Review

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Bioprinting for cancer research

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Bioprinting offers the ability to create highly complex 3D architectures with living cells. This cutting-edge technique has significantly gained popularity and applicability in several fields. Bioprinting methods have been developed to effectively and rapidly pattern living cells, biological macromolecules, and biomaterials. These technologies hold great potential for applications in cancer research. Bioprinted cancer models represent a significant improvement over previous 2D models by mimicking 3D complexity and facilitating physiologically relevant cell–cell and cell–matrix interactions. Here we review bioprinting methods based on inkjet, microextrusion, and laser technologies and compare 3D cancer models with 2D cancer models. We discuss bioprinted models that mimic the tumor microenvironment, providing a platform for deeper understanding of cancer pathology, anticancer drug screening, and cancer treatment development.

Application of bioprinting to cancer research

Cancer remains one of the most predominant life-threatening diseases in the world, with 14 million new cases of cancer and 8.2 million cancer-related deaths worldwide in 2012. The annual number of cases is predicted to rise from 14 million to 22 million over the next two decades [1]. The economic burden in the USA was US$88.7 billion in 2011 based on direct medical costs alone [American Cancer Society (2015) Economic impact of cancer (http://www.cancer.org/cancer/cancerbasics/economic-impact-of-cancer)]. There are hundreds of known types of cancer and the disease is highly complex even within a single cancer type, making the development of a single cure an astronomical task [2,3]. To gain a better understanding of cancer genesis and progression, there is a need for more complex and physiologically relevant 3D cancer models that closely mimic the *in vivo* tumor microenvironment. In light of these challenges, bioprinting offers the ability to form highly controllable cancer tissue models and shows potential to significantly accelerate cancer research.

2D cancer models are widely used for cancer research, contributing to our basic knowledge of cancer biology. Protein expression [4], gene expression [5], protein gradient profiles and cell signaling [6,7], migration [8], morphology [9], proliferation [10], viability [9], organization [9], and drug response [11,12] have been shown to differ between 2D and 3D cancer models [6,13]. Although 2D cultures offer hypothetical results regarding cancer pathogenesis, it is necessary to expose cancer cells to the cell–cell and cell–matrix interactions they would experience *in vivo* to achieve more physiologically relevant results. Thus, cancer studies using 3D models have achieved more accurate representations of cancer tissues in terms of tumor microenvironment and biological behavior with controlled spatial distribution of cells, which is crucial for developing early diagnosis and treatment strategies for cancer.

3D printing is an additive manufacturing process by which precursor materials are deposited layer by layer to form complex 3D geometries from computer-aided designs [14–16]. A notable advantage of 3D printing is that complex architectures may be printed with efficiency and customizability either on an industrial scale or on a desktop-printing scale. 3D printing has more recently been developed into a process called bioprinting in which living cells, extracellular matrix (ECM) components, biomaterials, and biochemical factors are printed onto a receiving substrate or liquid reservoir [17–20]. The interest in bioprinting has significantly grown within the scientific and medical communities due to several key advantages over previously accepted fabrication methods such as photolithography, soft lithography, and microstamping. These advantages include the ability to create geometrically complex scaffolds containing viable cells [18,19,21], efficiency, low cost [22], high throughput [23], precise reproducibility [18], and limited need for specialized training. High-throughput fabrication of 3D structures is currently limited with traditional microfabrication techniques that generate 2D building blocks and rely on layer-by-layer assembly to form 3D structures [24–32]. Current methods for co-culturing multiple cell types in desired configurations lack high-throughput capabilities, demanding multiple labor-intensive fabrication steps [23], but spatial patterning of different cell types or ECM components is possible using various 'bio-inks' for printing [33]. With these unique advantages, bioprinting offers a broad range of applications including biochemical surface patterning and *in situ* printing of biomaterials for wound healing as well as designing 3D tissue constructs for basic research,

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Table 1. Comparison of common bioprinting technologies

Performance metric	Microextrusion bioprinting	Laser-assisted bioprinting	Inkjet bioprinting	Refs
Throughput	Medium	Low to medium	High	[23]
Droplet size	5 μm to millimeters wide	>20–80 μm	50–300 μm	[23,55,88]
Spatial resolution	Medium	Medium to high	Medium	[23]
Single-cell encapsulation control	Medium	Medium to high	Low	[23]
Cell viability	40–80%	>95%	>85%	[55]
Cell density	High	Medium, 10^8 cells/ml	Low, $<10^6$ cells/ml	[55]
Material/hydrogel viscosity	30 mPa.s to > 600 kPa.s	1–300 mPa.s	<10 mPa.s	[37,55]
Gelation method	Chemical, ionic, enzymatic, photocrosslinking, shear thinning, thermal, pH	Ionic	Ionic, enzymatic, photocrosslinking, thermal	[89]
Gelation speed	Medium	High	High	[89]
Print/fabrication speed	High	Low	Medium	[89]
Printer cost	Medium	High	Low	[55]

regenerative medicine, disease modeling, or pharmaceutical research.

