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Review

A 3D Toolbox to Enhance Physiological Relevance of Human Tissue Models

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We discuss the current challenges and future prospects of flow-based organoid models and 3D self-assembling scaffolds. The existing paradigm of 3D culture suffers from a lack of control over organoid size and shape; can be an obstacle for cell harvesting and extended cellular and molecular analysis; and does not provide access to the function of exocrine glands. Moreover, existing organ-on-chip models are mostly composed of 2D extracellular matrix (ECM)-coated elastomeric membranes that do not mimic real organ architectures. A new comprehensive 3D toolbox for cell biology has emerged to address some of these issues. Advances in microfabrication and cell-culturing approaches enable the engineering of sophisticated models that mimic organ 3D architectures and physiological conditions, while supporting flow-based drug screening and secretomics-based diagnosis.

Trends

Microfluidics and microfabrication have revolutionized the way in which cells can be studied and manipulated in systems that are starting to provide 3D models and organ-on-chip devices.

Individual-organ models and multiple-organ interaction models address the issue of how microengineered approaches can faithfully reproduce key elements of physiologically relevant microenvironments.

The innovative technical nature of such 3D systems opens up exciting possibilities of answering several important fundamental biological questions that cannot be addressed with standard culture conditions.

In the race to closely mimic the structural and physiological functions of human tissues and organs, new possibilities have emerged in the form of 3D organ-level structures that integrate dynamic mechanical cues as well as chemical signals.

'Flat Earth' Approach

There is no doubt about the great importance and urgent need to develop new physiologically relevant 3D *in vitro* models for basic science and pharmacological research, including drug screening applications. A prime example is in cancer drug discovery, where the preclinical validation processes generally comprise a series of primary biochemical and cell-based assays using 2D cellular monolayers followed by evaluation in animal tumor models. However, this current paradigm suffers from a high rate of attrition, with < 10% of candidates that are identified by conventional high-throughput screening (HTS) approaches actually become licensed drugs [1,2]. This can be accounted for by the fact that the *in vitro* models used do not fully mimic the *in vivo* situation where cells are in a 3D environment.

Various 3D cellular models have been developed to reproduce the characteristics of the tumor microenvironment, with examples including mammary glands [3,4], prostate tissue, liver tissue, or others types of tissues, as reviewed by Li *et al*. [5]. All of the data collected indicate significant differences between cells grown in 3D and 2D in terms of cellular morphology, differentiation, migration properties, protein expression, and viability [3,5]. These observations confirm that the 3D context in which cells are seeded is crucial to drive them to a specific fate.

The need for 3D differentiation-relevant model systems is particularly well exemplified for generating functional glandular tissue units (or **organoids**, see Glossary) and investigating carcinoma, which is a major form of cancer that originates from the malignant transformation of

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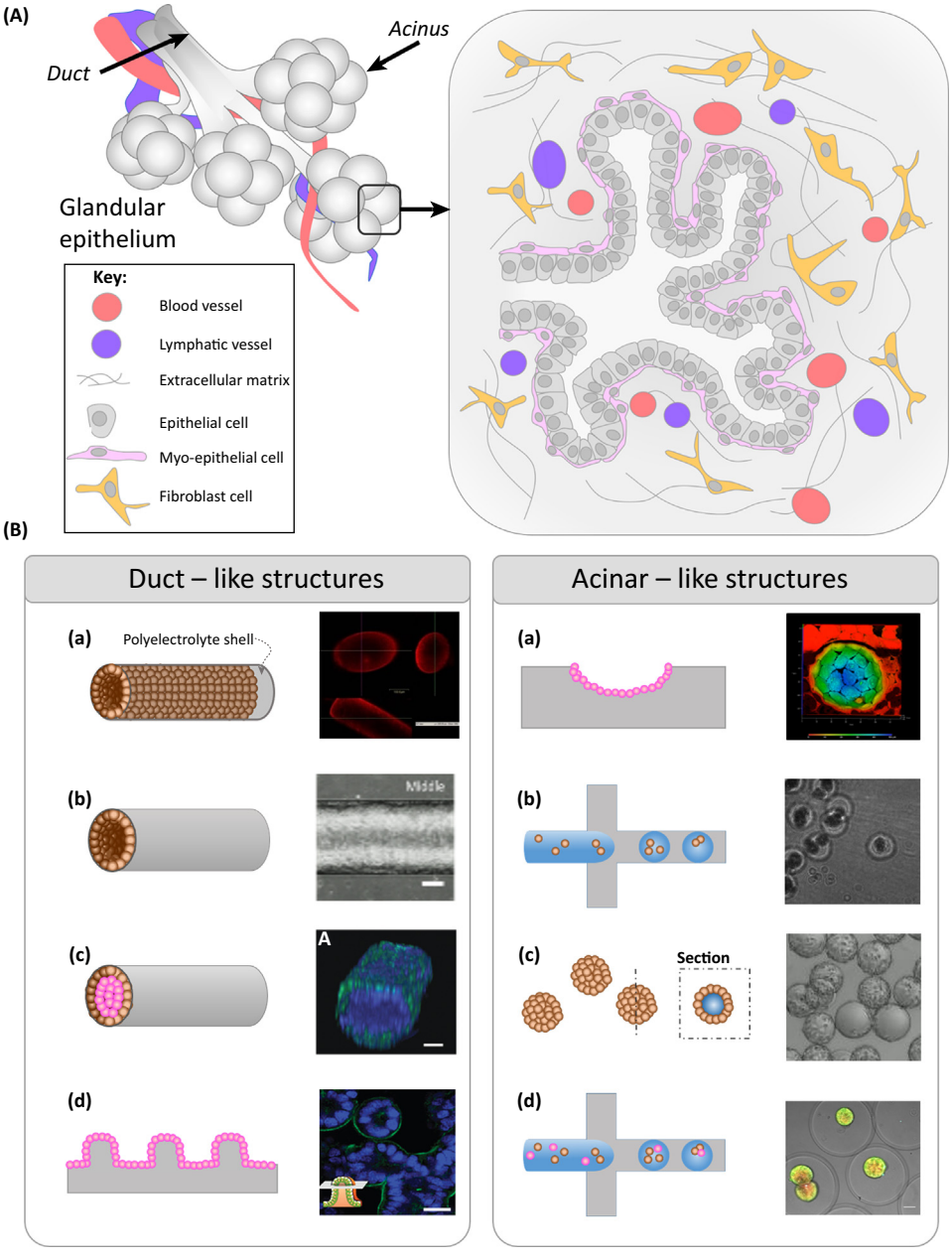

