Affinity-Based Approaches for Controlling Release of Therapeutic Proteins from Biopolymer Hydrogels

Proteins are powerful therapeutic agents but are challenging to effectively administer to the body. Their fragile 3D structures enable them to interact with other molecules in precise ways to regulate biochemical pathways. Most are administered parenterally, but natural clearance mechanisms limit the duration of their effect. Conventional controlled-release strategies typically used for small-molecule drugs often require harsh processing conditions that can cause irreversible protein denaturation. Affinity-based release systems incorporate therapeutic proteins into delivery vehicles under mild conditions, enabling higher bioactive drug loading. The rate of release is governed by diffusion and non-covalent interactions with the delivery vehicle. We designed a series of release strategies enabling flexible delivery from biopolymer-based hydrogels. Src homology 3 (SH3) binding peptides enabled the sustained release of rod-derived cone viability factor long (RdCVFL) fused with an SH3 domain from a hyaluronan methylcellulose-based hydrogel. Using finite element analysis, we modelled drug distribution to inform the delivery vehicle formulation. We learned that higher excess concentrations of binding peptide led to subtherapeutic levels of protein in the tissue, while lower concentrations did not extend the effective time of release. Importantly, we found that this affinity release strategy would deliver effective concentrations of RdCVFL-SH3 for 35 days. To enable multifactor release, we developed unique affibody binding partners through directed evolution that specifically bound to insulin-like growth factor 1 (IGF-1) and pigment epithelium-derived factor (PEDF). By varying the concentration of these protein binding partners, tunable and simultaneous release of both IGF-1 and PEDF from biopolymer hydrogels were achieved over the course of 7 days. This strategy enables the delivery of multicomponent therapies that are vital to the clinic. To streamline the

process of developing binding partners, we utilized ROSETTA to create a binding peptide for vascular endothelial derived growth factor (VEGF). We isolated a peptide within the complementarity-determining region by analyzing the complex between bevacizumab and VEGF. We further validated the interaction using biolayer interferometry. This approach enables the rapid discovery of binding peptides for affinity-based release. Overall, I demonstrated that affinity-based release systems effectively sustain the delivery of therapeutic proteins, and specific binding partners enable multicomponent controlled release strategies.