This review focuses on recent advances in the use of bioprinting technologies for cancer research, bioprinting physiologically relevant testing platforms for anticancer drug development, and computational modeling for improving bioprinting techniques.

Bioprinting techniques applied to 3D tumor models

Within the field of bioprinting, there are several strategies by which biological organization and complexity have been successfully modeled: inkjet-based [34,35], microextrusion [36–39], and laser-assisted bioprinting [40–44] (Table 1). Inkjet-based bioprinting involves generating droplets of bio-ink at the print head assisted by either a heater or a piezoelectric actuator (Figure 1A). Microextrusion bioprinting can be achieved using either pneumatic [36–39] or mechanical (piston or screw driven) forces [36–39,45–47] to extrude a continuous stream of a bio-ink (Figure 1B). Laser-assisted bioprinting can be conducted by two methods: laser guided or laser induced. In the laser-guided direct cell-printing method, a laser beam is directed into a cell suspension. The difference in refractive indices of cells and cell media enables a laser beam to trap and guide cells onto a receiving substrate [40,48] (Figure 1C). In the laser-induced bioprinting method, which is more common, a cell-laden hydrogel is deposited below a laser-absorbing layer that is used as a donor film and placed parallel to a receiving substrate (Figure 1D). Cell-encapsulating hydrogel droplets are transferred from the donor film to the receiving substrate due to the heat transfer from a laser pulse to the donor film and the pressure of a laser-induced vapor bubble [42,44,49,50]. Stereolithography, which involves curing a photoreactive material using light, has also been used for bioprinting. Digital micromirror projection printing uses a digital micro-mirror device to reflect UV light in a particular spatial pattern into a photopolymerizable macromer solution (Figure 2A) [51]. In this way, cells can be encapsulated in and seeded on 3D-patterned hydrogel scaffolds with a range of printable materials and control over microarchitecture and scaffold properties.

Two-step biofabrication

One method of bioprinting is a 'two-step' biofabrication method in which cell seeding is performed after 3D printing of the scaffold. Bioprinting can be used to generate precise biocompatible scaffolds for culturing cells with controllable structural features and composition. Digital micromirror device-based projection printing has been used to fabricate 3D polyethylene glycol (PEG) scaffolds with log-pile microarchitecture (Figure 2B–F) [52]. The elastic modulus of the scaffold was controlled by varying the PEG concentration without altering the structural or mechanical properties, allowing the effects of stiffness to be isolated and examined. Normal breast epithelial cells and Twist-transformed oncogenic cells were seeded onto the scaffold to study cell migration patterns. Cells cultured in 2D showed no statistical difference in migration on substrates with different stiffness. However, cells on 3D scaffolds demonstrated varying displacement, velocity, and path straightness depending on the scaffold stiffness and the presence of the Twist oncogene (Figure 2G–L). These results suggest that further research regarding cancer cell migration must be conducted in 3D systems.

One-step biofabrication

While 3D models can be generated via top-down methods by seeding cells into prefabricated scaffolds, there are limitations on controlling cell density, repeatability, spatial control, and scalability with this method [23]. In contrast to two-step bioprinting, one-step bioprinting methods print a mixture of hydrogel and cells, providing a more efficient way of fabricating 3D tissue models with less user input required [53]. A recent bioprinting technique has been shown to enable 3D patterning of human ovarian cancer (OVCAR-5) cells and normal fibroblasts on Matrigel™ with 3D complexity and spatial control over the microenvironment in terms of cell density and cell–cell distance [54]. This approach uses an automated XYZ stage with a dual ejector to position cell-encapsulating droplets at predefined locations on a substrate for high-throughput printing with high viability. OVCAR-5 cells were shown to proliferate and ultimately form acini (lobular structures) (Figure 3). Design parameters such as droplet ejection