Figure 1. How to Mimic Functional Glandular Tissue Units.

For a Figure360 author presentation of Figure 1, see http://dx.doi.org/10.1016/j.tibtech.2016.06.012#mmc1.

(A) Scheme of glandular tissue that forms common structural and functional acini and duct units. Enlarged scheme of a lumen-based glandular tissue, surrounded by extracellular cues. (B) Set of scaffolds to recreate key elements of glandular 3D structures. Microfabricated scaffolds and microfluidics-based 3D cell culture tools to mimic duct-like structures (left panel) and acinar-like structures (right panel). Left panel: (a) Open and closed microtubes with polyelectrolyte shell. Reproduced, with permission, from Ting JHY, PhD Thesis, University of Technology Sydney, 2008. (b) Microtubes lined with prostatic epithelial cells. Reproduced, with permission, from [29], and with mammary cells. (c) Reproduced, with permission, from [28]. (d) 3D scaffolds designed to mimic human intestinal villi. Reproduced, with permission, from [90]. Right panel: (a) Hemisphere-like structures (personal communication). (b) Microbead-based culture. Reproduced, with permission, from [45]. (c) Microcarrier-based culture. Reproduced, with permission, from [64]. (d) microencapsulated 3D cocultures. Reproduced, with permission, from [65].

epithelial cells. Epithelial organs form elaborate architectures consisting of ducts and **acini** in exocrine glands (Figure 1A). The role of extracellular cues in cellular differentiation, in particular the matrix and many other genetic and microenvironmental factors, is now well established [6]. The recent development of 3D cultures of epithelial cells that re-establish such physiological interactions from different organs (kidney, lung, prostate, salivary and mammary glands) in **Matrigel** offers real potential to create models for human acinar development, better than conventional 2D cultures [6]. In healthy tissues the architecture of the epithelium is controlled through a fine balance and synchronization of cellular proliferation, apoptosis, differentiation, and secretion. Genomic instability, as well as the complex interactions and forces acting in the tumoral microenvironment, significantly affect epithelial architecture and function during tumor progression [6,7].

In order to more closely mimic the physiological barriers of tissues and the dynamic environment of tissues and tumors, significant efforts have been made in the past decades to create a wide range of scaffolds to support 3D cell culture (see Trends).

Moving Beyond the Flat Earth Approach: from Microchips to Organs-on-a-Chip

The field of 3D cell culture first focused on biomaterials and matrices to engineer 3D spheroids, but it has more recently been extended to more sophisticated scaffolding methods that include microfabrication, microfluidics, 3D bioprinting, and 3D patterning [8] together with 3D imaging approaches [9]. The potential use of micro- and nanotechnologies in cancer research has previously been reported [10], with a particular focus on microarrays, soft lithography-based microfabricated well arrays, and 3D micropatterned substrates, which all offer precise control over cell cluster size and geometry but lack control over the dynamic (spatial and temporal) cell environment. To overcome this limitation more sophisticated approaches that implement these 3D cellular models in a dynamic microfluic format emerged in 2010 in the field of tumor biology research (for review, see [10]). In 2010, the feasibility of 3D microscale cell culture devices was however just being demonstrated and still required improvements.

Currently, 3D epithelial models mainly consist of entrapped cells within a gel, which precludes any functional analysis of their secretions due to the difficulty to harvest cells and to sample their luminal components. Moreover, these 3D epithelial models fail to mimic the *in vivo* double-layered architecture of acini, whereby luminal cells are surrounded by a myoepithelial cell layer in a basement membrane. Heterotypic 3D cell models using multiple cell types can fully mimic this histological complexity of a normal epithelial tissue [6].

