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Novel Approaches to the Study of Cardiovascular Development

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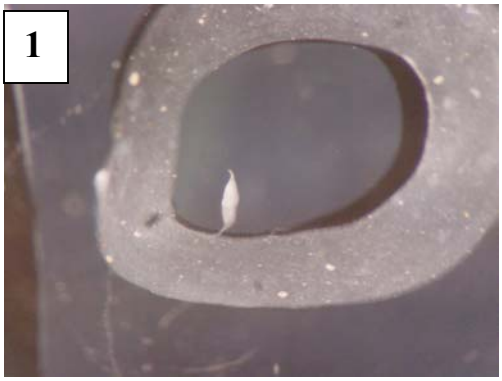
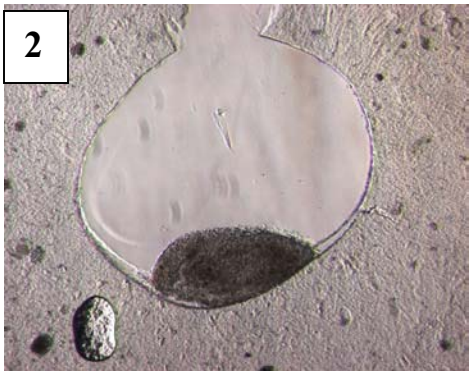
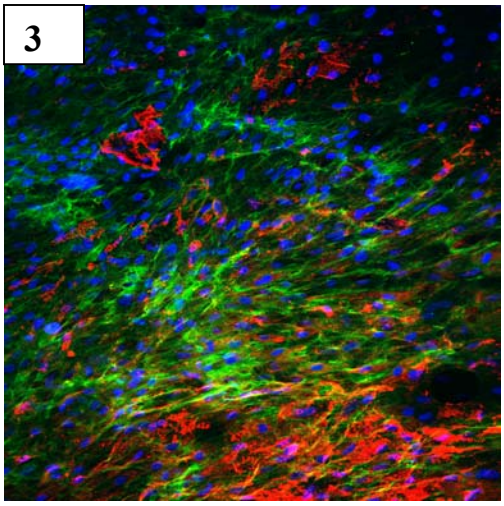
Cardiac malformations are the most common birth defect and cardiac disease is the leading cause of death in the industrialized world. In this study a new tissue engineering technique was developed that enables the construction of a three-dimensional (3-D) model system in which aspects of cardiac valve and vessel formation are recapitulated. The main component of this system is an engineered collagen tube substrate, on which developing cardiac tissues were allowed to differentiate. Valve anlagen, and cardiac vessel primordia (proepicardial tissues) were cultured on the tube scaffold with and without coculturing with embryonic cardiac myocytes so that the role cell/cell interactions could be determined. First, to investigate whether the 3-D collagen tube scaffold would be effective in sustaining atrioventricular canal (AV) valve development, stage 22 (day 5) chicken AV cushions were cultured within the lumen of collagen tubes with and without cardiac myocytes. Morphological and immunohistochemical analysis was carried out using electron and light microscopic techniques. AV canal explants grown within the collagen tubes retained their *in vivo* configuration and grew into leaflet-like structures (Fig.1) During normal valve development, the expression of periostin and fibrillin-2 are useful markers of valve differentiation. Both of these proteins are believed to be produced from the mesenchymal cells within the cushion. Staining of the cushion inside the tube showed the presence of both periostin and fibrillin-2. Periostin was observed throughout the cushion explant with the most intense staining at the periphery. This pattern of expression is analogous to that seen *in vivo* at closely approximated stages of development[1]. Experiments are ongoing to determine the role that myocytes play in this growth and development. Additionally, studies to determine if these cushions will remodel to become more leaflet in shape have begun (Fig. 2). The ability of proepicardial organ cells (PEO) to grow on the collagen tube scaffold was also investigated. Stage 17 (day 3) chicken and quail PEOs were isolated and incubated on the surface of collagen tubes seeded with or without rat embryonic myocytes. The PEO cells were processed for scanning electron microscopy and immunohistochemical analysis at days 3, 7, 14 and 21 of culture. These migrating PEO cells express matrix proteins such as fibronectin (FN), heparin sulphate proteoglycan (HSPG) and alpha cytokeratin. Collagen tubes containing PEO cells recapitulated the staining for cytokeratin and fibronectin. Interestingly, the staining for FN and cytokeratin were rarely co-localized (Fig.3). However both FN and cytokeratin were seen only where PEO cells had migrated over the surface of the tube. Scanning electron microscopy demonstrated that the ultrastructural makeup of these migrating PEO cells is also identical to that observed *in vivo*. Cultured PEO cells showed the ability to migrate over the tube in a sheet like fashion and maintained the typical apical microvilli appearance (Fig. 4). Studies presented here indicate that tissue engineering techniques are capable of taking *in vitro* development models into the third dimension, thus facilitating the investigation of the molecular mechanisms that regulate the morphogenesis of cardiac structures.

1. R. Goodwin et al., Developmental Dynamics, 2005. In press







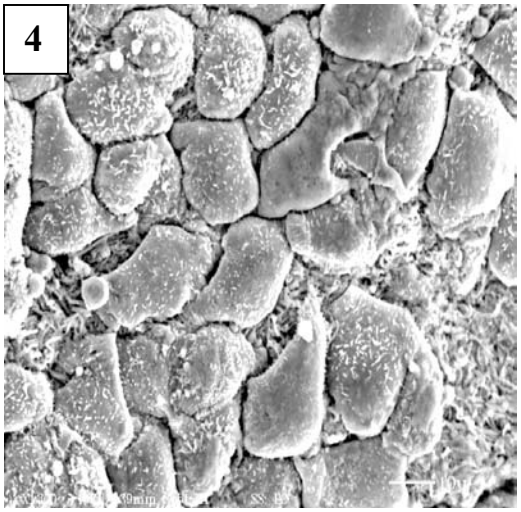


Fig. 1. AV canal explant grown in lumen of the collagen tube scaffold containing myocytes for 7 days. Notice the leaflet-like appearance.
Fig. 2. AV canal explant aggregate in the lumen of the tube scaffold. Notice the larger size of the aggregate.
Fig. 3. Confocal microcopy of PEO cells growing on the surface of the collagen tube lattice. The PEO cells are stained for cytokeratin (red) and fibronectin (green) and nuclei (blue) are shown. Virtually no overlap is observed in the two stains.
Fig. 4. Scanning electron microscopic image of PEO cells on the surface of the collagen tube lattice. The PEO cells are polygonal in shape and contain numerous apical microvilli.

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