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Microphysiological System Design: Simplicity Is Elegance

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Abstract

Design parameters for microphysiological systems (MPS) are driven by the need for new tools to answer questions focusing on human physiology in a robust and reliable manner. Within this perspective, engineering benchmarks and principles are identified to guide the construction of new devices in the MPS field, with emphasis placed on the design principles common to all tissues, as well as those unique to a subset of tissues. Leading organ replica technologies that recapitulate various functions of the brain, heart, intestine, and lung are highlighted as examples that meet the identified benchmarks and standards, with current barriers for large scale production and commercialization discussed. To reach their full potential and achieve widespread use, MPS will have to be recognized officially by government agencies, and toward this end, considerations of MPS as a potential regulatory tool are presented.

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nancy Allbritton and Yuli Wang have a financial interest in Altis Biosystems, Inc. The remaining authors declare no competing financial interests.

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Keywords

microphysiological system; organ-on-a-chip; high-throughput; microfabrication; primary cell culture

Introduction

Microphysiological systems (MPS), which include organs-on-chips and organ replicas, have undergone rapid development since their inception [1], driven by the need to replace animal models and recapitulate human physiology, thus paving the way for rapid discoveries in basic and pharmaceutical sciences, as well as personalized medicine [2]. The development of a single, modular and universal microphysiological platform for all organs or tissues represents a tantalizing goal; however, each carries a distinct set of requirements that are dependent on the tissue under study. Beyond ensuring that engineered platforms are capable of hosting healthy populations of cultured cells, engineers must also consider whether devices are scalable, manufacturable, reproducible, and predictive. Within this perspective, engineering benchmarks and design principles relevant to MPS are presented, and technologies that push current limitations of scalability and throughput are highlighted. Ultimately, new systems should be widely accessible and enable acquisition of high-content, reproducible information in a reliable format [3,4]. Moreover, medical and regulatory decisions are expected to benefit from this suite of technologies, in particular where endpoints of significance to human health can be obtained and validated across multiple laboratories.

Engineering benchmarks and design principles

In designing platforms that are not only physiologically relevant, but also scalable in manufacture and usage, it is helpful to distill each organ down into representative tissue subunits, from which dominant features can be identified and engineered into the organ-specific tissue replica. For example, the crypt or crypt/villus is the primary tissue subunit of the intestinal epithelium with its polarized architecture supported by *in vivo* biochemical gradients that regulate epithelial cell self-renewal and differentiation. The alveoli of the lung, in which an air-liquid interface is present, is responsible for gas exchange and is continuously subjected to rhythmic stretching that modulates cellular physiology. Likewise, the nephron of the kidney, sinusoids and lobules of the liver, and electrically connected, anisotropic tissue layers of the heart could be considered as the smallest derivative subunits of their respective organ that support its major function. Identifying these major subunits and their critical microenvironment features will enable design, construction, and operation of these tissue replicas to yield complex organ-level behavior and enable predictive experiments and assays. Additionally, these tissue subunits while recapitulating organ-level physiology do so with out the high construction complexity required to build a full organ.

Cultured cells are a core component of microphysiological systems, and thus, the choice of appropriate cell source should be a primary consideration during design (Fig. 1). The cells must be competent to interface with neighboring cells and/or tissue subunit components, in order to develop complex behaviors and display interactions that are not present in simpler

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culture systems. Human primary cells can retain a normal genome and physiology that closely mimic the *in vivo* phenotypes and functions of the original tissue, and thus are the preferred tissue source [5–8]. For many organs (e.g., blood, skin, intestine), primary cells are readily available from biopsies of healthy and disease state donors. However, in the case of organs in which biopsies are more difficult to obtain (e.g., brain, kidney, liver), primary cells can be isolated from surgically resected tissue or cadaveric donor organs. To address the finite lifespan of end-differentiated primary cells, tissue-specific, adult stem/progenitor cells are often used as the they have ability to renew themselves indefinitely under ideal growth conditions, and can be differentiated to yield some or all of the major specialized cell types of the *in vivo* tissue. Alternatively, primary somatic cells can be used to generate induced pluripotent stem cells (iPSCs), which thereafter may be differentiated into tissue-specific cell types. All primary cells require highly specialized growth conditions, which may include specific cytokines, metabolites, and growth factors that must be incorporated into the microdevice. Given that many stem cells can be long lived in culture, with some capable of being banked and frozen, genetically diverse biobanks can be established for population screens [9]. These attributes make stem cells a very attractive source to build microphysiological systems; however, robust protocols for stem cell maintenance and differentiation need to be established [10,11]. Despite the many advantages of using primary cells, it is acknowledged that immortalized cell lines can be used to build microphysiological systems, benefitting from their predictability, ease of culture, and low handling costs, despite having lost the complexities of the original tissue from which they were isolated.