A breakthrough envisioned for new tools would be to create a functional *in vitro* model that mimics the acinar and ductal structure of exocrine organs, while providing controlled growth conditions and access to their secretions (Figure 1B). In the race to more closely mimic the structural and physiological functions of human tissues and organs, new possibilities have emerged in the form of 3D organ-level structures that integrate dynamic mechanical cues (e.g., in the form of flow, shear stress, and stretchable substrate [11]), as well as chemical signals, also in the form of gradients [12,13]. One of the first reviews that exhaustively covered the field of bioengineered 3D microsystems and organ-on-chip technologies was published by Huh *et al*. [14] in 2011. This review highlighted the unprecedented potential medical applications of these tools in drug screening, toxicity prediction, disease modeling, and the perspective to build some complex human-like architectures linking one organ-on-chip to another. Since then, recent advances in the organ-on-chip field have been reviewed and they illustrate the ability of these platforms to control fluid flow and shear stress applied to cells, which enhances the differentiation, function, and long-term survival of many cell types [15–18]. The authors also addressed the point of how microengineered approaches can faithfully reproduce key elements of the

Glossary

Acini: in a normal exocrine gland, highly organized structures with a central lumen lined by polarized luminal epithelial cells, and surrounded by an outer layer of myoepithelial cells. Acini are the common structural and functional unit of glandular tissues (e.g., breast, prostate, pancreas, and salivary gland). Biofluids (e.g., milky secretions and seminal fluid) are secreted in the lumen of acini and collected through ductal structures.

Matrigel: a gelatinous protein cocktail derived from Engelbreth–Holm–Swarm mouse sarcoma commercialized under the name Matrigel. This complex material is one of the most commonly used supports for 3D cell culture and contains laminin, collagen IV, nidogen/entactin, and proteoglycan but also non-protein molecules such as glycosaminoglycans and other polysaccharides.

Organoids: can be generated from stem cells, from induced pluripotent stem cells (iPSs), from healthy mouse or human tissue cells, from cancerous cells, and circulating tumor cells. Organoids derived from healthy material contain differentiated cell types, whereas organoids derived from cancer tissue mimic the histology of the tumor. Established organoids closely resemble the *in vivo* organ and recapitulate the spatial organization, cell–cell interactions, cell–matrix interactions, and molecular pathways of real organs. Organoids are models of *in vivo* physiology, amenable to extended cultivation and used to study development, oncogenesis, and drug discovery.

Polyelectrolytes: thin multilayer films created by the adsorption of alternating layers of a cationic polyelectrolyte (positive charge) and an anionic polyelectrolyte (negative charge). The film is built up from the ionic attraction between the polyelectroytes of opposite charge, with no covalent bonds needing to be formed.

physiological microenvironment. However, as they also highlighted, the level of complexity of *in vitro* tissue models needs to relate to the *in vivo* situation by 'synthesizing minimal functional units . . . rather than to build a whole living organ.' As a next step for this organ-on-chip technology, biomimetic microsystems that include different interconnected organs have then been proposed to model dynamic physiological processes in a more comprehensive way [14,19–21].

Nonetheless, the next generation of organ-on-chip devices will probably benefit from emerging new technologies to go beyond the current limits of the existing organ-on-chip platforms composed of 2D flat membranes to provide a controlled, 3D spatial arrangement of cells. Most of the organ-on-chip platforms mimicking physiological barriers have been inspired by the compartmentalized structure initially proposed by Takayama *et al*. [22], which has recently been revisited by Huh *et al*. [23]. Compartmentalized platforms are composed of two microfluidics channels separated by a horizontal thin porous membrane, with cells being cultured on one side or both sides of this membrane. When the membrane is made from an elastomeric material, it can be stretched in a cyclic way to model surface strains that cells would be exposed to *in vivo*, for example, in the lung–alveola barrier [24] or the intestine barrier [25]. Cardiac 3D constructs (heart-on-chip) embedded in a gel and set in between two compartments have been also developed [12]. Liver-on-chip models show great promise for investigating drug bioactivation, drug clearance, susceptibility to drug-induced liver injury, nanoparticle or chemical toxicity, and production of reactive metabolites that can interact with other organs [13,17,26]. However, it has still not been possible to integrate a functional biliary outflow tract into these devices [17]. Other challenges must also be overcome for these platforms such as identifying alternative materials to polydimethylsiloxane, or integrating in-line sensors to monitor the cell microenvironment or analyze cell secretions, feedback controls, and real-time imaging [17], while preserving a good balance between complexity and practicality [18]. Although there have been outstanding recent successes, the organ-on-chip field remains in its infancy. For example, to date, 3D epithelial models for acinar structures lack physiologically relevant lumen structures, which hampers the access to secretions and hence the identification of new cancer biomarkers (see Outstanding Questions).

One example of a technological breakthrough is the reconstruction of 3D and hollow-shaped functional units of glandular tissues in a controlled microenvironment, in contrast to the widely used flat membranes as found in standard dishware, transwells, or even simple microchannels. A set of scaffolds (hemispheres, Matrigel beads, open and closed microtubes, or microcarriers) was developed to serve as key elements or building blocks for a 3D toolbox aimed at producing such 3D constructs (Figure 1B). Such a 3D toolbox will provide novel and exciting capabilities for fundamental research, tissue engineering, and drug discovery (Table 1, Key Table). So far, other specific 3D scaffolds have been designed to mimic human intestinal villi that were used for toxicity and drug screening [25] and for inflammation studies [27]. Similarly, 3D lumen-based *in vitro* mammary [28] or prostatic [29] duct models have been proposed to model invasive forms of cancer or exocrine function.

