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Hydrogels to model 3D in vitro microenvironment of tumor vascularization ☆

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ABSTRACT

A growing number of failing clinical trials for cancer therapy are substantiating the need to upgrade the current practice in culturing tumor cells and modeling tumor angiogenesis in vitro. Many attempts have been made to engineer vasculature in vitro by utilizing hydrogels, but the application of these tools in simulating in vivo tumor angiogenesis is still very new. In this review, we explore current use of hydrogels and their design parameters to engineer vasculogenesis and angiogenesis and to evaluate the angiogenic capability of cancerous cells and tissues. When coupled with other technologies such as lithography and three-dimensional printing, one can even create an advanced microvessel model as microfluidic channels to more accurately capture the native angiogenesis process.

Contents

1. Introduction . . . 0
2. Tumor vascularization mechanisms . . . 0
3. Engineering microenvironments for cancer cell growth and angiogenesis . . . 0
3.1. Natural hydrogel materials . . . 0
3.2. Synthetic hydrogel materials . . . 0
4. Modeling tumor angiogenesis in vitro . . . 0
4.1. Three-dimensional tumor angiogenesis assays . . . 0
4.2. Advanced biomimetic models for angiogenesis . . . 0
5. Considerations and future directions for drug screening and nanomaterial applications . . . 0
6. Conclusion . . . 0
Acknowledgments . . . 0
References . . . 0

1. Introduction

Despite the overall decreasing trend of the cancer mortality rate, over 1.6 million people in the U.S. are expected to suffer from cancer in 2013 with 580,000 estimated deaths [1]. In an effort to supersede the conventional treatments involving chemotherapy and radiation, various attempts have been made to discover new drugs with antitumor activity. However, clinical trials are very costly and often slowed down by high failure rates, commonly due to misguided preclinical models. Therefore, a more extensive analysis at the preclinical stage is required to more accurately predict the outcomes of clinical trials [2]. A growing number of researchers are now focusing on targeting biomarkers to accelerate the drug development process, minimize the cost, and maximize the benefit from early clinical trials [2,3].

Particularly, angiogenesis has been an attractive target for anti-cancer drugs [4]. As the unregulated tumor growth continues, exacerbated oxygen and nutrient deprivation turns tumors into the angiogenic phenotype, triggering the release of angiogenic growth factors and cytokines, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), to the microenvironment [5,6]. This dysregulated signaling pathway activates the nearby endothelial cells (EC) and perivascular cells, which ultimately results in the recruitment of new blood vessels to the area to support further tumor growth [6]. Eventually,

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these vessels would provide means for metastasis [7]. Inhibiting this angiogenic process has been one of the main foci of modern cancer research, but many of the recent clinical studies have reported various side effects of antiangiogenic therapies that utilize small molecule inhibitors (such as bevacizumab, sunitinib, and sorafenib), including hypertension, impaired wound healing, coagulation, and, in some cases, increased tumor activity and metastatic acceleration [8–12]. More importantly, currently observed benefits from this strategy are transient since tumors are capable of overcoming the anti-angiogenic condition by employing different pathways (for example, vasculogenesis, vascular mimicry and vessel co-option) to remodel their neighboring blood vessels [6,13–15].

More comprehensive investigation of tumor angiogenesis and identification of robust tumor angiogenic biomarkers are thus vital to developing viable cancer treatments. However, a lack of competent preclinical models often hinders successful subsequent clinical trials. Animal in vivo xenograft models are commonly used, but often cannot represent the disease sufficiently due to physical differences from humans. For example, tumors in a murine xenograft model grow relatively faster than human tumors, which results in immature blood vessels that cannot compare with tumorigenic vessels that have been established for a longer period of time [16,17]. In addition, key parameters that affect tumor progression, including oxygen tension, nutrient gradients, and mechanical forces, cannot be easily controlled and manipulated in these models [9]. Imaging tumor vasculature in vivo has been particularly challenging as well, making it difficult to evaluate the benefits from anti-angiogenic therapies [15,18]. To address these issues, investigators have been developing various alternative in vitro models for cancer cell growth and vascularization [19–24]. For this approach, the validity of a model would depend on how closely it can mimic the in vivo conditions. Up to this date, the majority of in vitro cancer studies have used two-dimensional (2D) monolayer cultures, where cells are usually grown on a plastic plane [25]. However, cell–cell and cell–extracellular matrix (ECM) interactions that are essential for tumor growth and angiogenesis cannot be recapitulated in 2D models, so these models may produce misleading results and provide wrong guidance for future clinical trials.

