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Corresponding Author: Dr. laura mayol,

Corresponding Author's Institution: University of Naples Federico II

First Author: laura mayol

Order of Authors: laura mayol; Teresa Silvestri; Sabato Fusco; Assunta Borzacchiello; Giuseppe De Rosa; Marco Biondi

Abstract: Posterior eye segment diseases are treated through monthly intravitreal injections, that evoke serious side effects. A promising approach to reduce injection frequency consists in producing biodegradable microspheres (MPs) releasing the protein in the vitreous body for long times. Moreover, a rational design of these MPs requires a discouraged diffusion/sedimentation within the intravitreal space, which are detrimental for the vision and the control over drug release kinetics. In this work, poly(lactic-co-glycolic acid) (PLGA)-based MPs encapsulating bovine serum albumin (BSA) were coated with hyaluronic acid (HA) at two molecular weights and tested for their release, diffusion and degradation features in simulated vitreous body (SVB). Results indicate that HA corona prolongs MP degradation time and BSA release. Furthermore, HA coating increased the affinity between MPs and SVB, thereby repressing device transport compared to control PLGA MPs. Results hold promise for the possible application of HA-decorated MPs for intravitreal injection of protein drugs.

***Highlights (for review)**

- Microparticles were decorated with two different molecular weights hyaluronic acid
- Hyaluronic acid corona discourage the diffusion of microparticles in the vitreous
- The molecular weight of the hyaluronic acid influence protein release kinetics
- Hyaluronic acid molecular weight influence the diffusion of the microparticles
- Hyaluronic acid molecular weight does not affect degradation time of microparticles

***Response to Reviewers**

Dear Editor, we are grateful for the accurate and thoughtful comments to our manuscript, which allowed us to significantly improve its quality. In the following, there is a point-by-point answer to referee considerations.

Reviewer #1: The authors revised the manuscript as per the reviewer's suggestions/ recommendations. Now the manuscript can be accepted for publication in this journal.

Reviewer #2: The authors have responded to my questions in a good manner.

I have only 2 additional questions:

1. the loading capacity of the BSA in PLGA is stated at 76% in table 1, while the text states 36% efficiency. Which one is correct?
We are sorry for the oversight that has been corrected.

2. Figure 4. Temporal release trends of BSA from MPs over time: the PLGA particles show a plateau from day 20, is this corresponding to 100% release? Could you convert the Y-axis also to percentage (or discuss how the doses in the Y-axis correspond to the BSA percentage loaded in the particles) to estimate which amount of the loaded dose has been released?

As suggested by the referee, we converted the Y-axis to percentage released.

Drug micro-carriers with a hyaluronic acid corona toward a diffusion-limited aggregation within the vitreous body

Laura Mayol[1,2,*], Teresa Silvestri[1], Sabato Fusco[2], Assunta Borzacchiello[3], Giuseppe De Rosa[1,2], Marco Biondi[1,2]

[1] Dipartimento di Farmacia, Università di Napoli Federico II, Via D. Montesano 49, Napoli, Italy.

[2] Interdisciplinary Research Centre on Biomaterials – CRIB – Università di Napoli Federico II – P.le Tecchio, 80, Napoli, Italy

[3] Istituto per i Materiali Compositi e Biomedici (IMCB-CNR), Università di Napoli Federico II, Viale J.F. Kennedy , Napoli, Italy

[*]Correspondence to:

Laura Mayol, Dipartimento di Farmacia, Scuola di Medicina e Chirurgia, Università di Napoli Federico II, Via D. Montesano 49, Napoli, Italy. Mail: laumayol@unina.it

Abstract

Posterior eye segment diseases are treated through monthly intravitreal injections, that evoke serious side effects. A promising approach to reduce injection frequency consists in producing biodegradable microspheres (MPs) releasing the protein in the vitreous body for long times. Moreover, a rational design of these MPs requires a discouraged diffusion/sedimentation within the intravitreal space, which are detrimental for the vision and the control over drug release kinetics. In this work, poly(lactic-co-glycolic acid) (PLGA)-based MPs encapsulating bovine serum albumin (BSA) were coated with hyaluronic acid (HA) at two molecular weights and tested for their release, diffusion and degradation features in simulated vitreous body (SVB). Results indicate that HA corona prolongs MP degradation time and BSA release. Furthermore, HA coating increased the affinity between MPs and SVB, thereby repressing device transport compared to control PLGA MPs. Results hold promise for the possible application of HA-decorated MPs for intravitreal injection of protein drugs.

Keywords: hyaluronic acid, microparticle, PLGA, poloxamer, diffusion, ocular delivery.