A second consideration for the development of *in vitro* tissue replicas is the organization of cell types into distinct regions that are reminiscent of *in vivo* tissue structure. Porous membranes are often used to place different cell types at opposing boundaries, thus generating an artificial tissue boundary analogous to that found *in vivo*. Microfabrication technologies such as 3D printing have the potential to build a variety of cell types with precise 3D architecture. To maintain cell viability, nutrient/oxygen supply and waste removal should be specifically considered for cell culture in microphysiological systems as they are usually in miniaturized formats with reduced media volume and non-convective geometries. Microfluidic perfusion [12], frequent medium replenishes [13,14], or integrations of mechanical mixers and gas reservoirs [15] have been used to facilitate the rates of nutrient/oxygen delivery and waste removal.

For adherent cells, extracellular matrix (ECM) is an important component to consider. Beyond providing binding sites for cell adhesion, material properties such as stiffness, viscoelasticity, and porosity should be considered to correctly recapitulate the cell phenotypes, gene expression and key functions of the target tissue. Tumor-cell-derived ECMs (*e.g.*, Matrigel) are frequently used for culture of 3D organoids; however, the composition of cell-derived ECMs are not well defined and vary batch to batch [16,17]. Purified protein or polysaccharide ECMs *(e.g.,* collagens, hyaluronic acids, alginate, and chitosan) are also used as matrices for 2D and 3D culture and are less costly compared to Matrigel. Moreover, as the biochemical properties and molecular structures of these purified, naturally-derived ECMs are well defined, the material properties are easier to tune than cell-derived ECMs, with preservation of cell compatibility. Synthetic polymer ECMs are also used in 2D and 3D culture when there is a necessity to tailor the material and structural

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properties of the matrix with the highest precision [18]. Dynamic modification such as photoreactive crosslinking [19,20], physical and chemical crosslinking [21], degradation [22], or enzyme driven modification [23,24] can be exploited to alter the mechanical properties [19,20] or create 3D microstructures [14,25]. However, protein coatings or chemical conjugation of cell binding moieties may be necessary to promote cell adhesion. Synthetic polymers in general are reproducibly manufactured, economical, and free of endotoxin or nucleases, but the cell behaviors including proliferation and cell phenotypes cannot always be fully replicated.

Implementing physiological features of the tissue (*e.g.*, shear forces, 3D microstructures, dynamic strains) or *in situ* sensing units (*e.g.*, electrical, mechanical, biochemical) for large scale use require materials and methods that are compatible with manufacturing methods (Fig. 2). Since the key features for successful tissue subunit recapitulation depend on the organ, material selection ideally should be target-tissue specific. However, there are manufacturing considerations that are universal across all *in vitro* tissue systems. All materials used that contact cells should be biocompatible, reproducibly manufacturable, and exhibit minimal variation within a batch or between batches. Absorption of small molecules should be mitigated for drug screening applications by choice of material or surface treatments and coatings. Compatibility with the standardized multiwell plates is advantageous for automation, and to fit into existing workflows and assay platforms. Optical transparency and low fluorescence background are required if the tissue properties will be evaluated by microscopic imaging. Examples of common chemical and physical attributes for tissue subunits to guide microphysiological device design are highlighted in Fig. 3.

Highlights of leading organ replica technologies

Hundreds of MPS devices exist throughout published literature, with a handful of commercial products under development. Within this section, we highlight several technologies developed in academic laboratories that push the limits of manufacturing and analytical scalability. Specifically, these technologies exhibit exceptional promise for automation of fabrication, tissue culture, and/or analytical assays, ultimately rendering the collection of high content information accessible to a greater number of end-users.

