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REVIEW ARTICLE

Applications of Bioengineered 3D Tissue and Tumor Organoids in Drug Development and Precision Medicine: Current and Future

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Abstract Over the past decade, advances in biomedical and tissue engineering technologies, such as cell culture techniques, biomaterials, and biofabrication, have driven increasingly widespread use of three-dimensional (3D) cell culture platforms and, subsequently, the use of organoids in a variety of research endeavors. Given the 3D nature of these organoid systems, and the frequent inclusion of extracellular matrix components, these constructs typically have more physiologically accurate cell–cell and cell–matrix interactions than traditional 2D cell cultures. As a result, 3D organoids can serve as better model systems than their 2D counterparts. Moreover, as organoids can be biofabricated from highly functional human cells, they have certain advantages over animal models, being human in nature and more easily manipulated in the laboratory. In this review, we describe such organoid technologies and their deployment in drug development and precision medicine efforts. Organoid technologies are rapidly being developed for these applications and now represent a wide variety of tissue types and diseases. Evidence is emerging that organoids are poised for widespread adoption, not only in academia but also in the pharmaceutical industry and in clinical diagnostic applications, positioning them as indispensable tools in medicine.

Key Points

Organoids are three-dimensional (3D) multi-cellular constructs of cells created in a variety of form factors, including spheroids, aggregates, hydrogel, or extracellular matrix (ECM)-encapsulated cells, among others.

The drug development pipeline stands to benefit from the integration of human organoids into compound screening with the introduction of a 3D human-based test component earlier in the development timeline.

A wide range of tissues and disease states can be modeled using organoids, providing a versatile set of technologies for more nuanced basic science research.

Creation of organoids from patient tissue and tumor biopsies will provide opportunities for personalized patient-specific approaches to the assessment of treatment efficacies in the laboratory before treatment is applied.

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1 Introduction

The use of bioengineered three-dimensional (3D) tissue and tumor organoids is common across numerous fields as it is becoming the gold standard for organ and tissue replication ex vivo [1–3]. Organoids are generally small-scale constructs of cells—much smaller than their in vivo counterparts—that are fabricated in the laboratory to serve as 3D representations of in vivo tissues and organs. These bioengineered platforms support a variety of applications, with implications in both the research and the clinical environment. Organoids allow for advancement of studies utilizing 3D environments in comparison to traditional two-dimensional (2D) cultures, which can be limiting when trying to replicate tissue-level physiology [4]. 3D culture allows for more nuanced control of cell–cell and cell–matrix interactions, stiffness, addition of biochemical factors, and modulation of tissue density, altogether allowing tailoring of the extracellular matrix (ECM) to fit the organ of interest [3]. For applications such as drug development and precision medicine, it is increasingly important that 3D culture systems be utilized to incorporate the many aspects of an in vivo tissue. Aspects such as multi-organ communications through organ-on-a-chip technologies and the addition of external factors, such as flow or physical forces, can be incorporated to better replicate the in vivo microenvironment [5, 6]. In recent years, studies have suggested that drug development has seen significant improvement in the diversity of assays available because of organoid systems and their in vivo-like properties [7]. Organoids are commonly used for studies of dose- and time-dependent drug compound toxicity. Such studies are being conducted on the targeted organ or tissue type as well as major organs such as the liver and heart, which often experience toxicity from drug treatments even when the compound in question is beneficial for the target organ or tissue.

Organoids have enabled the individual study of relevant tissue types to better understand dose- and time-related responses to drug compounds and toxins. Recent studies have provided evidence that the connection of multiple different tissue type organoids through microfluidic and "on-a-chip" devices has also allowed for more complete understanding of how the entire body may respond to drugs [3, 8, 9]. Each of the tissue types within the system can be studied in depth to understand their response and how they may play a role in the integrated response of other organoids [10]. An example of such an application is the screening of recalled drugs, which validated an organoid system in that the organoid platform exhibited negative side effects similar to those reported by the US FDA in human patients [3]. These systems are becoming more common in research investigating drugs being advanced into phase I clinical trials. Doses for administration and their effects over time on multiple human-derived organ systems can be studied before in vivo testing.

Further surpassing use in traditional tissue types, the immune system is being targeted for incorporation into multi-organoid systems, which could allow testing of immunotherapeutics and elucidate mechanisms of immune-mediated drug sensitivity or resistance [11, 12]. The use of organoids is also being explored in precision medicine applications. Such applications include disease diagnostics and prediction of cell behavior, personalized drug testing and selection [13], and tissue regeneration [14]. These organoids require tissue biospecimens from the patient but allow for quantitative results to be personalized and meaningful on an individual basis, thus providing clinically relevant and predictive data that can be actionable for improving patient outcomes. In this review, we highlight systems that have been specifically optimized for drug development and precision medicine applications utilizing organoid technology.

2 Organoid Technology

Organoids can be defined as 3D constructs comprising tissue-specific cells with the intention of recapitulating the cellular microenvironment; organoids may also include ECM components or biomaterials to achieve this aim [2]. Each organ-specific organoid often contains multiple cell types normally found within the target tissue [3]. The ratio of cell types is optimized to induce organoid function that can be measured using organ-specific biomarkers or other assays [10, 15, 16]. Tissues can be free formed through cell–cell aggregation in hanging drop or round-bottom non-adherent culture plates, which yield spheroids, or may be created using hydrogels in which the cells are embedded within synthetic polymers or ECM components. Spheroids are also commonly created and then embedded into hydrogels [17, 18]. With many methods for creation, the term organoid is broadly used for organ-specific 3D cultures.

The use of organoids over traditional 2D tissue culture has become broadly accepted in recent years as differences between 2D and 3D cultures in genotype, phenotype, and cellular behavior have been recognized [16, 19]. Each of these differences can contribute to cellular and tissue changes directly related to drug response, disease progression, and overall function [19]. These aspects are vital for the success of drug development platforms and precision medicine applications, as it is important to maintain in vivo-like behavior. The 3D cultures, if not formed through aggregation, are often created using biomaterials

that suspend cells in 3D within polymer or protein-networked matrices. Biomaterials have an advantage over spheroid approaches as they enable greater control of the organoid and organoid microenvironment regarding environmental and physical parameters, such as stiffness, addition of ECM components, and spatial organization of cell types [20]. The biomaterials used for tissue and tumor organoids are selected based on particular properties for a given tissue type. Biomaterials can offer different porosities, levels of stiffness, cell-adherent motifs, and viscosities, each of which can play a role in driving physiological cell and tissue function [21, 22]. Common biomaterials for use with organoids include collagen, hyaluronic acid, gelatin, and chitosan [23, 24]. These can be used as hydrogels in which cells are encapsulated during the formation of these matrices, or as scaffolds into which cells are directly seeded. Hydrogels capable of encapsulation can also often be tailored to biofabrication approaches such as bioprinting to improve the design and throughput of organoid creation. Hybrid approaches also exist, such as embedding aggregated tissue spheroids within hydrogels to form larger multi-colony (Fig. 1a) and highly functional tissue construct models [9].

