

Design of multimodal microgel populations for enhanced protein delivery: Investigations into light and redox responsive formulations

Paige J. LeValley

Advisor: April M. Kloxin

Committee Members: Kristi L. Kiick, Christopher J. Kloxin, Millicent O. Sullivan

Biologics are playing an increasingly important role in the treatment of a variety of diseases ranging from autoimmune diseases to cancers due to their high specificity and potency compared to their small molecule counterparts. These therapies represent the most advanced treatments available and, as such, the number of FDA approved biologics continues to grow. Despite their increased popularity within the medical field, there are still large-scale challenges associated with protein-based therapies including stringent storage conditions, limited delivery options, and costly therapeutic regimens. On top of this, proteins face therapeutic barriers *in vivo* that are inherent to the protein structure. For example, large proteins have difficulty penetrating dense tissues altering biodistribution, whereas small proteins suffer from short half-lives, limiting therapeutic time scales. These limitations have led to the need for large doses to be administered for therapeutic efficacy to be realized, which increases the probability of adverse side effects and costs experienced by the patient. In an attempt to address these challenges, protein delivery vehicles have been engineered to provide controlled, tunable, and localized delivery motifs.

Hydrogels have emerged as promising platforms for protein delivery as they can encapsulate large amounts of hydrophilic proteins and exhibit versatility in structure, size, and chemical nature. Further, hydrogel microparticles, or microgels, offer exciting opportunities for creating modular protein delivery platforms toward improved combination therapy regimens and personalized medicine. The overarching goal of this dissertation was to engineer multimodal redox and light responsive microgel populations to provide controlled and on-demand protein release profiles locally within sites of interest through tunable matrix degradation properties. This goal was achieved by addressing limitations in literature associated with *i*) the use of photolabile chemistries within hydrogel networks developed for controlled protein delivery, *ii*) the formation of injectable microgels using spontaneous click chemistries in combination with microfluidic flow focusing techniques, and *iii*) the design of cyclic peptides with functional handles for bioconjugation toward localization of therapies.

First, the light responsive behavior of biologically inert poly(ethylene glycol) (PEG)-based hydrogels containing different photolabile moieties within the network crosslinks were investigated for independent control of cleavage in response to exogenous (*e.g.*, light) vs. endogenous (*e.g.*, water) stimuli and achieving protein release over different timescales. Specifically, nitrobenzyl (NB) moieties, which readily degrade in response to long wavelength UV, with different labile bonds were synthesized and their photolytic and hydrolytic behavior as crosslinks within hydrogels was characterized. Four different cleavable bonds, NB-ester, NB-amide, NB-carbonate, and NB-carbamate, were investigated, in which the latter two had yet to be incorporated as crosslinks within hydrogels. The four different NB linkers were observed to exhibit a range of photolytic and hydrolytic properties that were utilized to control the release of model proteins in a sequential or combined manner from concentric circle hydrogels. Second, the NB-

carbamate was compared to a coumarin (CMR) photolabile moiety, which degrades in response to blue light, in both degradation and protein release properties. Hydrogels containing either NB or CMR moieties degraded rapidly in response to long wavelength UV or blue light, respectively. Further, both formulations were successfully degraded through a layer of porcine skin, a mimic for human skin, and able to effectively encapsulate and release a model bioactive antibody. These demonstrations highlight the importance in labile bond chemistry of photocleavable moieties for controlling degradation behavior and the potential utility of photolabile hydrogels as protein delivery vehicles.

Next, homogenous microgels that responded to either aqueous reducing conditions or light were formed using microfluidic flow focusing. Redox responsive microgels were successfully formed using a thiol-Michael addition between thiols and maleimides, where controlling the rate of hydrogel formation was determined to be key. Further, NB-carbamate containing microgels of the same size were formed through a strain promoted azide alkyne cycloaddition reaction, indicating microgel size is controlled by the microfluidic device design and not the polymerization chemistry. The redox responsive microgels were successfully degraded on clinically relevant time scales by incubation in high reducing, low reducing, or aqueous conditions. Light responsive microgels were degraded through irradiation with long wavelength UV light. Together, these microgels provide both sustained and on-demand degradation profiles, presenting future opportunities for combining these systems to create complex release profiles of multiple proteins.

Lastly, functional cyclic RGD peptides were synthesized to contain a functional handle for subsequent conjugations using orthogonal click reactions. The cyclic RGD was found to localize to breast cancer cell membranes indicating its potential use as a targeting peptide by conjugation to protein therapeutics or delivery vehicles. Collectively, this dissertation provides insights into the rational design of photolabile hydrogels for the on-demand delivery of protein therapeutics, offers design parameters for forming redox and light responsive microgels through spontaneous click chemistries, and presents a synthetic strategy for synthesizing cyclic peptides containing functional handles for localization or targeting of therapeutics to sites of interest. These strategies can be combined to create targeted multimodal microgel populations for the sustained, on-demand, and local release of protein therapeutics toward personalized medicine or improved therapeutic or vaccination regimens.