Miniaturized Bioreactors for Brain Organoids.

Brain organoids have shown potential for high throughput compatibility with *in vivo*-like tissue complexity. Typically, generation of brain organoids begins by forming an embryonic body from iPSC or ESC, followed by neuroectoderm induction and expansion, and finally differentiation leading to mature brain organoids. The initial embryonic body can be formed in a high throughput manner (*i.e.*, multiwell plates). However, as the organoids expand in size and cell number over time, static culture in multiwell plates often fails to provide efficient transport of oxygen and nutrients, leading to cell death in the long-term [26]. Qian and colleagues developed miniaturized spinning reactors, SpinΩ, to enable long term brain organoid culture (Fig. 4A). Representative regions include forebrain organoids containing all 6 cortical layers of the human fetal brain, as well as midbrain and hypothalamus organoids

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[27]. This system offers a cost-efficient and high-throughput compatible platform for delivering nutrients and oxygen, which may have applications for other cell and tissue types.

3D Printing of Cardiac Muscle Subunits.

Lind et. al. developed a cardiac MPS using a novel multimaterial three-dimensional (3D) printing method [28,29]. The group integrated multiple functional inks with different properties to grow multiple tissue constructs on a multiwell device with embedded sensors (Fig. 4B). The ability to print grooved micro-architectures using soft polydimethylsiloxane (PDMS) within each culture site facilitated the self-guided assembly of progenitor cells into electrically anisotropic layers of cardiac tissue. Multimaterial 3D printing incorporated electrically conductive, soft strain gauge sensors for a continuous, instantaneous, non-invasive readout. The cardiac tissue layers displayed physiologic functions, including contraction, in response to appropriate stimuli and increased or decreased contractility upon application of cardiac-active drugs. An advantage of this platform was the wide variety of printable polymer-based materials employed on a single, programmable fabrication platform and in particular the highly parallelizable embedded electric sensors to provide a continuous, near-instantaneous readout.

Hydrogel Molding for In Vitro Intestinal Crypts.

The development of organoid and monolayer culture methods for intestinal epithelial stem cells (IESCs) has enabled primary cell-derived, physiologically relevant, *in vitro* models of the intestines [13,14,30–37]. To create high-fidelity replicas of the microarchitecture and asymmetry of human colonic epithelium, arrays of crypt-like invaginations shaped from crosslinked collagen hydrogels mimicked the shape, size and density of human colon crypts [14] (Fig. 4C). Application of a growth factor gradient was sufficient to polarize the crypts so that the stem/proliferative cells were restricted at the base of the crypts (forming a stem cell niche) while various differentiated cell types were located at the luminal surface. The crypts responded appropriately to the luminal application of short-chain fatty acids or basal application of inflammatory cytokines. Platforms such as these enable replication of the intestinal microarchitecture, epithelial cell compartmentalization and polarity as well as key intestinal features such as chemical and gas gradients. Each cassette, containing <120 patterned crypts, exhibits great potential as a high-content and physiologically relevant cellular assay tool. While currently in 12-well format, innovations in automated scaffold fabrication strategies and 3D microscopy methods will maximize throughput.

Stretchable Thin Films to Mimic Lung Alveoli.

Generation of scalable models of the lung epithelium has presented a challenge in that appropriate mechanical forces must be simultaneously applied across all arrayed elements to simulate the rhythmic stretching and relaxation that occurs *in vivo* [38,39]. A lung-on-a-chip platform in which primary lung epithelial and endothelial cells were supported over a biomimetic collagen/elastin membrane covering the arrayed hexagonal holes of an electron microscopy grid, replicated lung alveoli both in the architecture and physical forces during breathing (P Zamprogno et al., bioRxiv doi: 10.1011/608919, Fig. 4D). Application of a cyclic negative pressure below the membrane enabled the *in vitro* alveoli to deflect in concert, experiencing radial strains similar to those found *in vivo*. Given that the dimensions

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of each hexagonal area (*i.e.*, 225 μm dia.) were nearly identical to those of *in vivo* lung alveoli (160 – 200 μm), the platform represented an impressive scalable mimic of its *in vivo* counterpart.

