ORGANOID TECHNOLOGY:

DISEASE MODELLING, DRUG DISCOVERY, AND PERSONALIZED MEDICINE

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Organoid Technology: Disease Modelling, Drug Discovery, and Personalized Medicine

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PREFACE

Organoids are miniature in vitro 3D models that mimic the near-physiological structure and function of the respective tissues and organs. Organoid bioengineering is a transdisciplinary approach that uses stem cells' capacity to self-renew, differentiate into several lineages, and self-organize into organoids. Using organoid bioengineering, scientists have employed induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and tissue-resident adult stem cells (ASCs) to generate these tiny tissue replicas. Several research teams have developed endodermal, mesodermal, and ectodermal organoids by manipulating stem cells in vitro. Numerous organoids may now be created, including those of the kidney, brain, lung, colon, intestine, breast, retina, and liver. Given the gap between animal-based models and human disease pathology, a paradigm change was required to simulate human diseases accurately. The 3D human organoid platform provides an unmatched opportunity to develop better models and get a more profound knowledge of human pathophysiology. Organoids provide information on human disease-related processes, such as disease-specific signaling alterations, cell-cell interactions, therapeutic target identification, therapeutic screening, and discovery.

Organoid technology has been used to model diseases across different organ systems, drug screening, and regenerative medicine. Recent advances, including the development of the novel organoid platform, engineering organoid complexity, disease modeling, introducing pathological aspects together, and drug discovery, have provided a ray of hope for human-specific therapeutic discovery. Patient-derived tumor organoids may be created from individual patients, and biobank, and used for therapeutic screening and personalized treatment. Significant progress is made toward large-scale organoid production. Novel technologies like high-resolution 3D imaging, genome editing, hybrid culture techniques, single-cell transcriptomics, microfluidics, organ on a chip, 3D printing, nanotechnology, and other cutting-edge technologies facilitate the development of physiologically accurate human disease models.

Significant numbers of animal-based preclinical studies leading to human clinical trials fail due to safety or efficacy concerns, raising the question of whether animal experiments can truly aid in the development of effective therapies and at what cost. Numerous alternatives, such as in vitro human-specific 3D microphysiological systems such as organoids and microfluidic-based organs-on-a-chip that closely mimic human physiology and architecture, have enabled cutting-edge animal-free research. Although optimistic, these technologies have not yet attained their summit, and a complete substitution of animal-based experiments may take decades. Scientists believe that addressing the obstacles of appropriate validation, proper standards, development of protocols for large-scale production, funding, regulatory rules, and ethical issues may enhance the use of human biology-based models, thereby improving the lives of humans and animals. In an endeavor to reduce animal dependence, in late December 2022, President Joseph Biden signed a law that novel therapeutics no longer require animal experimentation. After eight decades of medication safety regulation, this long-awaited action could help end animal experimentation and make therapeutic interventions that are tangible and effective. The purpose of these chapters is to shed light on the developing resources

addressing the concepts of organoids and disease models, including cancer. This book focuses on organ-specific organoids and disease modeling.

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CHAPTER 1

Bioengineering Organoids for Disease Modeling and Drug Discovery

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Abstract: Organoid technology has been used to model diseases across different organ systems, drug screening, and regenerative medicine. Organoid technology better mimics human physiology and can provide a better alternative to *in vivo* animal models. Recent advances in organoid technology, including developing the novel organoid platform, engineering complex organoids, and introducing pathological aspects, have provided significant progress toward producing miniaturized tissue or organs on a dish. Novel technologies like high-resolution 3D imaging, organ on a chip, 3D printing, gene manipulation, nanotechnology advances, and single-cell sequencing have led to a massive thrust in the organoid technology that can provide a unique insight into the behavior of stem cells, cater to preclinical research and theranostics (therapy plus diagnostics).

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Keywords: Bio-banking, Bioengineering, Cancer, Disease model, Drug screening, Infectious diseases, Organoid, Organ, Regenerative medicine, SARS-CoV2, Theranostics, 3D-printing.

INTRODUCTION

Since the late twentieth century, the preeminent way of modeling the physiology of human organs has been the usage of cell lines and animals. Global biological research has refined our perception of various cellular signal transductions, screening and identifying drug targets, and discovering novel drugs to combat dreadful diseases such as cancer, infectious diseases, and some non-communicable diseases. Their universal operations and application portray the historical dependence of today's biomedical research on these model systems [1]. The impeccable accuracy of the natural selection process and the *sine qua non* of evolutionarily conserved biological mechanisms across species have elicited the primary focus of researchers toward a few model organisms. They are generally rapidly growing species that can spawn a copious number of offspring in a short interval and can be propagated in a laboratory in a cost-effective manner, such as *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly), *Danio rerio* (zebrafish) and the common laboratory *Mus musculus* (mouse) and *Rattus norvegicus* (rat). As time passed, a common discovery thread became traceable while scanning the pathophysiology of maladies and various animal models of human diseases. Functional aspects of biological organisms were deciphered by genomic screens in invertebrates, with subsequent anatomization of evolutionarily conserved genetic aspects in mammalian models, which would pave their way to humans. These commonalities in principles and their applications have begotten an extensive mechanistic understanding of various human diseases [2].

Nevertheless, the triumph was dampened by the fact that the biophysical and physiological functions of the model organisms were not consistently replicated in humans owing to the human system's intrinsic complexity. Sometimes, it has been witnessed that extrapolating results from animal models to humans has become a significant bottleneck in drug discovery processes [1 - 3]. Moreover, recent insights into the phenomenon also reveal some biological processes specific to the human system alone (such as brain development, lung development, metabolism, and evaluation of drug efficacy) beyond the scope of modeling in animals. Obviously, the above challenges have prompted many attempts to model functions of human organs, which include stem cell differentiation in two-dimensions (2D) and three-dimensions (3D) with/without matrix, organoid bioengineering, bio-printing, and organ on a chip using microfluidics [4]. To bridge the disconnect between the actuality and models, a fecund technology,

organoid bioengineering, has recently substantially impacted global biomedical research. These essentially undifferentiated stem cell/ differentiated progeny/ cancer stem cell-derived 3D culture systems made the re-creation of precise tissue/ organ architecture and physiology possible.

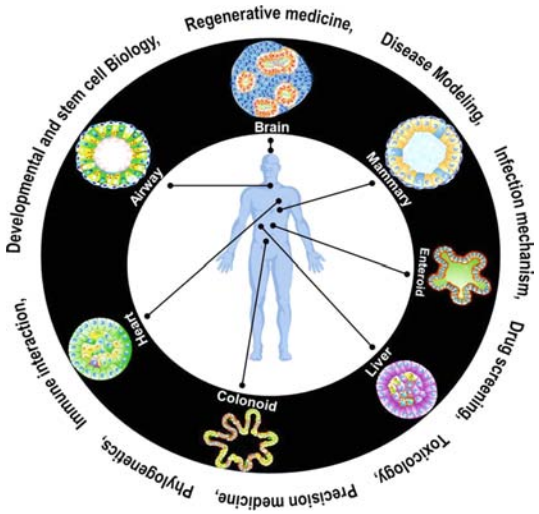

Fig. (1). Schematic showing the bioengineering of tissue-specific organoids and their use in many biomedical and commercial uses. Figure reproduced from reference 7.

