

Biofabrication Technologies for Developing In Vitro Tumor Models

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Abstract Despite having yielded extensive breakthroughs in cancer research, traditional 2D cell cultures have limitations in studying cancer progression and metastasis and screening therapeutic candidates. 3D systems can allow cells to grow, migrate, and interact with each other and the surrounding matrix, resulting in more realistic constructs. Furthermore, interactions between host tissue and developing tumors influence the susceptibility of tumors to drug treatments. The past decade has seen a rapid advancement of the application of 3D cellular systems to cancer research. These 3D tumor models, or tumor organoids, occupy a range of distinct form factors, each with their own strengths and weaknesses, and appropriateness for particular applications. In this chapter we highlight the major categories of tumor organoids and the methods by which they are biofabricated, aiming to provide the reader with an overview of the types of tumor organoids currently employed in cancer research applications.

Keywords Organoids • Biofabrication • Bioprinting • Tissue engineering • Tissue construct • Tumor-on-a-chip

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1 Introduction

Cancer research has been limited due to the inability to accurately model tumor progression and signaling mechanisms in a controlled environment. Animal models allow limited manipulation and study of these mechanisms, and are not necessarily predictive of results in humans [1]. Traditional *in vitro* 2D cultures fail to recapitulate the 3D microenvironment of *in vivo* tissues [2]. Drug diffusion kinetics vary dramatically, drug doses effective in 2D are often ineffective when scaled to patients, and cell-cell/cell-matrix interactions are inaccurate [3, 4]. Tissue culture dishes have three major differences from the tissue where the tumor was isolated: surface topography, surface stiffness, and a 2D rather than 3D architecture. As a consequence, 2D culture places a selective pressure on cells that could substantially alter their molecular and phenotypic properties. We recently demonstrated that on 2D tissue culture dishes, metastatic colorectal cancer (CRC) cells appeared epithelial, but when transitioned into a 3D liver organoid environment they “switched” to a mesenchymal and metastatic phenotype [5]. Such bioengineered platforms better mimic structure and cellular heterogeneity of *in vivo* tissue, and are therefore more suitable for cancer research. These models can be viable for long periods of time and develop functional properties similar to native tissues. They can also recapitulate the dynamic roles of cell–cell, cell–ECM, and mechanical interactions inside the tumor. Further incorporation of cells representative of the tumor stroma, such as endothelial cells and tumor fibroblasts, and physical matrix components, can mimic the *in vivo* tumor microenvironment. Thus, bioengineered tumors are an important resource for *in vitro* study of cancer and development of new and more effective therapies for patients in the clinic. In this chapter, we describe a variety of the technologies researchers employ to create, or biofabricate, 3D *in vitro* tumor models. These technologies range from the simple – cell aggregates or spheroids – to significantly more complex – bioprinted tumor constructs using extracellular matrix bioinks for example (Fig. 1).

2 Tumor Spheroids

Tumor spheroids represent perhaps some of the earliest forays into creating model systems of tumors with enhanced complexity compared to traditional 2D cell cultures on dishes or well plates. Spheroids are spherical cell aggregates formed by cell-cell adhesions during self-assembly. They are simple and generally quick to produce, thus their widespread appeal [6]. Cell spheroids are generally on the scale of several hundred microns at the maximum, as larger aggregate suffer from poor oxygen and nutrient transport into the center of the spheroid, often resulting in necrotic core regions. However, this feature can mimic necrotic core regions occasionally found in some tumors *in vivo*. Importantly, the 3D nature of tumor spheroids (and many of the other tumor organoid form factors discussed later) provide a

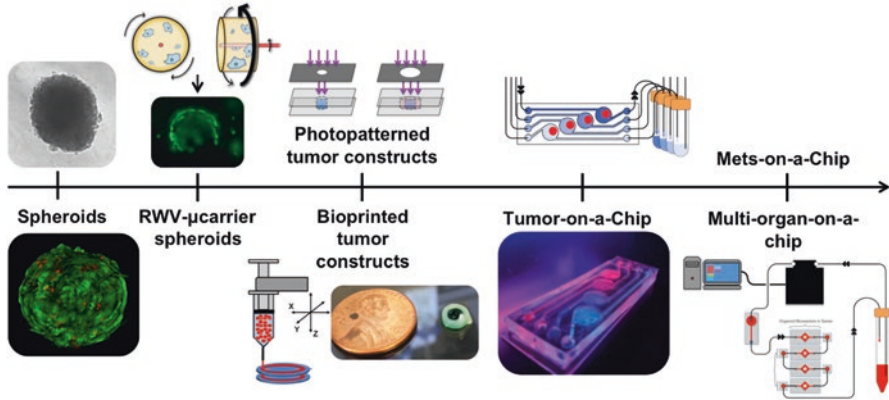


Fig. 1 Tumor organoids vary widely in complexity from simple spheroids to more complicated systems containing multiple organoids in a single platform

variety of characteristics that are superior to traditional 2D cell cultures. Spheroids allow for increased cell-cell interactions as well as interactions between cells and cell-secreted extracellular matrix (ECM) components [7]. Because of the transport limitations described above, spheroids can be used as models for testing drug diffusion kinetics. Likewise, lack of oxygen transport in larger spheroids can create hypoxic regions, which can be an important characteristic of certain types of tumors that drives a variety of end biological behaviors such as proliferation or genetic mutation. Spheroids can be comprised of a single cell population or formed using multiple cell populations, thereby providing opportunities to better model the heterogeneous nature of many tumors. All of these potential features result in model systems that often demonstrate increased chemoresistance to drugs, an outcome observed often in human patient, but less so in 2D cultures [8–10].

Generally, these types of cell-aggregated spheroids are formed using a hanging drop technique. Initially, this could be performed with no specialized equipment. Droplets of a cell suspension would be placed on the underside of a Petri dish or well plate cover and carefully flipped, resulting in drops hanging from the cover. Within these drops, with a lack of a physical substrate, cells aggregate and adhere to one another through cell-cell adhesions such as cadherins and tight junctions (Fig. 2a) [11]. This process has become more streamlined with the development of specific hanging drop plates containing wells with openings at the bottom. Cell suspensions are simply pipetted into each well, and the drop hangs through the bottom of the well. Following cell aggregation, the resulting spheroids can be collected in a 96 or 384 well plate beneath, corresponding to the wells above (Fig. 2b) [12]. More recently, researchers have begun to use ultra-low adhesion round bottom well plates to form spheroids. With the round bottom geometry of the wells, and the inability to adhere to the plastic surfaces in the wells, just as in hanging drop cultures, the cells aggregate with one another into spheroids (Fig. 2c) [13].

