Self-Assembling Peptide Nanofiber Hydrogels for Inductive Tissue Engineering Applications: Functional Protein Release Kinetics, In-Scaffold Diffusion Mechanisms and Eluted Protein Structure

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Statement of Purpose: Incorporating and controlling the presentation of therapeutically relevant molecules (i.e. cues for illiciting cellular response, drug release, etc) is of fundamental importance in the design of scaffolds for regenerative medicine applications. Self-assembling peptide nanofiber scaffolds present a uniquely suited material that are generally non-immunogenic, able to respond to external stimuli under various physiological conditions and maintain a high water content (i.e., ca. 99.5 % w/v): the latter may allow for the presentation of a wide range of molecules. Moreover, recent results have shown that charge effects largely dominate the release dynamics of small molecules from these hydrogels [1]. In order to enhance the therapeutic efficacy of these hydrogels it is vital that the release characteristics of functional proteins be understood.

To this end, RADA16 1%(w/v) self-assembling peptide scaffolds were loaded with a variety of proteins (pI = 4.6–8.5, MW = 14.3–160kDa). Release kinetics and in-scaffold diffusion mechanisms were determined using fluorescence correlation spectroscopy (FCS) techniques. Eluted protein structure was determined using circular dichroism (CD) and fluorescent spectroscopy (FSPEC) techniques. The release of these proteins, including IgG, in a functional state and the determination of in-scaffold diffusion mechanisms is considered a significant advance in the tailored design of peptide-based nanofiber scaffolds for regenerative medicine applications.

Materials and Methods: Proteins (Trypsin Inhibitor, Bovine Serum Albumin, Transferrin, Lactoferrin, Human Ig, Sigma-Aldrich) were used without further purification and labeled with Alexa-647. Unbound fluorophore was removed according to well-established protocols. RADA16 peptide 1% (w/v) was added to DI-H_2O water (Millipore), mixed with protein (5nM labeled, 5 mM unlabeled) and pipetted into a well of 384 plate. Nanofiber scaffolds were formed upon the addition of phosphate buffer (PB, 0.1 M, pH=7.2) and measurements taken for up to 60 hrs. FCS (MF-20, Olympus, Japan) was used to characterize in-scaffold diffusion mechanisms and concentration profiles. Released proteins were characterized using FCS, CD and FSPEC techniques to determine release rates and eluted structure.

Results/Discussion: Figure 1 summarizes the release rates of protein from nanofiber scaffolds to PBS. It is evident that the elution of protein from the scaffold is highly sensitive to the charge of the protein: proteins of drastically different size are released in a similar manner, but proteins of similar sizes are segregated based on charge. Moreover, it is evident that proteins as large as IgG (~160kDa) are also released from these scaffolds, and that IgG release continues for ~40 hrs.

To see if released proteins have aggregated, or their structures become significantly altered during release, their diffusivities before and after release were determined (Table 1). Significant denaturing or aggregation events are expected to influence diffusivity values. However, protein structures and their diffusivities remain unchanged during the release process. These results were confirmed from CD and FSPEC (not shown).

The similarities between the 'in-scaffold' and PBS protein diffusivities suggest that proteins are diffusing within large water domains that 'form' as peptides associate to build the nanofiber structure. 'In-scaffold' diffusion was not anomalous (results not shown).

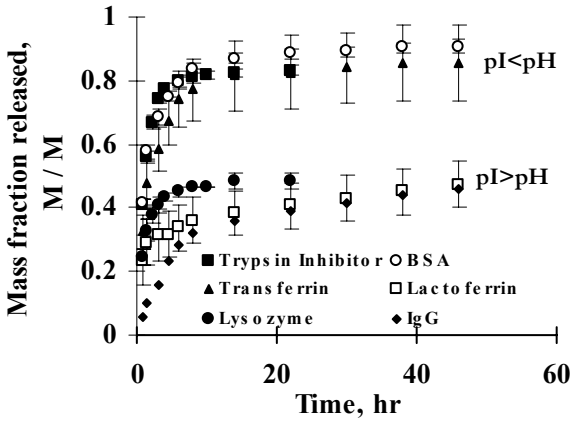

Figure 1. Protein mass fraction release profile.

Table 1. Protein diffusivities,determined using FCS (n>4).

Protein	Diffusivity, x 10^{10} m^2/s Native	In-scaffold	Eluted
Lysozyme	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.3
Trypsin Inhibitor	0.69 ± 0.05	0.66 ± 0.03	0.7 ± 0.1
BSA	0.5 ± 0.1	0.48 ± 0.02	0.5 ± 0.1
Transferrin	0.5 ± 0.1	0.4 ± 0.1	0.46 ± 0.07
Lactoferrin	0.5 ± 0.1	0.48 ± 0.07	0.5 ± 0.2
Human IgG	0.34 ± 0.05	0.3 ± 0.1	0.4 ± 0.2

Conclusions: Work is continuing, investigating ways of controlling the release kinetics through incorporating engineered peptides. However, the current elucidation of functional protein release characteristics from self-assembled peptide nanofiber scaffolds is considered a significant step towards developing these materials for inductive tissue engineering applications.

References: 1. Nagai Y. *et al., J of Controlled Release* 2006; 115: 18-25.