1. Introduction

Posterior eye segment diseases can be treated in principle through four routes of administration, i.e. topical, systemic, intraocular and periocular (Herrero-Vanrell et al., 2001). The systemic route requires high drug doses to achieve therapeutic drug levels in the eye and this correlates with a high risk of eliciting systemic adverse effects; the topical and periocular routes are progressively gaining more attention, but the intravitreal injection is still the most employed since the active molecules are directly inserted into the vitreous, thereby overcoming the numerous biological barriers hampering the access of drugs and molecules within the eye (Gaudana et al., 2010; Falavarjani et al., 2013). Unfortunately, even if intravitreal injection is actually a very effective route of administration, it educes serious drawbacks. First, drug distribution in the vitreous is non-uniform: for example, small molecules can rapidly diffuse through the vitreous, whereas the transport of larger molecules is restricted. Besides, intravitreal injection is intrinsically associated to pernicious adverse effects, such as cataracts, retinal detachment, and hemorrhages, and the risk of their occurrence is increasing with increasing number of injections (Herrero-Vanrell et al., 2000). Consequently, the use of biodegradable controlled drug delivery systems (DDS) based on FDA-approved biodegradable polyesters such as poly(lactic-co-glycolic acid) (PLGA) for intravitreal injection is very attractive. Specifically, micron-sized particulate DDS, such as microparticles (MPs), are beneficial since they allow the co-administration of more than one active molecule, and help avoiding the surgical procedures needed to reach the intraocular target site. More importantly, biodegradable MPs can host, protect and sustain the release kinetics of the encapsulated drug(s), thereby leading to a reduction of the frequency of intravitreal injections and of the concentration peaks of the active molecule(s) in the intravitreal space, hence involving a minor risk of adverse effect insurgence (Yasukawa T et al., 2011). A personalized therapy can be easily realized by tailoring the amount of administered MPs and, hereafter, drug dosing. Also, biodegradable PLGA-based MPs are well tolerated after periocular and intravitreal injections in both animals and humans.

Indeed, the systemic toxicity of PLGA is basically negligible since the polymer is easily hydrolyzed *in vivo* to lactic and glycolic acid, which are then eliminated as carbon dioxide and water via the Krebs cycle (Danhier et al., 2012). For these reasons, presumably a variety of microparticulate formulations for different ophthalmic therapeutic uses will soon be available in the clinical practice (Herrero-Vanrell et al., 2014). After injection, PLGA-based MPs can behave like an '*in situ*-forming' implant, since they easily undergo aggregation. This has two conflicting implications: on the one side, MP aggregation at the site of the injection allows a free visual axis within the vitreous body; on the other hand, MP aggregation results into a less effective control over drug release kinetics, and this is strongly unwished for intravitreal administration. Consequently, it is desirable that MPs are separated during all the drug release phase and motionless within the intravitreal space. The vitreous body has a gel structure, it is composed almost completely of water (97%) and three main components: collagens, proteoglycans and HA. Among its macromolecular components, HA is present in high amount and it is thought to control many vitreous biophysical properties such as its viscoelastic properties that enable it to resist sudden compression shocks, offering the best protection for the retina.

Here, we present the preparation of biodegradable PLGA-based MPs, whose surface properties have been tailored to make the devices motionless within the intravitreal space. More in detail, we have decorated the external surface of these polymeric MPs with hyaluronic acid (HA). HA is a natural mucoadhesive polysaccharide, widely used in drug delivery and biomedical field, composed of alternating D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc) repeating units linked together via β-(1,4) and β-(1,3) glycosidic junctions. In aqueous solution, HA can bind and interact with water molecules and also entangle with other HA macromolecules, by that being responsible for the viscoelastic behavior of many biological fluids besides vitreous body, such as synovial fluid, tears etc (Mayol et al., 2014; Borzacchiello et al., 2010). Thus, here we have speculated that, by increasing the chemical affinity of the MP surface with the surrounding body fluid, the diffusion of

88 the MP in the vitreous body would be discouraged as a consequence of HA macromolecules ability
89 to interact and entangle with one another.

90 The MPs here produced were loaded with bovine serum albumins (BSA) and fully characterized for
91 their technological features. Then, the degradation properties of the MPs were analyzed since this is
92 a crucial feature for their ability to prolong the release of the drug into the eye which, in turn, is
93 expected to decrease the frequency of injection and reduce the adverse effects due to drug
94 intravitreal injections. Finally, the influence of HA molecular weight on MP surface on both drug
95 release kinetics and MP diffusion in simulated vitreous body was explored.

96

97 **2. Materials and Methods**

98 *2.1. Materials*

99 HA with a weight-average molecular weight (MW) of 1600 kDa and 180 kDa was provided by
100 Altergon Italia s.r.l (Italy). Equimolar uncapped poly(D,L-lactic-co-glycolic acid) (PLGA)
101 (Resomer RG504H, Mw 40 kDa, inherent viscosity: 0.16-0.24 dL g^{-1} in acetone at 25 °C) was from
102 Evonik (Germany). Poloxamers (PEO_a-PPO_b-PEO_a), which are amphiphilic triblock polymers
103 containing different numbers of oxyethylene (a) and oxypropylene (b) units, were used. In this
104 work, poloxamers F127 (a = 100 and b = 65) and F68 (a = 76 and b = 29) from Lutrol (Basf,
105 Germany) were employed. All other chemicals, i.e. bovine bovine serum albumins (BSA), ethanol
106 (EtOH), acetone, dimethylsulfoxide (DMSO), dichloromethane (DCM), poly (vinyl alcohol) (PVA:
107 Mowiol 40-88) sodium azide, Agar, rhodamine, phosphate buffer salts, sodium and potassium
108 chloridrate (PBS) were obtained from Sigma–Aldrich (USA).

