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Injectable and tunable poly(ethylene glycol) analogue hydrogels based on poly(oligoethylene glycol methacrylate)†

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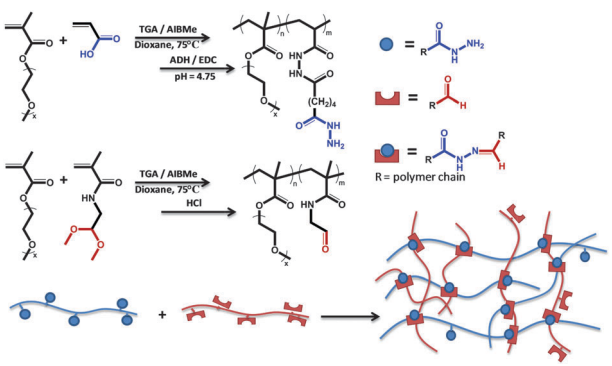
Injectable PEG-analogue hydrogels based on poly(oligoethylene glycol methacrylate) have been developed based on complementary hydrazide and aldehyde reactive linear polymer precursors. These hydrogels display the desired biological properties of PEG, form covalent networks *in situ* following injection, and are easily modulated for improved control over their functionality and physiochemical properties.

Poly(ethylene glycol) (PEG) hydrogels have been extensively studied as matrices for the controlled release of therapeutics and as scaffolds for promoting tissue regeneration.[1] PEG hydrogels are hydrophilic, non-cytotoxic and non-immunogenic and can effectively mask the material from the host's immune system.[2] A significant drawback of PEG, however, is that the polymer lacks chemical versatility given that functionalization is limited to the hydroxyl chain end(s).[3] PEG hydrogels are predominantly synthesized *via* step-growth polymerization of complimentary α,ω-functionalized PEG precursors using a range of different chemistries.[4] Although step-growth polymerization minimizes network non-idealities, chemical modification of linear PEG precursors is limited by the number of available reactive end groups. As such, there is increasing interest in polymers with similar (biological and physicochemical) properties that can be easily synthesized with improved control over the polymer functionality.[5]

Hereto, we prepared PEG-analogue hydrogels based on poly(oligoethylene glycol methacrylate) (POEGMA) that exhibit all of the desirable protein and cell-repellent properties of conventional PEG hydrogels while also being injectable, degradable, and easily chemically and mechanically tunable. The approach enables facile preparation of hydrogels with potential utility as injectable tissue engineering matrices, local drug delivery vehicles for small molecules or macromolecules, or joint lubricants, among other potential applications. POEGMA is synthesized *via* simple free radical copolymerization[6] and has been demonstrated to serve as an effective PEG analogue, exhibiting corresponding non-immunogenic, non-cytotoxic and protein repellent properties to PEG.[7] A number of POEGMA-based hydrogels have been reported to-date;[8] however, none are covalently cross-linked while also being injectable and degradable *in vivo*, significantly limiting their potential clinical application.

Our PEG-analogue hydrogels are based on hydrazide and aldehyde functionalized POEGMA precursors that rapidly form a hydrogel network *via* reversible hydrazone bond formation upon co-extrusion[9] (see Scheme 1). The injectable, *in situ* gelling nature of this system is useful to circumvent many of the issues concerning surgical implantation of bulk hydrogel-based materials. The hydrazide-functionalized POEGMA precursors ($PO_{100}H_y$) were synthesized by copolymerizing $OEGMA_{475}$ (EO repeat units, n = 8–9) with acrylic acid (AA) and subsequent post-polymerization modification using EDC chemistry with a large excess of adipic acid dihydrazide. The aldehyde-functionalized POEGMA precursors ($PO_{100}A_y$) were synthesized by copolymerizing $OEGMA_{475}$ with a functional acetal monomer (*N*-(2,2-dimethoxyethyl)methacrylamide, DMEMAm) and subsequently converting the acetal to the corresponding aldehyde by acid-catalyzed hydrolysis (Scheme 1).



Scheme 1 Preparation of injectable PEG analogue hydrogels.

