the choroid-retina and iris-ciliary body of healthy rabbits. The mean residence time of the drug included in the microspheres resulted 10 times higher than the CyS administered in solution.

9.3. Diabetic Rethinopathy (DR)

The evaluation of microparticulate carriers loaded with budesonide and celecoxib has been assayed for the treatment of diabetic retinopathy. Comparison between nano- (50 μg) and microparticles (75 μg), loaded with budesonide were performed after subconjunctival injection of particles in rats. In this study, microparticles (3.60 ± 0.01 μm) delivered the active substance in a more sustained fashion than nanoparticles (345 ± 2 nm) (Kompella et al., 2003). According to authors the nanosystems were removed more rapidly from the subconjunctival site of administration than microparticles. Nanoparticles, microparticles and budesonide in solution (75 μg) were administered in rat eyes. Different tissue levels (retina, vitreous, lens and cornea) were compared at different times after administration. On day 7 and 14 drug levels in the eyes treated with microspheres resulted higher compared with the solution and nanoparticles. Sustained release of celecoxib from PLGA (85:15) microspheres (1.11 ± 0.08 μm) was evaluated in a diabetic rat model (Amrite et al., 2006). A posterior subconjunctival injection of 0.05 mL of celecoxib-microsphere suspension (14.93% ± 0.21%) was usuful to inhibit diabetes-induced elevations in PEG2, VEGF and blood-retinal barrier leakage.

9.4 Macular edema (ME)

Macular edema (ME) is frequently treated with corticosteroids. Among them, triamcinolone is the most used.

9.4.1 Intravitreal administration

As previously cited, one of the first evaluations of PLGA microspheres in humans has been performed in patients suffering diffusse macular edema (Cardillo et al., 2006). Eyes treated with triamcinolone-loaded microspheres and showed marked decrease of retinal thickness as well as improved visual acuity for 12 months.

9.4.2 Periocular administration

Microspheres loaded with betamethasone appear under phase II/III clinical trial for the treatment of diabetic macular edema (Yasukawa et al., 2011). The microspheres are intended for sub-Tenon injection.

9.5 Acute Retinal Necrosis (ARN)

Viral infection is related to necrosis of retinal cells that can lead to irreversible blindness. Therapy for ARN usually involves intravenous or intravitreal administration of acyclovir. Although intravitreal administration of acyclovir has demonstrated to be more effective than the intravenous administration of the drug with fewer side effects, the relatively high dose required has untoward effects. Conte et al. (1997) developed controlled release microparticles from PLA and PLGA polymers. Particles were loaded with acyclovir using the spray-drying technique. The formulations were tested as an alternative to intravenous or intravitreous administration of the drug in solution in the treatment of acute retinal necrosis caused by virus injection. The drug was detected in the rabbit vitreous for 14 days after injection of D,L-PLA (28,000 g/mol) microparticles (0.5 mg) of 25 μm. Chowdhury and Mitra (2000) have described guanosine-loaded PLGA (75,000–100,000 g/mol) microspheres developed for a drug release of 1 week after intravitreal injection of the particles.

9.6 Cytomegalovirus retinitis (CMV)

CMV retinitis occurs in immunodeficiency patients and its progression can result in blindness from retinal detachment associated with retinal necrosis (Jab et al., 1989; Henry et al., 1987). Although intravitreal ganciclovir injections provide effective intraocular drug concentrations, frequent injections are required to maintain therapeutic drug levels.

With the objective to avoid frequent injections, Veloso et al. (1997) tested the antiviral effect of ganciclovir released from PLGA microspheres (300-500 μm) in rabbit eyes inoculated with the human cytomegalovirus (HCMV). Injection of ganciclovir-loaded microspheres (10 mg) prepared from PLGA 50:50 (inherent viscosity 0.39 dL/g) with 86.4 μg of ganciclovir/mg microspheres, controlled the progression of the disease in the HCMV-inoculated rabbit eyes. In treated eyes, vitritis, retinitis and optic neuritis decreased during the 14 days of the study, in contrast with control eyes. Immunofluorescence of virus antigen was not observed in treated eyes. Duvvuri et al. (2007) injected PLGA (different ratios of lactic: glycolic acid) microspheres prepared with ganciclovir and dispersed in a thermogelling solution of PLGA-PEG-PLGA in rabbit eyes. 60 μL of the microparticle formulation (196 μg of ganciclovir) was administered in rabbits. The formulation maintained mean vitreal concentrations of ganciclovir at approximately 0.8 μg/mL for 14 days, whereas direct injections maintained drug levels above 0.8 μg/mL for only 54 hours.