109 *2.2. Microparticle production*

MPs loaded with BSA were produced with a theoretical protein loading of 0.2% (0.20 mg of BSA *per* 100 mg of microparticles), using a slightly modified double emulsion–solvent evaporation technique. Briefly, 0.25 mL of an internal aqueous phase consisting of a BSA solution (0.4% w/v) in phosphate buffer (PBS, 1.420 g of Na_2HPO_4, 0.201 g of KCl and 7 g of NaCl in 1 L of purified H_2O) were poured into 2.5 mL of a 20% w/v organic solution of PLGA/F68/F127 (1:0.5:0.5 weight ratio) in dichloromethane (DCM). When necessary, 5 μL of a rhodamine solution in DCM (0.1% w/v) were added to the organic phase to obtain fluorescent particles used in diffusion studies. The two phases were then emulsified by a high-speed homogenizer (Diax 900 equipped with a 10G probe, Heidolph, Germany; 11,000 rpm, 2 min) and the obtained water-in-oil primary emulsion was poured in 40 mL of an external aqueous phase and further homogenized at 11,000 rpm (10G probe) for 2 minutes, to obtain the final double emulsion. The external aqueous phase contained 7.5 mg of F68, 7.5 mg of F127 and 30 mg of hyaluronic acid. In the case of control PLGA MPs, the organic phase was a 20% w/v solution of PLGA in DCM, while the external aqueous phase was produced without HA. The organic solvent was then evaporated overnight under magnetic stirring (MR 3001 K, Heidolph, Germany) at room temperature for MP hardening, which were finally centrifuged (5000 rpm, 4°C), washed with distilled water three times (Universal 16R, Hettich Zentrifugen, Germany) and lyophilized for 24 h (0.01 atm, -60 ° C) (Modulyo, Edwards, United Kingdom). As a control, PLGA MPs were produced without HA. In that case, the organic phase was composed of a 20% w/v solution of PLGA in DCM, and the external aqueous phase contained F68 and F127 poloxamers.

2.3. Microparticle morphology and size

Scanning electron microscopy (SEM) (Phenom XL, Phenom World, the Netherlands) was employed to analyze microsphere external shape and morphology after production. The samples were prepared by gold-sputtering under vacuum. The mean volume and size distribution of the produced MPs were determined by light scattering (Microtrac S3500 Series Particle Size Analyzer;

Montgomeryville, PA, USA). The tests were run by dispersing lyophilized MPs in MilliQ water containing 0.5% w/v PVA, which was used as a surface active agent to prevent MP aggregation during the measurements. The results and the standard deviation have been averaged on triplicate samples.

2.4. MP yield and entrapment efficiency

MP yield was gravimetrically obtained from the entire mass of MPs recovered after lyophilization ($p < 5$ hPa, 24 h; LyoQuest, Telstar, Japan). Direct method was employed to obtain the amount of BSA loaded into the MPs, by means of Bradford assay (Noble, Bailey; 2009). More in detail, for each experiment 10 mg of MPs were added to 500 µL of dichloromethane. Then, 100 µL of PBS (pH = 7.4) were added to the obtained organic solution and vortexed for 5 s to enhance an intimate contact between the aqueous and the organic phases. Afterwards, 50 µL of the aqueous solution were withdrawn and diluted within 950 µL of the Bradford reagent for 15 min. BSA was quantified *via* spectrophotometric assay (UV-1800, Shimadzu; $\lambda = 595$ nm, Figure 3.3), employing a quartz cuvette (6030-UV, d = 10 mm, Hellma Analytics). The linearity of the response was assessed in the 0.49-500 µg/mL BSA concentration range ($r^2 > 0.98$). Results are expressed as actual BSA loading (mg of loaded BSA *per* 100 mg of MPs), and BSA encapsulation efficiency (ratio of actual and theoretical BSA loading rate × 100) ± standard deviation of the values collected from at least three different batches.

2.5. Preparation of simulated vitreous body (SVB)

Simulated vitreous body (SVB), prepared following a previously published procedure (Kummer et al., 2007). Briefly, a solution composed of 0.4 g of Agar and 100 mL of water was lead to boiling up until complete Agar dissolution. Subsequently, the hot solution was mixed with 0.5 g of HA (1600 kDa) until a homogeneous mixture was obtained. A few drops of 0.02% w/v sodium azide

158 solution were added to the obtained solution as a preservative and, then, the solution was cooled to
159 room temperature for 24h.

160 *2.6. Rheological properties of SVB*

161 The viscoelastic properties of the prepared SVB were measured by a Kinexus rotational rheometer
162 (Malvern, USA). More in detail, small-amplitude oscillatory shear experiments were performed at
163 37°C in the 0.1 to 10 Hz oscillation frequency range, and at a strain amplitude at which linear
164 viscoelasticity was attained. The dynamic viscosity was, then, measured as a function of frequency.

165 *2.7 – BSA release kinetics*

166 The *in vitro* release profile of BSA from control PLGA MPs and HA-decorated MPs in SVB was
167 evaluated by the gel poured into a vial and adding the HA-decorated MPs at a 1:20 w/v ratio. The
168 MPs and the gel were accurately mixed until a uniform suspension was obtained. Subsequently, 1
169 mL of PBS at pH 7.4 was added on the gel containing the dispersed MPs. and placed at 37°C in a
170 thermostatic bath. At predetermined time points, 500 µL aliquots of the supernatant were withdrawn
171 and replaced with fresh PBS, and pH values were verified using litmus paper. 50 µL of solution
172 were withdrawn and placed in contact with 950 µL of Bradford and quantified by
173 spectrophotometric assay, as described in Section 2.4. The linearity of response was assessed in the
174 0.5-500 µg/mL BSA concentration range ($r^2 > 0.99$). The results were expressed in terms of the
175 percentage of released BSA ± the standard deviation of three replicates.

