In Situ forming poly(ethylene glycol)-based hydrogels *via* metal-free thiol-maleimide click chemistry

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Statement of Purpose: The incorporation of cells and sensitive compounds can be better facilitated without the presence of UV or other energy sources common in the formation of biomedical hydrogels. *In situ* forming poly(ethylene glycol) (PEG) based hydrogels were developed by the step-growth polymerization of maleimide- and thiol-terminated PEG macromers *via* metal-free Michael addition (**Fig. 1**). The effects of macromer concentration, pH, and the presence of biomolecule gelatin on gel formation were investigated. Swelling and degradation of the gels were characterized. It is hypothesized that the resulting network may have great potential in the development of novel *in situ* gel-forming drug delivery systems. Moreover, the incorporation of gelatin in the network renders it an ideal platform for further biofunctionalization to direct specific biological responses.

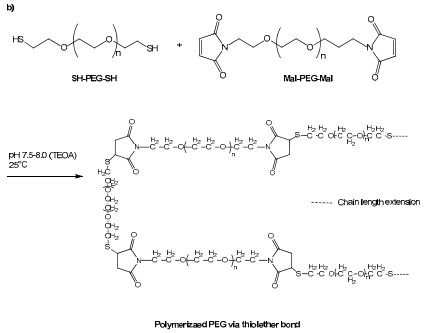

Figure 1 Synthetic scheme of PEG hydrogel *via* thiol-maleimide addition

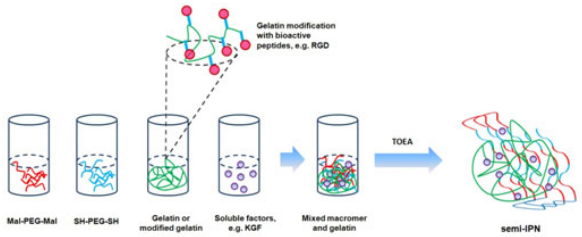

Figure 2 Mal-SH PEG gel formation

Methods: Hydrogels were prepared as shown in **Fig. 2**. The time of gelation was determined by the test tube inverting method. Polymer weight percent (wt%), pH effect, and the amount of gelatin incorporated were investigated. The equilibrium weight swelling ratios of various gels were evaluated in pH7.4 PBS at 37°C. FITC labeled dextran (FD-40, Mw ~40,000) was selected as a model solute to study the controlled release kinetics of macromolecules from the network prepared by thiol-maleimide click chemistry. Mal-SH PEG hydrogels were subject to cell adhesion studies on primary human monocyte, fibroblasts, and keratinocytes. The adherent cell densities on hydrogel surfaces were quantified and expressed as number of cells/mm^2 surface area *via* ImageJ.

Results/Discussion:

Gel formation Combining aqueous solutions of PEG-$(Mal)_2$ and PEG-$(SH)_2$ resulted in the formation of an entangled hydrogel. Gelation time ranged from minutes to days and was significantly affected by pH. Increasing macromer concentration led to a significant decrease in the gelation time, which might create more polymer entanglements during gel formation. The presence of gelatin perturbed the macromeric thiol-maleimide reaction and the subsequent gelation time. **Swelling and degradation** Mal-SH PEG hydrogels displayed consistent and increasing swelling through 7 days, with a maximum average weight swelling ratio of (23 ± 3), which is significantly higher than that of photopolymerized PEG hydrogel. Mal-SH sIPN showed significantly higher maximum swelling ratio than photopolymerized sIPN and mass loss was observed for both sIPN formulations after 24 h. ***In vitro* controlled release kinetics** FD-40 release from Mal-SH PEG hydrogel displayed a significant burst release with near 80% cumulative release at the 1h, due to the great swelling effect of click hydrogels and the rapid release of surface associated solutes. In contrast, sIPN showed a less significant burst effect, and a near zero-order release was observed from 0 to 4 h. It is expected that the release kinetics can be tailored by varying the amount of gelatin incorporated in the network as well as the PEG-to-gelatin weight ratio. **Cell adhesion** Adherent monocyte density on all surfaces decreased over the course of study associated with observable morphological changes over time. Adherent fibroblast and keratinocyte densities kept increasing on TCPS, but decreasing on photopolymerized PEG and Mal-SH PEG hydrogel surfaces, mostly due to the differences in protein adsorption behavior related to different material surface structures.

Conclusions: The PEG-based hydrogels was successfully synthesized *via* a metal-free thiol-maleimide reaction. pH, macromer concentration, and the presence of biomolecule gelatin showed significant effects on gel formation. Release studies using FD-40 indicated that the hydrogels has the potential as an *in situ* forming drug delivery matrix. Preliminary cell adhesion studies demonstrated that the networks were minimally adhesive to primary human monocytes, fibroblasts, keratinocytes thus providing an ideal platform for further biofunctionalization to direct specific biological response.

Acknowledgements
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