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BIOCOMPATIBILITY STUDIES

Ocular biocompatibility of polyquaternium 10 gel: functional and morphological results

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Abstract This paper deals with the characterization study of topical and intraocular biocompatibility and toxicity of cationic hydroxyethylcellulose Polyquaternium 10 (PQ10). It also evaluates the rheological properties of gels. The cytotoxicity assays were done in two cell lines: HEp-2 and VERO (human larynx epidermoid carcinoma cell and African green monkey kidney cells respectively). For the in vivo study, New Zealand albino rabbits were used. The in vitro cytotoxic activity of PQ10 shows no statistically significant differences in relation to the control of hydroxypropylmethylcellulose (HPMC) in any of the cell lines used in this study. Similarly, the signs of inflammation observed after treatment showed no significant difference between the groups of animals treated with the polymer compared to the control group. Normal histological characteristics were seen in both groups with no histological inflammatory reaction. After 1 month of the intracameral application of 2 % PQ10 (treatment group) or 0.3 % HPMC (control group), electroretinograms showed similar levels of a- and b-waves latencies and amplitude. In summary, PQ10 gel was well tolerated in these experiments, with proper monitoring, it could stand as a new alternative in the development of ophthalmic viscosurgical devices.

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1 Introduction

Functional biomaterials have been extensively employed in diverse research fields such as tissue engineering [1–3], cell therapy [4–6], drug delivery [7–9] and ophthalmic viscosurgical devices (OVDs) [10]. In recent years, hydrophilic polymer gels, or "hydrogels", have become an important class of materials for applications in nanotechnology, biotechnology, and medicine due to their unique material properties [11–13]. They can be prepared from a wide variety of natural and synthetic precursors ranging from commodity to designer chemicals, and as a result, can be readily commercialized [14]. Hydrogels are highly swelling in water and combine the ability of transporting molecular and nano-scale species throughout the material while maintaining solid-like mechanical properties [15]. This feature has led to the widespread use of hydrogels as a scaffolding material in biomedical applications including drug delivery, tissue engineering, and wound healing [13, 16–18].

One area in which the development of hydrogels completely changes the course of their evolution is Ophthalmology. The indications for OVDs, for example, have been expanded with the introduction of recently developed materials.

OVDs were first introduced to maintain space in the eye during the implantation of intraocular lenses. With the

development of different types of OVDs, additional indications have been included, such as protection of the corneal endothelium [19], retention of drugs and enlargement and stabilization of the pupil size, among other. Furthermore, ocular gels (OVDs currently employed), can also encompass an important use as drug delivery systems (DDS), either for the surface of the eye or for intraocular applications [20]. OVDs are widely available commercially. They present different rheological and physicochemical properties [21] given by the singular polymer composition. Some of the most common natural polymers used are gellan gum, alginic acid and xyloglucan [22]. The use of surfactants to induce changes in the conformation of cationic polysaccharides and to promote the formation of aggregates has also been proposed to obtain homogeneous aqueous dispersions and to modulate their rheological behavior [23–26]. High bioadhesive capacity [27], low toxicity [28] and long-term efficacy with adequate mechanical properties are important properties of these hydrophilic cationic polysaccharides that allow them to be particularly valuable as viscosurgical devices. Recently, a cationic hydroxymethylcellulose gel, the Polyquaternium 10 (PQ 10) (Fig. 1), has also been evaluated as a drug delivery device [29]. The aim of this study was to evaluate the biocompatibility, security and toxicity of a cationic cellulose derivative containing a quaternary amine group, PQ10, in topical and intraocular applications.

2 Materials and methods

2.1 Materials

High-viscosity cationic hydroxyethylcellulose PQ10, also known as Celquat® SC-230C, was kindly provided by The National Starch and Chemical Co. (Bridgewater, NJ). Stock solutions of 1, 2 and 4 % PQ10 were prepared in 20 mM phosphate buffer at pH 7.2 for biological assay and in citrate buffer pH 5 and tris buffer pH 8 for the solubility test. The sterilization of the solution was done by autoclaving for 30 min at 121 °C. No significant changes were observed after sterilization.

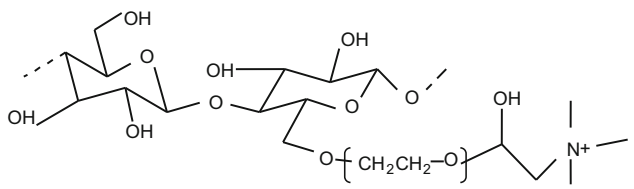

Fig. 1 Molecular structure of Polyquaternium 10 (PQ10)

HPMC 0.3 % lubricant eye drops and HPMC viscoelastic solution 2 % w/v were used as controls.

2.2 Spectral analysis

The spectral scanning of PQ10 at different concentration was determined by measuring absorbance in the range between 200 and 800 nm (Shimadzu UV-240) against a blank of distilled water.

2.3 Rheology

The rheological behavior of these samples was evaluated in triplicate at 25 °C in a Physica Rheometer Rheoplus/32, Anton Paar, Sanico equipped with an AR2500 data analyzer, fitted with a Peltier temperature control. A 50 mm cone-plate measuring geometry was used. Oscillatory shear responses (G′ or storage modulus, and G″ or loss modulus) were determined at 0.1 Pa over the frequency range of 0.1-100 rad s-1. The test conditions were within the linearity range of the viscoelastic properties. The zero shear viscosities (η°), complex viscosities (η^{*}), storage (G′) and loss (G″) modulus were determined for PQ10 and HPMC as control.