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† Electronic supplementary information (ESI) available: Detailed experimental procedures, chemical characterization and *in vitro* toxicity of the POEGMA precursors, as well as hydrogel swelling kinetics, degradation, rheology and histology. See DOI: 10.1039/c3cc48514e

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The degree of functionality of the $PO_{100}H_y$ and $PO_{100}A_y$ precursors was controlled by varying the AA and DMEMAm content from $y = 20$ to 40 mol% (ESI,† Table S1 and Fig. S1). The number-average molecular weight of the precursor polymers was controlled to be lower than 20×10^3 g mol^{-1}, well below the renal clearance limit of $40–50 \times 10^3$ g mol^{-1} to facilitate polymer elimination following gel degradation.[10]

POEGMA hydrogels were prepared by co-extruding $PO_{100}H_y$ and $PO_{100}A_y$ solutions in 10 mM PBS using a double-barrel syringe. Gelation occurs over time frames ranging from several hours ($\sim$8 h) to a few minutes ($<$10 min) depending on functional group content and polymer concentration (ESI,† Table S2). The hydrogels swell in PBS following preparation (Fig. 1E–J), indicating high water affinity. For POEGMA hydrogels prepared with precursors containing 25, 30 or 40 mol% functional groups (both hydrazide and aldehyde), the equilibrium mass-based swelling ratio (Q_m) is reached within 30 h (ESI,† Fig. S2) and decreases systematically with both the degree of chain functionalization and the concentration of precursor polymers used to prepare the hydrogel (Fig. 1A). This result suggests that facile tuning of the hydrogel water content at equilibrium is possible based on the types and concentrations of precursor polymers used (see ESI†).

The injectable POEGMA hydrogels are cross-linked through the formation of dynamic hydrazone bonds, which are reversible in an aqueous environment. Aqueous size exclusion chromatography of the degradation products of a hydrogel prepared from $PO_{100}H_{30}$ and $PO_{100}A_{30}$ confirmed that the molecular weight distribution (MWD) of the degradation products is directly analogous to the combined MWDs of both precursor polymers (see ESI,† Fig. S3). Consequently, the $PO_{100}H_y$ and $PO_{100}A_y$ polymers represent both the hydrogel precursors as well as the hydrogel degradation products. MTT assay results following exposure of 3T3 mouse fibroblasts to $PO_{100}H_y$ and $PO_{100}A_y$ confirm that these materials do not impart any significant *in vitro* toxicity up to concentrations of 2000 μg mL^{-1} (ESI,† Fig. S4), suggesting that both the precursors and degradation products of the hydrogels are compatible with cells.

The degradation rate of the POEGMA hydrogels is governed by the cross-link density and, by extension, the degree of functionalization and concentration of the $PO_{100}H_y$ and $PO_{100}A_y$ precursors (Fig. 1B). Hydrogels prepared with precursors at low concentrations ($<$125 mg mL^{-1}) or with low degrees of functionalization ($<$30 mol%) degrade in $<$1 min in 100 mM HCl, while hydrogels prepared with precursors at a high concentration (200 mg mL^{-1}) or with a high degree of functionalization (40 mol%) degrade significantly slower, requiring approximately 5 hours to fully degrade under acid-catalyzed conditions (Fig. 1B). Long-term incubation showed that the hydrazone cross-linked POEGMA hydrogels (150 mg mL^{-1} and 30 mol% reactive hydrazide and aldehyde groups) are stable for at least 5 months under physiological conditions *in vitro* but degrade within 4 weeks *in vivo* following subcutaneous injection in BALB/c mice.