Within ongoing efforts to develop organoid platforms indicative of human physiology that can be deployed for drug development and precision medicine, we will describe a wide variety of examples. We discuss a range of strategies, including integration of microfluidic devices, 3D-printed structures, and spheroids [9, 25]. We also discuss the use of microfluidic device technology for connecting organoids of different tissue origins to create a more complete body-on-a-chip, which, because of the systems biology approach, could be a significant advancement in the context of drug development and precision medicine technologies [3, 26]. Within these platforms, many elements are available for customization, and organ- and disease-specific tissue can be replicated for drug testing and precision medicine applications [8, 9]. Here, we highlight advancements in drug development and personalized medicine, focusing on the liver, cardiac systems, the lungs, and tumors as tissue models.

3 Organ Models for Drug Testing and Discovery

A major motivation for the development and utilization of organoid models is their potential impact on the drug discovery pipeline (Fig. 2). As previously described, 3D culture modalities are substantially more predictive and informative than 2D cell culture assays, of which modern drug discovery makes heavy use. In fact, compared with traditional 2D cell cultures, 3D models have been shown to be far more representative of key biomarkers in pathologies such as cancer [10, 27]. By incorporating 3D organoid models, candidate compounds can be screened more efficiently before in vivo testing, thereby increasing chances of success and reducing drug development costs. However, many drugs that pass through the entire drug development pipeline are commercialized and subsequently recalled from market after unforeseen toxicities. For instance, valdecoxib (tradename Bextra)—a non-steroidal anti-inflammatory drug (NSAID)—was recalled in 2005 because

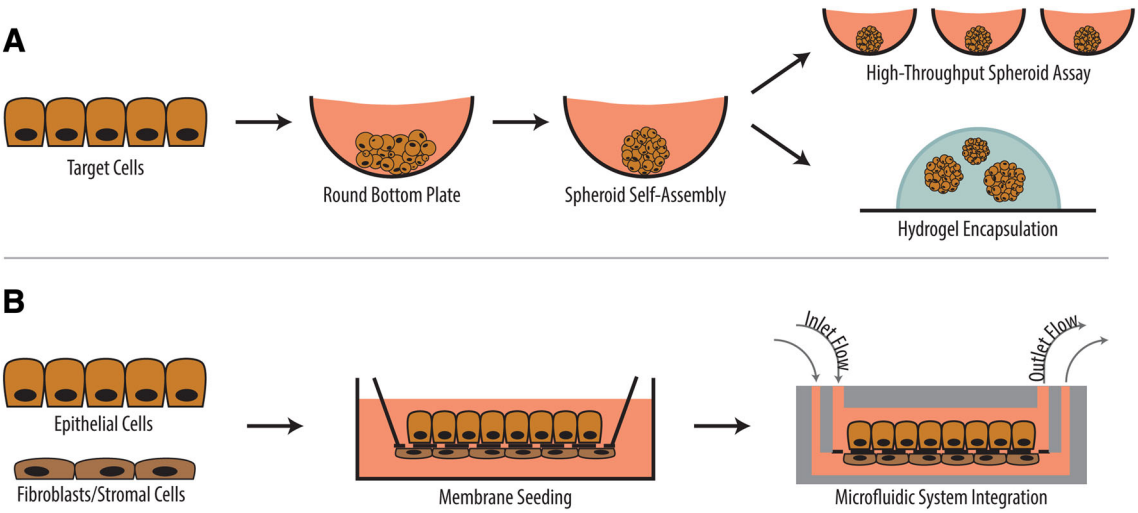

Fig. 1 Examples of organoid fabrication strategies. Cells with a variety of origins can be collected and transferred to a non-adherent, round-bottom well plate. Cells will aggregate because of gravity at the bottom of the well and create cell–cell adhesions, which eventually result in formation of a spheroid. Spheroids can then be used for high-throughput testing or encapsulated into hydrogels for high functionality (**a**). Airways or luminal tissues can be modeled using a membrane-based device. First, target epithelial and stromal cells are collected then seeded onto opposite sides of a membrane. Once attachment is achieved, cell-laden membranes can be integrated into microfluidic systems for standalone testing or use in body-on-a-chip platforms (**b**)

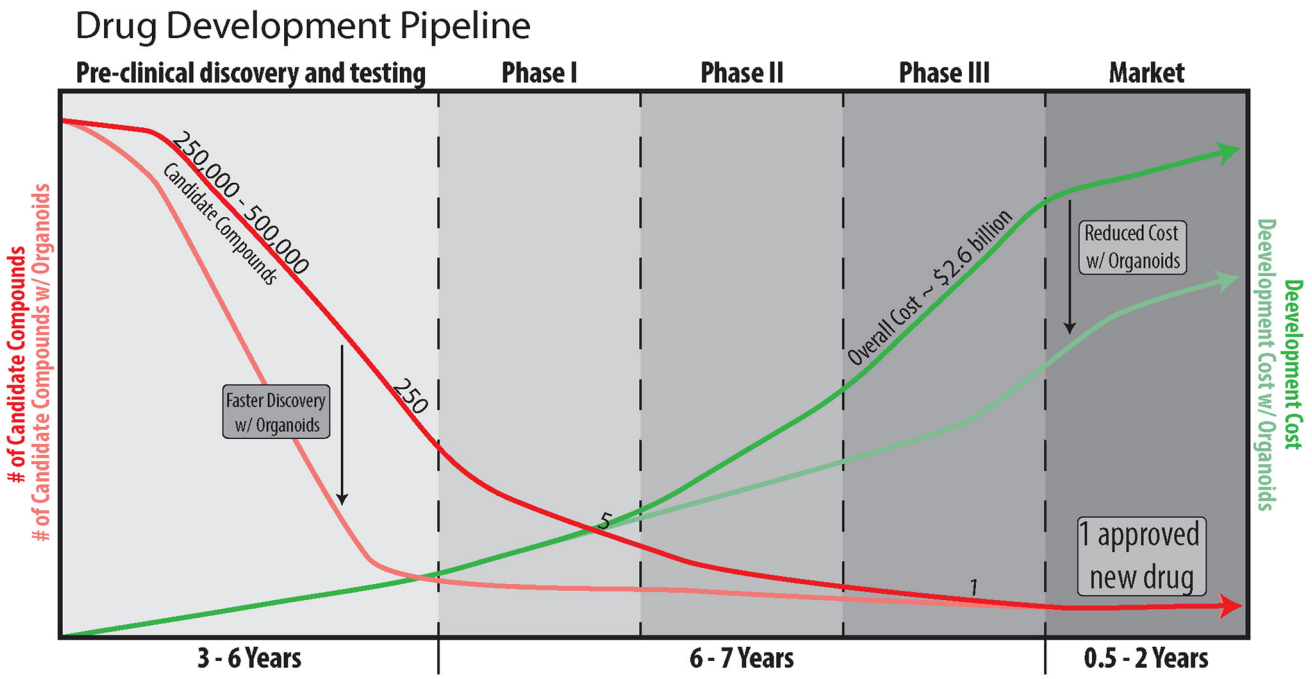

Fig. 2 Drug development costs and candidates versus time, with and without organoids. Integration of physiologically relevant three-dimensional (3D) organoid models could improve the drug development pipeline by eliminating toxic or ineffective compounds in pre-clinical testing. Phase I–III clinical trials are the primary cost driver of pharmaceutical development; organoids reduce the number of failed compounds making it to this point, thus reducing cost and increasing the number of approved drugs

of adverse cardiovascular effects that could result in stroke, myocardial infarction, or death [28]. Pemoline (tradename Cylert) was used to treat attention-deficit/hyperactivity disorder (ADHD)/attention deficit disorder (ADD) and was on the market for 30 years before being recalled because of liver toxicities [29]. Rapacuronium (tradename Raplon) was developed as a neuromuscular blocker for use in anesthesia but was recalled because of bronchospasms and sudden death [30–32]. Organoid models not only allow efficient testing in the target human organ system but also toxicity testing in organs such as the heart, liver, and lung, where unexpected complications can result in serious side effects and subsequent recall.