There is no denying that human-specific organoids have the unique potential to produce 3D cultures that closely mimic the human organ of choice and can represent human diseases more accurately than animal-based models [5, 6]. The most common feature of all organoids is attributed to the origin of organoids based on the recapitulation of various 3D-designed protocols of *in vitro* organogenesis from pluripotent or adult stem cells [6]. It is, therefore, limpid and conspicuous that upon analyzing the organoid generation, the scientific minds will extract precious mechanistic information regarding human development, organ regeneration, and disease modeling. 3D culture platforms, especially organoids/ spheroids also have demonstrated their potential and benefited human-specific disease modeling, pharmaceutical drug screening, and molecular medicine-based discovery. The perception of ramifications of its relevance has already triggered extensive application of this model in biomedical research, complementing the already existing ones [2, 4 - 6]. Fig. (**1**) presents a schematic view of tissue-specific organoids and their applications. The discovery and development of organoid technology are still in their juvenescence, compared to the existing cell/ animal-based models, with obstacles to circumvent. In this chapter, besides providing an overview of organoid technology, we shall briefly highlight the major advances in tissue-specific organoids, drug screening, organoid imaging,

CHAPTER 2

Organoid Technology: Disease Modeling, Drug Discovery, and Personalized Medicine

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Abstract: The last three decades have witnessed revolutionary growth in the fields of biomedical science and pioneering the same in regenerative medicine and disease modeling. Historically, biological research has been performed using 2-dimensional animal cell culture, but now we are switching to more intricate 3-dimensional models for better replicability of experimental results. Organoids are stem cell-derived 3D cell cultures that are the cornerstone of this new development. They retain the significant features of biological organs and have opened up new, previously not-thought-of avenues to steer research in personalized healthcare and disease modeling. The current chapter encapsulates how organoids came into the picture, addresses the current research occurring worldwide, and discusses futuristic aspects and applications. The significance of organoids in disease modeling is discussed in detail, and the following aspects, such as disease modeling in congenital conditions, cancer, infectious diseases, gene editing, and futuristic microfluidics, were elucidated. This chapter also covers the role of organoids in drug discovery. Drug discovery is a very time and money-intensive process, and many attempts have been made over the years to bring about change in the same. It has been noted that the development of many new drugs is being hindered due to the complexity of the human genome. This point has been elaborately discussed in this present chapter, along with the potential of organoids as a solution in high-throughput drug screening and personalized treatment. The chapter concludes with a look at how the COVID-19 pandemic has underpinned the use of organoids in drug research and disease modeling, and finally, it provides a summary of future research directions.

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Keywords: Drug discovery, Disease modeling, Organoids, 2-dimensional culture, 3-dimensional culture.

INTRODUCTION AND THE BASICS OF ORGANOIDS

The past decades have witnessed significant research and development in biomedical science concerning regenerative medicine, disease modeling, drug discovery, and drug repurposing. The advent of cutting-edge technologies in 3-dimensional (3D) biology, like spheroids, organoids, organs-on-chip, 3D hydrogels, 3D co-cultures, and 3D bioprinting, has delivered a powerful boost towards precision and personalized medicine. Earlier, 2-dimensional (2D) monolayer cultures were prominently used, but the existing lacunae and the concomitant development of 3D culture systems have limited their use. Adherent 2D cultures grow as stretched and flat adherent monolayers of cells in contrast to their original *in-vivo* morphology. Proliferation, apoptosis, differentiation, gene, and protein expression are some of the essential cellular processes that may be influenced by this altered morphology [1, 2]. 2D monocultures of cell lines often fail to simulate the *In vivo* cellular functions, tissue-specific cell-cell, and cell-matrix interactions as observed in 3D cultures [3].

Though 2D-based cell culture systems were the main-stay for drug discovery, the inherent drawbacks of the said system have led to the delayed progress of drug molecules through the clinical pipeline, and the majority of the pharmaceutical compounds failed in human clinical trials. Despite promising results in 2D cultures, the same could not be reproduced in clinical settings. Notable differences between 2D cultured *in-vitro* systems and actual *in-vivo* conditions may lead to differential responses to treatment [4]. These limitations have been overcome mainly by transitioning to 3D cell culture models. These 3D models have arisen methodologically and conceptually because of the classical re-aggregation experiments that demonstrated that cells segregated from embryonic organs have the capability to re-aggregate and re-constitute the original organ structure. This chapter intends to focus on organoids as 3D model systems to study disease modeling, drug discovery, and personalized medicine.

An inclusive definition will refer to ‘organoids’ as Minuscule replicas of corresponding biological organs or mini-organs, that are cultured as 3D structures derived either from pluripotent or organ-specific stem cells, supported on 3D gels constituting the extracellular matrix (ECM), fortified with specific nutrients, signaling molecules, and growth factors so that they harbor the ability to replicate their biological counterparts in terms of architecture complexity and physiological functions, as well as having the multi-lineage commitment and differentiation potential [5]. Till now, multiple human organoids like brain, kidney, lung,

stomach, liver, pancreas, endometrium, prostate, pancreas, thyroid, and retinal organoids have been produced [6] in different studies.

Before proceeding toward the various applications of organoids, it is imperative to understand how the microenvironment influences organoids. Engineering tissue-specific balanced and requisite microenvironment conditions is paramount to ensure the successful growth and development of an accurate organoid. The imbalance of any essential component may skew the organoid maturation and result in aberrant histomorphology, altered size, and cellular composition [7]. Organoids can be classified according to the type of stem cells [adult stem cells (ASCs) or pluripotent stem cells (PSCs)] used in their formation (Fig. **1**). PSCs (which give rise to three primary layers in the early embryo stage) can be re-activated by tuning and reprogramming them to form differentiated somatic cells. Induced Pluripotent Stem Cells (iPSCs) have great differentiation potential and have been progressively used for organoid cultures. Organoids may be described as "blank states" devoid of signals and need molecular cues to facilitate their growth and differentiation. Thus, delineating the role of extrinsic biochemical and physical cues is crucial for the development process. This enables a quantitative platform for the study of biological processes as significant experimental control can be established through their use [8].

The role of physical cues is to support cell attachment and maintain the structure for survivability [9]. Two types of techniques can be used for organoid development: i) Scaffold matrices and ii) Scaffold-free techniques. The matrigel derived from Engelbreth–Holm–Swarm mouse sarcomas is the most often utilized matrix. Four major basement membrane ECM proteins that make up the matrigel are: laminin (60 percent), collagen IV (30 percent), entactin (8 percent), and the heparin sulfate proteoglycan perlecan (2–3%) [10]. Scaffold-free techniques make optimal use of the self-aggregation properties of cells when kept in hanging drop microplates or low-adhesion plates to form spheroids and cell sheets [11]. Biochemical cues are the molecules that are responsible for the regulation (up or down-regulation) of the specific signaling pathways, which differ with the type of organoid cultured, the type of stem cell used (whether PSC or ASC), and the level of differentiation required [9]. Some of these biochemical cues are growth factors like insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), transforming growth factor (TGF), mitogen-activated protein kinase (MAPK), bone morphogenetic protein (BMP), RHO-associated protein kinase (ROCK), WNT-related integration site [7], and R-spondin (WNT regulator) [9]. Notch, Hedgehog, Wnt, and mTOR signaling pathways are frequently associated with and targeted in organoid technology. The physical and biochemical cues, including angiogenic growth factors, are transferred through

CHAPTER 3

Intestinal Organoid Bioengineering, Disease Modeling, and Drug Discovery

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Abstract: The intestinal organoid system is a unique *ex-vivo* representation of the complex and dynamic mammalian intestinal epithelium. Intestinal organoids are three-dimensional, crypt-villus structures with a central lumen that can be sourced from adult intestinal stem cells, embryonic stem cells as well as induced pluripotent stem cells. They serve as a bona fide model for not only understanding intestinal biology and development but also for disease modeling, regenerative therapeutics, and drug discovery. Organoids help bridge the gap in existing model systems by incorporating complex, spatial, and biological parameters such as cell-cell interactions, cell-matrix interactions, gut-microbe interactions, and other components of intestinal *in-vivo* physiology and pathology. In this chapter, we discuss the basic strategies to generate intestinal organoids and how different bioengineering approaches can be used to effectively model both genetic and infectious intestinal diseases to enhance their utility in research and therapeutics.

Keywords: Colon cancer, Colonoids, Cystic fibrosis, Disease modeling, Enteroids, Inflammatory bowel disease (IBD) modeling, Intestinal organoids, Intestinal stem cells, Organoid culture, Organoid engineering.

