Diffusive and Convective Transport of Proteins in Fibrin Gels

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The acute cause of heart attack and stroke is the formation of a thrombus (fibrin clot) in an artery and vein. For decades, researchers have studied how these clots can be safely eliminated from the blood vessels. Many methods for thrombus dissolution require delivery of enzymes or drugs to the clot. In recent years, the use of fibrin gels in medical applications, such as surgical glue and growth factor delivery to wound sites, has expanded extensively. Quantification of diffusion and convection of proteins and other bio-molecules within fibrin gels is necessary for estimating drug delivery from a fibrin gel, controlling temporal and spatial delivery of growth factors for tissue engineering applications, and predicting drug activated dissolution of thrombi.

The objective of our research is the creation of an experimental system using fluorescence microscopy to determine diffusive and convective transport rates of proteins such as Insulin-like Growth Factor (IGF-1) and Ribonuclease (RNase) in gels such as fibrin. Results are reported for 1) the diffusion coefficient of IGF-1, which has not been reported previously; 2) the hydrodynamic permeability of fibrin gels, and the flow-induced dispersion of RNase, a well-characterized protein, in the gels. The gels were 0.4% polymer and supported in a long (5 cm), thin (200 μm) rectangular capillary. Our system, which combines a controlled environment, fluorescence imaging and microfluidics allows the *in-situ* study of diffusional and convective transport of bio-molecules within model gels that mimic extracellular matrix.

Diffusion Coefficient

IGF-1 (MW = 7,600) and RNase (MW = 13,600) were covalently labeled with cyanine fluorescent dye (MW ≈ 800). The microcapillary containing the fibrin gel was fastened to a motorized microscope stage on a computer-controlled fluorescence microscope equipped with Cy3 and Cy5 filter sets, and a cooled CCD digital camera. Through microfluidic devices at the ends of the capillary, dye-labeled protein was injected and mixed into the gel-free region of the specimen. Time-lapse images were recorded at multiple fields of view as the labeled protein diffused across the gel/solution interface into the gel. By use of two-color imaging, the evolving gradients of both proteins were measured simultaneously in the same gel. The system is well modeled at time zero as a step function in protein concentration (a uniform level C0 in the gel-free region and 0 in the gel). Fick's equation for time dependent diffusion in one direction (along the length of the microcapillary) can be solved for this case. The concentration of the protein was measured versus time at various locations along the capillary and fit to the mathematical model. The agreement between the data and the model is very good. The diffusion coefficient determined from the data for RNase at 24°C (1.18 x 10^{-6} cm^2/s) is in very good agreement with literature results. The diffusion coefficient for IGF-1 was determined to be 1.55 x 10^{-6} cm^2/s, which is consistent with literature values for other proteins of comparable

molecular weight. To our knowledge, this is the first published value for the diffusion coefficient of IGF-1.

Hydrodynamic Permeability of Fibrin Gel

The Darcy permeability (k) is the coefficient relating the mean velocity of liquid to the applied pressure gradient divided by the viscosity of the liquid. It is difficult to measure with hydrogels because they are fragile and susceptible to collapse under pressure. We were successful in stabilizing fibrin gels (0.4% by volume fibrin) and obtaining values of k that describe the undeformed gel. The average value was $k = 7.5 \times 10^{-10}$ cm^2, which is consistent with gel fibers of radius 21 nm.

Dispersion of RNase

A pulse of protein broadens as it is convected through the gel. Using image processing, we computed the first and second moments of the pulse as it moved through the gel, and determined the mean velocity and dispersion coefficient of the protein. These experiments with dye-labeled RNase yielded velocities that were consistent with the mean solvent velocity. However, the dispersion coefficient was 3-4 times greater than the molecular diffusion coefficient. This result indicates that the fibrin gel had a heterogeneous microstructure. Given that the fibrin gels studied here were probably had a more consistent microstructure than real extracellular matrix, it is likely that the dispersion coefficient for proteins in real tissues is higher. This must be taken into account when modeling the transport of biological agents in tissues.

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