Development of Electrospun Hyaluronic Acid Scaffolds Containing Multivalent Peptide Conjugates

Nikhil A. Rode[1], Natalie C. Marks[2], and Kevin E. Healy[1,2]
[1]Department of Materials Science and Engineering, UC Berkeley, [2]Department of Bioengineering, UC Berkeley

Statement of Purpose: Heart disease is currently the leading cause of death worldwide. According to the World Health Organization, there are over 30 million myocardial infarctions every year and in 2008 ischemic heart disease caused 7.8 million deaths. These deaths are caused by the progressive thinning and weakening of the ventricular wall after MI, leading ultimately to heart failure. Current treatment options include surgical reconstruction of the ventricular wall and cell therapy. However, large defects cannot be repaired via surgery without the introduction of foreign, noncontractile material and injected cells demonstrate poor retention and survival . More recently, the use of cardiac patches to reinforce the ventricular wall has shown some success using materials such as PEUU[1] or electrospun PLGA with gelatin[2]. Our work presents a new material for use in a biodegradable cardiac patch using electrospun hyaluronic acid with multivalent conjugation of the bspRGD(15) peptide. This approach allows for the independent control of biological signaling via the conjugated peptides and the mechanical environment via electrospinning.

Methods: Hyaluronic acid was activated via carbodiimide and maleimide chemistry using EDC, NHS and EMCH. The maleimide activated HyA was purified via dialysis before reaction with the reduced peptide bspRGD(15) (Ac-CGGNGEPRGDTYRAY-NH_2). An aqueous solution of 3 wt% 1 MDa PEG with 0.5 wt% bspRGD(15)-HyA, 5 v% 200 Da PEGDA and 3 mg/mL Irgacure 2959 as a photoinitiator was mixed for electrospinning. PEG was used as a carrier polymer to ease the spinning of HyA and the concentrations were chosen based on an electrospinning morphological phase diagram. The solution was spun from a 22g blunt needle charged at +10.5 kV onto an aluminum foil target charged at -1.5 kV. Electrospun mats were crosslinked with 90 s of exposure to 7 mW/cm^2 365 nm UV light and lyophilized. Episomal human iPSCs (Conklin lab, UCSF) were differentiated on Synthemax II coated plate (Corning[3]). Cells were treated with Gsk3 inhibitor in RPMI-B27 minus insulin on day 0 for 24 h. On day 1, Gsk3 inhibitor was removed from the media composition. Wnt inhibitor was added to the media on days 3-5. On day 7 the media was exchanged for RPMI-B27 complete and exchanged every 3 days. Electrospun scaffolds were sterilized with 70% ethanol for 30 minutes prior to cell seeding. Differentiated human cardiomyocytes were seeded onto the fibers at 500,000 cells/mL and maintained in EB20 media. Cell seeded mats were imaged using a Nikon Eclipse TE300 Inverted Tissue Culture Microscope with a QImaging QICAM Fast 1394 Digital Camera and a FEI/Philips XL30 SEM in low vacuum.

Results: Electrospinning of the polymer solution resulted in the formation of well-defined sub-micron fibers. UV exposure rendered the fiber mats insoluble and stable in cell culture media for over 2 weeks. In the presence of bspRGD(15), cardiomyocytes seeded onto the electrospun fibers were capable of attachment and maintained cardiomyocyte phenotype. Cells tended to form clusters, though smaller numbers of cells existed throughout the scaffold. The cells began beating after two days and continued beating on the fibers for over two weeks with contractions becoming more pronounced with time. Long range beating capable of large scale deformation of the fiber mats was observed after 5 days. Due to the constant contractile force of the cells at the surface of the scaffold, the mat curled into helices after 1 week. Imaging under low vacuum SEM showed that in the presence of peptide the cells were capable of engaging with the substrate and exerting force on the fibers as seen in Figure 1. On scaffolds without peptide cells failed to generate force and did not significantly attach to the surface.

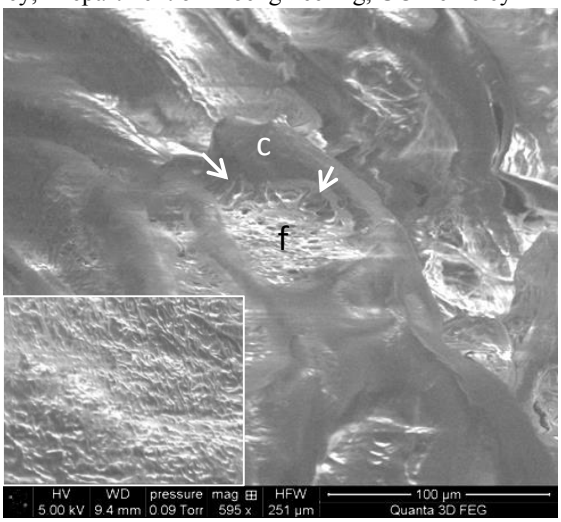

Figure 1. SEM images of cells on fibers with bspRGD. Cells on the fibers are visibly pulling on fibers in the mats, as shown by the white arrows. A cell cluster is labeled with a "c" and fibers with an "f". Inset: Fibers spun without peptide, same scale.

Conclusions: This study has demonstrated the utility of our novel material for use as a tissue engineering scaffold. Multivalent conjugation of bspRGD(15) to the HyA component of the electrospun fibers was used to program specific biological activity into the material such that it was capable of supporting cardiomyocyte viability and functionality for two weeks. By controlling the identity and quantity of the conjugated peptides it will be possible to tailor the biological signals presented. We can independently vary the macroscale mechanical properties and alignment of the electrospun fibers. We plan to modulate both of these properties for use in other applications, including use as a patch for chronic wounds.

References: 1. Fujimoto KL. J Cardiac Failure. 2012;18.
2. Prabhakaran MP. Biomed Mater. 2011;6.
3. Weber JL. Nature Methods. 2010;7.