Hydrogels Crosslinked by Photoactive Ruthenium Complex for Rapid Protein Release in Response to Visible Light

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Statement of Purpose: Dynamic, responsive materials that encapsulate drugs and therapeutics can serve as vehicles for drug delivery in response to external cues [1]. Hydrogels provide delivery platforms that offer aqueous encapsulation and controlled release through material design [2]. For example, hydrogels can be triggered in response to NIR when gold nanorods are encapsulated [3]. Here, we used a photoactive ruthenium polypyridyl complex with a di-aldehyde functionality (Ru-ald) to crosslink a hydrazide-functionalized hyaluronic acid (HA-adh) to form light-responsive hydrogels (Ru-HA). Upon exposure to visible light (400-500 nm), rapid dissociation within Ru-ald occured with high quantum efficiency, enabling triggered disassembly and payload release. We investigated the formation of microgels from these materials using microfluidic mixing devices and the encapsulation and release of a model enzyme payload, TEM1 β-lactamase (bLA).

Methods: HA-adh, functionalized with 40% of the disaccharide units modified with hydrazides, was combined at 3 wt% with 12 mM Ru-ald to form a hydrogel (Fig. 1A). Network assembly, viscoelastic properties, and disassembly in response to visible light irradiation were investigated through rheometry, and hydrogel degradation was observed both macro- and microscopically. bLA was included within gels at 0.25-0.75 mg/mL and the release was measured through its action on nitrocefin. To assess cytocompatibility, whole gels were incubated in the presence of fibroblasts for up to three days, as were degradation products at 0.01-1 mM concentration, and the cell population was assessed with an Alamar Blue assay. A PDMS-based microfluidic device was used to combine the gel components in droplets, which broke off at a t-junction and traveled through undulating channels to mix components prior to full gelation. Droplets were collected, allowed to fully crosslink, and the responses of the resulting microgels to visible light irradiation were assessed.

Results: Upon combination, HA-adh and Ru-ald reacted to form elastic hydrogels on the order of minutes, with full gelation occurring on the order of an hour to form a Ru-HA gel with G' ≈ 2 kPa and G'' ≈ 65 Pa. Upon exposure to 25 mW/cm^2 visible light (400-500 nm), rapid disassembly of the hydrogel occurred, as evidenced by G' dropping to < 13 Pa in under 30 s (Fig. 1B). Degradation of cylindrical macrogels (4 mm diameter, 0.5 mm thickness) was observed to occur in less than 10 minutes under constant agitation. Cells incubated in the presence of hydrogels showed no difference in proliferation when compared to the controls, and the degradation products showed no cytotoxic effects after incubation for 24 hours. As evidenced by its chromogenic activity on nitrocefin, bLA could be encapsulated and released as a bioactive payload. From macrogels, bLA could be released under continuous irradiation or dose-wise through intermittent irradiation (Fig. 1C). Microfluidics enabled the generation of monodisperse populations of microgels, with diameters of ~75 μm (Fig. 2A). Both macro- and microgels were stable in aqueous buffer for over a week. In comparison to macrogels, microgels responded even more rapidly to visible light irradiation, with full dissociation triggered by < 30 s visible light (Fig. 2B).

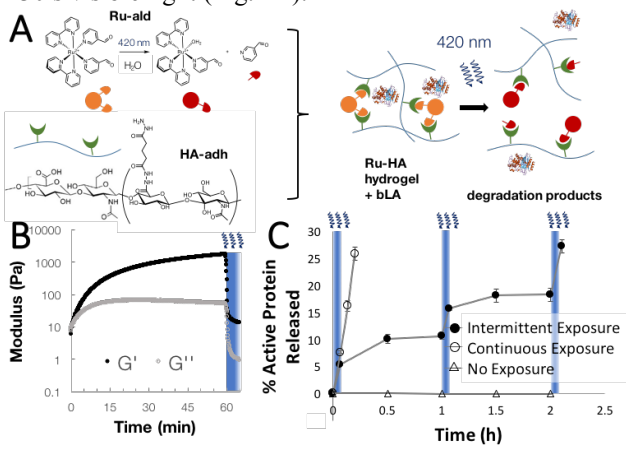

Figure 1. (A) Schematic representation of gel formation and degradation. (B) Rheologic measurement of gel formation (3 wt%) and rapid light-induced degradation (blue region). (C) Release of active enzyme under continuous or intermittent irradiation (4 min, every hour), intermittent exposures indicated.

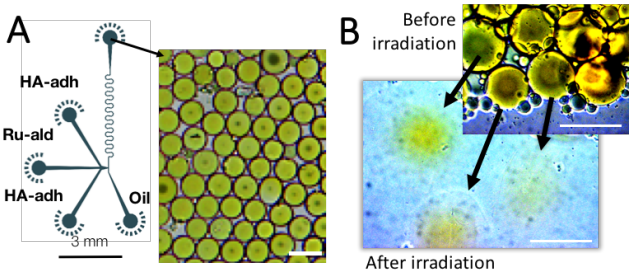

Figure 2. (A) Setup of microfluidic device and image of microgel population. (B) Image of microgels in water before 30 s irradiation (top) and after (bottom), with arrows indicating same gels. Scalebars 100 μm.

Conclusions: The Ru-HA gels developed here enabled rapid encapsulation and release of bioactive payloads in aqueous environments. The resulting gels were stable in the absence of light and cytocompatible. The high quantum efficiency of the Ru-based crosslinker allowed for sensitive enzymes to be released; thus, the Ru-crosslinked hydrogels might find widespread application in drug delivery and biomedical applications.

References: [1] Cohen Stuart MA. Nat Matls. 2010;9:101-113. [2] Hoare TR. Polymer. 2008;49:1993-2007. [3] Highley CB. Nanomed. 2016;11: 1579-1590.