Bioorthogonal Hyaluronate Hydrogels for Therapeutic Protein Delivery

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Statement of Purpose: Bioorthogonal chemistries enable new avenues for materials design when encapsulation of biologics without loss of their function is required. Recently, inverse electron demand Diels-Alder (IEDDA) chemistries have garnered interest for hydrogel synthesis applications in which bioorthogonality is paramount. In order to assess the applicability of IEDDA chemistries to designing hydrogel chemistries for therapeutic protein delivery, we have formed hyaluronate hydrogels that are crosslinked using IEDDA functionalities in the presence of an encapsulated antibody fragment (Fab) as a therapeutic delivery system. While generally considered orthogonal to biologics, a thorough evaluation of the bioorthogonality of IEDDA functionalities has been missing to date. In this work, we also assess a therapeutic fragment antibody (Fab) after exposure to several IEDDA functionalities by various analytical techniques to confirm the protein is unaffected by exposure to those chemistries.

Methods: Sodium hyaluronate was modified with varying levels of tetrazine functionalities and crosslinked in aqueous buffer without additional reagents via homobifunctional PEG-norbornene to produce HA-PEG hydrogels. Rheological assessments of hydrogel formation kinetics were performed with and without addition of Fab (up to 20 wt%) as an initial evaluation of orthogonality. The hydrogels were further characterized for swelling properties, hydrolytic and enzymatic degradation and release kinetics of the physically entrapped Fab. In addition, extensive biophysical characterization and physical and chemical stability assessments of the Fab molecule were carried out after exposure to model IEDDA small molecules containing tetrazine, norbornene and trans-cyclooctene functionalities. Analytic assays performed included size exclusion chromatography (SEC), ion exchange chromatography (IEC), capillary electrophoresis (CE-SDS), antigen-binding surface plasmon resonance (SPR) and mass spectroscopy (MS). Finally, Fab released from HA-PEG hydrogels was similarly assayed to ensure no loss in biological activity or off-target reactions after IEDDA chemistry exposure.

Results: IEDDA-mediated HA-PEG hydrogel formation was achieved with rapid kinetics at 23^{o}C; for typical formulations, gel formation (G'-G'' crossover) was measured within 1-4 minutes. At equilibrium, elastic moduli of 200 Pa to 3.8 kPa were achieved, depending on the final HA concentration and crosslinking density. As shown in Figure 1, gelation kinetics were unaffected by the presence of high amounts of Fab indicating crosslinking was selective and bioorthogonal. Crosslinking kinetics were also found to be faster than a benchmark Cu-catalyzed click chemistry (CuAAC)-based system. HA-PEG hydrogels degraded in the presence of hyaluronidase enzyme at a rate dependent on the crosslinking density and enzyme concentration, whereas hydrolytic degradation was minimal. Physically entrapped protein was released over approximately 24 hours owing to the large mesh size and fully hydrated nature of the hydrogel. Released protein was fully intact and biologically active, indicating it was unaffected by the IEDDA crosslinking reaction. Similarly, the Fab molecule was found to be largely unaffected by direct exposure to all IEDDA functionalities as determined by the various analytical techniques employed.

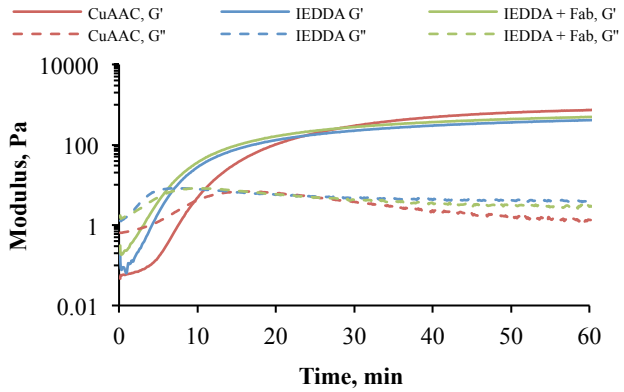

Figure 1. IEDDA-mediated HA-PEG hydrogel crosslinking kinetics—as assessed by oscillatory rheology—is unaffected by 20 wt% Fab loading and displays improved kinetics over CuAAC-mediated crosslinking.

Conclusions: CUAAC suffers from non-specific reactivity with biological molecules, limiting its use in applications that involve therapeutic proteins. In this work, we demonstrate that IEDDA chemistries are a viable alternative with vastly superior bioorthogonality. The ability to carry out IEDDA reactions in aqueous buffer, at room temperature and without impacting the biological activity of present therapeutic proteins may enable new avenues of hydrogel design. While the timescale of Fab release from HA-PEG hydrogels wasn't improved over previously reported systems, further optimization may be able to extend the half life of protein release, enabling facile synthesis of a long-acting delivery system.

References: Alge DL, Azagarsamy MA, Donohue DF, Anseth KS. Biomacromolecules. 2013; 14:949-953. Knall AC, Slugovc C. Chem Soc Rev. 2013;42:5131-5142.