



Special review series on 3D organotypic culture models: Introduction and historical perspective

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Abstract

Three dimensional (3D) organ-like (organotypic) culture models are a rapidly advancing area of in vitro biological science. In contrast to monolayer cell culture methods which were developed to achieve proliferation of animal cells in the beginning of in vitro biology, the advancements in 3D culture methods are designed to promote cellular differentiation, and to achieve in vivo-like 3D structure and organotypic functions. This project was conceived through the Society for In Vitro Biology to draw on the expertise of individual scientists with special expertise in organotypic cultures of selected tissues or associated interrogation methods to prepare individual-focused reviews in this series. This introductory manuscript will review the early achievements of animal cell culture in monolayer culture and the limitations of that approach to reproduce functioning organ systems. Among these are the nature and 3D architecture of the substrate on which or in which the cells are grown, physical and mechanical clues from the substrate, cell-cell interactions, and defined biochemical factors that trigger the induction of the 3D organotypic differentiation. The organoid culture requires a source of cells with proliferative capacity (ranging from tissue-derived stem or immortalized cells to the iPSC cultures), a suitable substrate or matrix with the mechanical and stimulatory properties appropriate for the organotypic construct and the necessary stimulation of the culture to drive differentiation of the cell population to form the functioning organotypic construct. Details for each type of organotypic construct will be provided in the following papers.

Keywords Organotypic culture · Functional differentiation · Biological matrix · iPSC

Introduction

Three dimensional (3D) organ-like (organotypic) culture models are a rapidly advancing area of in vitro biological science (Holloway *et al.* 2019). In contrast to monolayer cell culture methods which were developed to achieve proliferation of animal cells in the beginning of in vitro biology, new advancements in 3D culture methods are designed to promote cellular differentiation, and to achieve in vivo-like 3D structure and organotypic functions. 3D organotypic models may be composed of a single cell type, such as in simple reconstructed human epidermal, corneal, or airway epithelial

models (Cannon *et al.* 1994; Kaluzhny *et al.* 2011). More advanced co-culture models that consist of multiple cell types may include stromal components and/or other types of functional cells such as fibroblasts, pericytes, melanocytes, or immune cells. Examples of organotypic co-culture models include models of skin, airway, and intestine, as well as models of liver, heart, and neurological tissues (Cannon *et al.* 1994; Kaluzhny *et al.* 2011; Beauchamp *et al.* 2015; Meier *et al.* 2017; Aychunie *et al.* 2018; Trujillo and Muotri 2018). 3D organotypic models may be produced in a variety of forms. Epithelial barrier tissues are most often produced using micro-porous membrane scaffolds at the air-liquid interface (ALI) to compartmentalize the apical and basolateral surfaces. This format is highly useful for reproducing polarized structure and function and allows for in vivo-like exposure scenarios and chemical transport studies (OECD 2004; Kaluzhny *et al.* 2018; Neupane *et al.* 2020). Other types of tissue models, such as liver, cardiac, and neuronal constructs, make use of various methods for producing self-assembling spheroids or organoids (Trujillo and Muotri 2018). Both types of constructs are increasingly being incorporated into various types of

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organ-on-a-chip platforms that offer enhanced utility for reproducing mechanical cues and features such as fluid flow, shear forces, stretching, and organ-organ interactions that further promote organotypic differentiation and in vivo-like functions (Haring and Johnson 2020).

This project was conceived under the auspices of the Society for In Vitro Biology to draw from the experiences of individual authors who are experts in the development and application of specific 3D organotypic constructs. Each author has been asked to prepare a review that focuses on a specific tissue type in 3D organotypic culture. In part, the collection is intended as a teaching tool for researchers wishing to use these models in developmental biology, cancer research, drug discovery, toxicology, and regenerative medicine to name just a few applications. For any single organ or tissue, there may now be many 3D systems developed by academia and industry. The authors were asked to focus on a limited number of models for a specific tissue with an emphasis on more mature technologies that might be applied across laboratories. Such applications might also include the generation of data for the regulatory setting such as those detailed in the new Organization for Economic Cooperation and Development (OECD) in vitro test guidelines. The human cell-based 3D organotypic constructs provide test systems directly amenable to support the regulatory shift from strict reliance on animal models for prediction of human health effects as measured by the disruption of critical cellular metabolic pathways. This approach to predictive of toxicity was outlined in the Toxicology in the 21st Century Program (Andersen and Krewski 2010).