Considerations for commercialization

Though MPS carries tremendous potential to advance the areas of personalized medicine and drug development, there are significant hurdles that the field must overcome in order for MPS to be commercially viable and executable across laboratories [40]. Certain device features could make a technically capable method great for academic research and prototyping, yet hinder scale-up and widespread use. Concepts of scale often vastly differ between academic and industrial settings, with fabrication of 1 – 100 units considered sufficient for feasibility studies and/or peer-reviewed publication, though 100,000+ units potentially required for launch of a commercial product [41]. While the considerations below are not intended to be exhaustive, it is important that developers of MPS technology begin studying whether these issues are present and how to overcome them.

- *Sourcing of raw materials*. Raw materials that are to be included in the physical device or cassette, which may include plastics, polymers, and hydrogels, must not only be sourced for analytical assay compatibility, yet must also be done so at a large volume that meets good manufacturing practice (GMP) constraints, such as batch-to-batch reproducibility and the ability to apply robust standard operating procedures (SOP) during fabrication and assembly [42,43]. A commonly used material for organ-on-chip microfluidic devices is poly(dimethylsiloxane) (PDMS), which is biocompatible, widely accessible, and easy-to-use. However, attempted scaling of PDMS based devices reveals hidden issues and costs, including the need for large-volume fabrication of master replicas, and variabilities in obtaining high-resolution features during polymer molding [3,44]. Moreover, due to the high propensity of PDMS to absorb hydrophobic small molecules and proteins [45], antifouling solutions must be optimized and validated for each application and device.

- *Cell selection and validation*. Cells cultured within MPS are also subject to GMP compliance to provide assurance that any conclusions drawn are reliable and predictive, and thus must be selected, cultured, and validated accordingly. Geraghty et al. have provided a general protocol for acquisition, naming, and usage of new cell lines, with recommendations for avoiding pitfalls that could result in contamination or unreliable results [46]. Methods for scaled-up primary stem cell manufacturing are under active investigation [47], and it is generally accepted that all culture conditions should be fully defined and free of animal-derived substances [48–50]. With the above in mind, studies must be performed to determine the biological impact of donor-to-donor and passage-to-passage differences, which may inform decisions on whether multiple donors must be included for every assay and suitable “passage windows” in which these cells can be reliably utilized. While karyotyping can be a robust and informative method for quality control (QC), it is becoming clear that more in-depth

characterizations are required for assessment of genetic drift of stem cells throughout culture, which may include short tandem repeat profiling, copy number variation mapping, or whole-genome sequencing [46,51].

- *Assembly and quality control.* Given the complexity of most MPS, which integrate both living and non-biological components/mechanics, it is expected that some assembly will be required by the end-user (e.g., securing of all device components and seeding of cells), preventing a complete QC assessment of each assembled device by the manufacturer. Instead, QC will likely have to be based on thorough benchmarking of the separate components (e.g., genetic drift assessment of cells, size tolerance of machined components, coefficients of variation for embedded sensor responses), in accordance with the considerations for raw materials and cells outlined above. Further, this will necessitate providing detailed and unambiguous protocols for final MPS assembly and usage to the end-user, which will require continuous optimization and validation by the manufacturer.
- *Journey to the end-user.* Shipping of cell-based platforms over large distances and varying environmental conditions may present several concerns. What is the shelf life of each constituent? Must the devices and cells be shipped in an expedited fashion, under separate conditions, and are there federal transport agreements that must be agreed upon ahead of time? Once the device reaches the end-user, storage of each component much be clearly delineated, and/or device packaging must be designed in such a way that it is easy to use and store appropriately.

As suggested by these points, to reach their full potential these devices will ultimately have to meet regulatory guidelines where ruggedness and inter-laboratory repeatability are critical. The extensive testing that will be required may potentially need to be facilitated through public-private partnerships [52].

