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Synergistic Engineering: Organoids Meet Organs-on-a-Chip

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Organoid technology and organ-on-a-chip engineering have emerged as two distinct approaches for stem cell-derived 3D tissue preparation. Their strategic integration can address each approach's limitations and provide a path toward a superior, synergistic strategy of constructing tissues that will truly deliver on the promise of regenerative and precision medicine.

Recent advances in human pluripotent stem cell (hPSC) or tissue-resident adult stem cell (AdSC) biology have facilitated high-fidelity modeling of essentially any tissue in the human body. Three-dimensional (3D) cellular models offer greater predictivity of gene and protein expression, metabolic function, and physiological and functional readouts than standard two-dimensional (2D) cell culture models. Patient-derived stem cells cultured as 3D tissue models of human disease have the promise to revolutionize drug discovery and safety testing by uncovering disease mechanisms that will ultimately lead to effective cures. However, achieving high-fidelity 3D stem cell-derived tissues is still a major outstanding challenge. Two distinct approaches have emerged over the last several years: organoid technology, spearheaded largely by stem cell biologists, and organ-on-a-chip engineering, led mainly by bioengineers (Figure 1). The two fields use distinct techniques to achieve the same goal of high-fidelity 3D tissue generation.

Organoid Design Principles

Self-organization of differentiating cells has enabled the development of various epithelial and epithelial-mesenchymal organoids from hPSCs such as optic cup, endocrine tissues, gut, liver, pancreas, brain, kidney, and lung and retinal organoids, among many others (Lancaster and Knoblich, 2014). The main advantage of an organoid is structural sophistication of multiple lineages with an architecture resembling that of the original tissue via self-organization, albeit at a much smaller scale compared to the size of adult human organs (Figure 1). Most tissues generated in vitro possess fetal and relatively immature phenotypes; however, a series of studies have shown that upon transplantation of complex structures under specific circumstances, the tissues attain a certain level of maturity (Takebe et al., 2013). hAdSC-derived organoids have the advantages of establishing and maintaining a more mature phenotype compared with hPSC-derived organoids and have been used to generate robust, high-fidelity models of the digestive system, lung, mammary gland, prostate, and fallopian tissues, to name a few. However, as both organoid sources have their distinct advantages and disadvantages, this Forum will focus on organoids derived from hPSCs.

The conventional approach of guiding self-organization involves identifying a mechanism of "animal" organogenesis in vivo followed by deducing culture conditions for "human" organoid-genesis in vitro from hPSCs. Principles of multicellular self-organization involve three major distinct, but inter-dependent, processes: self-assembly (or sorting out), self-patterning, and self-morphogenesis (Sasai, 2013). Importantly, these processes are heavily dependent on the time, space, and context of the cells relative to each other and are, therefore, diachronic. Our knowledge of the self-organizing principles by which organ architecture develops through complex collective cell behavior is still limited. Despite the remarkable progress, it is still unclear how useful organoids will be for modeling complex human pathology toward future drug screening and precision medicine applications, as well as regenerative medicine. This is at least in part due to the stochastic nature of the self-assembly process, stabilized by endogenous intercellular interactions.

Organ-on-a-Chip Engineering Approaches

Engineering approaches enable a precise control of the geometry input and output flow conditions, nutrient supply and shear stress stimulation, and the local mechanical and electrical properties of the growing 3D tissues (Figures 1B, 1F, and 1J). Current organ-on-a-chip approaches mainly rely on combining pre-differentiated cells, often cell lines, in certain ratios to emulate the native tissue composition. This approach is based on basic engineering principles, in which a complex system is analyzed by breaking it into pieces, key functional features of the system are identified, design criteria are specified, and the simplified version of the system is synthetized according to the design criteria to fulfill the critical functions of the original system. The pioneering publication in the field created a lung-on-a-chip device using classical soft lithography and microfluidics to

