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TISSUE ENGINEERING PERFUSABLE CANCER MODELS

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Abstract

The effect of fluid flow on cancer progression is currently not well understood, highlighting the need for perfused tumor models to close this gap in knowledge. Enabling biological processes at the cellular level to be modeled with high spatiotemporal control, microfluidic tumor models have demonstrated applicability as platforms to study cell-cell interactions, effect of interstitial flow on tumor migration and the role of vascular barrier function. To account for the multi-scale nature of cancer growth and invasion, macroscale models are also necessary. The consideration of fluid dynamics within tumor models at both the micro- and macroscopic levels may greatly improve our ability to more fully mimic the tumor microenvironment.

Keywords

Tissue engineering; Cancer; Perfusion; Tumor models; Fluid flow

Introduction

In light of the inherent limitations of traditional two-dimensional (2D) tissue culture systems and animal models for preclinical drug testing and mechanistic studies, considerable progress has been made in the development of *ex vivo* three-dimensional (3D) tumor models that more fully recapitulate aspects of the *in vivo* tumor niche.[1,2] While the human tumor spheroid is currently the most commonly used 3D model, increasingly, enabling technologies developed in the tissue-engineering field are being leveraged to construct models that mimic the tumor microenvironment with greater fidelity. While these 3D models have appreciably advanced our understanding of the effects that the tissue architecture, extracellular matrix (ECM), and tumor-stroma interaction have on tumorigenesis, the impact of a generally neglected but significant component of the tumor microenvironment – fluid flow – remains largely undocumented.[3] Due to rapid and abnormal tumor-associated angiogenesis and the formation of a leaky and aberrant vasculature [4], the permeability of water and solutes within a tumor increases, resulting in a rise in interstitial fluid pressure and corresponding net convective flow out from the tumor mass into the surrounding tissue.[5,6] This interstitial fluid flow not only results in shear

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forces which may influence tumor cell proliferation [7], but also creates extracellular gradients of proteases and cytokines that may promote cancer invasion.[5,8-10]

Despite the increasing realization that fluid flow is an integral part of the tumor microenvironment, the contribution of this component to cancer progression remains to be fully elucidated. Notably, besides the need to better understand the repercussions of fluid flow in the tumor microenvironment, the inclusion of perfusion is also a potential strategy to overcome the diffusional limitations associated with most 3D tumor models.[1] To achieve this goal of recapitulating tumor-associated fluid dynamics, tight integration of the two most relevant engineering disciplines, microfluidics and tissue engineering, is necessary. This review will cover the current status in the development of microfluidic as well as macroscale tissue-engineered tumor models, and discuss the challenges involved in taking an interdisciplinary approach towards the development of perfused tissue-engineered tumor models.

Microfluidic Tumor Models

Since their advent, microfluidic systems have garnered the attention of the oncological community due to the possibility of modeling varying aspects of tumorigenesis characterized by a highly controlled spatiotemporal development, such as intercellular communication, metastasis and drug resistance. The laminar flow condition arising at the micro-scale presents the opportunity to mimic concentration gradients typical of the tumor niche [11,12], with better recapitulation of drug transport and transient biological processes occurring down to the single cell level.[13] This unique property, together with the potential for high-throughput and automation, has been leveraged in the last decade mainly for proteomic analyses [14], cell sorting [15], and pharmacodynamics studies [16], ultimately projecting microfluidic systems as highly advanced analytical tools for tumor biology research. One of the most prominent manifestations of this research direction is represented by the work of Jang et al., who developed a platform able to evaluate up to 100 different drug combinations, in line with the emerging importance of combinatorial drug therapy for chemoresistant patients.[17] The development of microfabrication techniques such as microcontact printing and soft lithography further enhanced microfluidic capabilities, that is, better control over topology and surface chemistry,[18] hence paving the way for more sophisticated mechanistic investigations of tumor biology.[13,19,20] Several groups have leveraged the preferential adhesion of endothelial cells and tumor cells on fibronectin and hyaluronic acid, respectively, to investigate tumor-vasculature interactions on microcontact-printed scaffolds with a particular focus on cancer cell motility associated with extravasation and metastasis.[21,22]

