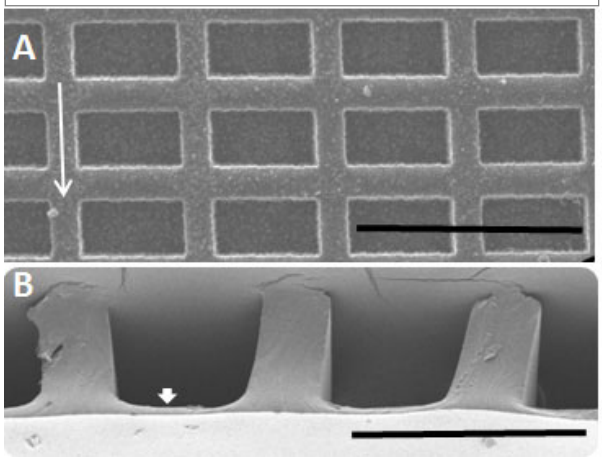
Effects Of Electrical Stimulation and Insulin-like Growth Factor On Heart Cells Cultured On A Microfabricated Degradable Elastomer

Hyoungshin Park[1], Benjamin L. Larson[2], Martin E. Kolewe[2], Gordana Vunjak-Novakovic[3], Lisa E. Freed[1,2]
1. Biomedical Microsystems Development Group, C.S. Draper Laboratory, Cambridge, MA; 2. Harvard-MIT Division of Health Sciences and Technology, Institute of Medical Sciences and Engineering, and Koch Institute, MIT, Cambridge, MA; 3. Department of Biomedical Engineering, Columbia University, New York, NY.

Statement of Purpose: Toward developing biologically sound models for the study of heart regeneration and disease, we engineered heart muscle by culturing heart cells on a MEMS fabricated poly(glycerol sebacate) (PGS) scaffold. This scaffold was selected to provide microscale structural features, anisotropic elastomeric material properties, and predictable biodegradation (1). Effects of electrical stimulation (ES), which enhances functional assembly of heart tissue (2), and supplemental insulin-like growth factor-I (IGF), which promotes cardiomyocyte growth and survival (3), were studied.
Methods: The PGS scaffolds, produced by replica molding from etched silicon wafers, were seeded with neonatal rat heart cells and studied in four experimental groups: Group 1 - without ES/without IGF, Group 2 - without ES/with IGF, Group 3 - with ES/without IGF, and Group 4 - with ES/with IGF. The ES (2 ms pulses, 1 Hz, 5 V/cm) was applied from culture day 3 through 8 in a direction parallel to the short axes of the rectangular wells of the PGS scaffold (Figure 1A, arrow). Heart cell morphology and two phenotypic markers (troponin-T and sarcomeric α-actinin) were assessed by confocal microscopy after immunostaining. Connexin-43 was assessed in immunohistochemistry. Sarcomeres were assessed by TEM. Matrix metalloprotease (MMP-2) expression was assessed by quantitative PCR. Construct contractile function was assessed by measuring excitation threshold (ET) and projection length (4). Apoptosis was assessed by TUNEL assay. Collagen deposition was assessed by Masson's trichrome stain. Data were evaluated by ANOVA and expressed as Mean ± SD.
Results: PGS scaffolds with rectangular wells (230±4 μm long, 105±9 μm wide, and 103±12 μm deep) separated by intervening struts (60±5 μm wide) and a thin underlying membrane (6.1±2.2 μm thick) were consistently produced by replica molding (Figure 1). Mechanical testing in two orthogonal directions confirmed that scaffold mechanical properties were anisotropic, with higher tensile modulus in the direction of the rectangular pore's long axis than its short axis ($p<0.001$). While the neonatal cells remained predominantly rounded on culture day 8, cardiac phenotypic markers were clearly present in all four groups. Supplemental IGF enhanced cell spreading and collagen deposition, and significantly reduced ET ($p<0.001$). Biomimetic ES, with or without IGF, induced the formation of cell bundles that were oriented in parallel to the direction of the electrical field. Formation of cell bundles was associated with a more than ten-fold increase in MMP-2 expression, suggesting that ES induced extracellular matrix remodeling. The combined presence of ES and IGF increased projection length, an index of cardiomyocyte contractile activity (4), with individual effects of each parameter and a significant interactive effect ($p<0.001$). Sarcomeres were best developed in the presence of both ES and IGF and somewhat developed with either ES or IGF, but were disorganized in the absence of either factor. Consistently, the gap junction protein connexin-43 was readily observed with both ES and IGF, less prevalent with either ES or IGF, and minimally present in the absence of either factor. Apoptosis was not observed if IGF or ES was present, but was observed if both factors were absent. Collagen deposition was observed if IGF or ES was present, but not if both were absent.

Figure 1. SEM of the scaffold, viewed (A) from above, scale bar: 500 μm, arrow indicates direction of applied ES; (B) in cross-section, scale bar: 200 μm; arrowhead indicates thin underlying PGS membrane.



Conclusions: A MEMS-fabricated elastomer with microscale structural features and anisotropic mechanical properties provided an *in vitro* platform to engineer contractile heart muscle containing cardiac specific molecular markers and sarcomeres. A systematic study demonstrated significant individual and interactive effects of two *in vitro* culture parameters: IGF and ES. The novel finding is that combined use of IGF and ES interactively affected the behavior of cardiomyocytes cultured at the surfaces and within the pores of the degradable scaffold. The resulting constructs may be applicable to medical applications wherein cell delivery and mechanical support are needed for the repair of damaged heart muscle.

References: (1) Neal, RA et al., Tissue Eng.-Part A. E-Pub, doi 10.1089 ten.TEA.2012.0330; (2) Tandon, N et al., Nature Prot. 2009; 4:155-173; (3) Davis, ME et al., PNAS USA 2006; 103:8155-8160; (4) Shim, J et al., J. Biomech. 2012; 45:832-841.

Funding: NIH R01-HL107503;