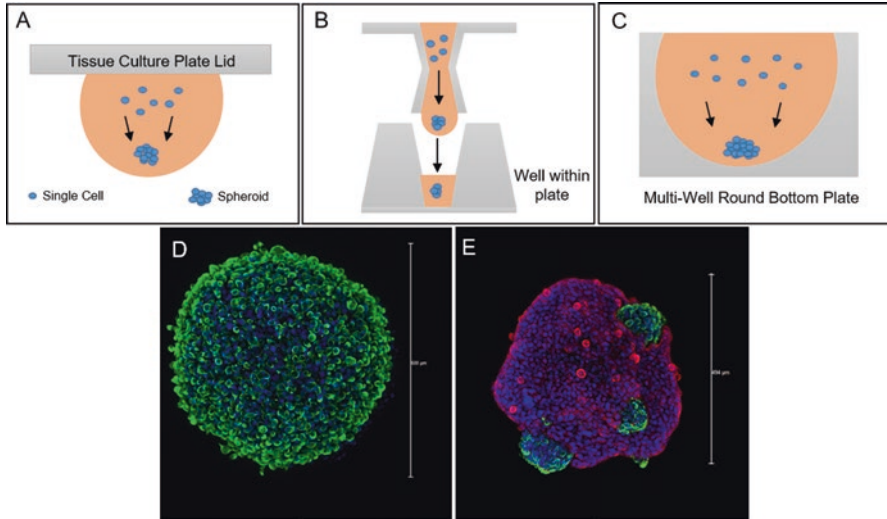


Fig. 2 Tumor spheroids. (a) Cells aggregate through cell-cell interactions in the hanging drop. (b) Hanging drop commercialized increased throughput well plate format. (c) Cell aggregation in round bottom plate. (d) Confocal image of a homogeneous mesenchymal stem cell (MSC) organoid and (e) a heterogeneous organoid with MSCs in *green* and BxPC3 pancreatic tumor cells in *red*

2.1 Homogeneous Spheroids

Homogeneous spheroids (Fig. 2d) are often used for the study of cancer cell self-assembly and their ability to interact with each other void of other cell types or surfaces. It has been shown that some cell types are unable to aggregate on their own, as they require additional signals or pathways unavailable through only themselves. This in itself has allowed researchers to better understand the requirements of various cancer types and the environmental factors. Surface tension has also been found to play a role in the development of tumor spheroids. This phenomenon was noticed as spheroids appeared to reduce in diameter however metabolic activity was sustained. It has been found that some cell types are more adherent to themselves than others and will form tight junctions reducing the overall size of the spheroid while maintaining a growing population. It has also been shown that over time single cell line tumor spheroids will dissociate and break apart, as they can no longer advantageously attach to each other. This too is specific to cell line and relies on external factors including how long the spheroids are kept in culture.

Drug treatments or external factors can be added to these single cell line tumor spheroids to study direct effects on the cancer aggregation. This allows for direct study of tumor cell behavior in reaction to the drug treatment when stromal cells are not present. Factors that directly influence the spheroid can also be added such as growth factors, conditioned media, or hypoxic conditions, which allow for the tumor spheroid to be better studied.

Currently, outside of academic research, pharmaceutical companies utilize spheroids in high throughput (384 well or greater) screening of compounds before advancing them to Phase I clinical trials. They have found spheroids to be advantageous because of their ability to better replicate an in vivo tumor over 2D culture and offer a platform from which more advance drug screens can be carried out.

2.2 Heterogeneous Spheroids

Heterogeneous spheroids have become of greater interest as cell types related to cancer types, but that are not necessarily tumor cells themselves, are added to improve the robustness of the model (Fig. 2E). Integration of additional cell types allows for natural cell assembly leading to more ideal replication of the tumor architecture. This self-assembly has been studied across many cancer types with numerous stromal cell additions. Tumor behavior is not uniform across all spheroids and can exhibit a variety of behaviors. The cancer cells have been shown to tightly grow together and force the stromal cells to grow as an outer shell around the center tumor spheroid, the cancer cells have also grown together with the stromal cells forming a second grouping grown together next to but attached to the tumor growth, and dispersal of the stromal cells within the tumor spheroid has also been shown. Each of these behaviors can be attributed to multiple factors including cell signaling, cell type adhesion propensity, and surface tension. Interestingly, it has been found that surface tension and cell viscosity play a role in the formation of spheroids, as the collection of cells, or the tissue itself, exhibits fluidic properties over time. This interesting phenomena occurring within the spheroids is that which contributes to cell organization or lack of organization with them. This also can play a role in the interconnection and adhesion of two spheroids grown of different cell lines that are then placed with each other to merge. Dependent on the fluidic properties of each of the cell types, they spheroids will merge in varying ways.

Drug studies are also widely preformed on co-cultured tumor spheroids offering insight into how a more complete tumor environment would react to drug. These studies can show how the tumor cells and stromal cells both behave in the presence of drug and if they play any role in the survival of each other. Heterogeneous spheroids are being further employed in applications such as body- and organ-on-a-chip where they can be placed into devices and studied with other spheroids or with drug via applied flow.

3 Biofabricated Tumor Constructs

Tumor spheroids have become a widely adopted tool for cancer biologists and engineers who wish to model cancer in 3D, using a relatively simple, yet effective methodology. However, this approach can be limited in some applications. For example, many primary tumor cells do not aggregate and self-assembly as easily. Additionally,

in the body, tumors do not exist in isolation; they reside in, on, or around other tissues. Therefore, in many studies, one may wish to integrate tumor cells with an extracellular matrix (ECM)-inspired environment or a surrogate for normal tissue. In these cases a wide variety of other technologies can be employed that range in complexity. Often these approaches employ other tools such as bioreactor or microfluidic systems to create supportive environments, or utilize biomaterials such as hydrogels and other scaffolds to serve as ECM analogs.

3.1 Rotating Wall Vessel

While hanging drop cultures employ gravity and a lack of adherent surfaces to form spherical organoids, a similar form factor of organoid can be biofabricated using a lack of gravity, or rather microgravity, and inclusion of adherent surfaces. Rotating wall vessel (RWV) bioreactor culture [14–19] is an established methodology that employs a rotating bioreactor to generate low fluid shear stress rotational forces, which simulates microgravity conditions. Simulated microgravity allows cells to self-aggregate into spheroids or to nucleate around microcarrier beads for adherent suspension cultures (Fig. 3a). To date, a wide variety of tissue types have been modeled as 3D organoids using RWV technology, including lung, colon, intestine, liver, vaginal epithelium, breast, and others [14–16, 18–22].

Our team employed this technology with the help of a custom-developed hydrogel microcarrier technology [19] to create tissue-tumor hybrid organoids. This platform is built on the combination of a modular hydrogel platform [23–25], which has been demonstrated extensively in the application of biofabricating tissue and tumor organoids [5, 19]. Our team recently published a study using this platform to create 3D organoids containing a hepatic cells line, HepG2, and metastatic colorectal cancer cells, HCT116 [5]. The organoids supported HCT116 tumor cell growth over time, induced expression of *in vivo-like* mesenchymal and metastatic markers, including active signaling pathways, and responded to chemotherapeutic drugs (Fig. 3b). More recently, we described new 3D liver tumor organoids comprised of more functional primary human hepatocytes, the same HCT116 colorectal cancer cells, and mesenchymal stem cells (MSC) as a surrogate for the stromal component of the liver, the liver stellate cells. We are using this tumor organoid model to observe tumor cell growth and tumor tissue maturation, and perform anti-cancer drug studies. Importantly, the cancer microenvironment is a complex space that contains stromal cells, a multitude of ECM components and proteins, as well as a plethora of signaling, paracrine, and growth factors. Together, these components of the microenvironment push and pull cancer cells between phenotypes and have a significant effect on the long-term progression of a tumor and response to therapy [26, 27]. With this platform, we were able to include a stromal component of the tissue-tumor environment in the form of MSCs. Liver-tumor organoids failed to grow in the absence of MSCs, indicating that the MSCs provide essential support for tumor growth, similar to hepatic stellate cells in liver cancer. By tracking

