Development of a Collagenase-Sensitive, Flexible Scaffold for Engineering of Urological and Other Soft Tissues

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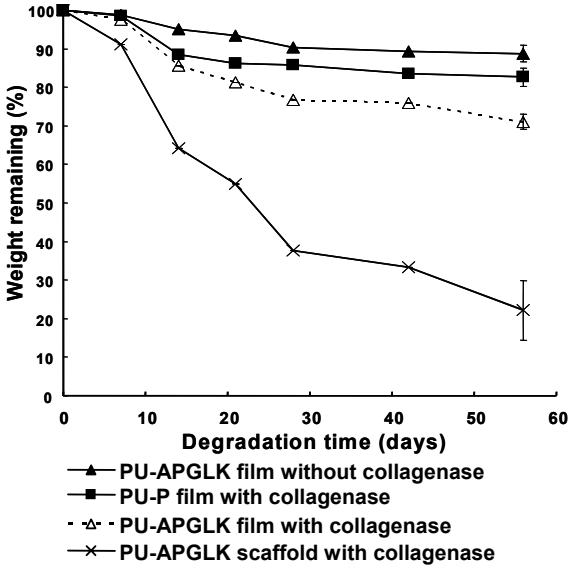
Introduction: Scaffolds for urological tissue engineering might be directly placed *in situ*, seeded with cells and placed, or cultured with cells in a specified regimen prior to placement. In each of these applications one might hypothesize that a scaffold possessing mechanical properties that approach that of the native tissue might perform better in ultimately integrating with tissue structures for augmentation or repair. Synthetic materials offer the opportunity to achieve desired mechanical properties while being processable into porous scaffolds. One limit of synthetic materials is that they commonly rely upon ester hydrolysis for degradation, unlike the enzymatic degradation inherent to native tissue restructuring. We report here on the development of an elastomeric polymer that incorporates sensitivity to collagenase degradation in addition to hydrolytic lability, and the processing of this material into a porous scaffold capable of supporting muscle-derived stem cell adhesion and growth.

Methods: The collagenase-sensitive polyurethane elastomer (PU-APGLK) was synthesized from polycaprolactone diol combined with butyldiisocyanate and chain extended with alanine-proline-glycine-leucine-lysine (APGLK). The collagenase-sensitivity of the APGL peptide has previously been confirmed in hydrogel applications.[1] For control purposes a polyurethane chain extended with putrescine (PU-P) was synthesized. Scaffolds were prepared by thermally induced phase separation from DMSO.[2] Degradation was measured at 37C in aqueous buffer with or without collagenase. Murine muscle derived stem cells (MDSC) were isolated by a pre-plate method and have been characterized previously.[3]

Results: The synthesized PU-APGLK film had a breaking strain of 847% and tensile strength of 25 MPa. Mass loss at 8wks from buffer-incubated films significantly increased from 11 to 29% when collagenase was present in the buffer. PU-APGLK film had significantly greater mass loss than control polymer PU-P in collagenase buffer ($p<0.001$; **Figure**). Degradation products showed no cytotoxicity in an endothelial cell cytotoxicity assay.



Porous scaffolds formed from PU-APGLK had open, interconnected pores with morphology dependent upon the polymer concentration and quenching temperature used in the processing. Pore sizes ranged from 20-240um and porosities from 85-90%. Scaffold mechanical properties also depended on processing with breaking tensile strains of 147-326%, and tensile strengths from 0.5-1.0 MPa. Scaffold incubation in collagenase buffer led to 78% mass loss at 8wks. MDSCs could be uniformly seeded into the scaffolds and growth to high cell densities after 14d of spinner flask culture was demonstrated.

Conclusions: A novel elastomeric polyurethane has been developed that possesses both hydrolytic and collagenase sensitive sequences. This material could be processed into porous scaffolds that retained reasonable strength and elastance and that supported stem cell adhesion and growth. This scaffold might find application in a variety of urological applications.

References:

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2. Guan J et al, *Biomaterials*, 2005; 26:3961.
3. Qu-Petersen et al. *J Cell Biol*, 2002; 157: 851.