The $PO_{100}H_y$ and $PO_{100}A_y$ precursors yield hydrogels with elastic moduli that can be tuned depending on the number of reactive functional groups as well as the precursor concentration. G' values range from 0.23 ± 0.02 kPa (150 mg mL^{-1}, 25 mol%) to 8.0 ± 1.0 kPa (150 mg mL^{-1}, 40 mol%) (Fig. 1C and D and ESI,† Fig. S5 and S6). For comparison, the G' of conventional PEG hydrogels prepared from multi-arm and di-functional PEG precursors at comparable concentrations typically ranges from 0.25 to 6.0 kPa,[4b,c,f] analogous to the range reported here. However, the modulus of these POEGMA hydrogels can be tuned independently of polymer concentration (and thus hydrogel osmotic pressure) if desired by modifying the precursor functionality, not possible using conventional PEG chemistry.

The biointerfacial properties of the injectable POEGMA hydrogels (prepared at 150 mg mL^{-1} with 30 mol% functional groups) were evaluated using protein adsorption (Fig. 2A and B) and cell adhesion (Fig. 2C–E) assays. POEGMA hydrogels were incubated with bovine serum albumin (BSA) and fibrinogen (Fib) at varying concentrations. BSA and Fib adsorption to the POEGMA hydrogel is maintained below 100 and 500 ng cm^{-2} respectively even upon exposure to a 2000 μg mL^{-1} protein solution (Fig. 2A and B), comparable to adsorption values reported for conventional PEGylated surfaces (see ESI,† Table S4).[11] Note that protein *absorption* is also likely to occur for POEGMA hydrogels, suggesting that the true protein adsorption on the hydrogel surface is likely even lower than reported in Fig. 2A and B. Furthermore, negligible adhesion of fibroblast cells (noted to adhere particularly strongly to many biomaterials due to their inherent ability to produce extracellular matrix)[12] is observed to the POEGMA hydrogels, with 0 ± 1 cells per mm^2 ($n = 6$) observed on POEGMA hydrogel interfaces (Fig. 2D and ESI,† Fig. S7) compared to 2664 ± 100 cells per mm^2 ($n = 6$) for

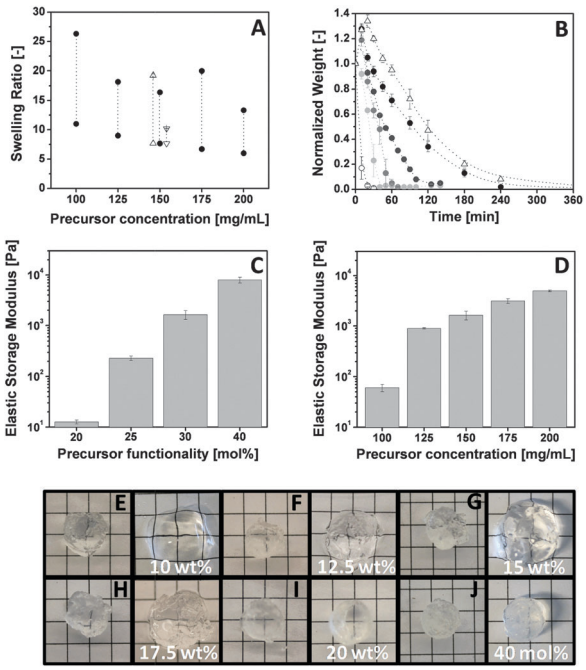

Fig. 1 Physiochemical characterization of injectable POEGMA hydrogels. (A) Bottom point = initial swelling and top point = equilibrium swelling (△) 25 mol%, (●) 30 mol% and (▽) 40 mol% hydrazides. (B) Degradation kinetics at 30 mol% functionality in 100 mM HCl (○) 100 mg mL^{-1}, (●) 125 mg mL^{-1}, (●) 150 mg mL^{-1}, (●) 175 mg mL^{-1}, (●) 200 mg mL^{-1} and (△) 150 mg mL^{-1} and 40 mol%. (C and D) Elastic storage modulus as a function of the precursor functionalization (C) and concentration (D). (E–J) Photographs of the unswollen and swollen hydrogels (grid = 0.5 mm × 0.5 mm).

