The Vitreous Model – a new in vitro test method simulating the vitreous body

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Abstract

The vitreous body has become an interesting location for the administration of injections or implants in modern ophthalmology. Such intravitreal dosage forms are loaded with active agents which are released over a prolonged period of time. A test method simulating the in vivo situation is needed to observe the release behaviour of these new dosage forms and the distribution of drug substances in the vitreous humor. To simulate this situation the Vitreous Model (VM) was developed.

The spherical glass corpus of the VM can be filled with a gel medium which is adapted to the in vivo situation. Gel insertion and sampling can be performed via a vent at the top. To simulate the movement of the eye the VM was placed on an altered orbital shaker. As vitreous substitute a modified polyacrylamide gel (PAA-gel), which consists of a 30% solution of acrylamide and standard Ringer buffer solution, was used. The water content, pH, relative density and refractive index of the PAA-gel were examined and compared to previous reported data for properties of human vitreous humor. For comparison the viscosity of the PAA-gel and porcine vitreous humor was determined experimentally. During a period of 3 hours the distribution behaviour of an injection of fluorescein sodium solution (1.25 mM) in the PAA-gel and in porcine vitreous humor was investigated by using the VM.

The water content (99%) and pH (7.4) of the PAA-gel were equal to the values reported for porcine and human vitreous humor. Furthermore, the values obtained for the relative density (1.0013) and refractive index (1.3385) showed good accuracies (deviation < 1%). Slight deviations were only found for the viscosity. Also in the distribution study a similar behaviour were detected. Along with the adapted shape of the VM, the new model seems suitable to perform in vitro experiments, which are capable of simulating in vivo conditions. Through the possibility of insertion of intravitreal injections and implants in the VM, biorelevant distribution and release studies may become feasible.

1 Introduction

Drug targeting to and substitution of the vitreous body is one of the most interesting topics in ophthalmic research1. Pathologic changes of the vitreous body like posterior vitreous detachment or macula edema are treated via approaches in which solutions, suspensions or drug-loaded implants are administered directly in the vitreous body1,2. In clinical practise silicone oils are currently used as vitreous substitutes3. Another possible substitute described in literature1 is a polyacrylamide gel. This approach passed into oblivion because of a lack of testing1. In this work a modified polyacrylamide gel (PAA-gel) was prepared and tested regarding its suitability to mimic the vitreous body in its physical and chemical properties for an in vitro test system. A number of innovative intravitreal implants are in the research pipeline4. Some of them have already been introduced to the market and the application frequency is increasing. One example is Ozurdex® (a Poly(D,L-lactide-co-glycolide) rod-shaped implant loaded with dexamethasone) which is used to treat macula edema5. For first clinical studies animal models like rabbit eyes or porcine eyes are commonly used to investigate the drug release and safety of such implants5. Porcine eyes would be the closest match since they are reported to be most similar to human concerning anatomical properties6. For this reason vitreous humor of porcine eyes were used to compare it to the vitreous substitute. Animal models entail much of disadvantages. The inhomogeneity of the tissues and anatomical differences in the interspecies comparison result in large variations and the transfer to humans is often not possible6. A test method simulating the in vivo situation is needed to observe the release behaviour of these new dosage forms and the consequential drug distribution in the vitreous body. On that account a new biorelevant test method, called the Vitreous Model (VM), was developed. In this study first distribution experiments were performed to evaluate the applicability of the new setup.

2 Methods

2.1 Vitreous Model

As vitreous substitute a modified polyacrylamide gel (PAA-gel) was used. To prepare the gel, a 30 percentage solution of acryl amide (Rotiphores®) was mixed with standard Ringer buffer solution. Afterwards ammonium persulfate (APS) and tetramethylthelyenedia-
mine (TEMED) were added to crosslink the acryl amide monomers. The crosslinking is essential for gelatinization of the mixture. Rotiphorese®, APS and TEMED were purchased from Carl Roth GmbH, Karlsruhe, Germany. The viscose solution was filled in a glass model (see below) where it gelatinized in approximately 10 minutes. The corpus of the Vitreous Model (VM) consists of a spherical glass vessel (figure 1) with a filling volume of approximately 4 cm³. Prior to gelatinization the PAA-solution can be inserted via a vent at the top of the vessel which can also be used for sampling. The spherical model can be disassembled along its equator line to remove the contents for analysis.

**Figure 1: Spherical glass vessel of the Vitreous Model**

2.2 Model characterization

The relative density, viscosity, pH and refractive index of the cross-linked polyacrylamide gel were quantified to check the similarities to human and porcine vitreous humor. The relative density was determined via filling and weighing the gel in a pycnometer (Pycnometer Superior, Marienfeld GmbH, Lauda Königshofen, Germany). The viscosity was measured by a rotation viscosimeter (RVDV-II+CP, Brookfield Engineering Laboratories, Massachusetts, USA). Furthermore the pH (pH meter FiveEasy FE20, Mettler Toledo, Gießen, Germany) and the refractive index (Abbé refractometer, Carl Zeiss Jena, Jena, Germany) were ascertained. Except of the values for the viscosities all mentioned properties for human vitreous humor were obtained by literature analysis. Porcine vitreous humor was used for comparison of the viscosity with the PAA-gel. The porcine vitreous body was extracted from fresh porcine eyes, which were obtained as animal byproducts from a registered slaughter facility.

