PERSPECTIVE

Advancing cancer research using bioprinting for tumor-on-a-chip platforms

Stephanie Knowlton[1], Ashwini Joshi[1], Bekir Yenilmez[2], Ibrahim Tarik Ozbolat[3,4], Chee Kai Chua[5], Ali Khademhosseini[6,7,8] and Savas Tasoglu[1,2*]

[1] Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06269, USA
[2] Department of Mechanical Engineering, University of Connecticut, Storrs, CT 06269, USA
[3] Engineering Science and Mechanics Department, Pennsylvania State University, University Park, PA 16802, USA
[4] The Huck Institute of the Life Sciences, Pennsylvania State University, University Park, PA 16802, USA
[5] Singapore Centre for 3D Printing (SC3DP), School of Mechanical and Aerospace Engineering, Nanyang Technological University (NTU), 50 Nanyang Avenue, Singapore 639798, Singapore
[6] Biomaterials Innovation Research Center, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[7] Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[8] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA

Abstract: There is an urgent for a novel approach to cancer research with 1.7 million new cases of cancer occurring every year in the United States of America. Tumor models offer promise as a useful platform for cancer research without the need for animal models, but there remains a challenge to fabricate a relevant model which mimics the structure, function and drug response of human tumors. Bioprinting can address this need by fabricating three-dimensional constructs that mimic tumor heterogeneity, vasculature and spheroid structures. Furthermore, bioprinting can be used to fabricate tissue constructs within microfluidic platforms, forming "tumor-on-a-chip" devices which are ideal for high-throughput testing in a biomimetic microenvironment. Applications of tumors-on-a-chip include facilitating basic research to better understand tumor development, structure and function as well as drug screening to improve the efficiency of cancer drug discovery.

Keywords: bioprinting, cancer, tumor-on-a-chip, microfabrication, microfluidics, drug screening

*Correspondence to: Savas Tasoglu, Department of Biomedical Engineering and Department of Mechanical Engineering, University of Connecticut, Storrs, CT, USA; Email: savas@engr.uconn.edu

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1. Clinical and Pharmaceutical Need for Three-Dimensional (3D) Tumor-on-a-chip Platforms

With an estimated ~1.7 million new cases of cancer occurring in the United States of America (USA) in 2016[1], there is a growing need for innovative cancer research approaches to develop more effective therapies. Rapid innovation in bioprinting technology has great potential in cancer research and therapy. Bioprinting enables fabrication of three-dimensional (3D) cancer models for basic science research and for testing pharmaceuticals and

therapies *in vitro*. Traditional two-dimensional (2D) approaches to cancer research have left significant gaps in our understanding of the disease as well as our ability to develop effective treatments. This is partly due to the inability of 2D cancer models to recapitulate the microenvironment of a tumor which exists in the human body. Past studies have demonstrated a significant difference in cell behavior between 2D and 3D models, specifically in terms of protein expression[2] and gradient profiles[3], drug response[4,5], as well as cell migration[6], morphology[7], proliferation[8] and viability[7]. Cell-cell and cell-matrix interactions are enhanced in 3D models compared to 2D, offering a more physiologically-relevant microenvironment. Bioprinting offers the ability to generate cancer models with 3D complexity in a high-throughput, reproducible manner which better reflects tumor anatomy, biology and function and will serve as a platform for further cancer research[9].

Integration of fabricated tissues into microfluidic devices has given rise to a new field of interest, called "organs-on-a-chip," adding a new level of complexity in the ability to model living organs *in vitro*. Use of microfluidic devices as a platform for tissue engineering offers several advantages over static culture[10,11]. Exposing tissues to continuous fluid flow over a prolonged time allows integration of dynamic mechanical cues into biomimetic systems. These cues, such as shear stress, are crucial to accurately mimic the physiological microenvironment in *in vitro* systems. In particular to tumor models, it has been shown that interstitial fluid flow in and around the tissue generates shear stress, which causes cell cycle arrest in tumor cell lines[12]. It has also been shown that cancer cells migrate along the direction of fluid streamlines in 3D scaffolds[13], further highlighting the importance of mechanical cues to modulate molecular signals, gene expression, and cell proliferation and migration. Moreover, due to the small dimensions of microfluidic channels, the flow in these devices is laminar, thus affording the ability to generate complex and highly controllable fluid flow regimes. For example, this capability enables generation of sustainable gradients of chemicals and biomolecules to study cell response to chemotactic stimuli. Chemotaxis is known to be important for tumor cell homing, which plays an integral role in cancer metastasis[14]. Lab-on-a-chip platforms not only recreate a biomimetic microenvironment, but also offer high throughput for systematic testing, such as drug screening[15].

