Reversible Dose-Based Drug Release Mediated by Azobenzene/α-Cyclodextrin Complexation to Study Myofibroblast Activation

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Statement of Purpose: The purpose of this project is to develop a hydrogel drug delivery system with reversible, photo-initiated soluble factor release to probe the chemical activation and deactivation of myofibroblasts. Prolonged activation of valvular interstitial cells (VICs) contributes to the progression of calcific aortic valve disease, the most frequently observed of valvular heart diseases. It has been shown that the myofibroblast phenotype is responsive to both mechanical and chemical stimuli. Hydrogels have become a popular cell culture substrate for the investigation of cellular response to many factors due to their physiologically-relevant moduli and potential for culture in 3 dimensions [1]. Specifically, PEG-based hydrogels have been shown to achieve moduli sufficiently low to maintain VICs in a quiescent state (5-10 kPa), which will allow us to better investigate the effects of soluble activating factors such as TGF-β [2]. The development of a reversible drug release hydrogel would allow for the probing of chemical activation of myofibroblasts and the reversibility of this response on soft hydrogels, and the chemical deactivation and coupled reversibility on stiff hydrogels. Here, we present a system composed of a hydrogel containing a cyclodextrin host and a protein functionalized with an azobenzene photoswitchable guest to temporally control the protein release profile (Figure 1A).

Methods: A peptide containing a terminal azobenzene derivative was synthesized using Fmoc-based solid phase peptide synthesis. This peptide, which contained an aspartate residue, was activated using N-Hydroxysuccinimide and used to functionalize bovine serum albumin (BSA), a model drug, along with NHS-Fluorescein (Pierce). Multi-arm poly(ethylene glycol)-acrylamide (PEG, 10 kDa molecular weight) was partially functionalized by Michael addition chemistry with α-CD that had been thiolated using Traut's reagent. Gels (10 weight percent) were formed using Michael addition chemistry between this PEG and multi-arm PEG thiol at pH 10 with 300 mM triethanolamine. Complexation between the azobenzene peptide and the α-CD-functionalized PEG was observed in solution using UV/Vis spectrometry before and after irradiation with 365nm light (5min, 5 mW/cm^2).

Results: Many azobenzene derivatives will form a host/guest complex with α-CD when they are in the trans configuration via hydrophobic interactions. Isomerization of the azobenzene to the cis configuration under irradiation with UV (320-365 nm) light disrupts this complexation by increasing the dipole of the azobenzene. The complexation creates an increase in the maximum absorbance at ~325 nm relative to the azobenzene alone as shown in the UV/Vis results in Figure 1B. Additionally, the isomerization of azobenzene triggered by irradiation with UV light (365 nm, 5 mW/cm^2) for five minutes, and the subsequent disruption of the host/guest complex results in a decrease in the absorbance maximum at ~325 nm. Studies of BSA release are in progress. The azobenzene functionalized BSA was successfully encapsulated in a PEG hydrogel with tethered CD moieties. The goal is to fix the soluble factor in the gel via host/guest complexation between the azobenzene and α-CD until irradiation disturbs the complex and allows for diffusion mediated release of the soluble factor (Figure 1A). Furthermore, irradiation with visible light (400-500 nm) will cause a cis-to-trans isomerization. This will allow for the reversible fixation of the soluble factor to the hydrogel or alteration of the drug release profile to slow release.

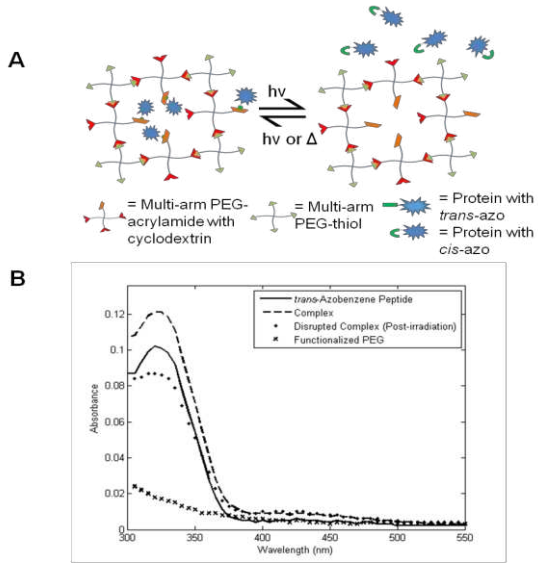

Figure 1. A) Hydrogel formulation with reversible host/guest complexation. B) UV/Vis data demonstrating host/guest complexation of azobenzene peptide (100 μM) and CD functionalized PEG (100 μM) in 10mM phosphate buffered saline.

Conclusions: The functionalization of PEG with α-CD and a peptide with azobenzene allows for reversible host/guest complex formation. Furthermore, BSA can be bifunctionalized with fluorescein for quantification and an azobenzene derivative for reversible fixation into hydrogels functionalized with α-CD. Future investigations will focus on the characterization of BSA release profiles from hydrogels of varying moduli. Once the gel system is optimized, VICs will be cultured on a soft gel containing TGF-β. Upon irradiation of the gel with UV light, it is anticipated that the TGF-β will be released, resulting in an activation of the VICs. Upon irradiation with visible light or thermal relaxation in the dark, the complexes will reform and the VICs will return to a quiescent state.

References:

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