9.7. Choroidal Neovascularization (CNV)

PLGA microspheres loaded with a kinase inhibitor PKC412 were periocularly injected in a porcine model of laser induced choroidal neovascularisation (CNV) obtained by laser photocoagulation (Saishin et al., 2003). After the rupture of Bruch's membrane in eight locations, 100 mg of microspheres were injected in the animals. Microspheres loaded with different amounts of PCK412 (25 or 50%) were compared to blank microspheres. After 10 days, the areas of CNV at Bruch's membrane rupture sites were noticeable lower for PCK412 microspheres. PCK412 levels in vitreous, retina and choroids were detected 20 days after periocular injection for the 50% PCK412 loaded microparticles.

9.8. Degenerative diseases affecting the optic nerve. Glaucoma.

Neuroprotection has been proposed as a therapeutic option for the treatment of glaucoma. The therapeutic approach focuses on promoting the survival of retinal ganglion cells (RGC) which can be achieved by the local sustained delivery of neurotrophic factors such as Glial cell line-Derived Neurotrophic Factor (GDNF). Jiang et al. (2007) investigated the potential of GDNF-loaded microspheres using the hypertonic saline model. In this work microspheres remain in the vitreous for at least six weeks. At 8 weeks following a second hypertonic saline injection, in retinas treated with

GDNF microspheres, the authors reported a decrease in nerve head cupping and an increase in the thickness of nerve fiber and inner plexiform layers. Xiao and Zhang, (2010) described a significant increased long-term retinal ganglion cell survival in the DBA/2J mouse glaucoma model after injection of microspheres loaded with GDNF. Checa-Casalengua et al. (2011) evaluated the efficacy of long-term delivery of low amounts of GDNF in an animal model of glaucoma. Microspheres were prepared from PLGA 50:50 (Mw 35,000 g/mol) and GDNF combined with vitamin E (vit E). The effects of GDNF/VitE microspheres on RGC survival were evaluated 10 weeks after injection by counting anti-NeuN positive cells in the ganglion cell layer (GCL) (figure 8). In eyes without IOP elevation, the average number of anti-NeuN positive cells were 67.4 $\pm$ 4.2/mm. After ten weeks of IOP elevation, a significant loss of the RGC (20.8 $\pm$ 2.1/mm) was observed for blank particles. GDNF/Vit E microspheres (0.64 ng GDNF/eye) resulted in a preservation of RGC (51.6 $\pm$ 3.2/mm). The positive effect of the novel GDNF/VitE formulation on the optic nerve axon survival was also demonstrated after comparing the number of axons in elevated IOP eyes compared to the number of axons in the contralateral eye without IOP elevation. The survival percentaje resulted significantly higher (72.68%) for GDNF/VitE microspheres compared to GDNF (36.58%), Vit E (30.65%) and blank microspheres (28.96%) groups. The neurotrophic factor in combination with Vit E released from the microspheres was effective for at least 10 weeks after a single intravitreal injection in an animal model of glaucoma. These results confirm the hypothesis that low amounts of GDNF maintained for a long time resulted effective in a chronic degenerative disease such as glaucoma.

9.9. Photoreceptors degeneration. Retinitis pigmentosa (RP).

Retinitis pigmentosa is an inherited disease that brings on retinal degeneration. This degenerative pathology causes vision impairment and often blindness. Patients undergo a gradual loss in vision because of the death of the rods and cones.

Systemic high dose of tauroursodeoxycholic acid (TUDCA) has demonstrated to prevent degeneration of photoreceptors in rd10 mice and P23H rat retina. PLGA microspheres (2-40 μm) loaded with TUDCA (drug: polymer ratio 2:10) were intravitreally injected in homozygous P23H line 20 days old rats. Rats received phosphate buffer solution (5% w/v) in the right eyes as control. Retinal function was assessed by electroretinogram at P80, P100 and P120. Scotopic a- and b-wave amplitudes were analyzed. Scotopic light-induced retinal responses registered at P80 showed a- and b-wave mean amplitudes significantly higher in TUDCA-PLGA injected eyes compared with vehicle-injected. Maximal differences were observed at the maximal amplitude

responses both in a- and b-waves. Significant differences in a- and b-wave amplitudes were not found at P100 and P120. This work suggested that PLGA microspheres loaded with TUDCA have a potential neuroprotective effect that could be useful to delay vision loss in retinitis pigmentosa (Herrero-Vanrell et al, 2011c).