The ultimate advancement in the applicability of microfluidics to oncology stems from the use of 3D scaffolds to more fully recapitulate the native tumor niche and incorporate the effects of matrix composition, structure and mechanical properties on tumor progression.[19,23,24] Mirroring the current trend in macroscale tumor models, biocompatible hydrogels such as collagen [25], alginate [24], and Matrigel™ [26], are popular scaffolds used to investigate the role of cell-cell, cell-ECM and hydrodynamic cues on tumor progression and drug efficacy within a microfluidic context. In one example illustrating the precise spatial control that can be achieved with microfluidic systems, Huang et al. demonstrated the ability to pattern separate, adjacent channels with distinct hydrogel types.[27] With this microfluidic configuration, tumor-derived macrophage cells encapsulated within Matrigel™ were observed to invade into contiguous metastatic breast cancer cell – laden collagen hydrogels and not blank gels, highlighting the potential utility of this system for systematic studies of cell-cell and cell-ECM interactions. In a similar compartmentalized platform, Sung et al. demonstrated the distance-dependent effects of breast cancer progression towards

the invasive phenotype using a 'Y'-shaped channel in a co-culture model of mammary epithelial cells and fibroblasts.[26]

Beyond being able to mimic the multicellular cross-talk within the tumor microenvironment, microfluidic culture systems have recently been shown to have utility in investigating the effect of interstitial flow on tumor cell migration. Using a microfluidic device consisting of two channels separated by a 3D collagen I matrix seeded with breast carcinoma cells, Polacheck et al. demonstrated the feasibility of generating tumor-relevant interstitial flow via the application of a hydrostatic pressure gradient across the construct.[28] This study demonstrated how the directional bias of migration along the streamline is determined not only by cell density, but also the interstitial flow rate. Interestingly, as the tendency for upstream migration of cells was found to correlate with high cell density and flow rates, the authors suggest the presence of an 'escape radius', where interstitial flow either guides tumor cells upstream to remain clustered with the tumor, or downstream towards the draining lymphatics or veins - the outcome being dependent on whether the cell is within or outside this 'escape radius'.[28] In another similar study, Haessler et al. not only demonstrated the heterogeneity of tumor cell migration in response to interstitial flow, but also the feasibility of studying the differential effects of flow on different subpopulations of cells within a heterogeneous tumor population.[29] Collectively, these mechanistic studies highlight the potential of using microfluidic culture models as tools to understand the role of interstitial flow in promoting cell migration, which may introduce novel approaches to treating metastatic disease.

A typical 'perfused' 3D cell culture system in the literature most often refers to the provision of a continuous flow of media for the supply of nutrients to and removal of wastes from the construct. However, a more precise distinction can be made to distinguish between systems where medium flows over the surface of the scaffold, as opposed to perfusion through the bulk of the scaffold.[30] Thus far, approaches to incorporate scaffold-based 3D cultures into microfluidic devices typically employ the use of hydrogels to immobilize cells for the formation of 3D structures where mass transport of solutes, primarily driven by diffusion, may not be efficient.[31] To maximize mass transfer, Toh et al. developed a microfluidic channel-based system consisting of an array of microfabricated pillars for cell immobilization and laminar flow complex coacervation reaction of polyelectrolytes for the formation of a thin layer of 3D matrix to support the cells.[32] Using carcinoma cell lines Hep G2 and MCF-7, the authors demonstrated that cells formed viable multicellular aggregates after 3 days of perfusion culture. In a similar gel-free approach to facilitate perfusion and mass transfer, the surfaces of C3A and A549 carcinoma cells were modified to contain free aldehyde groups, which reacted with an intercellular linker to form hydrazone covalent linkages between cells, facilitating cell aggregation within micropillar arrays.[33] While interesting, further investigation into the effect of fluid flow on the growth and survival of these cancer cells is warranted. Although diffusional limitations exist with the use of hydrogels as scaffolds, few in the literature have explored the possibility of using porous polymeric scaffolds to culture cancer cells within microfluidic systems. In order to evaluate the cytotoxicity of anti-cancer drugs together with liver metabolism, Ma et al. developed a micro-scale perfusion-based two-chamber system, where liver cells and glioblastoma multiforme brain cancer cells were separately cultured within two adjacent chambers housing porous poly(lactic acid) scaffolds.[34] The study demonstrated that while hepatic metabolism has a detrimental effect on the efficacy of the drug temozolomide, it is actually required for the prodrug ifosfamide to exert cytotoxicity on the cancer cells, suggesting the potential of this system for testing metabolism-dependent toxicity of anti-cancer drugs, an important aspect often overlooked in current *in vitro* systems.