2.4 Texture analysis

This study described the extrusion pattern as a measure of the force required to drive a viscous solution of PQ10 through a 27 gauge line. HPMC viscoelastic solution 2 % w/v ophthalmic solution served as a reference. The TAXT2 texturometer (Stable Micro Systems) equipped with a 25 kg load cell was used to determine the extrudability profiles. The texture analyzer was equipped with a fixed platform and a cylindrical acrylic probe with a diameter of 5 cm. The samples were transferred to a 1 ml syringe and the runs were made through a 27 gauge needle at a speed of 0.7 mm/s. Graphics show the average data from three measuring forces as a function of the distance obtained.

2.5 Cytotoxicity assessment

3-4,5-Dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) was employed as a colorimetric cytotoxicity assay to estimate the number of viable cells after contact with increasing concentration of PQ10. Reduction of the tetrazolium salt into a blue colored product (formazan) occurs only in metabolically active cells. By measuring the UV absorbance of the formazan at 570 nm with a reference wavelength at 650 nm, the percentage of viable cells was estimated. The cell lines used in the MTT assays were HEp-2 (ATCC CCL23), human larynx epidermoid carcinoma cells and VERO (ATCC CCL-81), African green

monkey kidney cells. Both lines were grown and maintained in minimum essential medium (MEM) with 1.5 g/l sodium bicarbonate, 2 mM L-glutamine, and 10 % of fetal bovine serum at 37 °C in humidified 5 % CO_2 atmosphere. All medium supplements were from Invitrogen.

Cells were seeded in 96-well microtiter plates in 100 μL culture medium at a concentration of $1{\cdot}10^4$ cells/mL and cultured for 24 h at 37 °C to 85 % confluence. The growth medium was then removed and 100 μL of PQ10 was added carefully to each well at final concentrations of 2, 1 and 0.5 % (eight replicates per sample). MEM without serum was added for negative control, and 10 % dimethyl sulfoxide (DMSO) for positive control. After 24 and 48 h of incubation at 37 °C, the cytotoxic effect was determined by observation using an inverted phase contrast microscope (Axiovert 135 M; Karl-Zeiss, Göttingen, Germany).

For the quantitative evaluation of cytotoxicity by MTT assay, cells were seeded in 96-well plates at a density of $1{\cdot}10^4$ cells per well. The cells were incubated for 24 h at 37 °C to 85 % confluence. After 24 h, the culture medium was removed from the wells and equal volumes of PQ10 serial dilutions were added to each well including 1, 0.5, 0.25 and 0.1 %. Wells containing culture medium without cells were used as blank value and the cells with MEM without serum were used as negative control presenting 100 % viability. The positive control was the cells treated with 10 % DMSO. After 24 h of incubation, MTT assay was performed to evaluate the cell viability. A solution of 20 μL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) in phosphate-buffered saline was added to each well. After a further incubation period of 3 h at 37 °C, the supernatant was aspirated, cells were washed with phosphate buffered saline (PBS – pH 7.5) to remove unreacted MTT and sample residues and formazan crystals were dissolved by 150 μL DMSO. The absorbance was measured at 540 nm using ELISA microplate reader (Anthos 2010 Microplate Reader). Cell viabilities were presented as the percentage of the absorbance of treated cells to the absorbance of cells cultured only with MEM. MTT assays were repeated in four separate experiments.

2.6 Experimental design of in vivo study

This experimental study was performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for use of animals in ophthalmic and vision research.

Eighteen male New Zealand albino rabbits of 1.5–2.5 kg body weight were randomly divided into three groups. Each group (A, B and C) comprised six rabbits. In all groups, the right eye (OD) was used to test PQ10, and the left eye (OS) served as control. In addition, group C were divided into two subgroups of three each, using regular PQ10 in one subgroup and PQ10 labeled with fluorescein on the other to study the retention of the substance from the AC.

In order to evaluate the biocompatibility of PQ10, three alternative routes were used: topical (group A), subconjuntival (group B) and intracameral (group C). HPMC 0.3 % lubricant eye drops and HPMC 2 % w/v viscoelastic solution were used as controls for group A (topical application) and for groups B and C (subconjuntival and intraocular application) respectively.

Rabbits were anesthetized by a topical application of 1 % tetracaine chlorhydrate. In addition, we used intramuscular xylazine hydrochloride (5 mg/kg of body weight) and ketamine hydrochloride (35 mg/kg of body weight) for group C.

In group A, one drop of PQ10 1 % to physiologic pH was instilled every 4 h in the conjunctival sac, keeping the eyelid closed for 30 s, to ensure proper distribution of the product.

In group B, a subconjunctival blister of 0.1 ml of PQ10 2 % was created by injecting the viscoelastic solution, through a 25G needle, in the superior bulbar conjunctiva of OD. A subconjunctival blister of HPMC viscoelastic solution 2 % w/v on OS served as control.

In group C, a corneal self-sealing incision was created in the OD with a 15° knife and PQ10 2 %, regular and labeled with fluorescein, was injected in the anterior chamber (AC). Both subgroups were treated under the same conditions.