3.1 Liver

Drug-induced liver damage is commonly associated with pharmaceuticals. The toxicity may be low level and manageable, such as that found with acetaminophen, or it can be severe enough to force recall from candidacy or market. Liver damage can manifest in many forms, including cell death, hepatitis, or fibrosis, and capturing all types of liver damage within a single model can be difficult. However, engineered models can be fabricated that output measurable liver function that can then be quantified and correlated with physiologic injury. For instance, micropatterned zones of ECM proteins were used to culture primary hepatocytes and 3T3-J2 fibroblasts in confluent co-cultures, which could then be used to measure the liver toxicity of a variety of known toxins by measuring decreases in metabolic activity [33]. The addition of fibroblasts increased the duration and magnitude of hepatocyte function as measured by mitochondrial metabolism and cytochrome p450 (CYP) activity, demonstrating the importance of cell–cell interaction and the incorporation of a stromal component for physiologic liver function [33]. Interestingly, a similar approach using rat instead of human hepatocytes displayed reduced output, highlighting the importance of using species-specific cells for testing [33, 34].

Although micropatterned models can be engineered to produce physiologic-like outputs, they still retain some of the drawbacks of traditional 2D culture; namely, they do not possess physiologic microarchitecture and do not replicate the unique diffusion parameters of 3D structures. Spheroid-based models bridge this gap by integrating cell–cell interactions and stromal components while utilizing a 3D format. Several studies show liver spheroids, from a variety of cell sources, outperform 2D cultures in terms of albumin secretion, CYP activity, and metabolism, measures that can be correlated to hepatotoxicity [9, 35–37]. Integration of non-parenchymal cells such as stellate, fibroblast, or mesenchymal stem cells also increase hepatocyte output and function, further cementing the importance of stromal interactions for liver functionality [15, 38, 39]. Spheroids fabricated using patients' liver cells displayed more homogenous protein expression than freshly isolated, disperse liver cells from the

same patient; indicating that the spheroid format produces consistent results that capture a patient's biology [35]. These same spheroids also replicated chronic drug-induced liver injury response: initial doses of fialuridine caused no toxicity at 48 h, but half-maximal effective concentration (EC_{-50}) values dropped to 100 nM after 4 weeks of exposure [35]. We have produced liver spheroids (composed of primary hepatocytes and Kupffer and hepatic stellate cells) that remain viable, with stable albumin and urea production, for at least 6 weeks, with significantly higher CYP activity when compared with 2D culture [9]. Because of their small, compact form factor, many spheroids can also be suspended in hydrogel droplets to increase functional output [9, 40].

Currently, spheroids comprising primary human hepatocytes are the gold standard for liver toxicity testing and drug screening [16]. However, new technologies utilizing hydrogels, microelectromechanical systems (MEMS), and circulating flow have innovated beyond the currently widely implemented spheroid approach. In our liver model, we embed primary human hepatocyte spheroids into hyaluronic acid and gelatin-based hydrogel modified to include liver-specific ECM extract [9]. The inclusion of ECM increases long-term hepatocyte viability, stabilizes albumin secretion, and supports CYP activity [21]. Our model can also be integrated into multi-tissue, body-on-a-chip systems to test drug or toxin kinetics in the context of multiple organs [8, 9].

3.2 Cardiac

Unlike the liver, the heart is primarily a mechanical organ system. It is mostly composed of cardiomyocytes, cardiac-specific muscle cells that produce the contractile force necessary for pumping blood. Cardiotoxicity can of course manifest when compounds damage or kill cardiac muscle tissue, which alters the heart's ability to pump, causing irregular beating [41, 42]. However, in reality and in the clinic, cardiotoxicity most commonly occurs when a drug or toxin modulates the activity or expression of key ion channels on the cell or mitochondrial membrane, which results in irregular beating activity or deterioration of cardiac tissue [43, 44]. In fact, pro-arrhythmic cardiac toxicity is the most common cause for withdrawal of commercial drugs [45]. Cardiac models for assessing toxicity must reproduce the electrical activity of cardiomyocytes and be sensitive to cytotoxic effects that would damage cardiac muscle cells. Almost all cardiac organoid models utilize cardiomyocytes as the main cell of interest but employ them in different form factors.

Sheets of cardiomyocytes are a low-complexity model for the heart that can capture its beating action. To produce beating sheets, cardiomyocytes must be aligned anisotropically, which can be achieved using engineered surface topography [46–48], microfabricated channels in polydimethylsiloxane (PDMS) or hydrogel [49–52], or micropatterning of ECM proteins [53]. Electrical activity of these sheets can then be measured using multi-electrode array (MEA) systems that can track conductance throughout the sheet to diagnose changes in electrophysiology in response to drug treatments. Disopyramide, lidocaine, and flecainide (Na^+ channel blockers) decreased conduction speed and increased refractory time in a cardiomyocyte cell sheet, whereas verapamil (Ca^{2+} channel blocker) showed the opposite effect—a result that mirrors the ventricle [54]. Another study used MEA analysis to show that conduction slowed in response to quinidine and propafenone (Na^+ channel blockers) as well as 1-heptanol (gap junction blocker that impedes cell–cell ion conduction) [55]. Although these studies demonstrate the viability of cell-sheet technologies for studying the electrophysiologic effects of drug-related cardiotoxicity, the 2D format means they may not be ideal for the assessment of muscle damage-related effects and are difficult to integrate into body-on-a-chip systems. The use of 3D organoids facilitates integration into on-chip systems and assessment of drug toxicity and external damage via tissue analysis over single-sheet layers.

Cardiac spheroids can be produced in a similar manner to liver spheroids, using low-adherence plates or hanging drop methodology [56]. We utilized spheroids produced from induced pluripotent stem cell (iPSC)-derived cardiomyocytes that were then embedded in fibrin hydrogels to generate a heart-on-a-chip model for integration into a "larger body on a chip" [9]. With this system, we measured cardiac output through optical beating analysis [57] and live–dead staining (Fig. 3); treatment with epinephrine increased the beating rate, which could then be blocked by adding propranolol. Others have used different 3D fabrication techniques, such as suspending cardiomyocytes in collagen–fibrin hydrogels that are allowed to crosslink around a pair of molded PDMS posts. The posts deflect under the contraction of the cardiomyocytes, yielding a mechanical measure of the tissue's function [58]. Digoxin and isoproterenol were tested on this system, and both modulated the force and temporal behavior of contractions. Another study based around a similar model tested a variety of pro-arrhythmic compounds and demonstrated concentration-dependent and reversible changes in beating [59].

