**A microfluidic tumor model to study the effects of oxygen level and 3-D culture on tumor angiogenesis**

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**Statement of Purpose:** Hypoxia driven up-regulation of vascular endothelial growth factor (VEGF) is traditionally considered the primary driver of tumor angiogenesis. However, tumors consist of a landscape of spatiotemporally varying oxygen level ($pO_2$), and other microenvironmental cues such as cell-cell and cell-extracellular matrix (ECM) interaction also play an important role in this process. We are interested in 3-D *in vitro* culture systems to decouple these chemical and physical microenvironmental cues in the context of tumor angiogenesis. Hydrogel scaffolds that either prevent or enable integrin engagement are used to study the effects of 3-D culture, cell-ECM interaction and $pO_2$ on pro-angiogenic tumor cell behavior. Using these biomaterials, we have developed microfluidic scaffolds to enable spatiotemporal control of the chemical environment within a model tumor, at physiologically relevant length scales. Data collected from these studies allows for a deconvolution of the effects of $pO_2$ from other microenvironmental cues. Implications of these observations are now being explored in heterotypic tumor models, incorporating both tumor and endothelial cells, that enable a direct study of tumor angiogenesis *in vitro*. This could lead both to an improved understanding of tumor angiogenesis, and improved cancer therapies.

**Methods:** We have developed thin (~200 µm) hydrogel discs to attain a basic understanding of the effects of 3D culture, cell-ECM engagement, and uniform $pO_2$ on pro-angiogenic signaling by oral squamous cell carcinoma cells (OSCC-3s). We generated quantitative values, via ELISA, for VEGF and interleukin-8 (IL-8) secretion as well as $O_2$ consumption in response to different uniform $pO_2$ (uniformity confirmed by $O_2$ consumption measurements, finite element modeling, and histological analysis) in both 2-D and 3-D contexts. 3-D cell-ECM engagement was either prevented or enabled by use of inert or RGD-modified alginate discs. Using lithographically defined microchannels embedded within 3-D tumor scaffolds we were then able to create spatiotemporal oxygen profiles relevant to growing tumors. Microfluidics were used to control oxygen level by varying dissolved $pO_2$ in delivered media. Phosphorescent nanobeads incorporated into the scaffold were used to image $pO_2$, while microfluidics performed double duty, acting as taps for collection and subsequent analysis of the pro-angiogenic factors secreted.

**Results:** From our 3-D disc cultures, we have determined that while hypoxic culture conditions (1% $pO_2$) up-regulate VEGF in both 2-D and 3-D formats (Fig. 1a), consistent with previous observations, the pattern is different for IL-8. While up-regulated by hypoxia in 2-D, IL-8 is increased significantly at ambient $O_2$ levels (17% $pO_2$) in 3-D. IL-8 is furthermore up-regulated by cell-ECM engagement provided by gel-incorporated RGD peptide sequences, indicating that normoxic $pO_2$ and cell-ECM engagement up-regulate IL-8 in an integrated manner in a 3-D context (Fig. 1b).

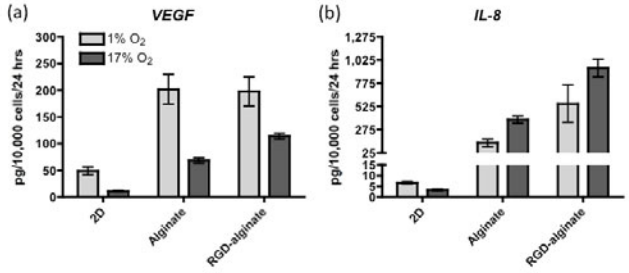


Figure 1. Comparison of OSCC-3 pro-angiogenic secretions in 2-D and 3-D culture. 3-D culture is performed in 200 µm thick non-modified and RGD-modified alginate, as a function of $pO_2$.

A double-network microfluidic scaffold was developed to mimic the adjacent hypoxic core and normoxic periphery of a growing tumor, and heterogeneous $pO_2$ was verified via phosphorescence imaging (Fig. 2a, increased phosphorescence emission correlates with reduced $pO_2$). Resulting differences in VEGF and IL-8 secretion, specifically elevation of IL-8 in the normoxic niche and with time, were demonstrated in this system (Fig. 2b).

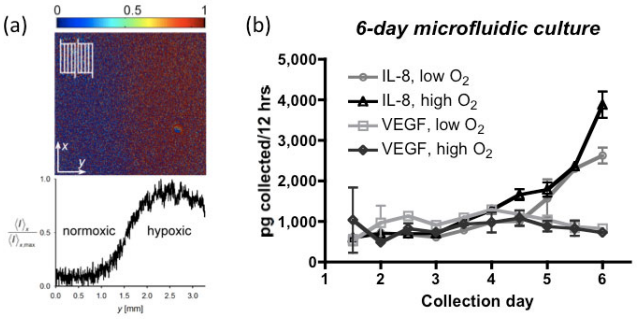


Figure 2. Culture of OSCC-3s in a microfluidic "double network" scaffold, and resulting pro-angiogenic signaling measured via the microfluidics operated as protein taps.

**Conclusions:** Our data indicates that the integrated interplay of 3-D culture, cell-ECM engagement, and $pO_2$ influences OSCC-3 pro-angiogenic signaling, with qualitative differences between VEGF and IL-8 regulation. An oxygen-sensing microfluidic tumor model has been developed that recapitulates an *in-vivo*-like oxygen profile, and that can be used to examine the pro-angiogenic influence of complex and physiologically relevant spatiotemporal variations in $pO_2$.

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