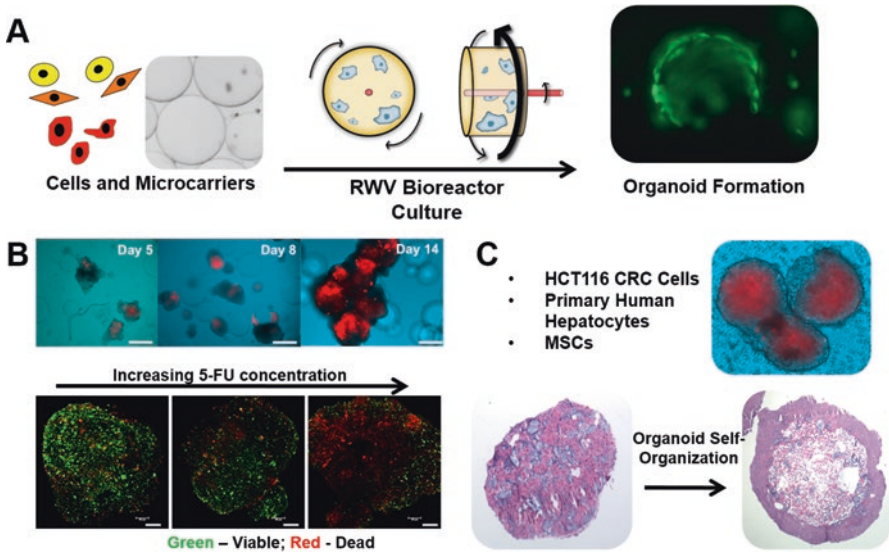


Fig. 3 Generation of tumor organoids in a rotating wall vessel (RWV) bioreactor system. (a) An RWV system with microcarriers supporting cell aggregation. (b) Using this technology, organoids can be formed such as liver organoids containing metastatic colorectal cancer cells that allow for screening of chemotherapy drugs. (c) Using stromal cell populations, phenomena such as self-organization into distinct stromal and tissue/tumor zones can be observed

fluorescently labeled HCT116 cells we demonstrated the active proliferation of the tumor cells when the MSCs were present. Besides supporting tumor cell growth in the liver organoids, inclusion of MSCs in these organoids resulted in tumor-like tissue organization and maturation. The MSCs migrated to the periphery of the organoid and created an organized shell-like tissue encapsulating the tumor cells and hepatocytes at the core of the organoid (Fig. 3c). Our results demonstrated not only a dose dependent response of the tumor liver organoid to a range of 5-FU drug concentrations but also that more organized organoids were less sensitive to the treatment, similar to results in many studies where the stroma can protect the tumor from therapy [28–30]. These results further demonstrate the capacity of the RWV bioreactor conditions to create a more physiologically relevant tumor models compared with tumor cells in cell culture dishes.

3.2 Three Dimensional Bioprinting

In the last decade and a half, bioprinting, once referred to as “organ printing” [31–34], has emerged as a tool with incredible potential in tissue engineering and regenerative medicine [35]. Bioprinting can be described as additive fabrication using biological building blocks that has the potential to build or pattern living 3D organ-like or tissue

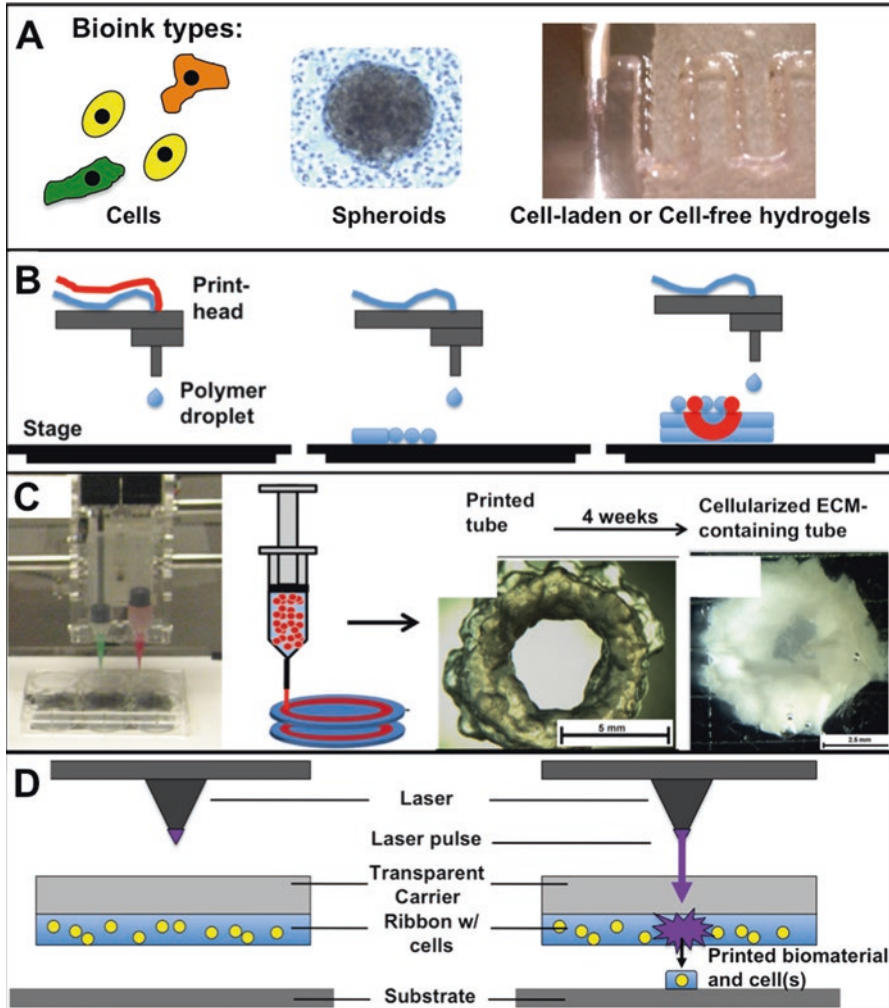


Fig. 4 Bioprinting. (a) Types of bioink. (b) Inkjet printing. (c) Extrusion printing. (d) Laser-induced forward transfer (LIFT) printing

structures [36]. In general, bioprinting employs a computer-controlled 3D printing device – the bioprinter – to accurately deposit cells and printable biomaterials into physiologically relevant biological structures. Different bioprinters have different capabilities, but generally are able to print cell aggregates, cells encapsulated in hydrogels or viscous fluids, cell-seeded microcarriers, or cell-free biomaterials – all of which can be referred to as “bioink” (Fig. 4a) [31, 37]. Biologically-derived 3D computer-aid design files, such as .stl or .dwg file formats, can be used to guide the placement of cells and bioinks into geometries that may mimic the macro-architecture of actual tissues and organs. Eventually, the ultimate goal of bioprinting is to print

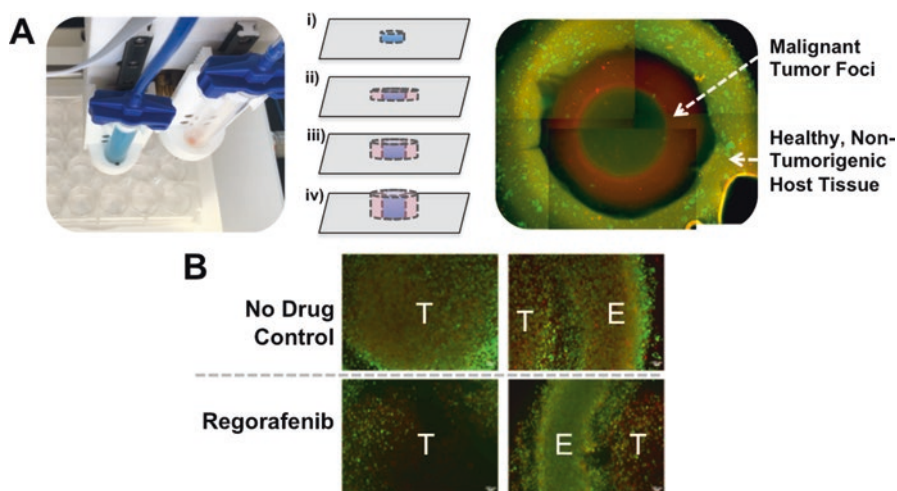
organs, which are subsequently matured into functional tissue constructs or organs [32, 38, 39]. To date, complete fully functional solid organs have not been printed, but this remains the primary long-term goal of bioprinting. However, small-scale tissue and tumor constructs are currently being implemented in a number of applications, including pathology modeling, drug development, and toxicology screening.