The procedures in groups B and C were performed under a surgical microscope (OPMI 6 CFC XY, Carl Zeiss, USA). Topical povidone-iodine 5.0 % was applied prior to starting the operation and moxifloxacin 0.5 % eye drops were supplied every 4 h for a week after surgery.

All groups were controlled by slit-lamp biomicroscopy (Haag-Streit AG, SL-7E; Topcon, Tokyo, Japan) at 12, 24, 48 and 72 h and once a day for a week. For those rabbits receiving the fluorescein-labeled PQ10 evaluation at the slit lamp, cobalt blue filter was used to monitor retention and elimination of the viscoelastic solutions. Intraocular pressure (IOP) was measured daily, for 3 days, with a Goldmann applanation tonometer (AT 900®, Haag-Streit AG, Switzerland). The mean of three readings was considered for each IOP measurement. Dilated indirect ophthalmoscopy was used for fundus examination. The pupils were dilated with one drop of 0.25 % tropicamide and phenylephrine ophthalmic solution.

All observations were performed by a blind examiner to treatment groups to minimize bias. The parameters considered by the blind observer were conjunctival congestion, corneal edema and the presence of flare or cells in the AC.

Severity of conjunctival injection was evaluated using a grading scale from 0 to 3 as previously described by Akpek

et al. [30], where 0 equals absence of injection and 1 means mild injection. Moderate injection with edema of the palpebral conjunctiva and hazy view of the deep tarsal vessels was graded as 2 and severe injection obscuring visualization of the deep tarsal vessels received 3 points.

2.7 Electroretinography

Under general anesthesia and with the pupils dilated as previously described, electroretinograms (ERGs) were recorded 1 week before and 30 days after injection of hydrogels into the AC (group C). A lid speculum was used and a child-sized electrode was placed on the rabbits' cornea.

A Grass xenon flash, which was attached to a Ganzfeld screen (PS22, Quincy, MA, USA), was used as a stimulus to elicit the ERG responses in the dark-adapted state. The maximum luminance was 75.4 cd s/m2, and neutral density (ND) filters were used to reduce the maximum stimulus intensity. The stimulus intensity was attenuated in 1.0-log unit steps (ND = 4.0–1.0). The stimulus duration was about 10 s.

The responses were amplified by an evoked potential measuring system (Neuropack Sigma MEB5508, Nihon Koden, Tokyo, Japan) with a band-pass filter (0.2–500 Hz for *a* and *b* waves, 50–500 Hz for oscillatory potentials (OP)). Two bipolar Burian-Allen-type contact lens electrodes were used to record ERG responses from both eyes simultaneously.

After 30 min of dark adaptation, the recording started with the weakest stimulus by placing the 4.0-log unit ND filter. The stimulus was increased stepwise to a 3.0 log unit.

Three responses were averaged at each light intensity for *a* and *b* waves. The amplitude of *a* wave was measured from baseline, and *b* wave from the trough of *a* wave to the peak of *b* wave. Photopic responses were recorded without a ND filter, providing stimulus every 1 s. The ten responses were averaged. The amplitude of the OP was measured from the tangent between the troughs. The amplitudes and implicit times were averaged for each illuminance of the stimulus for a comparison between the two eyes. A two-tailed paired *t* test was used to statistically analyze the results.

2.8 Histology

Two rabbits from group C were sacrificed at week 4 after the surgical intervention with an overdose of pentobarbital. Both of their eyes (OU) were enucleated for histological evaluation. The eyes were fixed in 5 % formaldehyde for at least 48 h before being embedded in paraffin and sectioned. Three consecutive Sects. (4-μm thickness each), were stained with hematoxylin and eosin (H&E) and examined by light microscopy.

2.9 Statistical analysis

Data were subjected to ANOVA statistical analysis using GraphPad Prism 3.0 version statistics program (GraphPad Software Inc., San Diego, CA). A Newman-Keuls multiple comparison test was used to compare the data within each experiment, and the unpaired *t* test was used to compare data between two variables. *P* values less than 0.05 were considered to be statistically significant.

3 Results

3.1 Study of the physicochemical properties of PQ10 polymer

PQ10 is a high viscosity cationic polymer compatible with surfactants, commonly used in a wide range of personal care products. This polymer has strong cationic characteristics in a wide range of pHs, displaying high solubility in aqueous solvents. It also offers significant thickening effects. In this sense, we observed a gelling time dependence of the polymer with the pH of the medium. Table 1 shows the time required to form a gel PQ10 4 % according to the pH and at two different temperatures, 25 and 90 °C. This result shows that at 25 °Cthe solubilization of PQ10 is slower as pH decreases, whereas at 90 °C the influence of pH is very low.

On the other hand, the spectral analysis of the dilute solutions (<1% w/v) showed that PQ10 has no absorbance between 200 and 800 nm (data not shown).