In addition to the use of microfluidic platforms to study tumor migration and drug response, another research focus has been the development of microfluidic tumor models for the study of tumor vascularization and anti-angiogenic therapy.[35] The use of endothelialized microfluidics has led to a deeper understanding of pathological vascular networks [36], elucidating the role of vascular barrier function [37], with successful description of tumor microvasculature and its effect on tumor progression.[25,38] Recent studies demonstrated the *in vitro* engineering of perfusable blood vessel analogues made by microfluidic channels to model and investigate tumor cell intravasation [37] and extravasation.[25] In one example, Zervantonakis et al. developed a microfluidic platform consisting of microchannels interconnected by a 3D ECM hydrogel, which enabled the real-time analysis of cancer cell invasion and intravasation into an endothelial monolayer.[37] Endothelial barrier function was found to be modulated by soluble biochemical factors and macrophages, which directly impacted the rate of tumor intravasation.[37] These attempts are however, limited in their ability to recreate the inherent characteristic properties of *in vivo* endothelia, due to the artificially-induced blood vessel morphogenesis. To overcome this problem, other microfluidic approaches have made important advances toward the formation of 3D capillary tubes in a more physiological manner, by inducing the in vitro formation of capillary networks as opposed to generating endothelial cell-lined channels, through the synergistic use of an alginate-based framework and medium perfusion.[39] A recent investigation by Kim et al. tried to recapitulate all these aspects within a perfusable microfluidic system, by combining heterotypic cell-cell interactions in 3D constructs together with the formation of interconnected networks of microvessels.[38] The high fidelity of this system resulted in vascular-specific response to shear stress of the endothelium, satisfactory recreation of angiogenesis and vasculogenesis, and perfusion of medium through the engineered microvessels.

In summary, this section demonstrates the broad applicability of microfluidic systems to model the manifold aspects of tumor progression. Notably, given the versatility offered by microfluidic systems, it is crucial to identify the fundamental parameters in each application so that the resulting microfluidic models are uniquely tailored to accurately reflect specific oncological mechanisms. For instance, while the co-culture of liver cells with cancer cells is necessary to simulate the dependency of anti-cancer drug efficacy on hepatic metabolism, the inclusion of liver cells may not be as critical in evaluating the migratory behavior of cancer cells in response to interstitial flow.[28,34]

Macroscale Perfused Tumor Models

Despite the exponential progress in the last decade, the so-called ‘world-to-chip’ problem continues to persist in the field of microfluidics.[40] Due to the inherent differences in scale, even the simplest microfluidic configuration may lead to differences in cellular response when compared to classical macroscale systems used for biological assays.[37,41] This discrepancy may be more apparent in modeling tumorigenesis, a highly complex process which engages both the micro- and macro-environment.[42] Accordingly, macroscale perfused tissue-engineered systems, such as flow perfusion bioreactors, may be a necessary affiliate to microfluidic culture systems in modeling tumor progression. However, given that the tumor engineering community is only beginning to appreciate the need for perfused macroscale models to mimic the biophysical properties of the tumor microenvironment and to overcome diffusional limitations,[1] literature in this area is still sparse. Amongst the few examples in the literature, Mishra et al. extrapolated the concept of organ reengineering to develop an *ex vivo* lung cancer model, by using a decellularized rat lung matrix as a scaffold to culture human lung cancer cells.[43] The authors demonstrated that the seeded lung cancer cells grown within the native matrix formed perfusable tumor nodules with similar

features to the original human lung cancer, suggesting the applicability of this *ex vivo* model for mechanistic studies of lung cancer progression.

Other potentially translatable techniques developed for perfusing normal tissue constructs to tumor engineering are currently only at the conception stage. Perfused macroscale systems have seen success in modeling normal tissues such as musculoskeletal [44-46] and cardiovascular tissues [47], where they have been used to engineer uniform constructs at the millimeter-scale in the presence of physiological stimuli such as electrical stimulation, shear stress and native tissue contraction.[47] Notably, even though these systems do not offer the same degree of spatiotemporal control as their microfluidic counterparts, precise mathematical modeling can be employed to predict flow conditions and correlate the cellular response to system specifications.[47,48] Leveraging similar porous polymeric scaffolds used for engineering perfusable normal tissues [46,49,50], the development of 3D biomimetic tumor models which recapitulate aspects of the tumor microenvironment has been reported.[51-53] In a landmark study, by culturing human oral squamous carcinoma cells within porous poly(lactic-co-glycolic acid) scaffolds, Fischbach et al. established a 3D engineered model that exhibits *in vivo*-like angiogenic characteristics and drug response.[51] More recently, our laboratory demonstrated the feasibility of employing scaffolds that are compatible with flow perfusion bioreactor systems and previously used for creating perfusable bone tissue constructs, to the development of *in vitro* bone tumors.[53] By culturing Ewing sarcoma cells within porous 3D electrospun poly(ε-caprolactone) scaffolds, we observed that the tumor cells were not only more resistant to traditional cytotoxic drugs but also exhibited remarkable differences in the expression pattern of the insulin-like growth factor-1 receptor/mammalian target of rapamycin pathway as compared to cells cultured in 2D monolayer.[53]