3.3 Lung

The lungs are similar to the heart in that they have a primarily mechanical function: increasing and decreasing volume to draw air in and out of the body. The alveolar membrane is the site of gas exchange in the body, where carbon dioxide is expelled and blood is replenished with oxygen. These two functions constitute breathing and are the most affected by drug-induced toxicity. Muscles in the lung and diaphragm can be damaged, resulting in decreased

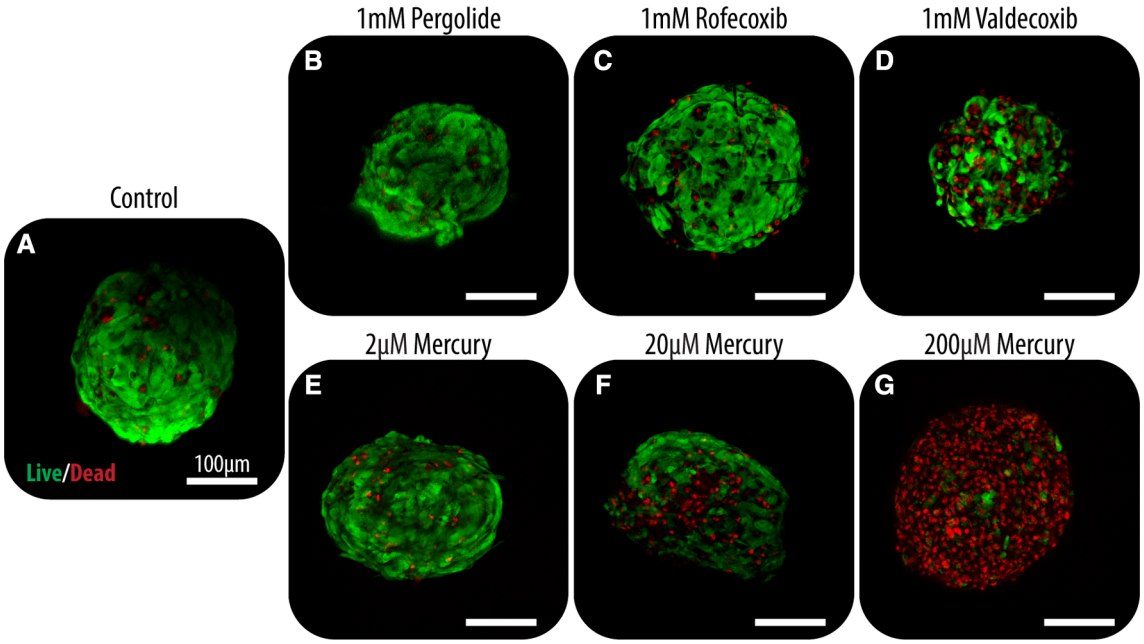

Fig. 3 Deployment of cardiac organoids for toxicity testing. Functional cardiac organoids can be used to screen a variety of agents to assess toxicity. For example, Drugs recalled by the US FDA because they caused heart failure demonstrate varying cardiotoxicity versus control (**a**). Pergolide (1 mM) (**b**) was withdrawn for causing valve disease. Rofecoxib (1 mM) (**c**) was recalled because of an increased risk of heart attack. Valdecoxib (1 mM) (**d**) was also removed from the market because of an increased risk of heart attack. The effects of metal exposure can also be assessed using organoid models. Cardiac organoids show dose responses to varying levels of mercury (2–200 μM) (**e**–**g**). Calcein AM-stained green cells are viable cells; ethidium homodimer-stained red cells are dead cells

lung volume or inconsistent contraction/relaxation, which causes bronchospasms. Tissue can become inflamed or fibrotic, causing breathing difficulties. The most common type of drug-induced damage to the lung is interstitial lung disease, which refers to a progressive scarring of lung tissue, decreasing the rate of gas exchange [60, 61]. An ideal model of the lung for drug screening and toxicity testing should be able to measure differences in gas exchange, breathing rate, and/or cell viability.

Many models of the lung use a Transwell system seeded with alveolar epithelial cells on one side of the membrane and endothelial cells on the other (Fig. 1b) [62, 63]. Systems like this are ideal for assessing the barrier function of the alveolar epithelium; one study used such a system to show that nanoparticle exposure decreased the integrity of the barrier [64]. Trans-epithelial electrical resistance (TEER) sensors can be used to accurately measure changes in barrier integrity in response to drug treatments in real time [65]. Another model used primary human alveolar type II cells cultured in Matrigel, which resulted in alveolar-like cysts [66]. When treated with forskolin, the cysts increased in size because of fluid secretion, indicating this model is sensitive to drugs that modulate fluid transport. We have produced a similar layered lung organoid using lung epithelial cells layered on fibroblasts and endothelial cells [9]. We integrated a TEER sensor into the system and used it to show that histamine exposure decreased resistance, indicating a dilation of the barrier—similar to the in vivo response. When exposed to bleomycin, a chemotherapeutic used for lymphoma, our lung organoids secreted interleukin (IL)-8, a lung-specific inflammatory marker [9].

The layered structure of the lung means it is feasible to construct a layered organoid within a microfluidic chip. Our model was integrated into a microfluidic system with several other organ models [9]. Benam et al. [67] fabricated a lung organoid with an air–liquid interface and integrated it into a microfluidic chip with epithelial cells and endothelial cells lining a membrane. They used this model to simulate both asthma and chronic obstructive pulmonary disease. Treatment with IL-13 increased the number of Goblet cells and increased the secretion of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF), two inflammatory cytokines, which approximates an asthmatic response. A study to replicate the breathing action of the lung on a microfluidic chip used built-in vacuum channels on either side of a cell-lined membrane. The vacuum channels could be used to stretch the membrane and exhibit strain on the cells [68]. Under cyclic strain, endothelial cells aligned, similar to vessels in vivo. Treatment with tumor necrosis factor (TNF)-α induced endothelial expression of inter-cellular adhesion molecule-1 (ICAM-1), an activation factor, which then recruited circulating neutrophils to the endothelial surface.

4 Organoid Models of Disease

A large proportion of drugs are developed to treat specific disease symptoms and etiologies and are discovered and tested using models of the disease of interest. These models range from genetically altered cell lines that express genotypes or phenotypes similar to the disease through to modified animals that approximate the disease. Although these methods have yielded impressive results, they are inherently low-level recapitulations of human disease: single-cell-type models do not capture tissue-level effects, and animals do not fully mimic human physiology. However, organoids represent highly functional models and are often designed to allow modification and tuning. Many researchers have followed this path to generate organoid-based models of disease, sometimes called disease-in-a-dish or disease-on-a-chip models [69]. They span the gamut from fibrosis to cancer to ischemia and allow both pharmaceutical companies and scientists to study diseases in a controlled, relevant manner.

