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Abstract. In this work, we designed and realized a new phantom able to mimic the principal mechanical, rheological, and physical cues of the human eye and that can be used as a common benchmark to validate new surgical procedures, innovative vitrectomes, and as a training system for surgeons. This phantom, in particular its synthetic humor vitreous, had the aim of reproducing diffusion properties of the natural eye and can be used as a system to evaluate the pharmacokinetics of drugs and optimization of their dose, limiting animal experiments. The eye phantom was built layer-by-layer starting from the sclera up to the retina, using low cost and easy to process polymers. The validation of the phantom was carried out by mechanical characterization of each layer, by diffusion test with commercial drugs into a purposely developed apparatus, and finally by a team of ophthalmic surgeons. Experiments demonstrated that polycaprolactone, polydimethylsiloxane, and gelatin, properly prepared, are the best materials to mimic the mechanical properties of sclera, choroid, and retina, respectively. A polyvinyl alcohol-gelatin polymeric system is the best for mimicking the viscosity of the human humor vitreous, even if the bevacizumab half-life is lower than in the human eye. [DOI: 10.1117/1.JBO.19.6.068001]

Keywords: bevacizumab; drug diffusion; eye phantom; mechanical properties; vitreous analogous; vitreous viscosity.

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1 Introduction

The eye is the main structure of the visual system and is located within the orbit, where it occupies approximately one-third of the volume and is composed mainly of blood vessels, muscles, and fat. Its wall consists of three concentric membranes: (i) the fibrous tunic, composed in front of the cornea and later on the sclera; (ii) the vascular tunic, or uvea, expressed earlier by the iris, centrally by the ciliary body, and subsequently by the choroid; (iii) the nervous tunic, or internal, formed exclusively by the retina. These structures surround the vitreous body, a transparent gel consisting of water and a network of collagen fibers, hyaluronic acid, and a small amount of soluble proteins.[1] With aging or with the onset of eye diseases, the eye structures may be subject to modification and their functionalities can thus degrade. Drugs with specific pharmacological and pharmacokinetic characteristics can be given topically, subconjunctivally, intraocularly, and systemically to treat a variety of ocular diseases.[2] Although this approach could be effective in some common and important inflammatory ophthalmic diseases, such as anterior scleritis, uveitis, and corneal graft rejection,[3] the study of drugs diffusion into the humor vitreous to analyze their pharmacokinetics is difficult and expensive. If it is not possible to intervene with a pharmacological therapy, it will be necessary to use vitreo-retinal surgery. The main techniques for the surgical treatment of vitreo-retinal diseases are the episcleral way and the vitrectomy. During the latter surgical procedure, the surgeon inserts small instruments into the eye, cuts the vitreous gel, and suctions it out. After removing the vitreous gel, the surgeon may treat the retina with a laser (photocoagulation), cut or remove fibrous or scar tissue from the retina, flatten areas where the retina has become detached, or repair tears or holes in the retina or macula. Vitrectomy is mainly used to treat otherwise untreatable diseases, such as retinal detachments, vitreous opacities, vitreous traction on the retina, and complications resulting from proliferative diabetic retinopathy. Vitrectomy, as do other surgical techniques, requires careful training. Considering that the physical properties of eye tissue degrade immediately after death, tests for pharmacokinetic and vitrectomy are usually performed on eyes taken from animal models. The main concern with this procedure regards the possibility of incongruities between the eyes of laboratory animals, such as rabbits, pigs, cattle, and the human eye. Moreover, animal tests are very expensive and pose ethical issues. For these reasons, the development of a common platform, such as a standard phantom, to test new ophthalmic surgery devices, the pharmacokinetics of new drugs, and, of course, to train surgeons is always more necessary and urgent. The use of a phantom is common in eye-related fields, especially to evaluate the dose of ionizing radiation in the eye (e.g., rachiteraphy[4]). Software to evaluate the potential dose is also numerous. Despite the large amount of phantoms and materials available for dosing, there are few reports about a phantom to be used in pharmacokinetics and vitrectomy. Generally, in fact, several research groups had focused their attention on vitreous humor substitutes to be used in clinical practice, considering viscosity as the main parameter to be replicated.[5,6] Pharmacokinetic aspects are not taken into account, and in any case, these substitutes are not framed into a well-organized eye phantom. An example of an

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eye phantom can be found in the research of Richa et al.,[7] where it was used to validate a vision-based proximity detection system in retinal surgery. In another paper, Jafri et al.[8] recognized the importance of an eye phantom to simulate the eye's behavior under ultrasound analysis for surgical training. It is, therefore, extremely useful to provide ophthalmic surgeons an eye substitute that allows them to optimize the various surgical parameters, evaluate the best conditions of operation, and determine pharmacokinetics and distribution of drugs or other small molecules. Thanks to a strong collaboration with ophthalmic surgeons, technical specifications and functional requirements of the eye phantom have been defined. Moreover, an eye must be easy to prepare and to use also by unskilled users, it has to be transparent, easy to handle, and, as much as possible, have a low cost. Thus, an ocular phantom has been designed and realized, reproducing the main features and composition of each layer of the human eye, knowing the mechanical and rheological properties of the various structures that compose it. Each structure has been realized using different polymers or their blends, for which the mechanical and/or rheological behavior has been characterized. Finally, a team of ophthalmic surgeons tested the realized phantom.