Given that promising advances have been achieved by the tumor engineering community towards the development of *in vivo*-like tumor constructs, one can imagine that the incorporation of actual perfusion into these technically perfusable tissue-engineered tumor constructs would greatly enhance our understanding of the interplay between architectural, biochemical and biophysical cues and their impact on tumor progression. Notably, while bioreactors in tissue engineering often serve only to support an intermediate tissue construct that would eventually be implanted *in* vivo for further tissue maturation to occur, the ultimate goal in tumor engineering is to *fully* recreate the tumor tissue *in vitro* to derive a fundamental and accurate understanding of cancer biology. Hence, efforts to develop macroscale perfused cancer models should not only focus on translating current bioreactor strategies in tissue engineering to tumor engineering, but also in the adaptation of these strategies to account for the need to fully model the tumor microenvironment *in vitro* with high fidelity.

Conclusions

A microfluidic approach to the development of tissue-engineered, perfused tumor models is evidently dominant in the literature. The strong relevance of microfluidic cultures in oncology is not surprising, given the recognized molecular basis of cancer and the inherent capacity of these systems to precisely model biological processes occurring at the cellular level. As microfluidic approaches become progressively versatile in accommodating more advanced tissue-engineered scaffolds, one can expect that these miniature systems are likely to meet the mammoth challenge of synergistically integrating both biochemical and biophysical cues together, with the capability to model tumor vasculature and interstitial flow.

On the other end, the hierarchical nature of tumorigenesis - evolving on multiple scales, from the subcellular to the organ level - has long been recognized, underscoring the importance of macroscale models as a necessary partner to those at the microscale. Furthermore, given the significant advances in tumor engineering in terms of recapitulating architectural cues and tumor-stroma interactions at the macroscale, a logical progression in this field is the introduction of perfusion strategies to more fully mimic the tumor microenvironment.

With both micro- and macro-approaches currently advancing in parallel and exchanging technology, one can envisage an ultimate convergence toward the development of perfused, *in vivo*-like tissues *in vitro*, potentially resulting in paradigm-changing breakthroughs in the evaluation of drug efficacy and mechanistic studies of tumor biology.

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Highlights

- Role of fluid flow in cancer progression remains inadequately elucidated.
- Recapitulation of fluid dynamics requires both microfluidic and macroscale models.
- Microfluidic models enable oncology investigations at the single-cell level.
- Macroscale models can reproducibly mimic the hierarchical nature of tumorigenesis.

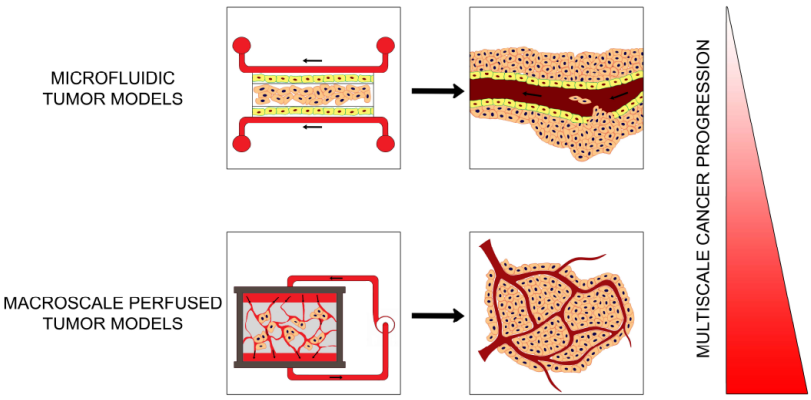

Figure 1.
Hierarchical nature of cancer progression. The integrated use of microfluidic and macroscale perfused models may synergistically enhance our understanding of fluid dynamics in tumorigenesis on multiscales.