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Two-layered injectable self-assembling peptide scaffold hydrogels for long-term sustained release of human antibodies

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ABSTRACT

The release kinetics for human immunoglobulin (IgG) through the permeable structure of nanofiber scaffold hydrogels consisting of the ac-$(RADA)_4$-$CONH_2$ and ac-$(KLDL)_3$-$CONH_2$ self-assembling peptides were studied during a period of 3 months. Self assembling peptides are a class of stimuli-responsive materials which undergo sol–gel transition in the presence of an electrolyte solution such as biological fluids and salts. IgG diffusivities decreased with increasing hydrogel nanofiber density providing a means to control the release kinetics. Two-layered hydrogel structures were created consisting of concentric spheres of ac-$(RADA)_4$-$CONH_2$ core and ac-$(KLDL)_3$-$CONH_2$ shell and the antibody diffusion profile was determined through the 'onion-like' architecture. Secondary and tertiary structure analyses as well as biological assays using single molecule analyses and quartz crystal microbalance of the released IgG showed that encapsulation and release did not affect the conformation of the antibody and the biological activity even after 3 months inside the hydrogel. The functionality of polyclonal human IgG to the phosphocholine antigen was determined and showed that IgG encapsulation and release did not affect the antibody binding efficacy to the antigen. Our experimental protocol allows for 100% IgG loading efficiency inside the hydrogel while the maximum amount of antibody loading depends solely on the solubility of the antibody in water because the peptide hydrogel consists of water up to 99.5%. Our results show that this fully biocompatible and injectable peptide hydrogel system may be used for controlled release applications as a carrier for therapeutic antibodies.

1. Introduction

The use of hydrogels as drug delivery carriers has been explored since the beginning of the controlled release era in the 1960s primarily focusing on synthetic polymer and animal-derived hydrogels. The first generation of hydrogels consisting of synthetic polymers did not represent an ideal system for biomedical applications due to: (i) component and degradation product toxicity (e.g., many polymers require the use of toxic cross-linkers, like glutaraldehyde, and other chemicals that pose a life threat whereas others such as polyglycolic–polylactic acid and its analogues during degradation release acids locally), (ii) post-gelation polymer swelling often causes pain in the host, and (iii) release of the active compound over brief periods of time due to the large pores of the polymer network. Furthermore, some animal-extracted biopolymers such as collagen and laminin [1,2] are not suitable for drug delivery applications in humans due to their origin and the risk of inflammatory host response from viruses, bacteria, and other unknown substances that may be present in the donor tissue. In response to the need of biocompatible polymer drug release systems, biodegradable polymer hydrogels were developed [3–5] whereas recent advancements in the purification of hydrogel material components such as hyaluronic acid and polysaccharides open new paths for applications in humans [6,7].

Previously, a nanofiber hydrogel consisting of the self-assembling peptide ac-$(RADA)_4$-$CONH_2$ (where R is arginine, A is alanine and D is aspartic acid) was studied for controlled release of small, model-drug molecules [8]. In a recent study, it was shown that proteins with different molecular weights and isoelectric points were slowly released through the ac-$(RADA)_4$-$CONH_2$ peptide hydrogel and the release kinetics were studied over a period of 3 days [9]. Self assembling peptides form a hydrogel inside the body upon interaction of the peptide water solution with biological fluids and therefore represent an injectable drug delivery system. Upon being introduced to electrolyte solutions, self-assembling peptides form nanofibers with diameters between 10 nm and 20 nm which are further organized to form a hydrogel containing water up to ~99.5% w/v and form pores with diameter 5–200 nm [9]. Peptide gelation does not require harmful materials, such as toxic cross-linkers, to initiate the sol–gel transition while the degradation products of the hydrogel are natural amino acids, which can be metabolized and reused by the body. The fact that the sol–gel transition occurs at physiological conditions facilitates mixing of the

Abbreviations: CD, circular dichroism; IgG, immunoglobulin; PC, phosphorylcholine; FCS, fluorescence correlation spectroscopy; QCM, quartz crystal microbalance.

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peptide solution with bioactive molecules and co-injection in a tissue-specific manner to form the drug delivery vehicle in the tissue. Peptide hydrogels are biocompatible, amenable to molecular design, and have been used in a number of tissue engineering applications including bone and cartilage reconstruction, neuronal and heart tissue regeneration, wound healing, angiogenesis, and hemostasis [10–12]. Self-assembling peptide hydrogels provide a platform that makes them ideal for a wide range of bionanomedical applications as they facilitate cell migration inside the hydrogel. Furthermore they are non-toxic, non-immunogenic, non-thrombogenic, biodegradable, and applicable to localized therapies through injection into a particular tissue [12].

Long-term administration of therapeutic antibodies may be beneficial for the treatment of many diseases such as cancer, where tumor cell-specific antibodies can be used to prevent cancer relapse or metastasis after radiotherapy, chemotherapy or surgery, rheumatoid arthritis, or viral diseases in individuals with compromised immune system [13]. Currently, long term treatment with therapeutic antibodies requires frequent injections due to the limited life time of immunoglobulins in the human body (i.e., half-life is between 2 and 23 days depending on the isotype), or intravenous injection which however, carries a risk of infection, vein rupture and extravasation, hypothermia, and patient discomfort. Due to the cost associated with the production of monoclonal antibodies, alternative approaches are required in which doses might be reduced and the bioavailability is enhanced. Local and sustained antibody release reduces the number of injections and lowers the administered dose thus minimizing the adverse toxic effects associated with administration of high doses of biologically active agents. Previously, the sustained delivery of functional antibodies has been investigated using 3–8 mm solid poly(ethylene-co-vinyl acetate) polymer matrices, which however had to be implanted or immobilized in contact with the mucus vaginal environment [14], implanted polyurethane hydrogel which released the IgG load within a few hours [15], implanted carboxymethylcellulose gel which also released the load in 6–9 h [16], and more recently using an implantable pH-responsive poly(methacrylic acid) (PMAA) hydrogel [17].

