Simulation of Drug Distribution in the Vitreous Body After Local Drug Application into Intact Vitreous Body and in Progress of Posterior Vitreous Detachment

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ABSTRACT: Intravitreal injections and drug-loaded implants are current approaches to treat diseases of the posterior eye. To investigate the release of active agents and their distribution in the vitreous body, a new test system was developed that enables a realistic simulation of eye motions. It is called the eye movement system (EyeMoS). In combination with a previously developed model containing a polyacrylamide gel as a substitute for the vitreous body, this new system enables the characterization of the influence of eye motions on drug distribution within the vitreous body. In the presented work, the distribution of fluorescence-tagged model drugs of different molecular weight within the simulated vitreous was examined under movement with the EyeMoS and without movement. By replacing a part of the gel in the simulated vitreous body with buffer, the influence of the progress of posterior vitreous detachment (PVD) on the distribution of these model substances was also studied. The results indicate that convective forces may be of predominate influence on initial drug distribution. The impact of these forces on drug transport increases with simulated progression of PVD. Using the EyeMoS, the investigation of release and distribution from intravitreal drug delivery systems becomes feasible under biorelevant conditions. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:517–526, 2014

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INTRODUCTION

Many patients with eye diseases (glaucoma, macula edema, etc.) have to apply eye drops several times a day. The compliance of these patients is often poor because in most cases disease progression occurs painlessly and patients tend to forget the administration.[1] An alternative to topically administered drugs are modern implants, loaded with active agents, which can be injected periocularly or intraocularly. Injections and implants can be placed, for example, subconjunctivally,[2] in the sub-Tenon's room[3] or intrasclerally.[4] The vitreous body is also a location for the administration that is attracting increasing attention.[5] With Ozurdex® (2010) and Iluvien® (2013), intravitreal implants have been introduced into the market to treat macula edema.[6–8]

An important step in the development of intravitreal dosage forms is the *in vitro* characterization of drug release and distribution. To analyze drug release from intravitreal dosage forms *in vitro*, incubation methods can be used. Other approaches include the adaptation of the compendial flow-through apparatus as reported by Browne and Kieselmann.[9] Such classical release setups in which the dosage form is immersed in a fully stirred compartment do not allow for the examination of evolving distributions. Knowledge regarding local distributions in the vitreous is however very important for these dosage forms as the site of action is located at the retina. The transport of substances in the gel-like vitreous body can occur through diffusion, sedimentation, or convection. Especially for substances with a low molecular weight diffusion has been discussed as the prevailing transport mechanism.[10] For this reason, the examination of diffusion-driven distributions in a simulated vitreous compartment is desirable. For such studies, a suitable vitreous substitute is needed to mimic the gel-like vitreous body. The use of silicone-based oils or hydrogels for this purpose has been investigated.[5,11–14] *In situ* cross-linking hydrogels, for example, consisting of polyacrylamide (PAA) or poly(ethylene) glycol, have been reported to be the most suitable to simulate the vitreous.[12–14] The shape and volume of the human vitreous body was often simulated using a glass corpus.[15] Furthermore, a potential impact of eye movement has been considered. Repetto et al.[15] developed a model to simulate saccadic eye movements and investigate the effect on the distribution in glycerol as vitreous substitute. They showed that it is difficult to mimic the transport dynamics of the vitreous body because of the natural complexity. Nevertheless, they observed intensive effects on distribution processes in the vitreous cavity and shear stresses along the surrounding membranes caused by the simulated saccadic eye movement. It was the aim of this work to develop an *in vitro* model for the evaluation of drug release and distribution upon intravitreal administration combining some of the approaches listed above under further approximation of selected test conditions to the situation *in vivo*. Particularly, the simulation of different types of natural eye movements including pursuit movements and saccadic motions and the impact of

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posterior vitreous detachment (PVD) were to be evaluated. To the authors' knowledge, these factors have not been included in *in vitro* studies before. PVD is a condition caused by the liquefaction of the vitreous beginning in childhood that progresses with age and may eventually cause the separation of the vitreous membrane from the retina. Because of this disease, the ratio of gel-like vitreous body and liquid vitreous portion changes intensively.[16] The consequence should be an increase in the influence of convective transport in the vitreous cavity. It must be expected that the situation for drug release and distribution is also changing because of this phenomenon. This important aspect is often neglected and is most likely not accounted for in animal models either, as typically very young animals are used.

The developed test system combines a previously reported gel-filled glass body called the vitreous model (VM)[13] with a newly developed eye movement system (EyeMoS).

With the combination of EyeMoS and VM, the shape, the volume, and four main movement types of the eye can be simulated. The concept allows for the investigation of the effects of different eye movement types on the distribution of an injected model substance in a modified PAA gel as vitreous substitute. Furthermore, the different compositions of a completely gel-like and a partially liquefied vitreous body can be simulated by replacing certain fractions of the gel with ringer buffer. In the work presented here, this new setup is described in detail; the movement schemes achieved with the EyeMoS are characterized and the first experiments regarding the distribution of fluorescent model substances are described.