INTRODUCTION

Multicellular organisms orchestrate cellular and tissue renewal by harnessing the regenerative and multi-differentiation potential of adult somatic stem cells for the maintenance of homeostasis. The human intestinal epithelium in particular, is well known to utilize the regenerative program of the intestinal stem cells (ISCs) in order to prevent the accumulation of genetic mutations and to maintain intestinal health and function. The intestinal epithelium is a crypt-villus structure that plays a crucial role in the absorption of water, nutrients and electrolytes as well as drug transport, metabolism, and hormone excretion. Due to this complex array of functionalities, it is constantly exposed to substances of varied compositions and

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toxicities. However, it has a protective mechanism in the form of its disposable epithelium, which sheds off intestinal epithelial cells cyclically. The ISCs then replenish and replace the intestinal epithelium every 3-4 days. These ISCs are housed at the bottom of the intestinal crypt and produce progenitor cells called transit-amplifying cells (TA cells) which proliferate and migrate up along the crypt-villus axis to differentiate into mature intestinal epithelial cells, which constitute the intestinal surface epithelium. These ISCs are armed with long-term self-renewal capacity and multipotent differentiation, which continue to regenerate the crypt-villus structure of the entire intestinal epithelium during the individual's lifetime [1].

Even though it has been difficult to recapitulate the multifactorial and complex intestinal crypt-villus mechanism in the laboratory, several preclinical models have been developed during this endeavor. These include the conventional models such as cell lines [2 - 6], Using chamber [7 - 9], and everted sac [10 - 12], as well as the more sophisticated models such as InTESTine™ [13, 14], microfluidic gut-on-chip [15 - 17], and intestinal organoids (IOs). These intestinal model systems have their advantages, applications, and limitations [18]. They have contributed to enhancing our understanding of intestinal epithelium and its myriad functions and mechanisms in health and disease. In this chapter, we will take a deep dive to understand the stem cell-derived *In vitro* model of the intestinal epithelium, IOs, which closely mimics the physiological 3D intestinal crypt-villus morphology. Previously published reports have used analogous terms such as 'enteroids', 'tumoroids', 'mini-gut organoids', colonoids' for *In vitro* cultured-3D-intestinal structures which we refer to as IOs in this chapter. Kindly note that 'intestinal' in the term intestinal organoids, encompasses both small and large intestine (colon) for the purpose of this chapter.

ESTABLISHMENT OF INTESTINAL ORGANOID CULTURE SYSTEM

Knowledge of the developmental biology of intestinal crypt-villus structure, the micro environmental factors, and signaling pathways involved in the same have equipped us with tools necessary to instruct somatic ISCs to self-renew, differentiate *ex-vivo*, and self-organize into three-dimensional (3D) structures that preserve the structural, molecular and functional identity of the original intestinal tissue. The cornerstone of intestinal organoid culture is the ability to isolate stem cell containing units, intestinal crypts, or a single population of stem cells and grow them with appropriate 'niche' culture conditions providing cell-cell and cell-extracellular matrix (ECM) cues to self-assemble into the parent tissue of origin in the culture dish.

Research and Development

Although some culture systems have been described, it has been challenging to establish *in vitro* propagation of adult somatic stem cell-derived organoids without inducing genetic transformations. One of the first of these organoid-type cultures was the rat intestinal crypt culture system, established and propagated for 1-2 weeks by seeding crypt epithelial cells with subepithelial fibroblasts on a Type I collagen-coated surface [19]. Notably, this population of rat intestinal crypts were not able to survive without the companion fibroblasts, which proved the importance of 'niche' signaling for the establishment of organoid cultures. Another study reported long-lasting maintenance of IOs by culturing neonatal intestinal epithelium within a niche comprising mesenchymal fibroblasts providing Wnt signaling cues using a novel, air-liquid interface culture system [20]. Although the existence of multipotent cells in intestinal epithelium has been known for a long time, confirmatory evidence of leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) positive crypt base columnar cells possessing the requisite properties of stemness, long-term self-renewal, and multipotent differentiation came from elegant fate-mapping experiments in mice [21]. In these experiments, lineage tracing of Lgr5+ expressing cells was visualized with a Cre-activated reporter (Lgr5-EGFP-IRES-CreER) [22]. Independent research groups also reported Bmi1, Hopx, and mTert expressing label-retaining cells as ISCs [23, 24]. Another decade of research using gene-targeting experiments in mice revealed that intestinal stem cell plasticity was determined more by niche signaling pathways than cell-intrinsic properties like cell position or marker expression [25 - 27]. This stem cell niche signaling in the intestinal epithelium was found to be dominated by Wnt signaling, the master regulator of proliferation and undifferentiated status of ISCs [28]. The binding of Wnt ligand to Frizzled and LRP5/6 receptor complexes suppress the APC/GSK-3/Axin leading to the nuclear translocation of β-catenin, where it forms the Tcf4/β-catenin complex. The Tcf4/β-catenin induces downstream expression of Wnt target genes such as Lgr5. R-spondin is the ligand of the Lgr5 receptor, which enables sustained activation of Wnt signaling. R-spondin-treated mice have been observed to develop crypt hyperplasia and high ISC numbers due to enhanced Wnt activation [29, 30]. In contrast, inhibition of bone morphogenetic protein signaling (BMP) is necessary for ISC renewal [31, 32]. It has been observed that BMP inhibitors such as Noggin or Gremlin induce crypt formation and increase ISCs. Notch signaling is another player in ISCs maintenance and regulates secretory lineage differentiation [33 - 35]. EGF signaling is also essential for intestinal stem cell renewal, and PI3K/Akt signaling induces hyperplastic changes in the intestinal epithelium [36 - 38].

CHAPTER 4

Bone Organoids: Current Approaches, Challenges, and Potential Applications

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Abstract: Organoids are complex three-dimensional microtissues formed by the self-organization of stem cells and aimed to mimic the structural and functional characteristics of human tissues. Bone comprises multiple cells with a mechanically rigid extracellular matrix (ECM). The diversity of a bone in terms of structure and complexity demands an ideal bone model with limited control of physico-chemical parameters. Potential applications of bone organoids can be seen in bone regeneration, and regulation mechanism studies and to address various bone-related disorders and defects. Approaches to creating bone organoids may include using mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), osteoblasts, and osteoclasts in addition to endothelial cells for the vasculature generation. Moreover, in bone organoids, ECM of biological origin materials that display close resemblance with the native bone ECM are preferred or may be generated using scaffold-free methods. The mechanical load should be the primary parameter that should be considered in the model. Since bone is considered hypoxic, organoid-based bone models should include an O2 regulation mechanism to achieve a physiologic hypoxic environment. Advanced cell isolation, tissue culture, and cell differentiation techniques, along with microfluidics and tissue engineering strategies, might lead to the production of physiologically relevant bone organoids. This chapter outlines prerequisites for bone organoid development and the potential applications of bone organoid-based models in various biomedical research domains. Additionally, their limitations and future perspectives are also explored.

Keywords: Bone organoids, Bone remodeling, Biomaterial, Biocompatible, Bone tissue engineering, Extracellular matrix, Hypoxic, Hydrogels, Mesenchymal stem cell, Microfluidic devices, Microtissues, Osteoblasts, Osteoclasts, Primary cells, Polymeric beads, Scaffold-free, Self-assembly, Spheroids, 3D printing, Vascularization.

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INTRODUCTION

Currently, animal models are used as a platform to establish proof-of-concept preclinical studies in order to develop treatment options. Nonetheless, the species-specific physiological and metabolic differences hinder the extrapolation of results obtained from animal experiments to be applicable to humans. There have been reports of significant failure rates of novel promising medicines in clinical studies, even though animal studies showed the desired results [1, 2]. Thus, biomedical approaches need to be reviewed to achieve a higher degree of relevance in physiology. With the advancement in *In vitro* cell culture systems, mainly driven by tissue engineering and regenerative medicine approaches, human physiology has been reflected to a greater extent. These approaches include the isolation of primary cells and their large-scale expansion, followed by the generation of a three-dimensional (3D) tissue biomimetics scaffold using biocompatible materials. Furthermore, advanced biomaterials, 3D culture, bioreactors, and efficient microfluidic systems have also been developed as promising *In vitro* systems, and as effective alternatives to animal testing [3, 4]. The mineralized structure of bone provides support, load bearing, and protection to the internal organs. Most bone models should recapitulate the biological and functional characteristics of bone, which are crucial to the respective research question. Hence an ideal bone model should adequately depict the investigated features of bone physiology. Organoids are comprised of 3D multicellular *In vitro* tissue construct formed either of pluripotent stem cells (PSCs) or adult stem cells (ASCs) by the process of self-organization that mimics the complex *In vivo* tissues and organs. This makes them very promising models for the study of human processes, structures and cell-tissue interface in the tissue culture dish.

