3-D Spheroid Model As A New Tool For Toxicity Testing

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Introduction

The fundamental obstacles in environmental safety and toxicology studies is the extrapolation of data and risk assessment from research conducted from *in vitro* cell culture to the more complex biological tissues found *in vivo*. A better tissue culture model of complex biological systems is necessary to more accurately predict adverse human health effects to environmental toxicants. Thus, there exists a need of creating biologically relevant *in vitro* tissue culture models for toxicity testing.

Objective

The goal of our study was to establish a "Vascular Sphere in Dish model" for toxicity screening based on a multi-cellular culture of human vascular cells. We developed a method for preparing a 3-dimensional (3-D) vascular spheroid that consisted of combining human microvascular endothelial cells, smooth muscle cells, and fibroblasts. Furthermore, we compared vascular spheroids grown with endothelial cells that overexpressed Id (inhibitor of DNA binding and differentiation) protein 3 (ID3). ID3 acts as positive regulator of cell growth and plays a major role in neovascularization.

Material & Methods

Human cerebral microvascular endothelial cell line, HCMEC/D3, were transduced with Precision LentiORF and selected with antibiotic for 30 days to develop a stable ID3 clone. The endothelial wild type cells are referred to as EC^{WT} and the ID3 clone are labeled as EC^{ID3+}.

Cells were suspended in serum-free DMEM/F12 (1:1) supplemented with B27®. Approximately 100-150 cells per well were seeded in an ultra low-attachment 24-well plate. HCMEC/D3 cells and pulmonary arterial smooth muscle cells (SMC) were cultured in specified ratios for a time sufficient to form the vascular spheres. Next, spheres were transferred into solid support and cultured for up to 28 days without B27 supplement in a defined fibrin matrix seeded with human dermal fibroblasts (HDF) and maintained in a culture medium comprised of DMEM/F12, Microvascular Growth Supplement, and Smooth Muscle Growth Supplement.

The 3-D spheroids were harvested followed by routine paraffin embedding, histological processing and staining.

Results

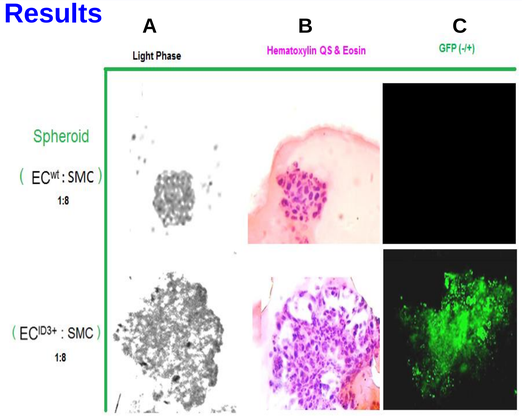

Fig 1. **Histological processing of EC:SMC vascular spheroid**. Optimized conditions of cell ratio of 1:8 (EC:SMC) grown in liquid suspension culture for 14 days then transferred into a solid matrix for 14 Days prior to histological processing. Spheroids embedded in fibrin matrix are easily processed using routine paraffin embedding techniques. **(A)** Light microscopy images of the spheroids. **(B)** Spheroid stained with eosin & Hematoxylin QS. X200. **(C)** Live confocal microscopy image of vascular spheroids before histological processing. Localization of EC^{ID3+} is shown by GFP expressing cells. X200. Using this model and histological procedure, spheroids grown with EC overexpressing ID3 showed a robust and larger vascular spheroid mass with internal vascular lumen compared to vascular spheroids grown with wild-type EC.

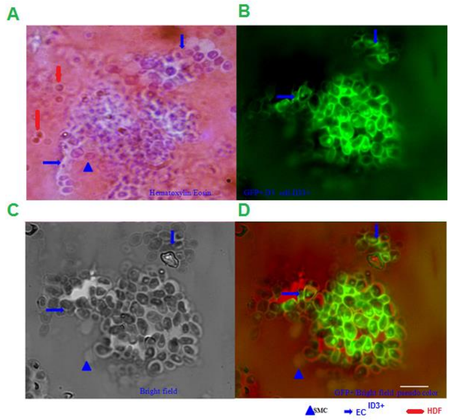

Fig 5. Histological processing of multi-cellular (EC^{ID3+}:SMC:HDF) vascular spheroid at 28 days. Tissues were histologically processed after 28 days to characterize the internal structure of sprouts and spheroid. **(A)** Hematoxylin stained slide showing morphology of spheroid. **(B)** confocal image, **(C)** Bright field, & **(D)** Merged photo of fluorescent and bright field images (B+C) with pseudocolor of red background. Scale=50µm. Vascular spheroids grown for 28 days under these conditions formed a viable core of EC^{ID3+} that were capable of forming tubes with a visible lumen and the tissue was stable enough to process using paraffin embedding methods.

Conclusions

- ID3 overexpressing endothelial cells cultured under specific conditions with SMCs and fibroblasts resulted in robust vascular spheroid growth that is easily histologically processed using standard paraffin embedding techniques.
- The vascular sphere in dish model is practical in studying either gain- or loss-of-function as shown in this study with the gene ID3.
- ID3 overexpression allows for robust vascular spheroids that may be grown for at least 28 days in cell culture. Chronic exposures to toxicants using this vascular sphere in dish model can provide novel measurements of toxicity that currently are unavailable.

Literature cited:

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