Influence of Siluron® insertion on model drug distribution in the simulated vitreous body

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Abstract: Biorelevant in vitro test systems may be helpful to understand the in vivo behaviour of modern intravitreal dosage forms such as implants and injections. The already presented Vitreous Model (VM) in combination with the Eye Movement System (EyeMoS) was used to simulate the situation after a vitrectomy in combination with Siluron® silicone oil (SO) insertion in vitro and to investigate the distribution of the model drug fluorescein sodium (FS) within the modified VM. The state after a vitrectomy was simulated in vitro by replacing half the volume of the gelled vitreous substitute by SO. Under consideration of simulated eye movements the position of SO towards the simulated vitreous body was examined. Furthermore, the influence of two different injection techniques was studied. On the one hand, FS was injected directly into the gel and on the other hand the injection was set through the gel in order to directly reach the SO. Independent of the injection technique, it was shown that the model drug distributed almost exclusively into the gel and not into the SO. This can be explained with the backflow of FS into the gel and the lack of solubility in the SO. Using the modified VM and EyeMoS, the in vitro characterization of drug release and distribution behaviour of intravitreal injections can be performed under consideration of a simulated vitrectomy.

Keywords: eye movement system; intravitreal distribution; silicone oil; vitrectomy; vitreous body; vitreous model.

1 Introduction

The use of Siluron® silicone oil (SO) as an intraocular tamponade has become a standard technique in vitreoretinal surgeries [1, 2]. Pathological changes of the vitreous due to retinal detachment, diabetic retinopathy or vitreous haemorrhage may require the partial removal of the vitreous body. Subsequently, SO serves as an internal tamponade to retinal holes, stabilizes the intraocular pressure and prevents a further retinal detachment by pressing the retina onto the pigment epithelium.

There is a lack of data about the behaviour of SO within the vitrectomized eye especially in combination with drug administration. A reliable tamponade effect acting on the retina is expected if the SO is located close to the target tissue. The effectiveness of this procedure is considered to depend on the shape, volume, weight and position of the SO bubble. Also, patients that have undergone vitrectomy and SO insertion are often treated with intravitreal drug injections. It is unclear how this exchange of the vitreous body against SO affects the local drug concentrations and the pharmacokinetics within the eye.

To gain a better understanding of the circumstances within vitrectomized eyes, an in vitro model was developed. This model consists of the previously developed and slightly modified combination of the Vitreous Model (VM [3]) and the Eye Movement System (EyeMoS [4]) with a partial substitution of the simulated vitreous against Siluron®.

2 Material and methods

2.1 Preparation of the simulated vitreous body

The conventional VM of glass [3] was combined with a new 3D printed VM (see Figure 1) in order to examine both quantitatively and visually distribution behaviour of...
In this in vitro study a modified polyacrylamide gel (PAAG) served as synthetic vitreous substitute [3]. The hydrogel consists of Rotiphorese®, ammoniumperoxodisulfate (APS) and tetramethylethylenediamine (TEMED®). All chemicals were of analytical grade and purchased from Carl Roth GmbH (Karlsruhe, Germany). The polyacrylamide, being in a liquid state, was filled into the VM. After gelation, the model was filled with silicone oil resulting in a ratio 50:50, v/v of gel:SO. Siluron® 5000 (FLUORONGmbH, Ulm, Germany), a high purity sterilized liquid, composed of repetitive polydimethylsiloxane units (CH₃)₃SiO-[Si(CH₃)₂O]ₙSi(CH₃)₃ was used for this purpose. It is a certified transparent medical device for intraocular use. The physicochemical properties of Siluron® 5000 in comparison to the natural vitreous body and the PAAG are shown in Table 1.

### Table 1: Comparison of the physicochemical properties of the natural vitreous body, polyacrylamide gel and Siluron® 5000.

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<tr>
<td>Density at 25 °C (g/cm³)</td>
<td>1.0053–1.0089</td>
<td>1.0013 ± 0.0014</td>
<td>0.97</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.3345–1.3348</td>
<td>1.3385</td>
<td>1.404</td>
</tr>
<tr>
<td>Miscibility with water</td>
<td>miscible</td>
<td>miscible</td>
<td>immiscible</td>
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The EyeMoS [4] has been designed to integrate the established VM and to simulate human eye movements in vitro. It is composed of six 3D printed holders, in which six VM can be clamped. With the software Nanopro 1.70.8.0, two multiphase motors (PD4 N6018L4204, Nanotec Electronic GmbH & Co. KG, Feldkirchen near Munich, Germany) were programmed to implement four different movement patterns (see Figure 1) and to combine them together (see Figure 1) to more adequately simulated movement patterns.

An aqueous solution of fluorescein sodium (FS, 2 mg/ml, injection volume of 20 µl) was injected via the IN-Stopper. A subject of investigation was the distribution of FS after a central injection into the PAAG (22 G needle). In another set of experiments, the injection spot was located in the half of the model occupied by the SO thus injecting through the PAAG (see Figure 1).