In order to acquire a deeper understanding of the behavior of PQ10, studies into the rheological behavior of PQ10 were assessed in relation to a commercial ophthalmic HPMC. Figure 2 represents the average curve where it shows that the zero-shear viscosity (η°) of PQ10 2 % is twice that of the commercial sample. This value is the apparent viscosity (quotient between shear stress and shear

Table 1 Time gel formation of 4 % PQ10 as a function of pH and at two temperatures, 25 and 90° C

pH Buffer	Time (min)	
	25 °C	90 °C
5	56 ± 3	4 ± 0.15
6	22 ± 1	3 < 0.15
7	6 ± 0.3	2 < 0.15
8	2 ± 0.15	2 < 0.15

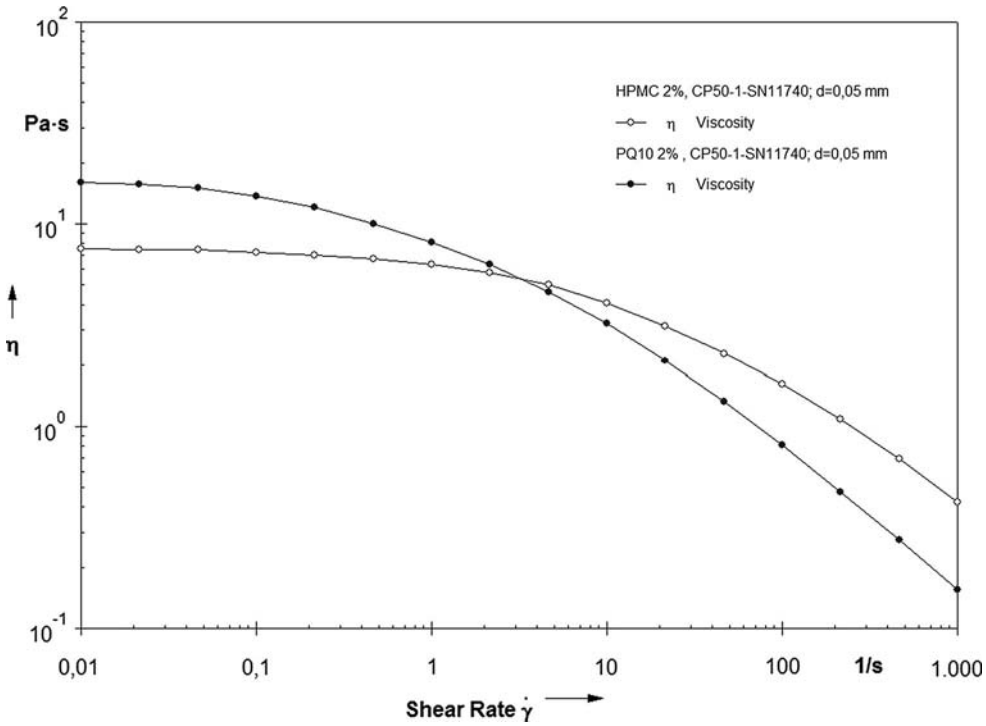

Fig. 2 Rheological analysis of PQ10 (*white circle*) and HPMC 2 % w/v (*black circle*)

rate) of the material in the limit of zero-shear rate, and represents the ability of the material to avoid sedimentation when stored. A high-zero shear viscosity is interpreted as the material will show homogeneous during long storage.

In a typical rheological experiment, we seek to measure G′ (ω) and G″ (ω). We make the measurements as a function of the frequency of oscillation (ω) since, whether a soft material is solid-like or liquid-like depends on the time scale at which it is deformed. PQ-10 shows the typical viscoelastic behavior of macromolecular homogeneous fluids (Fig. 3). The viscoelastic behavior of the system is characterized by the storage modulus, G′, and the loss modulus, G″, respectively characterizing the solid-like and fluid-like contributions to the measured stress response. Figure 3 shows that, at the lowest accessible frequencies, the response is viscous-like, with a loss modulus larger than the storage modulus; however, at the highest frequencies accessed, the storage modulus dominates the response, indicating solid-like PQ10 behavior. By contrast, for HPMC, the elastic modulus (G′) is always smaller than the viscous modulus (G″), which describes a more elastic than viscous behavior type. Figure 3 also shows that the complex viscosity (η*) of HPMC 2 % w/v is slightly higher than that of PQ10 2 %.

Finally, we also measured the force required to drive the gels through a needle of 27 gauges. Figure 4 shows the curves obtained with the average values of a solution of PQ10 2 % in association with HPMC viscoelastic solution 2 % w/v as a reference. We can observe that the average force is about 275 g for PQ10 2 % and 900 g for the control. In view of this, even with a value twice the viscosity of the formulation controlling, the PQ10 hydrogel shows much less resistance to the passage through the chosen route.

3.2 Cytotoxic assay

We carried out a qualitative evaluation of the potential cytotoxic effect of PQ10 with different cell lines. The results showed that increasing concentrations of PQ10 up to 2 % did not affect cell monolayers of Hep-2 and VERO at intervals of 24 and 48 h. Figure 5 shows the microphotography of monolayers incubated for 48 h with PQ10 2 % compared to the untreated controls, the cells exposed to PQ10 maintained normal morphology compared with negative control cells. They did not demonstrate any detrimental changes, such as reduction in cell growth, shrinkage or membrane blebbing. Dilutions of PQ10 at 1 and 0.5 % shows similar results (data not shown).