'Round-Earth' Approach: 3D Multicellular Assembly Engineering

Ideal 3D scaffolds that improve the modeling of 3D human tissue and tumor microenvironments should provide native spatial arrangement, adequate biomechanical properties, and biocompatibility. One approach to achieve those aims is to decellularize whole organs to create ECM-based scaffolds. Decellularized tissues have been used for several organs, including heart [30], blood vessels [31], lung [32], and liver [33], but the use of human or animal organs is bound by ethical constraints in addition to having a limited supply. Attempts to mimic native human organs have benefited from the knowledge gained from microengineered cell culture models to provide the biotechnological alternatives to improve modeling of 3D human tissue and tumor microenvironments. This pathway has at least two possible directions; both of which rely on the use of

Key Table

Table 1. Next Steps and Challenges in the Three Emerging Fields of 3D Organoid Applications: 3D Organoid Culture, 3D Bioprinting and Organs-on-Chip

	Tissue engineering and regenerative medicine	Fundamental research	Pharmacological assays–drug discovery	Refs
3D organoid culture	Better mimic mechanical properties and biochemical functionalities of whole living organs.	Better understand morphogenesis and physiopathology. Better control over tissue stroma/ microenvironment. Better reproduce spatiotemporal gradients of chemicals and O_2.	HTS/HCS in 3D organoids. 3D imaging	[45,53,63, 85,86]
3D bioprinting	Engineer thick and complex tissues with fully functional vasculature and innervation. Biocompatibility bioink/ materials/cells. Long time viability for remodeling and maturation. Tissue-specific design and engineering process. *In vivo* bioprinting to direct tissue repair.	Tissue maturation and functionality. Tissue shape and function mimicking. Cancer/disease models. Dynamic tissue function assessment.	Hollow non tubular organs (bladder) Solid organs (kidney).	[34,35,74, 75,84]
Organ-on-chip	Soft scaffolds seeded with cells as artificial organs. *In vivo* implantation. Grafts (heart, liver, kidney). Replacement therapies. Stimulate organ regeneration. Polymers with smart properties (e.g., temperature-sensitive; photoreactive). Cell-sheet technologies (with thicker patches and blood vessels). Transplanting tissues with beating cells.	To develop more complete pathophysiological biomimicry. To study polarized functions of various cells. To reconstitute a tissue–tissue interface. Compartmentalized microfluidics for cocultures. Interconnected compartments via microfluidics ('human-on-chip').	To develop drugs that are safer and more effective. Integrated devices within microfluidics to increase the level of functionality (scale-up). Organomimetic microdevices with integrated functionality. Use iPS and ES. Integrate sensors (optical, chemical, electrical, and mechanical). Toxicity studies. Would improve routes of drug delivery and drug formulation. Virtual human clinical trials.	[12–18,20,21, 23,24,26,27]

hybrid systems to combine biological cells and a microfabricated architecture. The first approach involves integrating organoids into microfabricated and/or microfluidic devices. The second approach involves implementing microfluidics within scaffolded organ models that are constructed from a wide range of available biomedical polymers. These biotechnological approaches can also benefit from the rapidly evolving field of 3D bioprinted organ scaffolds or biomimetic microdevices that recreate 3D organ functionalities *in vitro* [34,35].

3D Toolbox to Optimize Manipulation and Analysis of Organoids

Microfabrication

The convergence of cell biology with microfabrication techniques has introduced novel strategies and platforms to prepare 3D cellular models with a tight control on their size and size distribution, geometry, and cellular composition, while being suitable for their large-scale production. These strategies can be classified into several groups depending on the format of these cellular models. The first popular format relies on the use of a microfabricated array of microwells [36,37]; either constructed in a cell-repellent material [38] or coated with a cell-repellent layer [39] to prevent cell attachment and to force cellular aggregation into a microtissue. In an alternative approach, tissues with well-defined dimensions have been successfully prepared using simple chemical patterns combining cell-repellent areas and areas promoting cell attachment [40]. A popular conventional approach for microtissue production is the so-called hanging drop technique [41]. This technique, however, suffers from a lack of reproducibility and control of tissue size, while also being time-consuming and cumbersome. Microfabrication techniques have improved this technique by using highly parallelized platforms with footprints of standard microwell plates. Furthermore, these platforms are compatible with the use of liquid-handling robots for both the steps of cell seeding and medium refreshment, as well as for the delivery of therapeutic agents for drug screening assays [42,43]. As such, they have promoted the introduction of 3D models into preclinical applications, beyond the basic research and/or academic laboratories [44]. However, microtissues generated by the hanging-drop technique are limited in size; they usually lack matrices, and they do not mimic cell–cell interactions readily because this tissue-production process occurs through artificial aggregation of cells rather than favoring the clonal growth of the tumor-like spheroids [45], as in real tumors.

Over the past few decades, microengineering cells into tissues on biochips has been demonstrated using silicon microfabrication or soft-lithography techniques that are both utilized to process biomaterials into 3D scaffolds of well-defined shapes structures and architectures at the micro- and nanoscales [46]. Attempts to better mimic tissues and organs have been reported with small 3D gel-based building blocks that can self-assemble into larger 3D organ-like structures (for a review, see [14]) or be woven into 3D complex architectures [47]. More recently, alternative strategies exploiting material biofunctionalization have been explored using self-assembling molecules and supramolecular chemistry [48].