A number of bioprinting modalities exist, encompassing use of inkjet-like printers, extrusion devices, and laser-assisted devices. Inkjet printing (Fig. 4b), also referred to as drop-by-drop bioprinting, is one bioprinting approach that is being explored for creating 3-D biological structures, that is closely related to technologies used for cell patterning. Where basic cell patterning creates a 2-D pattern comprised of cells on a surface, by incorporating a hydrogel or other cell-friendly biomaterial, 3-D cellularized structures can be fabricated drop by drop [40, 41]. Extrusion-based deposition (Fig. 4c), generally from syringe-like equipment, is an additional approach for 3-D bioprinting that relies on the mechanical and temporal properties of the polymer materials being printed. In this modality, the properties of the printed polymer or hydrogel are used to facilitate extrusion through a syringe tip, commonly driven by pneumatic pressure or mechanical pistons controlled by a computer. Laser-induced forward transfer (LIFT)-based bioprinting (Fig. 4d) is a recently introduced method that has been adopted from other fields by researchers pursuing bioprinting [42, 43]. LIFT technology was initially developed for high resolution patterning of metals for use in areas such as computer chip fabrication. More recently it has been employed to create micropattern peptides, DNA, and cells. LIFT technology is comprised of a laser beam that is pulsed at desired time lengths and a donor material “ribbon” comprised of the printable material. This is supported on a transport layer such as gold or titanium that absorbs the laser energy and transfers it to the ribbon. When the laser pulses on the ribbon, the focused energy generates an incredibly small, high pressure bubble that propels a droplet of the donor material onto a collecting substrate and stage. By either moving the stage or the laser in relation to the ribbon, material can be patterned on the collecting substrate [44–46]. In the case of LIFT-based bioprinting, the ribbon may be comprised of a biopolymer or protein, and can contain cells within. In this scenario the laser pulse-driven ribbon droplets contain cells, which are then deposited in a pattern on the substrate to create cellular structures and patterns. The ability to print nearly a single cell per droplet [47], has positioned LIFT as a bioprinted modality with much potential in the future. Of these methods each has particular print speeds, resolution, cell densities, and cell viability outcomes that often must be considered when selecting a technology for a given application (Table 1) [35, 48–51].

To date, bioprinting has only for a short time been applied to generating tissue models or organoids. Some notable examples do exist, including using bioprinting to fabricate microfibrinous scaffolds to support myocardium and endothelial cells as a cardiac construct in a heart-on-a-chip platform [52]. In addition, our lab has developed a tissue specific hydrogel bioink system for bioprinting that can be used to match both the elastic modulus of a select soft tissue as well as the biochemical growth factor profile of that tissue. We demonstrated this by bioprinting primary human liver organoids [53, 54]. Fewer examples exist still in which bioprinting has

Table 1 Printing parameters for different bioprinting parameters, including resolution, print speed, and cell viability

Printer type	Resolution (μm)	Print speed	Cell viability (%)
Inkjet	>300	5×10^3 drops/s	75–90
Laser – assisted	10–30	9×10^{-8} mL/s	Not available as %
LIFT	30–100	10^2 drops/	95–100
Extrusion	5 μm to millimeters	10–50 $\mu\text{m/s}$	40–80

**Fig. 5** Bioprinted tumor organoids. (a) Multi-zoned tumor organoid printing. (b) Biofabrication of multiple zones allows generation of more *in vivo*-like models, in which the tumor resides inside healthy tissue. This allows drug screens to be performed that demonstrate the capability of targeting the tumor (T), not the healthy tissue (E). In (b), green indicates calcein AM-stained viable cells and red indicates ethidium homodimer-stained dead cells

been employed to create tumor organoids, although there is currently work occurring in this area. For instance model systems such as a 3D ovarian cancer coculture model [55] and 3D glioma stem cell-derived brain tumor models have been bioprinted for disease modeling and testing of drug susceptibility [56]. Currently, our team is adapting a long track record of bioprinting experience and bioink development towards printing both cell line-based tumor organoids and primary patient tumor-derived models, incorporating multi-zoned printing to incorporate not only the tumor, but also healthy surrounding tissue in which the tumor resides (Fig. 5a). This is an important feature in these models, as it now allows querying the tumor targeting capabilities of drugs that might be screened. For example, we have shown recently that a broad acting chemotherapeutic agent such as 5-FU can be effective against a tumor population, but also exhibits toxicity in the normal cells surrounding the tumor. Conversely, a drug such as regorafenib that targets a specific mutation of the epidermal growth factor receptor (EGFR) pathway [57] only found in the tumor population, can be effective against the tumor, while being less toxic to the healthy cells (Fig. 5b).

3.3 Photopatterning

An alternative strategy for fabricating 3D tissue and tumor constructs is through photopatterning. Specifically, as more and more microfluidic organ-on-a-chip systems evolve [58, 59], strategies that allow integration of 3D constructs inside these devices are becoming more important. The limitation stems from the nature of most microfluidic devices are inherently closed systems, with no direct access to the internal channels and chambers. To address these challenges of integrating 3D cell culture with on-a-chip platforms we have developed a methodology for their in situ fabrication [19] that utilizes widely employed hydrogel biomaterials comprised of photocrosslinkable hyaluronic acid and gelatin [20, 21]. Unlike conventional materials such as collagen, Matrigel, and alginate, these materials are easily integrated with variety of biofabrication techniques, including bioprinting as described above. Furthermore, since HA and gelatin are natural components of native ECM, it provides a truly biomimetic structure in the form of crosslinked HA polysaccharide chains and cell-adherent motifs in the form of hydrolytically degraded collagen gel [23, 53]. In the general approach to fabricating cell culture constructs, HA and gelatin components are mixed with target cells, as well as a crosslinker and photoinitiator to support thiol-acrylate photopolymerization via UV exposure [22, 23]. This mixture is introduced to all microfluidic chambers for a given cell/tissue type and patterning is then performed using a positive-tone photomask to define the shape and location of one or more polymerized construct (Fig. 6a). The cross-linked hydrogel is adherent to the top and bottom surfaces of the chamber, allowing it to be retained following a wash with clean buffer. This photopatterning can be performed simultaneously in an arbitrary number of independent microfluidic chambers. The resulting 3D cell culture constructs can subsequently be kept under circulating flow with long-term viability, and the total system is amenable to analytical investigation, including direct imaging, aliquot sampling, and biochemical administration, such as drug and toxicology screens [19]. Additional patterning (e.g. with additional cell types) can also be used to produce multi-component, concentric structures as well, enabling significant tissue construct complexity to be achieved (Fig. 6b). Notably, the hydrogel platform employed in our work also supports incorporation of components derived directly from tissue ECM, employing heparin pendant changes that can immobilize ECM-derived growth factors, presenting encapsulated cells with additional biomolecular factors *specific to the tissue* [53, 54, 60, 61]. Our studies have shown that such in vitro constructs recapitulate a broad range of physiological activities and reactions observed in vivo [7, 19], highlighting the biomimetic nature of the system. This type of patterning can be used across many types of crosslinkable materials that require activation via UV or visible light and is not restricted to the HA and gelatin gels described above. UV crosslinkable materials are considered those that include thiol groups that can react with free radicals to link with other thiol groups. Versatility is restricted only by materials that are crosslinkable and allows for optimization of each tumor microenvironment being studied. Overall, this fabrication approach is rapid, inexpensive, and modular, with straightforward potential to be expanded to massively parallel investigation.

