CellPress

Cell Stem Cell
Review

Engineering Stem Cell Self-organization to Build Better Organoids

Jonathan A. Brassard[1] and Matthias P. Lutolf[1,2,*]
[1]Laboratory of Stem Cell Bioengineering, Institute of Bioengineering, School of Life Sciences (SV) and School of Engineering (STI), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[2]Institute of Chemical Sciences and Engineering, School of Basic Science (SB), EPFL, Lausanne, Switzerland
*Correspondence: matthias.lutolf@epfl.ch
https://doi.org/10.1016/j.stem.2019.05.005

Organoids form through self-organization processes in which initially homogeneous populations of stem cells spontaneously break symmetry and undergo *in-vivo*-like pattern formation and morphogenesis, though the processes controlling this are poorly characterized. While these *in vitro* self-organized tissues far exceed the microscopic and functional complexity obtained by current tissue engineering technologies, they are non-physiological in shape and size and have limited function and lifespan. Here, we discuss how engineering efforts for guiding stem-cell-based development at multiple stages can form the basis for the assembly of highly complex and rationally designed self-organizing multicellular systems with increased robustness and physiological relevance.

Tightly regulated cellular self-organization programs orchestrate dynamic interactions between cells and their environments, ensuring the robustness of tissue and organ development, homeostasis, and regeneration in multicellular organisms. Because self-organization is an emergent property of an integrated multicellular system, complex patterning events cannot be simply explained by causal links between genes and the phenotypes at the level of single cells but rather must be understood at the population level through sequential iterations of local interactions between individual cells (or subsets of cells) and their environment. As such, self-organization depends on the intrinsic capacity of cells to sense, integrate, and respond to various systemic and local cues, such as morphogen gradients (Sagner and Briscoe, 2017), mechanical boundary conditions (Anlaş and Nelson, 2018), or cellular proliferation and environmental remodeling (Haupt and Minc, 2018). Therefore, self-organization is fueled by evolving interactions at the microscale that collectively result in changes at the macroscale. This implies that by providing an optimal and permissive environment and by adding key spatiotemporal cues to drive multicellular responses, it should also be possible to harness the cell's self-organization potential outside of an organism, i.e., *in vitro*.

Indeed, when cultured in a three-dimensional (3D) environment that provides permissive growth conditions but no external patterning blueprint, initially uniform groups of cells can display emergent behaviors akin to aspects of morphogenesis and organogenesis. In marked contrast to 2D mammalian cell cultures that normally acquire a flattened morphology, cells in 3D environments form more physiologically relevant multicellular structures. For example, mammary epithelial cell aggregates spontaneously self-organize into lumenized spherical structures, which can generate lactating mammary acini (Barcellos-Hoff et al., 1989) and even undergo branching morphogenesis (Nelson et al., 2006). 3D aggregates of salivary epithelial cells can likewise engage in budding and clefting processes, similar to those driving salivary gland development (Wei et al., 2007), and epithelial cysts constructed of canine kidney cells undergo tubulogenesis when uniformly exposed to hepatocyte growth factor (O'Brien et al., 2002).

The unique property of stem cells that allows them to both self-renew and differentiate into cell types from multiple lineages adds a layer of complexity to their behavior that has been harnessed to emulate features of organogenesis in cell culture. Seminal studies from the laboratories of Yoshiki Sasai (Eiraku et al., 2011) and Hans Clevers (Sato et al., 2009) revealed that, aside from directing stem cells to commit toward cell types of various lineages, these cells and their progeny seem to follow their innate developmental programs and self-organize into "organoids"—structures that mimic multiple histological and functional aspects of real tissues and organs, including preserving niches containing self-renewing stem cells. For instance, Sasai and colleagues recapitulated aspects of eye development *in vitro*, showing that mouse and human pluripotent stem cells (PSCs) can self-organize into a bilayered optic-cup-like structure when cultured in 3D (Eiraku et al., 2011; Nakano et al., 2012). Since the pioneering work of Sasai and colleagues, researchers have developed protocols for transforming aggregates of PSCs into organoid versions of multiple organs, including the brain, intestine, stomach, lung, liver, and kidney (Clevers, 2016; Lancaster and Knoblich, 2014; McCauley and Wells, 2017; Rossi et al., 2018).

Perhaps the archetypal organoid system has been described by Sato, Clevers, and colleagues. They demonstrated that adult $Lgr5^+$ intestinal stem cells (ISCs) embedded in 3D Matrigel and provided with uniform niche signals, including R-spondin1, Noggin, and EGF, not only survive and proliferate to produce ISC colonies, but subsequently undergo morphogenesis to form structures that approximate the adult small intestinal mucosa: crypt-like projections that radiate outward from a spherical epithelial structure surrounding a central lumen (Sato et al., 2009, 2011a). Importantly, these structures reconstitute the principal geometrical, architectural, and cellular hallmarks of the native epithelium. Notably, around the same time, Kuo and colleagues reported an air-liquid interface approach for the long-term 3D

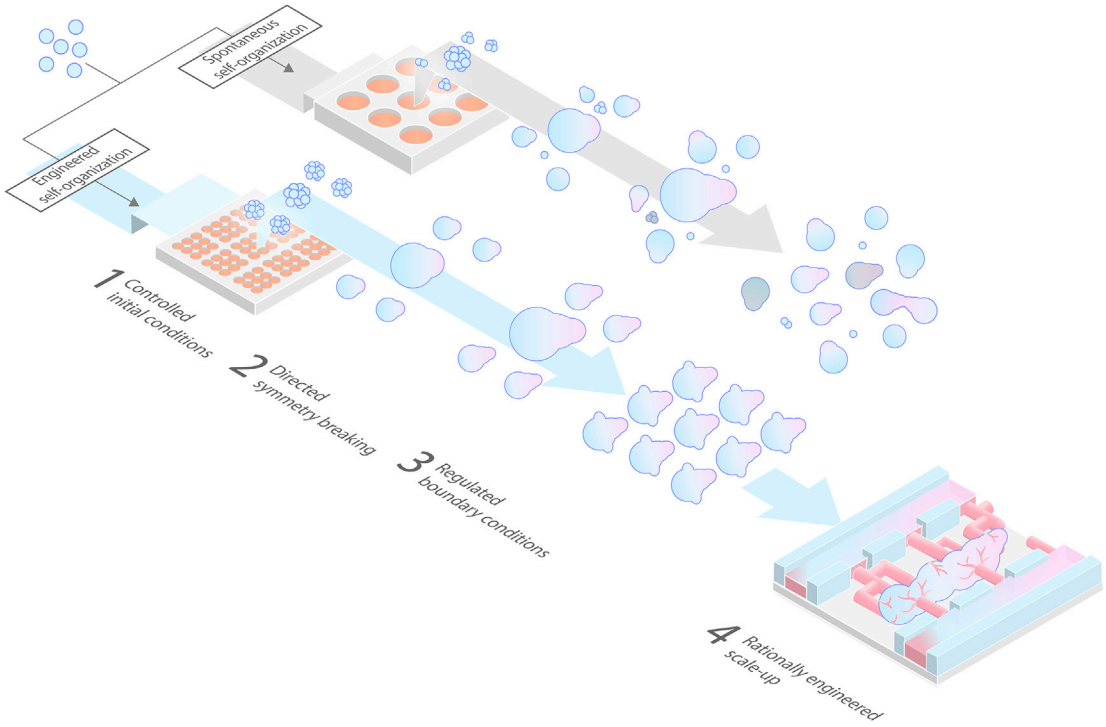

Figure 1. Engineering Self-Organization
Generating more complex multicellular systems will require a delicate balance between spontaneous self-organization and its precise modulation that could be accomplished by engineering the different stages of *in vitro* organogenesis. Detailed guidance over each level of development, from initial conditions to stem cell patterning, should provide the foundation for self-organized tissues that are highly functional and relevant in size and lifespan.

culture of intestinal organoids, comprising myofibroblasts that provided key niche signals (Ootani et al., 2009). Intestinal organoids, like other gastrointestinal organoids such as those derived from the colon (Jung et al., 2011; Sato et al., 2011b) or stomach (Barker et al., 2010), are powerful models for the study of basic adult stem cell (ASC) biology, tissue homeostasis and regeneration, and patient-specific diseases. These organoids could also serve as sources of tissue for autologous transplants.

The self-organizing processes that build organoids—requiring only minimal external cues and spatially homogeneous cocktails of growth factors—can generate impressive levels of tissue organization and functionality that cannot be matched by classical tissue engineering approaches relying primarily on a scaffold. At the same time, their full reliance on cell-autonomous self-organization lacking in pre-defined extrinsic patterning instructions may be their main weakness. This invariably introduces stochasticity in organoid formation and leads to heterogeneities in size, shape, and cell-type composition. Moreover, current organoid culture technology limits their size to the millimeter scale, precluding their widespread adoption for a wealth of applications, such as regenerative medicine.

In this review, we argue that self-organization can be engineered by spatiotemporally controlling cell-cell and cell-extracellular matrix (ECM) interactions and that this approach should allow researchers to push the limits of existing *in vitro* organogenesis. To bio-fabricate more functional and size-relevant tissues, the main challenges relate to controlling and scaling-up stem cell self-organization. We propose that this could be achieved by focusing on four major stages during the *in vitro* developmental process that are summarized in Figure 1: (1) controlling initial culture conditions, (2) directing symmetry breaking, (3) imposing boundary conditions to guide cellular self-patterning, and (4) rationally engineered scale-up.

Controlling Initial Culture Conditions

The inherent self-organization capacity of stem cells does not mean that elaborate tissues can be formed in any condition. Rather, it emphasizes the importance of the environment in steering the cellular development in a highly context-dependent manner. Indeed, what is generally referred to as self-organization in organoids may already be considered "directed" self-organization as, for example, mouse embryonic stem cell (ESC) aggregates can be cultured to form both optic cups (Eiraku et al., 2011) or gastruloids (Beccari et al., 2018) depending on the physicochemical cues provided. Small variations in the initial conditions are bound to have dramatic influences on the final patterning and morphogenesis outcome, since organogenesis and organoid formation are characteristically non-linear deterministic systems (Dahl-Jensen and Grapin-Botton, 2017). Consequently, controlling the initial conditions to improve organoid reproducibility and designing *in vitro* developmental trajectories toward desired patterning outcomes are of paramount importance. Indeed, because self-organization is iterative and cumulative, small deviations from the initial optimal condition can steer development away from a desired emergent behavior. This feature has been termed "stigmergy," which refers to an outcome of a

