Organs by design: can bioprinting meet self-organization?

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STRUCTURED ABSTRACT

Purpose of review: Engineering functional organs starting from stem or progenitor cells holds promise to address the urgent need for organ transplants. However, to date the development of complex organ structures remains an open challenge.

Recent findings: Among multiple approaches to organ regeneration which are being investigated, two main directions can be identified, namely (i) the patterned deposition of cells to impose specific structures, using bioprinting technologies, and (ii) the spontaneous development of organoids, according to principles of self-organization. In this review, we shortly describe the advantages and limitations of these paradigms and we discuss how they can synergize their positive features to better control and robustly develop organs from stem cells, towards organogenesis by design.

Summary: The outlined possibilities to bring together tools and concepts of bioprinting and self-organization will be relevant not only to generate implantable organs, but also to dissect fundamental mechanisms of organogenesis and to test therapeutic strategies in modelled pathological settings

Keywords: Organ engineering, Regenerative medicine, Organogenesis, Tissue Engineering

INTRODUCTION

Generating functional organs from living cells is promising to develop drug testing platforms, clinical grafts for regenerative medicine, and to investigate fundamental mechanisms of organogenesis. However, how to design and instruct development of large-scale, complex organs remains unknown.

Bioprinting relies on the possibility of depositing living cells according to defined patterns in a layer-by-layer fashion, potentially using a hydrogel or its precursor as a delivery agent. This paves the way for the generation of complex tissue mimics or even whole organ structures. The technology has emerged as an alternative to cells randomly embedded into a hydrogel, or placed in the voids of a porous scaffold. Despite eye-catching simulations of bioprinted organ-like structures and proofs-of-principle promises, such strategy has not yet convinced the feasibility of establishing and maintaining function over a relevant space- and time-scale.

Complementary to externally imposing a structure by design, the intrinsic ability of stem cells to self-organize can also be harnessed, as occurs during development. Organogenesis relies on efficient strategies selected by evolution, in which multiple tissues synergistically and stereotypically compartmentalize, deform, induce each other and grow while generating their own sustaining features (*e.g.*, vasculature). Organogenesis is also adaptive as it mitigates the effects of space and time perturbance in order to achieve a degree of robustness. Small *organoids* can now be formed in a dish *via* self-organization. However, assembling and growing them into large, complex organs remains a challenge (1).

Here, we will shortly review the respective advantages and limitations of bioprinting and self-organization. Subsequently, we will then consider how to synergize their positive features to better control and robustly develop organs from stem cells, towards organogenesis by design.

ADVANTAGES & LIMITATIONS OF BIOPRINTING

Bioprinting can theoretically precisely control the deposition of materials and cells in three dimensions. However, its precision strongly depends on the shape fidelity of the printed biological structures. In 2013, the concept of the "biofabrication window" was introduced (2), that underscored this central problem. Fabrication of complex living structures with high resolution dictates narrow boundaries for the physical properties of the printed matter, *i.e.,* the "bioink", while at the same time, the printed structure should facilitate the performance of embedded cells. Thus, bioprinting imposes opposing requirements on the properties of bioinks, and only limited classes of materials (*i.e.,* those displaying strong shear thinning behaviour) were deemed suitable.

However, over the recent years, this window has been significantly expanded by introducing new hydrogel platforms, such as those based on nanocomposites (3), interpenetrating networks (4), self-healing materials (5), and multi-material blends (6). The recent development of new bioprinting technologies have also extended the available toolbox for processing of these materials. Bioprinting involving the use of support baths (7), *in situ* crosslinking strategies (8), coaxial nozzles (9) or microfluidics (10), are examples of such novel approaches that go beyond inkjet printing and robotic dispensing and now also allow for the three-dimensional patterning of soft, cell-friendly hydrogel-based materials. Moreover, a trend towards the convergence of these bioprinting technologies and other high-precision additive manufacturing technologies, such as melt electrowriting, in a single fabrication process can be observed (11).

Bioprinting can thus introduce complex hierarchy and organisation within a 3D living construct. This includes layered patterns, as well as (pre-)vascular structures that can be decorated with peri-vascular cells and perfused with nutrient-rich medium to sustain the viability of larger structures that would not be able to survive based on diffusion alone. The process of bioprinting can, however, be time consuming, which may impact on the viability of the cells. Moreover, the ability to generate eye-catching simulations of organ-like structures does not mean that bioprinting will immediately yield a fully functional tissue- or organ-unit. The maturation phase of post-printing remodeling is a crucial step of the entire biofabrication process to favor the recapitulation of naturally and non-naturally occurring phenomenon of organ development (12). This tightly orchestrated process does rely on the self-organizing capacity of the embedded (and potentially endogenous) cells, and the specific cues required to steer this developmental process for each of the specific components of a tissue remain often unclear.

