Probing Hydrogel Transport Properties and Micro-structure

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Statement of Purpose: Knowledge of how soluble molecules diffuse through materials is important in order to efficiently design devices for controlled protein release, 3D scaffolds for cell growth, and artificial organs. Protein diffusion can be particularly impacted by the material micro-structure and thus this work has an implication for many advanced materials that are multicomponent, demonstrate dynamic phenomena (degradation, swelling) or are patterned. The purpose of this study was to apply Fluorescence Correlation Spectroscopy (FCS), a non-invasive optical technique, to study probe diffusion in cross-linked hydrogels, and then to compare the results to those derived from Fickian bulk diffusion method. The two methods, bulk diffusion and FCS, are complimentary in that they measure phenomena on disparate length-scales. The bulk diffusion method considers only the macro-environment of the gel, while FCS considers the micro-environment of the solute. Further, bulk diffusion studies are lengthy and require a large amount of protein. An average value for bulk diffusion is estimated without taking into account the heterogeneity of the system or possible solute-matrix interactions. FCS can probe very small volumes (sub to femtoliters) of samples containing nanomolar concentrations of fluorescent probes. A local diffusion coefficient is estimated which allows for probing matrix heterogeneity and the sensitivity of the measurement allows for probing protein-matrix and protein-protein interactions all of which are important both for cell growth and protein delivery.

Methods: 4-arm poly(ethylene glycol) vinyl sulfone (PEG-VS)[1] and PEG-dithiol ester[2] were synthesized in house. The % end group modification as shown by H^1NMR (CCl_3D) was 97% and 95%, respectively. The gels were formed by dissolving both reagents in 0.3 M triethanolamine (pH 8) at equimolar ratio of reactive groups. For bulk diffusion the amount of protein that diffused out of the gel was measured and this data was applied to a Fickian model of diffusion to calculate the effective diffusion coefficient[3]. Local diffusion, which is inversely proportional to Tau or the characteristic diffusion time, was estimated by FCS[4]. Lysozyme, BSA, Ig, and GFP served as model diffusion proteins and were used alone or labeled with Rhodamine Green. All studies were carried out at room temperature.

Results and Discussion: There is a distinct correlation between matrix pore size (which is proportional to polymer density), the solute size, and solute release behavior. Thus we considered two approaches towards comparing trends in results from bulk and FCS diffusion studies. First, cross-linked PEG hydrogels with 3 different polymer densities were fabricated, namely 5, 10, and 15 wt% PEG and diffusion of BSA was monitored. Both methods demonstrated that the diffusivity of BSA decreased with increased polymer density.

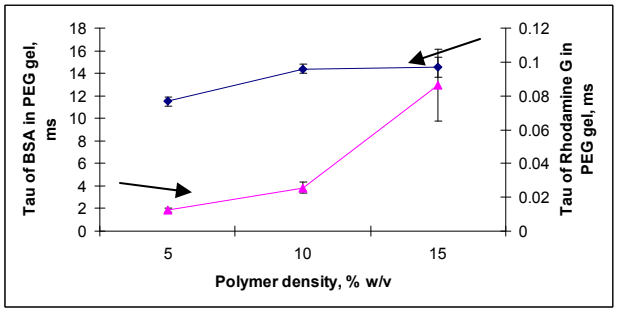

Figure 1: Dependence of BSA and Rhodamine Green fluorophore diffusion on polymer density as measured by FCS**.** Tau is the characteristic diffusion time and is inversely proportional to diffusivity.

A second study was performed with solutes of various sizes. The trends from FCS and bulk diffusion showed that the diffusivity of the solute decreased with increased solute size. An example of how characteristic diffusion time, as measured by FCS, is influenced by the polymer density and the solute size is shown in **Figure 1**. Further FCS studies were carried out to gain more detailed information on the gel structure and transport properties. Gel heterogeneity on the micro-scale was probed by measuring BSA diffusion at various gel locations. We found that the gel was homogeneous on micro-scale. The technique also allowed for studying protein-protein and protein-matrix interactions. We demonstrated that BSA did not aggregate in the PEG gel at concentrations between 0.04% and 4%, while Ig aggregated at concentrations >1%. Further we also found that BSA did not react with the PEG hydrogel during the cross-linking reaction but may have been physically trapped in the pores or cross-links. Finally, gelation and swelling behavior of the hydrogels were tested by measuring the change in BSA diffusivity as a function of time. The study revealed that the gelation occurred on the order of minutes and was pH dependent, whereas complete swelling was reached after 4 hours of equilibration in buffer.

Conclusions: In this work, we show that FCS can yield effective diffusion coefficients comparable to those derived from the bulk diffusion method. However, FCS allowed us to perform in-situ investigations of gel heterogeneity, protein-protein interactions (e.g. aggregation), protein-matrix interactions (e.g. chemical interactions during gelation) and gel dynamics (e.g. swelling behavior). We believe these studies are essential when designing devices for protein release or biomaterials to be used as cell scaffolds in tissue engineering.

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