3D bioprinting for oncology applications

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First draft submitted: 11 February 2019; Accepted for publication: 25 February 2019; Published online: 30 May 2019

Keywords: bioprinting • cancer • oncology • tumor microenvironment

Bioprinting for cancer modeling

Cancer is a leading cause of mortality and morbidity worldwide with approximately 18.1 million new cases and 9.6 million cancer-related deaths in 2018, affecting populations in all countries and regions [1]. Yet, it remains a grand challenge for the pharmaceutical industry to effectively develop anticancer drugs and, in clinical practices, to accurately select personalized therapeutics, making cancer one of the prime focal targets of biomedical research.

To this end, 3D bioprinting has emerged as a promising cluster of technologies attracting increasing multi-attention for its utilization in cancer tissue engineering, by allowing for recapitulation of the *in vivo* tumor microenvironment at high fidelity outside the human body. The motivation here is to improve our capacity in cancer modeling over existing strategies, in attempts to facilitate drug development as well as personalized therapeutic screening, especially for the latter where the use of 3D bioprinting may be conveniently combined with patient-derived cells and materials. Indeed, bioprinted 3D cancer models are anticipated to bridge the gap between conventional planar cell cultures and animals, and can possibly even replace animal models (e.g., patient-derived xenografts) in the case of personalized medicine [2,3].

Advantages of bioprinting for cancer modeling *in vitro*

Conventionally, cancer is modeled through monolayer cell cultures and in animals, which have collectively allowed us to make tremendous progress in our understanding of this deadly disease. Although animals are physiologically competent, they often fail to reproduce human behaviors and responses, and planar cell models are extremely simplified and not representative of what may happen *in vivo*.

In fact, in the native tumor microenvironment, which is highly complex, cells interact with other cells of same origin as well as different types and are in contact with a plethora of extracellular matrix molecules arranged in a volumetrically heterogeneous but hierarchical manner and are further evolving with time [4]. Therefore, 3D cell models are believed to better emulate the complex native tumor microenvironments although most of the existing biofabrication strategies have not been able to capture such complexity in cancer models at a satisfactory level. Yet, 3D bioprinting, featuring automated robotic processes capable of spatially assembling multiple types of cells and biomaterials with high reproducibility, almost perfectly tackles challenges faced by other biofabrication methods in building tightly controlled and well-defined structures of desired levels of complexity necessary to be incorporated into *in vitro* cancer models [2,3,5].

Applications of bioprinted cancer models

Tumor angiogenesis

One of the main difficulties in engineering 3D cancer models is the lack of vascular networks, which however, play a key role in transporting nutrients and oxygen to cells and therefore in cancer progression [6,7]. Bioprinting has a

unique advantage to integrate the vasculature with tumor models. For example, vascular structures were achieved via sacrificial bioprinting, where microchannels could be first formed within the hydrogel matrices through selective removal of the bioprinted fugitive bioinks, followed by seeding endothelial cells onto the interior surfaces of these microchannels to mimic the blood vessels; meanwhile, tumor microtissues could be encapsulated within the hydrogel matrices in the vicinity of the bioprinted microvessels, enabling investigations into tumor vascularization [8]. Other bioprinting strategies amenable to vascular integration include microfluidic bioprinting and stereolithography [9–11]. For the former, single- and multi-layered hollow tubular structures could be bioprinted in a single step, which when cellularized, would be a good extended model of standalone blood vessels [9,10]. For the latter, high-resolution vascular patterns could be formed to model the hierarchical and disordered tumor vascularization, with potential integration with multimaterial capacity [11].

Tumor microenvironment

The tumor microenvironment as a whole, containing many other components such as immune cells, cancer-associated fibroblasts, lymphocytes and extracellular matrix molecules, is equally important. It raises issues around precise compositional and structural controls, which can be aided through 3D bioprinting. For example, a recent model of a bioprinted minibrain consisting of glioblastoma cells and macrophages has been developed to study the dynamic interactions between these two cell types [12]. The model was based on two-step bioprinting process in which the hydrogel-based minibrain encapsulating RAW264.7 macrophages was first bioprinted to host an empty cavity, and then the second bioink containing GL261 glioblastoma cells was deposited into this cavity, providing an enabling *in vivo*-like microenvironment to allow for cross-talk between the two cell types. Indeed, the glioblastoma cells were found to actively recruit the immune cells and program them into glioma-associated macrophages, which in turn, promoted the invasive phenotype of the brain tumor cells. The unique advantages of 3D bioprinted tumor models will likely advance our understanding of tumor biology and its dynamic microenvironment.