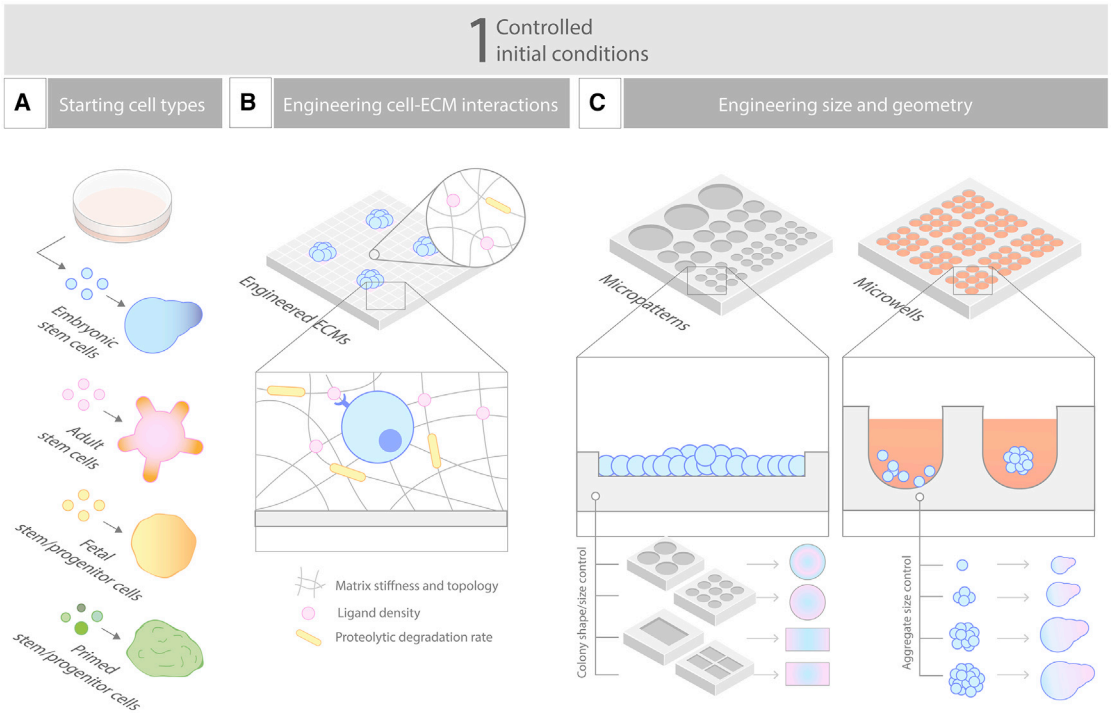

Figure 2. Controlling Initial Conditions

(A–C) Carefully designed starting conditions are critical for robust organoid formation, and these include (A) choosing the nature of the cells and their self-organization potential, (B) controlling their aggregation to a defined size and shape, and (C) engineering their environment to empower their ability to self-organize.

"stimulation" that is dependent on the history of previous "stimulations" (Sasai, 2013a). We believe this can be countered by identifying the optimal conditions for robust and elaborate self-organization by exploring the effect of the initial cell number and density (i.e., size of a stem cell aggregate), geometry, and microenvironment (Figure 2).

Cell Types and Cell States

The self-organization potential of a stem cell depends on its origin and stage of development (Rossi et al., 2018), thus representing an obvious first important consideration for organoid-based tissue engineering (Figure 2A). PSC-derived organoids are obtained by mimicking the presumptive sequential signaling interactions operating during *in vivo* development, whereas those derived from ASCs are obtained by providing signaling cues that operate in the respective adult tissues. PSCs possess a broader potency that allows the coordinated generation of cells from multiple germ layers. One striking example is the development of PSC-derived intestinal (Spence et al., 2011), gastric (McCracken et al., 2014), or liver organoids (Dye et al., 2015) composed of an epithelial layer covered by mesenchymal cells that co-evolve during organoid formation. Indeed, exploiting tissue-tissue crosstalk during organoid development may be a powerful strategy for increasing the complexity of organoids. However, despite their exciting potential, PSC-derived organoids have yet to be predictably developed, as exemplified by the fact that ESC aggregates can produce a seemingly random number of vesicles during optic cup formation (Eiraku et al., 2011) and that gastric organoids are formed within a larger, uncontrolled tissue aggregate (Noguchi et al., 2015).

In contrast, the potency of ASCs has already been restricted and is linked to their tissue of origin, so guiding ASC-organoid formation generally does not require extensive sequential switching through different lineage-specifying culture conditions. ASC-based organoids more closely recapitulate the homeostatic conditions and regenerative processes of the corresponding tissues, with a microscopic architecture closer to that of adult tissue (Clevers, 2016). Unfortunately, this restricted potential compared to PSCs means that ASCs lack the necessary tissue-tissue interactions to promote organ-level complexity, such as in the formation of organ buds derived from reciprocal epithelial and mesenchymal interactions (Ikeda et al., 2019). As another example, vilification of the small intestine is believed to be initiated by mesenchymal clustering beneath the intestinal epithelium and subsequent modification of the mechanobiological environment around the epithelium (Wells and Spence, 2014). Consequently, increasing the tissue complexity of ASC-derived organoids will require positioning multiple cell types in an environment in which the tissues can interact to properly self-organize. Of note, organoids have also been derived from mouse embryonic pancreatic progenitors (Greggio et al., 2013) and mouse fetal intestinal progenitors (Fordham et al., 2013; Navis et al., 2019; Yui et al., 2018). In addition to the advantages and disadvantages seen in ASC-derived organoids, such fetal organoids can be employed for example as models of developmental maturation (Navis et al., 2019).

An in-between approach is to partially differentiate PSCs toward a specific progenitor cell fate before aggregating them with cells of other lineages (Takebe et al., 2013). Such "primed"

stem/progenitor cells have the advantage of being still proliferative with a high differentiation potential, while their partial commitment could prevent the development of non-desired tissue structures. However, one important aspect to keep in mind in such a setting is that this "primed" cell population may be highly heterogeneous, which could compromise the robustness and reproducibility of the approach. Indeed, since multicellular responses depend on previous culture conditions (Sasai, 2013a), it is particularly important to carefully characterize the starting cell population, making use of technologies such as single-cell RNA sequencing (RNA-seq) (Camp et al., 2018).

ECM Engineering

One of the principal requirements for organoid culture is an environment that both confers the key biophysical and biochemical input signals while remaining permissive to self-organization. Most organoids developed to date have relied on ECMs derived from animals, such as Matrigel or collagens. However, the poorly defined composition, heterogeneous nature, and batch-to-batch variability of such matrices hinder the establishment of robust processes. Alternative approaches have focused on engineering synthetic stem cell microenvironments that can mimic key features of natural ECMs (Figure 2B). Particularly, 3D screening approaches can be used to synthesize and test hydrogels of varying stiffnesses, degradabilities, and bioactivities for their influence on stem cell fate (Ranga et al., 2014). This methodology has also been applied to stem-cell-based morphogenesis, where the study of poly(ethylene glycol) (PEG)-based hydrogel formulations decoupled the effect of parameters such as stiffness and ECM composition on neuroepithelial cyst patterning (Meinhardt et al., 2014; Ranga et al., 2016). Synthetic or semi-synthetic matrices have also been used to grow pancreatic organoids (Greggio et al., 2013) as well as intestinal organoids derived from PSCs (Cruz-Acuña et al., 2017) and ASCs (Gjorevski et al., 2016). Of note, the fabrication of defined matrices derived from naturally occurring materials, such as alginate (Capeling et al., 2019), fibrin (Broguiere et al., 2018), or recombinantly engineered ECMs (DiMarco et al., 2015) is another promising strategy to improve the translational relevance of organoids.

The optimal conditions for the growth and patterning of mouse intestinal organoids were interrogated using synthetic matrices with a modular ligand composition, stiffness, and degradability and were found to vary, with an intermediate stiffness promoting efficient stem cell expansion and a lower stiffness promoting crypt formation (Gjorevski et al., 2016). Of note, the covalent cross-linking of currently used synthetic hydrogels is not permissive to the growth of larger (i.e., millimeter-sized) tissues and generally restricts those morphogenetic processes that require extensive matrix displacement or remodeling (Blondel and Lutolf, 2019). Intriguingly, fine-tuning the dynamical softening of PEG hydrogels in mouse intestinal organoids could satisfy the requirements for both initial optimal stem cell expansion and subsequent organoid patterning (Gjorevski et al., 2016), but this might limit long-term culture because of the irreversible nature of the chemical degradation process. We thus postulate that ideal synthetic environments for organoid culture and larger-scale tissue engineering, apart from fulfilling the key signaling functions, should prevent the accumulation of excessive compressive forces in response to tissue growth and morphogenesis. Hydrogels that have been partially or completely physically crosslinked more realistically capture the viscoelasticity and dynamics of native ECMs. They should thus be well suited to relax in response to tissue-induced compressive stresses by breaking and subsequently rearranging their network, permitting cellular remodeling without compromising the stability of the material over time (McKinnon et al., 2014). Although they have not yet been used in the context of organoid culture, such dynamic hydrogels have been shown to influence stem cell fate (Chaudhuri et al., 2016) and hold great promise as tunable environments for self-organization. It should be noted that culture media supplemented with "soluble" ECM such as Matrigel (generally on the order of 2%–5%) can also support organoid formation and complex morphogenesis (Eiraku et al., 2011; Koehler et al., 2017; Suga et al., 2011). This minimal environment is permissive to self-organization but might restrict the emergence of structures that are highly dependent on ECM mechanics.

Geometry and Cellular Density

Even in the presence of a homogeneous matrix and soluble factor distribution, each individual cell within a stem cell collective is subject to a unique mechano-chemical microenvironment defined by the integration of all the interactions with its neighbors, the nanoscopic features of the surrounding environment, and the gradient of mechanical forces that are progressively exerted by surrounding cells (Vianello and Lutolf, 2019). Combined with the fact that cells at the periphery of an aggregate can interact with the ECM, this suggests that a tissue can, through individual cells, sense its boundary conditions and pattern in response to them (Figure 2C, left panel). This is beautifully exemplified by a set of experiments in two dimensions where human ESC (hESC) colonies were confined to circular adhesive micropatterns (typically from 0.7 to 1 mm in diameter) and stimulated with bone morphogenetic protein 4 (BMP4) (Etoc et al., 2016; Warmflash et al., 2014). In these culture conditions, hESC monolayers underwent a striking self-patterning process that resulted in concentric rings composed of domains of cells from different germ layers, recapitulating salient aspects of *in vivo* gastrulation. Reducing the size of the micropatterns resulted in the disappearance of the central fate, presumably because the differentiation control was established by the boundary of the colony. Indeed, cells situated at the edge of the colony and in a lower density environment had lower receptor lateralization and were thus more sensitive to BMP ligands (Etoc et al., 2016). Although size contributes to defining precise phenotypes in some situations, it generally does not act alone, but in conjunction with the modulation of biochemical signaling. For example, in a similar model of patterned hPSCs, it was shown that colony patterning was closely dependent on morphogen concentration and induction time (Tewary et al., 2017). Indeed, specific combinations of concentration and induction time rescue gastrulation-like patterning for sizes that were not responsive to previous BMP4 concentration. This underscores the interdependence between size and biochemical signaling, which should be taken into consideration to control larger-scale morphogenesis.

Size and geometry sensing via the differential availability of receptors and ligands or mechanics-triggered signaling, i.e., through YAP/TAZ mechanotransduction (Brusatin et al., 2018), are bound to play an even more critical role in a 3D tissue. Indeed, several reports have shown that the cellular density

and size of initial aggregates play a role in differentiation and self-organization in several cases including, but not limited to, embryoid body (EB) (Choi et al., 2010), optic cup (Eiraku et al., 2011), gastruloid (van den Brink et al., 2014), as well as adenohypophysis (Suga et al., 2011) and cerebral (Lancaster et al., 2017) organoids. For the adenohypophysis organoids, increasing the size of the initial aggregates alone was sufficient to enhance rostral head ectodermal induction in a similar proportion as direct BMP4 treatment (Suga et al., 2011). In this setup, the inhibition of BMP signaling antagonizes the formation of rostral head ectoderm, and endogenous BMP ligand expression was increased in larger cell aggregates. This points to a regulation of BMP signals dependent on the cell population size, with only a specific size range supporting the induction of a rostral head ectodermal domain. A key parameter in controlling self-organization in many systems may be this ability of thick tissues to both form local gradients of endogenous signals and limit the diffusion of externally supplied factors to its center.

