Creating Force-Responsive Polymeric Materials by Incorporating Synthetic Cryptic Crosslinking Sites

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Introduction: Crosslinked polymeric materials are an increasingly common and important class of materials, with applications ranging from protective coatings to dental implants. Traditional crosslinking methods such as ultraviolet or thermal curing have significant drawbacks such as poor penetration depth or prohibitively high temperatures respectively. Here, we present a new class of synthetic force-responsive materials by taking inspiration from fibronectin, a protein which contains hidden or cryptic sites that become exposed after mechanical stimulation[1]. We installed synthetic cryptic sites consisting of long pendant poly(ethylene glycol) (PEG) chains along the polymer backbone that creates a significant steric barrier and prevent some reactive moieties from spontaneously crosslinking. After applying mechanical force through ultrasonication, the cryptic sites are unshielded and form new interchain crosslinks, resulting in a crosslinked material (Figure 1A).

Materials and Methods: Polymers were synthesized *via* thermally initiated RAFT polymerization. Poly(ethylene glycol) methyl ether methacrylate (500 g/mol and 950 g/mol) (PEGMA500/PEGMA950), methyl methacrylate (MMA), glycidyl methacrylate (GMA), acetoacetoxyethyl methacrylate (AAEM), ethylene diamine (EDA), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPA), 2-(azo(1-cyano-1-methylethyl))-2-methylpropane nitrile (AIBN), 1-butanol (BuOH), 1,4-dioxane, and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich. Methacrylate monomers were passed through a basic alumina column to remove inhibitors. Polymer molecular weight and monomer incorporation were determined through ^{1}H NMR on a Brukers 400 MHz spectrometer. Storage modulus (G') was determined on a Malvern Kinexus rheometer. Measurements were run on a 20 mm plate at 1 Hz and 1% strain. Sonication was done on a Qsonica Sonicator Q500.

Results and Discussion: Control polymer poly(GMA-*co*-MMA) (GMA:MMA) were synthesized with a 1:1 molar ratio of GMA to MMA. Shielded polymer poly(GMA-*co*-PEGMA500) (GMA:PEGMA500) were synthesized with a 1:1 molar ratio of GMA to PEGMA500. Shielded polymer poly(GMA-*co*-PEGMA950) (GMA:PEGMA950) were synthesized with a 1:1 molar ratio of GMA to PEGMA950. Samples were dissolved into 1:1 BuOH:DMF so that the concentration of oxirane rings was 1.55 M in each sample. BuOH was chosen as a cosolvent to catalyze the ring opening reaction between oxirane and primary amines (-NH_2). EDA was mixed into each sample in an equimolar ratio between -NH_2 and oxirane. Shielded polymer poly(AAEM-*co*-PEGMA950) (AAEM:PEGMA950) was synthesized with 30 mol% AAEM and 70 mol% PEGMA950. Control polymer GMA:MMA that does not contain shielding monomers crosslinked in the presence of EDA at static conditions after 2.5 hours and reached an equilibrium modulus on the order of 10^5 Pa. On the other hand, shielded polymer GMA:PEGMA500 formed a gel after 8 hours and reached a final modulus on the order of 10^4 Pa, and shielded polymer GMA:PEGMA950 did not form a gel at all due to the steric effects of pendent PEG chains (Figure 1B). The shielded polymer AAEM:PEGMA950 showed an acceleration of crosslinking under the influence of sonication. It reached a higher modulus at the 1-hour timepoint compared to static samples, indicating that the mechanical force imparted by sonication is overcoming the steric effects of PEGMA950 pendent chains (Figure 1C).

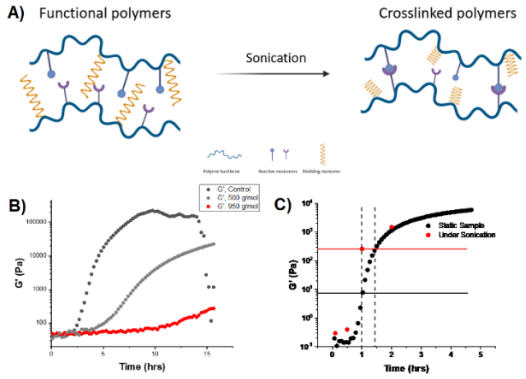

Figure 1: **A)** A cartoon representation of our steric shielding approach, sonication induces interchain crosslinks forming a solid material. Made with Biorender. **B)** Comparison of elastic modulus (G') between GMA:MMA (control, black), GMA:PEGMA500 (500 g/mol, grey), and GMA:PEGMA950 (950 g/mol, red) mixed with EDA crosslinker. The control sample transitions to a solid after ~2.5 hrs, whereas the steric shielding of the PEGMA500 delays the onset of gelation to ~8 hrs, and PEGMA950 prevents gelation entirely during the monitoring time. Decreasing G' of the control sample past the 10 hr mark is due to the sample breaking during measurement. **C)** Comparison of 30:70 AAEM:PEGMA950 during sonication (red) and static (1% strain rheological measurements, black). The sonicated sampled reaches a higher modulus at the 1-hour mark compared to the static sample, indicating that sonication is overcoming the steric barrier from the PEGMA950 side chains.

Conclusions: We have developed a new class of force-responsive polymeric materials in which the force responsiveness arises from steric interactions between pendent side chains and adjacent reactive polymers. This new class of force-responsive polymers has the potential for use in deep wound healing where ultraviolet light's penetration depth presents challenges and intense temperature is not tolerable.

(1) Klotzsch, E.; Smith, M. L.; Kubow, K. E.; Muntwyler, S.; Little, W. C.; Beyeler, F.; Gourdon, D.; Nelson, B. J.; Vogel, V. Fibronectin Forms the Most Extensible Biological Fibers Displaying Switchable Force-Exposed Cryptic Binding Sites. Proceedings of the National Academy of Sciences 2009, 106 (43), 18267–18272. https://doi.org/10.1073/pnas.0907518106.