4.1 Liver Fibrosis

Fibrosis of the liver is initiated by the hepatic stellate cells (HSCs), a resident fibroblast-like cell, or Kupffer cells, a resident macrophage, which, when activated, cause an increased secretion of ECM proteins in an abnormal arrangement resembling scar tissue. This excess tissue causes decreased functionality of parenchymal cells and can later lead to cirrhosis of the liver. Organoids modeling liver fibrosis have been developed and could be used to test drugs targeting liver fibrosis. Leite et al. [70] cultured hepatocyte-like cells (HepaRG) and primary human HSCs in spheroid format after which spheroids were treated with the pro-fibrotic compounds allyl alcohol and methotrexate, and subsequently the HSCs became activated to generate a fibrotic state. A similar spheroid-based system incorporated Kupffer cells in addition to HSCs and HepaRGs and was treated with transforming growth factor-β1, methotrexate, and thioacetamide to induce activation in both HSCs and Kupffer cells to start fibrosis [71]. Such systems rely on cell–cell and cell–matrix interactions and are ideal for use in 3D organoids as the disease state is directly related to changes in the tissue microenvironment and cell phenotype, which is best captured in 3D.

4.2 Cardiac Ischemia

Cardiac ischemia is a state of low oxygen perfusion in the cardiac tissue, during which the smooth muscle of the heart cannot function at a normal contractile level. Patients with this disease experience fatigue, shortness of breath, and—in severe cases—death. A variety of in vitro models of cardiac ischemia have been developed to better study this pathology. Katare et al. [72] used neonatal rat cardiomyocytes to produce rings of heart tissue that spontaneously contracted after pre-treatment with cyclical mechanical stretching. When the rings were subjected to hypoxic conditions, they exhibited conduction defects, connexin-43 deactivation, and loss of cell-survival protein expression—a response identical to that of adult heart tissue. Several older studies used hypoxic conditioning on simple 2D cardiomyocyte cultures to simulate the effects of ischemia on cardiac tissue [73, 74]. Both reported findings in line with physiologic responses to ischemia, indicating hypoxic exposure of cardiac organoids may be a promising, simple method of generating ischemic models.

4.3 Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene, resulting in viscous mucous buildup in the exocrine glands of the body. Over time, the gradual accumulation of liquid on these surfaces can block airways or lumen and trap bacteria, leading to rapid infection. The genetic origin of CF means most organoid models are similar to normal models but use cells with genetic abnormalities that exhibit CF-like phenotypes and behaviors in culture. Dekkers et al. [75] used cells with a Cftr F508del mutation and cultured them in Matrigel to produce ductal organoids. They then treated the organoids with VRT-325, Corr-4a, and VX-809 (CF-correcting drugs) and demonstrated that the organoid swelling could measure CF correction; such measurements are not possible in 2D models. Another study utilized similarly fabricated organoids and tested a CRISPR/Cas9 system to introduce a wild-type *CFTR* gene and restore normal function of the epithelial cells [76]. In this case, the use of CRISPR/Cas9 further demonstrated the value of organoid-based models of disease for the development and testing of novel, cutting-edge therapies.

4.4 Cancer

3D platforms can mimic the in vivo structure, cellular heterogeneity, cell–cell and cell–ECM roles, and mechanical interactions observed in tumors [77]. Further advances in microfluidics and microfabrication have led to organ/tumor-on-a-chip platforms with additional functionality [69, 78–80]. To date, a wide variety of cancer organoids and tumor-on-a-chip systems have been developed. Biofabricated organoids can be created using a wide range of methods related to placing cells in 3D suspension. Rotating wall vessel bioreactors allow cells to self-

aggregate around microcarrier beads that can be customized in composition to modulate cell adherence and behavior in 3D [81]. We have created several cancer organoids with this approach, including colorectal cancer metastases in liver, that have been used in both mechanistic and drug-screening studies [10, 15, 82]. Photopatterning strategies have been implemented to integrate 3D tissue and tumor constructs within microfluidic devices. Through exposure to ultraviolet (UV) or blue light, biomaterials with added crosslinking components can form solid structures through photomasks to yield defined shapes and locations in situ within microfluidic tumor-on-a-chip devices [83]. Harnessing control over the ECM components and adding healthy cells can yield organoids with more complex stroma and ECM architectures, which can influence tumor cell behavior [84]. Additional complexity and physiological relevance can be realized by creating multiple tissue and tumor organoids and combining them in a single closed system. This facilitates the study of phenomena such as metastasis, where events take place in two locations—a primary tumor site and a downstream tissue. Notably, we recently demonstrated such a system using a metastasis-on-a-chip device to model metastasis of colorectal cancer cells from a gut organoid to a liver organoid [27].

5 Precision Medicine

Precision medicine can be defined as individualized diagnosis and treatment using diagnostic and therapeutic strategies targeting patient- or disease-specific genetic, proteomic, and phenotypic characteristics [85]. Such innovations have become vital for the advancement of patient-oriented prognosis, diagnosis, and treatment. Organoids have become a tool within precision medicine efforts for many reasons. For instance, they require a minimal number of cells to replicate the in vivo microenvironment, and they can be used for many precision applications to determine specific primary cell and patient outcomes [9].

As described, many organ systems have been the basis for both healthy and diseased organoid models; however, they incorporate commercially available cells that may not represent a patient's unique physiology. Integrating patient cells into organoid models brings a patient's biology to the bench for diagnosis and prognosis (Fig. 4). These studies are advantageous over cell-line, or even commercial primary cell, disease studies as they offer insight into natural genetic variations, cell-type mixtures, and patient-specific behavior. Precision medicine can broadly concern methods, techniques, and analyses that yield exact results for individual patients and their disease state. Such studies no longer generalize disease but seek to more precisely understand its behavior and response to treatment to both benefit the patient and gain greater insight into disease.

Precision medicine strategies require the isolation of patient cells, integration into a model system, and subsequent experimental study. For personalized organoid development, tissue is isolated directly from the patient and processed for single-cell culture use. Isolation is commonly carried out with diseased tissue resections or biopsies. Models may also use human iPSC (hiPSC) techniques by gathering cells that are easy to isolate from patients, dedifferentiating the cells into hiPSCs, and differentiating the cultures into desired cell types for study. However, this type of culture can create its own challenges because of the nature of hiPSC: the differentiation process is often variable, and results can be unpredictable or unrepresentative of the disease state [86].

Thus far, few patient-oriented personalized organoid models have been developed for the study of disease behavior or their response to external stimuli. Although 3D models have been found to yield different and potentially more in vivo-like results than 2D cultures, most personalized models to date have been in 2D [87]. The gap between the use of patient-derived cells for personalized medicine and organoid culture is closing. Cancer-related models have started to integrate patient-isolated cells to recreate the in vivo microenvironment for drug screening and behavior prediction [88, 89]. However, non-cancer, disease-specific patient-derived organoids for personalized medicine applications are rarely developed or utilized. Few examples using patient-derived cells for personalized medicine in organoids exist for liver, cardiac, or lung organ systems.