In addition to size, the specific geometry used for confining stem cell aggregates, which can be spherical, cylindrical, rectangular, or various other combinations, affects the physical boundary conditions. The geometry may also drastically alter the shape of the diffusible gradients and modulate local mechanical cues or juxtacrine signaling, in turn affecting stem cell differentiation and patterning (Blin et al., 2018; McMurtrey, 2017; Ruiz and Chen, 2008). Therefore, a systematic control over the 3D geometry of the cell population and the resulting shape of the gradients of diffusible molecules could improve self-organization to achieve more stereotypical and complex morphogenetic processes, such as the organogenesis processes that initiate from a tubular epithelial tissue.

In a study of guided self-organization, a lower surface-to-volume ratio of EBs was shown to hinder the neuroectodermal development of brain organoids (Lancaster et al., 2017). Decreasing the size of the initial aggregates resulted in lower non-neural tissue specificity and smaller neuroepithelial buds, but increased relative induction of neural characteristics. This was presumably because the higher surface-to-volume ratio promoted the development of neuroectoderm at the periphery of the EB. Of note, decreasing the aggregate size below a certain threshold prevented robust organoid formation (Lancaster et al., 2017), accentuating the importance of cellular density for the emergence of higher-level complexity. Moreover, controlling the aspect ratio of the initial EB using poly(lactic-co-glycolic acid) (PLGA) microfilaments as a minimal internal scaffold could significantly enhance neuroectoderm formation and robustness across different cell lines and batches (Lancaster et al., 2017).

These results stress the importance of employing engineering-inspired approaches to guide self-organization using a defined initial cellular density and aggregate geometry. Microwell-based cell culture substrates, generated for example via photolithography or micropatterning, offer a very straightforward approach for controlling cellular aggregation and organoid formation (Figure 2C, right panel). Microwells have been microfabricated using a large range of materials, including poly(dimethylsiloxane) (PDMS), PEG, or agarose hydrogels (Ahn et al., 2016), and have been used to control the size of aggregates in various cellular systems, such as EBs (Hwang et al., 2009; Vrij et al., 2016b), islet organoids (Candiello et al., 2018), salivary gland stem cell aggregates (Shin et al., 2018), and kidney organoids (Czerniecki et al., 2018). Microwells can also be used to decipher the optimal ratio of interacting cell types in co-culture, as was demonstrated for example with embryonic organoids termed "blastoids" that are formed by aggregating ESCs and trophoblast stem cells (Rivron et al., 2018). Circular microwells of different diameters, however, only allow control over the size of spontaneously forming spherical aggregates and prevent immediate embedding of the cells. This issue can, for example, be addressed using sacrificial molding technologies where microstructures can be created inside stiffer gelatin and then transferred to softer hydrogels such as fibrin or Matrigel (Cerchiari et al., 2015). Alternatively, control over the initial geometry of 3D tissues inside the ECM has been achieved by directly stamping micropatterns into collagen (Nelson et al., 2006). Mammary epithelial cells fully embedded in collagen formed tubules constrained by the shape of the collagen gel. Interestingly, emergent branching from the tubules could be predicted based on the geometry of the micropatterns, suggesting that mechanical and geometrical cues play a critical role in symmetry breaking and morphogenesis (Nelson et al., 2006). It could be interesting to apply this approach to stem-cell-based self-organization to elucidate the role of geometry and mechanics on cell-fate decision-making and organoid patterning.

Directing Symmetry Breaking

To develop into bigger and more elaborate tissues, early stem cell aggregates composed of a relatively homogeneous population have to evolve into a complex system with heterogeneous components and cell-type patterns. The transformative step in this process is the first moment when one or few cells break the symmetry of the system by "spontaneously" changing their fate, shape, or function despite uniform conditions in the external environment. Symmetry breaking then modifies the microenvironment of neighboring cells to, in turn, provoke successive patterning events until the system reaches a new state. In intestinal organoids, for example, a divergent YAP1 activation state between cells at early stage initiates a Notch/Dll1 lateral inhibition event triggering Paneth cell formation (Serra et al., 2019), a key differentiation event that results in crypt formation and the maintenance of the stem cells by a niche (Farin et al., 2016). Interestingly, in the absence of this symmetry breaking event, the initial stem cell colony drifts toward a fully differentiated epithelial spheroid, resulting in the loss of the self-renewal and morphogenetic potential of the system (Serra et al., 2019). Apart from cell-to-cell variability, several other mechanisms can explain the initial perturbation in a homogeneous system, such as pattern-forming theories like Turing reaction-diffusion mechanisms (Mathison, 1952).

In stem cell aggregates, cells located at the surface and at the center are exposed to different physical parameters that can drastically affect the initial phase of self-organization. This creates boundary conditions that will promote symmetry breaking by affecting cell-cell and cell-ECM interactions, mechanical tension, and cytoskeletal rearrangement as well as diffusional gradients of endogenous and exogenous molecules (Kinney et al., 2014). Engineering these boundary conditions, e.g., by spatiotemporally controlling the presentation of mechanochemical cues to a precise subset of cells, or even a single cell, would

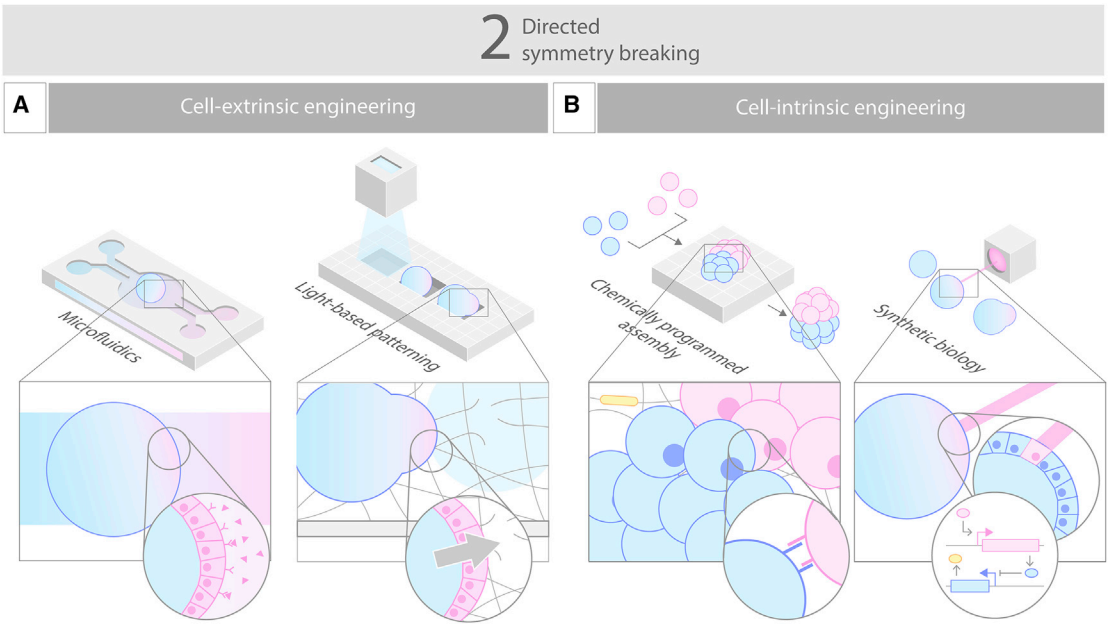

Figure 3. Directing Symmetry Breaking

(A) The cells' external environment can be precisely tailored using bioengineering approaches to locally modify various cues. Photopatterning can modify the biochemical and biophysical properties of the supporting matrix, while microfluidics can generate spatiotemporally adjustable morphogen gradients.

(B) The cell itself can be engineered via modification of its membrane properties or through synthetic biology approaches, respectively, resulting in programmed cell assembly or controlled gene expression.

thus provide directional guidance over symmetry breaking events (Figure 3). For example, current EB-based organoid derivation suffers from a profound lack of reproducibility, with success rates that can be as low as 30%–40% (Lancaster et al., 2017; Sasai, 2013b). Directed symmetry breaking via cell-extrinsic and cell-intrinsic engineering could improve the robustness of organoid technology and represents an essential part in the design of reproducible and scalable organoid cultures.

Cell-Extrinsic Control

Different engineering approaches have succeeded in locally controlling a broad range of biophysical and biochemical cues, such as the presence of soluble molecules and the mechanical properties of the ECM in cell cultures. Among these, microfluidics holds great promise for steering stem cell fate because of the high spatiotemporal control ensured by its laminar flow (Figure 3A, left panel). In an early example, a microfluidic device was fabricated to expose human colon organoids ("colonoids") to a steep gradient of Wnt3a and R-spondin1 (Attayek et al., 2016). In this setup, transit-amplifying cells were localized within a region of high Wnt3A/R-spondin1 and underwent crypt-like budding reminiscent of the native colon. Interestingly, single stem cells were found to be more responsive to the gradient than colonoids, suggesting that aggregates can already have an intrinsic pre-pattern, making it more difficult to re-orient their pattern with extrinsic cues. More complex microfluidic devices, often based on a "source and sink" principle, can recapitulate the complex gradients that play important roles in embryonic development and later tissue homeostasis. For example, neural tube formation *in vivo* is determined by two pairs of orthogonally positioned opposing gradients: patterning along the dorsal-ventral axis is induced by gradients of sonic hedgehog (SHH), bone morphogenetic protein (BMP), and wingless/integrated (WNT) ligands (Ribes and Briscoe, 2009), whereas antero-posterior patterning is simultaneously induced by gradients of retinoic acid (RA) and fibroblast growth factor (FGF) (Diez del Corral et al., 2003). By reproducing the complexity of these gradients, it was possible to control in space the differentiation of mouse embryonic stem cell (mESC) toward different fates (Demers et al., 2016). Additionally, culturing chick neural-plate extracts in a microfluidic chip displaying antiparallel gradients of SHH and BMP allowed locally controlled tissue patterning (Zagorski et al., 2017). In a culture of mouse ESCs under conditions favoring neural tube organoid formation (Meinhardt et al., 2014), the initial apicobasal polarization that precedes dorso-ventral patterning could be controlled by exposure to a gradient of leukemia inhibitory factor (LIF) (Tabata and Lutolf, 2017). Altogether, these data suggest that a reproducible *in vitro* model of neural tube development, or other intricately patterned multicellular systems, could be generated by leveraging microfluidic technologies combined with stem cell self-organization. Alternatively, morphogens can be locally delivered using microbeads in the culture to induce the asymmetrical distribution of proteins at the single-cell level (Habib et al., 2013), highlighting an interesting avenue for triggering symmetry breaking events in self-organizing multicellular systems.

Hydrogel photopatterning can provide even greater control over the spatiotemporal presentation of biophysical and biochemical cues to cells grown within engineered 3D matrices (Brown and Anseth, 2017; Figure 3A, right panel). A wealth of methods for the light-triggered modification of hydrogels have been developed, such as those based on protein-protein interactions (Wylie et al., 2011) or enzymatic crosslinking (Mosiewicz

et al., 2013), to attach cell-adhesive peptides, growth factors, antibodies, or other signaling cues. These approaches could be complemented with the photoactivated release of bioactive ligands already embedded within the hydrogel (Kloxin et al., 2009; Tsurkan et al., 2015) to potentially initiate symmetry breaking in developing organoids. To this end, the activity of Wnt and Notch ligands, which are essential for intestinal organoid crypt formation, could be spatiotemporally controlled through light to promote crypt formation in a predictable manner. Reversible light-mediated hydrogel modifications (DeForest and Tirrell, 2015) could also be used, with the clear advantage of promoting symmetry breaking events without compromising subsequent morphogenesis. Hydrogel softening has also been implicated in crypt initiation (Gjorevski et al., 2016), suggesting that hydrogel photodegradation (Kloxin et al., 2009; Lunzer et al., 2018) could be used to modify the mechanical environment around stem cell epithelia to locally control this process.