2 Materials and Methods

2.1 *Materials*

Polyvinyl alcohol (PVA), gelatin from porcine skin, and polycaprolactone (PCL) were purchased from Sigma-Aldrich® (St. Louis, Missouri). Bevacizumab, Avastin™ was obtained from Genetech (San Francisco). Sodium chloride was purchased from Carlo Erba Reagents® (Milano, Italy). Genipin was obtained from Challenge Bioproducts co., LTD (Yun-Lin Hsien, Taiwan). Sylgard® 184 and Sylgard® 527 polydimethylsiloxane (PDMS) elastomers were purchased from Dow Corning (Midland, Michigan). Disodium tetraborate decahydrate was purchased from Baker (Deventer, Holland). Deionized water was used throughout the study.

2.2 *Phantom Project: Specification and Design*

We developed the phantom by extracting the features and dimensions of each layer of the human eye from the literature and reproducing their biomechanical behaviors with synthetic and natural polymers. Like the human eye, the phantom is realized by combining several layers. Because the surgical space of interest during vitrectomy is the posterior part of the eye, the design was focused on the sclera, the choroid, the retina, and the humor vitreous. For readability, the sclera, the choroid, and the retina are treated in the first part of this section because their substitutes have been mechanically characterized following similar testing protocols. Then, the preparation and testing of the humor vitreous will be described.

2.3 *Sclera*

The sclera is characterized by an elastic modulus composed of 2.9 ± 1.4 MPa in the anterior part and 1.8 ± 1.1 MPa in the posterior part.[9] Generally, the maximum stress before failure is 13.89 ± 4.81 MPa.[10] Considering the aforementioned range of elastic moduli, a PCL-based polymeric system was used as a substitute to mimic sclera structure. To evaluate the exact polymer concentration to better fit the mechanical behavior of the natural sclera, polymeric films were prepared by dissolving PCL in chloroform in different percentages [1%—5%—10% (w/v)] and then were spun using the spin coater Delta10TT to obtain a homogeneous thickness.

2.4 *Choroid*

The choroid is a vascular layer, containing connective tissue, and lying between the retina and the sclera. The human choroid thickness is composed of between 0.1 mm in the anterior and up to 0.2 mm in the posterior parts of the eye.[11] Its mechanical properties were evaluated in several papers, and its average elastic modulus is around 0.6 ± 0.28 MPa,[12] but is significantly different from the posterior (higher) and the anterior (lower) parts. In the realized phantom, the choroid has been replaced with biocompatible silicone, PDMS, prepared in accord to the producer's specification by mixing the monomer and the activator in a ratio of 10:1. The previous experiments[13] demonstrate that the PDMS mixture of Sylgard® 527 and Sylgard® 184 had an elastic modulus similar to that of the choroid. For mechanical testing, film-shaped samples were obtained by spinning a mixture of monomer and activator with a Delta10TT spin coater.

2.5 *Retina*

The retina is the light-sensitive layer of tissue at the back of the inner eye.[14] Generally, its mechanical behavior is anisotropic and usually the material is nonhomogeneous. Globally, its elastic modulus is around 20 kPa;[15] very often, in the literature the "spring" elastic constant is indicated (2 N/m for bovine eye).[15] Gelatin crosslinked with genipin was used to mimic the biomechanical properties of the retina. Genipin is a natural crosslinker extracted from gardenia jasminoide ellis.[16,17] During polymerization, this polymeric system switches its color from transparent to light brown to intense blue. Starting from previous works,[18–20] we investigated the mechanical properties of 4% and 7.5% (w/v) gelatin in water crosslinked with different percentages of genipin [0.1% to 0.2% (w/v) with respect to the volume of the gelatin solution]. Samples were obtained by casting the uncrosslinked mixture into 24-multiwell plates.