This introductory paper is intended to provide a brief overview and historical perspective of some of the major achievements of the pioneers in early tissue culture who set the stage for the science as we know it today. These leaders also appreciated the limitations of the monolayer systems for cellular differentiation. These limitations were particularly evident in the efforts to produce functionally differentiated parenchymal cells of human liver, mammary, skin, and other organs. Based on work with many types of cells, it became clear that submerged monolayer culture conditions were missing essential influences that are required for functional differentiation. Among these are the nature and 3D architecture of the substrate on which or in which the cells are grown, physical and mechanical clues from the substrate, cell-cell interactions, and defined biochemical factors that trigger the induction of the 3D organotypic differentiation (Hayden 2020). In this introductory paper, we will not examine those influences in detail as they are often unique to the individual organ system in question. Those will be addressed in the individual 3D organoid manuscripts.

Historical perspective of cell culture methods development - Early developments

Plant and animal cell culture of all kinds is now a multi-billion dollar technology supporting academic, governmental, and industrial research and production. There are few aspects of biological science that are not touched by this technology. Things were quite different eighty some years ago. The early history of tissue culture is one of a relatively small group of scientists with diverse backgrounds and scientific goals building a new technology. This group included cell physiologists, cellular nutritionists, developmental biologists, virologists, and cancer biologists. In the days before commercial media and serum sources, just initiating and maintaining simple cultures without luxuriant microbial overgrowth was an achievement. As selective and increasingly defined media were developed to support the growth (replication) of distinct cell types from different species, it became clear that a mechanism to track these developments would be an advantage to all. In 1948, the National Research Council, under its Cellular, Physiology, Cytochemistry and Nutrition Committee, organized a meeting in Hershey Pennsylvania to bring together scientists from all facets of tissue culture and cellular nutrition. One result of this meeting was the founding of the Tissue Culture Committee. The first chairman of the committee was Dr. Keith Porter (Rockefeller Institute). From this committee rose the Tissue Culture Association in 1949 which is now the Society for In Vitro Biology (Schiff 1997).

The practice of tissue/cell culture grew rapidly with the availability of media formulations and the appreciation of the range of cell biology questions that could be addressed. Some examples are provided to illustrate the achievements in this early period:

- The ultrastructure of individual cells in monolayer culture was evaluated using the electron microscope (Porter *et al.* 1945).
- Cell culture allowed the detailed study of cell division and cell motility using time lapse cinematography (Pomerat 1958)
- Great advances in the understanding of cellular nutrition generally (Eagle 1955) and the selective requirements of cell types from different species was advanced by the development of selective media (Morgan *et al.* 1950; Ham and McKeenan 1979).
- The first human tumor cell line was developed in the laboratory of George Gey at Johns Hopkins (Gey *et al.* 1952). The HeLa cell has become one of the most widely studied cell line worldwide but unfortunately not all of those studies were intentional as the HeLa cell has become one of the most ubiquitous cross

contaminates of other cell lines and strains (Lucey *et al.* 2009).

- The importance of hormones and growth factors was elucidated in an effort to produce defined media (Waymouth and Reed 1965; Sato *et al.* 1970; Barnes and Sato 1980).