Differential mechanical stiffness and ECM protein presentation can also mimic important phenomena happening during early development. For example, a new model of amniotic tissue was developed where an implantation-like niche was mimicked by placing stem cells in between a soft tissue bed and a stiffer basement membrane (Shao et al., 2017). In this system, reproducing the implantation-like environment could efficiently steer the development of amnion-like tissue, with no need for maternal or extraembryonic sources.

Cell-Intrinsic Control

Because symmetry breaking arises from local interactions of individual cells, an alternative approach to manipulating it is to directly engineer the cells, through genome editing or chemically modifying the cellular membrane, to trigger changes in gene expression or adhesive properties, respectively. For example, the cell surface could be modified by incorporating DNA (Gartner and Bertozzi, 2009; Todhunter et al., 2015), aptamers (Zhao et al., 2011), or liposomes (O'Brien et al., 2015) acting as adhesion molecules for chemically programmed assembly (Figure 3B, left panel). The precise control over cell-cell interactions conferred by these methods enabled robust control over cell sorting and patterning (Gartner and Bertozzi, 2009). In adenohypophysis organoid formation, for example, the induction of hypophyseal tissue required the previous induction of oral ectoderm and hypothalamic neuroepithelium (Suga et al., 2011). The ensuing self-assembly of these two populations then triggered the emergence of Rathke's pouch-like vesicles. This self-organization process is, however, highly stochastic and resulted in an uncontrolled number and positioning of the vesicles. Artificially forcing cellular sorting with controlled layer formation should help to decipher the principal cues necessary for efficient fate induction in cases such as the pituitary gland where formation of tissue-tissue interfaces can steer self-driven morphogenesis.

In contrast with the above techniques that rely on an indirect modification of cell fate, synthetic biology is amenable to the direct alteration of cell-fate decisions by manipulating gene regulatory networks of individual or populations of cells (Figure 3B, right panel). Programming morphogenesis using genetic circuits is a rapidly evolving field (reviewed by Velazquez et al., 2018) in which several pioneering works have demonstrated the manipulation of symmetry breaking and cellular patterning. For example, CRISPR interference was used to induce mosaic knockdown of ROCK1 and CDH1, which regulate cortical tension and cell-cell adhesion, respectively (Libby et al., 2018). Differential expression of these genes in human induced pluripotent stem cells (hiPSC) resulted in the spontaneous formation of complex patterns including cellular segregation within the colony or the concentration of cells into a band at the colony periphery. Similar pattern emergence from an initially homogeneous population could be obtained by Cadherin-mediated cell sorting, where different populations of cells were engineered to express different cell-adhesion molecules (Cachat et al., 2016).

Another powerful approach is to genetically modify transcription factor expression to trigger morphogenesis events. Transient GATA6 expression in hiPSC colonies resulted in induced symmetry breaking followed by cellular patterning into three germ layers and further co-differentiation into multiple lineages (Guye et al., 2016). Similarly, the temporal induction of Nkx-1 and PAX8 forced EB differentiation toward thyroid follicle cells and guided their organization into thyroid follicles (Antonica et al., 2012). Interestingly, directly expressing these genes known to be critical for thyroid follicle emergence avoided the challenges arising from the lack of current knowledge of the signaling pathways controlling their expression.

In addition to using inducible transcription factor-expressing circuits, cell fate can also be controlled through the rewiring of cell sensing/response behaviors. Notch signaling has been successfully used as starting platform for this purpose (Morsut et al., 2016). The Notch extracellular domain can be engineered to recognize specific stimuli, and the intracellular notch transcriptional domain (NICD) can be replaced by a user-specified transcription factor or protein-protein interaction domain. A specially designed synthetic Notch (synNotch) system triggered the *trans*-differentiation of fibroblasts into myoblasts and epithelial-to-mesenchymal transition (Morsut et al., 2016). In a follow-up experiment, the synNotch system was used to control Cadherin-based adhesion to create a system with self-assembling properties and level of control over symmetry breaking and cell-type divergence (Toda et al., 2018). Although synthetic biology has yet to be used in stem cell-derived organoids, these impressive advances in programming morphogenesis are providing a critical basis for future adoption of this approach. It is expected that the engineering of cell-intrinsic properties will play an important role in developing the field of modern tissue engineering, either by advancing basic knowledge or by the biofabrication of more robust platforms for tissue self-organization.

Imposing Boundary Conditions to Guide Self-patterning

Once a self-organizing cytosystem has reached an asymmetric state, cell-autonomous guidance via self-patterning and self-sorting will drive tissue morphogenesis toward a new equilibrium. However, in fast-growing systems like organoids, the rapid increase in asymmetry results in abrupt changes in the boundary conditions of the population, consequently facilitating the establishment of microenvironmental gradients and promoting further symmetry breaking events. With ever-changing external cues affecting the cells, the system must sense and regulate its environment across scales to reach equilibrium and prevent aberrant growth. In multicellular organisms, this essential control over the boundary dynamics is mainly achieved by tissue-tissue

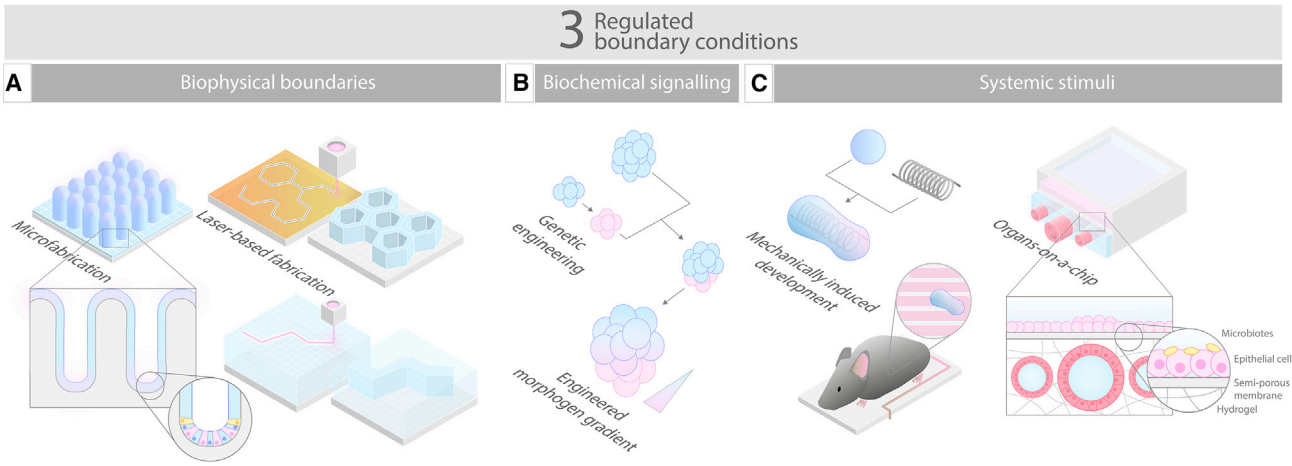

Figure 4. Imposing Boundary Conditions to Guide Self-patterning

(A) Engineering methodologies such as microfabrication and laser-based fabrication can be leveraged to reproduce native 3D ECM topography and organ microarchitecture.

(B) Mimicking biochemical signaling within tissues and at their interfaces can be leveraged to steer self-organization *in vitro*.

(C) Tissue-level physiological stimuli essential for cell patterning, such as mechanical forces, electrical stimulation, and microbiota interactions, can be simulated using micro-devices and organs-on-a-chip.

interactions that establish signaling interfaces and physical borders restricting growth. The reconstruction of these boundaries *in vitro* should increase our ability to rationally steer stem cell patterning and self-driven morphogenesis (Figure 4). In addition to genetic or microfluidic approaches, we believe that this can be achieved by mimicking the characteristic biochemical and physical cues observed during native organ development or by implementing systemic physico-chemical stimuli (e.g., tissue strain/compression or fluid flow) specific to a targeted organ.

Mimicking Biophysical Boundaries

Physical boundary conditions play a key role in the formation and long-term preservation of tissue architectures by providing individual cells with tissue-scale information on shape and topography. Indeed, at a single-cell level, it has long been known that the geometry and mechanics of a mammalian cell's microenvironment can profoundly influence cell behavior, including apoptosis (Chen et al., 1997), differentiation (Dupont et al., 2011; Engler et al., 2006), and proliferation (Murphy et al., 2014). Importantly, 2D geometrical confinement has been shown to guide the patterning of differentiating mouse ESCs via the location-dependent differential signal responsiveness of these cells (Blin et al., 2018). Therefore, biomimetic scaffolds that recapitulate the geometric boundary conditions seen *in vivo* could guide stem cell self-organization *in vitro* (Figure 4A, left panel). An early example in this direction was implemented based on the characteristics of the small intestinal epithelium, where stem cells reside at the bottom of small crypts that are intercalated in between large villus-regions of differentiated cells. Microfabrication and molding were used to generate an array of crypts and villi to recreate the topography of the intestine (Wang et al., 2017a). By combining this scaffold with gradients of morphogens known to maintain the crypt-villus axis, primary human ISCs formed an epithelium with an actively proliferating stem/progenitor cell compartment. A similar approach mimicking colonic epithelium architecture also recreated features of colon homeostasis (Wang et al., 2017b). Other promising methods have been used to create topographic features within hydrogels to control tissue architecture, such as "Intaglio-Void/Embed-Relief Topographic" molding (Stevens et al., 2013), sacrificial molding (Cerchiari et al., 2015), or laser photo-ablation (Brandenberg and Lutolf, 2016; Figure 4A, right panel).

The physical properties of a tissue depend on many factors, including the physicochemical characteristics of the ECM, cell-cell and cell-ECM interactions, as well as the supracellular tissue architecture. Differences in material properties within a tissue or at its interface can guide morphogenetic events based on existing active and passive forces (Stooke-Vaughan and Campàs, 2018). Intriguing experiments in zebrafish showed that body axis elongation is driven by changes in the tissues' physical properties, characterized by the posterior part undergoing a jamming transition from fluid-like to solid-like behavior (Mongera et al., 2018). At the growing extremity, corresponding to the mesodermal progenitor zone, the supracellular architecture is less compact, with more extracellular space and more active fluctuations of cell-cell interactions. As these cells move toward the presomitic mesoderm, cellular condensation and reduction of fluctuations in cell-cell interaction progressively results in modification of the material properties, effectively "solidifying" the tissue. Here, the interactions between the solid-like state and the "melted" state orchestrate tissue flow, with the solid part acting as a mechanical support. As such, differences in tissue properties can act as physical boundaries that steer self-organization, with fluid-to-solid transition maintaining tissue shape and solid-to-fluid transition promoting shape modifications. Approaches for controlling physical tissue boundaries to steer the spatial organization of multiple cell types are promising, as they could recapitulate interactions between different cell types and tissues during *in vivo* development. Models that reproduce key aspects of *in vivo* boundary conditions can, for example, be generated by the micropatterned co-culture of two epithelial populations (Javaherian et al., 2015). The knowledge derived from such models will be essential if we want to manipulate

tissue boundaries to both form and maintain tissue architecture and patterning despite the fluctuating external environment.

