Cell-Responsive Polyurethanes: Synthesis of Peptide-Based Polyol Soft Segments

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Statement of Purpose: Tissue engineering utilizes biodegradable scaffolds to sustain functionality during regeneration and act as a structural template for tissue organization. Biomaterial scaffolds featuring cell-responsive degradation enable synthetic polymers to integrate into native remodeling processes and thus promote enhanced tissue formation. We propose to develop a series of collagen-mimetic polyurethanes that combine the strength and tunability of synthetic elastomers with the cell-responsive degradation of native collagen. In this study, a biodegradable soft segment based on poly (ethylene glycol) (PEG) and a collagen-derived peptide sequence was synthesized.

Methods: *Synthesis:* All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used as received. The biodegradable peptide sequence (GPQGIWGQG) was purchased from the Baylor Protein Chemistry Core Laboratory where it was generated on an ABI 433A solid-phase synthesizer and analyzed by reverse phase HPLC and mass spectroscopy. Once received, the peptide was stored at -80°C and lyophilized prior to use. PEG diacid was synthesized and purified according to the method developed by Ma.[1] Briefly, succinic anhydride was added to PEG diol (2000 g/mol) in solution and ester formation was catalyzed by dimethylaminopyridine. This PEG diacid was then reacted with ethylene diamine to generate amine end groups. Standard Ninhydrin assay was used to confirm the presence of free amine groups, and FTIR spectroscopy was utilized to verify end group functionalization.

Peptide-PEG Multiblock Formation: First, the C-termini of the FMOC protected biodegradable peptide was activated with dicyclohexylcarbodiimide (DCC). An established peptide coupling procedure was then used to attach the PEG diamine to the activated peptide to generate a peptide-PEG-peptide triblock.[2] Next, the FMOC protecting group was removed with 20% piperidine in DMF. The resulting amine functionalized triblock was then reacted with DCC-activated PEG diacid to form a PEG-peptide-PEG-peptide-PEG multiblock. The polymer was precipitated in cold ether, centrifuged, stored at -80°C overnight, and finally lyophilized to obtain the final purified product. UV spectroscopy was used to monitor the presence of the FMOC protecting group by measuring absorbance at 275 nm. FTIR spectroscopy and nuclear magnetic resonance (NMR) were used to verify the chemical structure at each step and the final product.

Results: *Synthesis:* Successful synthesis of PEG diacid and PEG diamine was verified with FTIR spectroscopy, **Figure 1**. Specifically, an absorption peak corresponding to the stretching vibration of an ester carbonyl (C=O) group not found in the spectrum of PEG diol was observed in the spectrum of PEG diacid at 1728 cm^{-1}. In addition to this ester carbonyl band, the FTIR spectrum of PEG diamine revealed three additional peaks in the carbonyl region to indicate successful amine functionalization. These bands at 1691, 1650, and 1622 cm^{-1} resulted from the stretching vibrations of free, ordered and disordered hydrogen-bonded amide I carbonyl groups, respectively. In addition to FTIR spectroscopy, successful synthesis of PEG diamine was verified by a positive blue color with the Ninhydrin assay.

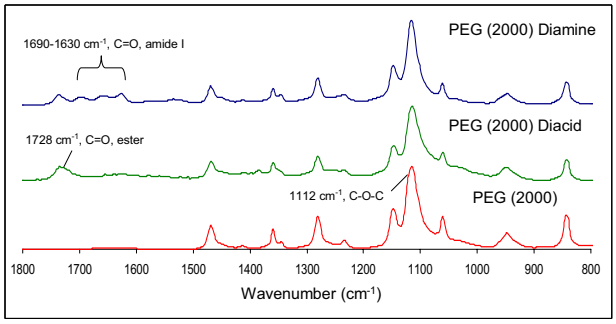

Figure 1: FTIR spectra of PEG functionalization.

Peptide-PEG Multiblock Formation: Successful coupling of the FMOC-protected peptide to the PEG diamine was first confirmed with UV spectroscopy. Subsequent deprotection of the peptide-PEG-peptide triblock was verified by the disappearance of the FMOC absorption peak at 275 nm. Formation of the PEG-peptide-PEG-peptide-PEG multiblock was then confirmed with FTIR spectroscopy, **Figure 2**. Dominant peaks in the carbonyl region, specifically at 1685, 1650, and 1627cm^{-1}, correspond to amide I groups present in the peptide sequence. Furthermore, the characteristic ether peak present in PEG, 1112 cm^{-1}, was observed in the FTIR spectrum for the multiblock. Further investigation of chemical structure using NMR is currently in progress.

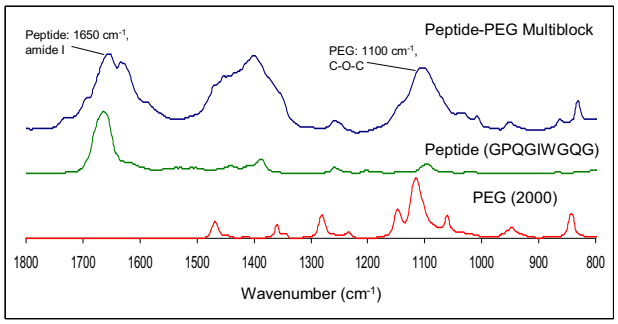

Figure 2: FTIR Spectra of PEG-Peptide-PEG-Peptide-PEG multiblock.

Conclusions: We have successfully synthesized a novel biodegradable soft segment based on alternating blocks of PEG and a collagen-derived peptide. The next step will be to incorporate this soft segment into polyurethanes to generate cell-responsive elastomers. Collagen-mimetic polymers are of particular interest in ligament tissue engineering where collagen is the central extracellular matrix protein responsible for mechanical strength.

References:

[1] Ma X. J. Biomed. Mater. Res. 1993; 27: 357-365.
[2] West JL. Macromolecules. 1999; 32: 241-244.