176 *2.8. Transport studies of MPs in SVB*

177 Transport properties of MPs in SVB have been studied through fluorescence measurements.
178 Fluorescent MPs loaded with rhodamine (Rhod) were produced as described in Section 2.2.
179 Subsequently, they have been evenly incorporated in the produced SVB (2% w/v) and 200 µL
180 aliquots of this dispersion were placed in the donor chamber of a 1 mL plastic syringe, preliminarily
181 cut in five equal sections (Figure 1). Consequently, the system was composed of one donor section

and four receiving sections. Adjacent sections were separated by a nylon web (approximately 0.5 mm average mesh aperture) to enhance an even separation between the sections.

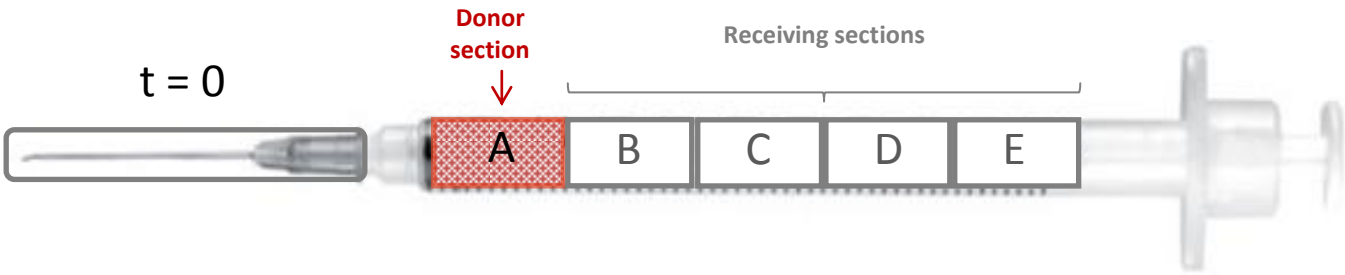


Figure 1. Schematic representation of the diffusion study apparatus.

All the syringes were kept in vertical position, with the tip accurately sealed to avoid SVB loss during the experiments. The top section of each syringe, acting as the donor compartment, contained a 4 mg/ml dispersion of Rhod-loaded MPs in SVB. Then, the syringes were placed at 37°C and, at scheduled time intervals, the syringes were withdrawn, and the single sections separated and immediately frozen at -20°C to anneal further transport. Then, the syringe was divided into single sections. The content of each section was dissolved in 2 mL NaOH 0.5 N and analyzed for Rhod content by spectrofluorimetric analysis at λ = 553 nm (Varian Cary Eclipse Fluorescence Spectrophotometer, Thermofisher, USA), using a quartz cuvette (6030-UV, d = 10 mm, Hellma Analytics, USA). The linearity of the response was assessed in the 0.125 – 2 mg/mL MP concentration range ($r^2 > 0.99$).

2.8. Mass loss kinetics of MPs

The rate of mass loss of the MP decorated with HA molecular weight equal to 180 or 1600 kDa were performed. In detail, 0,5% w/v suspensions of MPs in PBS were prepared and subsequently incubated at 37°C on a gently oscillating base (15 rpm) (Stovall Life Science Inc., USA). At scheduled time points, the samples were centrifuged (5000 rpm, 10 minutes) and the PBS

supernatant, containing the solubilized MP fractions, was withdrawn and replaced with an equal volume of fresh PBS. Then, the samples were lyophilized (LyoQuest, Telstar, Japan) and the residual weight was gravimetrically obtained, taking into account the mass of the salts, preliminarily determined by lyophilizing PBS solutions used as a control (n = 6). The results have been expressed as the mean residual mass percentage ± the standard deviations of at least three replicas.

$$100 \times \left(1 - \frac{MP\ Mass\ t_0}{Residual\ MP\ Mass}\right).$$

3. Results and discussions

The aim of this work was the development of a biodegradable MP platform designed to be motionless within the ocular vitreous body and able to sustain and control the drug release phase, thus reducing the frequency of intravitreal injections and, consequently, the occurrence of the associated risks (Adamson et al., 2016). The MPs decorated with HA were produced by a slightly modified double emulsion – solvent evaporation technique. HA was added to the external aqueous phase to promote its interaction with the organic phase containing the blend of lipophilic PLGA and amphiphilic poloxamers. During the second emulsification step, the droplets of the internal organic solution spontaneously orient the hydrophilic segments of the poloxamers towards the external aqueous phase containing HA, and this is expected to be decisive in promoting the HA arrangement on the surface of the MPs, driven by the lipophilicity gradient between the organic and the aqueous phase of the emulsion (Giarra et al., 2016), after the evaporation of the organic solvent. More in detail, HA was chosen because it is a major component of the vitreous body, and this is expected to increase MP affinity with the surrounding gel containing the same polysaccharide. In this work, it has been hypothesized that this affinity of composition between the surface of the MP and the vitreous body may hinder the diffusion/sedimentation and aggregation of MP within the ocular vitreous. A further objective of this work was to evaluate if the molecular weight of the HA used to decorate the surface can influence the degradation and diffusion of the MP in the simulated vitreous body (SVB), as well as on the release kinetics of the loaded protein. It must be underlined that, for a possible clinical application, the site of injection of MPs must be properly chosen to avoid the risk of vision blurring. In this perspective, a successful treatment based on intravitreal MP injection is strictly related on MP injection outside the visual path within the eye. For this reason, negligible diffusion features of MPs in the long time are necessary to ensure a significance of the treatment (Wang et al., 2014; Zhang et al., 2015). The SVB was prepared according to the procedures reported in the literature and, before the release and diffusion studies, we verified that its

rheological properties were close to those of the human vitreous body (data not shown) (Soman et al., 2003).

