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Biofabrication of Dynamic, 3-Dimensional, In vitro Models of Disease.

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Maintenance of homeostasis is dependent on numerous cell-cell and cell-ECM interactions that take place in a variety of microenvironments that are subject to a wide range of mechanical forces. A mechanistic understanding of these processes is necessary in order to use this knowledge for new and improved therapies. In general, two approaches, in vivo and in vitro, have been used to gain insight into the mechanisms that regulate pathological processes. In vivo approaches are ideal in most cases; however, experiments in whole organisms are often complex and it is difficult to control for all variables. On the other hand, traditional 2D tissue culture models do not have sufficient complexity to adequately model pathology. Thus, there is an enormous gap between simple 2D cell culture and in vivo models. With this as our motivation, we have created models of myocardial, valve, and coronary artery development using a variety of biofabrication techniques, [1-4]. These models have allowed for the elucidation of specific molecular mechanisms that drive the morphogenesis of these tissues [5,6]. We have also used these fabrication techniques in conjunction with a custom made fluid flow bioreactor to investigate the role of mechanical forces in cardiovascular fibrous development, which is extremely difficult to study in vivo [7,8]. These studies and others involving stem cell integration and differentiation have served to fill the gap between in vitro and in vivo approaches [9].

In the present study, we have developed a new fabrication technology that allows for the co-polymerization of collagen gels with mammalian primary cells. By adding this technology to our other fabrication protocols we have generated a dynamic, 3D, in vitro model of atherosclerosis, a common vascular disease in which endothelial, smooth muscle, and inflammatory cells form plaques. Our flexible manufacturing technologies allow for the generation of tubular vascular constructs that contain fibroblasts (cells of the adventitia), smooth muscle (cells of the media), and endothelium (cells of the intima). Using a computational fluid dynamics modeling approach, we designed a tube geometry with a central nozzle that is predicted to produce disturbed flow patterns that are associated with atherosclerotic plaque development. This tube geometry was used to investigate the role that hemodynamics plays in atherogenesis. This specific 3D geometry was then manufactured and characterized for growth and remodeling under static and flow conditions. **Figure 1** shows the different cell morphologies, the dynamic cell distribution, and the cell specific remodeling that was observed in the tube constructs over the course of these experiments.

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In another use of our new biofabrication technology, we have generated a 3D model of the foreign body response, which is a process that limits the form and function of implanted biomaterials [10]. In this model, a 3D printed mold is used in conjunction with our collagen/cell casting process to create a silicone implant that is coated with a collagen/cell polymer. Initial characterization of this model indicates that cells take on different cell morphologies, have a dynamic cell distribution, and deposit new type I collagen over a 14-day culture period (**Figure 2**). This model will be used to investigate mechanisms of the foreign body response and to screen potential new therapeutic interventions.

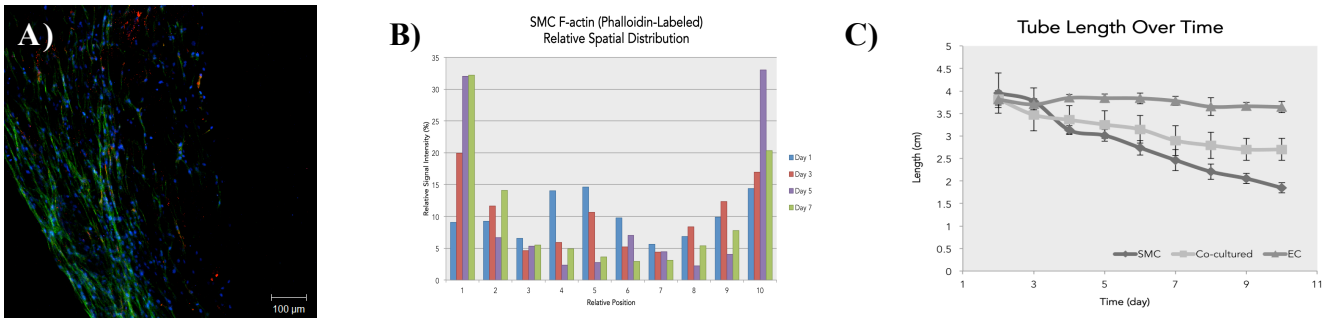

Figure 1. Cytotube Model of Atherosclerosis. Panel A is a confocal image of cells within the wall of the construct at day 7 (Green=F-actin, Blue=DAPI, Red=smooth muscle alpha actin). Panel B shows the distribution of cells over time. Panel C shows tube contraction over time is dependent on cell type.

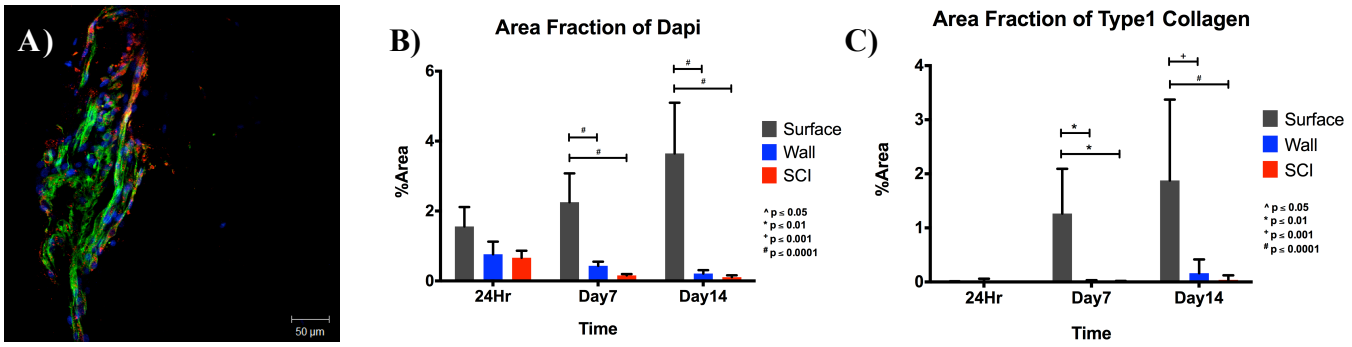

Figure 2. 3D Model of the Foreign Body Response. Panel A is a confocal image of cells within the wall of the construct at day 7 (Green=F-actin, Blue=DAPI, Red=Type I collagen). Panel B shows the distribution of cells over time. Panel C shows the distribution of Type I collagen within the construct.

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