Microfluidics-based 3D Cell Culture

Microfluidics has revolutionized the field of *in vitro* cellular models, also in a 3D configuration, by providing exquisite control over the physical and chemical microenvironment [14,45] (Figure 2). While microfluidics is still mainly developed by engineers, there are more and more biologists that can have access to microfluidic technology in the framework of interdisciplinary collaborations. Moreover, companies are now designing and selling devices with robust and user-friendly interfaces that are tailored for biologists. The combination of cellular supporting matrices and microfluidic technology offers unique opportunities for 3D cell culture and assays to be extended to organ-on-chip models. Microfluidic devices accurately control physical and chemical conditions (e.g., temperature, gas tension, medium composition, and concentration of soluble factors) at the microscale level, benefiting from the laminar flow configuration and the high surface-to-volume ratio in order to create *in vivo*-like conditions, while being compatible with

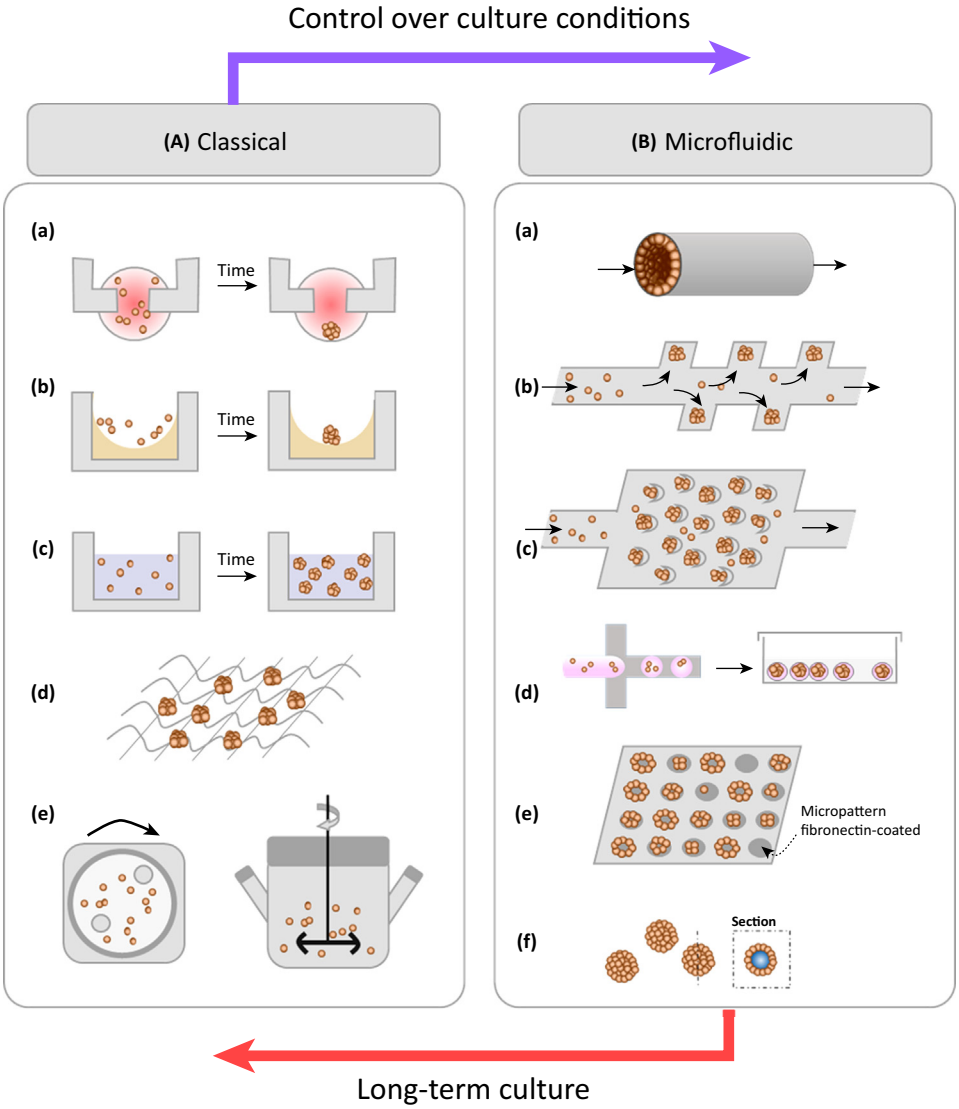

Figure 2. Classical and Microfluidic-based 3D Cell Culture Techniques. (A) Classical systems are (a) Hanging drop culture. (b) Cellular aggregation on nonadhesive surface (e.g., agarose). (c) Formation of organoids within physiologically relevant gels (collagen I, Matrigel, etc.) based on cellular division. (d) Scaffold-based culture. (e) Spinner flask culture and bioreactors. Modified, with permission, from [83]. (B) A broad variety of microsystems has been proposed including: (a) circular channel cell culture with a possibility of perfusion [29]; (b) flow-based formation of spheroids within channels micropockets [87]; (c) using microfabricated channels traps [88]; (d) microbead-based culture based on cell encapsulation within hydrogels (agarose, alginate, Matrigel, collagen, etc.) [45]; (e) micropatterning techniques to spatially constrain cellular growth into multicellular aggregates [89]; (f) microcarrier-based culture [64].

flow-based assays [49,50]. Additionally, microfluidic platforms have proven to be suited for stratified co-culture, complex gradient formation, and medium perfusion [50–52], or simultaneous exposure of an embryonic body (EB) to different solutions, by benefiting from the laminar configuration of the flow [53]. The latter configuration has particularly been applied to induce controlled differentiation of an EB simultaneously exposed to two different culture media. Moreover, microfluidic devices only require small amounts of both samples and cells, while allowing the combination of cell culture with on-line sampling such as for direct analysis of metabolites [54].

