AFFINITY HYDROGELS: TAILORED PROTEIN DELIVERY FROM PERMISSIVE TISSUE ENGINEERING MATRICES

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Introduction

Sustained release of protein therapeutics from synthetic matrices is a critical technology for tissue engineering. Hydrogels incorporating biomimetic species such as heparin have been used previously to lower the release rates of basic growth factors such as nerve growth factor (NGF), and basic fibroblast growth factor (bFGF).[1] However, the non-specific electrostatic interactions between positively charged proteins and anionic heparin oligomers that result in sustained protein delivery can be unpredictable in complex biological environments and produce release profiles that are difficult to control. The uncertainty and low degree of specificity of heparin-protein binding explain why these materials have not been exploited for tunable dual-protein delivery. Protein immobilization via metal-ion-mediated affinity binding, on the other hand, is highly specific and stable under a broad range of physiologically relevant conditions. In addition to its widespread use in protein purification, this binding mechanism has been used to fabricate stimuli-sensitive hydrogels[2] and functionalize organic and inorganic surfaces.[3] It has also been used to increase protein bioavailability during photoencapsulation.[4] However, the use of this binding mechanism in achieving sustained delivery of multiple proteins from homogeneously crosslinked hydrogels has not been investigated. In this abstract, we present a novel *affinity hydrogel* platform containing well-defined protein-binding moieties immobilized within an otherwise inert matrix. By manipulating the concentration and chemistry of these bioactive sites, the sustained release of multiple target proteins can be readily and independently tailored.

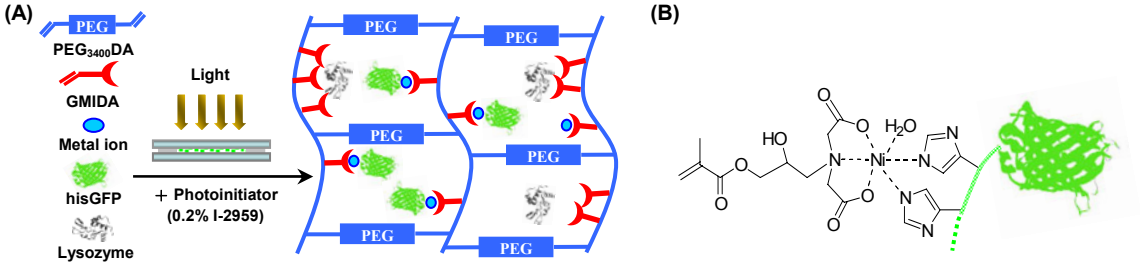

Figure 1. (A) *In situ* photopolymerization of homogeneous affinity hydrogel. An aqueous mixture of PEG3400DA, GMIDA, metal ions, proteins, and photoinitiator is reacted to form the gel under ambient conditions. Selective yet reversible protein-GMIDA binding permits sustained protein delivery from the otherwise highly permeable matrix. (B) Schematic for GMIDA-hisGFP chelation mediated by Ni^{2+}.

Results and Discussion

We have prepared PEG-*co*-GMIDA affinity hydrogels for dual-protein delivery by copolymerizing poly(ethylene glycol) diacrylate (PEGDA, 3.4 kDa) macromers with methacrylated iminodiacetic acid (GMIDA)[9] (Figure 1A). Model proteins including lysozyme (from Sigma, 14kDa) and hexahistidine-tagged green fluorescent protein (hisGFP, 27.4kDa) were mixed with the prepolymer solution and uniformly encapsulated within PEG-*co*-GMIDA hydrogels during photoinitiated polymerization and crosslinking. The one-step gel fabrication in aqueous solution maintains bioactivity and bioavailability of the hydrophilic proteins.[10] When complexed with divalent metal ions, GMIDA units act as synthetic protein receptors (Figure 1B), decreasing protein release rates by reversibly

sequestering soluble his-tagged proteins in a manner similar to the immobilization of proteins by native extracellular matrix.[11]

The effectiveness of obtaining sustained protein release via GMIDA incorporation was first demonstrated by quantifying his-tagged protein (hisGFP) delivery from PEG-*co*-GMIDA networks. Two parameters were evaluated including receptor-protein molar ratio (R_G) and receptor-protein affinity. As shown in Figure 2A, when GMIDA(Ni^{2+}) complex was copolymerized into the PEG networks, the release rate of hisGFP greatly decreased with increasing receptor concentration. When no GMIDA(Ni^{2+}) was used, 80% of the protein diffused out of the hydrogel within one hour. However, when 9.4mM GMIDA(Ni^{2+}) receptor ($R_G = 100$) was copolymerized into the network, the time required to release 80% of the protein was delayed to 24 hours, indicating over an order of magnitude decrease in release rate. The burst-release behavior commonly observed with traditional hydrogel matrices is also greatly decreased in the presence of GMIDA. As R_G increases the protein release rate and burst effect decrease because GMIDA-hisGFP binding equilibrium dictates that a greater fraction of encapsulated protein is reversibly immobilized to the insoluble PEG-*co*-GMIDA network at any given time. This conclusion of reaction-diffusion controlled release is supported by the fact that the swelling of the described hydrogel formulations does not change upon incorporation of GMIDA, indicating that the observed release behavior is not due to a decrease in the soluble protein diffusion coefficient.