Outlook: Regulatory approval and broad adoption

In the United States, regulatory science is “the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products” [53]. Since regulatory science is used to make decisions that can have life-and-death consequences for large numbers of people, it employs consensus and time-proven methods. Within this context, the FDA has developed innovative approaches to advance regulatory science, such as its Advancing Regulatory Science Initiative and Critical Path Initiative. Section #1 of the FDA’s “Strategic Plan for Regulatory Science” addresses the need to *modernize toxicology to enhance product safety* [53]. One of the aims of MPS to-date has been to supplement or even replace *in vivo* toxicology testing. FDA’s “Predictive Toxicology Roadmap” also discusses MPS as a promising new technology [54]. FDA, DARPA and NIH-NCATS have made significant contributions to these efforts through a collaborative “Tissue Chip” program with the ambitious goal of developing a “human-body-on-a-chip” for drug screening [55]. Outside of the United States, high enthusiasm exists for advancing MPS technology, with the European Union (EU) funded Project ORCHID

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(Organ-on-Chip In Development) advocating for increased awareness of organ-on-chip technology and providing a roadmap for organ-on-chip adoption throughout the EU [56,57].

MPS have potential applications in regulatory science that go far beyond toxicology testing. In particular, the ability of MPS to recapitulate a significant functional aspect of an organ or system can benefit preclinical performance and safety testing of medical devices, reducing or replacing the burden of animal testing [58]. With the potential for application to so many different medical products across multiple government agencies, a useful thought experiment is to ask what qualities MPS should embody to be highly successful tools for regulatory science. All regulatory tools have basic requirements such as precision, reproducibility, and robustness [59,60], but what are some particular areas that could be challenging or problematic for MPS that deserve further research and attention early in development? A few to consider are:

- *Determining which biological signals are clinically important for human health.* There are many possible endpoints that can be measured in an MPS, and it will be important to conclude which ones can consistently relate to effects in humans.
- *Challenges of representing the whole with a small sample size.* Micro-sized samples often do not contain a large enough population to be representative of the distribution of all cell behaviors, *etc.* in an organ or system. This would likely be caught in reproducibility testing, but may only show up as a problem when certain test variables are employed which select for sub-populations.
- *Challenges in comparing data with large numbers of variables that impact the outcome.* We have found that the more input variables are involved in determining the outcome of an *in vitro* test, the less likely that it is useful across multiple companies or products because of the inability to reproduce every one of those inputs in testing. With a small number of variables some assumptions and corrections can be made, but with MPS, which are highly complex systems, it will be essential to have very fine control over many parameters. This stresses the urgent nature of having consensus guidelines for competing technologies with similar measurement goals.

It will be important for MPS to make the transition from answering research driven questions to satisfying requirements pertaining to regulatory decision making. Mature, robust MPS platforms with a significant public health benefit will catalyze continuing public-private collaborations and facilitate future regulatory application.

