

New Dimensions in Vascular Engineering: Opportunities for Cancer Biology

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Angiogenesis is a fundamental prerequisite for tissue growth and thus an attractive target for cancer therapeutics. However, current efforts to halt tumor growth using antiangiogenic agents have been met with limited success. A reason for this may be that studies aimed at understanding tissue and organ formation have to this point utilized two-dimensional cell culture techniques, which fail to faithfully mimic the pathological architecture of disease in an *in vivo* context. In this issue of *Tissue Engineering*, the work of Fischbach-Teschl's group manipulate such variables as oxygen concentration, culture three-dimensionality, and cell–extracellular matrix interactions to more closely approximate the biophysical and biochemical microenvironment of tumor angiogenesis. In this article, we discuss how novel tissue engineering platforms provide a framework for the study of tumorigenesis under pathophysiologically relevant *in vitro* culture conditions.

ANGIOGENESIS IS A critical step in cancer development, whereby tumors establish dedicated blood vessels to facilitate growth, invasion, and metastasis.¹ This complex and highly regulated process, which involves the migration and proliferation of endothelial cells (ECs), is driven by tumor-cell-derived pro-angiogenic signals, yielding continued tumor growth and metastasis. Once a tumor acquires a dedicated blood supply, it facilitates growth and spread of cancer cells to other organs. Additional studies confirm that malignant transformation is associated with the upregulation of tumor-specific factors that selectively induce the assembly of tumor vessels.² Several studies have suggested that angiogenesis is in fact the rate-limiting step in tumor growth and progression.^{3,4} Recently, the idea was introduced that ECs do not only function as the passive building blocks of blood vessels, but also comprise a vascular niche that nurtures tumor growth and initiates tissue regeneration directly through elaboration of specific growth factors.⁵

Normal blood vessels and tumor-induced blood vessels differ greatly in morphology and function. Normal neoangiogenic vessels recruit pericytes and vascular smooth muscle cells to the ECs to stabilize these vessels.⁶ By contrast, tumor-induced blood vessels are often less stable, disorganized, and leaky. They lack a hierarchical arrangement, have irregular diameters, and follow random branching patterns. Tumor growth in tissues leads to increasing hydrostatic and solid pressures, inducing tumor cell quiescence and necrosis, as well as blood vessel collapse.

Current efforts in regenerative medicine aimed at recreating the unique microenvironments surrounding both normal and tumor-driven neoangiogenesis have been met with limited success. The principal difficulty stems from the lack of a physiological reproduction of growth factor gradients, which are critical to create a pro-angiogenic niche. Traditional static two-dimensional cell culture systems reduce the biological relevance and complexity of dynamic tissue architectures. For these reasons, three-dimensional (3D) tissue constructs better reflect native biophysical and biochemical environments, and hence have become a focus of recent investigations.⁷

The complexity of angiogenesis suggests the existence of multiple controls, some of which may be better evaluated with bioengineering approaches. To improve the angiogenic capability of engineered tissues, design of scaffolds provides a spatio-temporally controlled delivery of cells and/or growth factors in both *in vitro* and *in vivo* models. To create a microenvironment that more accurately mimics a tumor microenvironment, 3D models have been engineered to recreate the *in vivo* tumor niche by growing cells in polymeric scaffolds.^{8,9}

In this issue, Fischbach-Teschl and colleagues,¹⁰ in their article titled "Oxygen-Controlled 3D Cultures to Analyze Tumor Angiogenesis," report on a 3D microscale tumor model to define the distinct importance of oxygen concentration, culture dimensionality, and cell–extracellular matrix interactions on the angiogenic capability of carcinoma cells.

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Their studies were focused on defining the distinct roles of oxygen and 3D cell–extracellular matrix interactions in pathologically relevant culture conditions *in vitro*. Such systems are aimed at modulating the angiogenic potential of tumor cells, and hence improving our understanding of tumor angiogenesis.

Malignant transformation occurs within the context of a dynamically evolving stroma in three dimensions. In contrast to normal healthy tissues, the vasculature of solid tumors has a limited reach, resulting in pockets of low oxygen concentration. This causes the tumor's hypoxic core to become necrotic, with a surrounding layer of cells whose exposure to hypoxia triggers a cascade of hypoxia-inducible factor-1 and vascular-endothelial-growth-factor-mediated signaling events that initiate tumor neovascularization. Three-dimensional cultures may be utilized to study the mechanisms of cancer cell signaling in this distinctive tumor hypoxic microenvironment. We have recently succeeded in generating stable cocultures of vascular cells in a honeycomb alginate scaffold (with an average channel diameter of 300 μm) that can self-organize into capillary-like structures. The porous 3D alginate depots containing the cells, in a serum-free condition, were further exposed to laminar flow to recapitulate the vasculature *in vivo*. The scaffold remained intact with the cells remaining adhered to it and aligned in the direction of flow, demonstrating its suitability for establishing durable angiogenic modules that may ultimately enhance organ revascularization or model tumor neoangiogenesis.¹¹

A major unresolved issue concerning the interaction of cancer cells with endothelium is the requirement for a source of viable, stable ECs *in vitro*. The ability of human embryonic stem cells (hESCs) to self-renew and to generate ECs for therapeutic revascularization has also been recently demonstrated. hESCs, in addition to their self-renewal capacity, have the potential for large-scale differentiation into any adult cell type *in vitro*,¹² and are thus an attractive alternative source of ECs. The emergence of ECs from differentiating hESCs in real time was monitored by an EC-specific genetic reporter, whereby the vascular endothelial cadherin (VE-Cad) promoter drives expression of green fluorescent protein (hVPr-GFP).¹³ This novel approach for vascular monitoring in combination with the ability to grow cells on 3D platforms will allow for closer scrutiny of tumor growth while examining the contribution of the hVPr-GFP+ ECs to the neoangiogenic process.

Additionally, biologically compatible scaffolds enable *in-vitro*-generated vascular cells to receive and respond to important mechanical stimuli in three dimensions.

Mechanical signals regulate multiple cellular processes and thus have numerous effects on tissue development (including blood vessels) and in disease processes. Such stimuli, such as hemodynamic shear stress, influence the cytoskeleton assembly directly, thereby translating the mechanical signal into changes in biochemical signaling pathways (i.e., mechanotransduction). It is therefore important to elucidate cellular mechanochemical interactions in 3D to improve engineered tissue models and to better investigate these processes *in vivo*.

Some may argue that 3D cultures fail to accurately reproduce the complexity of tumor cell biology *in vivo*. However, if cell cultures in three dimensions increase the survival and/or the expansion of cancer cells from solid tumors, they may offer utility in the preclinical analyses of the molecular

mechanisms underlying tumor biology. In summary, biomaterials are used in a variety of tissue engineering and drug delivery projects to promote angiogenesis, and hence influence the regeneration of tissues and organs in the body. Such approaches will enable us to grow, or engineer, long-lasting tissues and organs using 3D depots, which may serve to guide new tissue formation therapeutically in the body or *in vitro* as physiological models to study disease pathogenesis.

Insights gained from 3D platforms may improve our understanding of cancer and contribute to the development of antiangiogenic therapies. Can tissue engineering transform the cancer field by providing innovative tools to study tumorigenesis under pathologically relevant culture conditions? This is a question that only time and intensive investigation can answer.

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