2.3 Distribution study

The PAA-gel was filled in the glass model. After completed gelatinization, 15μl of 1.25 mM fluorescein sodium solution (as model substance) were centrally injected into the simulated vitreous via a microliter syringe. The model was put on an altered orbital shaker. The shaker was set to 180 U/min thus moving the model circular whereas the top of the glass vessel was fixed yielding a rolling movement as a rather simulation of the eye movement (figure 2). After 3 hours the model was removed and frozen. In frozen state the “globe” was divided in 4 parts (figure 2). For analysis the samples were defrosted and incubated with acetone. After 12 hours incubation, segregation of the acetone phase and subsequent evaporation of the acetone, the eluted fluorescein sodium was taken up with Ringer solution and quantitatively analyzed via fluorescence reader (Varioskan Flash Multimode Reader; Thermo Scientific /Fisher, Waltham, Massachusetts, USA) by an excitation wavelength of 485 nm and emission wavelength of 538 nm. All experiments were performed as triplicates. For comparison the same procedures were performed with porcine vitreous humor. As blank value samples were frozen and prepared without movement direct after the injection of the solution.

**Figure 2: Experimental setup for the distribution study and segmentation of the frozen gel for analysis (a: fixation of the VM on the altered orbital shaker; b: circular rotating movement of the fixed VM).**

3 Results

3.1 Model characterization

The pH of the PAA-gel corresponds to the literature values of the human vitreous humor¹. For the relative density, viscosity and refractive index slight deviations were observed. Data is presented in table 1. By using the Ringer buffer solution a composition of electrolytes and water content comparable to vitreous humor was obtained.

Regarding the physical and chemical properties of the PAA-gel, the substitute seems to be suitable to perform *in vitro* experiments, which are capable to simulate *in vivo* conditions.

**Table 1:** Comparison of the natural vitreous humor and the PAA-gel; experimental data for density and viscosity presented as mean ± standard deviation (n=3).

<table>
<thead>
<tr>
<th>property</th>
<th>human/porcine</th>
<th>PAA-gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>water content</td>
<td>98-99%¹</td>
<td>99%</td>
</tr>
<tr>
<td>pH</td>
<td>7.4¹</td>
<td>7.4</td>
</tr>
<tr>
<td>density</td>
<td>1.0053 – 1.0089 g/cm³¹</td>
<td>1.0013 ± 0.0014 g/cm³</td>
</tr>
<tr>
<td>refractive index</td>
<td>1.3345-1.3348¹</td>
<td>1.3385</td>
</tr>
<tr>
<td>viscosity</td>
<td>606.0 ± 9.6</td>
<td>699.2 ± 66.9</td>
</tr>
</tbody>
</table>

¹ From literature.
With approximately 4 cm³ the volume of the new glass model is consistent with the volume of an average human vitreous body³.

3.2 Distribution study

The fluorescein sodium solution was injected in the center of the VM via the vent on the top. With both filling media, PAA-gel or porcine vitreous humor, most of the injected solution moved back along the puncture duct when retrieving the needle of the microliter syringe. The result was a concentrated spot in the middle with a stripe in direction of the vent (figure 3). This phenomenon is already known and described¹. After 3 hours the spot had disappeared and an increasing homogenization could be observed (figure 3).

![Figure 3: Fluorescein sodium solution in the puncture duct when retrieving the needle just after injection (A); Vitreous Model after 3 hours movement (B).](image)

For the blank value of both filling media 99% were obtained at the site of injection (parts 1+2 in figure 2). After 3 hours of movement most of the injected solution (75%) was still found in these parts of the porcine vitreous humor. With an amount of 81% a similar result was obtained for the PAA-gel (figure 3+4). Only a low quantity diffused into the anterior part (quadrants 3+4 in figure 2) of the gel. The relative standard deviation was in a range of 0.3 to 14 percentages. The detected amounts of fluorescein sodium in the 4 quadrants (figure 2) were similar in the comparison of the PAA-gel and the porcine vitreous body (figure 4).

![Distribution of the fluorescein sodium in the PAA-gel and porcine vitreous humor](image)

As published previously⁸, the position of the injection plays an important role for the distribution and the therapeutic effect. The slow distribution of the fluorescein sodium solution during the incubation period of 3 to 12 hours reinforces this statement. Therefore the implantation should be set close to the target to quickly reach a therapeutic concentration at the site of action. Structures like collagen fibers or proteins are currently not included in the PAA-gel. Influences of these structures could be an explanation for the slight deviation and might be more important for active ingredients with different chemical properties, such as high protein binding. Another limitation of the VM is the closed circulation. In vivo an exchange of aqueous humor between the vitreous humor and its environment occurs. However, this exchange is a quite slow process. The good accordances of the results obtained for fluorescein sodium in PAA-gel with the porcine vitreous humor show the applicability of the VM to simulate the vitreous body. Distribution processes in the vitreous body can be strongly influenced by convection processes which are driven by the eye movement. Therefore physiological movement patterns have to be included in the model in future.

4 Conclusion

The investigated properties of the PAA-gel cross-linked in the glass vessel are very similar to the properties reported for human vitreous humor. The direct comparison between the PAA-gel and the porcine vitreous body in the distribution experiments with the model drug showed only minor deviations. Modification approaches will be to adapt the eye movement and the composition of the model even closer to in vivo conditions and mimic the age-related liquefaction of the vitreous body. The number of drug loaded intravitreal implants will increase and in vitro models characterizing the drug release are required. With the VM the simulation of the vitreous body becomes feasible in a simplified in vitro model.

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6 References