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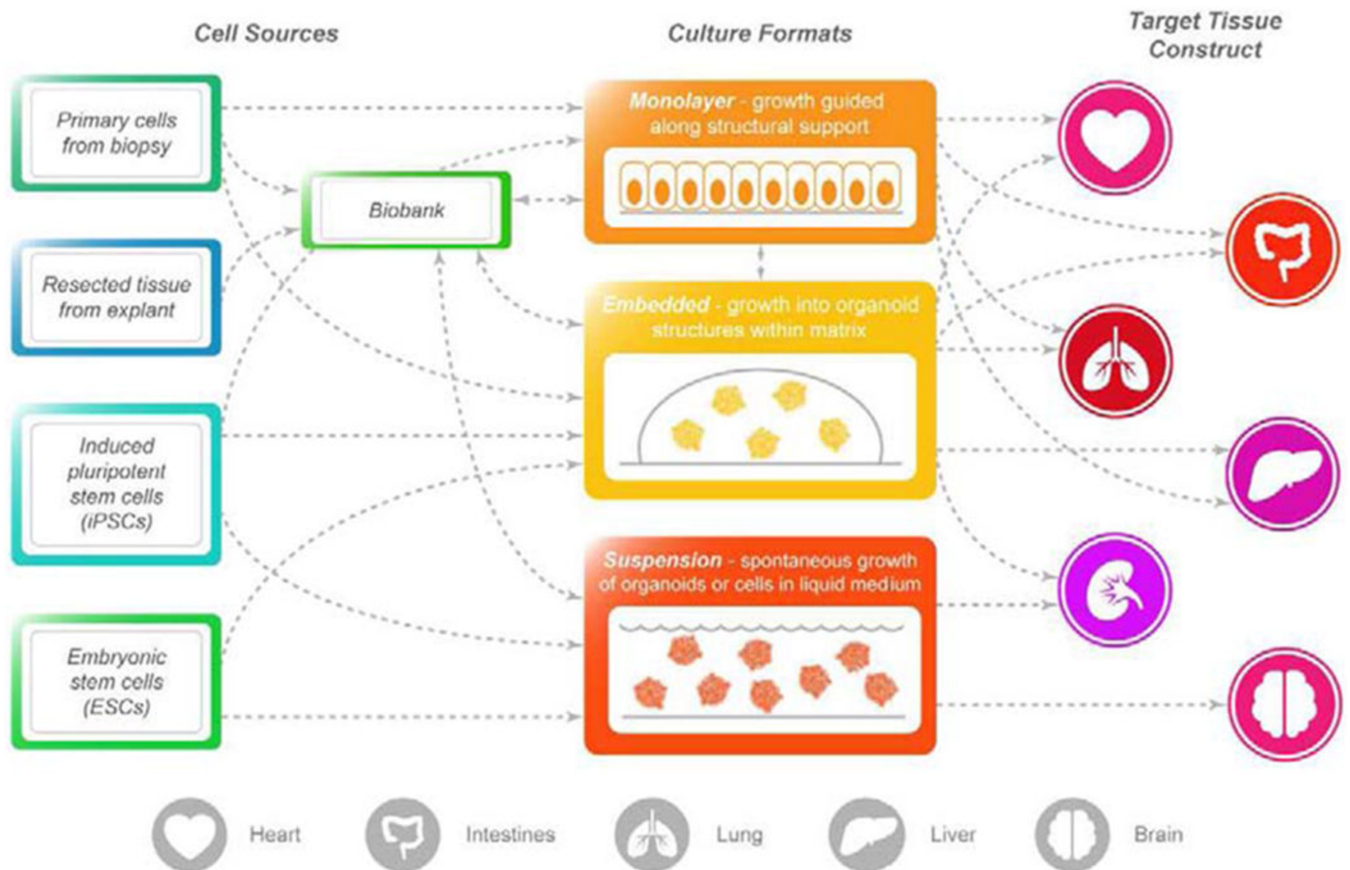

Figure 1.
Sources of tissue and example culture schemes to expand the primary cells. Diagrams indicate potential routes that can be taken for expansion of primary cells for culture within microphysiological systems that recapitulate functions of specific organs.

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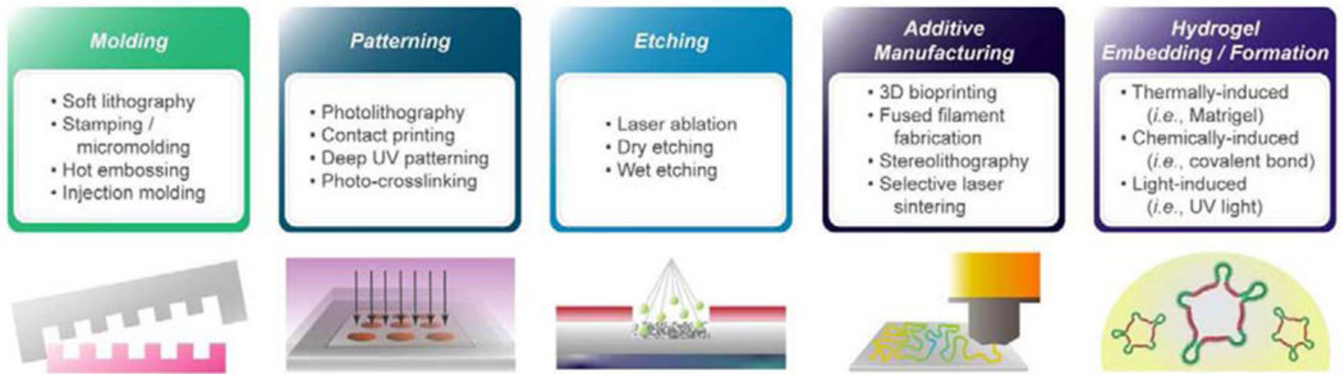

Figure 2.
Example manufacturing categories and techniques for microphysiological systems.