3.1. MP characterisation

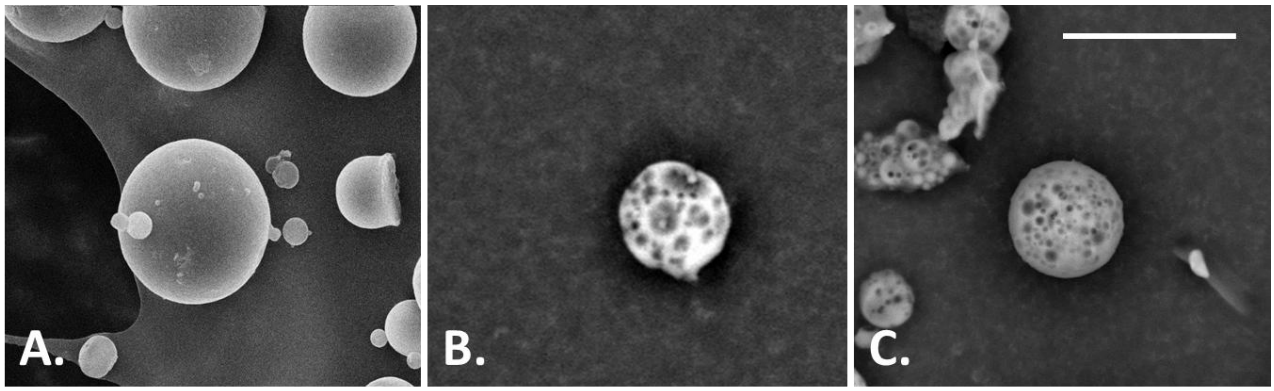
In table 1, the acronyms, mean diameter and BSA entrapment efficiency of the prepared formulations are summarized.

Formulation	Hyaluronic acid molecular weight [kDa]	Mean diameter [µm] (± S.D.)*	BSA entrapment efficiency [%] (± S.D.)
PLGA	-	8.3 (±1.7)	76 (±4)
HA180	180	9.0 (±1.2)	85 (±11)
HA1600	1600	8.7 (±1.1)	88 (±12)

* Standard deviation is calculated on 10 different batches

Table 1.: Overall technological features of the produced MPs.

As shown in Table 1, the adopted formulation conditions allowed to obtain MPs with a mean diameter around 9 µm, able to encapsulate BSA with > 76% efficiency in all cases. As clearly revealed by SEM images, the produced PLGA MPs were spherical with a smooth surface (figure 2A) while HA containing formulations presented a porous surface (figures 2 B and C).



Figures 2: Representative SEM images of (A) PLGA (B) HA180 and (C) HA 1600 MPs. The bar is 10 μm.

3.2. Degradation studies of MPs

As described in the Section 2.8, the mass loss/degradation kinetics have been observed through a 0.5% w/v MPs suspension in PBS incubated at 37°C. At scheduled time points, the residual mass of the MPs was gravimetrically obtained on the lyophilized residue, taking into account the salts mass.

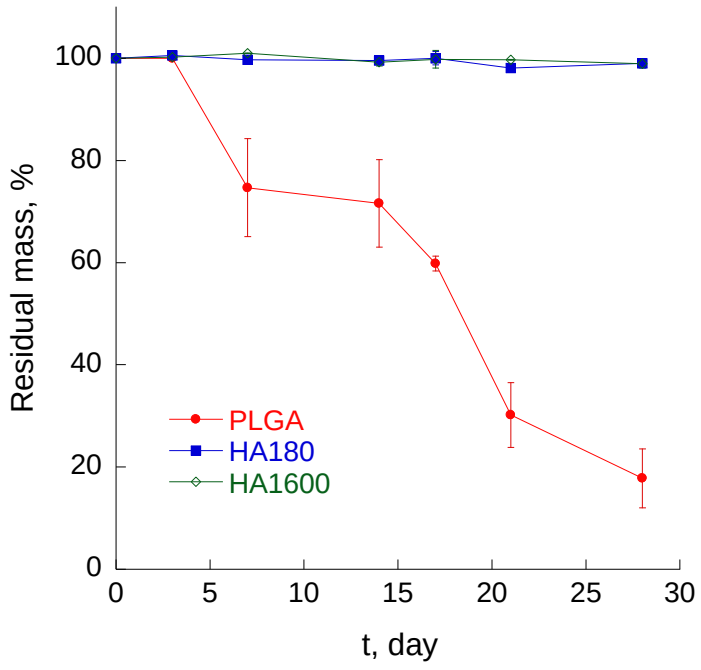


Figure 3: MP time trend of mass loss.

Per cent changes in MP mass were calculated as the rate of material loss during the incubation of the devices over a 4-week course *in vitro*. The trend of MP mass loss is presented in Fig. 3.