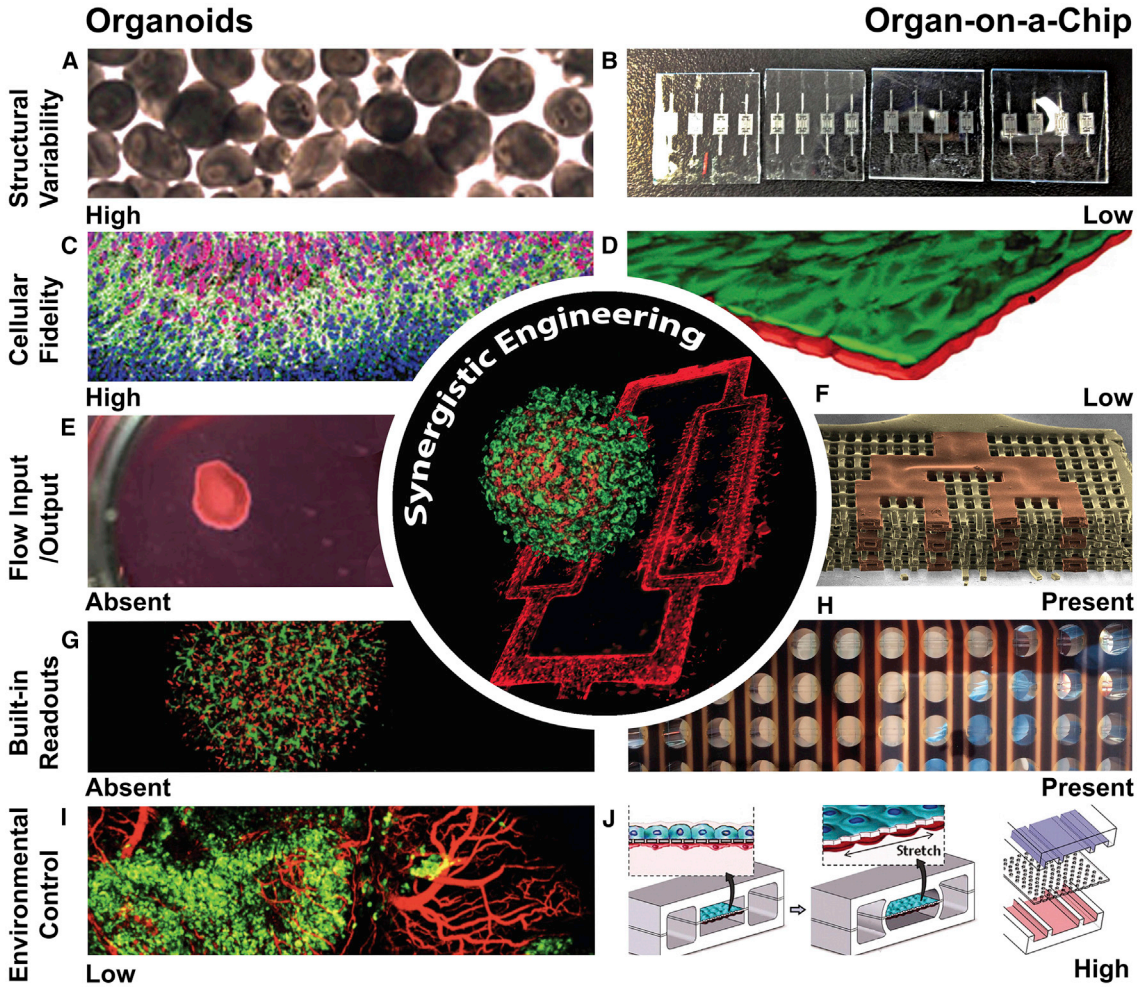

Figure 1. Synergistic Engineering to Overcome Challenges in Organoid Technology and Organ-on-a-Chip Engineering
(A) Liver organoids.
(B) AngioChip scaffold production (Zhang et al., 2016).
(C) Brain organoids (Lancaster et al., 2017).
(D) Lung-on-a-chip device (Huh et al., 2010).
(E) Liver organoid (Takebe et al., 2013).
(F) AngioChip structure (Zhang et al., 2016).
(G) Liver organoid (Takebe et al., 2013).
(H) Electrodes on a 96-well plate.
(I) Liver organoid (Takebe et al., 2013).
(J) Lung-on-a-chip device (Huh et al., 2010).
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reproduce a critical functional alveolar-capillary interface of the human lung (Huh et al., 2010) (Figure 1J). The polydimethylsiloxane (PDMS) device contained a flat, permeable membrane seeded with a human alveolar epithelial cell line and microvascular endothelial cells (Figure 1D). The channels at the top and bottom of the membrane provided precise input of culture media; application of vacuum to the side channels enabled mechanical stimulation, akin to breathing (Huh et al., 2010), enabling researchers to emulate pulmonary inflammation and infection (Figure 1J).

Since the publication of this first lung-on-a-chip paper in 2010, the field has rapidly expanded from 11 publications with the keyword Organ-on-a-Chip in 2010 to 540 publications in 2016. We can now broadly group these approaches into three distinct categories: (1) modeling barrier function within each organ (where lung-on-a-chip work belongs), (2) modeling functionality of the parenchymal tissue, and (3) modeling the systematic interaction between various tissues and organs of the body (i.e., body-on-a-chip).

The barrier functions of gut, kidney proximal tubule, and small airway have all been reproduced using the device originally developed for lung-on-a-chip application (Huh et al., 2010), and the blood-brain barrier was reproduced in modified devices. In the parenchymal-tissue-on-a-chip category, a major focus has been on reproducing the key functional properties of the liver (metabolism and drug conversion) and cardiac tissue