After a movement activity of 1, 4, 8, 24 and 48 h the VM was removed from the EyeMoS and deep-frozen (-19°C). Since the SO did not freeze opposed to the PAAG, both phases were separated, incubated with 4 ml modified Ringer buffer solution and analysed by fluorescence spectrometry (Varioskan Flash, Thermo Scientific, Waltham, USA) with an excitation wavelength of 485 nm and an emission wave length of 538 nm.
3 Results and discussion

3.1 Simulation of vitrectomized vitreous bodies

By the use of the modified VM with a partial replacement of PAAG by SO as substitute (50:50, v/v), an *in vitro* model of the vitrectomized eye was successfully implemented.

*In vivo*, a reliable tamponade effect is expected if the SO is located close to the retina and is not dislocated during eye movement. During the period of 72 h with simulated eye movements, the positions of SO and PAAG in the simulated vitrectomized eye did not change. Neither fast nor slow simulated eye movements caused a change in the shape and size of the SO bubble. There was no phase distribution or formation of droplets. This finding is in accordance with the results obtained by Hillier et al. [6] who also investigated the behaviour of SO in simulated vitreous *in vitro*.

A limitation of the presented *in vitro* system is the missing of anatomical elements of the eye, for example the lens, which may influence the position and behaviour of the SO. However, the model allows for first experiments regarding the distribution of injected drug between the two compartments (simulated vitreous and SO).

3.2 *In vitro* distribution testing

The fluorescent model drug was chosen to visually track the distribution in the simulated vitreous body. After the injection of FS into the PAAG, the already described injection channel [3, 7] was still detectable *in vitro*. Within 1 h of simulated eye movement, FS diffused from the clearly defined injection channel to peripheral regions of PAAG (see Figure 2A). An optically homogeneous distribution of FS within the PAAG was found after nearly 24 h (see Figure 2C). There was no distribution of FS into the SO. FS was only detected to a remarkable extent within the gel phase (see Figure 3). The injection through PAAG into SO could not be conducted in the way as it was intended. The PAAG covered the tip of the injection needle dislocating the boundary between the gel and the SO with the needle movement. This effect may have been caused by the surface texture of the injection needle and the rheological properties of the PAAG as well as the SO. Therefore an injection through the PAAG directly into the SO was not feasible. The injection was performed at the same spot in spite of the dislocated boundary between the phases. During the injection of FS, the solution flowed back along the puncture duct and was distributed along the phase boundary between the PAAG and the SO. The examination of drug distribution after the injection through the PAAG into the SO lead to comparable results as the direct injection into the PAAG (see Figure 4). Almost all of the injected FS was distributed to the PAAG. The only difference was the initial spreading of FS at the interface of the PAAG and the SO instead of the injection channel when the injection was set directly into the PAAG.
Based on the results, it is conceivable that the injection into a vitrectomized eye represents a special situation in vitro as well as in vivo. The described effects may also occur in vivo, because the PAAG is adjusted in some of its physicochemical properties to the human vitreous body [3] and both, the SO and the type of injection needle, are in general clinical use. If an intravitreal injection or implant becomes necessary following a vitrectomy with SO insertion, it should be considered that SO may possibly influence the injection process. Consequently, an existing SO bubble may also affect the intravitreal distribution and the pharmacokinetic profile of intravitreal injections [6]. It is assumed that the position of the injection [8] influence the safety and efficacy of the dosage form. Further the nature of the intravitreal area in which the injection is applied, for example a juvenile, liquefied or vitrectomized vitreous body with SO as substitute, may be of great importance [4]. FS is a highly water soluble model drug. However, it may be expected that clinically relevant drugs also may not distribute in the SO. In this case it may be speculated that drug transfer to the retina which should be in direct contact with the SO might also be very limited.

The in vitro distribution behaviour of different dissolved or suspended dosage forms containing clinically relevant drugs such as lipophilic corticosteroids, and the in vivo relevance of the observed effects will have to be examined in further studies.

4 Conclusion

Frequently occurring problems after vitrectomy are inflammatory or neovascularization diseases, which are post-operatively treated with intravitreal injections. To obtain a better understanding of the effectiveness of drugs and dosage forms in vitrectomized eyes, biorelevant in vitro systems can be used for initial estimations. Using the Eye-MoS and the modified VM it was possible to simulate the state of the vitreous body after vitrectomy in vitro and to examine the behaviour of SO within a moving model. No mixing of the components or dislocation of SO was observed during a time period of 72 h. The first outcomes in terms of a simulated vitrectomized vitreous body indicated the importance of SO to the initial distribution of FS, based on different injection techniques. After injecting directly into the PAAG, an injection channel was formed, in which the FS was initially distributed. An intended application through the PAAG into the SO resulted in a distribution of the model substance along the interface of the SO and the PAAG. In contrast to the different initial location of FS, the resulting distribution of FS after several hours was comparable in both cases. Almost no FS was detected within the SO but nearly the entire amount within the PAAG. Further studies, in vitro as well as in vivo, are necessary to gain detailed information about pharmacokinetics within a vitrectomized vitreous body.

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References