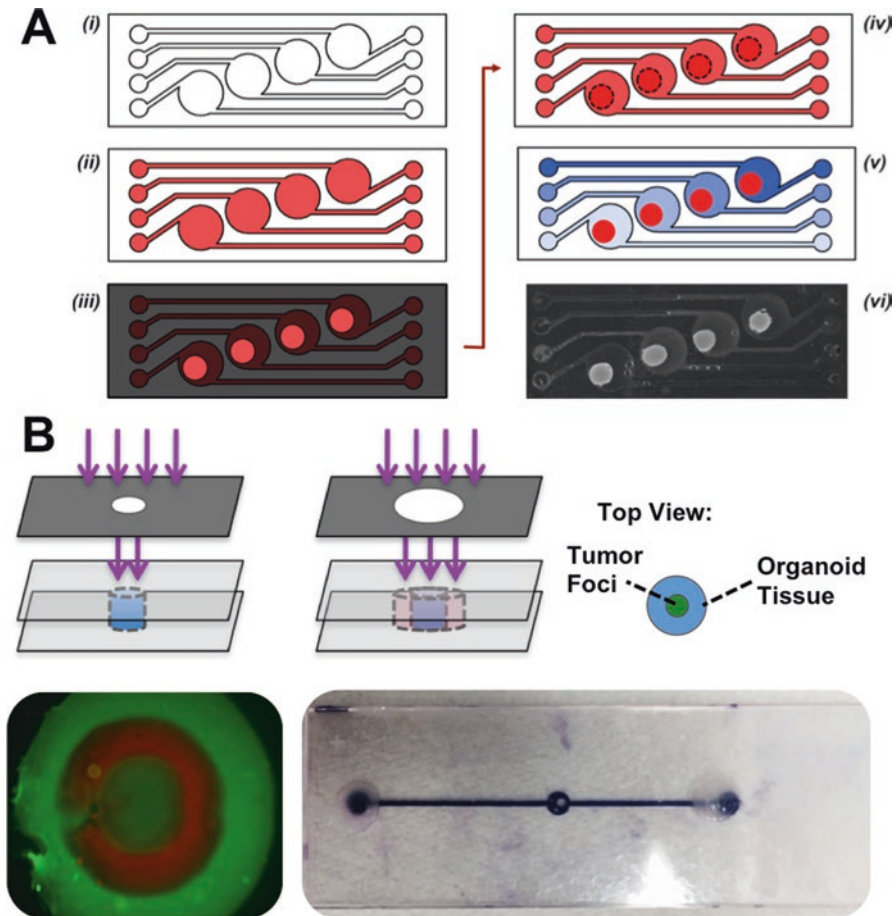


Fig. 6 Photopatterning of hydrogels to create organoids. (a) In situ photopatterning within a microfluidic device. A photo-crosslinkable hydrogel precursor solution with cells is injected into the device organoid chambers. A photomask is placed above with apertures allows light exposure to photopolymerize select regions. The uncrosslinked material is washed out leaving 3D organoids within the device chambers. (b) Multi-stage photopatterning allows biofabrication of more complex, multi-zoned structures, such as the concentric circle structure shown. These can be fabricated as incredibly small structures, such as the clear circle shown in the middle of the microfluidic device. Blue dye is used to visualize the organoid

3.4 Tumor-on-a-Chip

Further development of bioengineered organ microengineering [62, 63] combined with microfluidic device fabrication has resulted in a growing library of organ-on-a-chip technologies. These kinds of on-a-chip devices and systems can take on a wide variety of forms, from single cell analysis devices to multi-organoid housing systems that can be employed for drug testing, toxicology [64], high throughput screens

[65], and disease modeling (Fig. 7a) [66]. These platforms bring together a variety of important parameters that allow better mimicry of in vivo conditions, including 3D architecture, cell-cell/cell-matrix interactions, circulation, and integration of multiple tissues within one platform. With the biofabrication technologies previously described, tumor-on-a-chip devices can be fabricated in a multitude of ways, which allow for many devices created in parallel for high-throughput screening and research. The tumors organoids are often contained within spheroids or bioinks and placed within the devices and then sealed, resulting in contained systems, which may have open or closed fluidic perfusion. Within these systems, adequate nutrients and oxygen are supplied and external factors can be administered to treat and study the tumor. This technology has been advanced to reduce the overall size of these devices allowing for them to be produced in industry and research on plates as small as 384 wells. With these advances a greater number of studies are able to be conducted in parallel [65].

3.5 *Metastasis-on-a-Chip*

A metastasis-on-a-chip platform, comprised of tumor foci within multiple host tissue constructs, is a concept that to our knowledge has not been widely explored outside our laboratory and our collaborators' laboratories. Metastasis here, is defined as the migration of tumor cells from a primary source to a secondary tissue that is not physically connected to the source, but requires a circulatory system connecting the two sites. This is unlike the study of tumor cells simply in circulation. In the past decade, with the growth of the organ-on-a-chip field, there have also been advances in on-a-chip devices that assess specific scenarios of metastasis. For example, the Kamm group has developed a device that includes endothelium barrier tissue and an adjacent bone construct, facilitating modeling of intravasation of breast cancer cells from circulation into bone [67, 68]. Other platforms of recent years include devices for assessing the effects of interstitial pressure on cell migration [69], multi-channel microfluidic devices that aim to investigate the processes through which aggregated tumor cells migrate through a collagen gels and endothelium [70], and a platforms for increased throughput screening anti-angiogenic drugs [71]. However, there has been an obvious lack of technologies that aim to integrate both primary and metastatic sites, and the features in between (i.e. endothelium and circulatory system) in one device. To address this gap, we employed biofabricated tissue and tumor organoids integrated into a multi-chambered microfluidic device in which the chambers were connected by circulating perfusion (Fig. 7b). By providing circulating flow through the organoid system, we can achieve the dissemination of CRC cells from a colon organoid into circulation, after which metastatic cells can colonize the liver organoid downstream (Fig. 7c) [25]. This model was one the first in vitro models of metastasis recapitulating migration from a 3D primary tissue to a 3D target tissue. This is important and novel because phenotype of cells in the originating malignant tumors and metastases often vary in invasiveness due to genetic