Several organoid models consist of various cell types that mimic their *In vivo* counterparts and are still an area of ongoing research. Bone remodeling *In vivo* necessitates the interaction between osteoblasts (OBs) and osteoclasts (OCs), and the process is directed by extrinsic stimuli like mechanical load and the effect of hormones like the parathyroid hormone. Therefore, a system designed for studying the remodeling demands the inclusion of at least OBs, OCs and external factors. Since bone extracellular matrix (ECM) resorption by the OCs is a physiological process driven by enzyme and pH changes, thus a suitable matrix is desirable for regulated degradation of the bone matrix constituents while preserving the integrity of the available scaffold [5]. Moreover, besides having differences in anatomical properties, like the cortical (or compact) and cancellous (trabecular or spongy) bone, it also possesses differences in mechanical properties due to variances in orientation and alignment of collagen fibers although they have similar material composition. Such differences, along with dissimilarities in anatomical structures and vasculature, have not been reproduced in existing

methods. In addition, selecting an appropriate ECM is crucial for developing bone organoids.

Depending on culture time and biomechanics, there are several methods for the generation of bone-like ECM using various 3D cell culture systems. Utilizing 3D bioprinting to create multicellular bone organoids is a recent advancement in the field. A suitable bioink resembling the native bone ECM is needed for 3D printing to generate desired anatomical features. The parameters such as mechanical actuation, perfusion, and the exchange of gas are provided by the bioreactor for generating complex bone models. The exclusion of xenogeneic substances and materials is done in order to avoid side effects on cells. Bone organoids mimic complex human bone, and their treatment response to therapeutic targets can significantly deliver more accurate translation results to human physiology and pathology. Due to their compact size, bone organoids can be a promising platform for various potential applications depicted in Fig. (**1**).

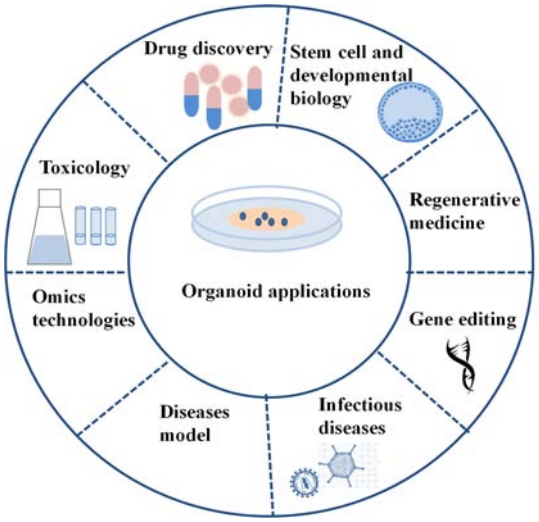

Fig. (1). Various potential applications of organoids

Physiology of Bone and Key Parameters

Bone is a mineralized, strong, and rigid connective tissue that provides structural support for the musculoskeletal system. Components of bone include ECM, a composite material with several specialized cell types. The bone ECM comprises inorganic minerals (~ 60%), organic matrix (~ 30%), water, and lipids [6]. Increased mechanical stability of the ECM is attributed to the bone mineral part known as hydroxyapatite (HA), formed largely of calcium and phosphorus. The organic component confers flexibility and elasticity and primarily consists of type I collagen. Histologically two different classes of bone tissue: namely, cortical (or

CHAPTER 5

Cardiac Organoids: Promises and Future Challenges

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Abstract: The development of state-of-the-art, *In vitro* three-dimensional organoid culture methodologies, represents a quantum leap in stem cell technology and tissue engineering. In contrast to traditional two-dimensional cell culture and rodent models, organoids generated from patient-derived cells can dramatically increase the precision and relevance of *In vitro* approaches to model human development and disease. Currently, the most well-established organoid systems are those for the intestine, brain, bone, kidney, and eye. Surprisingly, research using cardiac organoids, or "cardioids, is still in a nascent phase, lagging significantly behind these other, more mature, tissue-specific organoid platforms. Consequently, there is an ongoing need to develop more robust and reproducible protocols capable of yielding self-organizing cardiac organoids that assemble following valid cardiogenic principles, recapitulating the microanatomy and cellular hierarchy observed during *In vivo* cardiac development. Cardiovascular disease is currently the primary cause of death in developed countries, and its prevalence is growing worldwide. Improved cardiac organoid technologies have the potential to facilitate and enhance the application of the new wave of personalized medicine aimed at addressing cardiovascular disease. This book chapter will discuss the development of cardiac organoid research, its present state, and future challenges in detail.

Keywords: Cardiac organoids, Cardiovascular disease, Embryogenesis, Personalized medicine.

INTRODUCTION

The journey from a single totipotent cell to a complex, multicellular adult organism is an elegantly coordinated process of self-renewal, proliferation,

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differentiation, self-organization, and self-assembly. Provided with the proper microenvironment, dissociated cells can reassemble to regenerate differentiated structures, including a variety of embryonic organs, adult tissues, and even entire organisms. Taking advantage of these properties, researchers have developed "organoids" or mini organs. An organoid is an *In vitro* 3D micro-tissue structure that recapitulates fundamental, *In vivo* properties of the corresponding parental organ. As such, organoids can provide a flexible *In vitro* platform for experimental manipulation of specific aspects of tissue and organ biology in a controlled environment isolated from the influence and complexity of the intact organism [1, 2]. Although growing cultured cells in a 3D matrix is an age-old technique, the term "organoid" is relatively new to the scientific community and is used to denote cellular assemblies derived from either pluripotent stem cells (PSCs) or adult stem cells (ASCs). The PSCs used to generate organoids can be either embryonic stem cells (ES) or induced pluripotent stem cells (iPSCs). In contrast, ASCs can come from a variety of organs or tissues and may therefore already be partially committed toward specific cell or tissue lineages. Although the self-organization and self-assembly processes that govern organoid formation *In vitro* are not identical to those that occur during *In vivo* embryonic organ development, they can accurately model key phenotypic features. It is important to note that tissue morphology is not simply a direct manifestation of the genetic code but is the outcome of numerous interactive dialogues and iterative readjustments between the underlying genetic template and the biophysical processes that ultimately shape the complex tissue architecture [3]. During organoid development, the regenerative potential of the starting pool of stem cells, their interactions with one another, and the experimental environment, ultimately determine the characteristics of the growing structure.

For more than a decade, starting with either PSCs or ASCs, it has been possible to derive complex tissue-like structures that recapitulate essential properties of the brain, retina, intestine, and other organs [4]. However, the development of cardiac organoid technology to model heart development has lagged behind those of other tissue-specific organoids. This chapter highlights cardiac organoid research, current development, application, and future challenges. We also highlight and compare various tools and methodologies available for human organoid research. We also provide useful information encouraging basic biologists to consider using this emerging platform to study human cardiac pathophysiology.

BACKGROUND OF ORGANOIDS

3D Culture Models

3D cell culture initially evolved from techniques culturing suspension cell cultures, with or without the introduction of a scaffold to bypass direct contact with plastic culture surfaces. In this context, a scaffold is a bio-mimetic extracellular matrix (ECM) used to model an *In vivo* cellular niche. Feeder cells have also been used as a scaffold to support cell growth. Feeder cells are a layer of live cells that have been rendered growth-arrested but provide cell-cell contacts and secrete growth factors that help the cells of interest to proliferate. Feeders differ from a coculture system because only the cell type of interest is capable of proliferating. In terms of cell-free scaffolds, Matrigel™, produced by Corning Life Sciences, is one of the most often used. It is derived from a complex mixture of proteins secreted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells [5]. Many adhesive proteins in Matrigel™ mimic the extracellular environment, providing mechano-transduction and ECM-like signals to the cells. In contrast, scaffold-free 3D cell culture techniques primarily rely on hanging drop culture methods using a combination of a well-defined culture medium, gravity, and surface tension [6]. Culturing of organoids at an "air-liquid interface" has also been used successfully by initially growing cells, or layers of cells and feeder cells submerged in a defined medium, then allowing the media to gradually evaporate or be removed, exposing cells to the air in a way that can stimulate cell polarization and differentiation [7, 8].