The use of microfluidics has proven to be a powerful approach to study collective cancer cell migration in multicellular spheroids in response to controlled chemotactic gradients [55], to screen antiangiogenesis drugs [56], or to identify new drugs for immunotherapy. Altogether, a key advantage offered by microfluidics is its ability to monitor in real-time multiple cell-type interactions in 3D, while providing tight control of the cell microenvironment [57].

More recently, droplet microfluidics has been successfully exploited for the high-throughput production of microtissues, with the technique of encapsulating a controlled number of cells in culture medium or hydrogel with or without a protective shell [58–61]. Using the same platform, long hydrogel-based fibers have been produced, which have subsequently been assembled or woven into more complex 3D cellular models [47]. Droplet microfluidics has also allowed the encapsulation of cells in a Matrigel matrix as microbeads to produce 3D cell constructs of well-defined size, to enable the spontaneous evolution into individual acini [45]. Following up on this technology, Dolega *et al*. demonstrated that Matrigel spherical bioreactors allow regulation of the cell microenvironment in well-defined microbead sizes, together with morphogenetic analysis at the single cell level, with greater reproducibility than has been reported previously for standard 3D culture systems. This droplet microfluidic tissue production platform and several morphological criteria have led to two key discoveries in the field of acinar development: (i) a single cell can generate a prostatic acinus; and (ii) acinus formation is properly initiated and differentiation is sustained so as to produce one architecturally accurate lumen-containing structure. Insofar as the relative formation of acini versus spheroids can be used as cancer models, such a droplet microfluidic approach could provide a much-needed high throughput. In comparison, even if current microfluidics 3D culture approaches are becoming increasingly mature, so far, they most often still preclude large screening assays; often due to difficulties in properly imaging the 3D structures. Encapsulated cells within a controlled Matrigel environment remain as floating objects that can easily be aspirated, dispensed, recovered for further analysis and sorted by large-particle fluorescence-assisted cell sorting (FACS). This capability opens exciting possibilities for single acinus handling and analysis. Development-controlling gene screening in single model acini would indeed undoubtedly help to identify genes that are essential in both the normal development of glandular tissue and carcinogenesis [45].

The advantage of using microfluidics to closely mimic the natural ductal structures that connect acini in exocrine glands has led to the creation of circular microtubes, which serve as scaffolds to support the growth of epithelial cells from exocrine glands [29]. When grown in those microtubes, the cells did not detach under physiological shear rates; therefore, this model is directly applicable to collect cellular secretions or to study cellular responses to shear stress inside small tubes. Furthermore, this work brought new insights into the influence of the 3D character on prostate cell morphology and proliferation. Immunostaining and microscopy observations have indeed revealed that microchannels coated with either Matrigel or a single layer of poly(sodium styrene sulfonate) promoted the adhesion and proliferation of epithelial cells. In contrast, a single layer of polyallylamine hydrochloride as coating of the lumen clearly slowed down cell proliferation and spreading [62].

A common trend in the field of microfluidics is to focus on the spatial and temporal control of the cell environment. The physiological relevance of these systems also relies on the ability for cells to survive for a long period of time and to reach a steady state to eventually yield the targeted tissue structure [63]. For instance, for cells that would be exposed to a flow *in vivo*, applying a continuous flow in the *in vitro* model is required in order to mimic physiological conditions. In the case of epithelial secretory cells, which are highly sensitive to shear stress and chemical and mechanical cues, a benchtop microfluidic bioreactor has been developed for continuous microcarrier-based cell culture [64]. Microcarriers (i.e., microbeads supporting a well-defined number of cells) were used as a growth support for anchorage-dependent mammalian prostatic

cells. Microcarriers provide convenient surfaces for increasing the yield of cells from standard monolayer culture vessels and perfusion chambers. Interestingly, the combination of microcarriers and microfluidics provides an adequate and controlled microenvironment for continuous benchtop cell culture to be performed outside an incubator, which is not achievable by most of the current (3D) cell culture systems. Along the same line, alginate microencapsulation based on continuous agitation bioreactor was recently exploited for 3D coculture of cancerous breast cells with fibroblasts forming organoids by artificial cell aggregation [65].

3D Toolbox to Create Artificial Scaffolded Organs

Considerable progress has been made in the engineering of materials for 3D *in vitro* culture systems [6], and recently a classification of 3D matrices and scaffolds was proposed by Ravi *et al*., who also discussed their advantages and possible applications [66]. Other groups have reviewed certain types of biomaterials for specific applications that include differentiation studies, drug discovery, or cancer research [67].

Gel-based Scaffolds

A wide range of gel-supported scaffolds (biologically inert polymers such as alginate, agarose or chitosan) or biologically active polymers (such as collagen I, hyaluronan, and silk fibroin [68]) have been introduced in recent decades to better suit *in vitro* cell culture in terms of mimicking tissue structure, mechanical properties, and function. These systems contrast with their gel-free, spheroid-based counterparts that have proven to be excellent models for avascular tumor tissues, so they are therefore routinely used for large drug screening assays [69].