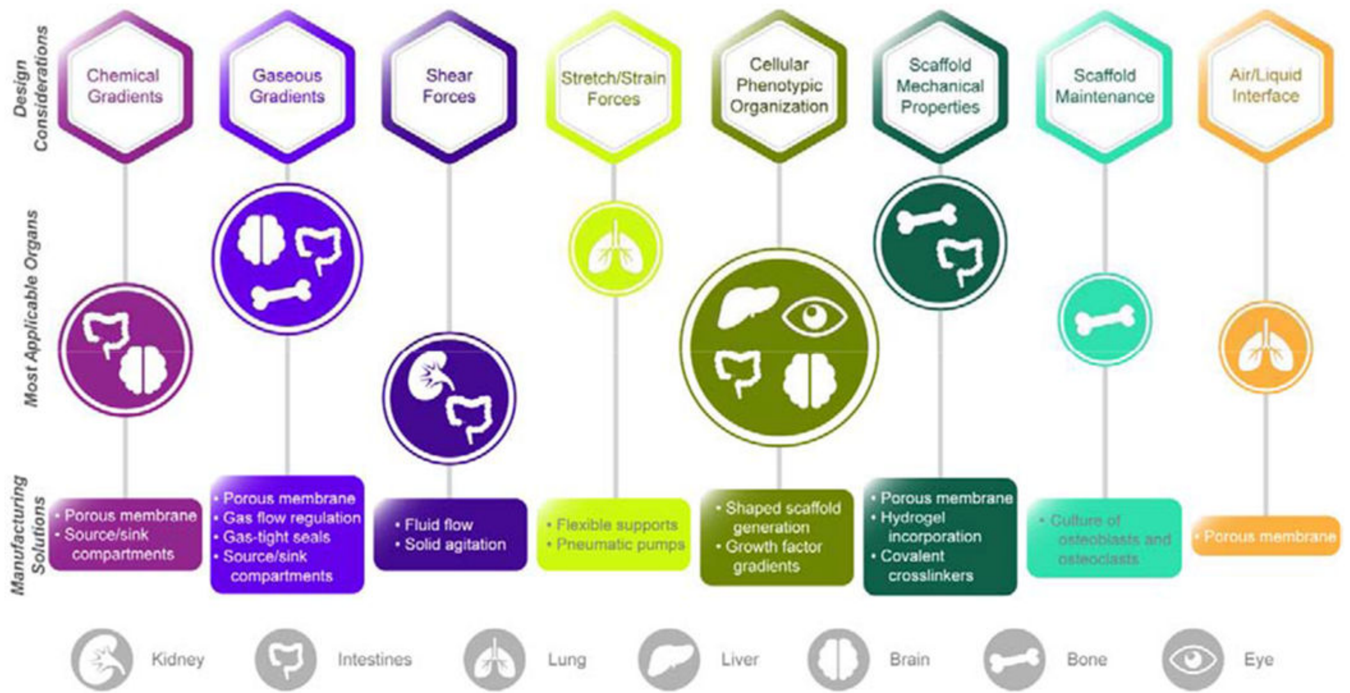

Figure 3.

Design considerations and commonly utilized solutions for the manufacturing of microphysiological systems, with representative organs pertinent to each category highlighted in the central row.

