Protein Interactions with Biological Matrices: A Fluorescence Correlation Spectroscopy Investigation

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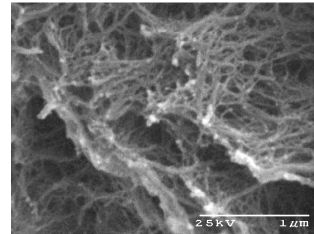
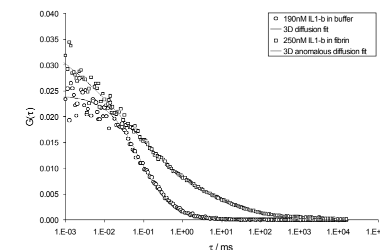
Statement of Purpose: Protein interactions with biomaterial surfaces are known to be a critical determinant in the performance of medical devices, but little is known about the interactions of proteins with three-dimensional matrices. We have developed fluorescence correlation spectroscopy (FCS) as a technique capable of providing detailed, molecular-level information on the interactions of soluble proteins with complex, three-dimensional matrices [1]. We present here an investigation of the interactions of important soluble proteins, such as growth factors, cytokines, and serum albumin, with common biological matrices used for tissue engineering, including type I collagen, fibrin, and hyaluronic acid. These investigations will provide the basis for understanding the impact of protein-matrix interactions on the performance of tissue-engineered medical products.

Methods: A sample preparation for investigating interleukin-1β (IL-1β) interactions with fibrin matrices is as follows. Lyophilized recombinant human IL-1β (100μg, Peprotech) was reconstituted in a sodium phosphate buffer (pH 8.5) to a final concentration of 0.1 mg/ml. A twenty-fold molar excess of the thiol-reactive Alexa Fluor® 488 C_5-maleimide thiol reactive fluorescent dye (Invitrogen Detection Technologies) was added to the reconstituted protein solution which was subsequently stirred for two hours at room temperature. The labeled protein solution was split into eight 125 μl aliquots which were centrifuged through separate Quick Spin® Protein Columns (Roche Applied Sciences) which contained a solution of G-25 sephadex separation medium in phosphate buffer to remove any un-reacted dye. Each column had been pre-passivated with 125 μl of a 100μM solution of bovine serum albumin in phosphate buffer to minimize potential losses of the labeled cytokine. The eluted protein solutions were recombined and a ten-fold molar excess of bovine serum albumin in phosphate buffer was added as a carrier. Fibrin matrices were prepared by adding 60μl of 380nM labeled IL-1β to a mixture of 30 μl of human α-thrombin (50 units/ml, Enzyme Research Laboratories, IN) and 5μl Factor XIII (100 units/ml, Enzyme Research Laboratories) in a 0.5ml microfuge tube. The solution was gently vortexed and spun-down several times to ensure complete mixing of the components. Then 60 μL fibrinogen (18.08 mg/ml, Enzyme Research Laboratories) was added to the top of the solution which was subsequently spun-down so that the fibrinogen would permeate the mixture and cross-link as it came into contact with the activated Factor XIII. Matrices were processed using critical-point drying and characterized structurally with scanning electron microscopy. Fluorescence correlation spectroscopy was performed using a custom-built spectrometer using excitation provided by a titanium:sapphire laser (Coherent) that is focused into an inverted microscope (Zeiss). Samples were loaded into a perfusion chamber and fluorescence was epi-collected and measured with two avalanche photodiodes (EG&G). Fluorescence intensity was analyzed with a commercial correlator (ALV).

Results / Discussion: The fibrin matrix has the expected fibrillar structure, as shown in Figure 1. The matrix has a characteristic mesh size of 200 nm. In FCS, the excitation volume has a characteristic length scale of 1 mm, indicating a single measurement averages over approximately 100 cells in the fibrin structure.



The correlation function for IL-1β in buffer and in fibrin is shown in Figure 2. The correlation function is a representation of the probability that if the probe molecule is in the excitation volume at time zero, it remains there at a time τ later. In buffer, IL-1β displays standard diffusional dynamics, with a diffusion constant of 1.6 x 10^{-7} cm^2/s. In fibrin, IL-1β displays complex dynamics, which are attributed to interactions with the matrix. These are a mixture of excluded volume interactions and binding interactions. Sahni et al. measured an equilibrium binding constant of 2.3 nM for IL-1b in fibrin using radiolabeling techniques [2], and the interactions observed in FCS are a molecular-level representation of this. However, the environment encountered by soluble proteins in three-dimensional matrices is complex, and FCS provides information on a spectrum of interactions. Comparisons between proteins that are thought to bind to these matrices with those, such as albumin, that do not, will provide information on the importance of excluded volume versus binding interactions.



Conclusions: Fluorescence correlation spectroscopy is capable of providing detailed measurements of the interactions of soluble proteins with matrices used in tissue-engineering applications. Future work will focus on understanding relationship between protein-matrix interactions and biomaterial performance.

References:

[1] Fitzpatrick JAJ, Yamada KM, Washburn NR. Biophys J. *Submitted.*
[2] Sahni A, Guo M, Sahni SK, Francis CW. Blood. 2004;104;409-414.