The gel-based options described above represent a simplified support model compared to the physiological ECM that forms the framework to which cells are attached *in vivo*. The ECM is a complex mixture of proteins that are required for cell growth and differentiation, and interactions of cells with the basement membrane are essential to regulate cell behavior. Recently, Capulli *et al*. reviewed how natural ECMs can be an excellent source of inspiration to design tissue engineering scaffolds by guiding the self-assembly of cells into distinct functional tissues [70]. Furthermore, Girdhari and Weimin provided an exhaustive overview of the most recent advances in scaffolding techniques; this review focusing however on breast cancer research [71].

Currently, only the naturally derived hydrogels like collagens (I and IV) and Matrigel are capable to fully support cell differentiation and real 3D environment [8], so they are therefore relevant for many applications. Nonetheless, Matrigel suffers from poor component definition, low mechanical flexibility, and batch-to-batch variations. It is worth considering how to identify straightforward, reliable, and reproducible approaches to fabricate scaffolds that can satisfy all the constraints posed by biologically derived matrices. To overcome the problems presented by Matrigel, a general trend is to develop more defined environments [72]. Soft scaffolds blending a variety of synthetic materials (e.g., polyacrylamide, polyethylene glycol–fibrinogen, and polylactic acid) have been seeded with cells to mimic artificial organs [72]. They are attractive because they have mechanical and transport properties more similar to native tissues and, for instance, they enabled 3D liver aggregates to be built within open channels [73]. Additional advantages are that these compounds are designed to be compatible with 3D bioprinting and UV patterning to stabilize the structures [73] and to build micrometer-scale substructure [74]. Synthetic self-assembling peptides have also been shown to be ideal candidates as inks for bioprinting and biofabrication [75].

Self-Assembling Scaffolds

Biomimetic strategies based on 3D self-assembling **polyelectrolytes** (PEs) produced using the layer-by-layer (LbL) technique have been introduced to provide 3D scaffolds for epithelial cell growth [29,62] (Figure 3). PEs are polymers whose repeating units bear an electrolyte group,