Quantitative evaluation of cytotoxicity was conducted by MTT assay, which is an assay of metabolism of methyltetrazolium salt by mitochondrial dehydrogenase of active cells into formazan crystals. The results of MTT assay are shown in Table 2. After 24 h of incubation, high viability values of 97–99 % were obtained for all the

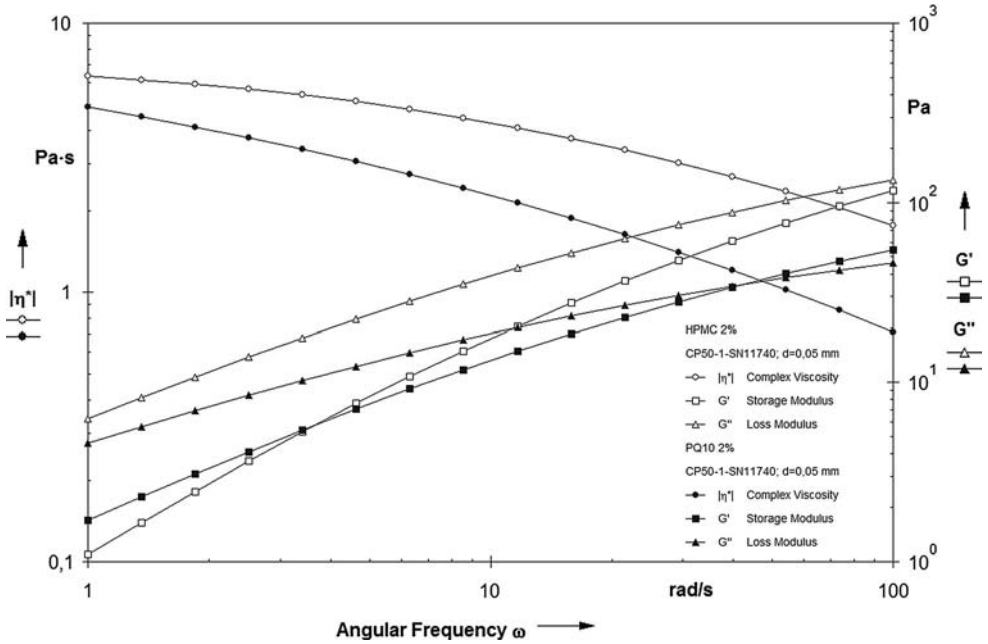

Fig. 3 Rheological behavior of PQ10 2 % (*white circle*) and HPMC 2 % w/v (*black circle*)

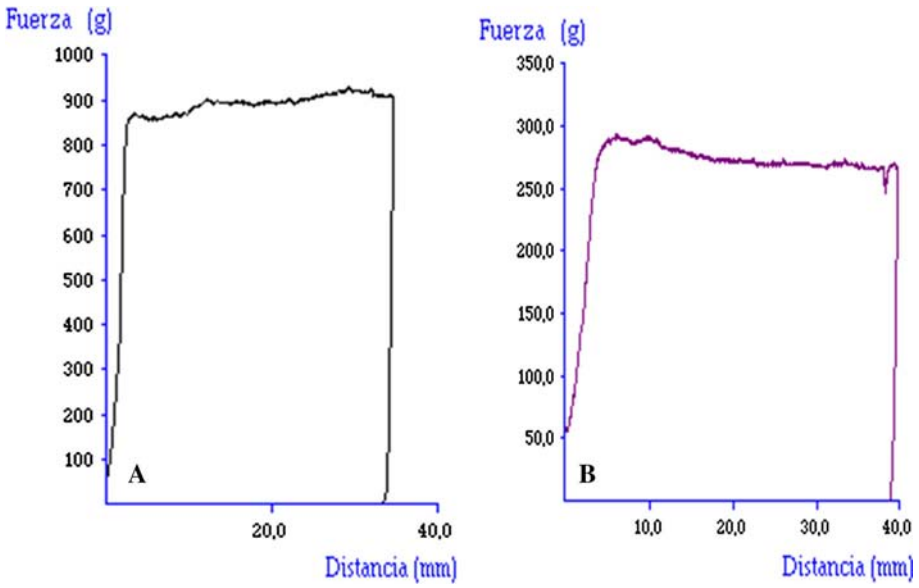

Fig. 4 Extrusion graph showing the capacity of the two viscous solutions used: **a** HPMC 2 % w/v the average force measurement was about 900 g and **b** showed viscous solution of PQ10 2 % with an average force of 275 g

investigated concentrations, in contrast to that observed with the positive control (DMSO), where cell remainder viability after treatment did not reach 30 %. Each experimental value represents the average of four different experiments; standard deviation at each point was below 10 %.

3.3 Eye examination

Slit lamp observation revealed that none of the rabbits from group A evidenced signs of conjunctival congestion or keratitis, nor conjunctival or corneal toxicity. There were no significant differences with the left eyes used as controls.