Autocatalytic PLGA degradation can be schematized as follows: (i) initially, water invades the polymeric matrix due to MP porous structure which allows a fast water penetration (Tamada et al., 1992) and polymer plasticization (Blasi et al., 2005); (ii) then, hydrolysis-triggered PLGA degradation at chain backbone takes place and, progressively, soluble acidic by-products are formed; (iii) the produced acids catalyze degradation, thereby furthering the formation of additional soluble acidic degradation products (Joshi, Himmelstein, 1991). It must be specified that, during degradation, molecular weight steadily decreases, while device mass loss takes place when polymer molecules reach a critical molecular weight below which PLGA oligomers are soluble.

The collected data display that in 28 days control PLGA MPs lost > 80% of their initial mass while, in the same time frame, the overall mass of both HA-containing formulations was roughly constant, with a loss of material around few per cent points. This indicates that, only in HA-containing devices, the molecular weight of PLGA is still above the dissolution threshold value and, hence, that the degradation rate for both formulations is definitely lower. Actually, poloxamers presence in the bulk of MPs can be associated to a dilution of acidic degradation products, along with a buffering effect which slow down the autocatalytic degradation of MPs. Furthermore, it must be pointed out that MPs containing poloxamers are more hydrophilic compared to the devices made up of bare PLGA and this is associated to a sensible enhancement of acidic degradation by-products diffusion outwards to the surrounding gel. Furthermore, the results depicted in Fig. 3 show that the presence of different molecular weight HA molecules on the surface has seemingly no effect on the percentage of degradation in this first stage of HA containing MP degradation.

3.3. Release studies of MPs in SVB

In order to study the release features of the produced MPs decorated with HA in the ocular vitreous body, control PLGA and HA-decorated MPs have been dispersed in SVB and the resulting MP-loaded gel placed on the bottom of a vial, covered with PBS and incubated at 37 °C. The presence of SVB ensures the interactions between the HA chains exposed upon MP surface and the HA molecules contained in SVB. At scheduled time points, spectrophotometric analysis has allowed to assess whether the molecular weight of HA coronas influenced drug release kinetics of BSA from MPs. In figure 4, the release profiles of BSA from PLGA-based MPs, with or without external HA (at 180 or 1600 kDa) corona, are reported.

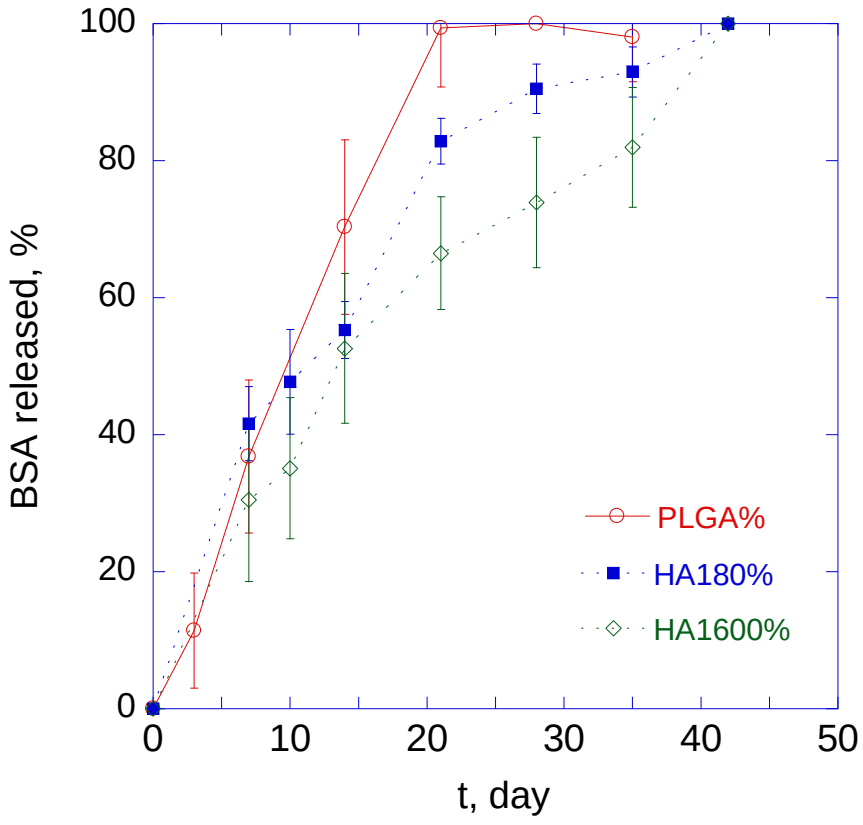


Figure 4. Temporal release trends of BSA from MPs over time.

It is known that drug release from PLGA-based drug delivery systems is governed by a complex mechanism deriving from the combination of drug diffusion through the polymeric matrix and the concomitant polymer bulk degradation (Blasi *et al.*, 2005). Results indicate that PLGA MPs can

295 prolong BSA release phase for three weeks with a release curve, in this time frame, quite
296 superimposable to that obtained from HA1600 formulation and slower compared to HA180
297 formulation. Differently, both formulations with HA present qualitatively similar trends of protein
298 release kinetics, with a prolonged release phase for at least six weeks. Specifically, the amount of
299 protein released by HA1600 MPs was found to be lower compared to that released by HA180.
300 Therefore, a prolonged degradation time of MPs, due to the presence of poloxamers in the
301 formulation, contribute to sustain the release of the loaded drug molecule(s), thereby reducing the
302 frequency of intravitreal injections and the related risk of adverse effects. The different profile of
303 HA 180 and HA 1600 MPs can be related to the HA arrangement on MP surface. Indeed, high
304 molecular weight HA is constituted by longer chains which can form physical entanglements. This
305 feature may hinder the diffusion of the drug, thus slowing down drug release kinetics.