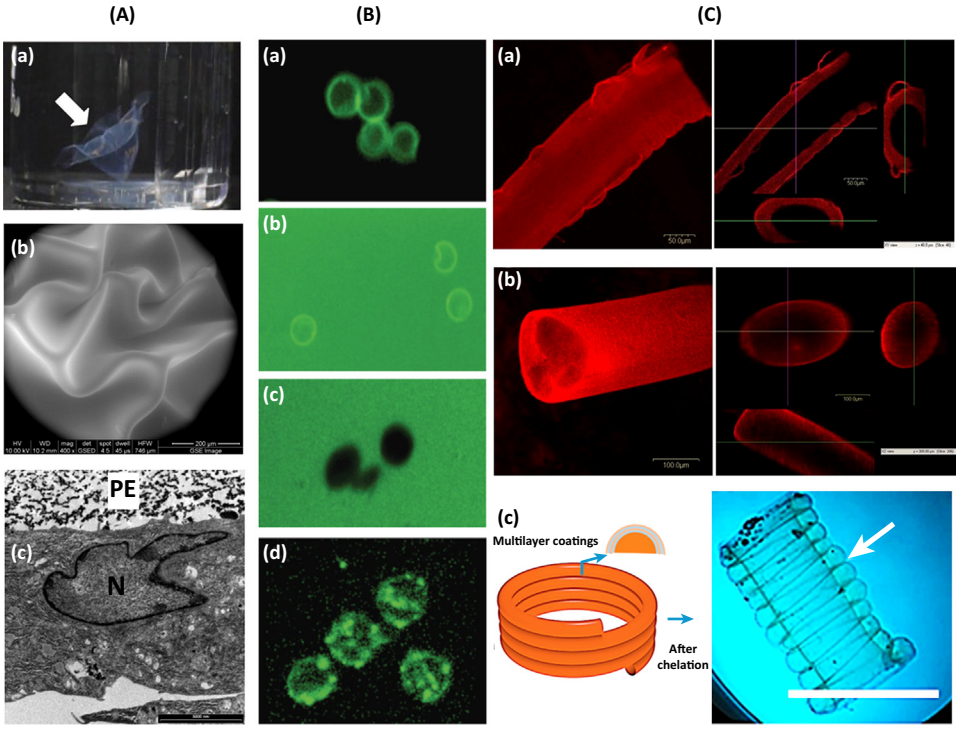

Figure 3. (A) Demonstration of the robust nature of a composite agarose/polyelectrolyte membrane (PEM). (a) An unconstrained PEM film is indicated by the white arrows, which keeps its integrity but is prone to folding in response to the thermal convection currents from the addition of hot aliquots of Milli-Q water. Reproduced, with permission, from [79]. (b) A hollow capsule made from nine layers of poly(sodium styrene sulfonate)/polyallylamine hydrochloride and imaged using environmental scanning electron microscopy (ESEM). The capsule was strong enough to withstand the ESEM vacuum and only partially collapsed. (c) Transmission electron microscopic image of a 3T3-L1 cell adhering to a polyelectrolyte capsule (PE). The white arrows show processes from the cell to anchor in the PE. The nucleus (N) of the cell is also visible. Reproduced, with permission, from Ting JHY, PhD Thesis, University of Technology Sydney, 2008. (B) Confocal microscopy images of hollow microcapsules in the presence of dextran–fluorescein isothiocyanate (FITC). (a) Microcapsules loaded with dextran–FITC. After washing, the microcapsules retained their fluorescence, which indicates that the lipid membrane formed an effective insulating barrier to seal the microcapsules. (b) Direct addition of dextran–FITC to a solution of uncoated microcapsules suspended in phosphate-buffered saline (PBS) resulted in immediate uptake of the dye, demonstrating the permeability of the polyelectrolyte for large neutral species in PBS. (c) Addition of dextran–FITC to previously lipid-coated microcapsules did not lead to fluorescence in the coated microcapsules. (d) Discrete lipid-coated capsules showing fluorescence from streptavidin A–FITC staining with biotinylated gramicidine A. Reproduced, with permission, from [81]. (C) Free-standing 3D microstructures of polyelectrolytes. (a) Hollow tube with open ends made from nine layers of poly(sodium styrene sulfonate)/polyallylamine hydrochloride (PSS/PAH), stained with rhodamine, and imaged using a confocal microscope. The left panel shows the composite image of the z-stack sections and the right panel shows the cross-sections of the tube. (b) Hollow tube with close ends made from nine layers of PSS/PAH, stained with rhodamine, and imaged using a confocal microscope. The left panel shows the composite image of the z-stack sections and the right panel shows the cross-sections of the tube. Reproduced, with permission, from Ting JHY, PhD Thesis, University of Technology Sydney, 2008. (c) Production of spiral tube of polyelectrolyte membrane coating a core of alginate. The right panel is an optical image of the hollow tube of polyelectrolyte after removal of the alginate by 0.05 M EDTA solution. The white arrow indicates the single winding points all over the construct. Scale bar is 1 cm. Reproduced, with permission, from [80].

which makes the substance electrically conductive. These groups dissociate in aqueous solutions so that the polymers become charged. A key advantage of PEs is their versatility and suitability to coat an infinite variety of shapes. Furthermore, such PE multilayer films can be adsorbed onto a wide variety of surfaces, including plastic, glass, latex, colloidal particles, and bacterial or mammalian cells [76]. Such unique biologically inspired approaches were specifically developed to design novel biomimetic membranes [77]. The versatility of PEs for creating

nanostructured membranes in 3D shapes provides a physiologically relevant approach to construct organ and tissue models. These models are not restricted to any particular matrix, such as Matrigel. For example, PEs have been utilized for the nanoassembly of supporting PE membranes as cellular scaffolds, thereby extending previously considered research applications [78]. This PE technology opens new avenues for inhibiting cancer progression by providing a controllable cell culture microenvironment. For example, a positively charged PE film several nanometers in thickness can alter the adhesion and reduce the proliferation rate of cancerous epithelial prostatic cells (PC3, a prostate cell line for advanced cancer). Significantly, the clustering phenotype of PC3 cells was shown to be impeded by that nanostructured film [62]. Some reports have highlighted that PE films can modulate cellular adhesion, proliferation, and differentiation, and they are therefore particularly attractive for tissue engineering and biomedical applications [79]. By modulating the surface charge of the PE films and controling the mechanical and biophysical properties of the support on which the films are placed, it is possible to fine-tune the response of tumor cells and their growth [62]. PE films have also been adapted to fabricate a permeable interpenetrating hydrogel of nanoscale thickness that is still sufficiently rigid to function as a free-standing biomimetic membrane planar support [77]. The versatility of the LbL deposition technique is also demonstrated in the production of a self-supporting 3D spiral macrostructure that was stabilized by a coating with a multilayer PE film [80] (Figure 3). More generally, PE films form attractive permeable substrates to support lipid bilayers, with great promise in biomedical applications for thin film coatings, micropatterning, nanobioreactors, and the realization of artificial cells [81], drug delivery systems [76], and other biomimetic membranes [82].

Concluding Remarks and Future Perspectives

Microfabricated systems such as lab-on-a-chip devices or sophisticated organ-on-chip platforms offer the opportunity to grow cells under conditions that maintain normal 3D environmental cues while reproducing organ function. A 3D toolbox based on the combined use of nano- and microtechnologies, along with organoid models, may constitute new weapons for multiomics and high-throughput analysis in the arsenal that is available to biologists and oncologists, because they offer many more possibilities than the mere miniaturization of standard assays. It is likely that a reference format combining several advantages of each approach (e.g., encapsulation, new PE-based matrix, or 3D scaffolds) will emerge in the future (see Outstanding Questions). New directions towards PE coated-3D scaffolds combined with microfluidics are highly attractive to design a chip for *in vitro* functional assays of exocrine glands and for novel screening platforms for biomarkers identification. More generally, such 3D tools are likely to be instrumental in addressing the next big challenge for the field: the full validation of physiologically relevant models.

Outstanding Questions

How can the next generation of organ-on-chip devices benefit from emerging new technologies to create controlled 3D spatial arrangements of cells that go beyond the existing limits of current organs-on-chips that are composed of 2D flat membranes?

How can the development of next-generation organ-on-chip devices be enhanced by integrating new 3D biomimetic tools that have emerged, such as microfluidics, 3D printing, biomimetic membranes, and innovative scaffolds?

How can the next generation of organ-on-chip devices be based on the reconstruction of 3D and hollow-shaped components of glandular tissues in a controlled microenvironment?

How can the next generation of organ-on-chip devices best mimic organ 3D architectures and support secretomics-based diagnosis and flow-based drug screening?

How can the next generation of organ-on-chip devices be best engineered to accelerate investigations of carcinogenesis, identify new cancer biomarkers and develop new approaches for medical diagnosis?

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