A couple of hours after subconjuntival injections, animals from group B showed a slight congestion in the conjunctival area of both eyes, treated and controlled, although this signal disappeared completely 24 h after the procedure. This effect can be explained by the trauma produced by handling the subconjuntival space and inducing tissue dissection when injecting the gels. Furthermore, it should be noted that the blebs in the eyes treated with HPMC completely disappeared after 72 h, while the blisters of the eyes injected with PQ10 remained

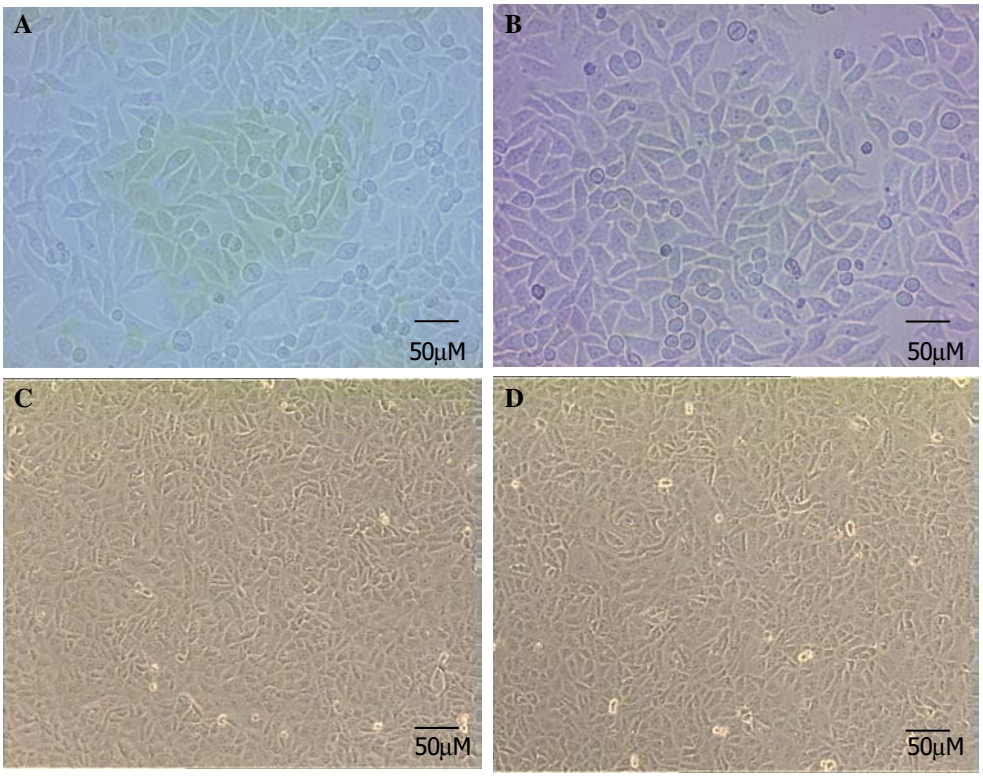

Fig. 5 Hep2 cell control (**a**) and after 48 h of incubation with 2 % PQ10 (**b**), and VERO cell control (**c**) and after 48 h of incubation with 2 % SC (**d**)

Table 2 MTT cytotoxicity assay showing percentage cell viability over a concentration range of SC-230 on Hep-2 and VERO cells

PQ10 concentration (%)	Hep-2 cell viability (%)	VERO cell viability (%)
0	100 ± 12	100 ± 10
0.1	98 ± 13	98 ± 11
0.25	99 ± 11	99 ± 13
0.5	99 ± 12	99 ± 12
1.0	97 ± 13	98 ± 13

an average of 7 ± 1 day without any sign of inflammation (data not shown).

In group C, after intracameral injection of both, PQ10 2 % and HPMC viscoelastic solution 2 % w/v, we observed a gradual increase in corneal swelling and conjunctival congestion in both groups of rabbits, as shown in Fig. 6. Furthermore, the presence of these signs increased as IOP rose, to gradually decrease as IOP returned to baseline at 24 h (Figs. 6, 7, 8). In summary, these results show no significant differences in responses after intracameral administration of PQ10 with respect to HPMC. A mild corneal edema remained for 2 days after surgery in all animals. The fact that both hydrogels absorb large amounts of water produces a rapid swelling when the substances comes into contact with the aqueous humor that could affect the output pathway with increased aqueous humor resistance to flow and consequent elevation of IOP, producing transient corneal edema.

In the fluorescein-labeled polymer subgroup, we observed a homogeneous staining of the corneal endothelium 6 h after injection of the substance in the AC, which decreased significantly after 12 h to almost completely disappear after 36 h in both treated and controlled eyes. This observation could demonstrate an analogous outflow pathway of both gels. Thus, we can deduce that 2 % PQ10 follows the same elimination path as that used by the aqueous humor, explaining the increase in IOP in the first hour of permanence of the polymer in the AC. The difficulty of the molecule to pass through the trabecular meshwork diminished the excretion of aqueous humor by increasing the output resistance, hence, producing a raise in IOP. No significant difference ($P > 0.06$) was observed