2.6 *Mechanical Testing*

Mechanical characterization of polymeric materials to mimic sclera, choroid, and retina was performed with a uniaxial testing machine Zwick-Roell Z005. PCL and PDMS were shaped in a film with ~5-mm width and a 25-mm length with a thickness of 500 μm. Samples were mechanically characterized by a tensile test until failure with a deformation rate equal to 0.2% s^{-1}. Gelatin crosslinked with genipin samples was shaped into cylindrical samples with a 13-mm diameter and 5-mm height and mechanically characterized by compression test, until 20% deformation, with a deformation rate equal to 0.2% s^{-1}. All tests were performed in triplicate.

2.7 *Vitreous Humor*

The vitreous body constitutes two-thirds of the total amount of the eye and it owes its transparency to its low molecular and cellular content.[1] Various anatomical regions have been defined in it, including the central vitreous, the basal vitreous, the vitreous cortex, the vitreoretinal interface, and the zonules. The central vitreous contains the canal of cloquet, a central tubular structure constituting the main residual of the primary embryonic vitreous. The basal vitreous is composed of collagen fibrils

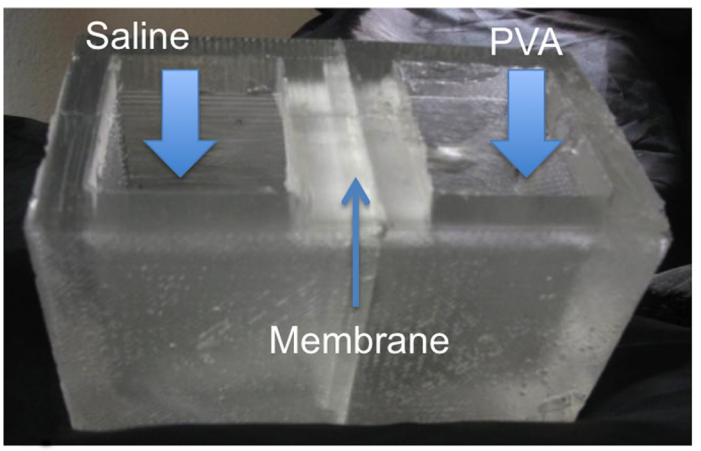
packed together and strongly fixed to the retina and to the pars plana. The third element of the vitreous body is a thin layer of vitreous gel that encloses the central gel; they are discernible by the different orientation of the base collagen fibers.[21] Zonular fibers consist of fibrillin-containing microfibrils. The preparation of vitreous humor consists of 2.5% (w/v) gelatin in water mixed with a PVA solution at different percentages [from 10% up to 40% (w/v)].[22–24] The solution is stirred moderately at 70°C to ensure the complete homogenization of dispersion; 50 μl of HCl 1 M are then added and the mixture is stirred at 100 ± 5 rpm speed for 30 min at 50°C, to allow the esterification reaction between PVA and gelatine.[25] Unlike the results described by Pal et al.,[26] the sample was not dried, and we obtained a homogeneous and transparent gel. Gelatin and HCl percentages were maintained fixed.

2.8 *Rheological Characterization*

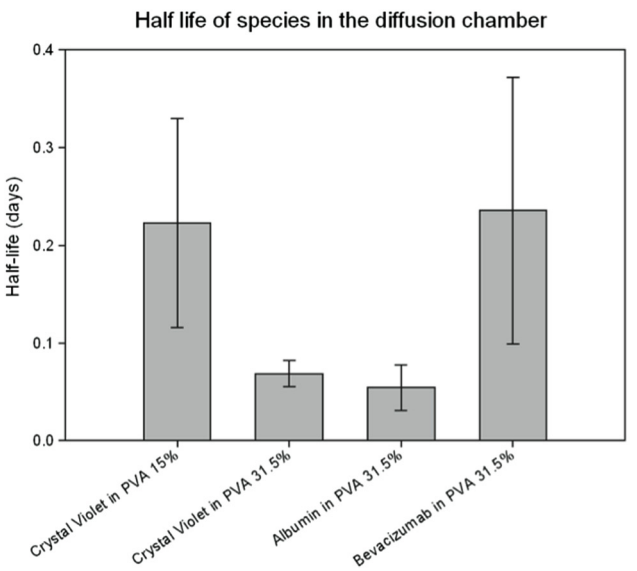
PVA-gelatin mixtures were characterized from the rheological point of view by varying the PVA-gelatin ratio as previously indicated. The dynamic viscosity of each sample was evaluated using a cone-plate rotary rheometer Rheostress RS 150 Haake with a measurement body cone-plate P61 and C60 in order to determine the PVA percentage reproducing a viscosity comparable to the human vitreous one, equal to 4000 cP.[27] The tests were carried out at room temperature and at 35°C to simulate typical eye temperature in order to evaluate the temperature influence on viscosity.