The application of cell culture to virology contributed greatly to one of the most impactful public health achievements during this period. The contributions of Enders, Robbins, and Weller to the development of efficient (high cell and virus yields) industrial scale cell culture processes provided the means to produce quantities of poliomyelitis virus for vaccine development (Salk 1955). For their extensive contributions to the field of tissue culture and virology, they were awarded the Nobel Prize in Physiology or Medicine in 1954 (Enders *et al.* 1980). There was also a desire to produce virus stocks for vaccine production in normal human cells in order to reduce concerns about adventitious viruses in the production cells. This need was answered with the development of the human lung fibroblast strain WI-38 (Hayflick and Moorhead 1961; Hayflick *et al.* 1962). Not only was this cell strain used extensively in virus production; it also was instrumental in demonstrating the finite life span (ability to replicate) of normal diploid cells in culture. This cell strain became an important tool in the study of cellular aging (Hayflick 1965). These impressive series of achievements occurred over a period of only about thirty years.

Several basic principles of cell biology *in vitro* were elucidated during this period that would provide important guidance in the development of 3D organotypic constructs. Among these was the demonstration of specific nutrient requirements of cells (both epithelial and stromal) from specific species (Barnes and Sato 1980) (Ham and McKeehan 1979). The inverse relationship between cell replication and differentiation was demonstrated for many cells *in vitro*. Hepatocytes are a good example of this effect (Rana *et al.* 1994). 2D systems were designed to focus on replication for population expansion which often precluded retention of the differentiated phenotype. Non-tumorigenic cells were found to require attachment to a substrate (i.e., plastic or glass) to proliferate in culture (termed anchorage-dependent growth) in contrast to tumorigenic cells which could grow without attachment in soft agar (anchorage-independent) (Grinnell 1978). The development of non-rigid substrates that supported anchorage-dependent growth was a major step forward in 3D organoid technology (Holloway *et al.* 2019). Finally, growth factors were identified and produced in quantity. These growth factors served to direct the cellular maturation and differentiation. Many of these growth factors were discovered by their action *in vitro* (Sato *et al.* 1970).

Limitations of early 2D models drive development of 3D organotypic models

The development of methods for proliferating and maintaining cells in 2D monolayer culture were groundbreaking events in *in vitro* biology. However, cells in 2D monolayer or suspension culture showed numerous limitations. The study of organ-specific epithelial cells began with isolating the cells from organ explants which were plated on plastic or other solid substrates. However, as cells began to proliferate, the cultures soon lost their differentiated phenotype. Along with the loss of differentiation, many important organ-specific features and functional attributes that researchers hoped to study are also lost. For example, airway epithelial cells in 2D submerged monolayer culture do not form cilia or secrete mucus. Epidermal keratinocytes do not form differentiated stratified layers with barrier function. Hepatocytes quickly lose most of their drug metabolizing capabilities. Thus, these undifferentiated cells have limited utility for many practical applications in toxicology, drug development, and basic research that requires differentiated phenotypes.

The reasons underlying the loss of differentiated function in the cultures were the focus of much discussion. Was it a change in the parenchymal cells themselves (de-differentiation)? Alternatively, was the loss of differentiated function due to cell selection in culture where the epithelial cells were overgrown by the stromal cells? Between these two “extremes,” what other factors might also impact differentiated function in the cultures? Did the binding to a solid substrate prevent the required polarization of the cells seen in the native organ? Were medium requirements for proliferation vs. differentiation fundamentally opposed? What was the role of the tissue stroma in epithelial differentiation and the structure of the organ? Were stromal requirements specific to the organ in questions? A number of *in vitro* systems were proposed to address these questions. A major impediment to this work was the difficulty in maintaining 3D cultures and particularly mixed cultures of epithelium and stroma.

To address the potential cell selection as the basis for loss of differentiated function, Sato *et al.* (1960) used primary rat liver cell cultures. The authors had noted similarities in cell morphology among undifferentiated cultures derived from several different organs. This suggested that a similar cell type had overgrown the different cultures. If de-differentiation was responsible for the loss of function, then the loss of selective functions should be a gradual process whereas if the population was overgrown by a non-parenchymal cell type, the loss should be more rapid and complete. They addressed this question using day-old rat hepatocyte cultures. They produced a series of antisera that were raised against either adult rat hepatocytes or cells of non-hepatocyte cultures (day-old rat kidney). Samples of the antisera were also absorbed with either adult hepatocytes or cells from hepatectomized day-old rats