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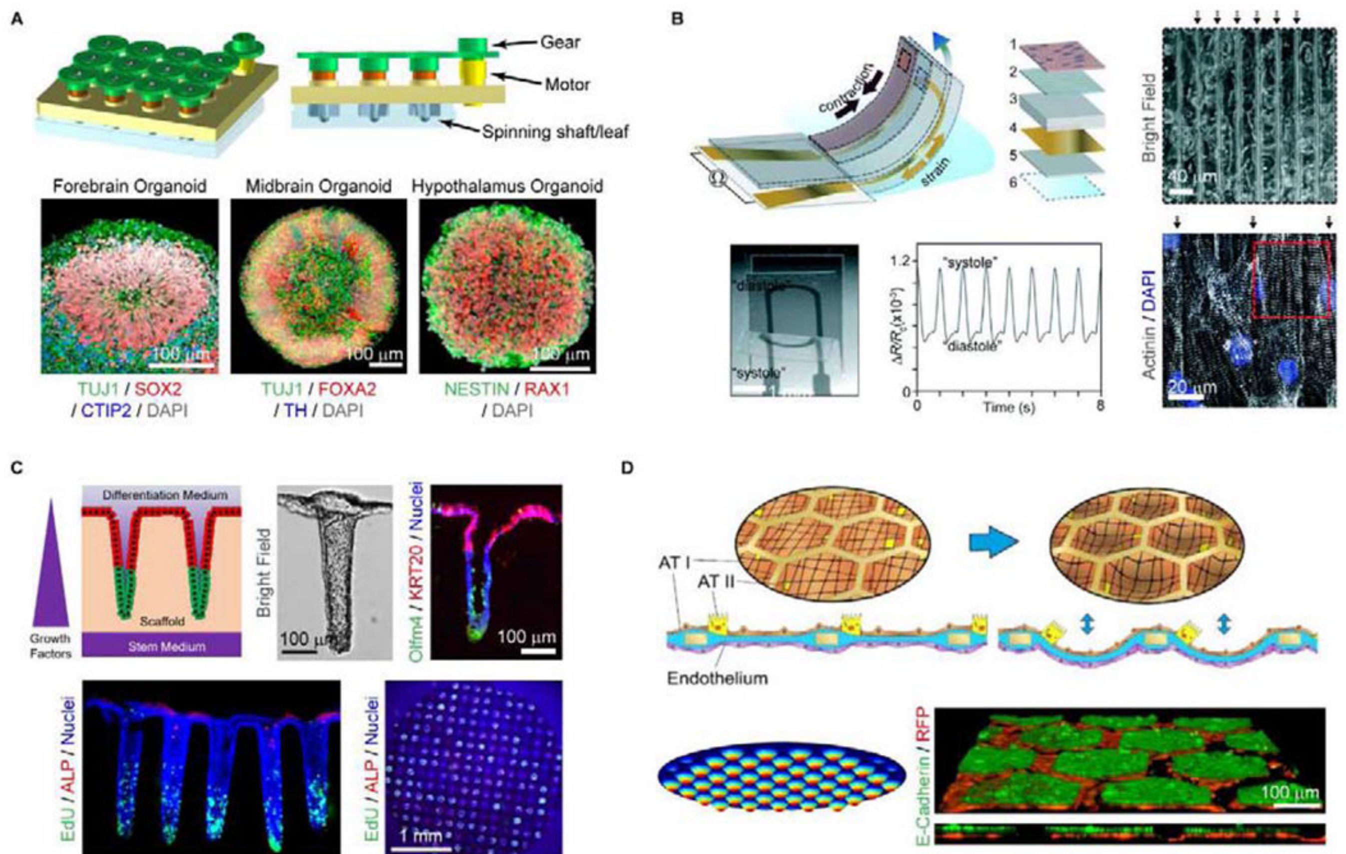

Figure 4.

Recent technologies pushing current limitations of scalability and throughput. A) Diagram of SpinΩ bioreactor used for the formation of brain organoids. Region-specific organoids grown within the bioreactor are displayed via confocal microscopy in lower panels. Reproduced from Ref. [27] with permission from Elsevier. B) Schematic of instrumented cardiac microphysiological device, capable of measuring cardiac stresses within cell incubator environments in real-time. Reproduced from Ref. [29] with permission from The Royal Society of Chemistry. C) Generation of *in vitro* human colonic crypt arrays by application of chemical gradients across shaped collagen hydrogels. Reproduced from Ref. [11] with permission from Elsevier. D) Formation of lung alveoli replicas over a collagen/ elastin membrane suspended within a gold mesh. The segregated membrane areas, with primary co-cultured lung epithelial (E-cadherin^{+}) and endothelial (RFP^{+}) cells, can be stretched and analyzed in parallel. Reproduced from P Zamprogno, et al., bioRxiv doi: 10.1011/608919, with permission from the corresponding author.