2.9 *Diffusion Parameter Characterization*

During the present work, a permeation silicone chamber was purposely designed and realized, with the aim of studying the diffusion of drugs in the vitreous substitutes and, secondary, of understanding if an injection into this vitreous substitute can mimic elimination kinetics after intravitreal injection into the human eye [Fig. 1(a)]. The two chambers are separated by a membrane with a porosity that allows the passage of the drug, but not of the hydrogel. Preliminary experiments in diffusion tests led to the choice of the Advantec MFS (Dublin,



(a)



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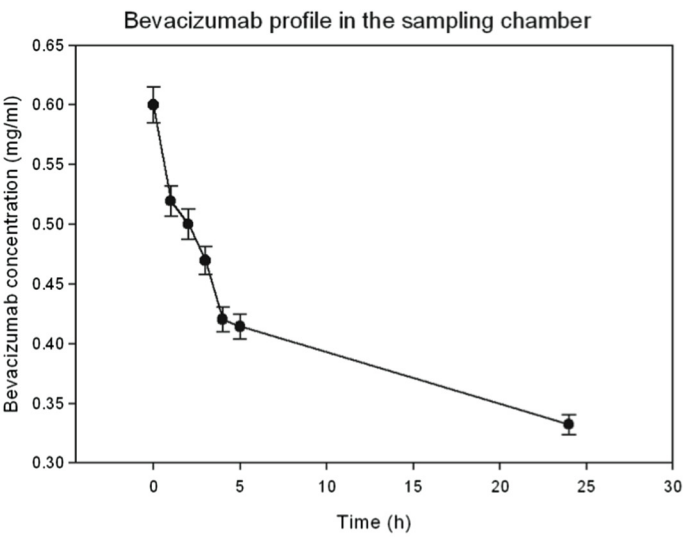

(c)

Fig. 1 Diffusion tests in artificial vitreous. (a) Permeation silicone chamber with its functional parts; (b) Estimation of various substances half-life using an exponential decay function fit to raw data; (c) Bevacizumab profile versus time in the diffusion chamber. Error bars in the graphs represent sample standard deviations.

California) membrane of cellulose esters with a 0.45 μm porosity as a separator membrane. Permeation chambers were made of biocompatible silicone Sylgard® 184 and both have 4-ml internal volumes. One of the chambers was filled with saline solution, whereas the other with a mixture of 31.5% (w/v) PVA solution in deionized water and 2.5% (w/v) gelatin in deionized water. This concentration was chosen after rheological characterization (see Sec. 3). Bevacizumab, a humanized monoclonal antibody used in ophthalmology for the treatment of neovascularization in diseases such as diabetic retinopathy and age-related macular degeneration, was used as a target drug to characterized diffusion parameters.[28] The dose used during eye-care vitrectomy is 100 μl of a commercial solution, which corresponds to 25 mg/ml. This treatment produces, in the eye, a drug concentration of 0.6 mg ml^{-1}. The experiments reproduced the surgical procedure: the drug was injected in the same concentration in one of the two chambers, and then the diffusion was analysed, evaluating the concentration in the other chamber. 100 μl of bevacizumab were injected in the saline-filled chamber. Starting from that moment, a 200 μl sample was taken from the liquid compartment after 1, 2, 3, 4, 5, and 24 h, in order to observe the disappearance of the drug from the chamber, and so evaluate the diffusion time into the hydrogel. This design was due to the fact that it was tricky to observe and sample the compound diffusion in the hydrogel itself. Each sample was then analyzed using the spectrofluorimeter FLUOstar Omega BMG LABTECH (Ortenberg, Germany), after a calibration procedure.

2.10 *Phantom Realization Techniques*

The phantom presented in this work is characterized by a layer-by-layer structure. Each layer mimics its natural counterpart in terms of biomechanical properties (Fig. 2). Furthermore, the phantom should be (i) transparent; (ii) easy to fabricate, to handle, and to use; (iii) not expensive. Because most surgical problems are related to posterior part of the eye, the phantom was focused on this part and thus it is characterized by a hemispherical geometry. To obtain these requirements, the entire phantom was built following a sequential procedure. First of all an ocular