2. Advantages of Bioprinting for Tumor-on-a-chip Fabrication

2.1 Mimicking Tumor Heterogeneity

To mimic the tumor microenvironment, 3D-printed tissues must mimic various features of *in vivo* tumors, including heterogeneous distribution of several different cell types and biomolecules, in order to serve as a physiologically-relevant model for cancer research. With the bioprinting technology, cell-aggregate based bioinks can contain multiple cell types[16] such as cancer-associated fibroblasts, immune cells and endothelial cells that create vascular networks[17]. Bioprinting has been used to fabricate a 3D co-culture tumor model comprised of cancer and fibroblast cells with a high degree of spatial control over the microenvironment[18]. It is also important to consider the heterogeneous distribution of biologically-relevant proteins and growth factors in the tissue scaffold, which are essential to control cell signaling, proliferation, and migration[19]. For example, biomolecule gradients which may signal cancer metastasis[20] can be recreated using bioprinting techniques. In summary, bioprinting provides a method to mimic the heterogeneous tumor microenvironment *in vitro* with a high level of precision, throughput and reproducibility.

2.2 Modeling Tumor Vasculature

Tumor vasculature differs greatly from the vessels that supply healthy tissue, specifically in the heterogeneity, permeability, multi-directional blood flow, and irregular distribution throughout the tumor[21]. These abnormalities can be mimicked by using 3D-printed vascular networks which can be further utilized to test and compare the behavior of healthy and abnormal vasculature under different conditions and therapies. In one study, 25, 45, and 120 micron channels were 3D printed based on micro-computed tomography (μCT) scans of rat capillaries[22]. This biomimetic chip was used to observe the differences in cancer cell migration through vessels of different sizes.

Understanding tumor vasculature is also crucial to understanding drug delivery to tumors and developing effective chemotherapeutics. The leaky and poorly-organized blood vessels supplying tumors significantly impact drug delivery[23]. This makes it difficult to test drugs in alternative tissue models due to differences in drug permeability through normal vasculature compared to leaky vessels. However, in future bioprinting

applications, these diseased vascular structures may be replicated *in vitro* using bioprinting in order to test targeted therapies and assess drug delivery.

2.3 Forming Tumor Spheroids

Tumor spheroids are known to closely resemble the tumor microenvironment[24,25] and express the biochemical gradients associated with tumor growth[24]. Thus, tumor spheroids are widely used to study cancer processes and therapies[25]. Recently, 3D projection printing was used to fabricate concave polyethylene glycol (PEG) hydrogel structures that facilitated the growth and viability of tumor spheroids in the long term[25]. In this study, the properties of a breast cancer spheroid grown to day 10 closely matched the hypoxic and necrotic properties expected of a tumor spheroid. These spheroids were stained for HIF-1α, a marker for hypoxia, and found to contain the characteristic hypoxic core that prompts further tumor growth *in vivo*. The 3D-printed concave hydrogel structures are a promising low-cost, reproducible platform for long-term spheroid culture and high-throughput cancer studies.

3. Bioprinting for Tumor-on-a-chip Models

3.1 Modeling Tumors in Microfluidic Platforms

Tumor models in microfluidic platforms have demonstrated promising results in studying cancer growth, metastasis and treatments *in vitro*. One study generated a device, dubbed "disease-on-a-chip," to grow phenotypically normal breast epithelial tissue, which modeled mammary ducts and mimicked the development of tumor nodules within a breast tissue environment[26]. That study showed that tumor nodules within the biomimetic platform displayed morphological and anti-cancer drug sensitivity differences compared to cultures on flat surfaces. Another study demonstrated the ability to model natural fluidic streams using continuous laminar flow in microfluidic chips[27]. The microfluidic chips in this work enabled studies on the effect of shear stress on tumor cell metastasis and ovarian cancer nodule formation. Results showed flow-induced changes in E-cadherin protein expression and an increase in vimentin leading to increased metastatic potential. Tumor models have been also used in screening for optimal nanoparticle transport for nanoparticle-based therapies[28,29].

3.2 Bioprinting-assisted Fabrication in Microfluidic Platforms

In light of the demonstrated potential to generate biomimetic tumor models via bioprinting, it is important to consider practical fabrication approaches for bioprinting within microfluidic platforms. 3D microorgans have been generated via direct cell writing into microfluidic circuits which were fabricated using standard soft-lithography techniques using PDMS followed by bonding of the PDMS channels to a glass slide[30]. One study compared two approaches for introducing cells into microfluidic devices fabricated via precision extrusion deposition and replica molding[31]. In one approach, cells were placed directly into the exposed channels of the replica-molded microfluidic channels and then covered with a PDMS cover component. In an alternative approach, cells were guided to form networks along open channel walls and then embedded fully in PDMS to produce a leak-resistant open channel network with a simplified fabrication method. Another proposed fabrication technique involves digital micro-mirroring to fabricate the channel structure combined with multi-nozzle biological deposition to print cells into the channels of the device[32]. Bioprinting has also been performed in parallel with the chip fabrication using an integrated solid freeform fabrication system, reducing the need for photomasks and eliminating the long fabrication process and harsh chemicals traditionally used for fabrication[33]. The platform utilized a four print-head system, each capable of 3D motion: a photopolymer head to deposit photoresist for the chip architecture; a photolighographic head to crosslink the photoresist after deposition; a plasma treatment head to treat channels with helium and oxygen plasma prior to cell deposition; and a biologics head for cell deposition into the microchannels. This approach has been applied to generate a cancer co-culture model within a microfluidic environment.