(Ca^{2+} handling and contractile properties) (Nunes et al., 2013; Zhang et al., 2016), since these are most often affected by drug toxicities. The presence of a stromal cell type (e.g., fibroblast) together with a main cell type (e.g., cardiomyocyte or hepatocyte) in 3D culture is commonly required in order to facilitate matrix remodeling and tissue formation (Zhang et al., 2016). However, in terms of cellular heterogeneity, phenotype fidelity, and physiologically relevant complexity, organ-on-a-chip devices remain inferior to organoids (Figure 1).

Organ-on-a-chip technology also offers the promise to strategically integrate multiple organ or tissue compartments to simulate the human body and make predictions about the pharmacokinetics of new drugs, as recently demonstrated with a pumpless 14-compartment body-on-a-chip system (Miller and Shuler, 2016). The human body absorbs, distributes, metabolizes, and eliminates (known as ADME) drugs across multiple organs. Mimicking this physiological complexity takes more than simply linking various cellular environments via microfluidic lines. Proportional scaling of each organ model is required to accurately reflect the actual physiological relationships between them. Allometric scaling (according to organ mass and surface area), functional scaling (according to organ-specific physiological functions and outputs), and methods based on organ volume and blood flow residence time are the three common approaches. However, every approach has specific focus and limitations, making it difficult to capture the multi-dimensionality of the human body. For instance, the functional activity of some organs (kidney and lung) correlates to tissue surface while others (liver and heart) correlate more strongly to tissue mass.

Synergistic Engineering

We propose that a superior approach to high-fidelity human organ modeling from stem cells will arise by finding the right balance of techniques at the intersection of organoid and organ-on-a-chip approaches (Figure 1). The synergistic engineering model relies on some degree of stochasticity, highlighting the need for investigation of precise self-organizing mechanisms combined with recently evolving technical advancements in bioengineering.