(representing all the other cells antigenic markers except those of hepatocytes). They used these antisera to treat their hepatocyte cultures at various stages in culture. Several key findings emerged. The treatment of initial inoculum of day-old hepatocytes showed the clear presence of hepatocytes within the cell suspension, but with time in culture the hepatocyte markers were lost. Furthermore, if the initial inoculum was treated with anti-hepatocyte antibodies and complement, the cultures grew out in about the same number of cells whether the antisera was unabsorbed, absorbed with adult hepatocytes, or hepatectomized day-old rats. Alternatively, if the inoculum was treated with antibodies raised to non-hepatocyte cultured cells and complement, few if any cell grew out into the cultures except when the antisera was pre-absorbed with hepatectomized day-old rat cells. These and other data showed that the cells that grew out to produce the cultures were in fact stromal cells which we now know to be fibroblasts. Anyone who has done primary culture, particularly from tissues with slow growing parenchymal cells, will recognize this phenomenon.

Not all loss of function in culture could be attributed to loss of the parenchymal cells themselves. To evaluate the ability of cells from 2D cultures to undergo organized growth and differentiation, several systems were employed. One system that proved useful was an *in vivo* mouse mammary gland model developed by K. DeOme (UC Berkeley). The mouse mammary gland grows from the nipples into the mammary fat pads under the influences of the hormones of puberty. It further differentiates under the hormones of pregnancy to make alveolar structures which make milk. If the nipple is excised before puberty, a gland-free fat pad remains as an ideal site for subsequent transplantation of isolated tissue or cells. Thus, the mammary epithelium may be followed in the absence of competing epithelia (DeOme *et al.* 1959). Normal mouse mammary epithelium can be digested free of the surrounding fat pad and placed into monolayer culture where it grows to confluence and can be passaged. In monolayer culture, it does not differentiate to make organized structures or to make milk proteins following exposure to the hormones of pregnancy. However, if the cultured cells are implanted into the gland-free mammary mouse fat pad, they proliferate (like the epithelium from the nipple) to make ducts and associated structures. Furthermore, when the recipient becomes pregnant, the glands further differentiate and make milk (Daniel and DeOme 1965). Because the mammary fat pad stroma was intact, it could interact with the transplanted epithelium in the normal fashion.

These and other results demonstrated that the tissue stroma is critical for parenchymal cell differentiation and function. In mixed cultures of stroma from one organ with epithelium from another, Cunha and Lung were able to demonstrate that the organ structure was directed by the stroma while the biochemical differentiation was a function of the source of the

epithelium (Cunha and Lung 1979). In a similar fashion, the structural differentiation of the mammary gland is directed by the stroma resident within the mammary fat pad (Daniel *et al.* 1983). Finally, mouse mammary cells could be induced to make milk proteins *in vitro* if they were plated on flexible collagen gels that were similar in compliance to the native stromal tissue, and allowed to form polarization cuboidal cells (Shannon and Pitelka 1981). In summary, these studies conclusively showed that these epithelial cells cultured *in vitro* had not lost the capacity to differentiate into the parenchyma of the organ nor the ability to undergo final induction of differentiated function, but rather were missing the inductive influence of the tissue-specific *in vivo* stromal environment and hormones.

Once it had become clear that the de-differentiation of cells in monolayer culture was a reversible process, researchers began to turn their attention to developing systems for restoring and reconstructing the *in vivo*-like environment to allow organotypic differentiation *in vitro*. We now know that many factors are involved in directing differentiation and de-differentiation of *in vitro* cell cultures, including medium composition (e.g., high vs. low Ca, hormones, and growth factors), scaffolds that allow polarization (e.g., microporous membranes), and matrix components with *in vivo*-like physical properties. These factors, some of which are fairly general (e.g., low vs. high Ca²⁺ medium), and many that are organ-specific, will be discussed broadly in the “Overview of the critical factors in generating 3D organotypic constructs cell sources for production of organotypic models” section below and in detail in the individual papers dedicated to specific organ systems in this Special Issue.