Mimicking Biochemical Signaling at Interfaces

During development, gradients of signaling molecules can guide morphogenesis by providing positional information that will trigger differential gene expression in neighboring or distant cells. These signaling molecules are dispersed within the tissue by diffusion or cell-based mechanisms (Sagner and Briscoe, 2017), creating biochemical boundary conditions that will guide tissue growth, differentiation, and patterning. In the classic view, signaling gradients are generated by the production of morphogens from an initially asymmetric and localized cell source, followed by the integration of their subsequent diffusion and clearance (by degradation and cellular uptake) across the environment. Interestingly, sharp and robust gradients of morphogens can also form within a wide region composed of homogeneous cells via a process called self-organized shuttling, where the morphogen is co-produced with its shuttle, a molecule which facilitates the diffusion of the bound morphogen (Haskel-Ittah et al., 2012). Besides the shape and steepness, morphogen gradients possess many characteristics that can be sensed by the tissue. Indeed, it was demonstrated that patterning of PSC colonies can also depend on the induction time as well as the rate of change of the morphogen concentration (Heemskerk et al., 2019; Tewary et al., 2017). This illustrates the importance of the dynamic nature of biochemical boundary conditions, with cells getting progressively more responsive with longer exposure, responding differently to distinct rates of change of the same morphogen. Regulating the various processes leading to gradient formation and understanding how their dynamics affect stem cell patterning should allow us to engineer emerging gradients within evolving tissues to effectively steer self-organization.

In an elegant study, human brain organoids were engineered by embedding a small aggregate of inducible SHH-expressing hPSCs within a larger spheroid of wild-type hPSCs (Cederquist et al., 2019). Upon induction, the incorporated SHH-producing cells generated a gradient of diffusive SHH, acting as a signaling center to steer the spatial organization of distinct presumptive forebrain domains. Strikingly, microscopic architecture of different regions could develop within the same organoid, with rosette-like morphologies recapitulating the architecture seen in forebrain organoids, and thin and extended neuroepithelia mimicking the organization observed in 3D culture of hypothalamic tissue. Introduction of asymmetric morphogenetic cues during early organoid development is a promising approach to mimic the biochemical boundary conditions that are established during development (Figure 4B).

Control of tissue growth and properties by morphogen gradients can subsequently affect morphogen production, creating an intricate feedback mechanism that is integral to self-organization processes. Thus, in the context of development and organogenesis, physical and chemical boundary conditions should be considered in light of their coupled interactions. For example, morphogen-induced growth can cause dilution and advection of these same morphogens, a process that could further affect the gradient shape or the growth rate of the tissue. A model was developed to take these interactions into account, where homogeneous growth and exact scaling of signaling molecule distribution are co-dependent and derived from critical feedback strength (Aguilar-Hidalgo et al., 2018). Another model encompasses mechano-chemical patterning, using morphogen reaction and diffusion combined with a two-phase poroelastic rheology, where one phase of the tissue corresponds to the extracellular fluid space and the other corresponds to the cellular network (Recho et al., 2019). In the case where an increase in morphogen results in an increase in cell volume, stable patterns can be formed with only one morphogen. Indeed, in this scenario, cells pump extracellular fluid to increase their volume, changing the local properties of the material and creating a large-scale flow that will further enrich the morphogen in the already concentrated region. Using this model, it was shown that tissue mechanics can be coupled with morphogen gradients. Efforts to increase our understanding of signaling gradients and their interplay with physical effectors will allow us to predict and control their influence on self-organization, fostering the development of engineering technologies to modulate them.

Mimicking Systemic Stimuli

During *in vivo* tissue morphogenesis, some important stimuli are unrelated to the immediate niche environment and are more systemic in nature, including mechanical inputs induced by tissue strain and compression, fluid flow, electrical stimulation, or microbiota interactions. Based on this notion, researchers have started to engineer such long-range physiological stimuli with the goal of improving organoid development and function. For example, a nitinol spring was used to probe the effect of mechanical strain on the maturation of human intestinal organoids implanted *in vivo* (Poling et al., 2018; Figure 4C, left panel). Strikingly, these mechanically guided organoids demonstrated improved architecture with longer villous projections and deeper crypts as well as an increased maturation compared to the control transplanted without the spring. Strain was also found to improve the mechanical properties of the tissues, as evidenced by a thicker smooth muscle layer around the organoid and a higher tensile capacity. Another study created a microfluidic device to generate pulsatile stretching of micropatterned neuroectoderm (Xue et al., 2018). Mechanical forces could directly activate BMP signaling and guide self-organization, promoting a neural plate border at the expense of neuroepithelial differentiation. Other notable results have highlighted the role of systemic external stimuli in differentiation and morphogenesis, such as an increased antimicrobial peptide production and barrier function in human intestinal organoids microinjected with bacteria (Hill et al., 2017), an increased maturation of electrically stimulated hiPSC-derived cardiomyocytes (Nunes et al., 2013; Ronaldson-Bouchard et al., 2018), or improved platelet biogenesis in response to turbulence (Ito et al., 2018). Deciphering and exploiting the multiplexed inputs that cells and tissues are able to sense, compute, and transform into morphogenetic outputs is critical if researchers want to harness the full potential of self-organization to engineer better tissues.

Organs-on-a-chip are promising tools for probing these interactions because they can both control large-scale stimuli that are complex in nature and isolate their effect on tissue growth and homeostasis (Bhatia and Ingber, 2014). Particularly, these devices can readily incorporate precise flow control and spatially controlled co-cultures (Figure 4C, right panel). In one implementation, ISCs were co-cultured with endothelial cells on separate

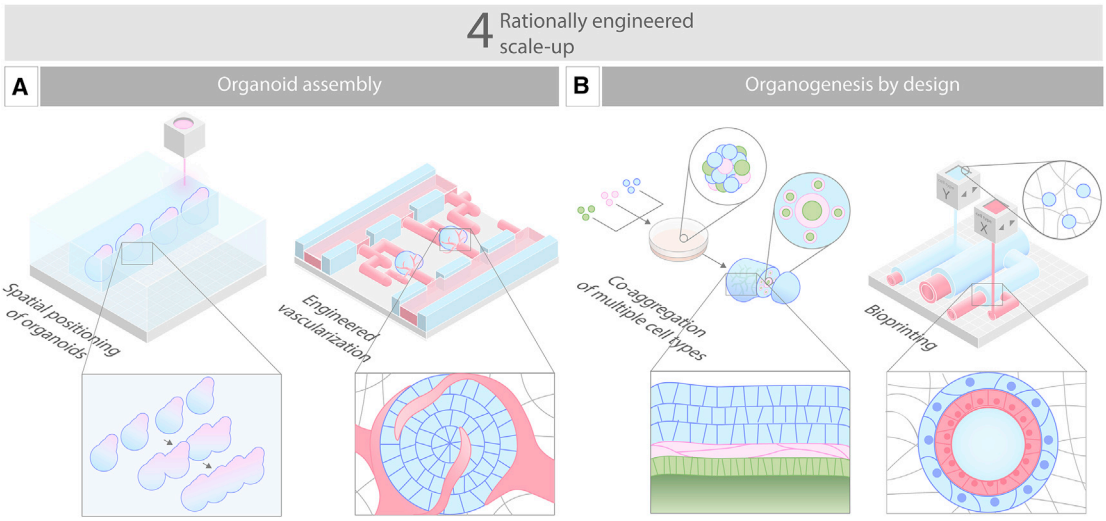

Figure 5. Rationally Designed Scale-up

(A) Depending on the target organ to be produced, several approaches can be used to take advantage of the self-organization program of cells. Organoids can be used as building blocks for the generation of organ-specific macroscopic geometries or can be assembled within a vascularized artificial organ.

(B) Increasing the complexity of *in vitro* organogenesis can be achieved by guiding the co-evolution of various tissues within an evolving system. Multiple cell-type-controlled aggregation and bioprinting represent powerful methods for regulating the spatiotemporal tissue-tissue interactions necessary to yield intricate biological emergence.

sides of a permeable membrane within a perfusable chip (Kasendra et al., 2018; Workman et al., 2017). Continuous flow increased the thickness of the intestinal epithelium, and spontaneous, densely organized, villus-like structures formed to cover the surface of the membrane. Additionally, the easy lumen accessibility provided by the organ-on-a-chip culture allowed for probing the epithelial responses to biological stimuli. Microfluidic systems can also be adapted to incorporate organoids to study the manipulation of their environment. In one example, exposing PSC-derived kidney organoids to shear stress by modulating the flow inside the chip was shown to enhance vasculogenesis (Homan et al., 2019). In the future, interfacing the control afforded by organs-on-a-chip with the microfabrication of biomimetic scaffolds and organoid technologies will give researchers powerful platforms to both isolate and merge the biophysical and biochemical cues that steer complex morphogenesis (Ingber, 2018).

Rationally Designed Scale-up

Size-relevant tissue engineering methodologies have historically depended on seeding or embedding cells in biomaterial scaffolds that could be easily processed to approximate the shape and composition of a targeted tissue or organ. Although these scaffolding technologies have had some impressive success in engineering "simpler" tissues such as bone or cartilage, reproducing organs as complex as the liver or the pancreas have proved more difficult. Indeed, reproducing the intricate architecture and cellular composition of these organs by spatiotemporal manipulation of different cell types, ECM components, and diffusible signaling factors would require the development and parallelization of technologies that are not currently available—and might never be. Instead, taking advantage of a stem cell's potential for self-organization, rather than focusing on punctual control over cell and ECM positioning, could yield living tissues better representing their native counterparts (Laurent et al., 2017). However, extensive organoid growth beyond a few millimeters is currently hindered by diffusion limitations and incomplete maturation, which is presumably due to the lack of critical factors essential for efficient and complete self-driven morphogenesis. Below, we describe two emerging and complementary scale-crossing strategies for the efficient scale-up of self-organized tissue mimetics (Figure 5). We postulate that already formed organoids can serve as specialized building blocks for the multiscale assembly of physiologically relevant artificial organs, and that control over *in vitro* organogenesis can be increased by the spatiotemporal assembly of single cells in a designed configuration based on knowledge of stem cell patterning and tissue-tissue interactions.

Multiscale Assembly of Organoids

Organs are composed of a combination of various cell types, packed together at high density and a relatively small proportion of supportive ECM constituents, i.e., highly complex 3D living matter that are difficult to reproduce by scaffold- or encapsulation-based technologies. One technology that shows promise is multiscale tissue assembly (Figure 5A, left panel), a stepwise process characterized by the formation of dense cellular units, their stacking to form larger-scale geometries, and their fusion during subsequent remodeling (Guven et al., 2015). Each unit can be produced separately to serve as the dynamic building block of a previously designed construct. This concept has been applied to cell sheets (Haraguchi et al., 2012; Kim et al., 2017; Tsuda et al., 2007) and tissue spheroids (Jakab et al., 2004). Some building blocks with more complex geometries have been produced, using smaller cell spheroids assembly inside defined microfabricated structures (Vrij et al., 2016a). In cell-sheet engineering, cell culture takes place on thermoresponsive surfaces where cells can be grown into a coherent tissue that is later detached without perturbing cell-cell junctions

or cell-surface proteins (Haraguchi et al., 2012). This method was used for the alternate stacking of different cell-specific sheets to generate vascularized beating cardiac and liver tissues (Haraguchi et al., 2012; Kim et al., 2017). Alternatively, spherical aggregates of cells obtained through condensation were placed in close proximity to promote fusion and later the formation of large and uniform tissues (Jakab et al., 2004). Importantly, bioprinting was used to scale up spheroid assembly and to print single- or double-layered branched vascular tubes (Norotte et al., 2009). These various experiments demonstrate how a bottom-up assembly approach can be powerful for scaling-up tissue biofabrication, provided that the initial building blocks are sufficiently dynamic to remodel their environment after initial arrangement.