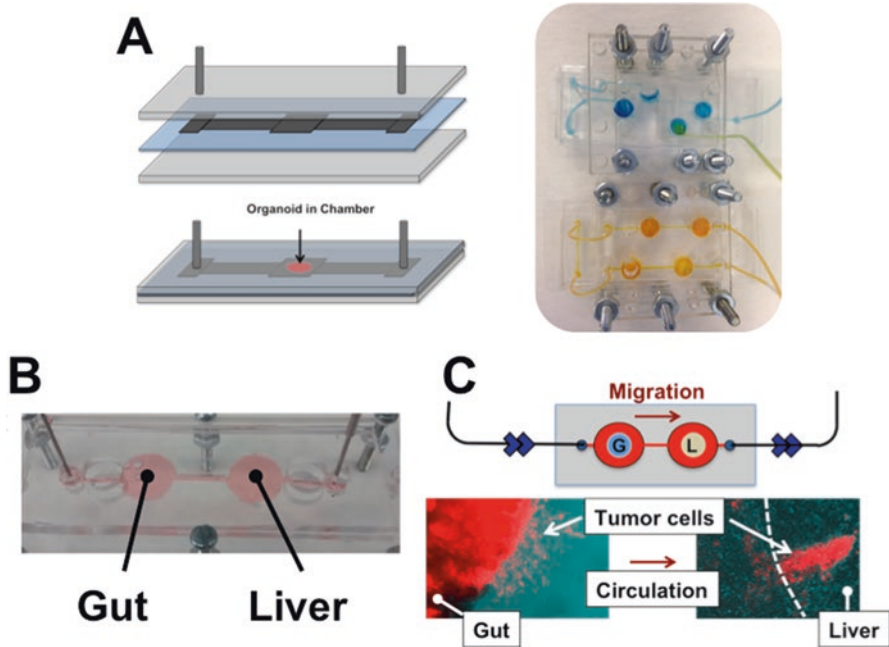


Fig. 7 Cancer-on-a-chip. (a) Examples of generic organ-on-a-chip devices. An organoid is housed within a chamber connected to a perfused fluidic system. Linking together of organoid chambers results in a device supporting multiple organoids onboard a single device. (b) A metastasis-on-a-chip device, consisting of 2 organoids for modeling colorectal cancer metastasis from the gut to liver

profiles that influence functions such as MMP secretion and stemness [72, 73]. This makes the ability to study both sites and microenvironments extremely important. Notably, we also showed that while metastatic cells metastasized in our system, less malignant non-metastatic tumor cells did not, suggesting that these types of systems can discriminate between different classes of tumors [25]. This opens up potential for new studies that can focus on the mechanistic side of metastasis, or on the other hand, facilitate screening of candidate agents with anti-metastatic properties.

4 Patient-Specific Tumor Models for Personalized Medicine

Precision oncology, whereby tumor DNA is sequenced to identify actionable gene mutations, is poised to become a standard clinical practice for therapeutic decision making of cancer treatment [74–76]. However, in practice, the utility of precision medicine is less clear [77]; even after identification of key mutations, oncologists are often left with several drug options, and for some patients there is no one definitive treatment solution, thus creating a need to further develop a model system to help predict the personalized response to anti-cancer drugs [78, 79]. Novel

technologies, capable of extending the diagnostic utility of tissue specimens are critically needed for robust assessment of therapeutic biomarkers and validation of these biomarkers as actionable targets. Moreover, there is a great variability in the biologic behavior of cancer based on histologic type, grade and volume of disease. This variability is currently addressed through precision medicine analysis, by relating genetic mutations to chemotherapy options. However, the efficacy of a given treatment in a specific patient is often unknown. Within research, patient derived xenografts are also used to study patient tumor progression and drug treatment response. These models are lacking in that they require immunodeficient mice to place the biopsies or tumor samples in which causes them to become infiltrated with cells from the mouse. The cells also adapt to their new environment and genetic drift has been shown from the initial samples again making them less ideal. In the clinic, after identification of a mutation through precision medicine, given the unknown impact of the specific mutation on tumor biology and the equally unknown effect of chemotherapy options on the specific cellular phenotype, a modification of a predetermined fixed treatment strategy is a rare event. Bioengineered tumor models derived from patient tumor biospecimens may be more easily attained and less expensive than PDX models, and provide a powerful tool for screening potential therapeutic agents and determining the most efficacious and safest therapy for a particular patient. This is a very new area of tumor organoids, but is one that holds incredible potential for improving cancer patient outcomes.

Such personalized tumor models are currently being developed within our laboratory. With regular access to primary patient samples from biopsies and complete resections, we have been able to dissociate the tumor masses into single cells and use biomaterials and bioinks to biofabricate patient-specific 3D tumor organoids (Fig. 8a) that remain viable in the laboratory (Fig. 8b). Using both bioprinting and photopatterning methods, we have created platforms for testing drugs on these personalized tumor organoids. Figure 8c shows an example of such a drug screening scenario in which tumor organoids created from a gastrointestinal tumor biospecimen responded well to 5-fluorouracil and oxaliplatin (anti-proliferative agents), and regorafenib, an agent targeting a particular EGFR. However, these organoids did not respond to the combination therapy of trametinib and dabrafenib, a therapy often used to treat tumors with a different EGFR mutation profile [57]. These platforms are creating the opportunity for personalized drug treatment optimization in patients that have unclear genetic data that does not respond to standard treatments. We are also able to confirm our models by treating the patient tumor organoids with drugs that the patient responded to in the clinic. Additionally, genetic data can be paired with the patient tumor organoids to study genetic drift and relation to drug response. These tools are still being optimized but show promise for future personalized medicine applications. We anticipate one day having the technology and capabilities to determine the most effective therapy, and just as importantly, the safest therapy, for a given patient prior to any actual treatment administration in the clinic (Fig. 8d). We hope this significantly improves outcomes in patients afflicted with cancer.

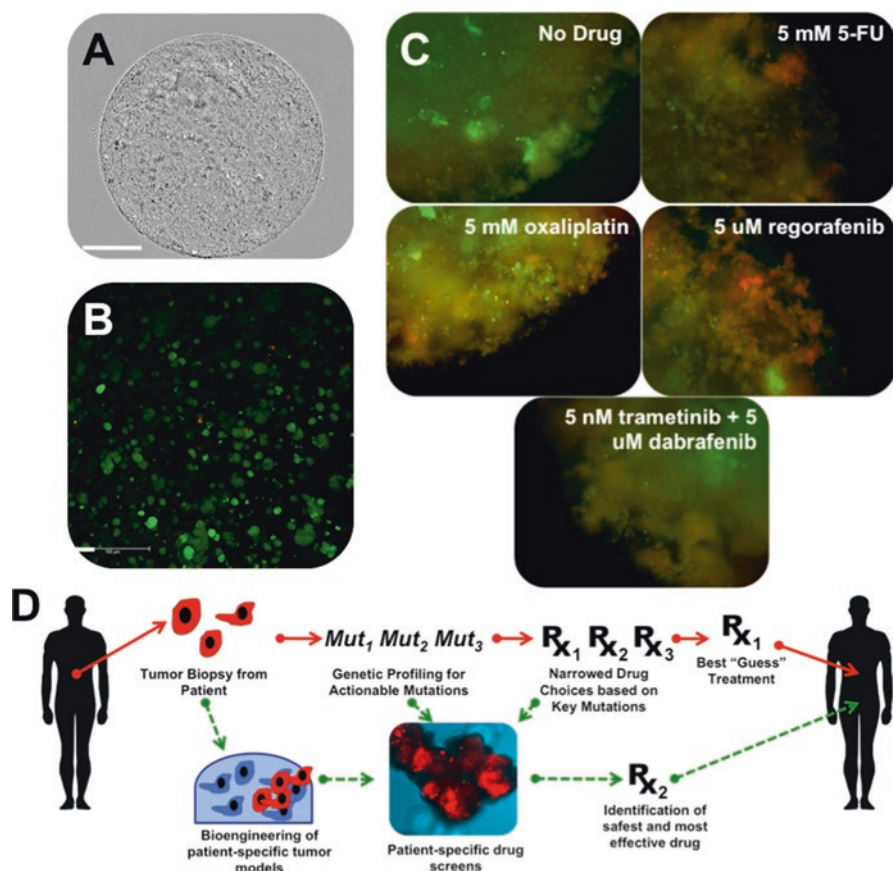


Fig. 8 Patient-derived tumor organoids for personalized medicine. (a) Tumor organoids comprised of patient tumor-derived cells encapsulated in 3D extracellular matrix hydrogel constructs (b) remain viable in culture (*Green* – viable cells; *Red* – dead cells). (c) An example of an in vitro drug screen using these patient-specific tumor organoids. These organoids were more responsive to 5-fluorouracil, oxaliplatin, and regorafenib than a combined treatment of trametinib and dabrafenib (*Green* – viable cells; *Red* – dead cells). (d) Typically, precision medicine works by using the patient tumor biospecimen to run genetic analysis with the goal being to identify actionable mutations for which there are drugs available (*red arrows*). However, often multiple mutations and drugs are identified and there is still no clear answer to which will yield the best result for the patient. Patient-specific tumor organoids (*green arrows*) can be used to supplement genetic analyses by allowing drug screens to be performed prior to administration of therapy in the patient, thereby identifying the most effective and safest drug or drug combination