Overview of the critical factors in generating 3D organotypic constructs - Cell sources for production of organotypic models

A requirement for development of *in vitro* organotypic models is a readily available source of appropriate cells. The cells used to produce 3D organotypic constructs range from tissue-derived epithelial and stromal cells or organ-derived stem cells to induced pluripotent stem cells (iPSC) (Hayden 2020). An example of cells used to produce epidermal constructs is the human keratinocyte. As primary cultures, these cells have relatively limited expansion potential in culture but with the addition of cholera toxin or other factors to stimulate cyclic AMP production (Green 1978), the population can be expanded to produce large numbers of epidermal constructs without transforming the cells. Normal cells in culture may also be selectively transformed into immortal cell lines by the introduction of viral oncogenes. While this approach can produce an immortal cell line with greatly enhanced or

even unlimited expansion potential, phenotypic changes induced by the immortalization process and/or subsequent extensive passaging may be considerable. A very promising technique for expanding a cell population with minimal phenotypic effects is introduction of exogenous hTERT to “immortalize” the primary human cells (Lee *et al.* 2004). The hTERT gene codes for the catalytic subunit of the telomerase which can prevent telomere shortening. This action prevents the telomerase-controlled cell senescence and produces a continuous cell line. Of particular interest is the observation that the hTERT-treated cells often retain most of their “normal cell” phenotypic properties.

Primary cultures of many types of human epithelial, stromal, and endothelial cells may now be readily obtained from clinical specimens or from commercial cell suppliers. These types of cells proliferate well enough in culture that obtaining adequate numbers of cells to support large-scale research projects is often feasible. Defined culture media, supplements, growth factors, and hormones that support either growth or differentiation of many types of human cells are also readily available from commercial sources. However, obtaining adequate numbers of cells from tissues composed of cell types that do not replicate in primary in vitro culture, such as cardiomyocytes, hepatocytes, neurons, and pancreatic islets, has been a particular challenge.

The world of tissue culture changed radically with the introduction of methods to produce iPSC cultures from differentiated somatic cells. Creation of iPSCs is based on selectively inducing the expression of four transcription factors in the target cells (Takahashi and Yamanaka 2006; Takahashi *et al.* 2007a, 2007b; Yu *et al.* 2007; Sayed *et al.* 2016). Initial protocols used retroviral vector systems to integrate the transcription factors into the host genome. This approach was very successful for the generation of iPSC cultures for research, but there were concerns regarding use of these cells for regenerative medicine. Protocols that do not require the integration into the host genome were developed to eliminate this issue (Fusake *et al.* 2009; Warren *et al.* 2010). These iPSC cultures are a major boon to regenerative medicine as tissue constructs can be produced from the patient’s own somatic cells. iPSCs also provide an important source for generation of cells for use in in vitro model development, particularly for cells mentioned above that are limited by lack of in vitro proliferation potential (i.e., cardiomyocytes, hepatocytes, neuron, etc.). The process of developing differentiated cultures from somatic cells has been further refined to allow direct reprogramming from the somatic cells into the desired cell type with specific transcription factors. This process bypasses the initial reprogramming to the iPSC phenotype (Ieda 2013).