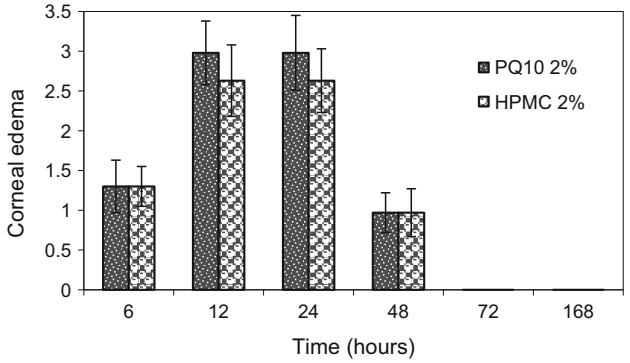

Fig. 6 Evolution of corneal edema en function of time

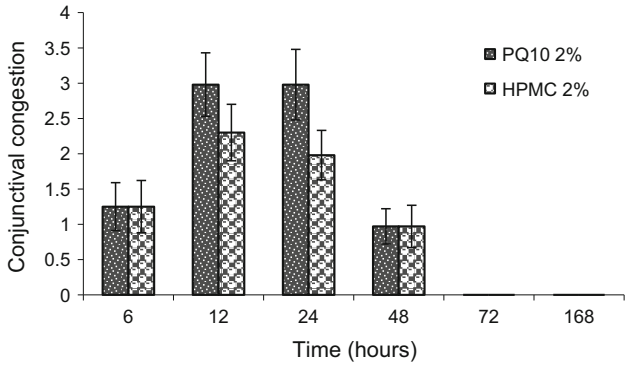

Fig. 7 Presence of conjunctival congestion as a function of time

when comparing the mean IOP value between the commercial HPMC viscoelastic solution 2 % w/v and PQ10 2 % groups at each time point (Fig. 8). In both groups, IOP returned to baseline within 24 h. It should be noted that only a slight increase in IOP was observed 6 h after surgery; the rise was probably not significant due to the loss of aqueous humor through the corneal/limbal incision. Within postoperative day one, IOP was maintained at a higher level probably due to the swollen hydrogels that would increase the aqueous humor outflow resistance at the trabecular meshwork. With the beginning of gel dissolution, the IOP values decreased and remained at baseline for 7 days (mean IOP 5.2 mmHg). See Fig. 8.

During the follow-up examinations, no cataract, corneal neovascularization, stromal disease or sign of endophthalmitis were observed at the slit-lamp after the introduction of PQ10 2 % and HPMC viscoelastic solution 2 % w/v into the AC. No evidence of vitritis, uveitis, retinitis or endophthalmitis was observed in the fundus exam either.

3.4 Electroretinographic and histological findings

ERG showed no statistically significant difference of latency and/or amplitude of *a* and *b* waves between the

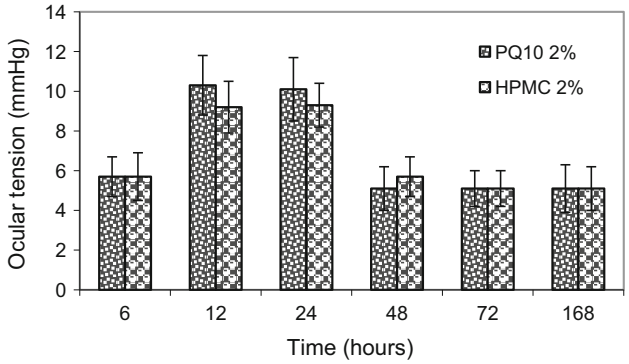

Fig. 8 Rise in intraocular pressure (IOP) next to the injection of PQ10 and HPMC viscoelastic solution 2 % w/v

eyes injected with PQ10 and the left controlled eyes (supplementary material). However, it was observed that the values obtained in both eyes, right and left, were slightly decreased compared to the ERG of animals that did not undergone any experiment. This temporary electroretinographic effect is probably attributed to a slight retinal hypoxia induced by the transient intraocular hypertension (data not shown).

3.5 Histology

The results of histopathologic examination of eyes injected with PQ10 or with HPMC by light microscopy after enucleation showed no significant changes in the inner retina (data not shown) or any other structures of the eyes, like the cornea (supplementary materials). Similarly, no reduction of tissue or presence of inflammatory cells was observed in the ciliary body (Fig. 9).

4 Discussion

Since the introduction of Healon (sodium hyaluronate 1 %) in 1979 [10], viscosurgical devices (OVDs) have become valuable tools in many ophthalmic applications. In this sense, the characterization of tissue response to biomaterials is one of the most relevant factors.

In this study we demonstrate that cationic polymer PQ10 shows important physicochemical and rheological properties that suggest that this polymer could be suitable for viscosurgical uses. Viscoelastic materials show responses containing two in-phase and out-of-phase contributions; these contributions reveal the extent of solid-like and liquid-like behavior. As a consequence, the total stress response reveals a phase shift with respect to the applied strain deformation that lies between that of solids and liquids. PQ10 showed a clear viscoelastic behavior, presenting the characteristic crossover point between the