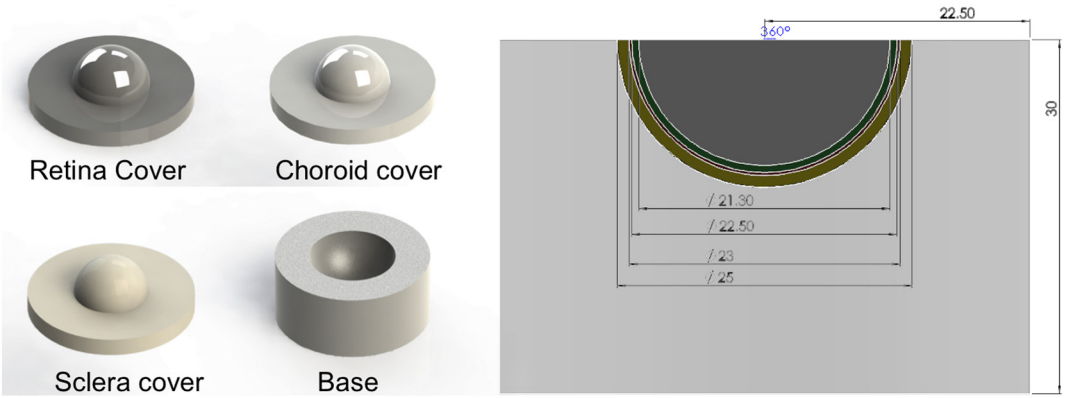

Fig. 2 Sketch of the various parts composing the phantom. (a) View of the covers used for making the different eye parts. As explained in the text an eye part is made for compression moulding of polymer between the base and a specific cover; (b) various phantom parts: sclera (yellow), choroid (purple), retina (green), and vitreous (gray). The BASE (Plexiglas) represents the ocular bone cavity.

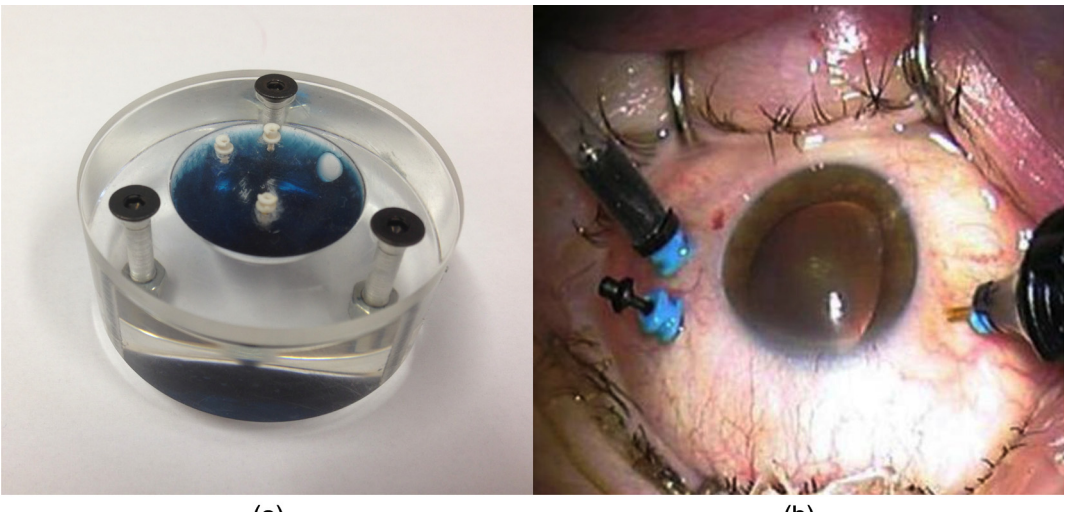

Fig. 3 Phantom versus eye. As it is possible to see the insertion scheme of trocar and vitrectomes in the eye phantom (a) is the same than in real eye (b).

cavity, obtained by Plexiglas computer numerical control (CNC) machining, was chosen to mimic the bone structures. This cavity, which is transparent, has a radius of 12.5 mm, and hosted all the other layers. In this cavity, a layer of PCL with a thickness of 1 mm, representing the sclera, was obtained by compression molding. The closure of the mold needed to obtain the desired layer was fabricated by CNC machining. However, as shown in Sec. 3, this layer was not transparent. An alternative ocular cavity was fabricated with a radius equal to 11.5 mm, eliminating the layer that mimicked the sclera. This second version of the phantom was developed on the basis of surgeons' requirements in order to have an eye phantom also transparent on its lateral side. The PCL layer is white allowing vision only from top of the phantom or with an optic fiber. The choroid was fabricated following the same principle of the sclera, by compression molding. In this case, a closure of appropriate dimensions pushed the PDMS onto the previous layer. Also, the retina was built in the same way, just changing the mold counterpart and, of course, the material. On the retina analogue layer, a casting of vitreous humor analogous completes the fabrication procedure. A transparent cover, fixed with screws to the "ocular cavity," sealed the system. The cover featured three openings to better emulate the surgical scenario: an opening for the vitrectomy probe, a second one for the optic fiber, and a third one to supply a physiological solution to the eye environment, positioned as in Fig. 3. If desired, a PDMS (Sylgard® 184) layer can substitute for this cover.