Tumor metastasis

Through 3D bioprinting, the resulting tumor models would further enable more faithful studies on metastasis, a leading cause of cancer-associated mortality [13]. Although still preliminary, several studies are already present. For example, a breast cancer bone metastasis model was reported, which integrated 3D printed nanomaterials and utilized a table-top stereolithography-based 3D bioprinting technique to create an *in vitro* bone matrix, offering a biomimetic niche for investigating breast cancer bone invasion using MDA-MB-231 breast cancer cells and human bone marrow-derived mesenchymal stem cells within the printed 3D bone matrix [14]. In another interesting example, breast cancer cells were bioprinted onto *ex vivo* cultured rat mesenteric tissues using laser direct-write to study metastasis through time-lapse imaging [15]. This hybrid strategy can potentially help us observe both the native living tissue constructs and the deposited tumor cells, combining advantages of bioprinting with the competent *in vivo* tissue microenvironment.

Anticancer drug development & therapeutic screening

The use of 3D bioprinted cancer models provides a promising platform to innovate anticancer therapeutic agents. Drug development for cancer has been experiencing low success rates for decades with over 95% candidate drugs failing to enter the market [16]. For this, not only is bioprinting useful in generating cancer models of high biological and physiological relevancy, but it can also allow for construction of biomimetic models of normal tissues to create linked systems for simultaneous screening of both efficacy and side effects.

A model was produced by a co-extrusion bioprinting method in a single step, which was used to study the interactions between breast cancer cells encapsulated in the peptide-conjugated alginate fibers and macrophages within the channels, and explored the effects of drug treatment in a clinically visible manner [17] (also see [12] where both chemo- and immunotherapeutic agents were evaluated). Moreover, a 3D bioprinted model of *HER2*-positive breast cancer embedded in a matrix of adipose-derived mesenchymal stem cells (ADMSCs) was used to examine its response to doxorubicin and associated drug resistance [18]. This model may improve oversimplified planar co-cultures for studying interactions between ADMSCs and breast cancer cells, which are oftentimes insufficient in mimicking the breast cancer microenvironment leading to biased observations on the doses of anticancer drugs.

Perspective

One emerging future direction in the development of bioprinting for oncology applications is the use of patients' own cells, such as cancer cells, stromal cells and immune cells, for the creation of personalized 3D cancer models. Although 3D bioprinted cancer models allow the integration of various cell types in defined structures to precisely mimic the *in vivo* tumor microenvironments, ways to unify the different culture conditions are a remaining challenge.

Another challenge lies in the scaling of the bioprinted models in relation to their native counterparts. Although ways to scale include that based on tissue mass, volume or metabolic activity [19], it would perhaps ultimately be tumor type- and microenvironment-specific, so that accurate drug dosing, metabolism and toxic effects can be reproduced.

The final remark goes to the design of the bioinks. A bioink must be printable (suitable viscosity), crosslinkable (physically or chemically), biocompatible and bioactive. While bioactivity can be achieved through the use of natural polymers, synthetic materials can bring conveniently tunable mechanical properties; hence, a common approach relies on the combination of both components [2]. However, the tumor microenvironment is highly complex and stage- and tumor-type dependent, making it tempting to consider the use of conserved native matrices, such as the decellularized extracellular matrices [20,21], to serve as bioinks for the construction of tumor models with better phenotypic conservation.

Conclusion

In summary, cancer models featuring *in vivo*-like microenvironments made possible by 3D bioprinting have become an enabling tool for screening anticancer drugs and personalizing treatment regimens for individual cancer patients, as well as an important platform to study cancer biology and pathology.

Financial & competing interests disclosure

This work was supported by funds from the NIH (K99CA201603, R00CA201603, R21EB025270, R01EB028143) and the New England Anti-Vivisection Society. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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