Self-organization is an essential developmental characteristic during embryogenesis, and this same property is fundamental to organoid technology. In 1907, Henry Peters Wilson first demonstrated the capacity for self-organization by regenerating an entire, mature organism from dissociated sponge cells [9]. Numerous research groups built on these initial dissociation-reaggregation studies by demonstrating the ability of dissociated amphibian pronephros [10] and chick embryos [11] to regenerate various organs. Organoid research and the concept of self-organization gained further momentum following significant advances in the field of pluripotent stem cell biology because of the potential for modeling human disease and personalized medicine [12 - 15]. In 1987, Li *et al.* [16], for the first time, demonstrated that ECM secreted from EHS (now known as Matrigel™) induced mammary epithelial cells to self-organize into 3D duct-like structures complete with lumens and the ability to secrete milk proteins, a stark contrast to their behavior in a 2D cell culture system. Another seminal experiment demonstrated that alveolar type II epithelial cells sustain a differentiated form in a 3D ECM environment in contrast to their undifferentiated state when cultured in a 2D system [17]. These findings are consistent with the concept of cell-matrix

CHAPTER 6

Respiratory System-Based *In Vitro* Antiviral Drug Repurposing Strategies for Sars-Cov-2

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Abstract: To date, no known drug therapy is available for COVID-19. Further, the complicated vaccination processes like limited infrastructure, insufficient know-how, and regulatory restrictions on vaccines caused this pandemic episode more badly. Due to the lack of ready-to-use vaccination, millions of people have been severely infected by SARS-CoV-2. Additionally, the increasing contagion risk of the SARS-CoV-2 variants makes drug repurposing studies more critical. Conventionally, antiviral drug repurposing has been conducted on two-dimensional (2D) cell culture systems or *in vivo*-based experimental setups. Recently, *In vitro* three-dimensional (3D) cell culture techniques have proven more coherent in mimicking host-pathogen interactions and exploring or repurposing drugs than other 2D cell culture methods. 3D culture techniques like organoids, bioprinting, and microfluidics/organ-on-a-chip have just been started to mimic the natural microenvironment respiratory system infected with SARS-CoV-2. These techniques avoid the need for animals in agreement with the 3R principles (Replacement, Reduction, and Refinement) to enhance animal welfare. Herein, SARS-CoV-2-host interaction and 3D cell culture techniques have been

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proposed for drug screening and repurposing models through representative examples. This study will frame tissue engineering strategies for studying SARS-CoV-2 infection and enlightening host-virus interactions.

Keywords: SARS-CoV-2, 3D cell culture model, Antiviral, Alveolar tissue, Drug repurposing, Drug screening, Drug screening, Infection.

INTRODUCTION

Every once a year, new infectious pathogens may appear and are only detected when an epidemic or pandemic occurs. Early detection and drug repurposing studies may reduce the number of deaths related to these contagious diseases. Notably, *in vitro* conditions for modeling respiratory system diseases still requires more attention due to the lack of fundamental knowledge on the prognosis of such diseases. Globally, researchers are showing a great effort to find the treatments to bring the COVID-19 pandemic under control. COVID-19 patients develop Acute Respiratory Distress Syndrome (ARDS) with renal failure, pericarditis, and disseminated intravascular coagulation (DIC), including death. Several clinical trials and computer-aided drug design direction studies have focused on detecting the hidden benefits of any existing drugs to improve survival or control COVID-19 [1].

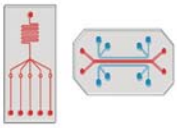
The drug repurposing strategies were found to be more effective in accelerating infectious control modalities compared to the long and complex process of vaccine or drug development [1, 2]. Drug repurposing strategies are of great importance to accelerate the R&D process without any preclinical steps compared with conventional drug discovery approaches. Additionally, this process has the potential to reduce the total development costs and discovery time involved in drug development [3]. Nevertheless, the platforms like computational and experimental approaches can be adapted to vaccine efficacy studies as they are intended for human-specific design [4, 5]. Such repurposing studies may aid in optimizing the diagnosis process for COVID-19 and support healthcare systems [6].

For several decades, many 2D and 3D *In vitro* cell culture techniques have been developed for drug repurposing. To check the alarming spread of COVID-19, both Food and Drug Administration (FDA) approved and unapproved drugs have been tested to cure this disease [7]. The studies have reported that 3D models show more consistency with real scenarios due to physical, chemical, mechanical, and biological similarities with the native tissue than 2D models [8 - 10].

Table **1** shows 2D and 3D cell culture techniques and their properties. The air-liquid interface (ALI) models significantly contribute while mimicking the natural

environment of organs such as skin, lungs, and brain [11 - 13]. However, organotypic models have drawbacks, such as limited cellular diversity and life terms compared to spherical culture techniques [14]. Compared to 2D models, 3D cell culturing strategies such as spherical cultures (organoids), organ-on-a-chip systems, and bioprinting technologies can combine tissue engineering and virology studies to construct ideal platforms to model drug repurposing systems [15].

Table 1. Comparison of cell culture techniques for modeling the respiratory system based on COVID-19.

Techniques	Advantages	Disadvantages
Air-Liquid Interface 	- Mimics in vivo systems better than 2D cultures as it consists of ECM* - Ability to tune the mechanical &biochemical properties of the gel/scaffold - Mechanical and biochemical signaling support for cells - High reproducibility - Co-culture ability - Ease of using pathogen transfection studies	- Additional prep-time needed - Relatively high-degree of cell-matrix vs. cell-cell interactions - Diffuculties of imaging and integrating to HCS** systems
Spharical Cultures  Cell cluster 3D	- Compact, multicellular aggregates - Well-known and established technology for spheroids based on the traditional Harrison's hanging drop technique - Ease of adapting to HTS*** and HCS platforms - Sensitive size control for spherical models - Not need to expertise and high quality equipments - Suitable for 3D tissue models - Co-culture ability - Reproducibility	- Expertise for imaging process - Require sensitive culture conditions - Susceptibility to mass transport limitations - Non-automated culture techniques - Labor intensive culture protocols at times
Microfluidics / Organ-on-chip 	- In order to mimic physiological conditions, combines microfabrication and tissue engineering approaches - Ability to manipulate and stimulate the cells, tissues, and ECM by using physicochemical, electrical, and mechanical factors - Provide interconnection among different types of tissues - Reducing reagents and supplies consumption	- Technology readiness level 4 or 5 and limited commercialized products - Required highly-specialized techniques and expertise - Hard to adapt to HTS - High-cost consumables - Lack of standardization/validation

Abbreviations; ECM: Extracellular matrix; HTS: High Throughput System; HCS: High Content System.