3 Results and Discussion

3.1 *Mechanical Characterization*

Figures 4(a) and 4(b) show the results of the mechanical characterization of the sclera substitute. A PCL film, obtained from a starting concentration of 5% (w/v) in chloroform, represented the polymeric system that better mimicked the biomechanical behavior of the sclera, and thus this concentration was chosen. A lower concentration (1% w/v) was too weak to be accurately tested. As already stated in the text, and as reported by Palchesko et al.,[13] PDMS films prepared with a mixture of 40% Sylgard® 184% and 60% Sylgard® 527 show an elastic modulus of ~0.6 MPa, very close to the one of the choroid. The gelatin crosslinked with genipin characterization is shown in Figs. 4(c) and 4(d), and according to the literature data, the retina behavior is better mimicked by the sample with a 4% (w/v) gelatin plus 0.2% (w/v) genipin.

3.2 *Rheological Characterization*

The tests were carried out at room temperature and at 35°C in order to simulate the physiological temperature of the eye. Each experiment was replicated at two different temperatures, evaluating the influence of temperature on viscosity. Subsequently, the data were processed using a linear regression model. In the obtained rheogram, the shear stress, expressed in pascals, and the shear rate, expressed in s^{-1}, are linearly related.

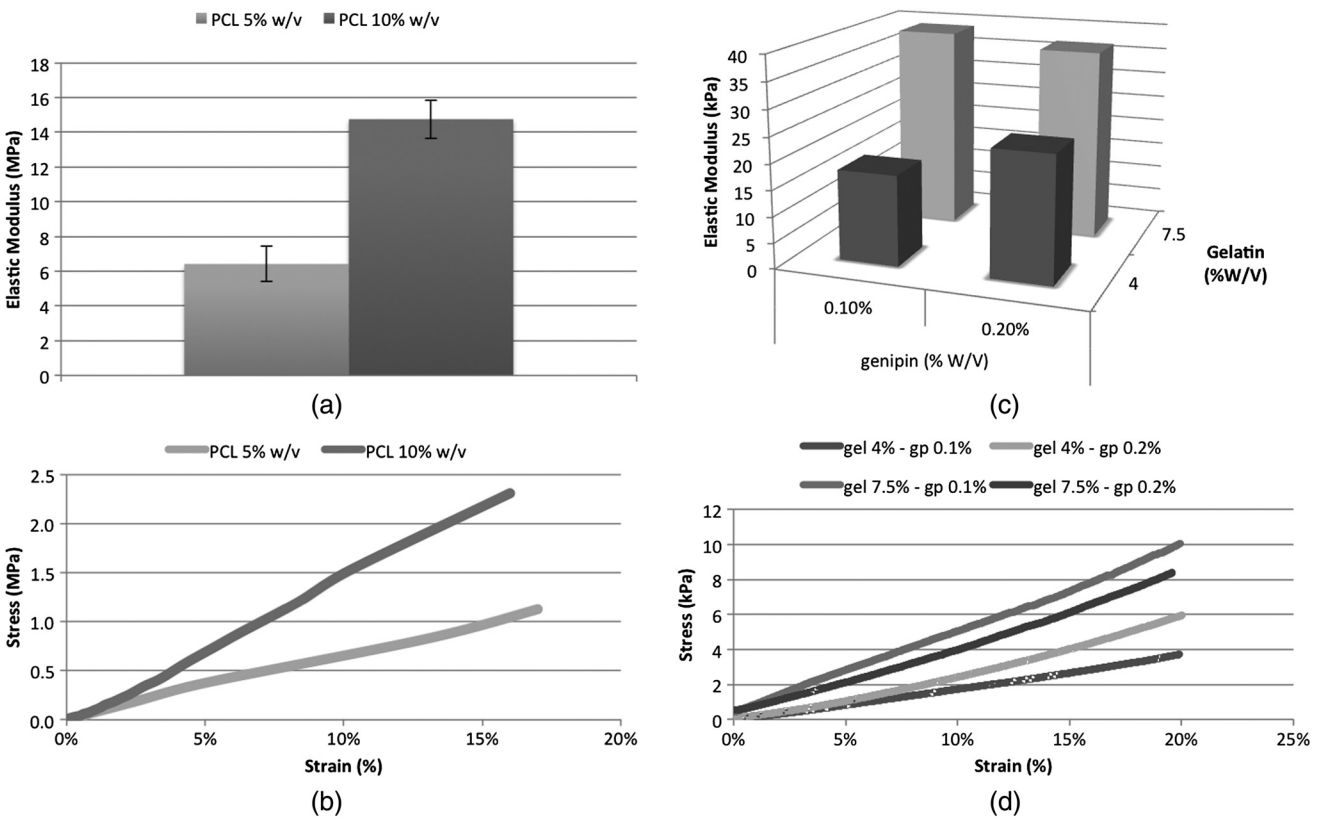

Fig. 4 Mechanical characterization of eye phantom materials. (a) Elastic modulus of polycaprolactone (PCL) at various concentrations. The 5% was found to be the best material in order to mimic the properties of sclera; (b) stress–strain curves relative to different PCL concentrations; (c) elastic modulus of gelatin–genipin construct at different gelatin and genipin concentration. It was found that gelatin 4% (w/v)—genipin 0.2% (w/v) was the best formulation in order to match retina properties; (d) stress–strain curves relative to different gelatin and genipin concentrations.

