Bioactive Protein Photorelease from Hydrogels via Tissue-penetrating Green Light

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Statement of Purpose: Over the past decade, photoresponsive biomaterials have birthed a surge of innovation in targeted drug delivery and 4D cell culture. Compared to other material-modifying stimuli (e.g., pH, enzymes, heat), light is a particularly powerful stimulus, uniquely affording spatiotemporal control in potentially wavelength-orthogonal manner.[1] Despite its established promise in laboratory settings, significant challenges remain in pushing these technologies to the clinic. Current photosensitive materials are constrained by their reliance on high-energy UV light, which is poorly penetrant through human tissue, limiting the depths to which they may be used for *in vivo* treatment. In this work, I will highlight our recent successes in photoreleasing site-specifically modified proteins from stable hydrogel biomaterials while retaining native bioactivity using tissue-penetrating visible light ($\lambda \leq 530$ nm) (**Figure 1**).

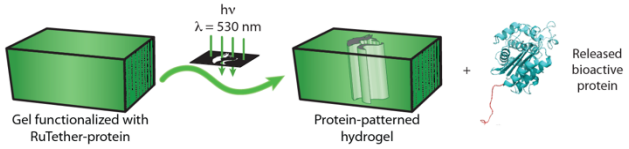

Figure 1. Hydrogels decorated with proteins of interest via RuTether respond rapidly to visible light, permitting patterned gel creation and concomitant release of bioactive species.

Methods: Building on our recently reported methods to create hydrogel biomaterials that undergo rapid photodegradation in response to low-energy visible light[2], we sought to establish methods to photorelease protein cargos from photostable hydrogel biomaterials using tissue-penetrating visible light. Towards this, we created a new highly photosensitive ruthenium polypyridyl-based tether that contained two reactive moieties: one for site-specific conjugation onto proteins of interest and another for direct incorporation into hydrogels. This molecule (referred to as "RuTether") was found to be hydrolytically stable, cytocompatible, and well suited for site-specific conjugation to protein cargo via sortase transpeptidation[3]. RuTether photocleavage kinetics were assessed through ^{1}H nuclear magnetic resonance (NMR), mass spectrometry, and via analytical high-pressure liquid chromatography (HPLC) following a variety of light conditions (λ = 530 nm, t = 0 – 5 min, I = 0 – 5 mW cm^{-2}). RuTether was chemoenzymatically fused to model fluorescent proteins, enzymes, and growth factors via sortase-tagged expressed protein ligation (STEPL)[3]. Following incorporation into poly(ethyelene glycol) (PEG)-based hydrogels, broadly available light sources were used to pattern protein release from biomaterials including commercially available LEDs, common laser lines on a confocal microscope, and a femtosecond-pulsed laser. Pork belly was used as a complex tissue mimic to assess material photoresponsiveness at varying tissue thicknesses and exposure times.

Results: RuTether was successfully appended onto the C-termini of several model proteins with excellent efficiency, as confirmed by whole-protein mass spectrometry, via STEPL. RuTether-modified proteins were readily incorporated into PEG-based hydrogels at user-defined concentrations (0 – 100 μM) via strain-promoted azide-alkyne bioorthogonal click chemistry. As both a small molecule and as a protein tether, RuTether demonstrated exceptionally high photoresponsiveness under visible light exposure (≤530 nm), permitting protein release from hydrogels with as little as 15 mJ/cm^2 – a remarkable improvement compared with the standard light dose required when using classical chromophores such as *ortho*-nitrobenzyl (~8,000 mJ/cm^2).[3] This high efficiency permitted protein release through up to 2 cm of complex tissue using green light. RuTether was found to be cytocompatible, both before and after light exposure, for encapsulated mammalian cells. These findings demonstrate RuTether's unique potential in release bioactive proteins from tissue-transplanted biomaterial constructs using low-dosed and low-energy visible light.

Conclusions: Through synthesis and characterization of a new photolabile protein-biomaterial linker, we demonstrate the ability to pattern bioactive protein release from hydrogel biomaterials for the first time using tissue-penetrating green light. RuTether is exceptionally photoresponsive, cytocompatible, and amenable to a variety of photolithographic processing. Uniquely, the reported approach preserves protein bioactivity while enabling direct photorelease of protein cargo through up to 2 cm of tissue.

References:

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