CHAPTER 7

Organoids: New Research Tool in Cancer Diagnostics and Therapeutics

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Abstract: Cancer remains the leading cause of mortality in the world, despite several cutting-edge technologies and established therapeutic regimens for cancer treatment. Therefore, the key to developing accurate and effective therapeutics is having a comprehensive knowledge of these complex molecular events. Patient-derived organoids (PDOs) represent a perfect model for studying cancer drug resistance and therapy. These cancer organoid models are cheaper alternatives to xenograft models and traditional two-dimensional (2D) cell culture model systems. All cancer organoid models are developed using iPSC-derived spheroids and tumor cells from different sources, which are then processed on a matrigel scaffold to get cancer organoids. The major advantage of these model systems is that they can recapitulate many functional and genetic characteristics of the same tumor tissues "*in vitro*". These cancer organoids can be passaged, frozen, and preserved for further high-throughput screening analysis. PDOs are powerful tools for evaluating mutational profiles and testing cancer drugs for personalized therapy. Cancer organoids can also be used to study tumor microenvironment cell types by co-culturing the required cell types involved in the process of transformation, which allows us to study tumor microenvironment and tissue-tissue interactions in the tumor development and metastasis process. This leads to more accurate predictions of the process of tumor development and evaluation of responses of cancer drug-resistance in a particular patient to develop personalized therapies for cancer. However, several limitations to these cancer organoid models must be addressed and resolved to get a perfect system for cancer drug evaluation. Several scientists are working on it by developing standardized protocols and reagents to generate individual tissue organoids. It is hoped that major developments in technologies, such as organoids-on-chips, 3D bio-printing, and advanced imaging techniques, will improve the handling of these organoids more precisely. Further CRISPR-Cas9-based gene editing technology allows us to bioengineer normal organoids by introducing any combination of cancer gene alterations to derive cancer

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organoids. In this review, we focused on the development and improvement of various normal and cancer organoids for targeted tissues such as the lung, breast, colon, liver, and kidney and their use as model systems for cancer drug discovery and personalized therapy. We have also highlighted some of the uses of the latest technologies, such as microfluidics chips and 3D bioprinting, for deriving better cancer organoids-based *in vitro* models for future research on cancer therapeutics.

Keywords: Breast cancer, Colon cancer, Cancer organoids, Lung cancer, Personalised therapy, Tumour Microenvironment (TME), 3D organoid model system.

INTRODUCTION

GLOBOCAN 2020 has reported approximately 20 million new cancer cases and almost 10 million people dying from cancer in 2020 globally [1]. Thus, cancer has become one of the leading causes of death worldwide, even though there are many innovative technologies as well as therapeutics protocols established for the cure of cancer [2]. However, there are still many issues that must be addressed in order to improve cancer therapy. So far, cancer has been treated as a global and homogeneous disease, and tumors are considered a uniform population of cells. Thus, the exact understanding of these complex phenomena is the most critical fact in designing precise and efficient therapies. During cancer progression, tumors become highly heterogeneous, having a mixed population of cancer cells with different molecular features with diverse responsiveness to various cancer therapies. The heterogeneity of tumor cells is the key factor responsible for the generation of resistant phenotypes and a critical challenge in managing cancer therapies. Therefore most cancer research programs focus on finding new and efficient therapies that can target cancer stem cells and related drug-resistant cells to reduce drug resistance in these cancer therapeutics. Thus, several different technologies are currently under evaluation to find specific targeted cancer drugs to cure cancer.

For many decades, two-dimensional (2D) cell cultures were considered as the best model system for drug screening and biological assays [3]. These culture systems mainly involve monolayer culture, which grows in flat layers on plastic surfaces. These model systems do not mimic the *in vivo* cell organization due to the lack of cell-cell/cell-matrix interactions in the original tissue architecture and the microenvironment structure around it. Therefore three-dimensional (3D) cell cultures have recently been introduced as a pioneering platform, allowing cell growth and interaction with each other in 3D. Recent advances in 3D culture-based organoid technology have opened new avenues for developing novel *in vitro* model systems resembling human cancer models [4]. These organoid cancer models play an important role in the development of cancer therapeutics to get

precise treatment for metastatic cancer patients. Normal organoids can be developed from embryonic stem cells (ESCs) and adult stem cells (ASCs), which recapitulate the original organ architecture of the same organ. These normal organoids can be genetically modified by various innovative gene editing technologies to get disease-specific organoids for therapeutic drug screening and the development of personalized treatment regimes. In this book chapter, we highlight the development of cancer organoids and their use in monitoring cancer therapeutics for metastatic cancer patients. It also explores recent advances in the development of 3D cell culture model systems for developing various organoid cultures for particular cancers on their application in a pharmacological analysis. Finally, special emphasis is paid to organoid bioengineering concepts, 3D constructs that mimic the 3D architecture of intact organs, and the associated technologies to study the exact mechanism of action of therapeutic drugs on the inhibition of cancer growth for a better cure for this disease.

STEM CELLS AND THEIR APPLICATION IN ORGANOIDS RESEARCH

Rheinwald and Green *et al.*, 1975 first cultured human diploid epidermal cells in serial culture using 3T3 lethally irradiated cells as a feeder layer. As epidermal cells required the presence of fibroblast cells for their growth and to initiate colony formation, they have used lethally irradiated 3T3 cells with appropriate density for this culture [5]. Rheinwald and Green have further shown that each colony consists of keratinocytes which ultimately form a stratified squamous epithelium, where dividing cells are confined at the lowest layers. Hydrocortisone was added to the growth medium to make the colony morphology distinctive and maintain proliferation at a slightly greater rate. These epidermal cells have a finite culture lifetime ranging from 20-50 cell generations. Thereafter several studies have developed human cell lines and used them as 2D culture model systems for various drug discovery programs and in various research programs in the field of biomedical sciences [6]. Organoids are the latest innovation in the field of tissue culture technology and stem cell research. Organoids are 3-dimensional tissue culture systems that originate from specific stem cells and recapitulate the structural and functional properties of human organs. These organoids can be established from Adult Stem Cells (ASCs), Embryonic Stem Cells (ESC), and induced Pluripotency Stem Cells (iPSCs). Human organoids have become an excellent model system for studying the exact cellular and molecular mechanism of normal and human diseases and screening drugs for their precise therapeutic use to better cure diseases [7]. Thus, organoid technology has played an important role in the fields of oncology, neurology, and infectious diseases. However, there are many challenges regarding protocol development, cost, and the total time

CHAPTER 8

Current Advances in the use of Tumor Organoids in Lung Cancer Modeling and Precision Oncology

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Abstract: Lung epithelium involves adult stem or progenitor cells that possess self-renewal, differentiation, and self-organizing potential and form the concoction of tissue-specific organoids. Researchers have used genetically modified lung organoids to study different aspects of lung tumorigenesis. Another approach is the patient-derived lung organoid to create a more representative lung cancer model with the tumor microenvironment, extracellular matrix, and immune component. The *In vitro* patient-derived organoids histologically and functionally mimic the related parent tumors. Lung cancer organoids and organoid-co-cultures can be used to dissect difficult-t--answer questions, especially regarding human lung cancer. Lung cancer organoids are

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used not only for understanding tumor biology but also to undertake biomarker studies, and drug screening, evaluate immunotherapeutics, and target tumor microenvironment, and personalized medicine. Lung organoids can also be used to create organoid biobanks for future gene-specific pre-clinical trials and evaluation. This chapter will present an overview of the therapeutic areas in which lung cancer organoids are transforming therapeutic discovery and development, followed by a discussion of future prospects.

Keywords: Cancer modeling, Drug discovery, Patient-derived xenograft, Tumor microenvironment, Tumor organoid, Therapy.

INTRODUCTION

Lung cancer with an expected 1.8 million fatalities, is still the most significant cause of cancer-related mortality worldwide, affecting both smokers and nonsmokers, despite early identification and treatment advances. Men have the highest incidence and fatality rates for lung cancer, while women rank third in incidence and second in mortality [1]. Lung cancer is heterogeneous with numerous genetic and epigenetic alterations and has the lowest five-year survival rate (10 to 20%) among all malignancies [2]. Eighty to eighty-five percent of lung malignancies can be histologically categorized as non-small cell lung cancer (NSCLC), and approximately fifteen percent are small cell lung cancer (SCLC). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most prevalent subtypes of NSCLC [3, 4]. Lung cancer patients have a poor prognosis and survival rate due to a lack of early detection and ineffective treatments. The treatment options for lung cancer are surgery, chemotherapy, radiation, or targeted therapy, but survival is dismal. Treatment exhibits poor therapeutic indices, varied health implications, and chemotherapeutic toxicity [2]. The accuracy, efficacy, and success are limited by tumor cell resistance, immune suppression, and inter-subject variation. For lung cancer therapeutics to be successful, tailored cancer models that accurately depict the heterogeneity of tumors mimicking that of the patient's tumor are required immediately [4, 5]. Recent advances in 3D culture techniques, single-cell transcriptomics, pharmacogenetics, imaging techniques, and immunotherapy have inspired better therapeutic strategies [4, 6, 7].