Therefore, the vitreous substitute exhibited Newtonian fluid properties. As expected, an inverse correlation between temperature and viscosity was found, as shown in Fig. 5(a). In order to determine the amount of PVA able to mimic the healthy human humor vitreous viscosity, the graphic reported in Fig. 5(b) was built, examining the results obtained at 35°C. Using the exponential equation shown on the graph, the percentage of PVA able to reproduce a viscosity equal to 4000 cP was 31.5% (w/v). To confirm the obtained results, ophthalmic surgeons tested the sample. Based on information provided by ophthalmologists, it was also interesting to determine the best vitreous analogous for pathological vitreous conditions, which is normally encountered during vitrectomy. Further tests have been carried out with 10% (w/v) and 15% (w/v) of PVA solution in deionized water. Those concentrations were more conciliatory than others in mimicking vitrectomy conditions, but nevertheless were not adequately transparent.

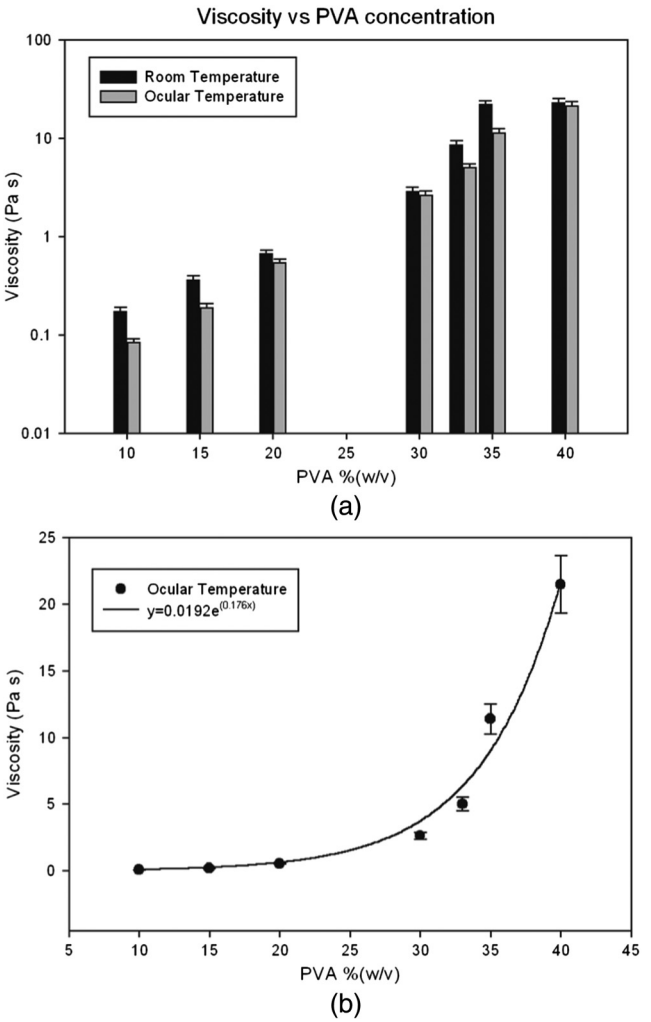

Fig. 5 Rheological characterization of phantom vitreous humor. (a) Viscosity of gels composed of polyvinyl alcohol (PVA) and gelatin as function of PVA concentration at room and ocular temperatures. The viscosity of the mixture decreases with temperature; (b) Curve fitting of viscosity versus PVA concentration at ocular temperature. In order to obtain the 4000 cP required to match the eye vitreous humor viscosity the needed PVA concentration is 31.5% (w/v). Error bars in the graphs represent sample standard deviations.

3.3 *Diffusion Results*

In Fig. 1(c), it is possible to see the concentration profile of bevacizumab into the sampling chamber. It was possible to estimate bevacizumab half-life ($T^{1/2}$) into the vitreous substitute thanks to a regression to a mono-exponential curve. The bevacizumab $T^{1/2}$ in the gel system made by PVA 31.5% (w/v) in deionized water plus gelatin 2.5% (w/v) was revealed to be too low ($T^{1/2}$ 0.235 days) compared to the rabbit and the human $T^{1/2}$. However, the absence of ocular structure like a retina and aqueous humor could be a reason for the lower than expected $T^{1/2}$. The phantom $T^{1/2}$ could be lower than the real eye one also because of multiple factors: unwanted mixing effects produced during the sampling; adsorption/absorption effects on the walls of the silicone chamber, which could lead to a faster dye rate of disappearance from the saline chamber.

