Final Report on "Intravitreal Drug Delivery: Experimentation and Mathematical Modeling of Ocular Fluid Dynamics and Transport Phenomena"

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1. Background

Eye diseases such as Age-related Macular Degeneration (AMD) and Diabetic Retinopathy (DR) affect a large segment of the US population, especially the aging. While many advances have been made for drug development, proper delivery to specific ocular regions such as the retina is still a significant medical, pharmaceutical and fluid dynamics problem. Among the challenges is the development of drug delivery methods that target the retina and minimize the losses to unaffected regions. This is from the standpoint of both conservation of the drug and minimizing unnecessary toxicity. The objectives of the proposed research program are to acquire an understanding of the transport mechanisms in the human eye for the purpose of targeted drug delivery to the posterior chamber. As mentioned, the retinal region is prone to diseases and physiologically very complex, thus very challenging for treatments. There is additional challenge that with age, syneresis develops, i.e., the normally gelatinous vitreous develops liquid pockets that enlarge in time. This introduces heterogeneities in the system that are mathematically challenging for analytical modeling of fluid and solute transport in the eye.

1.2 ONGOING RESEARCH AND GOALS

The current program consists of collaboration between VSoE and CHLA for experimentation and mathematical modeling of surrogate transport through the vitreous humor by diffusive and convective means. The experimental part consists of MRI visualization of surrogate drugs that also serve as contrast agents. The short-term goal is to firmly establish the physics of fluid transport in the vitreous humor, and how the transport processes serve to move large and small drug molecules towards the retina. For the purpose of mathematical modeling, it is necessary to accurately measure the diffusive and convective transport properties of this medium. The goal from a theoretical standpoint is to develop a comprehensive mathematical model that encompasses the various mechanisms participating in transport process, and tune in conjunction with the extensive experiments towards predicting the effective drug deposition rates at the desired ocular locations.

2. New Outcomes and Impact

2.1 EXPERIMENTAL PROCEDURE:

Extensive experiments have been carried out with ex-vivo fresh whole bovine eyes placed in specially prepared moulds. With the use of two MRI contrast agents: Magnevist® ($C_{28}H_{54}GdN_5O_{20}$) and gadolinium-Albumin. Here, the latter is a significantly larger molecule 67.5 kDa as compared to 938 Da for Magnevist), and one of the aims our experiments was to establish the lower diffusion rate of the larger molecule. In addition, we have created syneresis in some of the eyes to examine the effect of liquefaction on diffusion rates. To understand the effect of syneresis, the condition was created by injecting enzymes. A proteolytic enzyme cocktail consisting of hyaluronidase, collagenase, and plasmin (EMD Biosciences, Inc., San Diego, CA) was used to induce syneresis. The enzymes were injected close to the retina in every case. With the contrast agent injected at the center of the eye, enzymes were deliberately injected off-center to see any asymmetries in the surrogate distribution attributable to syneresis.

2.2 RESULTS

The MRI signal intensity was interpreted in terms of concentration with the use of calibration phantoms that were prepared in saline solution loaded with various concentration levels of the contrast agent. The phantoms were used with every imaging procedure to ensure consistency of signal intensity to concentration conversion. The raw images showing the degree of permeation of the contrast agent have been obtained for various conditions. The images have been color-mapped for various intensity signals to indicate concentration levels. Figure 1 exhibits a sequence of images where Magnevist® was used as a contrast agent. The images represent the propagation of the surrogate (which is also the contrast agent) to a selected threshold of the MRI signal. The penetration towards the retina seen on the third through the sixth images appears to indicate the effect of syneretic liquefaction created by the enzymes.

For the purpose of the **Diffusion Coefficient Measurement,** no enzymes were used, and the spherical symmetry of the Gd-DTPA (Magnevist) distribution was achieved. The nearly perfect spherically symmetric images (see Figure 2) serve to provide accurate measurement of the diffusion coefficient on the basis of the mathematical model. In fact, the distributions of the surrogate in space and time very closely resemble that from a point-source spike, as long as we consider times to be short enough before the effects of the lens and the wall of the eyeball interfere with the symmetry. In Figure 3, we give the plots of the concentration distribution which have a typical Gaussian profile given by

$$c(r,t) = \frac{M}{8(\pi D t)^{\frac{3}{2}}} e^{-\frac{r^2}{4Dt}}$$

where M is the total amount of the Gd-DTPA administered in moles, D is the diffusion coefficient, r is the radial distance from the point of injection, and t is the time. By fitting the data and requiring a fit as per the above equation, the diffusion coefficient value was extracted at various time points, and found to have the average value of 2.84×10^{-6} cm^2/s .
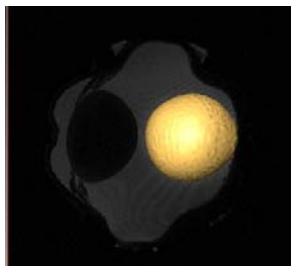

