Affinity Hydrogels for Controlling Protein Release via Intermolecular Hybridization

Boonchoy Soontornworajit, Jing Zhou, Mark Battig, Yong Wang
Department of Chemical, Materials & Biomolecular Engineering, University of Connecticut, Storrs, CT 06269

Statement of Purpose: Protein drugs hold great promise for the treatment of various human diseases. However, efficient and safe delivery of protein drugs is a long-standing challenge in the field of drug delivery. Many protein delivery systems suffer from problems including the rapid release of protein drugs, the inefficiency of controlling the release of multiple proteins, and the involvement of toxic molecules and/or harsh conditions during the preparation of protein delivery systems. Thus, we studied a novel and biocompatible system for controlling protein release. The model system is made of agarose hydrogel and nucleic acid aptamers. The aptamers can entrap multiple protein drugs inside hydrogels because of their affinity and specificity. Importantly, the pegylated molecular triggers can penetrate the hydrogel and to release the bound protein drugs with desired kinetics via an intermolecular hybridization-based mechanism.

Methods: Agarose hydrogel was used as a model. Two aptamers that recognize PDGF and VEGF were used to functionalize the hydrogel. The preparation of the aptamer-functionalized hydrogel was based on the physical entrapment of aptamer-modified particles in the hydrogel network. The aptamers were investigated by the prediction of their secondary structures and the analysis of their binding affinities. The hydrogel was examined by both an AR-G2 rheometer and microscopy imaging. The aptamer functionalization and protein release were characterized by flow cytometry and ELISA, respectively. To enable the triggered release, the complementary oligonucleotides of the aptamers were functionalized with polyethylene glycol.

Results. Aptamers could be immobilized on the surface of the particles with a density of 56,000/particle. The efficiency of protein entrapment could reach more than 90%. The microscopic observation showed no particle aggregation due to the surface functionalization with both aptamers and proteins. In addition, the particles were well distributed in the agarose hydrogel. The rheology examination showed that the storage and loss moduli of the hydrogel were not significantly affected in the presence of the particles. Because the preparation of the hydrogel does not need any crosslinking between the polymer chains and the particles, these results indicate that any type of hydrogel can be functionalized with aptamers by using this approach.

To examine if the molecular triggers were able to hybridize with the aptamers, both surface plasmon resonance and gel imaging were performed. The surface plasmon resonance showed that the molecular triggers could bind to the aptamers and enable the fast dissociation of aptamer-protein complexes. In addition, the dissociation kinetics was a function of the molecular weight of polyethylene glycol. In addition, the gel imaging analysis showed that the molecular triggers could penetrate the hydrogels rapidly and bind to the aptamers.

After examining the functionality of the hydrogel, we performed a series of experiments to test the profiles of protein release. In the presence of the aptamer-functionalized particles, the proteins were released very slowly. During the first day, less than 5% of the proteins were released from the aptamer-functionalized hydrogels (**Figure 1**). Afterwards, the average daily release rate was less than 1%. In contrary, more than 70 to 80% of loaded proteins were released from the control hydrogels. These results clearly showed that the aptamers could effectively entrap protein drugs in the hydrogels. The pegylated molecular triggers were further used to trigger the release of the entrapped proteins. After a one-hour triggering, the daily release rate could reach 20 to 50% (**Figure 2**). The total amount of the released proteins was dependent on the molecular weight of polyethylene glycol, the triggering time, and the concentration of the molecular triggers.

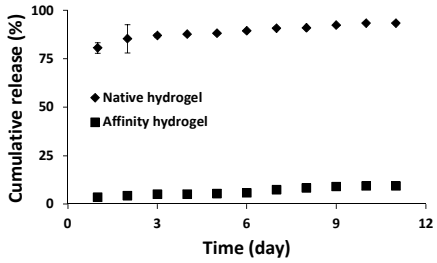

Figure 1. Sustained protein release from hydrogels in the absence of molecular triggers.

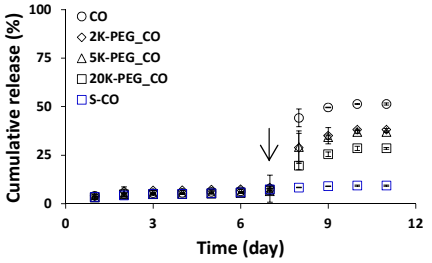

Figure 2. Triggered protein release. CO: molecular trigger; PEG_CO: pegylated CO; S-CO: scrambled CO. The arrows show the time point of stimulating the hydrogels with the molecular triggers.

Conclusions: Aptamers can be used to develop novel affinity hydrogel. The affinity hydrogel can prevent the rapid release of multiple proteins because the affinity of the aptamers. Importantly, pegylated molecular triggers can penetrate the hydrogels to release the bound protein drugs with different release kinetics. The authors greatly acknowledge the support from the NSF (CAREER Award Program DMR-0955358; CBET-0967512) to Y.W. and the Royal Thai Government Scholarship to B.S.