ADVANTAGES & LIMITATIONS OF SELF-ORGANIZATION

In the *conceptus*, organs form from pools of stem cells that set apart, in specific spatio-temporal patterns, supporting progenitors and mature cells. Altogether, these structures form compartmented tissues whose interactions and developmental histories fuel the progression of organ progenitors of increasing complexity. Stem cells can unleash a genetically-encoded program in a dish, under specific physico-chemical conditions, to form multicellular structures mimicking some features of organs (13). This revealed a previously underestimated potential for

stem cells to develop *in vitro*. Such *organoids* can be produced in large numbers and mimic various organs (*e.g.*, liver (14), brain (15), bone (16) and stomach (17)). Taking into account the modular nature of organs functional units, *organoids* are considered both as building blocks and embryonic seeds for organ engineering.

Currently, provided that adequate stem cells are available, the practical challenge in forming *organoids* is to find the initial conditions that are sufficient to unleash their intrinsic program. This involves recreating microenvironments using signaling molecules that are usually inspired by developmental trajectories, sometimes combined with hydrogels with a specific rigidity and functional moieties (18), and microsystems to precisely pool and confine cell types (19–21). Combinatorial statistics of these initial conditions proved valuable to systematically explore the landscape of parameters (22,23). Overall, this approach aims to develop effective, chemically-defined culture conditions that coax stem cells to recapitulate spatio-temporal windows of development up to the millimeter-scale. For example, *gastruloids* are millimeter-scale aggregates of embryonic stem cells that elongate and form spatially arranged *primordia* (organ precursors) for mesodermal, endodermal, and spinal cord derivatives (24).

Control over the initial conditions (*e.g.*, initial transcriptomic and epigenetic states of the stem cells, size and geometry of multicellular aggregates, mechanical and biochemical microenvironments) is, however, often loose, leading to a lack of reproducibility (25). Also, *organoids* formed from pluripotent stem cells reflect a fetal tissue rather than an adult one, due to a lack of knowledge on how to accelerate maturation and aging in a dish (25). In addition to these challenges, current approaches are insufficient to create structures with the size and complexity of organs. This is because organs grow within the dynamic, interactive environment of an embryo that, beyond the intrinsic genetic program, also embroils the synergistic growth of multiple, sometimes transient, tissues, and the co-development of sustaining features (*e.g.*, immune, hormonal, vascular, and nervous systems (26–28)). For example, the pituitary gland forms *via* the synergistic interactions of two layered tissues, the neuroectoderm and oral ectoderm, that progressively fold, deform and grow into the final organ. Only parts of these processes can be recapitulated in a dish using current approaches (29,30). The complex and dynamic context necessary to further support organogenesis might be engineered *in vitro* by spatio-temporal delivery of soluble factors (*e.g.*, using microfluidics (31,32)) or dynamic changes of the properties of the hydrogel (33). However, here we argue that these approaches, although necessary, will not be sufficient to form large-scale, complex organs in a dish.

PERSPECTIVE FOR COMBINATION OF STRATEGIES

From the short above descriptions of bioprinting and self-organization, it is clear that the two approaches are conceptually based on different principles and methodologically relying on different sets of tools. Despite that, we here propose how they could be synergistically combined to advance knowledge and practical solutions towards proper organ engineering. Organs result from a developmental history. Their formation from stem cells necessitates the progressive interaction, remodeling, and growth of progenitor structures rather than the end-point combination of separately-generated differentiated cell types. Some of these progenitor structures can be purely supportive and act transiently (*e.g.,* similar to the placenta), while some others are seeds

that symbiotically grow, morph and merge into the final organ. Such symbiotic culture of multiple *organoids* mimicking regional progenitors already proved useful to trigger large-scale, complex developmental processes *in a dish*, especially to model the brain (34–36).

The size and the geometry of the developing tissues are of crucial importance. They create the internal gradients of morphogens, mechanical forces, local densities and deformations that constrain developmental processes. Such sizes and geometries previously proved to be sufficient to generate internal patterns of cellular behaviors in three-dimensional microfabricated tissues (37–41). While current *organoids* mostly start from a spherical shape of loosely-defined dimensions, fabrication techniques including bioprinting should help to impose specific geometric designs and sizes in order to break the symmetry of the tissue and impose conditions prone to further self-patterning (Fig 1A). One of the challenges is to determine which initial priming conditions are sufficient for tissue progenitors to predictably grow and morph into organs. Here, the versatility of biofabrication methods should prove useful to systematically screen and explore the design principles triggering predictable behaviors. Technologies (42) including bioprinting could spatially pattern pools of cells within hydrogel volumes and according to numerous designs, to systematically explore the rules triggering subsequent interactions and remodeling by self-organization of the preformed *organoids*.

