Injectable, Degradable and Biocompatible Poly(oligoethylene glycol methacrylate) Hydrogels

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ABSTRACT SUMMARY
A highly tunable, in situ-gelling hydrogel platform based on complementary reactive poly(oligoethylene glycol methacrylate) precursors (POEGMA) has been developed. The physiochemical and biointerfacial properties of these covalently cross-linked POEGMA hydrogels can be systematically varied by changing the composition of the reactive precursors while maintaining high cell viability both in vitro and in vivo. We will demonstrate that these POEGMA hydrogels provide an promising alternative to poly(ethylene glycol) (PEG) for use as biomaterial scaffolds or drug delivery vehicles.

INTRODUCTION
Poly(ethylene glycol) (PEG) hydrogels have been widely studied as synthetic matrices for the controlled release of therapeutics and tissue regeneration. Many PEG-based biomaterials have been developed and applied in clinical settings, taking advantage of the hydrophilic, non-immunogenic, and non-cytotoxic properties of PEG. However, PEG is a relatively unreactive polymer and its chemical modification is limited to the reactive end groups. Consequently, there is increasing interest in polymers with similar biological and physicochemical properties that offer improved control over the chemical composition and/or allow for more facile chemical modification.

The limitations of PEG can be addressed by the use of poly(oligoethylene glycol glycol methacrylate) (POEGMA). POEGMA can be synthesized by facile free radical polymerization, offering improved control over the polymer’s chemical composition (by simple copolymerization) and morphology. Furthermore, POEGMA polymers can be made thermoresponsive, with their LCST behavior in water determined by copolymerizing OEGMA monomers with varying ethylene oxide chain lengths (n). Finally, POEGMA-based materials display many of the advantageous biological properties that have made PEG so widely applied in (bio)materials design.

EXPERIMENTAL METHODS
We have prepared injectable POEGMA-based hydrogels based on reversible hydrazone bond formation between POEGMA precursors functionalized with aldehyde and hydrazide groups. Hydrzone crosslinking eliminates the need for UV, heat, catalysts or (photo)initiators to induce gelation, facilitating hydrogel formation quickly (within seconds to minutes) in vivo following injection. Three POEGMA hydrogels with an LCST well below (PO₀), close to (PO₁₀) and significantly higher than (PO₁₀₀) the physiological temperature were prepared.

Physiological properties (i.e. gelation time, swelling, elastic modulus and thermostresponsivity) are measured using vial inversion tests, gravimetry, and rheological measurements respectively. Biological properties such as protein adsorption (assessed using fluorescent-labeled proteins), cell adhesion (using 3T3 mouse fibroblasts as model cells), drug release (using fluorescein-labeled bovine serum albumin and fibronectin as model drugs), and in vivo histology (following subcutaneous injection into a mouse model) were also assessed.

RESULTS AND DISCUSSION
Control over the LCST of the POEGMA hydrogels (Fig. 1) is achieved by systematically varying the copolymerization ratio of OEGMA monomers with n=2 and n=8-9 during precursor synthesis. The large LCST range (22°C for n=2 to >100°C for n=8-9) available for the reactive precursors provides an advantage over the use of our earlier reported injectable hydrogels based on N-isopropylacrylamide (PNIPAAm). The PO₁₀ hydrogel (prepared from precursors containing 90 mol% n=2 and 10 mol% n=8-9 monomers) shows a clear temperature transition at ~32-33°C; conversely, PO₁₀₀ (100 mol% n=8-9 monomer) has LCST>60°C.

Fig. 1 Thermoresponsive properties of the POEGMA hydrogels. Graph displays the decrease in water content as a function of the temperature (○) PO₀, (■) PO₁₀ and (▲) PO₁₀₀. The photos display the physical appearance of the hydrogels at 20°C, 37°C and 60°C (grid = 5 mm x 5 mm). The relative weight of the hydrogels (W / W₀) is denoted underneath each photo.
Although the theoretical cross-link density of each hydrogel is similar (all precursors were prepared with 30 mol% reactive aldehyde and hydrazide groups), the mechanical properties vary significantly. The elastic storage modulus of PO\textsubscript{0} hydrogels is ~1 order of magnitude higher than that of PO\textsubscript{100} hydrogels. Analogously, macroscopic gelation occurs significantly faster for the PO\textsubscript{0} hydrogels (~5 s), when compared to the PO\textsubscript{10} (20 s) and the PO\textsubscript{100} hydrogels (~20 min). The gelation time and elastic storage modulus of the POEGMA hydrogels can be controlled by varying either the concentration of reactive groups or by varying the precursor concentration. Consequently, the mechanical properties of the hydrogels can be readily matched to a range of tissues (e.g. adipose, neural, muscle, cartilage, or bone tissue).

The PO\textsubscript{10} hydrogel collapses when incubated at 37°C, trapping the proteins within the gels; conversely, the PO\textsubscript{100} hydrogel swells at 37°C which enables fast release. Improved performance can be achieved by mixing polymer precursors with different LCST values at intermediate ratios, enabling sustained release (up to 14 μg/week) with minimal long-term entrapment up to 40 days (Fig. 2B).

In vitro MTT assays showed that neither the precursors nor the hydrogels were cytotoxic. Similar to PEG, negligible protein adsorption and cell adhesion is observed for the PO\textsubscript{10} and PO\textsubscript{100} hydrogels. Consequently, in vivo studies on BALB-c mice show successful implantation with a mild inflammatory response at both the acute and chronic time points. (Fig. 3). Interestingly, PO\textsubscript{0} hydrogels (LCST < physiological temperature) do not show any of these favorable biological properties, illustrating the importance of the LCST of the precursors in the ultimate fate of POEGMA hydrogels.

BSA and fibrinogen release from the PO\textsubscript{10} and PO\textsubscript{100} hydrogels (Fig. 2), shows substantial differences. The low LCST hydrogel (PO\textsubscript{10}) shows an initial burst followed by slow release. The high LCST hydrogel (PO\textsubscript{100}) shows similar burst release until all the protein is completely released. The rate of release is governed by the temperature response.

**CONCLUSIONS**

POEGMA hydrogels can be considered suitable analogues for PEG while offering the additional advantage of broader control over the chemical composition of the hydrogel, key for drug delivery applications of these materials.

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**REFERENCES:**