

Injectable self-assembling peptide hydrogel for tissue-specific controlled release applications of functional proteins

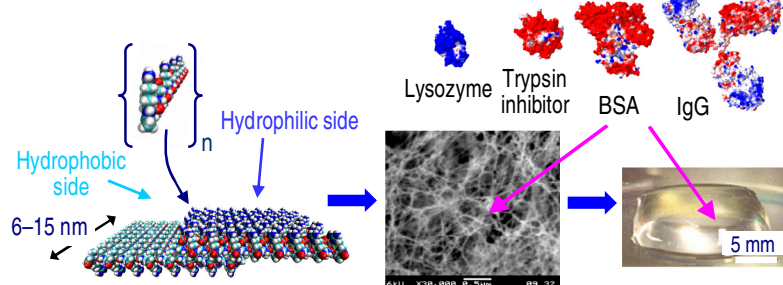
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Introduction

Successful implementation of hydrogels in controlled drug release applications depends on component and degradation product toxicity, inflammatory host response, and controlled and sustainable release of the active compound over prolonged periods of time. Despite the intense research conducted on myriad natural and synthetic materials (i.e., polyglycolic-poly-lactic acid, agarose, collagen, alginate, etc.) all of these challenges have not been resolved yet.

In 1993, we discovered that a class of peptides comprised of alternating hydrophobic and hydrophilic amino acids spontaneously self-organize into nanofibers with diameters of 10 – 20 nm upon being introduced to electrolyte solutions. These nanofibers further organize to form highly hydrated hydrogels (up to ~99.5% w/v water), with pore sizes between 5 – 200 nm.

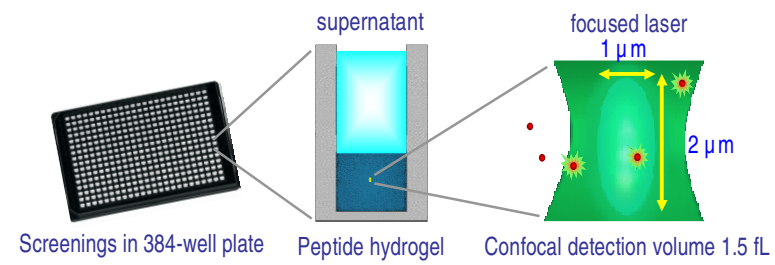
Peptide hydrogels do not use harmful chemicals (e.g., toxic cross-linkers, etc.) to initiate the sol-gel transformation, the degradation products are natural amino acids, they are easy to use, non-toxic, non-immunogenic, biodegradable, and applicable to localized therapies through injection to a particular tissue.



Materials & Methods

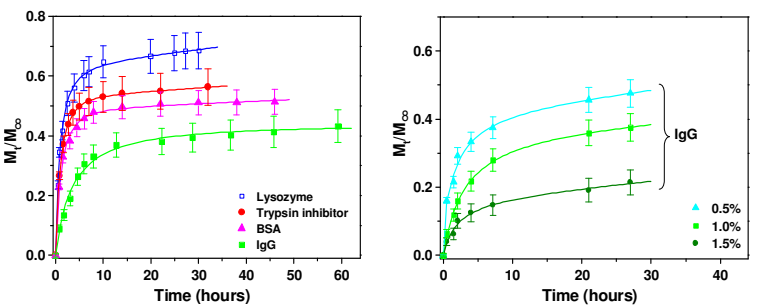
In the presence of electrolyte containing solutions, such as biological fluids, the ac-(RADA)₄-CONH₂ self-assembling peptide forms a hydrogel. The release kinetics of proteins with therapeutic interest through this peptide hydrogel were studied using the single molecule fluorescence correlation spectroscopy (FCS) technique (FluoPoint, Olympus). Concentrations and diffusion coefficients of the proteins were determined both inside the hydrogel and in samples removed from the supernatant. The diffusivity values obtained were compared to those calculated using the Stokes-Einstein equation, empirical, Fickian-based diffusion models and the literature.

To ascertain structural integrity of the released proteins we used circular dichroism (CD) and fluorescent spectroscopy. Furthermore, bioassays were conducted to verify the protein functionality using a quartz crystal microbalance (QCM, Attana, Sweden).



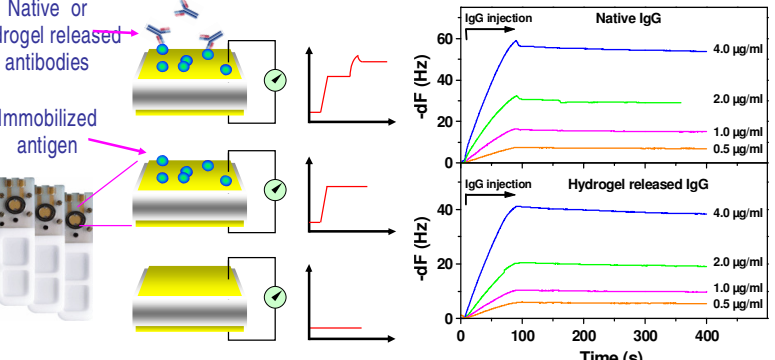
Results

Protein release through the peptide hydrogel seemed to asymptotically reach a plateau value after approximately 30 – 50 hours depending on the protein. Large proteins were retained inside the hydrogel scaffold for longer times compared to smaller proteins. Protein diffusion depended on the density of the nanofiber network of the hydrogel.



Using the single molecule FCS technique we calculated protein diffusivities both inside the hydrogel and in solution.

CD and fluorescence spectroscopy analyses showed that encapsulation and release did not affect the conformational characteristics of the released proteins. The functionality of the released monoclonal antibody was studied using QCM. The affinity constants of the native and hydrogel released antibodies for the antigen were calculated upon immobilization of the antigen on the QCM biosensor chip. Criteria for antibody functionality were the kinetics of binding and the affinity constants for the antigen. Upon interaction with the antigen, kinetic analysis showed that the association and dissociation rate constants for the native antibody were similar to those observed for the hydrogel released antibody. Furthermore, data analysis returned similar binding constants for native and hydrogel release antibody.



This biocompatible and injectable designer self-assembling peptide hydrogel system may be a useful carrier for the presentation of functional proteins and therapeutic antibodies for sustained release applications.

References

Zhang SG, Holmes T, Lockshin C, Rich A (1993) Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc Natl Acad Sci USA* 90:3334-3338.
 Koutsopoulos S, Unsworth LD, Nagai Y, Zhang S (2009) Controlled release of functional proteins through designer self-assembling peptide nanofiber hydrogel scaffold. *Proc Natl Acad Sci USA*, 106, 4623-4628.