Optimization of isolation and culture conditions of endothelial cells from human heart

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Purpose: Endothelial cells have an essential role in the maintenance of cardiac function. Cardiac endothelial cells in response of different cardiovascular risk factors become activated and this activation is often followed by endothelial dysfunction. The change of endothelial phenotype may influence interactions with surrounding cardiac fibroblasts, myocytes, pericytes.

The aim of our work is to produce in vitro culture system with high cell purity to investigate endothelial cells in different heart diseases. Poor quality of these tissue samples indicates the optimization of isolation and culture conditions for both cardiac microvascular and endocardial endothelial cells (CMVE and EE cells).

Methods: Human heart samples were obtained from valve surgery or explanted hearts during transplantation. CMVE cells were isolated from left ventricle with collagenase and trypsin digestion. EE cells were digested with collagenase from mitral valve. After separation the pellets were seeded to culture dishes and grown in a medium containing endothelial cell growth supplement. These basic methods would be changed as follows. The time of enzymatic digestion was changed. The collected cell suspensions were preincubated to allow fibroblasts to attach to the bottom of culture dish. The decanting medium was centrifuged repeatedly and collected cells were cultured in specific endothelial medium completed with sera from different origins. CMVE cells were treated with puromycin to eliminate pericytes. Cells were seeded on different artificial extracellular surface components (collagen, laminin, gelatin, fibronectin). The CMVE and EE cells were characterized by uptake of acetylated LDL and immunostaining of von Willebrand factor, VE-cadherin and CD31. Fibroblasts were identified by vimentin staining.

Results: Primary cells isolated with basic methods exhibited different cell morphologies, they were multilayered and only the 4% and 10% of the cells were endothelial cells, CMVE and EE respectively, separated by fluorescent-activated cell sorting (FACS). The attachment of fibroblasts and the puromycin treatment were effective in the primary cell cultures. Numerous cell colonies were observed within 2-3 days on gelatin coated surface and confluence was achieved within 10-14 days.

Conclusion: With use of these culture conditions the endothelial cell rate is increased in primary cultures and further separation by FACS or immunomagnetic methods will be more effective. These optimized cell culture systems provide possibility to investigate endothelial functions in different heart diseases.