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3D printing for the development of *in vitro* cancer models

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Abstract

In vitro engineering of tumor milieus is complex because cancer progression and metastasis involve spatio-temporally evolving cell–matrix interactions, myriad interactions between tumor cells and auxiliary cells, hypoxic cores, leaky unorganized vasculature and a host of signaling molecules. Recent advances in 3D printing approaches enable the precise placement of cells, bioactive factors and biomaterials, thus permitting the recapitulation of several features associated with the *in vivo* tumor microenvironment. 3D printed *in vitro* tumor models can serve as robust platforms to study mechanisms of disease progression, enable high throughput screening of drugs and aid the development of next generation molecular therapies. This focused review discusses the importance and relevance of 3D printing technologies in building tumor models *in vitro*. Several recent 3D printed cancer models are discussed, as also the evolution and features of next-generation models.

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Introduction

Studying the development and metastasis of cancer, and the subsequent development of therapies, are both very challenging due to the complex interplay of several biological factors that contribute to disease progression [1,2]. Traditional approaches to understanding cancer progression have largely involved animal models and 2D cultures. While animal models (e.g., xenograft models, chemically induced models and genetically manipulated models) have been widely used for pre-clinical testing [3], they are expensive, cumbersome and often fail to recapitulate critical aspects of human tissues due to inter-species biological differences [4,5]. As a result, they can lead to erroneous predictions with regard to the ultimate efficacy of drugs in clinical trials [5–7]. Animal models also do not easily permit the systematic investigation of the contributions of specific microenvironmental factors towards influencing cancer cell migration and metastasis. While some of these limitations are overcome with 2D cultures of diseased human cells, critical features of tumors such as cell-extracellular matrix (ECM) interactions and hypoxic cores are not accurately captured in monolayer cultures [8,9]. Moreover, cell morphology, gene/protein expression, proliferation/migration and drug susceptibilities are different in 2D and 3D cultures [8,10–12].

Capturing the heterogeneous composition and organization of tumor microenvironments requires precise spatio-temporal control over matrix properties, the presence of multiple bioactive factors, leaky vasculature and paracrine interactions between multiple cell types involved in cancer progression [13–15]. Advances in tissue engineering paradigms have demonstrated that biomaterial matrices can mimic the *in vivo* tissue microenvironment and consequently direct cell fate by promoting cell–matrix interactions [16]. Importantly, the mechano-chemical properties of matrices can be precisely tuned by an appropriate choice of biomaterial, fabrication technique and processing conditions [17]. These advances strongly indicate that biomaterial matrices can be used for developing 3D *in vivo* like tumor microenvironments that can potentially overcome the limitations with current cancer models. In turn, engineered tumors can be used for understanding cell-ECM interactions, investigating mechanisms of disease progression and assessing drug efficacies. When coupled with bioreactors [18], 3D models can potentially maintain long-term phenotypic functionality and also accurately predict drug responses.

The objective of this concise review is to present advances, primarily over the last five years, in the development of *in vitro* cancer models based on 3D printed biomaterial platforms. 3D printing approaches are particularly attractive for engineering the tumor microenvironment because they permit multi-level spatial control of cell organization and placement of materials/