Figure 1: Color map of bovine eye image sequence with Magnevest® as contrast agent. 30 microliters of Gd-DTPA (10 mM/L) was injected at the center of the eye. Close to the retina, an enzyme cocktail (hyaluronidase, plasmin, and collagenase) was injected about 1 hour after Magnevist. Images show that the contrast agent distribution favor transport to the syneretic region. The medium-to-high concentration contours penetrate that region.

Figure 2: Spherically-symmetric image of Gd-DTPA 3.4 hrs after injection

2.2 IMPACT: The important preliminary results that have emanated from this study indicate that the presence of liquid pockets affect the drug transport by convective transport enhancement. Therefore, liquid regions, whether created or naturally existing, impact the drug delivery rate and need to be properly evaluated for mathematical modeling. The modeling process demands accurate transport property values and for this purpose, the diffusion experiments have been perfected to the point of delivering very spherical concentration data images to fit the simple mathematical model accurately giving the value of D. The knowledge gained from this investigation has provided scientific information to design new experiments build successful mathematical models for ocular transport phenomena.

Figure 3: Gaussian distribution on Gd-DTPA in bovine eye vitreous at various times. This plot has been used to calculate the diffusion coefficient. The horizontal scale is in mm. The vertical is mM/mL.

Future Direction

The work described here has provided sufficient preliminary data to an industrial sponsor (Allergan, Inc.) to continue with additional support for further expansion of the project. New funding for 2012 has been provided to study water-transport effects in relation to glaucoma, and particular attention is being paid to flow processes in the anterior segment (aqueous humor) of the eye. Experimental investigation on the impact of water transport towards drug delivery is also being conducted. The results have also provided new insights into the transport processes involving macromolecules sufficient background materials for an NIH proposal. This will be submitted in June 2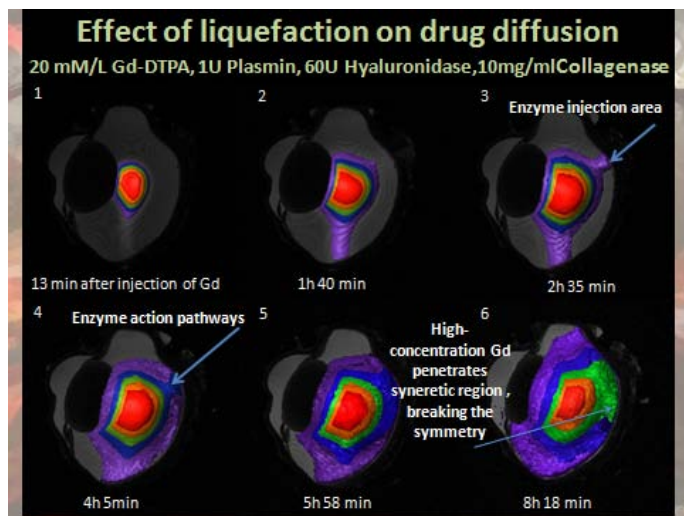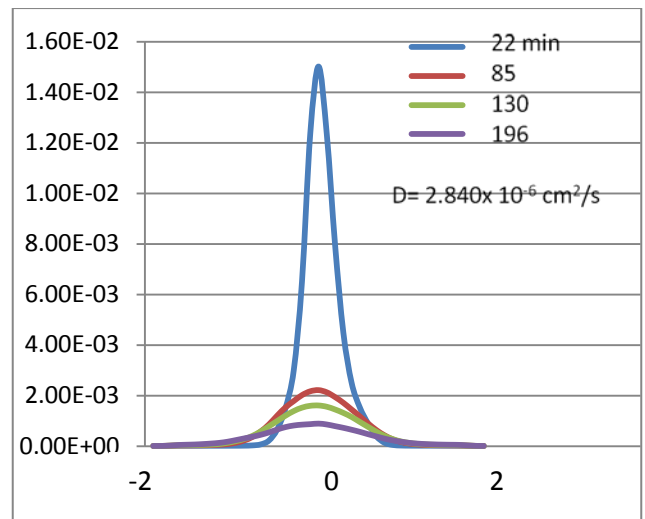012 as a joint USC-CHLA effort.