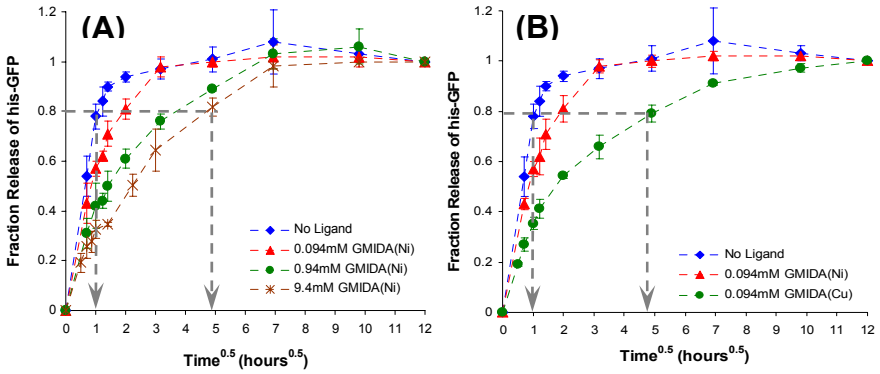

Figure 2. Sustained release of hisGFP from PEG-*co*-GMIDA hydrogels. The release rate of hisGFP decreases with increasing (A) [GMIDA(Ni)]-hisGFP ratio (R_G), and (B) GMIDA-hisGFP affinity.

For a given value of R_G, the fraction of his-tagged protein bound to the immobilized complex should also increase as the protein-GMIDA binding affinity increases. The binding affinity of PEG-*co*-GMIDA hydrogels for encapsulated proteins can be readily tailored through exchange of chelated metal ions. For example, it has been shown that IDA-Cu^{2+}, compared to IDA-Ni^{2+}, provides a higher affinity for 6×his-tagged proteins.[12] As shown in Figure 2B, 80% of encapsulated hisGFP was released in 4 hours from gels prepared with 0.094mM GMIDA(Ni^{2+}) ($R_G = 1$); however, this time-point was extended six-fold to 24 hours when the same amount of GMIDA(Cu^{2+}) was used.

Based on additional data obtained from lysozyme release studies, the applicability of the PEG-*co*-GMIDA affinity hydrogel formulation for dual-delivery of his-tagged and native protein was evaluated. We hypothesized that the release rate of hisGFP could be independently controlled without altering the lysozyme release profile based on the fact that GMIDA-metal ion complexes only bind strongly to his-tagged proteins. Figure 3A shows the dual-protein release results obtained by increasing receptor affinity through the complexation of Ni^{2+} with copolymerized GMIDA. Since the affinity binding between his-tagged proteins and GMIDA receptors is mediated by metal ions such as nickel, one expectedly sees a sharp decrease in hisGFP release rate when nickel ions are added. However, due to significantly weaker GMIDA interactions with non his-tagged proteins, lysozyme release profiles do not change.

The effects of GMIDA(Ni^{2+}) concentration on dual-protein release are shown in Figure 3B. Once again, release of non his- tagged protein (lysozyme) was not affected while hisGFP release decreased significantly as receptor concentration increased from0.94mM (R_G = 10) to 7mM (R_G = 74). Taken together, these data suggest that release of his-tagged protein from a mixture of encapsulated proteins can be independently regulated by simply adjusting the concentration or chemistry of copolymerized GMIDA-metal ion complexes. The release rate of native proteins can be controlled by adjusting traditional network parameters such as crosslinking density.

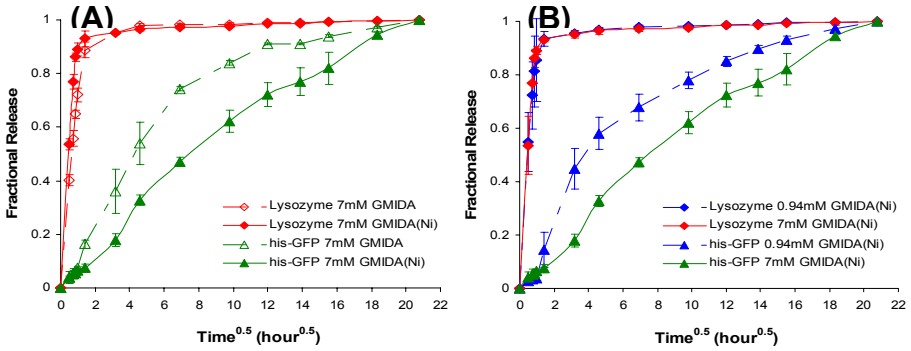

Figure 3. Dual release of hisGFP and lysozyme from PEG-*co*-GMIDA hydrogels as a function of (A) GMIDA-protein affinity and (B) GMIDA concentration.

Conclusions

In conclusion, homogeneous affinity hydrogels bearing well-defined concentrations of metal-chelating sites that reversibly bind to therapeutic proteins under physiological conditions have been prepared. Utilizing this strategy for simultaneous delivery of multiple proteins eliminates the need for multi-step matrix fabrication, increases the bioactivity and bioavailability of the delivered proteins, and, most importantly, achieves independent temporal control over the sustained release of his-tagged proteins such as hisGFP for up to several weeks. The versatility of this delivery scheme will be further improved in the future through the synthesis and incorporation of additional receptors with unique affinities for native proteins. With these enhancements, this hydrogel chemistry is expected to have broad application in the fields of controlled release and tissue engineering.

References

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