The existing lung cancer models, *i.e.*, 2D tumor cell culture and animal models suffer from multiple challenges, including the absence of verified driver genetic mutations, stepwise progression, ambiguous cell of origin, lack of human immune components, difficulties replicating the complex lung tumor microenvironment (TME) and predicting therapeutic response [8]. In recent years, the price of new anti-cancer medications has risen partly due to the increased complexity and limited success of clinical trials, especially in lung cancer. Therefore, the

discovery of lung cancer treatments has faced a severe setback. Recent developments in 3D culture systems constitute an invaluable set of tools for studying cell biology, especially cancer. A 3D cell culture is an artificially created smart environment *In vitro* wherein biological cells may grow or interact in 3D [6]. Organoids are a type of 3D cell culture system and are self-renewing, self-organizing 3D cell aggregates produced from primary tissue-derived adult stem cells (ASCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and tumor cells. It has been demonstrated that tissue-resident ASCs, ESCs, and iPSCs may self-organize into 3D architectures that resemble *In vivo* organs such as the breast, lung, colorectum, stomach, liver, pancreas, ovary, prostate, and brain. Organoids have a limited population of self-renewing stem cells that can develop into all major cell lineages, can be co-cultured with niche components, cryopreserved, and grown indefinitely. Organoids mimic the corresponding tissue's histological, genetic, and physiological state and are amenable to genetic modifications, making them highly relevant for basic and translational research applications. Though efficient, regular organoids lack the entire spectrum of cells and components, especially the immune repertoire seen in a patient's tumor, which is a disadvantage.

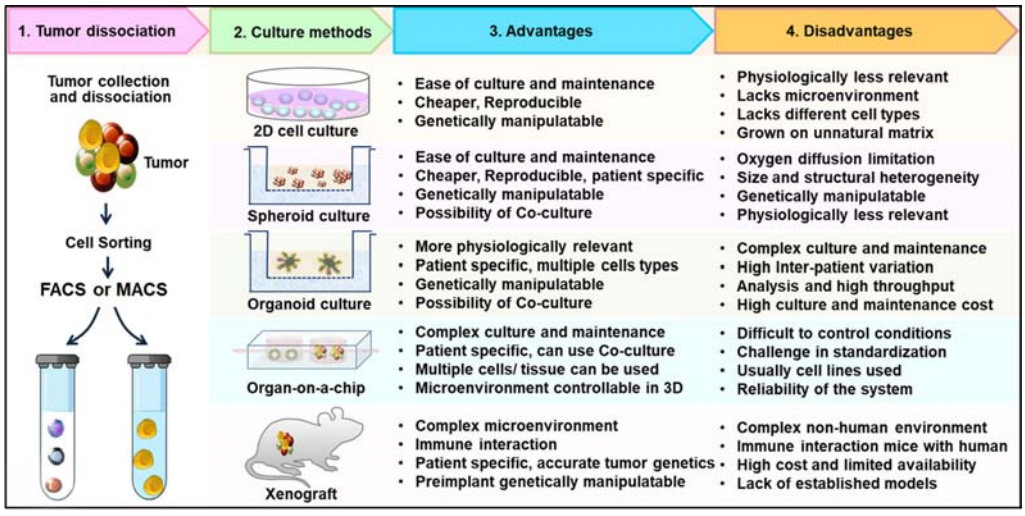

Fig. (1). Models used in cancer research. Patient-derived xenograft or cancer cells can be cultured using multiple model systems like 2D cell culture, spheroid culture, organoid culture, organ-on-a-chip, and rodent xenograft models. This figure also discusses the advantages and disadvantages of the different models. The figure is inspired by [10].

Tumoroids, on the other hand, are generated directly using freshly isolated patient tumor tissue and preserve the cellular complexity of the TME and native extracellular matrix (ECM), in contrast to stem cell-derived organoids. Patient-

CHAPTER 9

Additive Manufacturing and Organoids

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Abstract: Additive manufacturing (AM) is a rapid and efficient process of creating complex geometries or structures using a digital three-dimensional (3D) printing process. AM has many diverse applications in aerospace, automotive, defense, manufacturing industries, education, and research, most notably in the healthcare and bio-medical industries. 3D bioprinting allows us to create tissue-specific architecture with precise geometries limited to conventional fabrication methods. In this chapter, we have discussed the generalized process of 3D printing of objects in various organoid cultures, focusing on the advantages and limitations of AM technology. Further, we have discussed the major challenges and future direction in the context of organoid bioprinting.

Keywords: Additive Manufacturing, Bioprinting, Organoid culture, Tissue mimetics, 3D printing.

INTRODUCTION

Additive manufacturing (AM) or additive layer manufacturing (ALM) is the industrial production name for three-dimensional (3D) printing [1]. AM is a computer-controlled technique that progressively deposits different layers of materials to create a complex structural prototype. The process starts with generating a 3D computer model of a prototype obtained from the Computer-Aided Design (CAD) system and is split and cut using computer software. This process first creates a two-dimensional (2D) outline that determines whether or

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not material is added to each layer. Then every material layer is processed sequentially and compiled or stalked from the bottom to the top portion of the final structure.

AM is a rapid prototyping technology that allows the creation of bespoke parts with complex geometries using the digital process [2]. The use of 3D CAD at the beginning of the product designing process and conversion of the data throughout AM is coherent, making design alterations quick, error-free, and efficient during the conversion or translation of the design intent. Another significant advantage of AM is that it reduces the number of steps involved, decreasing material waste. A reduction in material waste reduces the price of high-value components. Unlike conventional complex designing methods, AM machine production is done in a single step irrespective of the complexity of the product's design [3, 4], thereby reducing the lead times and providing improved strength and durability to the final product. Reduction in labor costs and shortening of the supply chain make AM a revolutionary market process.

These advantages not only make an excellent socio-economic and environmental (controlling resource consumption, use of hazardous material, pollution) impact [5]but also enhance product quality and society's wellbeing [6]. It is anticipated that by 2025, the worldwide market for AM products may bloom up to 230-550 billion dollars due to its great potential in medical, aerospace, and tools manufacturing [7].

AM has many diverse applications in aerospace, automotive, defense, manufacturing industries, education, and research, most notably in the healthcare and biomedical industries.AM technologies are used in the healthcare and medical sector to fabricate patient-specific medical constructs such as anatomical implants, surgery tools, or those, prostheses, dental casts, tissue engineering scaffolds, drug delivery devices, *etc* [8]. In addition to the biofabrication of these advanced medical devices, materials, structural grafts, and prosthetic devices, the use of AM also encompasses a versatile and expanding array of technologies like organs and organoids synthesis by selectively distributing cells, bioactive materials, and cytokines which are otherwise difficult to fabricate due to their complex geometries and heterogeneous material distribution [9]. In 3D bioprinting, cells, growth factors, and biomaterials are combined in a 3D printer to create a biological component to mimic the properties of tissues and organs. Layer-by-layer construction with 3D bioprinting facilitates the creation of highly biomimetic and robust *In vitro* models for scientific research, therapeutics, drug delivery systems, and even organoid and tissue printing. Herein, we discuss the principles and methodologies used for constructing different organoids concerning AM technologies.

HISTORY OF ADDITIVE MANUFACTURING

The origin of AM dates back to almost 150 years to the concepts of topography and photo sculpture. These technologies use the principle of layer-wise 'cutting and slacking' to build a free-form object. From creating a 3D replica of an object, including human forms, capturing simultaneous surround photography [10] to the wax plates layering method of producing molds for the topographical maps, artisans have been using 3D modeling since the eighteenth century. It was in 1950 when writer Raymond Jones first described the3D printing as a ‘molecular jet’ in his story “Tools and Trade” in The Astounding Science Fiction Anthology [11]; the same was brought into a concept by David Jones (1974), in his regular column named ‘Ariadne’ in the journal 'New Scientist [12].

