Engineering Angiogenesis by Hypoxia-Induced Signaling: Adopting a Physiological Approach

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INTRODUCTION: Successful engineering of tissues with clinically relevant size and complexity critically depends on their *in vitro* pre-vascularization which can promote cell survival, differentiation and rapid vascularization post-implantation. However, mimicking *in vitro* the physiological complexity of a vascular network currently presents major obstacles[1]. In this study we tested the hypothesis that a hypoxia-induced signaling (HIS) - cell population can generate the complete angiogenic cascade necessary for inducing endothelial cell sprouting and tubule formation within a 3D construct.

METHODS: HUVECs and human-dermal-fibroblasts (HDFs) were co-cultured in 3D spiral or flat collagen constructs[2] for 1 or 2 weeks, with no direct contact between the two cell types. HDFs were seeded at high density ($23x10^6$cells/ml) or low density ($1x10^6$ cells/ml) in spiral and flat constructs, respectively. HUVEC-only constructs served as controls. Constructs were cultured in the presence or absence of anti-VEGF neutralizing antibody. O_2 tension within constructs was monitored using an optical fibre-based system[3]. ELISA was used to quantify HIF-Iα and VEGF in 5 and 10 day cultures.

RESULTS: Cell O_2 consumption in high-HDF-density co-cultures resulted in hypoxic O_2 levels (<3%) in the HDF region's core, within 24hrs. In high-HDF-density co-cultures HUVECs formed CD31 and von-Willebrand factor positive capillary-like structures (CLS) with lumens and invaded the HDF region at 1 week. There was a significant increase in the number of sprouts from 1 to 2 weeks which correlated with a reduction in the number of endothelial-cell clusters. No CLS formation was observed in HUVEC-only cultures or in low-HDF-density co-cultures (no hypoxic stimulus). HIF-Iα was present in high-HDF-density co-cultures at 5 and 10 days, while VEGF levels increased by 7 fold from 5 to 10 days. No HIF-Iα or VEGF were detected in HUVEC-only cultures. Anti-VEGF neutralizing antibody reduced sprout length by 50% in high-HDF-density co-cultures.

DISCUSSION & CONCLUSIONS:

While it is widely accepted that long-term exposure of cells to hypoxia can be detrimental to cell viability, the results of this study indicate that hypoxia can be employed as a physiological signal for inducing an angiogenic response within a 3D tissue construct. Here we show that the angiogenic response was accompanied by up-regulation of two critical, hypoxia-inducible angiogenic factors, HIF-Iα and VEGF. However, the use of hypoxia as the primary angiogenic signal would be expected to trigger the complete angiogenic cascade required for a physiological angiogenic response. The ability to spatially localize the hypoxic signal within a 3D tissue construct could be an invaluable tool for engineering angiogenesis *in vitro* or for pre-conditioning constructs prior to implantation.

We propose that a HIS - cell population could rapidly and physiologically induce an angiogenic response within a 3D tissue construct.

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