DEVELOPMENT OF AN IN VITRO MODEL OF QUAIL VASCULOGENESIS

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Blood supply to virtually all tissues depends on endothelial cells, which form the linings of blood vessels. Growth and formation of endothelial cells occurs in two ways. Vasculogenesis describes the initial proliferation and differentiation of early embryonic cells to form blood vessels. From there, additional growth and movement of endothelial cells from pre-existing blood vessels is referred to as angiogenesis. This project was designed to isolate primary quail endothelial cells from early embryos and to establish a three-dimensional system to study the ability of the cells to assemble into tube networks. The long-term goal of this study is to develop a new *in vitro* endothelial morphogenesis system where both *in vitro* and *in vivo* results can be directly correlated. First, we isolated the quail chorioallantoic membrane at embryonic day five and digested the tissue with a combination of collagenase and trypsin. We removed debris and then added the cell suspension to dishes in the presence of endothelial cell growth medium. We allowed the cell population to proliferate for about one week and after this time, cell suspensions were incubated on dishes coated with QH1 antibody, which recognizes a quail endothelial cell specific antigen. This allowed us to selectively capture the endothelial cells, as they adhere to the antibody-coated dish. We then grew them in culture for a period of time to expand them. After expansion of the cells, they were examined for expression of CD31 and QH1 to prove that they were strongly enriched for endothelial cells. Using these quail endothelial cells, we suspended them in three-dimensional collagen gels with a cytokine mixture and assayed for their ability to assemble into tubes. We show that this endothelial cell population is able to form tube networks, further suggesting their endothelial origin.