5 Conclusions

Cancer research as a whole, and in particular, development of new, effective therapeutic agents, has been limited due to the inability to accurately model tumor biology, progression, and signaling mechanisms in a controlled environment. Animal

models allow limited manipulation and study of these mechanisms, and are not necessarily predictive of results in humans [1]. Traditional in vitro 2D cultures fail to recapitulate the 3D microenvironment of in vivo tissues [2]. Drug diffusion kinetics vary dramatically, drug doses effective in 2D are often ineffective when scaled to patients, and cell-cell/cell-matrix interactions are inaccurate [3, 4]. Furthermore, 2D tissue culture dishes have three major differences from the tissue where the tumor was isolated: surface topography, surface stiffness, and a 2D rather than 3D architecture, which can force alterations of their molecular and phenotypic properties. Bioengineered tumor organoid technologies provide an immense opportunity to change how researchers study cancer and design studies aimed at identifying new treatment options for patients. As described in this chapter, tumor organoids vary greatly in geometry and form factor, cellularity, which combinations of cells are present if more than one population is utilized, inclusion and composition of extracellular matrix components, and how the organoids are formed or biofabricated. Having worked with the range of these organoid types, our philosophy is that no single platform is necessarily the best overarching superior technology. For instance, hanging drop spheroids are simple and inexpensive to fabricate, but often lack in complexity. Conversely, bioprinted tissue-tumor hybrid constructs in a metastasis-on-a-chip system may offer the most complexity in terms of recapitulating cell-matrix interactions, circulation, and multiple tissue sites, but may at this point in time be more difficult to run in a high throughput setting. As such, the different tumor organoid types described here can provide a toolset for researchers, from which a particular organoid form factor can be drawn to address a particular problem or ask a particular question. Nevertheless, tumor organoid technology as a whole provides a significantly improved platform for cancer research compared to traditional approaches, and will in all likelihood continue to advance in a rapid pace in the near future.

References

1. Bhattacharya S, Zhang Q, Carmichael PL, Boekelheide K, Andersen ME (2011) Toxicity testing in the 21 century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. *PLoS One* 6:e20887
2. Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R (2004) The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. *J Biomol Screen* 9:273–285
3. Ho WJ et al (2010) Incorporation of multicellular spheroids into 3-D polymeric scaffolds provides an improved tumor model for screening anticancer drugs. *Cancer Sci* 101:2637–2643
4. Drewitz M et al (2011) Towards automated production and drug sensitivity testing using scaffold-free spherical tumor microtissues. *Biotechnol J* 6:1488–1496
5. Skardal A, Devarasetty M, Rodman C, Atala A, Soker S (2015) Liver-tumor hybrid Organoids for modeling tumor growth and drug response in vitro. *Ann Biomed Eng* 43:2361–2373
6. Mehta G, Hsiao AY, Ingram M, Luker GD, Takayama S (2012) Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. *J Control Release* 164:192–204

7. Hirschhaeuser F et al (2010) Multicellular tumor spheroids: an underestimated tool is catching up again. *J Biotechnol* 148:3–15
8. Torisawa YS, Takagi A, Shiku H, Yasukawa T, Matsue TA (2005) Multicellular spheroid-based drug sensitivity test by scanning electrochemical microscopy. *Oncol Rep* 13:1107–1112
9. Kelm JM, Fussenegger M (2004) Microscale tissue engineering using gravity-enforced cell assembly. *Trends Biotechnol* 22:195–202
10. Lin RZ, Chang HY (2008) Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnol J* 3:1172–1184
11. Amann A et al (2014) Development of an innovative 3D cell culture system to study tumour-stroma interactions in non-small cell lung cancer cells. *PLoS One* 9:e92511
12. Messner S, Agarkova I, Moritz W, Kelm JM (2013) Multi-cell type human liver microtissues for hepatotoxicity testing. *Arch Toxicol* 87:209–213
13. Ivanov DP et al (2014) Multiplexing spheroid volume, resazurin and acid phosphatase viability assays for high-throughput screening of tumour spheroids and stem cell neurospheres. *PLoS One* 9:e103817
14. Barrila J et al (2010) Organotypic 3D cell culture models: using the rotating wall vessel to study host-pathogen interactions. *Nat Rev Microbiol* 8:791–801
15. Carterson AJ et al (2005) A549 lung epithelial cells grown as three-dimensional aggregates: alternative tissue culture model for *Pseudomonas Aeruginosa* pathogenesis. *Infect Immun* 73:1129–1140
16. Honer zu Bentrup K et al (2006) Three-dimensional organotypic models of human colonic epithelium to study the early stages of enteric salmonellosis. *Microbes Infect* 8:1813–1825
17. Nickerson CA, Ott CM (2004) A new dimension in modeling infectious disease. *ASM News* 70:169–175
18. Nickerson CA, Richter EG, Ott CM (2007) Studying host-pathogen interactions in 3-D: organotypic models for infectious disease and drug development. *J Neuroimmune Pharmacol* 2:26–31
19. Skardal A, Sarker SF, Crabbe A, Nickerson CA, Prestwich GD (2010) The generation of 3-D tissue models based on hyaluronan hydrogel-coated microcarriers within a rotating wall vessel bioreactor. *Biomaterials* 31:8426–8435
20. Barrila J et al (2010) 3-D cell culture models: innovative and predictive platforms for studying human disease pathways and drug design. *Nat Rev Microbiol* 8:791–801
21. Hjelm BE, Berta AN, Nickerson CA, Arntzen CJ, Herbst-Kralovetz MM (2010) Development and characterization of a three-dimensional organotypic human vaginal epithelial cell model. *Biol Reprod* 82:617–627
22. Nickerson CA et al (2001) Three-dimensional tissue assemblies: novel models for the study of salmonella enterica serovar Typhimurium pathogenesis. *Infect Immun* 69:7106–7120
23. Skardal A, Zhang J, Prestwich GD (2010) Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. *Biomaterials* 31:6173–6181
24. Serban MA, Prestwich GD (2008) Modular extracellular matrices: solutions for the puzzle. *Methods* 45:93–98
25. Skardal A, Devarasetty M, Forsythe SD, Atala A, Soker S (2016) A reductionist metastasis-on-a-chip platform for in vitro tumor progression modeling and drug screening. *Biotechnol Bioeng* 113(9):2020–2032
26. Fidler IJ, Kim SJ, Langley RR (2007) The role of the organ microenvironment in the biology and therapy of cancer metastasis. *J Cell Biochem* 101:927–936
27. Langley RR, Fidler IJ (2007) Tumor cell-organ microenvironment interactions in the pathogenesis of cancer metastasis. *Endocr Rev* 28:297–321
28. Majidinia M, Yousefi B (2017) Breast tumor stroma: a driving force in the development of resistance to therapies. *Chem Biol Drug Des* 89(3):309–318
29. Dauer P, Nomura A, Saluja A, Banerjee S (2017) Microenvironment in determining chemoresistance in pancreatic cancer: neighborhood matters. *Pancreatol* 17:7–12
30. Bar-Natan M et al (2017) Bone marrow stroma protects myeloma cells from cytotoxic damage via induction of the oncoprotein MUC1. *Br J Haematol* 176(6):929–938

31. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR (2003) Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol* 21:157–161
32. Mironov V, Kasyanov V, Drake C, Markwald RR (2008) Organ printing: promises and challenges. *Regen Med* 3:93–103
33. Mironov V et al (2009) Organ printing: tissue spheroids as building blocks. *Biomaterials* 30:2164–2174
34. Prestwich GD (2007) Organ printing. *Chem Biol* 2:B33–B40
35. Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. *Nat Biotechnol* 32:773–785
36. Visconti RP et al (2010) Towards organ printing: engineering an intra-organ branched vascular tree. *Expert Opin Biol Ther* 10:409–420
37. Fedorovich NE et al (2007) Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. *Tissue Eng* 13:1905–1925
38. Boland T, Mironov V, Gutowska A, Roth EA, Markwald RR (2003) Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. *Anat Rec A Discov Mol Cell Evol Biol* 272:497–502
39. Derby B (2012) Printing and prototyping of tissues and scaffolds. *Science* 338:921–926
40. Catros S et al (2011) Laser-assisted bioprinting for creating on-demand patterns of human osteoprogenitor cells and nano-hydroxyapatite. *Biofabrication* 3:025001
41. Guillotin B, Guillemot F (2011) Cell patterning technologies for organotypic tissue fabrication. *Trends Biotechnol* 29:183–190
42. Bohandy J, Kim B, Adrian F (1986) Metal deposition from a supported metal film using an excimer laser. *J Appl Phys* 60:1538
43. Barron JA, Ringeisen BR, Kim H, Spargo BJ, Chrisey DB (2004) Application of laser printing to mammalian cells. *Thin Solid Films* 453:383–387
44. Chrisey DB (2000) MATERIALS PROCESSING: the power of direct writing. *Science* 289:879–881
45. Colina M, Serra P, Fernandez-Pradas JM, Sevilla L, Morenza JL (2005) DNA deposition through laser induced forward transfer. *Biosens Bioelectron* 20:1638–1642
46. Dinca V et al (2008) Directed three-dimensional patterning of self-assembled peptide fibrils. *Nano Lett* 8:538–543
47. Guillotin B et al (2010) Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials* 31:7250–7256
48. Holzl K et al (2016) Bioink properties before, during and after 3D bioprinting. *Biofabrication* 8:032002
49. Skardal A, Atala A (2015) Biomaterials for integration with 3-d bioprinting. *Ann Biomed Eng* 43:730–746
50. Li J, Chen M, Fan X, Zhou H (2016) Recent advances in bioprinting techniques: approaches, applications and future prospects. *J Transl Med* 14:271
51. Malda J et al (2013) 25th anniversary article: engineering hydrogels for biofabrication. *Adv Mater* 25:5011–5028
52. Zhang YS et al (2016) Bioprinting 3D microfibrinous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. *Biomaterials* 110:45–59
53. Skardal A et al (2015) A hydrogel bioink toolkit for mimicking native tissue biochemical and mechanical properties in bioprinted tissue constructs. *Acta Biomater* 25:24–34
54. Skardal A et al (2016) Bioprinting Cellularized constructs using a tissue-specific hydrogel Bioink. *J Vis Exp* (110):e53606
55. Xu F et al (2011) A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. *Biotechnol J* 6:204–212
56. Dai X, Ma C, Lan Q, Xu T (2016) 3D bioprinted glioma stem cells for brain tumor model and applications of drug susceptibility. *Biofabrication* 8:045005
57. Tran NH et al (2015) Precision medicine in colorectal cancer: the molecular profile alters treatment strategies. *Ther Adv Med Oncol* 7:252–262
58. Bhise NS et al (2014) Organ-on-a-chip platforms for studying drug delivery systems. *J Control Release* 190:82–93
59. Esch EW, Bahinski A, Huh D (2015) Organs-on-chips at the frontiers of drug discovery. *Nat Rev Drug Discov* 14:248–260

60. Skardal A et al (2016) A tunable hydrogel system for long-term release of cell-secreted cytokines and bioprinted in situ wound cell delivery. *J Biomed Mater Res B Appl Biomater*
61. Skardal A et al (2012) Tissue specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function. *Biomaterials* 33:4565–4575
62. Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. *Trends Cell Biol* 21:745–754
63. Ghaemmaghami AM, Hancock MJ, Harrington H, Kaji H, Khademhosseini A (2012) Biomimetic tissues on a chip for drug discovery. *Drug Discov Today* 7(3–4):173–181
64. Skardal A, Devarasetty M, Soker S, Hall AR (2015) In situ patterned micro 3D liver constructs for parallel toxicology testing in a fluidic device. *Biofabrication* 7:031001
65. Phan DT et al (2017) A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. *Lab Chip* 17:511–520
66. Skardal A, Shupe T, Atala A (2016) Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling. *Drug Discov Today* 21:1399–1411
67. Bersini S et al (2014) A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone. *Biomaterials* 35:2454–2461
68. Bersini S, Jeon JS, Moretti M, Kamm RD (2014) In vitro models of the metastatic cascade: from local invasion to extravasation. *Drug Discov Today* 19:735–742
69. Polacheck WJ, German AE, Mammoto A, Ingber DE, Kamm RD (2014) Mechanotransduction of fluid stresses governs 3D cell migration. *Proc Natl Acad Sci U S A* 111:2447–2452
70. Niu Y, Bai J, Kamm RD, Wang Y, Wang C (2014) Validating antimetastatic effects of natural products in an engineered microfluidic platform mimicking tumor microenvironment. *Mol Pharm* 11:2022–2029
71. Kim C, Kasuya J, Jeon J, Chung S, Kamm RD (2015) A quantitative microfluidic angiogenesis screen for studying anti-angiogenic therapeutic drugs. *Lab Chip* 15:301–310
72. Karakiulakis G et al (1997) Increased type IV collagen-degrading activity in metastases originating from primary tumors of the human colon. *Invasion Metastasis* 17:158–168
73. Franci C et al (2013) Biomarkers of residual disease, disseminated tumor cells, and metastases in the MMTV-PyMT breast cancer model. *PLoS One* 8:e58183
74. Glade Bender J, Verma A, Schiffman JD (2015) Translating genomic discoveries to the clinic in pediatric oncology. *Curr Opin Pediatr* 27:34–43
75. Andre F et al (2014) Prioritizing targets for precision cancer medicine. *Ann Oncol: official journal of the European Society for Medical Oncology / ESMO* 25:2295–2303
76. Roychowdhury S, Chinnaiyan AM (2014) Translating genomics for precision cancer medicine. *Annu Rev Genomics Hum Genet* 15:395–415
77. Hayes DF, Schott AF (2015) Personalized medicine: genomics trials in oncology. *Trans Am Clin Climatol Assoc* 126:133–143
78. Cantrell MA, Kuo CJ (2015) Organoid modeling for cancer precision medicine. *Genome Med* 7:32
79. Gao D et al (2014) Organoid cultures derived from patients with advanced prostate cancer. *Cell* 159:176–187