Extracellular Matrix Derived Gel for Cardiac Tissue Engineering Applications

Donald O. Freytes[1,2], Kimimasa Tobita[2,5], Joseph P. Tinney[5], Bradley B. Keller[2,5], John W. Wainwright[1,2], Jeffrey Martin[4], Sachin S. Velankar[4], Stephen F. Badylak[1,2,3]

Department of Bioengineering[1], McGowan Institute for Regenerative Medicine[2], Department of Surgery[3], Department of Chemical and Petroleum Engineering[4], and Department of Pediatrics[5], University of Pittsburgh, Pittsburgh PA

Statement of Purpose: Heart failure remains one of the major causes of death in the Western population. In ischemic cardiomyopathies, the myocardium losses contractility, and scar tissue is formed. Current research has focused on the delivery of bio-inductive agents and/or cells to restore myocardial function and improve circulation. Porcine extracellular matrix (ECM) scaffolds have been used for the repair of a variety of tissues leading to constructive remodeling without scar formation. One such ECM scaffold, called urinary bladder matrix (UBM), has been used as a ventricular patch resulting in constructive remodeling of ventricular wall defects[1,2]. Although the 2-D sheet form of UBM is suitable for ventricular wall restoration, surgical placement of the UBM requires an invasive procedure. A gel form of UBM could be delivered via minimally invasive methods as a bio-inductive scaffold to repair damaged myocardium. The objective of the present study was to characterize the rheological properties of a UBM derived gel and the *in vitro* response of cardiomyocytes when cultured on the UBM gels.

Methods: The preparation of UBM has been previously described[3]. UBM scaffolds were composed of the basement membrane plus the subjacent tunica propria of the porcine urinary bladder. UBM sheets were disinfected, lyophilized, and powdered.

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 formed by raising the pH, ionic strength, and temperature to physiological range.

Rheological tests were performed with a TA Instruments AR2000 stress-controlled rheometer using a 40mm-diameter parallel plate geometry and a Peltier cell. The samples were loaded into the rheometer while maintaining a temperature of 15°C. The sample edge was protected from evaporation by applying mineral oil. The temperature was then set to 37°C to induce gelation. The linear viscoelastic properties of the gel were measured by performing a frequency sweep between 15.9 Hz and 0.08 Hz at 37°C and 5% strain.

Hamburger-Hamilton stage 31 (day 7) white leghorn chicken embryonic cardiomyocytes (CMs) were harvested as previously described[4]. CMs were cultured in DMEM supplemented with 10% FBS, 1% antibiotic, and 1% chick embryo extract. Gels were formed as previously metioned in 96 well plates and the CMs were seeded on the surface of the gels and cultured for 5 days.

Results/Discussion: The dynamic viscosities of UBM and collagen gels are shown in Figure 1. UBM at a concentration of 6 mg/ml was found to have similar dynamic viscosity values when compared to collagen gels at a concentration of 3 mg/ml.

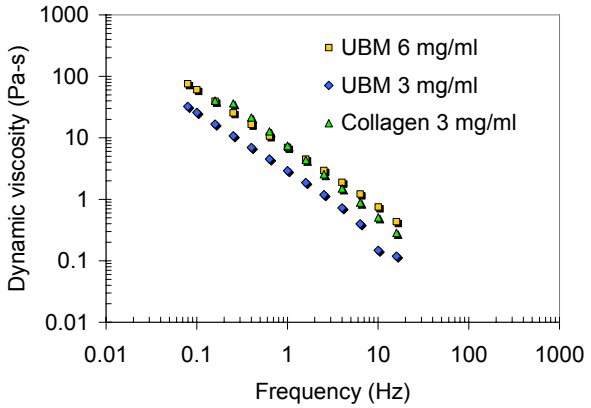

Figure 1: The dynamic viscosity of UBM and collagen type I gels.

CMs seeded on UBM gels showed substantial branching from the aggregates when compared to CMs cultured on collagen gels. The characteristic morphology of CMs on UBM gels is shown on Figure 2. After 5 days in culture, CMs spontaneously contracted when cultured on UBM gels (3mg/ml) without stimulation.

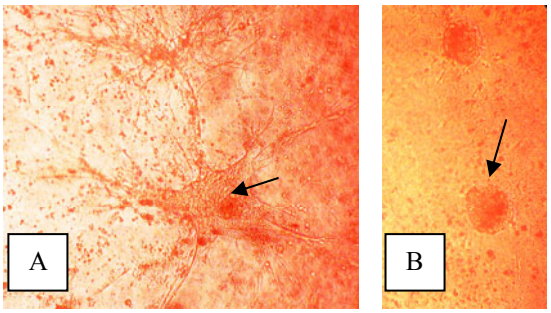

Figure 2: CMs cultured on a (A) UBM Gel (6mg/ml) and (B) collagen gel (3mg/ml) for 5 days. Arrow shows the CMs. 10x Magnification.

Conclusions: The present data shows that UBM derived gels can be formed with similar dynamic viscosity to collagen gels and that UBM gels support the growth of chicken embryonic CMs. The results show the potential for future investigations examining UBM gels as a substrate for an engineered cardiac tissue.

References: 1. Badylak SF. Cell Trans 2006; 15(supp1) S29-S40; 2. Robinson KA. Circulation 2005; 112[suppl I]:I-135-I-143]; 3. Freytes DO. Biomaterials 2004; 25(12):2353-61; 4. Tobita K. Am J Physiol Heart Circ Physiol 2006; 291:H1829-H1837.