Affinity Hydrogels for Sustained Protein Delivery

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Statement of Purpose: Over the past few decades, advances in hydrogel technologies have spurred development in many biomedical applications including controlled delivery of biomacromolecules. Of particular interest are hydrogels with programmable, specific affinities for protein therapeutics[1,2]. The general mechanisms employed in these studies to prevent rapid protein release are non-specific electrostatic interactions between encapsulated proteins and immobilized ionic species such as heparin. However, non-specific binding of polyelectrolytes can be unstable and produce release profiles that are difficult to predict and/or control in complex biological environments.

Here, we describe an affinity hydrogel formulation for achieving sustained protein release based on specific protein-ligand binding. A synthetic ligand (methacrylated iminodiacetic acid, GMIDA) was copolymerized within poly(ethylene glycol) (PEG) hydrogel networks to form PEG-*co*-GMIDA affinity hydrogels. The use of PEG retains hydrophilicity and biocompatibility of the hydrogels while GMIDA, in conjunction with divalent metal ions, provides protein-binding sites to delay the release rates of a model recombinant protein, hexahistidine-tagged green fluorescent protein (hisGFP).

Methods: Poly(ethylene glycol) diacrylate macromer (PEGDA, 3.4kDa) was synthesized by reacting PEG with acryloyl chloride[3]. The biomimetic ligand, methacrylated iminodiacetic acid (GMIDA), was synthesized by reacting glycidyl methacrylate with iminodiacetic acid[4]. PEGDA and GMIDA were copolymerized via photo-polymerization to obtain affinity PEG-*co*-GMIDA hydrogels (gel weight ~40mg, gel thickness ~0.6mm). 6xhisGFPuv plasmids were transformed and expressed in *E. Coli.*[5] to obtain recombinant hisGFP which was mixed (0.25wt%) into the hydrogel precursor solution prior to photopolymerization. In vitro protein release was performed in pH 8.0, 10mM HEPES buffer at 37°C. The concentration of released hisGFP was determined by fluorescence measurement and fluorescamine assay.

Results/Discussion: To circumvent initial burst release and fast protein diffusion commonly seen with highly swollen hydrogel networks, GMIDA monomers were synthesized and copolymerized into PEG hydrogels to act as immobile ligand and sustain release of his-tagged proteins through protein-specific affinity binding. Two parameters were evaluated including ligand-protein molar ratio (*R*) and ligand-protein affinity. As shown in Figure 1A, when GMIDA(Ni^{2+}) complex was copolymerized into the PEG networks, the release rate of hisGFP greatly decreased with increasing ligand concentration. A reaction-diffusion model base on protein-ligand reversible binding was developed to predict protein release from the affinity hydrogels. Model predictions indicate that as *R* increases the protein release rate decreases because a greater fraction of encapsulated protein is reversibly bound to the crosslinked polymer network (Fig. 1A).

While experimental results and model predictions agree at low ligand-protein ratios, protein is released faster at high ligand concentration (R=100) than model calculations predict. This most likely occurs due to limited incorporation of ligand monomers during polymerization. The limitation presented by using high ligand concentrations can be overcome, however, by using low concentrations of higher affinity ligands. Model predictions indicate that protein release rates decrease as ligand-protein affinity increases (Fig. 1B). It has been shown previously that IDA-Cu^{2+}, compared to IDA-Ni^{2+}, provides a higher affinity for 6×his-tagged proteins[6]. Prediction of slower hisGFP release agrees well with experimental data when GMIDA(Cu^{2+}) is used as a higher affinity ligand.

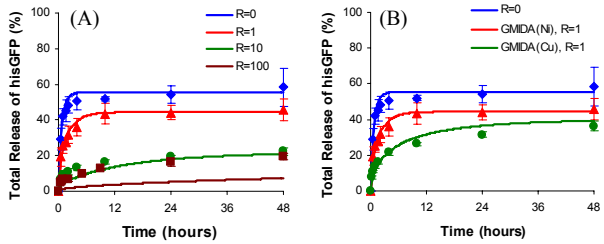

Figure 1. Sustained release of hisGFP from PEG-*co*-GMIDA hydrogels. The release rate of hisGFP decreases with increasing (A) [GMIDA(Ni)]-hisGFP ratio (*R*), and (B) GMIDA-hisGFP affinity. Symbols and curves are experimental data and theoretical predictions, respectively.

Conclusions: We have developed an affinity hydrogel system for actively controlling protein release rates based on specific ligand-protein binding. The versatility, tunability, and predictability of this release system indicate its strong potential to advance the field of biotherapeutic delivery.

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