We report here the sustained release of human immunoglobulin (pI 7.1, MW 146 kDa) through injectable ac-$(RADA)_4$-$CONH_2$ and ac-$(KLDL)_3$-$CONH_2$ self assembling peptide hydrogels (Fig. 1) over a period of 3 months and the release kinetics of IgG through the hydrogels. The self assembling peptide system will gel upon injection intramuscularly, subcutaneously, in the void space of the brain, in the knee joint, or in any other tissue and release therein the therapeutic antibody. This peptide hydrogel system allows for 100% loading efficiency of IgG inside hydrogels whereas due to the consistency of the hydrogel (i.e., contains water up to 99.5%) the maximum amount of antibody loading depends solely on the solubility of the antibody in water. To examine if the processes involved in incorporating and releasing the antibody from the peptide hydrogels affect its conformation and function, the released antibody was analyzed using circular dichroism (CD), fluorescent spectroscopy and immunoassays to verify its biological activity. The functionality of IgG was monitored using single molecule fluorescence correlation spectroscopy (FCS) and quartz crystal microbalance (QCM) biosensor techniques. The presentation of functional antibodies with therapeutic properties is important for sustained delivery biomedical applications.

2. Materials and methods

2.1. Chemicals and reagents

The ac-$(RADA)_4$-$CONH_2$ and ac-$(KLDL)_3$-$CONH_2$ peptides were obtained in powder form from CPC Scientific (Sunnyvale, CA, USA) and were characterized at the MIT Biopolymers Lab (Cambridge, MA, USA). Human polyclonal IgG was purchased from Sigma-Aldrich (St. Louis, MO, USA). The pI of human IgG was 7.1 as determined by isoelectric focusing gel electrophoresis in a PhastSystem, using IPG strips and protein standards (Bio-Rad Laboratories, Hercules, CA) and the IPGphor system (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.2. IgG release experiments

Peptide hydrogels were formed using well-established protocols [8–11]. Briefly, the ac-$(RADA)_4$-$CONH_2$ and ac-$(KLDL)_3$-$CONH_2$ peptides were dissolved in deionized water and ultrasonicated using a probe sonicator for 30 min prior to use. The peptide solution in water was mixed with phosphate buffered saline (PBS, pH = 7.4) containing IgG at a final concentration of 5 μM. 40 μl of the solution mixture (i.e., peptide in PBS containing IgG) was transferred into 200-μl polypropylene tubes and gelation occurred in less than 10 min. Subsequently, 70 μl of PBS was slowly added to the 40 μl of the hydrogel which contained 5 μM IgG. To satisfy the perfect-sink conditions and allow for the determination of the protein release profile, at each time point 40 μl of the supernatant was replaced with the same volume of fresh PBS. During the course of the measurements the hydrogel volume did not change and therefore, the IgG release could not be due to hydrogel degradation or swelling. Control experiments did not show detectable adsorption of IgG on the surface of the polypropylene tubes.

Formation of a two-layer hydrogel architecture involved a two-step gelation process. The solution mixture containing 1.0% w/v ac-$(RADA)_4$-$CONH_2$ peptide and 5 μM IgG in PBS was introduced in a syringe and pushed through the needle to form a drop hanging at the tip of the needle. Then we waited 10 min to allow for gelation. The self-assembly process resulted in a hydrogel with spherical geometry. Then, using a different syringe containing a solution mixture of freshly prepared 0.6% w/v ac-$(KLDL)_3$-$CONH_2$ peptide in PBS we carefully allowed the drop of the ac-$(KLDL)_3$-$CONH_2$ solution to come in contact and encapsulate the preformed ac-$(RADA)_4$-$CONH_2$ peptide hydrogel thus creating an 'onion-like' two-layered hydrogel structure (Fig. 2). The

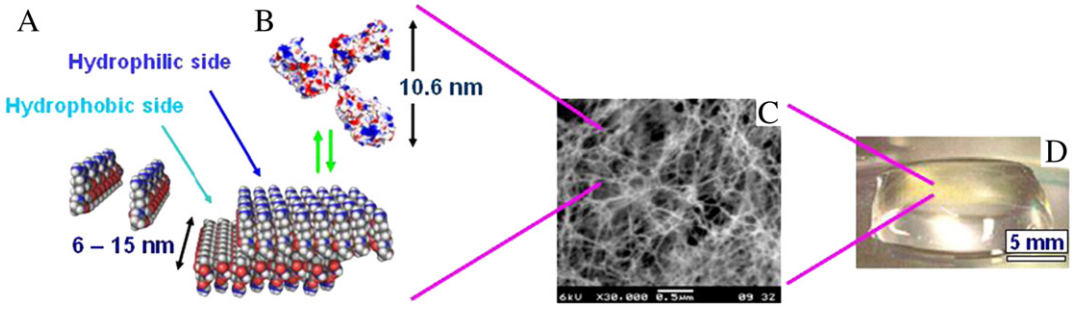

Fig. 1. Graphical representation of (A) the ac-$(RADA)_4$-$CONH_2$ peptide monomer, and of the peptide nanofiber, (B) the IgG molecule, (C) electron microscopy image of the peptide nanofibers, and (D) macroscopic image of the hydrogel. Color scheme for IgG and peptides: positively charged (blue), negatively charged (red), and hydrophobic (grey).