306 *3.4. Diffusion studies of fluorescent MPs*

307 MP size exceeds the vitreous mesh size (about 500 nm) and this clearly discourages their free
308 diffusion into the posterior segment of the eye (Xu et al., 2013). Thus, only MP sedimentation can
309 occur in a long time frame (up to some months of a sustained drug release), as a consequence of
310 viscous flow of macromolecules constituting the hydrogel matrix of the vitreous body. Hereafter,
311 the syringe technique was used to evaluate transport properties of MPs into SVB, thus mimicking
312 their sedimentation after the intravitreal injection of MPs. More in detail, diffusion tests of
313 fluorescent HA1600 and HA180 MPs in SVB were performed and compared to the transport
314 properties of MPs without HA corona (namely PLGA MPs) of comparable size. Results of diffusion
315 tests are presented in figures 5, 6 and 7.

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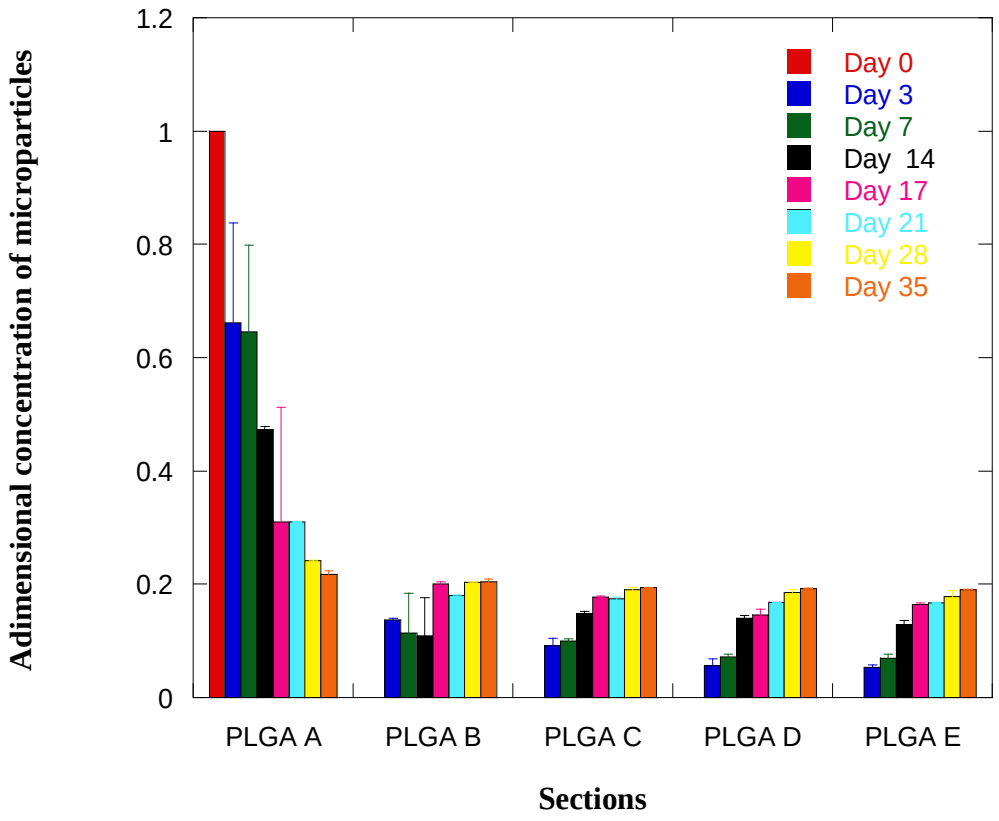

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319 *Figure 5: Diffusion test of PLGA MPs.*

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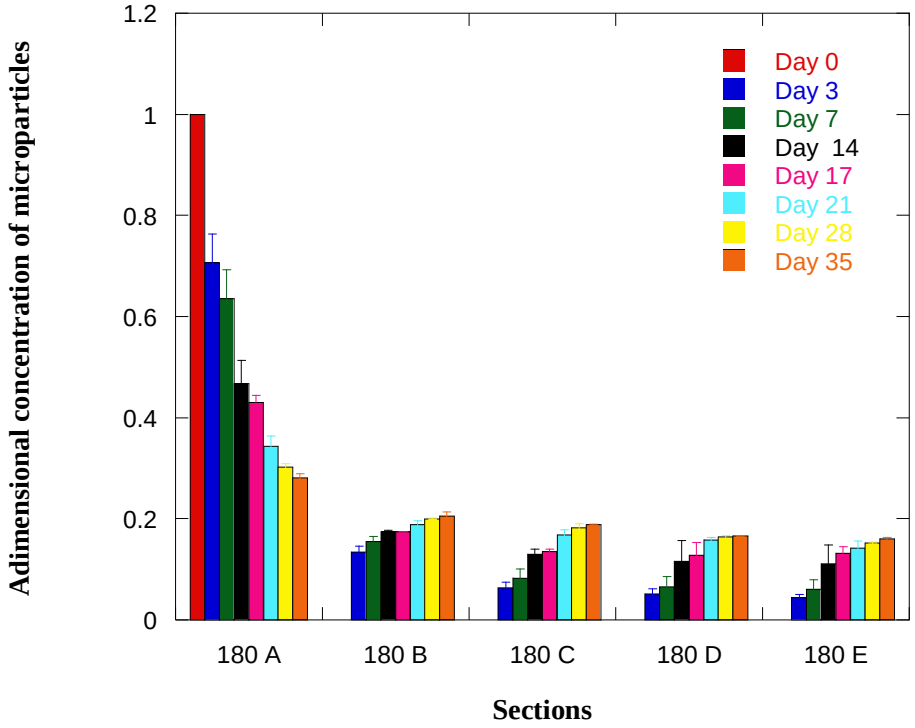