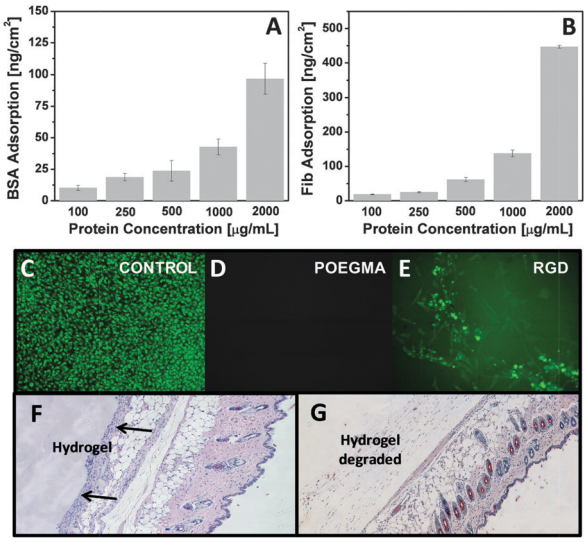

Fig. 2 Biological properties of injectable POEGMA hydrogels (based on $PO_{100}H_{30}$ and $PO_{100}A_{30}$, 15 wt%). (A and B) Adsorption of BSA (A) and fibrinogen (B). (C–E) Fibroblast adhesion after 7 days to a polystyrene control (C), POEGMA hydrogel (D) and an RGD-functionalized hydrogel (E). (F and G) Acute (F) and chronic (G) *in vivo* response.

tissue culture polystyrene interfaces (Fig. 2C) after 7 days of incubation. The presence of reactive functional groups in the POEGMA hydrogel precursor polymers facilitates facile additional tuning of cell–hydrogel interactions if desired. For example, $PO_{100}A_{30}$-RGD precursors (average of 1 RGD sequence per chain) were synthesized to prepare POEGMA hydrogels containing 1.2 mM RGD, a comparable concentration to other RGD functionalized hydrogels reported previously.[13] At this concentration, RGD promotes an increase in 3T3 mouse fibroblast adhesion to the injectable POEGMA hydrogel, as 91 ± 10 cells per mm^2 (n = 4) adhere (Fig. 2E) and subsequently proliferate (ESI,† Fig. S7). Note that the average degree of RGD functionalization can be increased independently of the cross-link density simply by functionalizing the precursors with a larger initial fraction of aldehyde groups. Thus, the injectable POEGMA hydrogels reported herein can either suppress or support cell adhesion, consistent with other PEG-based hydrogels reported in literature that are typically non-injectable and significantly more limited in terms of compositional diversity.

The *in vivo* response to the POEGMA precursors and hydrogel was evaluated by subcutaneous injection using a double-barrel syringe in BALB/c mice. Hydrogels were formed *in situ* in the subcutaneous space of the mice with minimal leukocytotic infiltration at the hydrogel-tissue interface (<100 cells per mm^2), suggesting a relatively mild acute inflammatory response 3 days post-injection (Fig. 2F). The POEGMA hydrogel fully degraded after one month (Fig. 2G), with no signs of chronic inflammation (*i.e.* macrophages, foreign body giant cells, granulation tissue) or fibrous capsule formation observed histopathologically. This result suggests that injectable POEGMA hydrogels are well-tolerated *in vivo*, again analogous to conventional PEG hydrogels. It should be emphasized that *in vitro* degradation studies suggest that the degradation lifetime of the POEGMA hydrogels can be tuned based on the number of cross-linkable groups incorporated in the hydrogel (Fig. 1B); in this sense, hydrogel clearance kinetics *in vivo* may also be engineered.

In conclusion, we have prepared injectable, hydrazone cross-linked hydrogels based on POEGMA that exhibit the same favourable biological properties of conventional PEG-based hydrogels (*i.e.* facilitating minimal protein adsorption, no significant cellular adhesion, and no significant chronic inflammatory responses *in vivo*) while offering the significant advantages of facile synthesis, rapid *in situ* gelation following injection, tunable mechanics, tunable degradation times, and excellent control over chemical composition and functionalizability. These results suggest the potential of these POEGMA-based hydrogels as a platform for the design of engineered hydrogels for a variety of biomedical applications now served by conventional PEG hydrogels.

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