Customized thiol-ene photo-click inks: tuning the mechanical properties based on the -ene moiety

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INTRODUCTION

The design of multi-stimuli-responsive biomaterial-inks is gaining increasing interest aiming at easily tuning hydrogel properties during the extrusion process and fabricating multi-functional constructs. Temperature and light irradiation are the most widely exploited green stimuli to provide construct primary and secondary stability, respectively. More recently, Vis-light is taking advantage over UV-light irradiation being a more cell-friendly light source and requiring more cytocompatible type II photo-initiators compared to type I. Vis-light thiol-ene photo-click chemistry represents a powerful tool, ensuring high spatio-temporal control and biorthogonal networks. Hence, thiol-ene chemistry can successfully open the way to finely tune construct mechanical properties. This work aimed at engineering two poly(ether urethane) (PEU)-based thiol-ene inks and investigating the influence of the –ene moieties on the photo-crosslinking mechanism and hydrogel mechanical properties. More in detail, an amphiphilic PEU bearing -NH groups was first synthesized; then, it was functionalized through green water-based reactions to graft photo-sensitive moieties (i.e., thiol, acrylate and norbornene moieties). Thiol-acrylate and thiol-norbornene hydrogels were thoroughly characterized in terms of thermo- and photo-responsiveness. Furthermore, their processability as biomaterial-inks was demonstrated through the 3D extrusion of constructs of different geometries. Lastly, their suitability as drug carrier was assessed through in vitro tests in view of thiol-ene ink exploitation in drug delivery applications.

MATERIALS AND METHODS

The PEU was synthesized through a two-step procedure and subjected to an acidic treatment to expose secondary amino groups [1]. Carbodiimide-mediated chemistry was exploited for PEU functionalization to expose thiol (S-PEU), acrylate (A-PEU) and norbornene (NB-PEU) moieties. PEUs were characterized by Size Exclusion Chromatography (SEC), Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopies, and Orange II Sodium Salt and Ellman colorimetric assays. Thiol-ene hydrogels were photo-irradiated at 525 nm (80k Lux, 30s) in the presence of Eosin Y and Triethanolamine (TEOA) and their mechanical properties were studied through rheology. Drug release capability was assessed through in vitro Ibuprofen (IBU) release tests. Hydrogel processability as biomaterial-inks was studied by extruding differently-shaped structures, while system cytocompatibility was assessed according to the ISO regulation.

RESULTS AND DISCUSSION

SEC, IR and Orange assay first assessed the successful synthesis of a high molecular weight PEU ($\bar{M}_n$ 22kDa) bearing 4.5×10E20 ± 1.8×10E19 -NH/gpolymer. Then, the successful carbodiimide-mediated functionalization was proved through the Ellman test and NMR spectroscopy, giving approx. 10E19 thiol and acrylate groups/gpolymer and 10E17 norbornene moieties/gpolymer. NMR spectra showed the almost complete consumption of photo-sensitive moieties upon irradiation with green light. Compared to not-irradiated samples, photo-irradiated inks exhibited a significantly higher elastic behavior (i.e., storage/loss moduli crossover frequency ω@ 30 °C = 8 rad/s vs. 4 rad/s for S-PEU/A-PEU and 3 rad/s vs. 1.4 rad/s for S-PEU/NB-PEU) and improved hydrogel resistance to applied strain (i.e., critical deformation ε = 18.6% vs. 11.6% for S-PEU/A-PEU and 11.6% vs. 7.2% for S-PEU/NB-PEU). Furthermore, TEOA addition resulted in improved mechanical properties only for S-PEU/A-PEU, thus demonstrating the -ene-dependent photo-crosslinking mechanism. Hydrogel capability to keep the shape over time was proved through the fabrication of 3D structures (Fig.1). IBU release from photo-irradiated systems demonstrated the absence of Vis-light-induced degradation phenomena. Lastly, hydrogels were found to be cytocompatible according to the ISO 10993-5.

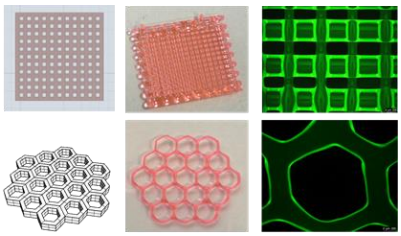

Figure 1. Representative images of bi-layer structures of different geometries: CAD models, photographs and microscopic images.

CONCLUSIONS

The versatility of PEU and carbodiimide chemistries was exploited to synthesize three different PEUs and their aqueous formulations were blended to develop two thiol-ene photo-click inks. We have demonstrated that the final construct mechanical properties can be tuned based on the –ene moiety.

REFERENCES

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