Substrate and matrix requirements of organotypic models

Development of 3D organoid constructs often requires a matrix or substrate other than the very rigid polystyrene of standard tissue culture plastic vessels. This we have known for decades. The matrix may also provide essential triggers for the expression of the organotypic phenotype. One of the first examples of such matrix was Matrigel (Corning, Corning, NY), the extracellular matrix proteoglycan mixture from the Engelbreth-Holm-Swarm mouse sarcoma cell line (Kibbey 1994). To produce the 3D organoid construct, the cells are embedded in a Matrigel scaffold and then the construct is overlaid with medium containing the appropriate growth and differentiating factors and cytokines to promote differentiation and expansion (Nguyen *et al.* 2020). Since Matrigel is the product of a murine tumor line, its precise composition can vary from batch to batch. In certain applications, these differences may not be significant. In other constructs, the difference can be noticeable. Furthermore, for application of constructs to regenerative medicine, the presence of xenogeneic material in the construct is undesirable. To address these issues and to provide more precise control over the chemical and mechanical properties of the matrix, a number of defined extracellular matrix preparations have been developed (Holloway *et al.* 2019). These hydrogels include collagen type 1 (Yui *et al.* 2018), fibrin (Brogiere *et al.* 2018), laminin (Holloway *et al.* 2019), and propylene glycol (Gjorevski *et al.* 2016). The chemical and physical properties of these hydrogels can be modified as needed by micropatterning, changes in stiffness, and selective layering.

The ECM components may be used independent of a secondary scaffold to create a 3D organoid that mimics the organ or tissue itself in the form of a spheroid-like construct. These organoids can be produced by several techniques such as hanging drop cultures, micromolding, ultra-low attachment well plates, or spinner flask cultures. The micromold and ultra-low attachment well plate techniques can produce a large number of very uniform-sized constructs while the spinner flask systems are amenable to large-scale production of spheroid 3D organoid cultures. These constructs are “self-organizing” and their size is limited by the diffusion of gases and nutrients into the construct, generally about 500 μm in cross-section. Examples of 3D organotypic spheroid tissue models include hepatic, neuronal, cardiac, pancreas, and tumor models (Fig. 1).

Other tissues, including many epithelial and endothelial models, are better modeled with an underlying scaffold on which the organoid can spread and develop cell and tissue polarization. Several of the 3D organoid construct systems reviewed in this series rely on a semipermeable membrane as their scaffold. This approach provides several advantages for modeling epithelial tissues. The semipermeable membrane

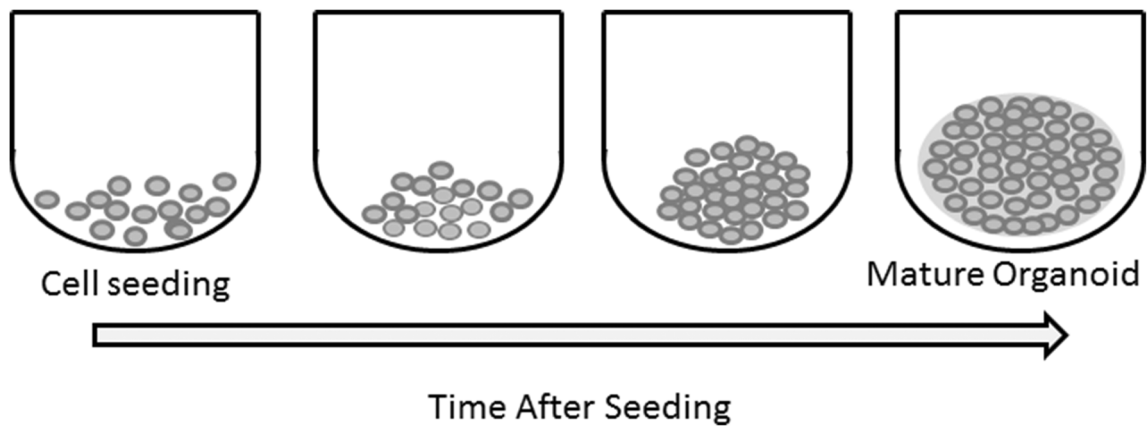


Figure 1. Spheroid organotypic construct: cells are seeded into a low adhesion micromold, allowed to spontaneously differentiate and self-assemble into the mature spheroid organotypic construct.

