Bioengineering of the Embryonic Cardiovascular Progenitor Cell Niche Using Electrospun Nanofibrous Scaffolds

Jessica M. Gluck[1], Richard J. Shemin[1], Sepideh Heydarkhan-Hagvall[1]
[1]Cardiovascular Tissue Engineering Laboratory, Department of Surgery, David Geffen School of Medicine at UCLA

Statement of Purpose: Tissue engineered structures are fast becoming a viable solution for not only the treatment of heart disease by replacing diseased cardiovascular tissue, but also as a means to further understand the complex nature of cardiovascular tissue development. We have previously seen that common extracellular matrix (ECM) proteins have varying effects on the terminal differentiation of murine embryonic stem (ES) cells[1]. It has also been previously thought that three-dimensional (3D) cell culture environments more closely mimic the in vivo native environment. In an effort to elucidate the mechanism through which the complex ECM 3D microenvironment enhances cardiovascular differentiation of ES cells, we have examined the production of cardiac progenitor cells (CPCs) from undifferentiated ES cells in varying 2D and 3D conditions with and without a 3D microenvironment in the form of electrospun nanofibrous scaffolds.

Methods: Co-axial bicomponent electrospun scaffolds were created from a core of 5% polyurethane (PU) and a sheath of a hybrid mix of 10% polycaprolactone-10%gelatin [2]. Briefly, the solutions were loaded into a double syringe system with a 25G blunt edge needle for the core solution and a 18G needle for the sheath solution. An applied voltage of 30kV was used with a flow rate of 70μL/min and a grounded collection plate 16.5cm away. The scaffolds were cut into 1cm x 1cm squares and sterilized with 70% ethanol. Protein coatings were applied to a standard 24-well culture dish in commonly-used concentrations: 0.1% gelatin, collagen IV (5μg/cm^2), laminin (5μg/cm^2), fibronectin (5μg/cm^2), and vitronectin (50ng/cm^2). The same protein concentrations were used for both 2D and 3D conditions. Each 2D or 3D condition was seeded with 500,000 undifferentiated mES cells. Both 2D and 3D conditions were also treated with the small molecule IQ-1, which has been shown to enhance CPC differentiation in 2D culture conditions previously. ES cells were expanded in standard cell culture conditions using a combination of ESGRO and KO-MEM media. Cells were kept in an undifferentiated state until ready for seeding on protein coatings. Once seeded, the cells were maintained in differentiation media, α-MEM media supplemented with 10% FBS (heat-inactivated), 1% Penicillin/Streptomycin, 1% MEM non-essential amino acids (100x), 1% L-glutamine, 0.2% HEPES (2mM), and 0.1% 2-mercaptoethanol. Analysis of the ES cells included initial biocompatibility testing to ensure lack of cytotoxicity and fluorescent activated cell sorting (FACS) analysis for CPC cell markers at various time points.

Results: Initial cytotoxicity assays confirm the biocompatible nature of the protein coatings and electrospun nanofibrous scaffolds. These studies have also confirmed the prolonged presence of the protein coatings for the duration of the current study, up to 9 days of culture. FACS analysis shows a gradual decrease in CPC population when exposed to collagen IV in 2D vs. 3D conditions. However, collagen IV in 2D conditions do exhibit high levels of CPCs. Laminin and vitronectin 3D conditions support higher amounts of CPCs as compared to their 2D counterparts. IQ-1 seems to show an inhibitory effect when added to 3D culture conditions, especially as seen in the collagen IV condition. Further results include examination at 5, 7, and 9 days in differentiation media and varying concentrations of IQ-1.

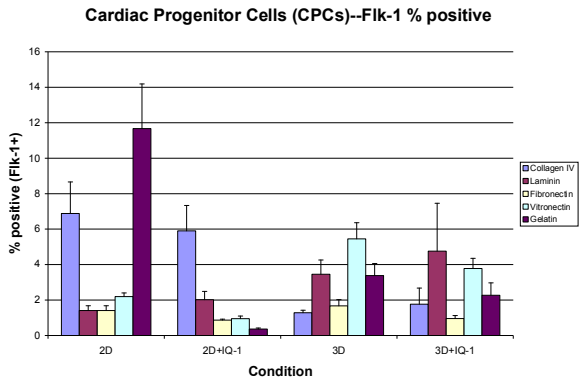

Figure 1: Cardiac progenitor cells (CPCs) as determined by Flk-1 marker from varying 2D and 3D culture conditions after 4 days as determined via FACS analysis.

Conclusions: As previously reported[1] CPCs as identified by the vascular endothelial growth factor, receptor 2 (VEGF-R2), Flk-1 have shown greater differentiation potential when exposed to various ECM proteins. Additionally, a 3D microenvironment further enhances this differentiation potential. In order to determine the mechanism by which a 3D microenvironment enhances a cellular response, we have combined both the 3D microenvironment of the electrospun nanofibrous scaffolds with various ECM proteins we have identified as influential. We have seen that the 3D microenvironment has the ability to enhance the amount of CPCs present after at least 4 days in differentiation conditions. Further study will be required to determine the appropriate in vitro microenvironment to fully recapitulate the cardiovascular stem cell niche present in the heart and understand the specific cellular mechanisms of how these ECM proteins are able to enhance CPC populations.

References:

1. Schenke-Layland K, Stem Cells, 2007; 25:1529-1538.
2. Heydarkhan-Hagvall S, Biomaterials, 2008;29:2907-2914