

NG2 Expression of HUVEC and hASC in Coculture

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Introduction: It has been shown that HASCs have the ability to home to sites of injury and participate in vascular growth and remodeling through secretion of pro-angiogenic growth factors and differentiation into endothelial cells. These cells, when injected *in vivo*, also have the ability to exhibit a perivascular phenotype where they assume close contact with microvessels on the abluminal surface. In a Transwell coculture model, it is possible to identify any association of hASCs and other cell types such as HUVECs or hSMCs mediated by contact or soluble factors. It is, therefore, the thrust of this study to determine whether physical contact or soluble factors influence hASCs into assuming a pericyte phenotype.

Methods: Dil-labeled hASCs and HUVECs were cocultured on either side of a Transwell membrane system in cell-cell contact arrangement using early passage cells (P3 and P2, respectively) seeded at 10k cells/cm² and 70k cells/cm², respectively. Cells were cultured in DMEM/F12 with 10% FBS for 24 hours before being fixed with 0.4% paraformaldehyde and stained for PECAM and NG2. Images were then analyzed with a confocal microscope and quantified by counting cells in apparent contact, and cells showing positive NG2 immunofluorescence.

Results: Approximately the same percentages of HUVECs and hASCs exhibited NG2 positive staining (45.5% and 53.5%, respectively), when cocultured on either side of the membrane (N=2). 11.1% of all hASCs observed were in direct morphological contact with HUVECs, and 66.7% of these cells expressed NG2 positive staining.

Conclusions: The observed percentages of NG2 expression for HUVECs and hASCs, especially in cases where cells were in apparent contact, suggest that there is a correlation between hASC/HUVEC contact coculture and pericyte recruitment, thus implicating hASCs as an active participant in blood vessel function and stability.