In the therapy of ocular diseases, a wide range of drugs with various molecular masses are locally administered into the vitreous body. On the one hand, there are active agents such as dexamethasone (DX) with a comparably low molecular mass, and on the other hand, large molecules such as antibodies are available.[17] Examples for large molecules are the IgG-antibody bevacizumab (150 kDa) as well as ranibizumab, a monoclonal antibody fragment with a molecular mass of 40 kDa, which are injected intravitreally to treat age-related macular degeneration.[18] To account for this diversity, model substances, with different molecular weights (350–150,000 Da), antibodies and the therapeutically used drug substance DX were examined to obtain information on the influences of the molecular weight on distribution in the vitreous body.

MATERIALS AND METHODS

Materials

Dexamethasone, fluorescein sodium (FS), fluorescein isothiocyanate–dextran with molecular masses of 4 kDa (FITC 4), 40 kDa (FITC 40), and 150 kDa (FITC 150), and methanol were obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany). Rotiphorese®, ammoniumperoxo disulfate (APS) and tetramethylethylenediamine (TEMED) were purchased from AppliChem GmbH (Darmstadt, Germany). The goat anti-human IgG (H+L)–FITC antibody (IgG antibody) was supplied by Dianova GmbH (Hamburg, Germany). All chemicals were of analytical grade.

Methods

EyeMoS and VM

The EyeMoS was created to mimic the natural eye movement. The system was designed to accommodate the previously introduced VM.[13] The combination of these two systems allows the examination of drug distribution in the simulated vitreous body under simulation of eye movement. The VM consists of a spherical glass corpus with a vent (Fig. 1). The corpus can be disassembled along its equator to remove the content for analysis. The shape and volume (4 cm^3 were adapted to the human vitreous body. For distribution experiments, the VM was mounted in the holder of the EyeMoS in a way that the longitudinal axis of the VM was oriented along the z-axis (Fig. 2b). To simulate drug distribution in the vitreous, the VM is filled with a modified PAA gel[13]. Ten milliliter of the PAA gel consists of 9.23 mL standard ringer buffer, 0.67 mL of a 30% solution of acryl amide and bisacryl amide (Rotiphorese®), 0.1 mL TEMED, and 0.01 mL a 1% APS solution. These components are carefully mixed initiating the cross-linking of the acryl amide monomers, poured into the glass corpus of the VM and allowed to solidify for 15 min.

To simulate natural movements of the eye, the VM can be embedded in a holder at the center of the EyeMoS (Fig. 2). At each motion shaft, servo motors (MKS DS 6125e and Savox SC 1257Tg) are assembled to move the holder periodically along the x- and y-axes (Fig. 1). The servo motors are processor controlled (Arduino Uno 65139). The open source software Arduino (www.arduino.cc) enables the definition of freely programmable

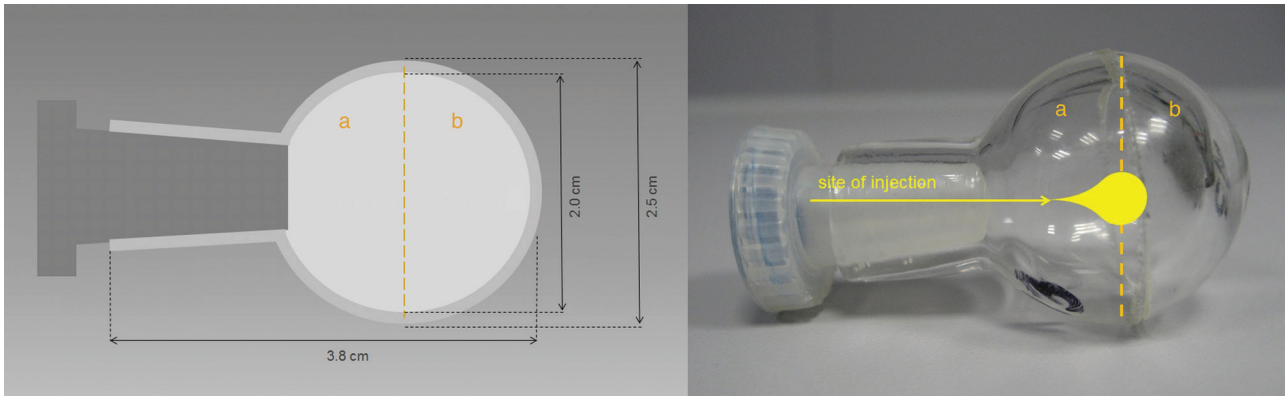

Figure 1. The VM: (a) schematic view, (b) image with scheme of the injection position and separation into two parts (a + b) of the vitreous substitute for quantitative analysis.

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