5.1 Liver Precision Medicine Applications

As discussed, numerous organoid models for healthy and diseased liver are advantageous for drug development. Many of the components of those systems can be integrated into precision medicine applications for use with patient-derived cells. One example of precision medicine applications related to the liver is the work by Sampaziotis et al. [90]. With a focus on bile duct-related diseases, they have been able to directly differentiate hiPSCs into cholangiocyte-like cells (CLCs) [90]. Such cells exhibit functional behavior similar to that of cholangiocytes, the epithelial cells of the bile duct in the liver, and thus can be leveraged in modeling Alagille syndrome, polycystic liver disease (PLD), and CF-associated cholangiopathy. Once differentiated, CLCs of both healthy patients and those with PLD were placed into organoids made of Matrigel (30- or 50-µl droplets) in which proliferation was notably increased and structures with cilia were observed. The organoids were

characterized and shown to be representative of in vivo cholangiocytes. Importantly, both healthy and diseased conditions were treated with octreotide, a treatment used in clinic to reduce cyst size in PLD. Drug treatment reduced the organoid size of both healthy and diseased models and showed a statistically significant response compared with untreated organoids. Responses of both models to octreotide validated expression of secretin receptor and somatostatin receptor 2 and their role in organoid growth and replicated the in vivo drug results with PLD patient cells. Using the same methods of differentiation into CLCs from hiPSC, cells from healthy patients and those with CF were

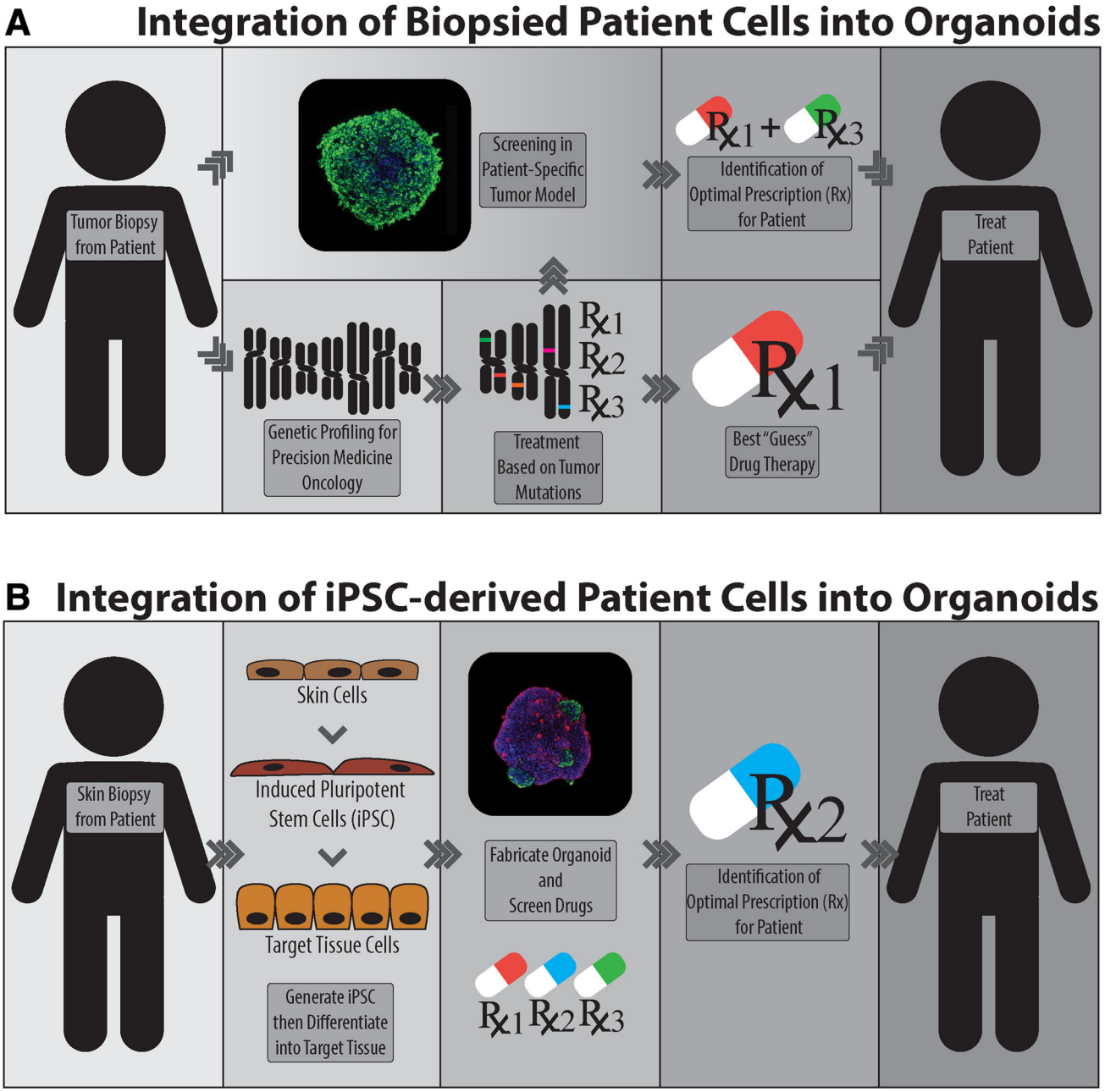

Fig. 4 Illustration of precision medicine strategies. Precision medicine relies on using patient-derived cells to screen a variety of therapeutics in vitro, the results of which can be used for treating the patient. A tumor biopsy can be used for genetic profiling as is done currently, but—in parallel—organoids can be fabricated from the patient's cells. Genetic testing can dictate which therapies or prescription drugs (Rx) to look toward, but all options can be screened to isolate the optimal choice for patient treatment (upper panel). Minimally invasive skin biopsies can be retrieved, digested to isolate skin cells, then dedifferentiated into induced pluripotent stem cells (iPSC), which are then redifferentiated into a target tissue cell type. Organoids can be created from these target tissue cells and screened with a variety of drugs to find the therapy that generates the best response for the patient (lower panel)

placed into organoids, and VX809—an experimental CF drug—was administered. Organoid size was increased with treatment, which represented efficacy as function and fluid secretion were improved. Success of the cultures relied heavily on the organoid structures and the ability of the cells to reorganize within the Matrigel to create relevant structures. Drug efficacy was additionally determined based on changes in organoid size as it was representative of change in cell structure size. This precision medicine application allows for patient-specific disease study and treatment prediction regarding PLD and CF, with development for others.

A second example of precision medicine in liver function and disease has been developed by Ma et al. [91], with a focus on 3D bioprinting patient-derived hepatic-like cells with relevant stromal cells and structural patterns. They focused on the integration of hexagonal structures in series to mimic the hepatic lobule structures seen in vivo. Patient hiPSC-derived hepatic cells were cultured with endothelial and mesenchymal support cells within 3D printed structures. Glycidal methacrylate-hyaluronic acid (GMHA) and GelMA were combined 1:1 to encapsulate cells, and the functionality of cultures was tracked for ≥ 15 days. The authors found that the bioprinted multi-cell cultures maintained albumin secretion and urea production level better than did 2D monolayers and the hiPSC-derived hepatic cells alone in 3D. Five CYP enzymes responsible for drug metabolism were also quantified to determine the ability of the system to respond to drugs, and basal CYP activity levels were highest in the multi-cellular 3D bioprinted structure. This precision medicine application placed focus on the organoid structure and multi-cellular interactions. Through the use of patient-derived cells and appropriate stromal cells, the model is improving in vitro replication of patient-specific liver behavior and response to drugs for precision medicine applications.