321
322 *Figure 6: Diffusion test of HA180 MPs.*

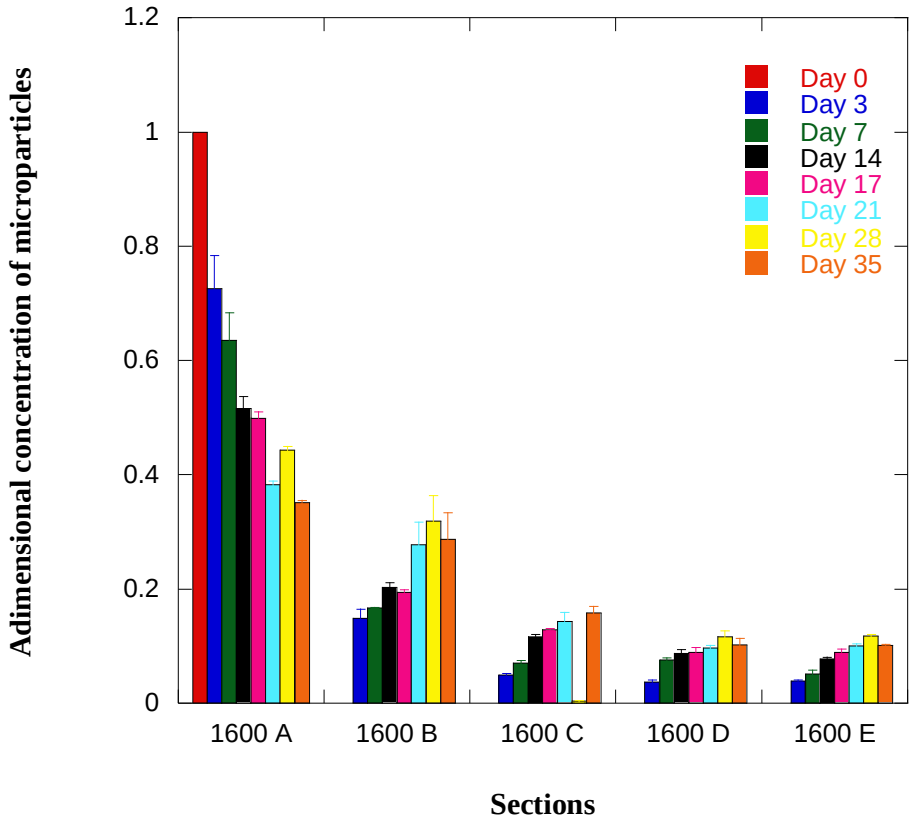

Figure 7: Diffusion test of HA1600 MPs.

The obtained results have shown that the MPs made up of bare PLGA diffuse in SVB faster than those decorated superficially with HA and also that, by increasing the molecular weight of the latter on MP surface corona, MP diffusion through the various sections in which the syringe has been previously divided is further hampered. More in detail, after 35 days, bare PLGA MPs diffuse from section A of the syringe (in which the MPs were initially loaded) to section E and are uniformly distributed thought the syringe sections . Differently, after 35 days in section E there are ~16% and ~10% of HA180 and HA1600 MPs initially loaded in section A, respectively. The difference in diffusivity between MP formulations can be confidently attributed to the presence of HA on the surface of MPs. In fact, it is possible to hypothesize that HA exposes the terminal of the polymeric chain outside the MP, thus favoring the interactions with the simulated vitreous body, containing the same polysaccharide, and thus slowing down the transport of the MPs. Furthermore, the different conformations assumed by HA of different molecular weight affects both the number and

the entities of the physical entanglements made with HA macromolecules present in the simulated vitreous, thus further modulating the diffusion kinetics of MPs. Therefore, HA1600 MPs, exposing longer HA chains on their surface, show a more hindered transport in SVB. This is expected to limit their aggregation once injected intravitreally, thus improving their ability to control and prolong drug release.

4. Conclusions

The aim of this work was the production of biodegradable PLGA-based MPs, with tailored surface properties so as to be motionless within the vitreal space. More in detail, MP surface has been decorated with HA with different molecular weight (180 and 1600 KDa), which is also a main constituent of the vitreous body. The chemical composition affinity between MP surface and the vitreous body proved to be efficacious in slowing down MP diffusion into SVB and the different length of HA chains on MP surface, proved to be an effective tool to further modulate the transport of MPs in SVB. Moreover, HA MPs presented a prolonged degradation time compared to bare PLGA MPs that, in turn, contribute to sustain the release of the loaded drug molecule(s), thereby reducing the frequency of intravitreal injections and the related risk of adverse effects. The different molecular weight of the biopolymer on MP corona does not greatly affect the degradation time of the MPs while can modulate the amount of protein released by the devices. Taken altogether, these outcomes shows the attractiveness of these systems for the intraocular delivery of therapeutic proteins.

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