Enhancing Fidelity and Reproducibility

Despite the enormous potential of organoids as in vitro model systems, recent single-cell transcriptional profiling highlighted challenges for improving the variability and fidelity in the generation of multicellular components that appear more sporadically. In organ-on-a-chip engineering, reduced variability comes at the expense of fidelity. Cellular composition in organ-on-a-chip devices is oversimplified, as most contain only a couple of cell types from the main tissue and the cell lines often have limited physiological relevance (Figure 1). Organ-on-a-chip devices frequently have limited structural resemblance at the sub-tissue level, which could be complemented through self-organization of organoids. Notably, overcoming variability and increasing fidelity in 3D cell culture relates partly to niche factors. Specifically, deductive optimization of key niche factors, including the geometric control of cell-cell and cell-ECM contact, nutrient supply, and local biophysical and electrical stimulation, may greatly minimize batch effects and offer a point of control in 3D cell culture. Ideally, directed cell differentiation and organization can then occur within the tailored organ-on-a-chip environment (Figure 1). For example, common epithelial organoids such as rectal organoids comprise cell spheroids with hollow lumens, whereas a true rectum is a tubular structure. Through synergistic engineering, hollow tubular rectal organoids may be created in the future. A recent example illustrating a synergy between organoids and bioengineering includes the use of a (lactide-co-glycolide) copolymer (PLGA) microfiber to guide generation of elongated embryoid bodies for production of cerebral organoids (Lancaster et al., 2017).

Addressing Fetal and Relatively Immature Phenotype

Organoids still exhibit immature characteristics and typically lack important neighboring components, highlighting the need for further synergistic efforts to improve the phenotypic fidelity with normal organs. Maturity levels of differentiating cell cultures can be somewhat improved simply by longer cultivation times, sometimes reaching up to 6 months, which make using differentiated cells in drug screening and personalized medicine impractical. Unfortunately, organ-on-a-chip devices also do not achieve fully mature adult phenotypes, although maturity levels have been enhanced through electrical (Nunes et al., 2013) or mechanical stimulation (Huh et al., 2010). In this particular case, the field is only likely to move forward if organoid technology is combined with a bioengineering approach, as neither field alone seems capable of overcoming this fundamental limitation. This new synergistic environment for directed cell differentiation may comprise a scaffold with optimal matrix composition, geometry, and electromechanical properties.

Increasing Spatiotemporal Control of 3D Tissue Generation

Current organoids rely heavily on a high level of default robustness for the generation of a precisely organized tissue architecture. During spontaneous differentiation, organoids of various shapes and sizes are created (Figure 1A). In contrast, organ-on-a-chip approaches place predifferentiated cells at precise locations, often in an artificial manner (Figures 1D and 1F). Toward advanced 3D tissues and organs, it is essential to introduce more complex interactions and attain spatiotemporal (4D) control under defined environments at cellular or tissue levels. At the cellular scale, a recently developed organoid self-condensation method, wherein mesenchymal cells orchestrate larger (>millimeter) 3D condensate formation prior to 4D self-organization, will be useful for the integration of multiple progenitors, including vascular and immune lineages (Takebe et al., 2015). Moreover, developmental organ-organ boundary programs are critical in organogenesis and will be further improved by more precise spatiotemporal control at the tissue-tissue level compared with what can be achieved by standard bioengineering methods such as microfabrication. Additional enhancements require further niche engineering by defining optimal biochemical factors, matrix composition, mechanics, and medium and gas conditions, which can be achieved through a bioengineering approach. Thus, given the major challenges related to functional access to the surrounding organs (liver-biliary, kidney-ureter, lung-airway, CNS-PNS, etc.), building inter-tissue or inter-organ communications into current systems is needed to address the issues of

reconstructing organ-organ boundaries, and this might be achieved using techniques such as AngioChip, which enables inter-organ communication via a common vasculature (Zhang et al., 2016) (Figure 1F).