Organoids offer an exciting opportunity for multiscale assembly, as they represent more relevant building blocks than spheroids that are made from cell populations with restricted morphogenetic and differentiation potential, such as immortalized cell lines. We believe that most of the technologies applied to spheroids can be translated to organoids to generate tissues with the microscopic architecture of organoids that are organized in a configuration reminiscent of native tissue. For example, intestinal organoid fusion has been shown to be promoted by growth and has enabled the formation of a centimeter-scale interconnected epithelial tube (Sachs et al., 2017). Precise spatial manipulation by bioprinting or programmed assembly could allow more robust formation of epithelial tubes via bottom-up assembly (Figure 5B, right panel). Organoid fusion has also been applied to human PSC-derived spheroids resembling specific regions of the brain (Birey et al., 2017; Xiang et al., 2017, 2019). In one of these studies, spheroids were formed to model either dorsal or ventral forebrain and were then assembled inside a conical tube (Birey et al., 2017). Spheroid fusion generated a microsystem wherein the migration of interneurons toward the cerebral cortex could be modeled. A similar migration of interneurons was also observed between fused spheroids modeling the medial ganglionic eminence and the cortex (Xiang et al., 2017), showing that organoid assembly could be an adaptable strategy both to scale up tissue size as well as to increase complexity through interactions between building blocks having a different (tissue) identity. One important consideration for organoid assembly is the creation of a physiological interface between the merging building blocks. Spheroids and epithelial organoids, identically derived and cultured, can fuse together and generate a seamless interface, following a process that may mimic tissue regeneration. In contrast, processes aiming at recapitulating developmental tissue-tissue interactions through building blocks of different identity may require careful timing and optimized conditions for initial growth and development. We expect that proper integration will depend on whether the building blocks are sufficiently responsive to endogenous and engineered cues to efficiently remodel the interface.

The large-scale assembly of organoids could also benefit from advances in engineering vasculature (Grebenyuk and Ranga, 2019). Indeed, a functional vascular network supporting interconnecting organoids could reproduce the function of organs whose architecture is derived from the assembly of small tissue modules (Figure 5A, right panel). Although this approach has not yet been adopted for organoid culture, several groups have shown that encapsulating cells around an engineered vasculature is possible (Kim et al., 2013; Miller et al., 2012; Yajima et al., 2018). In one interesting approach, HepG2 cells were densely packed into a linear structure mimicking the hepatic cord seen *in vivo* and were then assembled into bundles surrounded by endothelial cells that could be packed into a perfusable chamber (Yajima et al., 2018). Progress in engineering vascular networks connected to densely packed organoids could alleviate some of the nutrient diffusion limitations in the large-scale assembly of self-organized building blocks.

Engineering newer and simpler methods for assembling organoids and increasing their robustness will lead to their broader adaptation. It is expected that the spatial control necessary to reproduce the complex geometry essential for mimicking real organs will progress along with advancements in bioprinting, robotic cell/liquid handling, and other cell-positioning technologies. However, one critical hurdle still to be addressed is the lack of reproducibility in organoid formation. Methods for high-throughput production and for the selection of ideal starting materials based on stringent criteria (e.g., through imaging-based approaches) will have to be developed to provide a reproducible foundation for tissue assembly. By adopting a similar approach, one group showed that early intestinal spheroids could be sorted based on size and cell number to increase organoid formation efficiency and the homogeneity of the purified population (Arora et al., 2017). Meticulous organoid selection, in combination with improved reproducibility, e.g., via directed symmetry breaking events and forced cell patterning, is expected to produce the quality organoids mandatory for real-life applications, especially for screening in drug discovery and personalized medicine.

Organogenesis by Design

One other approach for producing more elaborate tissues is to increase the complexity of the cellular interactome leading to self-organization instead of focusing on using initially homogeneous populations to generate organoids controlled to their minute details (Figure 5B, left panel). For certain applications like whole-organ fabrication, ''perfect'' control over very fine organization, such as the exact number of crypts in an intestine or the precise positioning of each alveolar unit in a lung, is unnecessary to recapitulate function. Besides, too many engineering constraints during development or maturation could reduce the plasticity and adaptability of the tissue and interfere with homeostasis and regeneration. With these considerations in mind, we expect that increasing the intricacy of the starting conditions, combined with well-balanced external guidance, could improve current *in vitro* organogenesis.

The complexity of an emergent system can be described as a function of the number of potentially interacting elements, the level of heterogeneity of these elements, and their interconnectivity. This direct link between self-organization and individual element number could partially explain why a minimum number of cells is necessary for the formation of some organoids (van den Brink et al., 2014; Lancaster et al., 2017) or for more elaborate morphogenesis in others (Sasai, 2013a). Indeed, limiting the amount of starting material might preclude the formation of the necessary signaling centers or required differentiation events, as is exemplified by the disappearance of the central fate of 2D human gastruloids cultured on smaller micropatterns

(Warmflash et al., 2014). Additionally, it has been shown that heterogeneity in cell populations promoted the simultaneous co-induction of tissues from multiple lineages. By engineering a hiPSC population with heterogeneous GATA6 expression, a complex liver-bud-like tissue was generated without relying on the traditional sequential differentiation using varied cocktails of cytokines (Guye et al., 2016). Co-differentiation of stromal and parenchymal compartments mimicked niche-like interactions and further stimulated the generation of a large assortment of cell fates, such as hepatocytic, hematopoietic, and endothelial. These results support the idea that increased heterogeneity can promote biological emergence through interactions that are absent or poorly controlled in current organoid derivation protocols. It will be important in the future to characterize how initial heterogeneity in the expression of a single gene can be translated into heterogeneity in the cell-differentiation process—the latter most likely driving increased complexity via the emergence of tissue-tissue interactions.

The importance of tissue interconnectivity in organogenesis is perhaps best illustrated by the constant interplay between organ-specific mesenchyme and their corresponding epithelium. For example, co-induction of ectoderm and mesoderm enabled the development of a skin organoid model that could recapitulate hair follicle formation (Lee et al., 2018). A mouse PSC culture protocol was used to generate 3D structures with a stratified epidermis, dermis, and even an adipocyte-containing hypodermis layer. Of significant note, the tissue-tissue interactions at the dermis-epidermis interface encouraged dermal fibroblast condensation and the consequent initiation of hair-follicle development. Furthermore, signs of the induction of other dermal cellular compartments were observed, including arrector pili muscle, touch-sensing Merkel cells, and melanocytes derived from neural-crest-like cells (Lee et al., 2018). Although organoids generated by multilineage induction are poorly characterized, their cellular variety and architectural complexity exceed what is commonly seen in organoids derived from an approach focused on a single germ layer. A broader application of this strategy is likely to drive important improvements in developmental biology-inspired organogenesis.

Applying the same concept of organogenesis guided by multilineage interactions, complex oral organs were fabricated by leveraging epithelial-mesenchymal cross-talk and its inherent ability to guide organ germ formation at the interface (Ikeda et al., 2019). This bioengineering strategy consisted of mixing epithelial and mesenchymal stem cells isolated from the mouse embryo and injecting them into collagen gels. The tissue-tissue cooperation created within the aggregate mimicked the organ-forming field development seen *in vivo* and resulted in bud formation with specific organ evolution. This concept was applied to a broad range of organs, including teeth (Ikeda et al., 2009), hair follicles (Toyoshima et al., 2012), and secretory glands (Hirayama et al., 2013). Alternatively, the aggregation of other cell types can form organoids with improved architecture or functions. Pioneering work demonstrated that the *in vitro* co-culture of mesenchymal stem cells and endothelial cells with PSC-derived hepatic progenitors directed the formation of liver-bud-like 3D clusters due to organotypic interactions, with enhanced hepatic differentiation and function when transplanted *in vivo* (Takebe et al., 2013). This technique was then applied to tissues of various origins to prove that it could be a potent way to favor organoid vascularization (Takebe et al., 2015).

Altogether, these advances reveal several parameters governing the complexity of self-organization and suggest that their potential could be harnessed to guide sophisticated organogenesis *in vitro*. The advancement of technologies to spatiotemporally direct the co-development of multiple tissues will likely be the key to overcoming the size limitation seen in current organoid models. Several methods have already been developed to control cellular spatial organization, such as minimal scaffolding (Lancaster et al., 2017), bioprinting (Bhattacharjee et al., 2015), or programmed cell assembly (Todhunter et al., 2015). Particularly, cells could be functionalized with oligonucleotides to program their adhesion on a surface or their assembly with other cells to form dense tissues (Todhunter et al., 2015). This approach was shown to be compatible with ECM-based culture and for the deposition of multiple cell types, which could then be used to construct elaborate networks of co-evolving tissues. Freeform embedded bioprinting is an equally promising strategy due to its capacity for depositing a high density of cells in an environment permissive to cellular migration and remodeling (Bhattacharjee et al., 2015, 2016). The adaptation of these methods to self-organizing stem cells and organoids would provide powerful tools for the future of designed organogenesis.

Perspectives for Engineering Self-organization

The last decade has seen numerous breakthroughs in the field of stem cell biology. Particularly, the revelation that the self-organizing nature of stem cells could be exploited to produce organoids that resemble native tissues has bolstered the idea that whole organs could be reproduced *in vitro*. The impressive first steps that have been made, however, have been impeded by the uncontrolled nature of the current self-organized systems, including drawbacks such as a high variability, low level of maturation, and an inability to grow constructs bigger than a few millimeters. Combining the morphogenetic potential of cellular ensembles with engineering tools to direct their interactions has emerged as an encouraging approach to overcome these limitations (Kamm et al., 2018; Laurent et al., 2017). Early works on controlling the development of organoids by precise environmental manipulation through microtechnologies, advanced biomaterials, and other bioengineering technologies have already yielded relevant design parameters and morphogenetic rules that underlie multicellular self-organization. It is expected that these engineering tools, combined with advances in 4D imaging, synthetic biology, and organs-on-chips, will allow researchers to develop better model systems to tackle important fundamental questions related to how cells build tissues. Uncovering these mechanisms necessitates relatively simplified multicellular systems, where the multiple variables can each be isolated and manipulated to ask fundamental questions about the rules governing self-organization. Engineering these simpler systems will ultimately allow the transformation of the stochastic *in vitro* developmental processes into deterministic ones, representing a crucial step for the derivation of suitable physical and mathematical models of multicellular self-organization. In turn, these models, including *in silico* simulations, will provide fundamental knowledge to engineer better organoids, creating a multidisciplinary feedback loop where the development of simple systems

and their subsequent engineering will provide the basis for the generation of systems with more complex emergent behaviors, composition, and improved functionalities.

Current organoids are ideally suited to inquire on symmetry breaking processes (Serra et al., 2019) and on how self-organization can guide cell-fate decision making and patterning but have yet to faithfully reproduce organ functionality or macroscopic architecture. Design constraints should thus focus on controlling the emergence of these intrinsic biological characteristics, using progressively more intricate multicellular systems as we draw from the growing expertise of many disciplines. We have discussed how the critical stages of tissue development can each be engineered to potentially unlock the full morphogenetic potential of self-organized stem cell systems. Because biological emergence is determined by the summation of iterative interactions between individual cells, controlling the initial conditions and the various cues leading to symmetry breaking is of paramount importance for increasing the robustness of organoid formation. Additionally, the cellular environment must be judiciously regulated to provide the essential physiological signals to steer stem cell self-patterning without restricting later morphogenesis events. This delicate balance between driving natural tissue self-organization and providing artificial boundary conditions represents a critical stepping stone for the success of next-generation organoids. Indeed, based on our knowledge of *in vivo* development and, in particular, tissue-tissue co-evolution, the reconstruction of each targeted organ will need to follow a distinct and rationally designed procedure, i.e., a set of "guidance" steps to engineer self-organization based on the necessary and minimal criteria for subsequent function or transplantation.

