DOI: 10.1017/S1431927605507086
Microsc Microanal 11(Suppl 2), 2005

Tissue Engineered Heart Tube Using Embryonic Tissues

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We have developed an engineered contractile heart tube. The engineered heart tube consist of an aligned tubular collagen scaffold, that has been sequentially seeded with neonatal rat cardiac myocytes, neonatal rat cardiac fibroblasts, embryonic valve cushion cells from chick, and quail pro-epicardial organ cells. These cells and tissues have been cultured on the collagen tubes and have been shown to continue the development and maturation processes similar to in vivo development. We have shown that our novel tubular hearts are contractile; contain the proper cell types, ECM, and receptors found in normal heart tissue. The tubes can be maintained in culture for up to 3 months and maintain their native architecture.

We have selected type I collagen for use as our scaffold material because of its superior biocompatibility properties, FDA approval status (generally considered safe) and our belief that it provides important biochemical and biomechanical signals to the cells to influence cellular phenotype. We have constructed our scaffold as a tube of aligned collagen fibrils. Briefly, the tubes are created from bovine type I collagen recovered from cowhide. The recovered collagen is fed to a counter rotating cone extruder. The collagen passes between counter rotating cones then between a fixed plug and top plate to form a continuous tube. The tube is extruded into anhydrous ammonia and air mixed atmosphere that polymerizes the collagen. We further treat the tubes in water and $NaHCO_3$. We have successfully cultured fibroblasts on the collagen tubes. Figure 1 panel A is a photomicrograph of neonatal rat fibroblasts cultured on the collagen tubes. It is thought that fibroblast cells are required for long-term culture of these tubular hearts to maintain the condition of the extracellular matrix. Under our unique culture conditions, neonatal cardiac myocytes will attach and grow on these collagen tubes. The cells align with the collagen pattern and express an in vivo like phenotype. Figure 1 panel B is a confocal micrograph of several layers of aligned cardiac myocytes attached to the collagen tube. This image shows the multiple layers of cells following the collagen pattern. The cells display this behavior both inside and outside the tubes. However, they prefer to populate the lumen of the tubes.

We have cultured Chick AV cushion tissue within the lumen of muscle cell seeded tubes. The cushion tissue continues its developmental program and forms a valve like structure within the lumen of the collagen tube. Figure 1 panel C is a light micrograph taken 7 days after transplantation of stage 22 chick cushion tissue into rat cardiomyocyte seeded tubes. Finally, we have cultured stage 17 quail Pro-Epicardial Organ, PEO, cells transplanted onto the outer surface of the collagen tube. Unique to our in vitro system, the Quail PEO cells proliferate and cover the surface of the collagen tube that has been seeded with rat cardiac myocytes. The PEO cells then begin to form the tube like structures as seen in the micrograph in figure 1 panel D. This behavior is similar to that seen in vivo during vasculogenesis of the developing heart.