Precision medicine regarding liver tissue replication has previously been challenging because of the limited functionality and survival of primary hepatocytes. However, with the development of hiPSC-derived hepatocytes and similar cells, such as CLCs and liver-like biomaterials [21, 92–94], researchers have been better able to replicate the in vivo microenvironment and pursue diagnosis and optimal treatment in vitro. In addition to the 3D organoid models described, numerous 2D patient-derived models have similarly utilized hiPSCs. In both preceding models, the 3D culture element was necessary for success of the liver function and drug response, lending favor to 3D over 2D systems in future precision medicine.

5.2 Cardiac Precision Medicine Applications

Cardiac models have also been intensively developed for precision medicine applications in both 2D and 3D. One way in which cardiac organoids are being pursued is through the use of hiPSC-derived cardiomyocytes (hiPSC-CM). hiPSC-CM are important to the study of cardiac organs because patient cardiac tissue is not attainable while a patient is alive. Because of this, patient-related precision medicine regarding cardiac tissue is exclusively focused on the use of hiPSCs. Mathur et al. [95] created a cardiac-based micro-device for the culture of hiPSC-CM, which can also take electrical measurements and video record cultures. The device has three primary features to improve in vivo-like tissue replication: (1) aligned 3D tissue structure, (2) microcirculation, and (3) shear flow protection of the tissue with diffusive transport. Cells were perfused into the channel and then grown as a 3D culture. Cultures grew for a minimum of 5 days, after which a drug was administered. After 30 min, beating data were recorded. Isoproterenol, verapamil, metoprolol, and E4031 (in clinical trials) were administered, each of which has been shown to increase or decrease the heart rate clinically. A dose response was shown for each treatment, from which the half-maximal inhibition (IC_{50}) could be determined. Further, using clinical data, the authors were able to compare the dose response to that in patients to validate their system and model. This example includes microfluidic applications with patient-derived cells in pursuit of a more in vivo-like system. With the incorporation of flow, cardiac cells were able to align with each other and experience microcirculation with diffusion of nutrients without experiencing shearing that may influence behavior. These complexities may allow for cultures to better replicate the in vivo tissue microenvironment compared with static culture, ultimately improving precision medicine and personalized drug response.

Similarly, Zhang et al. [96] created an organoid system in which human umbilical vein endothelial cells (HUVEC) were bioprinted within an alginate/GelMa bioink to create a scaffold. The printed scaffold containing HUVECs was then seeded with hiPSC-CM, which created vessel- and fiber-like structures within the printed scaffold. Preliminary drug testing using doxorubicin was carried out over 6 days, in which the recorded heart rate decreased over time and with higher doses. Few other examples exist of organoid culture of patient-derived cardiomyocytes; however, cardiac-related diseases for precision medicine are being intensely studied in 2D. An example of this that would translate to organoids is work by Carvajal-Vegara et al. [97], who investigated cardiac behavior with patient-derived hiPSC-CM with a known genetic mutation in the *PTPN11* gene (specifically, T468M mutation) related to

LEOPARD syndrome, a developmental disorder characterized by skin, facial, and cardiac abnormalities [98]. The derived cells were grown in 2D and characterized for disease phenotype in comparison with healthy control hiPSC-CM. Phenotypic changes were seen between the patient-derived cells with and without LEOPARD syndrome. In the future, the functionality of diseased versus healthy patient-derived cells will allow for greater understanding of the impact mutations have on specific organs and response to treatment, which will improve patient care.

5.3 Lung Precision Medicine Applications

Interest in lung-related precision medicine is growing. With advancements in biological understanding and engineering ability, micro-devices for the investigation of disease behavior and treatment are being more readily developed. Wilkinson et al. [99] has developed such a system for the advancement of lung organoids for future use in disease modeling. The model was designed to replicate distal lung alveolar sacs in vitro and was to be accomplished using organoid culture with two cell types: patient-derived hiPSC along the mesenchymal lineage and isolated human lung fibroblast cells. Cell selection was based on trying to replicate idiopathic pulmonary fibrosis (IPF), which is a lung disease that causes irreversible scarring. Organoids were made using a rotating bioreactor and alginate beads for the cells to adhere to. Organoids contained fibroblasts alone or fibroblasts and mesenchymal cells combined. The authors found that organoids formed lung-like tissue through cell–bead, cell–cell, and bead–bead interactions within the bioreactor. Organoids expressed collagen I and alpha-smooth muscle actin and exhibited contraction over time. Levels of expression were similar to those seen in 2D for IPF disease remodeling, and contraction further demonstrated the remodeling ability of the cells. The authors considered the main benefit of this work to be the high throughput capability. As organoids are made in 96-well plates, many replicates can be made at one time for drug screening assays. This work would be advantageous with precision medicine applications as it would enable patient-derived cells to be screened in a large assay format to determine changes in behavior of the disease or response to drug treatment. Within the context of lung cancer, our group has worked to develop tumor organoids derived from biospecimens removed from patients with cancer, with the goal of further expanding these cells in vitro in biomaterial environments that mimic in vivo conditions, without suffering from genetic drift. Thus, we could increase the total cell number of biospecimen-derived cells, thereby increasing the size of personalized drug screens and other assays that might be used to guide therapy.

5.4 Organoids in Cancer Precision 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 regarding cancer treatment [100–102]. However, in practice, the utility of precision medicine is less defined [103]; after identifying key mutations, oncologists are left with several drug options, and—for some patients—there is no one definitive treatment solution, which still leaves treatment as the oncologist's best guess. This creates a need to further develop a model system to help predict the personalized response to anti-cancer drugs [104, 105]. Novel technologies capable of extending the diagnostic utility of tissue specimens are critical for the screening of therapeutic biomarkers and validation of such as actionable targets. Moreover, the biologic behavior of cancer varies widely according to histologic type, grade, location, and tumor size. This variability is currently addressed through genetic precision medicine analysis by relating genetic mutations to chemotherapy options. However, the efficacy of a given treatment in a specific patient is often unknown, as only the mutations are being addressed rather than total tumor behavior.

Within research, patient-derived xenografts are also used to study patient tumor progression and drug treatment response ex vivo through the injection of patient tumors into a mouse model. These models are lacking in that they require immune-deficient mice in which to place the biopsies or tumor samples, which causes them to become infiltrated with cells from the mouse, rendering the samples no longer 'patient-only' [106]. The cells also adapt to their new environment, and genetic drift from the initial samples has been shown, making them less ideal [107]. Therefore, 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 rare and gives little power to current precision medicine approaches [107]. Bioengineered tumor models derived from patient tumor biospecimens may provide a powerful tool for screening potential therapeutic agents and determining the most efficacious and safest therapy for a specific patient while also providing insight into tumor behavior and progression ex vivo [108]. This is a new area for tumor organoids but holds incredible potential for improving outcomes for patients with cancer.