may be selected by pore size and/or chemical composition and supplied affixed to the bottom of a rigid plastic tube which is held in a multiwell plate for easier handling. Examples of these culture systems are the Millicell (MilliporeSigma, St. Louis, MO) or Transwell (Corning). The membrane may be coated with an ECM mixture appropriate for the cell type. The undifferentiated cells can be seeded and the population increased within the well until the growth factors are changed and differentiation of the epithelium begun. The critical step is the raising of the epithelial layer to the air-liquid interface to induce differentiation (Li *et al.* 2016; Chen and Schoen 2019). This is achieved by removing the culture medium from the top compartment of the culture insert. Under these conditions, basal cells form tight junctions and show basal-apical polarization and expression of tissue appropriate transporters (Ayehunie *et al.* 2018). Skin epidermal keratinocytes undergo

structural differentiation and the expression of keratin characteristic of skin epidermis under the influences of certain specific inducers on one hand (Cannon *et al.* 1994) while in the absence of those inducers, differentiation occurs without cornification (as would be seen in the corneal epithelium) (Kaluzhny *et al.* 2018). Interestingly, tissues such as the intestinal or vaginal epithelium respond to this induction even though these tissues do not experience an air-liquid interface during development (Chen and Schoen 2019) (Fig. 2).

When precise placement of specific cell types and/or matrix is required to produce the construct, bioprinting methods have been developed to layer the cells and associated matrix in a 3D architecture. The combination of a single cell type, its appropriate matrix (ECM), and required growth factors are often referred to as bioinks and the printers can be loaded with a selection of bioinks just like an inkjet printer with color

Figure 2. Air-liquid interface (ALI) organotypic culture modeling the skin: cells are seeded into culture inserts and allowed to proliferate before being brought to the air-liquid interface to differentiate.

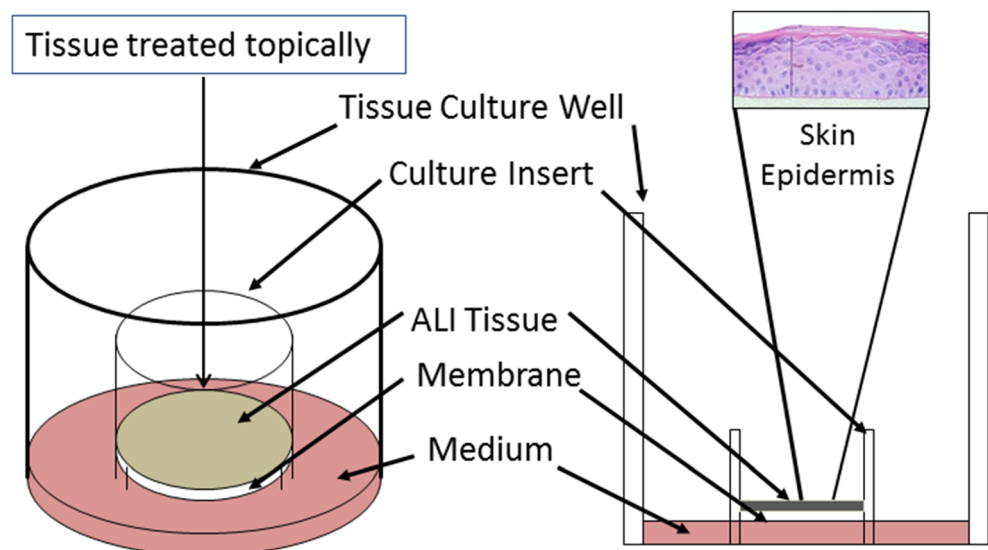
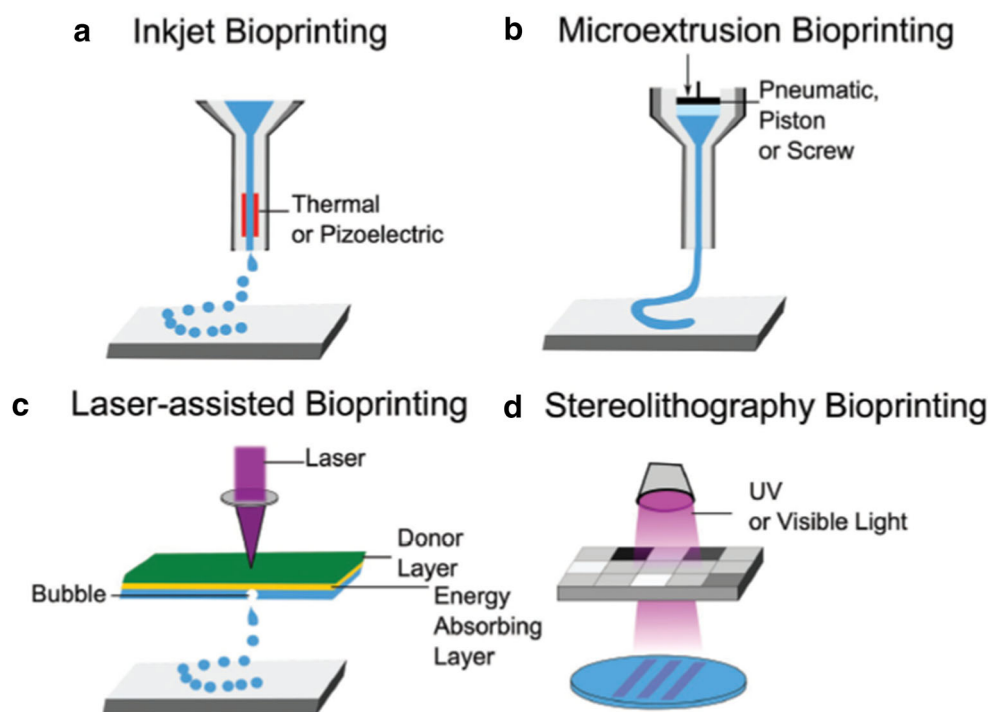