In fact, growing numbers of cancer studies are now utilizing three-dimensional (3D) culture models, and, not surprisingly, many have observed significantly distinct responses compared to the traditional 2D models. By encouraging cell–cell and cell–ECM interactions, 3D models support increased release of vascular growth factors, increased aggressiveness and metastatic potential, slower proliferation, increased resistance to anti-cancer drugs and radiation therapy, and physiological gene-expression profiles, all of which are characteristics of tumor cells in vivo [24–32]. In addition, integrin-mediated cell attachment to the 3D matrix and remodeling of ECM via matrix metalloproteinase (MMPs) are critical for proliferation and survival for both tumor cells and ECs [27,33]. Specifically for tumor angiogenesis, the remodeled ECM and immobilized molecular cues from tumor cells support EC recruitment and morphogenesis that leads to vascularization around the tissue [6,33]. It has also been shown that ECs respond to different topographies, geometries, and the mechanical stiffnesses of their 3D microenvironment. In their physiological environment, vessels exist as multi-cellular tubes with hollow lumens of circular cross-section, where ECs are polarized to interact with the ECM surrounding the vessels and respond to the shear stress from the fluid flow inside the lumens [33,34]. Together with shear stress, 3D geometrical cues have shown to contribute to the alignment and the elongation of the ECs inside the vessels, which directly relate to cell function and survival in vivo and cannot be observed in a static 2D culture [35–38]. In addition, we have recently demonstrated in vitro that the 3D curvature on which the ECs are grown results in circumferential ECM deposition and organization [39]. These observations demonstrate the advantages of utilizing 3D architectural designs in vitro to model the physiological microenvironments of various tissues in vitro. These models are prevalent in the field of tissue engineering, which has allowed researchers to design systems that mimic the physiological cell–cell and cell–ECM interactions of a variety of tissue types [21, 40–42]. Since tumor vascularization occurs within a 3D physiological environment just like other tissues, similar engineering principles and techniques can be applied to the model in order to study cancer biology.

Hydrogels are hydrophilic polymeric networks that are commonly used for creating 3D in vitro models of tissues. Hydrogels provide means of tuning the mechanical strength and chemical structures of the cellular microenvironment. Studies have shown that different stiffnesses of gels created by varying crosslinking densities can effect the proliferation, survival, and migration of the embedded cells and can also cue differentiation of stem cells to specific lineages [43–45]. In addition, hydrogels can be chemically modified to present cell-attaching sites (such as RGD amino acid sequence) and MMP-degradable sites which are crucial for tumor progression, endothelial migration, and, ultimately, tumor angiogenesis [6,28,45,46]. Recently, hydrogels have been incorporated with other technologies such as lithography, microfabrication, and microfluidics to develop complex blood vessels, which show promise for more advanced and clinically relevant tumor angiogenesis models [47–49].

The importance of 3D in vitro models is becoming evident as more and more studies benefit from the tunable platform by hydrogels that gives us more control over the microenvironment of a tissue. Here, we first review the mechanisms of tumor vascularization, and explore natural and synthetic hydrogels and design parameters commonly employed to form tumors and create vasculatures in vitro. We then examine hydrogel-based angiogenesis assays that are currently being used in cancer studies and move on to explore recent advanced in vitro models that recapitulate tumor angiogenesis from microvascular networks.

2. Tumor vascularization mechanisms

Angiogenesis is an intricate process that involves cell–ECM interaction and cell–cell interaction not only between ECs, but also between ECs and other cell types such as mural cells (pericytes and smooth muscle cells), fibroblasts, and inflammatory cells. It has been one of the key topics for cancer biology for decades due to its close association with tumor development, maintenance, and survival. The dysregulated nature of cancer growth provides unique features to tumor-associated blood vessels that may be critical for cancer therapies and should be sufficiently replicated in in vitro models to obtain better guidance for clinical trials. In this section, we briefly describe biomolecular and cellular mechanisms of tumor vascularization.

Initially, a tumor can grow with passive diffusion of oxygen and nutrients from the surrounding stroma without any support from blood vessels. However, as the tumor lesion grows to 1–2 mm^3, the cells at its core start to experience hypoxia and nutrient deprivation and accumulate hypoxia inducible factors (HIFs) such as HIF-1α, which triggers a phenotypic transition known as the angiogenic switch [50,51]. Activation of the pathway leads to overexpression of cytokines, growth factors, and other soluble factors that breaks the balance between pro- and antiangiogenic factors. This dysregulated cascade ultimately recruits new blood vessels to the tumor site. The generalized overview of tumor angiogenesis is illustrated in Fig. 1.

The most well-understood tumor angiogenic signaling pathways involve VEGF, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and angiopoietin (Ang), which are intricately coordinated and overlapped. Tumor angiogenesis begins with activation of pericytes by tumor-secreted VEGF and Ang-2, which leads to the detachment of the cells from the vessel and acquiring more proliferative phenotype [8,52]. The ECs at these sites thus are exposed to the cytokines and growth factors secreted by tumor cells and activated pericytes as well as to the interstitial collagen-rich ECM as the basement membrane is

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