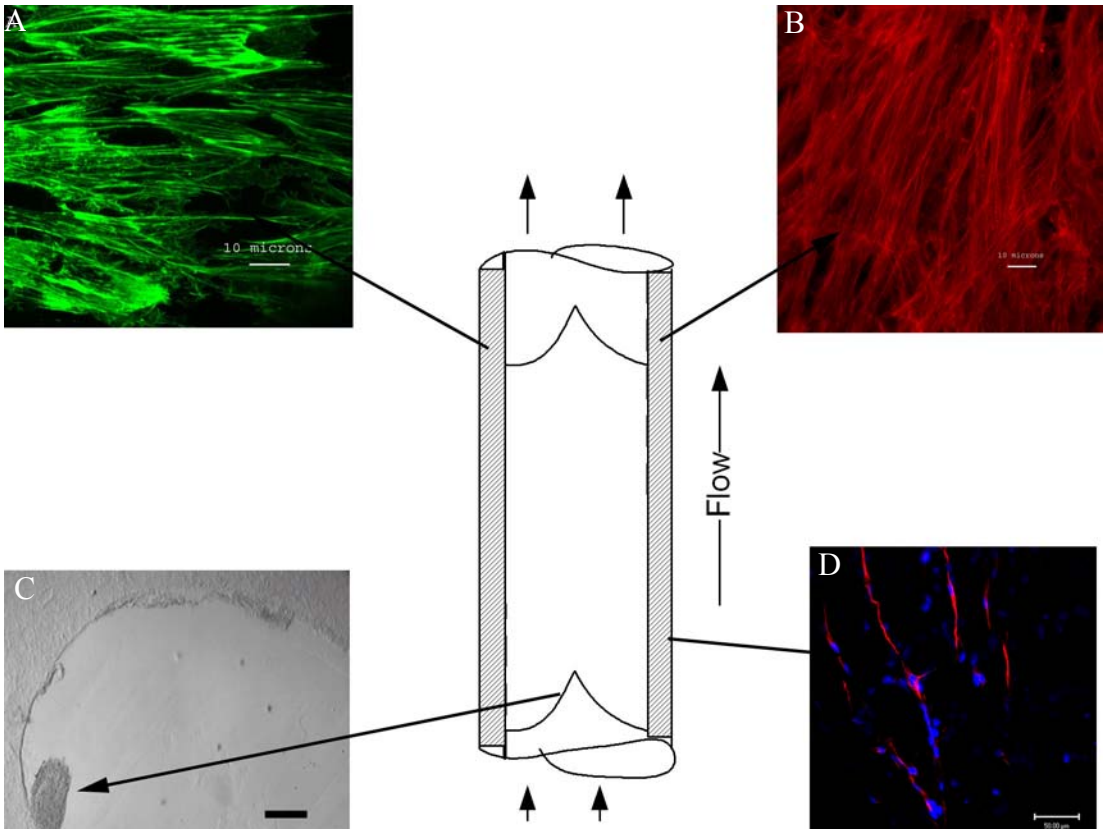

Figure 1 is a drawing of the engineered heart tube. The tube is designed to direct flow through the lumen unidirectionally. Panel A is a confocal micrograph of fibroblasts cultured within the wall of the tube. The f-actin components of the fibroblast's cytoskeleton are stained with Alexa 488 phalloidin. The image was taken using a BioRad® 1024ES laser scanning confocal microscope equipped with a 60x oil immersion lens. Box size is 1024 by 1024 pixels. Fibroblasts will populate the tube lumen, tube wall and to a lesser extent the outer surface of the tube. In some instances, fibroblasts will migrate through the wall of the tube and preferentially populate the lumen. Panel B is a confocal micrograph of neonatal rat cardiomyocytes populating the lumen of the heart tube. The same microscope and setup were used in this panel as in panel A. The alignment of the collagen fibrils causes the myocytes to adopt an in vivo like phenotype and multilayer. This image is a composite of a seven cell layers thick z series. Panel C shows the chick AV cushion tissue developing into a valve like structure within the lumen of the collagen tube. This image was taken 7 days after transplantation of stage 22 chick cushion tissue using a Nikon Optiphot-2® microscope equipped with a Zeiss Axicam® camera. Box size is 1024 by 1024 pixels. Panel D is a confocal micrograph of stage 17 quail Pro-Epicardial Organ, PEO, transplanted onto the outer surface of the collagen tube. The red is a marker specifically for quail cells, QH-1. The blue are DAPI stained cell nuclei. The Quail PEO cells proliferate and cover the surface of the collagen tube that has been seeded with rat cardiac myocytes. The PEO cells then begin to form the tube like structures as seen in the micrograph. This behavior is similar to that seen in vivo during vasculogenesis of the developing heart. This image was taken using a Zeiss LSM 510® confocal microscope equipped with a 40x water immersion lens. The box size is 1024 by 1024 pixels.

https://doi.org/10.1017/S1431927605507086 Published online by Cambridge University Press