Clearly, numerous difficult challenges will have to be overcome before we can reproduce efficient organogenesis in the laboratory. Specifically, technologies to guide symmetry breaking and cell patterning will have to be parallelized and improved to robustly and simultaneously control the necessary cues across multiple scales and multiple organoids. Perhaps more importantly, novel technologies will have to be developed to enhance control over multiple tissue-tissue interactions and to scale up the assembly of dynamic building blocks without compromising self-organization. Nevertheless, overcoming these limitations promises to revolutionize tissue engineering and will surely transform regenerative medicine.

ACKNOWLEDGMENTS

The authors thank Stefano Vianello for invaluable work on illustrations and Andrea Manfrin and Giuliana Rossi for helpful feedback on the manuscript. M.P.L.'s work in the area of organoid biology and technology is supported by the Swiss National Science Foundation (#310030_179447), École Polytechnique Fédérale de Lausanne (EPFL), the European Union's Horizon 2020 Research and Innovation Programme (INTENS, #668294), the Personalized Health and Related Technologies Initiative from the ETH Board, the National Center of Competence in Research (NCCR) "Bio-Inspired Materials," the "FreeNovation" funding program of the Novartis Research Foundation, the Marie Skłodowska-Curie European Training Network "EUROoC" (H2020-MSCA-ITN-2018), and the Vienna Science and Technology Fund (WWTF). The authors apologize to all the scientists whose work they could not cite owing to space restrictions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Aguilar-Hidalgo, D., Werner, S., Wartlick, O., González-Gaitán, M., Friedrich, B.M., and Jülicher, F. (2018). Critical Point in Self-Organized Tissue Growth. Phys. Rev. Lett. *120*, 198102.

Ahn, K., Kim, S.H., Lee, G.H., Lee, S., Heo, Y.S., and Park, J.Y. (2016). Features of Microsystems for Cultivation and Characterization of Stem Cells with the Aim of Regenerative Therapy. Stem Cells Int. *2016*, 6023132.

Anlaş, A.A., and Nelson, C.M. (2018). Tissue mechanics regulates form, function, and dysfunction. Curr. Opin. Cell Biol. *54*, 98–105.

Antonica, F., Kasprzyk, D.F., Opitz, R., Iacovino, M., Liao, X.H., Dumitrescu, A.M., Refetoff, S., Peremans, K., Manto, M., Kyba, M., and Costagliola, S. (2012). Generation of functional thyroid from embryonic stem cells. Nature *491*, 66–71.

Arora, N., Imran Alsous, J., Guggenheim, J.W., Mak, M., Munera, J., Wells, J.M., Kamm, R.D., Asada, H.H., Shvartsman, S.Y., and Griffith, L.G. (2017). A process engineering approach to increase organoid yield. Development *144*, 1128–1136.

Attayek, P.J., Ahmad, A.A., Wang, Y., Williamson, I., Sims, C.E., Magness, S.T., and Allbritton, N.L. (2016). In vitro polarization of colonoids to create an intestinal stem cell compartment. PLoS ONE *11*, e0153795.

Barcellos-Hoff, M.H., Aggeler, J., Ram, T.G., and Bissell, M.J. (1989). Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. Development *105*, 223–235.

Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H.J., van Es, J.H., Sato, T., Stange, D.E., Begthel, H., van den Born, M., et al. (2010). Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell *6*, 25–36.

Beccari, L., Moris, N., Girgin, M., Turner, D.A., Baillie-Johnson, P., Cossy, A.C., Lutolf, M.P., Duboule, D., and Arias, A.M. (2018). Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids. Nature *562*, 272–276.

Bhatia, S.N., and Ingber, D.E. (2014). Microfluidic organs-on-chips. Nat. Biotechnol. *32*, 760–772.

Bhattacharjee, T., Zehnder, S.M., Rowe, K.G., Jain, S., Nixon, R.M., Sawyer, W.G., and Angelini, T.E. (2015). Writing in the granular gel medium. Sci. Adv. *1*, e1500655.

Bhattacharjee, T., Gil, C.J., Marshall, S.L., Urueña, J.M., O'Bryan, C.S., Carstens, M., Keselowsky, B., Palmer, G.D., Ghivizzani, S., Gibbs, C.P., et al. (2016). Liquid-like Solids Support Cells in 3D. ACS Biomater. Sci. Eng. *2*, 1787–1795.

Birey, F., Andersen, J., Makinson, C.D., Islam, S., Wei, W., Huber, N., Fan, H.C., Metzler, K.R.C., Panagiotakos, G., Thom, N., et al. (2017). Assembly of functionally integrated human forebrain spheroids. Nature *545*, 54–59.

Blin, G., Wisniewski, D., Picart, C., Thery, M., Puceat, M., and Lowell, S. (2018). Geometrical confinement controls the asymmetric patterning of brachyury in cultures of pluripotent cells. Development. Published online September 21, 2018. https://doi.org/10.1242/dev.166025.

Blondel, D., and Lutolf, M.P. (2019). Bioinspired Hydrogels for 3D Organoid Culture. Chimia (Aarau) *73*, 81–85.

Brandenberg, N., and Lutolf, M.P. (2016). In Situ Patterning of Microfluidic Networks in 3D Cell-Laden Hydrogels. Adv. Mater *28*, 7450–7456.

Broguiere, N., Isenmann, L., Hirt, C., Ringel, T., Placzek, S., Cavalli, E., Ringnalda, F., Villiger, L., Züllig, R., Lehmann, R., et al. (2018). Growth of Epithelial Organoids in a Defined Hydrogel. Adv. Mater. *30*, e1801621.

Brown, T.E., and Anseth, K.S. (2017). Spatiotemporal hydrogel biomaterials for regenerative medicine. Chem. Soc. Rev. *46*, 6532–6552.

Brusatin, G., Panciera, T., Gandin, A., Citron, A., and Piccolo, S. (2018). Biomaterials and engineered microenvironments to control YAP/TAZ-dependent cell behaviour. Nat. Mater. *17*, 1063–1075.

Cachat, E., Liu, W., Martin, K.C., Yuan, X., Yin, H., Hohenstein, P., and Davies, J.A. (2016). 2- and 3-dimensional synthetic large-scale de novo patterning by mammalian cells through phase separation. Sci. Rep. *6*, 20664.

Camp, J.G., Wollny, D., and Treutlein, B. (2018). Single-cell genomics to guide human stem cell and tissue engineering. Nat. Methods *15*, 661–667.

Candiello, J., Grandhi, T.S.P., Goh, S.K., Vaidya, V., Lemmon-Kishi, M., Eliato, K.R., Ros, R., Kumta, P.N., Rege, K., and Banerjee, I. (2018). 3D heterogeneous islet organoid generation from human embryonic stem cells using a novel engineered hydrogel platform. Biomaterials *177*, 27–39.

Capeling, M.M., Czerwinski, M., Huang, S., Tsai, Y.H., Wu, A., Nagy, M.S., Juliar, B., Sundaram, N., Song, Y., Han, W.M., et al. (2019). Nonadhesive Alginate Hydrogels Support Growth of Pluripotent Stem Cell-Derived Intestinal Organoids. Stem Cell Reports *12*, 381–394.

Cederquist, G.Y., Asciolla, J.J., Tchieu, J., Walsh, R.M., Cornacchia, D., Resh, M.D., and Studer, L. (2019). Specification of positional identity in forebrain organoids. Nat. Biotechnol. *37*, 436–444.

Cerchiari, A., Garbe, J.C., Todhunter, M.E., Jee, N.Y., Pinney, J.R., LaBarge, M.A., Desai, T.A., and Gartner, Z.J. (2015). Formation of spatially and geometrically controlled three-dimensional tissues in soft gels by sacrificial micromolding. Tissue Eng. Part C Methods *21*, 541–547.

Chaudhuri, O., Gu, L., Klumpers, D., Darnell, M., Bencherif, S.A., Weaver, J.C., Huebsch, N., Lee, H.P., Lippens, E., Duda, G.N., and Mooney, D.J. (2016). Hydrogels with tunable stress relaxation regulate stem cell fate and activity. Nat. Mater. *15*, 326–334.

Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., and Ingber, D.E. (1997). Geometric control of cell life and death. Science *276*, 1425–1428.

Choi, Y.Y., Chung, B.G., Lee, D.H., Khademhosseini, A., Kim, J.H., and Lee, S.H. (2010). Controlled-size embryoid body formation in concave microwell arrays. Biomaterials *31*, 4296–4303.

Clevers, H. (2016). Modeling Development and Disease with Organoids. Cell *165*, 1586–1597.

Cruz-Acuña, R., Quirós, M., Farkas, A.E., Dedhia, P.H., Huang, S., Siuda, D., García-Hernández, V., Miller, A.J., Spence, J.R., Nusrat, A., and García, A.J. (2017). Synthetic hydrogels for human intestinal organoid generation and colonic wound repair. Nat. Cell Biol. *19*, 1326–1335.

Czerniecki, S.M., Cruz, N.M., Harder, J.L., Menon, R., Annis, J., Otto, E.A., Gulieva, R.E., Islas, L.V., Kim, Y.K., Tran, L.M., et al. (2018). High-Throughput Screening Enhances Kidney Organoid Differentiation from Human Pluripotent Stem Cells and Enables Automated Multidimensional Phenotyping. Cell Stem Cell *22*, 929–940.

Dahl-Jensen, S., and Grapin-Botton, A. (2017). The physics of organoids: a biophysical approach to understanding organogenesis. Development *144*, 946–951.

DeForest, C.A., and Tirrell, D.A. (2015). A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. Nat. Mater. *14*, 523–531.

Demers, C.J., Soundararajan, P., Chennampally, P., Cox, G.A., Briscoe, J., Collins, S.D., and Smith, R.L. (2016). Development-on-chip: in vitro neural tube patterning with a microfluidic device. Development *143*, 1884–1892.

Diez del Corral, R., Olivera-Martinez, I., Goriely, A., Gale, E., Maden, M., and Storey, K. (2003). Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron *40*, 65–79.

DiMarco, R.L., Dewi, R.E., Bernal, G., Kuo, C., and Heilshorn, S.C. (2015). Protein-engineered scaffolds for in vitro 3D culture of primary adult intestinal organoids. Biomater. Sci. *3*, 1376–1385.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., et al. (2011). Role of YAP/TAZ in mechanotransduction. Nature *474*, 179–183.

Dye, B.R., Hill, D.R., Ferguson, M.A.H., Tsai, Y.H., Nagy, M.S., Dyal, R., Wells, J.M., Mayhew, C.N., Nattiv, R., Klein, O.D., et al. (2015). In vitro generation of human pluripotent stem cell derived lung organoids. eLife. Published online March 24, 2015. https://doi.org/10.7554/eLife.05098.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature *472*, 51–56.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell *126*, 677–689.

Etoc, F., Metzger, J., Ruzo, A., Kirst, C., Yoney, A., Ozair, M.Z., Brivanlou, A.H., and Siggia, E.D. (2016). A Balance between Secreted Inhibitors and Edge Sensing Controls Gastruloid Self-Organization. Dev. Cell *39*, 302–315.

Farin, H.F., Jordens, I., Mosa, M.H., Basak, O., Korving, J., Tauriello, D.V.F., de Punder, K., Angers, S., Peters, P.J., Maurice, M.M., and Clevers, H. (2016). Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. Nature *530*, 340–343.