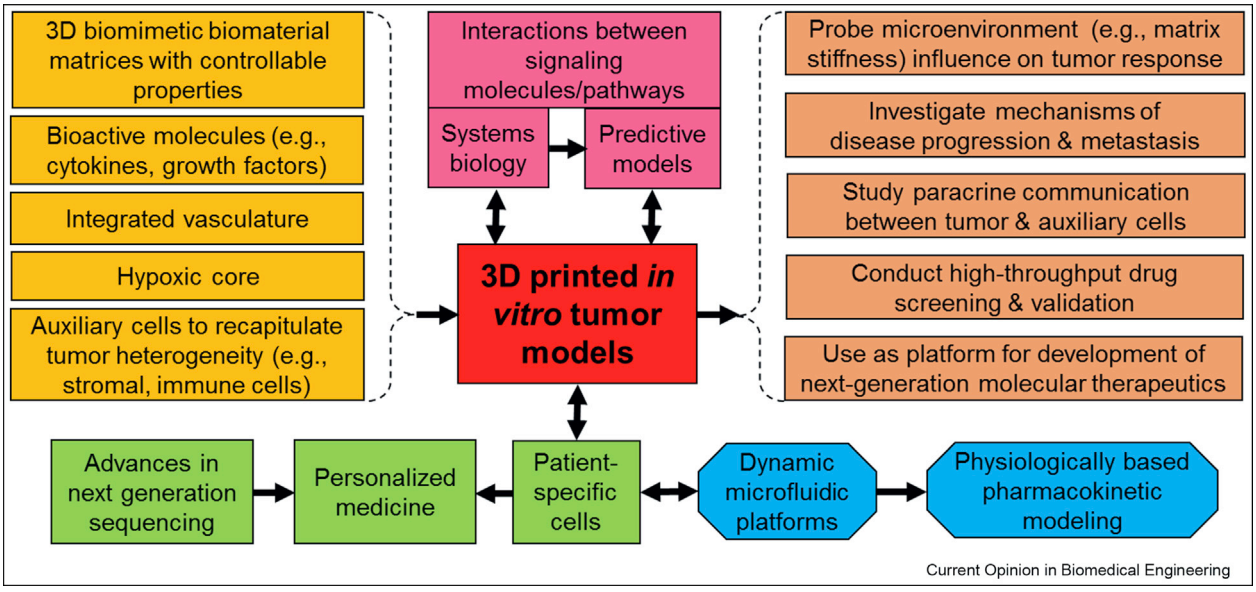
biomolecules [19], thus allowing precise recapitulation of ECM properties. Recent advances in 3D printing technologies also facilitate the delivery of various cell types and polymers into a single construct with the concomitant incorporation of micro-channels that aid nutrient and oxygen diffusion [20]. In addition, 3D printing approaches readily lend themselves to integration with other allied scaffold platforms (e.g., microspheres, nanofibers) [21,22]. These advantages and the relevance of 3D printing in tumor modeling are elaborated in the next section. Thereafter, we discuss several recent studies reporting the use of 3D printed platforms to investigate tumor spheroid formation, cell migration/metastasis, cell–cell communication and drug testing. Finally, we conclude with recommendations for future innovations in 3D printed *in vitro* cancer models. A schematic of the development and applications of 3D tumor models is presented in Figure 1.

3D printing technologies: merits, advances and relevance in tumor modeling

3D printing offers the unique ability to create architecturally and compositionally complex biomimetic microenvironments with high reproducibility. In traditional bottom-up approaches, polymers have been printed using extrusion-based techniques such as fused filament fabrication to create scaffolds with controllable porosity [23,24]. Although they can possess high mechanical strength, such scaffolds suffer from low resolution and must be cultured with cells post-fabrication. In addition to the limited choice of printing materials, high temperatures used during fused filament fabrication disallow *in situ* incorporation of cells and sensitive biomolecules [25]. In contrast, 3D bioprinting approaches such as direct-ink writing [26,27] and inkjet-printing [28] enable the direct incorporation of cells. Although inkjet printing provides high-throughput, it can result in needle clogging at high ink viscosities and potentially expose cells to high shear forces [19]. Another popular and rapid technique that allows the *in situ* incorporation of cells is stereolithography [29,30], which also provides high resolution and easy control of matrix properties. However, monomer toxicity and the use of ultraviolet radiation for curing are potential concerns for long-term cell viability. Recent advances in laser-direct-write printing have enabled the bioprinting of continuous 3D microstructures comprised of overlapping microbeads (with incorporated cells) whose architecture and composition at the individual-microbead level can be controlled [31]. The aqueous cores of these microbeads can promote rapid cell proliferation and also overcome diffusion limitations associated with conventional encapsulation.

One of the key considerations in 3D bioprinting is the choice of ink, which is usually a hydrogel-based formulation. Hydrogels must be biocompatible, easily printable and should result in robust cross-linked structures with sufficient integrity post-printing [32]. Since the tumor microenvironment is a complex matrix whose composition and mechanics not only evolve spatially but also change with the type of tumor and the stage of disease [33], the choice of bioink plays a critical role in recreating an *in vivo*-like tumor environment. While naturally available materials such as collagen, fibrin, gelatin and matrigel possess properties similar to ECM, synthetic polymers such as polyethylene glycol (PEG) and pluronics offer greater control over matrix properties

Figure 1



Schematic depicting the development of 3D *in vitro* cancer models and their applications.