Figure 3. Examples of different bioprinting methods. (a) Inkjet bioprinters; (b) microextrusion bioprinters; (c) laser-assisted bioprinting; (d) stereolithography bioprinters (Foyt *et al.* 2018).



cartridges. Bioprinting allows the production of complex tissue architecture for both hard and soft tissue 3D organoid constructs (Kolesky *et al.* 2018; Xia *et al.* 2018) (Fig. 3).

General design of the reviews

There are many reviews of 3D organotypic test systems for both biological assessment and regenerative medicine in the literature. The reviews prepared under this project are focused more on the use of 3D organotypic constructs as test systems. They are intended to address a series of questions that would assist potential users as to how the individual systems might be applied to the study of various biological questions. The reviews will cover 3D organotypic constructs for the airway, cornea, intestine, kidney, neuronal, oral, skin, and tumor cells, and interrogation techniques. The review authors were asked to address the following points:

- It is understood that the intact organ would likely be much more complex than the 3D organoid construct. What are the key cell types *in vivo* that will be included in the *in vitro* construct? What contribution do they make to the overall biological response?
- What is the subset of biological functions of the *in vivo* tissue/organ that are intended to be modeled by the *in vitro* construct?
- What are the necessary structural and functional characteristics of the 3D organoid construct necessary to model

the desired biological properties? These elements could include the cell types represented and their state of differentiation and function. These properties might also include xenobiotic metabolic competence, selective transport, or barrier properties (i.e., skin epithelium).

- What exposure scenarios have been employed to effectively replicate *in vivo* (physiological) exposures for the desired biological property under study?
- What endpoint measures are/can be used to measure tissue changes effectively? How can these endpoints be quantitatively interrogated? One review will focus specifically on this question.
- Are there “prediction models” developed to relate the quantitative changes in the endpoints from the 3D organoid constructs with quantitative changes *in vivo*? If a quantitative prediction model is not applicable or has yet to be developed, what qualitative models are available?
- Given the complexity of the 3D organoid constructs (either commercial products or user derived), what quality control measures are appropriate/necessary to assure consistency of the constructs over time and across users?
- What is the current state of scientific development to use the 3D organoid constructs to replace certain current *in vivo* models or protocols (strengths and weaknesses)?

Acknowledgments We wish to thank the authors of the individual manuscripts focused on specific organotypic constructs in the review series.

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