Andrieu-Soler et al. (2005) developed PLGA microspheres loaded with recombinant human glial cell line-derived neurotrophic factor (rhGDNF). The particles controlled the release of the biotechnological product (10 ng/day) for 3 months and resulted efficient preventing retinal degeneration in an animal model of retinosis (rd1) for 17 days. A significant delay of rod photoreceptor degeneration was observed in mice receiving the rhGDNF-loaded microspheres compared to either untreated mice or mice receiving blank or inactivated rhGDNF microspheres.

9.10. Age-Related Macular Degeneration (AMD)

Therapeutic angiogenesis via local delivery of protein drugs is one of the approaches to treat exudated age related macular degeneration. Anti-vascular endothelial growth factors (bevacizumab, ranibizumab and aflibercept) have been employed to reduce the neovascularisation in the eye (van Wijngaarden et al., 2008). Carrasquillo et al. (2003) prepared PLGA microspheres containing anti-vascular endothelial growth factor (anti-VEGF) RNA aptamer. The biotechnological product was encapsulated after freeze-drying with and without trehalose. In these conditions the inhibition showed by the aptamer was, in general, enhanced when it was co-lyophilized with trehalose. The microspheres released the aptamer in an average rate of 2 µg/day. The particles were loaded into a device and placed on the sclera of Dutch belted rabbits. The feasibility of delivering the bioactive anti-VEGF aptamer in a controlled manner was demonstrated.

9.11. Prevention of intraocular inflammation, infection and post-surgical fibrosis after cataract surgery

Ocular inflammation and infection after cataract surgery can be prevented with a co-administration of steroids and antibiotic agents. A treatment including PLGA ciprofloxacin-loaded microspheres and a solution of triamcinolone was compared with the topical administration of prednisolone (1%) and ciprofloxacin (3%). Efficacy of both treatmens was evaluated after 4 weeks (anterior chamber, cell and flare, conjunctival erythema, ciliary flush or symptons of ocular inflammation). The authors stated the same therapeutic response with both pharmacological therapies after cataract surgery

(Paganelli et al., 2009).

Gomes do Santos et al. (2006) prepared nanosized complexes of antisense TGF-β2 phosphorothioate oligonucleotides encapsulated in PLGA microspheres. The authors called the microparticulate systems “Trojan” microspheres. A suspension of 4-5mg of microspheres in 100 μL of a viscosizing agent (sodium hyaluronate 1.35%) was administered by subconjuctival injection in a rabbit experimental model of filtering glaucoma surgery. The authors described a prevention of post-surgical fibrosis following trabeculectomy for 42 days.

9.12. Microspheres for Regenerative Medicine. Co-trasplantation of microspheres with Retinal Progenitor Cells (RGCs).

Failure of the adult mammalian retina to regenerate can be partly attributed to the barrier formed by inhibitory extracellular matrix (ECM) and cell adhesion molecules, such as CD44 and neurocan. After degeneration, this mentioned barrier separates a subretinal graft from integrating into the host retina. Matrix metalloproteinase 2 (MMP2) can promote host-donor integration by degrading these molecules. Retinal combination of PLGA microspheres loaded with MMP2 and retinal progenitor cells (RPCs) have been assayed to enhance cellular integration and promote retinal repopulation (Yao et al., 2010). To this purpose, PLGA microspheres (2-20 μm) loaded with MMP2 and RPCs were co-transplanted to the subretinal space of adult retinal degenerative Rho−/− mice. Highly porous microspheres loaded with MMP2 were prepared, as fast release of the bioactive agent was needed. Following the delivery of MMP2 from microspheres, significant degradation of CD44 and neurocan at the outer surface of the degenerative retina without disruption of the host retinal architecture was observed. Furthermore, no changes in the differentiation characteristics of RPCs were shown due to the microspheres. The results suggest the co-transplantation of MMP2 microspheres and RPCs as a practical and effective strategy for retinal repair.