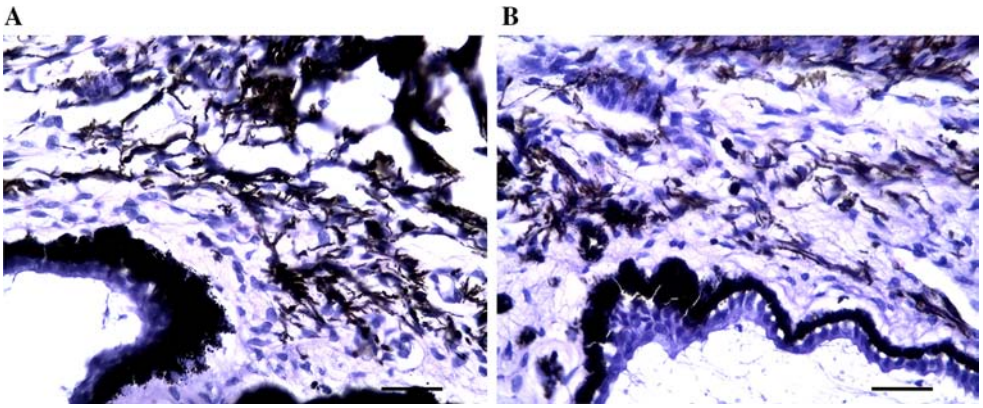

Fig. 9 After enucleation histology of the CB was evaluated by light microscopy using hematoxylin/eosin. Microphotograph of ciliary body (CB) of treated (**a**) and non-treated/control (**b**) rabbits respectively. No inflammatory cells were observed in any of the assessed groups (magnification ×300). *Scale bar* represents 20 μm

elastic modulus (G′) and viscous modulus (G″). By contrast, a viscous solution of HPMC 2 % w/v did not show the crossover between (G′) and (G″) suggesting a different behavior, such as a viscous gel only. This variation in the rheological properties may be useful to improve the application of PQ10. It should be noted that, although PQ10 has higher viscosity than that of HPMC at the same concentration, the results obtained from the texturometer show that it requires less extrusion force to pass through a 27 gauge needle, facilitating the handling of the samples for intraocular injection. This behavior may be related to the rheological results described above, which show that PQ10 has a typical viscoelastic behavior while HPMC behaves as a viscous material. In addition, the rheological behavior of PQ10 does not change after one autoclave cycle of 30 min at 1 atm, adding the further advantage of resorting to a simple, low-cost method, such as the autoclave, for the sterilization of the samples in their final containers.

In our study, we evaluated the local effects of PQ10 administered by three different routes: topical, subconjunctival and intraocular. Slit-lamp biomicroscopy was carried out to evaluate the presence of AC cells or flare, and the status of the cornea, iris and lens. We found that the subconjunctival injection of 2 % PQ10 was well tolerated, with only mild inflammatory reaction lasting for 24 h, easily explained by the trauma produced by the handling. Another important observation of this study was that the bleb produced by the 2 % PQ10 solution remained for a significantly longer period of time than that produced by HPMC viscoelastic solution 2 % w/v. This could be explained by the different metabolism of the two polymers, leading to a longer lasting effect of PQ10 compared with the control group.

Because different inflammatory responses are elicited in different types of tissues, the biocompatibility assessment of foreign implants in immune privileged sites of the body is of great significance. Among various immune privileged tissues, the AC of the eye has several advantages over other tissues as an implantation for the study of the biocompatibility of materials, including excellent tissue sensitivity and clarity, convenient access to view, simple implantation procedures and reliable ophthalmic parameters. In this regard, we have shown that 2 % PQ10 causes a transient increase in intraocular pressure that lasts no longer than 24 h, with the corresponding ciliary congestion and corneal edema that characterizes the state of ocular hypertension. However, the eyes injected with the fluorescent polymer showed the complete disappearance of the substance after 36 h and our results would indicate that both fluxes (PQ10 and the aqueous humor) follow the same route.

Our results indicate that the short-term elevated IOP induced by gel implantation may lead to significant transient corneal edema, as demonstrated by slit-lamp biomicroscopy. Nonetheless, a high IOP maintained for a period equal to or greater than 72 h has been reported to be required to damage the corneal endothelium by reducing its cell count [31]. As a result, a rise in pressure lasting up to 2 days does not lower endothelial cell density. In addition, the PQ10 2 % solution caused the same response in terms of corneal edema and intraocular inflammation as HPMC.

The histopathological examination of the eyes injected with PQ10, as well as the HPMC, showed no changes in the inner retina (data not shown), cornea or ciliary body. Moreover, no reduction of tissue or presence of inflammatory cells was observed in the ciliary body or the cornea. Similarly, no cytotoxic effects were found in the cell cultures incubated with the gel in the in vitro assays. Thus, these results demonstrate that PQ10 exhibits no toxic effects, or the fact that toxicity is too low to be able to be detected by any of the methodologies used.

One of the most important findings of this experimental study involves the results of the functional studies, performed by measuring the evoked potential that showed values within the normal range for both groups of rabbits, even after 1 month of treatment with intraocular applications of 2 % PQ10.

Finally, another interesting property of PQ10 with potential biotechnological application in further biomedical areas is the relationship of hydration and gelation of PQ10 with pH and temperature. We observed that the gelling time of the polymer decreases with the increase in pH but only at 25 °C; at higher temperatures, this dependence is lost. Considering the different applications of more concentrated solutions of PQ10, where high viscosity hampers the handling of the samples, this feature of polymer gelification as a function of pH could be used to design formulations that allow injected mixes freshly prepared at pH 5, where PQ10 has a shorter gelation time, leaving complete gelling in the injection site.

5 Conclusion

The rheological study of PQ10 evaluated in this research showed that the hydrogel behaves similar to those currently used, with some potential advantages such as residence time at the site of application, which is essential in this type of formulation. Furthermore, in vitro and in vivo studies demonstrate that hydrogel is particularly well-tolerated.

Therefore, a better understanding of the safety, rheology, stability and other design criteria for this new hydrogel brings us closer to the development of new OVDs, despite the need of long-term follow-up.

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