A Novel Platform for Controlled On Demand Multi-Protein Delivery

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Statement of Purpose: Recent advancements in the field of biotechnology have led to the development of abundant recombinant protein/peptide-based drugs such as vaccines, enzymes, and hormones[1]. Along with their isolation and purification, optimizing a delivery mechanism continues to be the primary obstacle for the clinical introduction of many protein-based drugs[2]. Tissue development and regeneration are regulated by a cascade of events with which certain growth factors and hormones are temporally and spatially associated[3]. To mimic and assist these physiological events in the target tissue, it may be useful to deliver multiple proteins at different time points. Here, we present a novel platform for on-demand multi-protein delivery. We report the design of novel hydrogel beads capable of triggered release of two different proteins on demand.

Methods: Pierce NHS-activated Agarose Resin (Thermo Scientific) was amine terminated using Ethylenediamine (pH=7.5) and left soaking overnight at 4°C. 100 mg of transformed beads were washed and resuspended in 10mL of 1 mg/mL of desired protein, FITC-BSA (Sigma) and Texas Red BSA (Invitrogen), solution at 4°C for one hour. Then 2mM of the cross-linker of choice, DTSSP or Sulfo-EGS (Thermo Scientific), was added to the beads and mixed at 4°C overnight. Before use, the beads were washed five times over 24 hours, and were then aliquoted for use. Protein conjugated agarose beads (4mg) were added to centrifuge tubes and the relevant cleaving agent (Cysteine for DTSSP or hydroxylamine for Sulfo EGS (Sigma)) was added to a final concentration desired. The tube was then filled to a final volume of 500µL, and the beads were incubated over a different time interval at 37°C to allow for release of protein. The concentration of the released protein was evaluated using its fluorescent intensity. For functional studies, two model enzymes (lysozyme and alkaline phosphatase (sigma)) were used and their functionality evaluated by performing their relevant assays. ELISA, Bradford protein assay, or Western Blot was performed on the selected samples to further characterize the released protein

Results: Two thiol- and hydroxylamine- cleavable crosslinkers were used to conjugate the two model proteins: FITC-BSA and Texas Red-BSA. By performing functionality and toxicity studies, the effective dose range of cleaving agents was determined. The toxicity levels of the cleaving agents are shown to be 50mM for cysteine and 500mM for hydroxyl amine. We also demonstrated that our cleaving agents can release the proteins from the agarose platform in a dose-dependent manner (Fig 1.). Our data showed that these two cleaving agents do not interfere with each other and act on their corresponding crosslinker independently. Furthermore, the model functional proteins, lysozyme and alkaline phosphatase, maintained their enzymatic activity which was evaluated by lysozyme and p-Nitrophenyl phosphate assay kits, respectively.

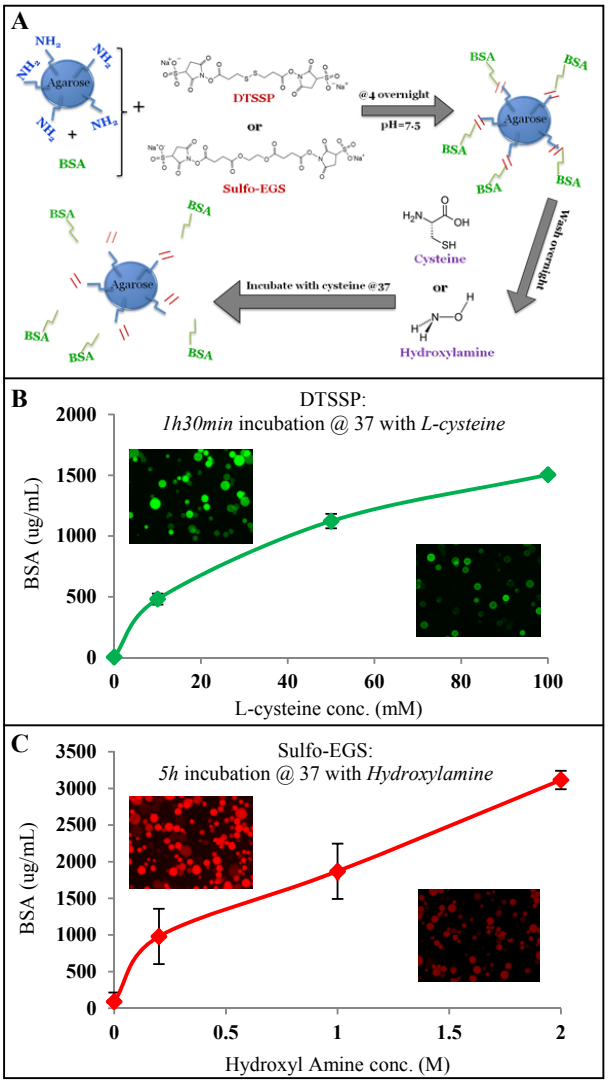

Figure 1. A) Schematic for conjugation and release of the protein to and from the agarose beads. Amount of release protein B) using DTSSP/Cysteine C) using Sulfo-EGS-hydroxylamine cleavable chemistry. Inserted images shows the amount of remaining fluorescently tagged protein on the surface of the agarose beads in different cleaving conditions.

Conclusions: This novel delivery system can potentially enable the orchestration of complex multi-phase regeneration processes which demand spatially and temporally organized signaling by multiple growth factors. The on-going direction for this system is exploring its capability for on-demand multi-protein delivery in an *in vivo* model.

References:

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