10. Current and future developments

Local administration of microparticulate systems can be considered as a therapeutic strategy to overcome the limitation of systemically administered drugs. The main goal in the development of microparticles for the treatment of retinal diseases and other intraocular pathologies has been to obtain long-acting injectable drug formulations and specific targeting options of the therapeutic molecule. Biodegradable microspheres represent an effective alternative to repeated intraocular injections. Administration of

microparticles is performed easily after their suspension in an aqueous vehicle. Injection of microparticles is performed without the need for surgical procedures. As most of the diseases affecting the back of the eye are chronical and multifactorial, microspheres are especially promising in this field. They can be loaded with more than one active substance and complemented with the inclusion of additives with pharmacological properties. Personalized therapy can be easily achieved by changing the amount of administered microspheres. After injection, PLA and PLGA microspheres suffer aggregation, behaving like an implant. Contrary to the non-biodegradable devices, PLA and PLGA microspheres disappear from the site of administration after delivering the drug. Furthermore, microspheres prepared from the aforementioned biomaterials are well tolerated after periocular and intravitreal injections in animals and humans. Biodegradable microspheres are also potential tools in regenerative medicine for retinal repair. According to the results reported, presumably a variety of microparticulate formulations for different ophthalmic therapeutic uses will be available in the clinical practice in the near future.

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FIGURE CAPTIONS

Figure 1. Strategies to avoid frequent intraocular administrations. Drug delivery systems: biodegradable and non-biodegradable implants and biodegradable microspheres.

Figure 2. Structure of microparticles (microspheres and microcapsules). In the microspheres, the active substance is dispersed in a polymeric network (matrix system), while microcapsules are formed by a core containing the drug, which is surrounded by a layer composed of a polymer or a mixture of several polymers (reservoir system).

Figure 3. According to the solvent extraction/evaporation method, microparticles are prepared from O/W, W/O/W or S/O/W emulsions. O/W: The polymer is first dissolved in a volatile solvent in which the drug is incorporated. This solution is then emulsified with a non-miscible solvent to form microparticles. W/O/W: A first emulsion (W_1/O) is formed between anadjuvant aqueous solution of the active substance and an organic polymeric solution. A second emulsion ($W_1/O/W_2$) is then prepared by the addition of the W_1/O emulsion to an aqueous external phase. S/O/W: The active substance in solid state is dispersed in an organic polymeric solution (S/O) and then added to an aqueous external phase to form microparticles. In all cases, microparticles are formed in the presence of an emulgent to ensure their formation and maintenance during the process. After the organic solvent removal, mature particles are recovered by filtration and dried.

Figure 4. PLGA (50:50, Resomer® 502) microspheres loaded with dexamethasone prepared by a solvent extraction/evaporation method from an O/W emulsion.

Figure 5. Local administration of microspheres for the treatment of retinal and other intraocular diseases: intravitreal, subconjuntival, subretinal, sub-Tenon and suprachoroidal injections.

Figure 6. Eye fundus photographs obtained at 40 days after the injection of 10 mg of unloaded (B) and dexamethasone-loaded PLGA microspheres (C) in an animal model of uveitis. An untreated group was used as control (A). From Barcia et al., 2009, with permission of Elsevier.

Figure 7. In-vitro release rate of GDNF from PLGA microspheres (ng GDNF/mg microspheres) and SEM images of particles at different time points of the study. During the first 2 weeks of incubation, particles kept a smooth surface. After 4 weeks, the erosion of the surface of microparticles was evident, and 2 weeks later they started to aggregate. After 8 weeks of the release assay, microparticles were completely aggregated.

Figure 8. GDNF/Vit E microspheres (0.64 ng GDNF/eye) increased retinal ganglion cells survival and axonal integrity in an animal model of glaucoma. A-E: effects of GDNF/VitE microspheres treatment on the survival of RGCs. (A) Normal retina without IOP elevation. GDNF/Vit E microspheres treatment (B) resulted in the preservation of RCGs compared with GDNF (C), Vitamin E (D) and blank microspheres (E). From Checa-Casalengua et al., 2011, with permission of Elsevier. F-O: effects of GDNF/Vit E microspheres treatment on axon survival due to chronic IOP elevation: normal ON axons without IOP elevation (F). GDNF/Vit E microspheres treatment (G) resulted in a preservation of axons compare with GDNF (H), Vit E (I) and blank microspheres (J). Corresponding representative EM photos (K-O). F-J: magnification 1000×; K-O: magnification 7100×. Quantitative analysis of GDNF/Vit E microspheres treatment on the survival of RGC (P). Chronic IOP elevation resulted in a significant loss of the RGC ($p < 0.01$). GDNF/Vit E microspheres treatment significantly increased the RGC survival compared GDNF, Vit E and blank microspheres treatment ($p < 0.01$). Quantitative analysis of GDNF microspheres treatment on the ON axon survival (Q). GDNF/Vit E microspheres treatment increased the survival percentage compared with GDNF, Vit E, and blank microspheres treatment ($p < 0.01$). From Checa-Casalengua et al., 2011. With permission of Elsevier.