Porcine Urinary Bladder Matrix Derived Gel for Tissue Engineering Applications

Donald O. Freytes[1], Annie S. Lee[2], Stephen F. Badylak[1,3].
[1]University of Pittsburgh, Department of Bioengineering, Pittsburgh, PA 15219
[2]University of Pittsburgh Medical School, Department of Otolaryngology, Pittsburgh PA 15219
[3]University of Pittsburgh Medical School, Department of Surgery, Pittsburgh PA 15219

Statement of Purpose: Extracellular matrix (ECM) derived scaffolds have been studied in both pre-clinical and clinical trials and have been successfully used in more than 300,000 patients. After implantation, ECM scaffolds are degraded by host cells while inducing site specific remodeling of the replaced tissue[1]. However, ECM scaffolds are limited by the inherent geometrical and structural properties of the native tissue. A gel form of ECM scaffolds represents an alternative configuration that would allow the delivery of the ECM via minimally invasive methods (e.g. injection) to locations such as the internal urinary sphincter and myocardium. The purpose of the present study was to investigate the ability of an ECM gel derived from the porcine urinary bladder to support the adhesion and growth of rat aortic smooth muscle cells *in vitro*.

Methods: The preparation of UBM has been previously described[2]. Briefly, porcine urinary bladders were harvested and the tunica serosa, tunica muscularis externa, tunica submucosa, and most of the tunica muscularis mucosa were mechanically removed. The resulting biomaterial was composed of the basement membrane plus the subjacent tunica propria. This bi-laminate structure was referred to as urinary bladder matrix or UBM. UBM sheets were disinfected for two hours in a 0.1% (v/v) peracetic acid solution. UBM sheets were lyophilized or lyophilized and powdered after processing.

Gels: One gram of lyophilized UBM powder and 100 mg of pepsin were mixed in 100 mL of 0.01 M HCl and kept at a constant stir for ~48 hrs at room temperature (25ºC). UBM and collagen type I gels were made by bringing the pH and the ionic strength to physiological range using PBS.

SMC Adhesion: Rat smooth muscle cells (SMCs) were harvested as previously described[3]. SMCs were seeded onto 6 mm disks of collagen type I gels, UBM gels, and lyophilized UBM sheets in triplicates at a density of $0.125x10^6$ cells/cm^2 (in serum free DMEM) for 30 minutes in 96 well plates. Non adherent cells were removed and the activity of the attached cells was determined using the MTT assay by following the manufacturer's instructions.

SMC Proliferation: SMCs were seeded onto 6 mm disks of collagen type I gels, UBM gels, and UBM lyophilized sheets in triplicates at a density of $0.125x10^6$ cells/cm^2 in DMEM with 10% fetal bovine serum in 96 well plates. After 48hrs, the cellular activity was measured using the MTT assay by following the manufacturer's instructions.

SMC Long term Growth: SMCs were seeded onto collagen type I gels, UBM gels, and UBM lyophilized sheets at $0.5x10^6$ cells/cm^2 in DMEM (10% fetal bovine serum) and incubated for 7 days. Media was changed every 2-3 days. Samples were fixed in 10% formalin and stained with H&E.

Results / Discussion: SMCs showed an increase in adherence to collagen type I gels, UBM gels, and UBM lyophilized sheets compared to tissue culture plastic (TCP) as shown in Figure 1.

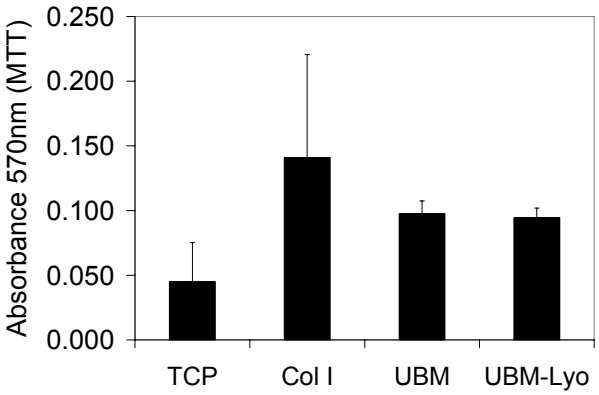

Figure 1: Results from the rat smooth muscle cell adhesion assay (mean ± SD for n=2).

After the 48hr incubation period, there were very small differences in cellular activity between TCP, collagen type I gels, UBM gels, and UBM lyophilized sheets. SMCs formed a confluent multilayer after the 7 day incubation period on both UBM gels and UBM lyophilized sheets.

Conclusion: The present data shows that lyophilized UBM and UBM gels support adhesion and growth of rat smooth muscle cells *in vitro*. The data also shows the potential for future investigations examining the *in vivo* activity of UBM gels as an alternative form of the UBM scaffold.

References:

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