4. Conclusion and Future Perspectives

Incorporation of bioprinted tumor models into lab-on-a-chip platforms presents a promising direction for cancer research, offering the ability to mimic physiological, mechanical and chemical cues and conduct high-throughput studies[15]. Novel bioprinting techniques are essential to precisely fabricate tumor constructs in lab-on-a-chip platforms. A promising application for this technology is high-throughput drug screening of anti-cancer drugs using microfluidic-based tumor-on-a-chip models. Bioprinted cancer models offer several advantages over animal and human models to test drugs. As obtaining FDA approval for a new drug costs a great deal of time (up to 15 years) and

money (US $2.6 billion)[34,35], there is a need for alternative options in preclinical drug testing[36]. A low-cost, reproducible model that mimics tumors, including the microenvironment, cell distribution and vasculature, would allow high-throughput drug screening prior to clinical trials as an efficient alternative to animal models. Such a bioprinted model has already been reported for cervical cancer[5]. Additionally, bioprinted models can be used to test other materials relevant to drug delivery, such as scaffolds for releasing signals[37] and polymer microspheres for biodegradation studies[38].

Although there is room for further innovation in bioprinting, this approach shows great promise for efficient generation of biomimetic tumor models to further advance and accelerate cancer research. A unique advantage of bioprinting compared to other microfabrication techniques is the ability to precisely control the spatial arrangement of cells and complex tissue architectures with ease[39–42]. The technology offers high throughput and excellent reproducibility, generating cancer tissue models which closely mimic the structure and function of tumors *in vivo*, including tumor heterogeneity and vascular structures. With rapid advances in bioprinting technology for cancer models, there is potential to expand our basic understanding of cancer and develop effective therapies.

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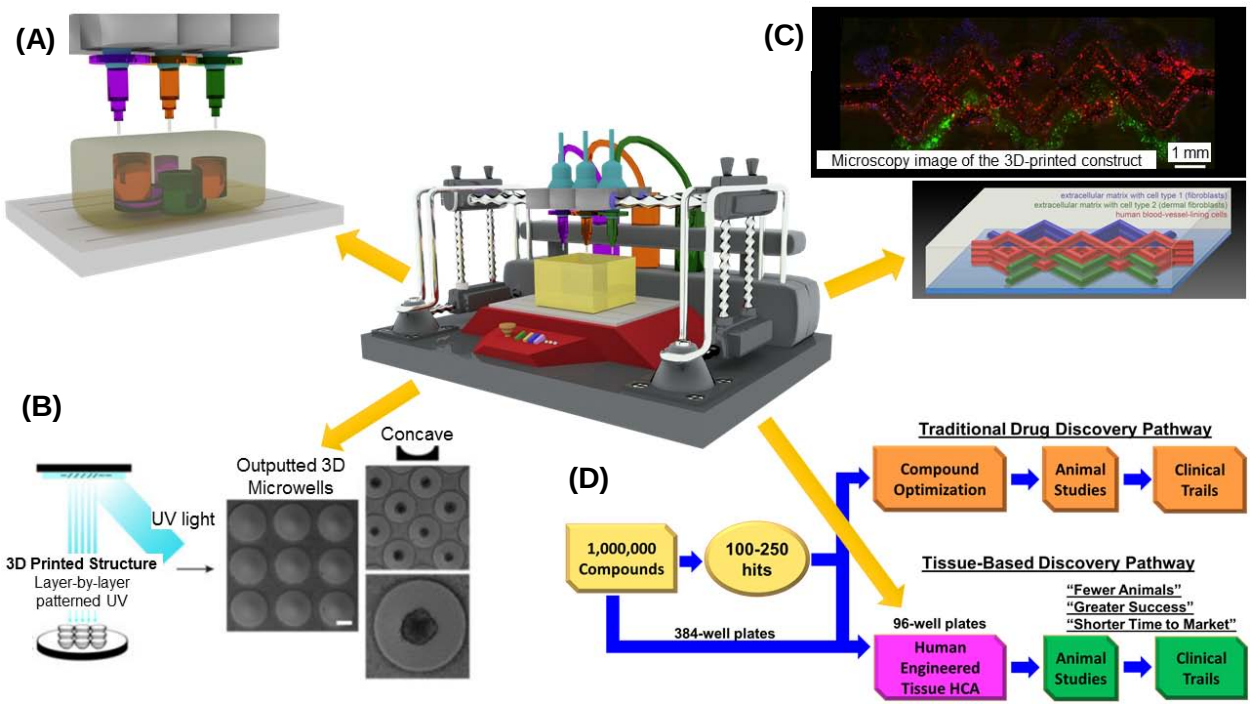

Figure 1. Advancing cancer research using bioprinting. **(A)** 3D bioprinting of heterogeneous tissues. **(B)** 3D printing of 3D microwells to facilitate spheroid formation. Reproduced with permission from[25]. **(C)** 3D bioprinting of vascularized tissue models. Reproduced with permission from[43]. **(D)** Traditional drug discovery pathway compared to a tissue-based discovery pathway enabled by bioprinting. Adapted from[44].

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