PEG-Norbornene Polycaprolactone Blends for Covalent Peptide Photopatterning

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Statement of Purpose:

Covalent patterning of bioactive factors onto hard biomaterials is challenging. Although microcontact printing using PDMS stamps can be used to immobilize small molecules with spatial fidelity,[1] they are not chemically bound to the surface and can detach over time. Click chemistry involves highly specific reactions between two entities that can be leveraged for robust surface modifications.[2] For example, Gramlich et al. used light-mediated thiol-norbornene click chemistry to covalently attach thiolated peptides onto norbornene-modified hyaluronic acid hydrogels.[3] Since light is needed for the thiol-norbornene click reaction, photomasks can be used to spatially pattern thiolated peptides onto norbornene hydrogels. Despite the versatility, specificity, and simplicity of click chemistry reactions, they have not been used to covalently modify hard biomaterials. Here, polycaprolactone (PCL) was blended with norbornene-modified poly(ethylene glycol) (PEG-Nor) to create PCL-Nor, a hard biomaterial amenable to covalent photopatterning with thiolated peptides. To showcase click chemistry reactions onto hard biomaterials, PCL-Nor sheets and nanofibers were formed and covalently photopatterned with thiolated rhodamine peptides.

Methods:

PCL-Nor Sheet Synthesis: PCL (4% w/v) was mixed with PEG-Nor (0.4% w/v) and dissolved in dichloromethane (DCM) overnight. The solution was then poured into a glass beaker and allowed to solidify to form thin sheets. For mechanical testing, PCL-Nor sheets were cut into cylinders (6 mm ø, 1 mm h) and compressed at a constant strain rate of 10%/min. Young's moduli (n>5/sample) were determined by calculating the slope of stress-strain curves between 10% and 20% strain. *PCL-Nor Nanofiber Synthesis:* PCL (18% w/v) was mixed with PEG-Nor (1.8% w/v) and dissolved in DCM and dimethylformamide (DMF) at a 3:1 ratio overnight in a scintillation vial. The PCL-Nor solution was then electrospun at a voltage of 10 kV under a draw ratio of 1. The presence of norbornene in PCL-Nor blends was confirmed using ^{1}H NMR. *Thiolated Peptide Synthesis:* a thiolated rhodamine peptide with an aspartic acid spacer (PeptideRed) was synthesized in-house using a solid-state peptide synthesizer (sequence: CDDDK-Rhodamine B). The thiol in cysteine "C" was used to click the fluorescent peptide onto PCL-Nor scaffolds using light. *Photopatterning scaffolds with red peptide:* PCL-Nor Sheets and Nanofibers were incubated in 0.2 mM PeptideRed and irradiated with ultraviolet light (15 mW/cm^2, 1 min onto sheets, and 10 mW/cm^2 1 to 3 min onto nanofibers). A stripe-patterned photomask was used to selectively bind different regions with PeptideRed. Photopatterned Sheets and Nanofibers were imaged with a confocal microscope.

Results:

By combining PCL with PEG-Nor, PCL-Nor blends were created (Figure 1A). Using ^{1}H NMR, the presence of norbornenes was confirmed by seeing peaks specific to norbornene (blue square) alongside PCL-specific peaks (black square) (Figure 1B). Quantification of the areas under these curves determined the amount of PEG-Nor in PCL-Nor to be ~9.6%, which confirms that the majority of the norbornenes remained within the blends. Compression testing shows no significant difference between PCL and PCL-Nor sheets (~1,500 kPa, Fig. 1C). PeptideRed was photopatterned onto PCL-Nor sheets using a stripe photomask (Figure 1D). PeptideRed stripes were also photopatterened perpendicular to the direction of aligned fibers. By varying light exposure time (left → right), a decrease in fluorescence (indicative of PeptideRed tethering) can be observed (Figure 1E).

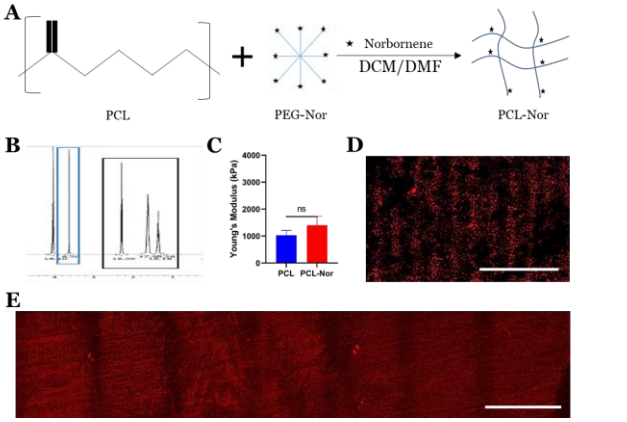

Figure 1. (A) Schematic for forming PCL-Nor blends. (B) 1H NMR spectra of PCL-Nor show PCL backbone (black square) and a norbornene peak from PEG-Nor (blue square). (C) Compression testing shows a comparable Young's Modulus between cylindrical PCL and PCL-Nor sheets. PCL-Nor (D) sheet and (E) aligned nanofibers are photopatterned with PeptideRed using a striped photomask. ns denotes no statistical difference and error bars are the standard deviation (n > 5 samples per group. Scale bars: (D) 500 µm; (E) 1 mm.

Blending PCL with PEG-Nor is a simple technique used to create a new class of hard biomaterials amenable to click functionalization. Since the thiol-norbornene chemistry relies on light, it can be used to spatially (via photomasks) and temporally (via user-defined light exposure) covalently tether thiolated molecules. This allows for the creation of complex biomaterial systems with defined microarchitecture (nanofibers, porous scaffolds) and spatial (patterns, gradient) bioactive modifications.

References:

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