Pluripotent and Vascular Stem Cell Responses to Oxygen Depletion

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Statement of Purpose: It is well established that the variations in O_2 levels affect the aerobic respiration of cells and the regulation of multiple genes due to HIF1-alpha accumulation in the cell. Oxygen tension has been shown to be a key factor during vasculogenesis and angiogenesis. Therefore, the effect of O_2 tension should be clearly understood in order to guide the *in vitro* growth and differentiation of vascular cells cultured in two –dimensional (2D) and 3D settings.

In the first part of this study we examined the responses of human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), endothelial progenitor cells (hEPCs) and umbilical vein endothelial cells (HUVECs) to atmospheric (20%), physiological (5%), and hypoxic (1%) O_2 levels in terms of their growth, oxygen uptake rates (OUR), and changes in gene expression. In the second part, we computed and tested oxygen transport in collagen type I gels as a tool to examine oxygen-controlled 3D tube formation.

Methods:

DO Measurements: Dissolved oxygen (DO) levels were monitored using sensor dish readers (SDR). The SDR is capable of reading DO levels from an immobilized fluorescent patch on a 6-well plate.

qRT-PCR: The expression of *VEGF*, *GLUT-1*, *Ang1*, *BNIP3* *BNIP3L*, and *Ang2* in cells cultured in 1% and 5% O_2 relative to cells cultured in 20% O_2 was determined using two-step qRT-PCR. Results were normalized to β-Actin or HPRT1.

Cell Cycle Analysis: To examine the effects of hypoxia on the cell cycle after 24 hours of exposure to hypoxia, cells were fixed in 70% ethanol and subsequently stained with propidium iodine. Flow cytometry was performed on stained samples.

3D Collagen Gel: HUVECs were encapsulated into a 2.5mg/ml collagen I gel at a concentration of 2 million cells per mL. Crosslinking was induced by the addition of 5 N NaOH.

Staining and Microscopy: The morphologic changes and tube formation of HUVECs were investigated using both light microscopy and confocal fluorescent microscopy. Cells were stained with Phalloidin to visualize actin filaments and DAPI to visualize nuclei. To show decreases in oxygen levels within the scaffold, the gel was stained with either ruthenium or pimonidazole.

Results: We found lower oxygen uptake rates with respect to decreased dissolved oxygen (DO) availability in all cell types. Figure 1(a) shows the OUR of hESCs and iPSCs at 1%, 5%, and 20% O_2.

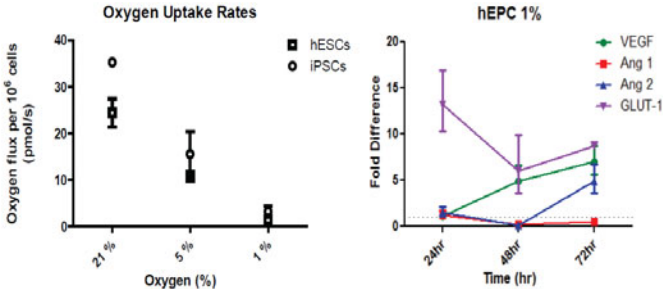

Figure1. (a) Oxygen uptake rate of cells at different O_2 tensions, (b) Gene expression profile of hEPCs cultured in 1% O_2

Results from qRT-PCR indicated that *VEGF* and *GLUT-1* remain highly upregulated over 3 days of culture in 1% O_2 (Fig.1b). Upregulation of *Ang1* and *Ang2* genes was also observed in some cultures. Pro-apoptotic genes *BNIP3* and *BNIP3L* were highly upregulated after 24 hours.

Flow cytometry results revealed that although these pro-apoptotic genes are highly upregulated, there is no evidence of apoptosis.

3D tube formation was observed when HUVECs were encapsulated in a collagen gel within 3 days in 20% O_2 (Figure 2).

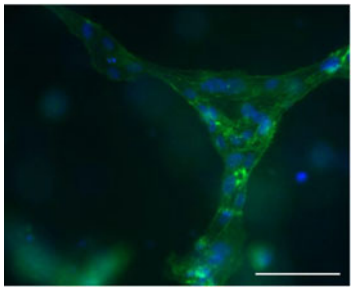

Figure 2. HUVECs tube formation in 3D collagen gel after 3 days: Phalloidin (green) and DAPI (blue). Scale bar is 10μm

The spatial O_2 levels through a collagen gel were estimated theoretically and tested using oxygen staining of the collagen gel and DO readings at the bottom of the gel. Current studies focus on correlating cellular responses to 3D O_2 tensions.

Conclusions: These results demonstrate adaptation of all cell types to hypoxia but different cellular responses. Further examinations with 3D cultures suggest a correlation between tubular formation and the O_2 availability in the collagen gel. We conclude that O_2 is a key regulatory molecule that should be monitored and controlled *in vitro* to obtain targeted cellular responses.