It is important to state that is particularly difficult to evaluate the diffusion front within a thick hydrogel. No local sampling is possible without destroying the gel, and the measurements done by destroying the gel are unreliable. We proposed this hybrid saline-gel solution in order to have better reliability on measurements of a single-gel sample. The various $T^{1/2}$ s are shown in Fig. 1(b). It is interesting to compare the obtained values to recent studies of human and animal models. In the study by Abrishami et al.,[29] New-Zealand albino rabbits were used to see any increase in bioavailability of the encapsulated nanoliposomes and bevacizumab as received. Each rabbit was treated with two forms of bevacizumab, one kind for each eye; afterward the rabbits were sacrificed at an established time and the vitreous humor was analyzed with an ELISA test. The bevacizumab-encapsulated nanoliposomes $T^{1/2}$ was 4.32 days. In another study by Nomoto et al.,[30] the bevacizumab elimination kinetics in rabbits' eyes after topical, intravitreal end subconjunctival administration were verified. The half-life, found in the vitreous humor after intravitreal injection, was 5.95 days. The study conducted by Bakri et al.[28] shows a different value in the $T^{1/2}$ equal to 4.88 days, in contrast with the previous studies. Studies carried out by Zhu et al.[31] show trend data on the elimination of bevacizumab into the human intravitreal cavity that lead to an estimation of $T^{1/2}$ equal to, respectively, 6.7 and 10 days. The half-life difference between humans and rabbits was mainly due to the different volume of the vitreous humor, which in rabbits is about one-third of that of humans. This explains the need of creating a model of vitreous humor consistent with the structural characteristics of humans and animals.

3.4 *Ophthalmology Control*

The main critical point in the design and fabrication of the whole phantom was the vitreous humor. All vitreous humor samples prepared in this study have been tested in the phantom with the inclusion of membranes by ophthalmology surgeons. The 31.5% (w/v) PVA plus gelatin 2.5% (w/v) in deionized water was recognized as the best humor vitreous substitute, but more interestingly, the humor vitreous analogue with 15% (w/v) PVA solution was the optimal concentration for reproducing eye pathological conditions.

3.5 *Phantom Final Embodiment*

The properties of each layer chosen for the phantom are summarized in Table 1. The final embodiment of the phantom is depicted in Fig. 3. From this figure, it is possible to see all

Table 1 Materials chosen for mimicking human eye.

Tissue	Material
Bone	Plexiglas
Sclera	5% (w/v) PCL in chloroform
Choroid	Polydimethylsiloxane
Retina	4% (w/v) Gelatin in deionized water + 0.2% (w/v) genipin
Vitreous humor	31.5% (w/v) polyvinyl alcohol in deionized water + gelatin 2.5% (w/v) in deionized water

the various layers that form the phantom, and in particular, the three apertures in the top part as required by some of the surgeons that tested the system.

4 Conclusions

The first purpose of this study was to create a phantom that was able to reproduce the mechanical characteristics of different physiological structures present in the human eye, specifically the sclera, choroid, retina, and finally the vitreous humor. As introduced in the text, this type of phantom could represent a common platform for drug dosing, surgical benchmarks, and surgeons' training. Other applications can be found not only in the clinical field (such as testing of ultrasound technology), but also in basic research, such as the evaluation of oxygenation of ocular tissues. The synthetic vitreous humors created seemed a valid prototype. In particular, the humor vitreous substitute containing 15% (w/v) PVA solution in deionized water, despite a lower viscosity than human vitreous humor, produced very interesting results because it was able to mimic the eye environment that the surgeon finds during a vitrectomy. In fact, during this surgical procedure, the eye is supplied by physiological solution and therefore the vitreous humor present lower viscosity than the real one. For this reason, this composition was useful as a system for the evaluation of vitrectomy devices. The determination of the pharmacokinetics is an expensive procedure requiring animal models, which should introduce incongruities due to biological variability of the cavies and due to differences between animal eyes and the human one. With the proposed phantom, we tried to overcome this problem by providing an easy and reproducible method to estimate the drug diffusion inside the human eye without destroying the gel sample at each measurement. The experimental setup consisted of two separated chambers, one for drug injection and one for sampling respectively: as discussed, the sampling procedure can affect the results due to unwanted mixing effects. Despite the experimental limitations which resulted in a shorter half-life of bevacizumab, the drug that we selected as a template, the proposed approach should open a new way for a faster screening of the elimination kinetics of intravitreal drugs, thanks to its flexibility and reproducibility.

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