Hybrid Fibrous Scaffolds from a Synthetic Elastomer and Urinary Bladder Matrix

John J. Stankus[1,3], Donald O. Freytes[2,3], Jianjun Guan[3], Stephen F. Badylak[2,3], William R. Wagner[1,2,3]
Departments of [1]Chemical Engineering, [2]Bioengineering and
[3]The McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219

Introduction

Scaffolds derived from the extracellular matrix (ECM) of tissues have been successfully used in a broad array of clinical applications, but remain limited for some applications by their inherent mechanical properties. Synthetic scaffolds can be molecularly designed for desired mechanical properties, biodegradability, and processability, but are limited in terms of the breadth of bioactivity that can be prescribed. Our objective was to create a hybrid matrix that imparted the mechanical properties of a biodegradable elastomer to ECM and that could be processed into a microporous scaffold format. Specifically, we combined a biodegradable poly(ester urethane) urea (PEUU) with porcine urinary bladder matrix (UBM) and utilized electrospinning to create elastic hybrid scaffolds that were characterized for their tensile properties, biodegradation rates and cytocompatibility.

Materials and Methods

PEUU was synthesized from polycaprolactone diol and 1,4-diisocyanatobutane with chain extension by putrescine as previously reported.[1] For UBM preparation, porcine urinary bladders were harvested and the tunica serosa, tunica muscularis externa, tunica submucosa, and most of the tunica muscularis interna were mechanically removed.[2] This resulted in a bi-laminate structure of basement membrane plus the subjacent tunica propria referred to as UBM. UBM sheets were disinfected for 2h in a 0.1% (v/v) peracetic acid solution and then lyophilized and powdered. Lyophilized UBM powder (1g) and 100mg of pepsin were mixed in 100mL of 0.01 M HCl, kept at a constant stir for 48h at room temperature, and lyophilized after the digestion process.

PEUU and UBM were blended in hexafluoro-isopropanol (0, 25, 50, 75 solute wt% PEUU) prior to electrospinning. PEUU was electrospun over a 15-cm distance using a 1 mL/min feed rate and by charging the solution at 10kV and the aluminum target at -10kV.[3] Electron microscopy was utilized to characterize scaffold fiber morphologies. Tensile testing was completed according to ASTM D638-98. *In vitro s*caffold mass loss was measured in PBS at 37°C over 2 wks. Rat vascular smooth muscle cells (SMCs) were seeded onto scaffolds at a density of 2.0 x 10^5 cells/mL and cell adhesion was quantified 1d after seeding using the MTT mitochondrial activity assay. For confocal microscopy, cells were fixed, permeabilized and stained with rhodamine phalloidin (f-actin) and draq-5 (nuclei).

Results

Electrospun PEUU/UBM scaffolds at all concentrations studied possessed continuous fiber morphologies, typified by PEUU/UBM (50/50), and supported spread cell morphologies (**Figure**). Cell adhesion was similar to tissue culture polystyrene for all samples except those containing 75% UBM, which had significantly higher adhesion (151% of TCPS, $p<0.05$). Scaffolds possessed tensile strengths ranging from

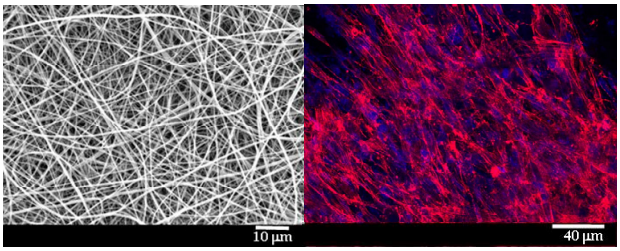

Figure. *Electron micrograph of electrospun PEUU/UBM (50/50) (left) and confocal micrograph of SMC morphology on the same scaffold type (right). (red = f-actin, blue = nuclei)*

2-13 MPa and breaking strains from 38-220% with values decreasing with higher UBM content **(Table)**. Mass loss increased at higher UBM content with values ranging from 18% to 66% at 2wks for UBM based samples.

Table. *Tensile properties of PEUU/UBM scaffolds.*

% PEUU / %UBM	Tensile Strength (MPa)	Breaking Strain (%)
100/0	13.0 ± 4.0	220 ± 80
75/25	11.8 ± 0.7	141 ± 11
50/50	4.9 ± 1.6	85 ± 28
25/75	2.0 ± 0.1	38 ± 1

Conclusions

Urinary bladder matrix extract was combined with a biodegradable and cytocompatible polyurethane through electrospinning to create elastomeric nanofiber scaffolds. This hybrid material combination resulted in increased mechanical robustness and flexibility from the synthetic component and increased cell adhesion and degradation rates from the natural component. These hybrid scaffolds have potential to be utilized in soft tissue engineering applications were increased strength and elastance may be required.

References

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3. Stankus JJ et al. *J Biomed Mater Res* 70:603 (2004).