The first patent detailing 3D printing along with rapid prototyping and regulated on-demand manufacturing of specific patterns was filed by Johannes Gottwald in 1971 for the continuous Inkjet metal material device known as Liquid Metal Recorder, which was used for metal fabrication of a reusable surface [13]. However, the development of AM equipment and materials began in the 1980s. Hideo Kodama, in April 1980, invented two additive methods for the production of 3D plastic molds by using photo-hardening thermoset polymer [14]. A patent for the fabrication method of articles by sequential deposition was granted to Raytheon Technologies Corporation in 1982, which includes hundreds of thousands of layers of powdered metal and a source of laser energy [15]. Bill Matsers (the year 1984) patent for Computer Automated Manufacturing Process and the system was the first on record 3D printing patent in history at the United States Patent Trademark Office (USPTO). This invention further laid the foundation for the 3D printing system today [16]. Robert Howard developed a color inkjet 2D printer in 1984, which was further commercialized as Pixelmaster in 1986. Chuck Hall, in 1984 filed a patent for the Stereolithography (SLA) apparatus [17], and in 1986, his company 3D Systems became the first 3D printing company in the world to release the first commercial 3D printer named 'SLA-1' in 1987 [18].

The practical origin of 3D printing can be traced back to Professor Emanuel Sachs of Massachusetts Institute of Technology (MIT), with 3D printing as a powder bed process using standard and custom inkjet print heads in 1993. This achievement was made possible by spreading powder and binder material that selectively joins the powder to create a layer with the help of inkjet printing [19]. In the same year, Solidscape Inc. introduced a high-precision polymer jet fabrication system that used soluble support structures classified as the “dot-o--dot” technique. Here, the printer uses a predetermined path on which it deposits printing material point-wise to construct the cross-sectional area of the object or

CHAPTER 10

Large-Scale Organoid Culture for High Throughput Drug Screening

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Abstract: Despite several limitations, two-dimensional cell culture has been widely used in drug and drug-related compound selection and screening studies. A more recent approach of using three-dimensional (3D) organoid culture enables researchers with a more robust and accurate model for drug screening. Numerous studies have reported the successful use of stem cells, including induced pluripotent stem cells (iPSCs) and adult stem cells, for organoid generation to predict therapy response in various disease conditions, including cancer. The development of high-throughput drug screening and organoids-on-a-chip technology can advance the use of patient-derived organoids in clinical practice and facilitate therapeutic decision-making. Although organoids are in complaisant with high-throughput screenings, extensive manipulation studies are required by current methods.

Keywords: High-throughput drug screening, Organoid culture, Stem cells, 3D culture.

INTRODUCTION

Today, the world has acknowledged the fundamental power of clinical research. This global health emergency has highlighted the significance of global health and its impact on the socioeconomic status by reminding us that health risks can even transcend national boundaries. With this global pandemic, researchers, scientific policymakers, and regulatory authorities have understood the importance of rapid decisions to initiate clinical studies and rapid implementation of drugs in regular practice. Moreover, as opposed to the current scenario where almost 10% of the total drugs were implemented in routine practice [1], it is essential to develop and

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standardize methodologies to reduce high costs and minimize time to identify suitable drug candidates. At the same time, current research methods require two-dimensional (2D) cell culture applications and animal models, mainly for drug screening and other toxicological studies [2, 3]. However, researchers reported several challenges in drug discovery applications; like maintenance of species-specific cell lines for a longer duration without contamination, the phenotypical and genotypical difference in several cell lines obtained from different interrelated species, as well as the inability to control the microenvironment, thereby regulating the epigenetic status of various cell lines, *etc.* These challenges are further responsible for failure in predicted outcomes, unanticipated toxicity issues that can be translated to humans, and further increasing time and cost in developing new drug candidates. Studies have acknowledged that drug development can be bettered by mimicking the three-dimensional (3D) structure of the cellular microenvironment to regulate its complex dynamics and identify effective target sites [3 - 5]. These studies have also proposed that the science of cell culture in current practice does not account for these proposals, leading to the failure of new drug implementation. It should be noted that advances in technology and cell culture science have developed such a 3D microenvironment *In vitro* [6]. Accordingly, the table below represents the comparative analysis of the advantages of using 3D cell culture over its 2D counterparts.

Table 1. Comparative Analysis of issues encountered in 2D cell culture vis-a-vis 3D organoid development

Type	Actual Representation of Human Tissue	Ethical Issues	Cost of Treatment	Maintenance	Risk	Assay Development
Animal Model	Low	Low	High	Medium	Low	Possible
2D Culture	Low	Low	Low	High	High	Not Possible
Organoid	High	Low	Low	High	Medium	Possible

With the advent of technology and recent scientific development, it is possible to develop different organs on a microfabricated chip. In simpler language, these chips are designed with the help of different primary cells obtained from human tissues to form 3D cellular aggregates incorporated in a microfluidic device [3, 4]. However, many issues need to be sorted out, further limiting the potential commercial use of these systems, which can further be noted as a lack of exact representation of human tissues due to the presence of specific matrix proteins [8, 9]. Moreover, certain studies also confirmed that the organ-on-a-chip (OOAC) models developed with the help of primary cells exhibit a limited capacity for self-regeneration and organization capacity to mimic tissue of interest.

In this regard, recently, the generation of OOAC models with the help of human induced pluripotent stem cells (hiPSCs) received tremendous acknowledgment; due to the self-regenerating capacity of stem cells and, importantly, their ability to copy exact structural and functional aspects of human tissues in a 3D cell culture setup [10]. Studies have also proposed that integrating these stem cells with microfluidic devices is an added advantage for the more realistic development of healthy and diseased models (Fig. **1**). Regarding organ-on-a-chip development, some of the characteristic features of stem cells, like the ability to transform into any tissue-specific cell lineage, self-renewal, indefinite proliferation, *etc.*, are advantageous to tackle challenges encountered previously with primary cells Fig. (**2**) [11, 12]. Certain studies have revealed the mechanism of fetal development by generating strong patterning signals that are released through a special signaling mechanism and have inductive effects on a wide range of tissue-specific cells. The same mechanism has been detected in hiPSCs, promoting them as the most suitable candidates for organoid generation [13]. Scientists are thus hopeful about the ability of hiPSCs to generate disease-specific models, further helping in strategizing personalized medicines as the foundation of the 21st-century healthcare [14].

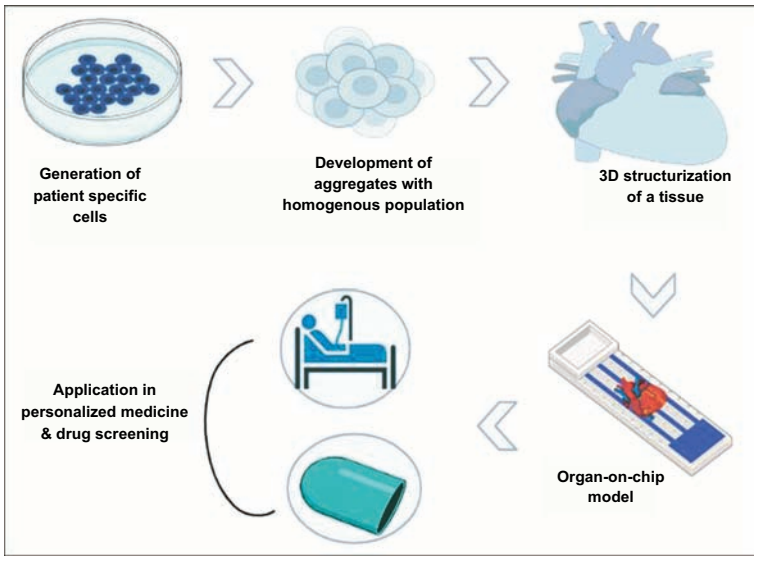

Fig. (1). Development of organ-on-a-chip model with induced pluripotent stem cells as crucial tools in personalized medicine and drug discovery.

Accordingly, the current chapter is intended to review the science behind the production of the organ-on-a-chip model with the help of hiPSCs, their regulated differentiation to specifically committed spheroids, and the controlled development of aggregates to provide adequate cues with homogenous cellular

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