Tumor models focusing on personalized precision medicine are being developed within our laboratory. With regular access to primary patient samples from biopsies and complete resections, we have been able to dissociate the masses to single cells and re-culture them in 3D hydrogel. Using biofabrication methods such as bioprinting and photopatterning, we have created platforms for testing

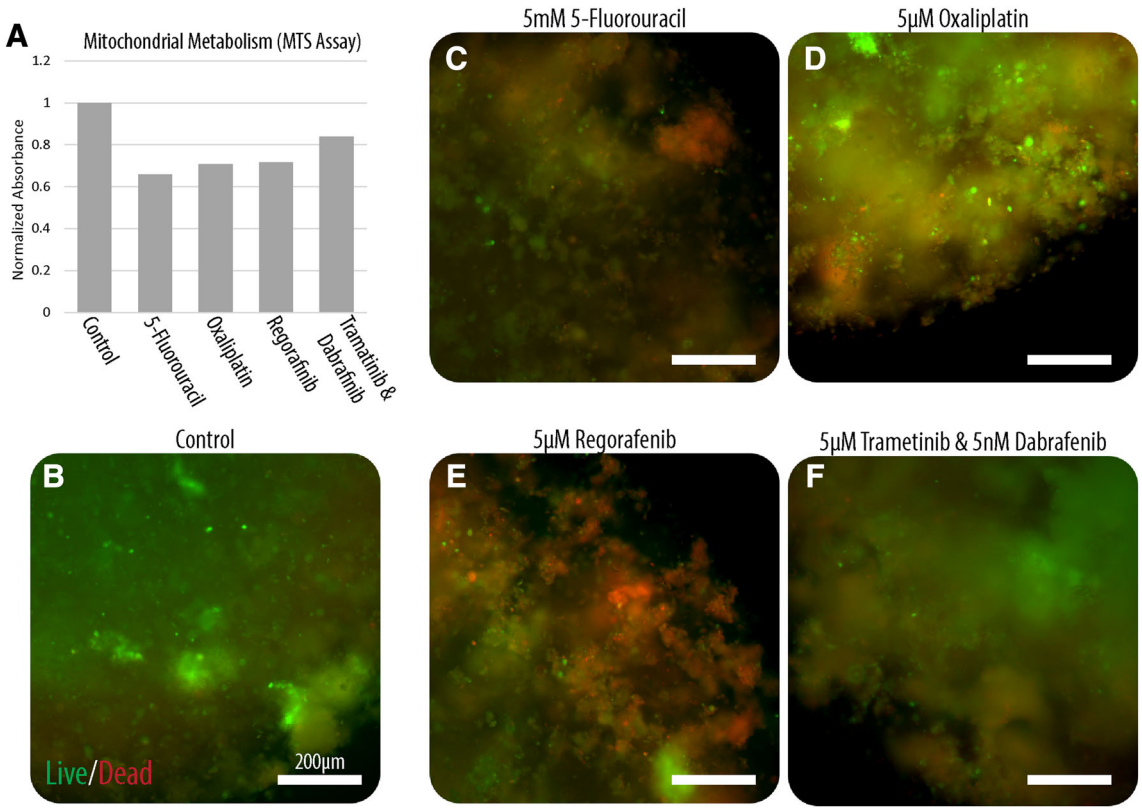

Fig. 5 Precision medicine screening of patient abdominal wall tumor biopsy. Cells were isolated from an abdominal tumor from a single patient and placed into three-dimensional (3D) hydrogel-based organoids for 7 days. Chemotherapies were administered on day 7 in culture. **a** After 3 days of treatment, an MTS (mitochondrial metabolism) assay was run on treated and untreated conditions to determine the relative change in culture metabolism. **b–f** Live/dead viability/cytotoxicity assays (Thermo Fisher) were also performed, and organoids were imaged to show qualitative differences in live and dead cells. **b** Control, **c** 5-fluorouracil (5-FU), **d** oxaliplatin, **e** regorafenib, **f** trametinib and dabrafenib. Calcein AM-stained green cells are viable cells; ethidium homodimer-stained red cells are dead cells

drugs on patient tumor organoids (Fig. 5). These platforms are creating the opportunity for personalized drug treatment in patients with unclear genetic data who do not respond to standard treatments, and they ensure the best treatment for those with known genetic mutations. We can also validate our models by treating the patient tumor organoids with drugs to which they are known to respond. Additionally, genetic data can be paired with the patient tumor organoids to study genetic drift and relation to drug response, as we have been able to culture viable organoids for many weeks.

5.5 Future Perspectives

Improvements in the development of organ and disease models have enabled the advancement of personalized medicine applications through the implementation of patient-derived cells. In the future, close relationships between medical and research facilities will enable patient tissue to be used within systems. With increased availability of patient samples for the study of disease, a greater understanding of disease behavior within a variety of patients can be developed, which will offer the opportunity to improve diagnostics and treatment plans based on cell and microenvironment behavior. This work will be invaluable to researchers pursing a mechanistic understanding of disease and will advance the development of relevant therapies. For such advancements to be made, improved cell extraction, processes to develop and differentiate iPSCs, and specific microenvironment factors must be achieved. Second, patient-based drug screening can be conducted at the clinical level to determine the best treatments for individual patients. These biological and engineering techniques can be transitioned into patient-centric applications that yield data for clinicians to interpret and utilize for improved care.

6 Conclusion

Organoids create a unique opportunity for the study of disease development, behavior, and therapeutic response as well as for the advancement of personalized medicine applications. The 3D models for drug testing and discovery

are of paramount importance in understanding drug mechanisms of action and tissue responses. Individual organ response and organ–organ interactions can be studied under treatment and model in vivo-like behavior more effectively than 2D culture systems can. Additionally, specific disease states can be modeled and used for the investigation of long-term progression and potential drug treatment. Further, precision medicine applications can leverage disease models using patient-derived cells. Organoids can be patient specific and allow for personalized disease and treatment modeling. Here, we have shown examples of liver, cardiac, and lung organoids used in drug development and precision medicine applications. Extensive consideration is given to developing healthy organoids of each tissue type for replication of function and tissue behavior. Healthy models can then be screened using toxins or drug treatments designed to affect other target organs that may have off-target effects. Disease models can further be designed based on advances in healthy models from which experimental drug studies can be conducted. Using patient-derived cells, models can be created to study individual diseases and their progression and to conduct drug screens to yield the best individual treatments. In both drug development and precision medicine applications, 3D organoid culture is allowing us to create more complex tissue-like constructs and make well-informed decisions with patients.

Compliance with Ethical Standards

Conflict of interest Mahesh Devarasetty and Andrea Mazzocchi have no conflicts of interest that are directly relevant to the content of this review. Aleksander Skardal is an inventor of several patents on organoid technologies for drug screening, disease modeling, and personalized medicine.

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