Higher-Throughput Readouts

Organ-on-a-chip systems are amendable to higher-throughput multiplexed sensing systems through microscopy, microfluorimetry, mechanical measurements, multiple electrode arrays, and other analytical systems, yet are subject to cellular fidelity challenges (Figure 1). Organoid systems that achieve the higher-order function of 3D tissues closely resembling in vivo organ architecture generally lack a screenable readout. Recently, researchers analyzed photosensitive cells in brain organoids by incorporating a high-density silicon microelectrode sensing system, illustrating the benefits of combining high-throughput readout sensors with organoids to further investigate complex cellular interactions (Quadrato et al., 2017). Integrated approaches will thus facilitate the development of phenotypic screening platforms for drug discovery and development applications with automated functional readouts.

Enabling Human Organoid Trial in a Dish

Future synergistic engineering approaches will demonstrate the value of human organoid trial (HoT) in a dish, a strategy of using in vitro-derived human organoids, with facile interrogation capability, to elucidate personalized pathology mechanisms and understand drug reactions underlying individual variations in humans. To realize the full potential of HoT, several obstacles need to be overcome: specifically, the length of cultivation time and oversensitive drug responses. In many cases, it takes months to build 3D tissues starting from stem cells or primary cell samples (e.g., tumor biopsies). The time accounts for cell expansion, differentiation, and 3D tissue assembly. If the tissues are required to inform clinical decisions, reduction in cultivation time is required, likely through appropriate niche engineering and biophysical stimulation. Extensive screening is also needed to prove that 3D tissues, both organoids and organs-on-a-chip, provide proper information about physiologically relevant drug doses. Effective in vivo doses often differ from those determined in vitro due to the issues related to drug distribution as well as auto-protective effects of 3D cell communities that are not observed in 2D culture. In principle, the organ-on-a-chip approach enables precise control of drug input and distribution to the tissue (Figures 1B and 1F), which, coupled with the cellular fidelity of organoids (Figure 1C) in a synergistic engineering strategy, holds promise to both shorten the cultivation time and achieve appropriate dose responses.

Scalability

A minimum therapeutic threshold of 10^8–10^9 cells is needed for replacement in most vital organs such as the liver and heart, although fewer cells are needed in some organs such as the brain, which requires $\sim 10^3$ cells. Producing organoids at the scale needed for therapeutic application is currently difficult as large-scale expansion of undifferentiated cells is first necessary, followed by scalable generation of 3D tissues. For transplantation of multi-cellular, functional structures, organ scale will likely need to be increased. Currently, most organoids are at the micrometer to millimeter scale, whereas human organs are at the centimeter scale, with defined vascular inputs and outputs for blood flow and branching vasculature. The field of organoids alone seems unlikely to overcome this issue. Bioengineers have worked for over a decade to overcome the issue of tissue and organ scale, and although the problem is not fully solved, recent advances pave the way toward successful generation of millimeter to centimeter scale tissues and organs (Figure 1F). In principle, increasing organ scale could be achieved by guiding stem cell differentiation and self-organization on microfluidic scaffolds with defined inputs, outputs, and branching vasculature (Zhang et al., 2016).

Conclusions

Delineating the precise context of developmental crosstalk at the cellular, tissue, and niche levels during organogenesis is critical to augment the potential of 3D tissues to model human phenotypes in a dish. Realistic drug screening applications require higher-throughput phenotypic readouts that are currently not available in organoid technologies, as well as high cellular fidelity that is currently lacking in organ-on-a-chip approaches. We envision that the convergence of the two approaches, through synergistic engineering, will thereby improve modeling and screening of complex human pathology, enable holistic mechanistic understanding of the dynamic nature of a self-developing system, and facilitate the discovery of effective treatments against irrecoverable diseases. Synergistic engineering will also enable us to overcome the issue of scale for both cell expansion and organ size that are required for therapeutic applications.

CONFLICTS OF INTEREST

M.R. and B.Z. are among the co-founders of TARA Biosystems, and they hold equity in this company. T.T. has served on scientific advisory boards for Healios.

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