Through a bottom-up, self-organization approach (43), it is also possible to generate a large number of small and complementary organoid units from progenitor cells (Fig. 1B). Bioprinting, along with other technologies (42) would be a very valuable tool to pattern these units over large scales. One could envision for example, the patterned deposition of numerous organoids to form one progenitor organ, in spatio-temporal configurations prone to trigger subsequent development. As an example, tissue progenitors could be assembled that mimic aspects of the embryonic neuroectoderm and oral ectoderm from which the pituitary gland originates. These initial conditions mimicking aspects of a specific embryonic stage or less natural configurations should aim at triggering the development of the final functional organs (*e.g.,* a pituitary gland). This approach would thus not target to set the definitive structure of the organ, but rather to impose high-order starting conditions amenable to predictable remodeling over large scales.

Currently, engineered tissues are often constrained and unable to naturally contract, deform or grow in order to develop their own architecture. In contrast, natural organ progenitors often undergo extensive three-dimensional remodeling and are only partially appended. As such, exploiting the intrinsic potential of stem cells to recapitulate organogenesis implies the formation of large-scale constructs partly free-standing, meaning only partially attached to a surface. Two microfabrication approaches previously showed that patterning aggregates of cells into geometric wells (43) or with specific designs within a hydrogel (44) is sufficient to induce predictable and complex self-deformations over large scales. Bioprinting of cells or *organoids* into free-standing structures or within loosely appended hydrogel volumes will contribute to unleash the potential for tissue progenitors to undergo large-scale, programmable, three-dimensional remodeling.

The initial configuration will be specific to the intended final structure. However, organogenesis tend to re-iterate and recycle a limited number of designs in different combinations (*e.g.,* the curling and the invagination of an epithelial sheet). It should thus be possible to define general rules promoting the development of progenitors into functional large-scale structures. These rules will be partially inspired by phenomenon naturally occurring during human embryogenesis and homeostasis, but might also explore roads that are not currently favored by natural selection.

CONCLUSION

Generating large-scale, complex organs *in vitro* remains undone. To this end, technological innovations are necessary, such as those in the field of bioprinting, but these should focus on unleashing the intrinsic potential of stem cells to undergo development in a dish. Here, we argue that a new approach based on the latest technologies and inspired by natural organogenesis, including the effects of, for example, tissue size, geometry, deformations, and molecular and mechanical inductions may lead to manufactured tissue progenitors prone to develop into large-scale, complex organs including sustaining features (*e.g.*, immune, hormonal, vascular, and nervous systems).

This approach will unravel design principles of development that remain currently inaccessible due to the intertwined processes of organogenesis within an integral embryo. It could pinpoint the levels of autonomy (*e.g.*, genetically encoded) and integration (*e.g.*, molecular induction) of developmental processes, and scaling influence. Such fundamental knowledge will lay down the basic rules needed to form and regenerate organs by design. In the long term, the resulting organs may effectively recapitulate complex natural functions, but may also include designed features including non-natural capabilities for spontaneous regeneration.

KEY POINTS

1. The technological tools and biological principles of bioprinting and self-organization could be synergistically combined to advance knowledge and practical solutions towards organogenesis by design.
2. Specific combinations of progenitor cells can be bioprinted into organoids and allowed to self-organize, leading to organ progenitors with the potential to recapitulate full organ development.
3. Alternatively, self-organization of progenitor cell suspensions can lead to structures of intermediate complexity, which can then be bioprinted into organ progenitors to instruct the complex processes of organogenesis.

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Figure Legend

Figure 1. The potential combinations of bioprinting and self-organization. **(A)** Specific combinations of progenitor cells can be bioprinted into organoids of defined sizes and geometries according to patterns designed to biologically prime their development. Bringing together these possibly different structures and allowing them to interact and self-organize would lead to organ progenitors with the potential to recapitulate full organ development. **(B)** Progenitor cell suspensions can be allowed to self-organize into millimeter-scale structures, possibly in different compositions and properties depending on the combinations and proportions of starting cells. The resulting structures of intermediate complexity would then be bioprinted into organ progenitors, with geometries and patterns designed to instruct the complex, large-scale processes of organogenesis (Drawings by Dr. M. Filippi)