Fordham, R.P., Yui, S., Hannan, N.R.F., Soendergaard, C., Madgwick, A., Schweiger, P.J., Nielsen, O.H., Vallier, L., Pedersen, R.A., Nakamura, T., et al. (2013). Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. Cell Stem Cell *13*, 734–744.

Gartner, Z.J., and Bertozzi, C.R. (2009). Programmed assembly of 3-dimensional microtissues with defined cellular connectivity. Proc. Natl. Acad. Sci. USA *106*, 4606–4610.

Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M.E., Ordóñez-Morán, P., Clevers, H., and Lutolf, M.P. (2016). Designer matrices for intestinal stem cell and organoid culture. Nature *539*, 560–564.

Grebenyuk, S., and Ranga, A. (2019). Engineering Organoid Vascularization. Front. Bioeng. Biotechnol. *7*, 39.

Greggio, C., De Franceschi, F., Figueiredo-Larsen, M., Gobaa, S., Ranga, A., Semb, H., Lutolf, M., and Grapin-Botton, A. (2013). Artificial three-dimensional niches deconstruct pancreas development in vitro. Development *140*, 4452–4462.

Guven, S., Chen, P., Inci, F., Tasoglu, S., Erkmen, B., and Demirci, U. (2015). Multiscale assembly for tissue engineering and regenerative medicine. Trends Biotechnol. *33*, 269–279.

Guye, P., Ebrahimkhani, M.R., Kipniss, N., Velazquez, J.J., Schoenfeld, E., Kiani, S., Griffith, L.G., and Weiss, R. (2016). Genetically engineering self-organization of human pluripotent stem cells into a liver bud-like tissue using Gata6. Nat. Commun. *7*, 10243.

Habib, S.J., Chen, B.C., Tsai, F.C., Anastassiadis, K., Meyer, T., Betzig, E., and Nusse, R. (2013). A localized Wnt signal orients asymmetric stem cell division in vitro. Science *339*, 1445–1448.

Haraguchi, Y., Shimizu, T., Sasagawa, T., Sekine, H., Sakaguchi, K., Kikuchi, T., Sekine, W., Sekiya, S., Yamato, M., Umezu, M., and Okano, T. (2012). Fabrication of functional three-dimensional tissues by stacking cell sheets in vitro. Nat. Protoc. *7*, 850–858.

Haskel-Ittah, M., Ben-Zvi, D., Branski-Arieli, M., Schejter, E.D., Shilo, B.Z., and Barkai, N. (2012). Self-organized shuttling: generating sharp dorsoventral polarity in the early Drosophila embryo. Cell *150*, 1016–1028.

Haupt, A., and Minc, N. (2018). How cells sense their own shape - mechanisms to probe cell geometry and their implications in cellular organization and function. J. Cell Sci. *131*, jcs214015.

Heemskerk, I., Burt, K., Miller, M., Chhabra, S., Guerra, M.C., Liu, L., and Warmflash, A. (2019). Rapid changes in morphogen concentration control self-organized patterning in human embryonic stem cells. eLife *8*, e40526.

Hill, D.R., Huang, S., Nagy, M.S., Yadagiri, V.K., Fields, C., Mukherjee, D., Bons, B., Dedhia, P.H., Chin, A.M., Tsai, Y.H., et al. (2017). Bacterial colonization stimulates a complex physiological response in the immature human intestinal epithelium. eLife. Published online November 7, 2017. https://doi.org/10.7554/eLife.29132.

Hirayama, M., Ogawa, M., Oshima, M., Sekine, Y., Ishida, K., Yamashita, K., Ikeda, K., Shimmura, S., Kawakita, T., Tsubota, K., and Tsuji, T. (2013). Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. Nat. Commun. *4*, 2497.

Homan, K.A., Gupta, N., Kroll, K.T., Kolesky, D.B., Skylar-Scott, M., Miyoshi, T., Mau, D., Valerius, M.T., Ferrante, T., Bonventre, J.V., et al. (2019). Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat. Methods *16*, 255–262.

Hwang, Y.S., Chung, B.G., Ortmann, D., Hattori, N., Moeller, H.C., and Khademhosseini, A. (2009). Microwell-mediated control of embryoid body size regulates embryonic stem cell fate via differential expression of WNT5a and WNT11. Proc. Natl. Acad. Sci. USA *106*, 16978–16983.

Ikeda, E., Morita, R., Nakao, K., Ishida, K., Nakamura, T., Takano-Yamamoto, T., Ogawa, M., Mizuno, M., Kasugai, S., and Tsuji, T. (2009). Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc. Natl. Acad. Sci. USA *106*, 13475–13480.

Ikeda, E., Ogawa, M., Takeo, M., and Tsuji, T. (2019). Functional ectodermal organ regeneration as the next generation of organ replacement therapy. Open Biol. *9*, 190010.

Ingber, D.E. (2018). Developmentally inspired human "organs on chips.". Development. Published online May 18, 2018. https://doi.org/10.1242/dev.156125.

Ito, Y., Nakamura, S., Sugimoto, N., Shigemori, T., Kato, Y., Ohno, M., Sakuma, S., Ito, K., Kumon, H., Hirose, H., et al. (2018). Turbulence Activates Platelet Biogenesis to Enable Clinical Scale Ex Vivo Production. Cell *174*, 636–648.

Jakab, K., Neagu, A., Mironov, V., Markwald, R.R., and Forgacs, G. (2004). Engineering biological structures of prescribed shape using self-assembling multicellular systems. Proc. Natl. Acad. Sci. USA *101*, 2864–2869.

Javaherian, S., D'Arcangelo, E., Slater, B., Zulueta-Coarasa, T., Fernandez-Gonzalez, R., and McGuigan, A.P. (2015). An in vitro model of tissue boundary formation for dissecting the contribution of different boundary forming mechanisms. Integr. Biol. *7*, 298–312.

Jung, P., Sato, T., Merlos-Suárez, A., Barriga, F.M., Iglesias, M., Rossell, D., Auer, H., Gallardo, M., Blasco, M.A., Sancho, E., et al. (2011). Isolation and in vitro expansion of human colonic stem cells. Nat. Med. *17*, 1225–1227.

Kamm, R.D., Bashir, R., Arora, N., Dar, R.D., Gillette, M.U., Griffith, L.G., Kemp, M.L., Kinlaw, K., Levin, M., Martin, A.C., et al. (2018). Perspective: The promise of multi-cellular engineered living systems. APL Bioeng. *2*, 040901.

Kasendra, M., Tovaglieri, A., Sontheimer-Phelps, A., Jalili-Firoozinezhad, S., Bein, A., Chalkiadaki, A., Scholl, W., Zhang, C., Rickner, H., Richmond, C.A., et al. (2018). Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci. Rep. *8*, 2871.

Kim, S., Lee, H., Chung, M., and Jeon, N.L. (2013). Engineering of functional, perfusable 3D microvascular networks on a chip. Lab Chip *13*, 1489–1500.

Kim, K., Utoh, R., Ohashi, K., Kikuchi, T., and Okano, T. (2017). Fabrication of functional 3D hepatic tissues with polarized hepatocytes by stacking endothelial cell sheets in vitro. J. Tissue Eng. Regen. Med. *11*, 2071–2080.

Kinney, M.A., Hookway, T.A., Wang, Y., and McDevitt, T.C. (2014). Engineering three-dimensional stem cell morphogenesis for the development of tissue models and scalable regenerative therapeutics. Ann. Biomed. Eng. *42*, 352–367.

Kloxin, A.M., Kasko, A.M., Salinas, C.N., and Anseth, K.S. (2009). Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science *324*, 59–63.

Koehler, K.R., Nie, J., Longworth-Mills, E., Liu, X.P., Lee, J., Holt, J.R., and Hashino, E. (2017). Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. Nat. Biotechnol. *35*, 583–589.

Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. Science *345*. Published online July 18, 2014. https://doi.org/10.1126/science.1247125.

Lancaster, M.A., Corsini, N.S., Wolfinger, S., Gustafson, E.H., Phillips, A.W., Burkard, T.R., Otani, T., Livesey, F.J., and Knoblich, J.A. (2017). Guided self-organization and cortical plate formation in human brain organoids. Nat. Biotechnol. *35*, 659–666.

Laurent, J., Blin, G., Chatelain, F., Vanneaux, V., Fuchs, A., Larghero, J., and Théry, M. (2017). Convergence of microengineering and cellular self-organization towards functional tissue manufacturing. Nat. Biomed. Eng. *1*, 939–956.

Lee, J., Böscke, R., Tang, P.C., Hartman, B.H., Heller, S., and Koehler, K.R. (2018). Hair Follicle Development in Mouse Pluripotent Stem Cell-Derived Skin Organoids. Cell Rep. *22*, 242–254.

Libby, A.R., Joy, D.A., So, P.L., Mandegar, M.A., Muncie, J.M., Mendoza-Camacho, F.N., Weaver, V.M., Conklin, B.R., and McDevitt, T.C. (2018). Spatiotemporal mosaic self-patterning of pluripotent stem cells using CRISPR interference. eLife. Published online October 9, 2018. https://doi.org/10.7554/eLife.36045.

Lunzer, M., Shi, L., Andriotis, O.G., Gruber, P., Markovic, M., Thurner, P.J., Ossipov, D., Liska, R., and Ovsianikov, A. (2018). A Modular Approach to Sensitized Two-Photon Patterning of Photodegradable Hydrogels. Angew. Chem. Int. Ed. Engl. *57*, 15122–15127.

Mathison, T.A. (1952). The chemical basis of morphogenesis. Philos. Trans. R. Soc. Lond. B Biol. Sci. *237*, 37–72.

McCauley, H.A., and Wells, J.M. (2017). Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. Development *144*, 958–962.

McCracken, K.W., Catá, E.M., Crawford, C.M., Sinagoga, K.L., Schumacher, M., Rockich, B.E., Tsai, Y.H., Mayhew, C.N., Spence, J.R., Zavros, Y., and Wells, J.M. (2014). Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature *516*, 400–404.

McKinnon, D.D., Domaille, D.W., Cha, J.N., and Anseth, K.S. (2014). Biophysically defined and cytocompatible covalently adaptable networks as viscoelastic 3D cell culture systems. Adv. Mater. *26*, 865–872.

McMurtrey, R.J. (2017). Roles of Diffusion Dynamics in Stem Cell Signaling and Three-Dimensional Tissue Development. Stem Cells Dev. *26*, 1293–1303.

Meinhardt, A., Eberle, D., Tazaki, A., Ranga, A., Niesche, M., Wilsch-Bräuninger, M., Stec, A., Schackert, G., Lutolf, M., and Tanaka, E.M. (2014). 3D reconstitution of the patterned neural tube from embryonic stem cells. Stem Cell Reports *3*, 987–999.

Miller, J.S., Stevens, K.R., Yang, M.T., Baker, B.M., Nguyen, D.H.T., Cohen, D.M., Toro, E., Chen, A.A., Galie, P.A., Yu, X., et al. (2012). Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat. Mater. *11*, 768–774.

Mongera, A., Rowghanian, P., Gustafson, H.J., Shelton, E., Kealhofer, D.A., Carn, E.K., Serwane, F., Lucio, A.A., Giammona, J., and Campàs, O. (2018). A fluid-to-solid jamming transition underlies vertebrate body axis elongation. Nature *561*, 401–405.

Morsut, L., Roybal, K.T., Xiong, X., Gordley, R.M., Coyle, S.M., Thomson, M., and Lim, W.A. (2016). Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. Cell *164*, 780–791.

Mosiewicz, K.A., Kolb, L., van der Vlies, A.J., Martino, M.M., Lienemann, P.S., Hubbell, J.A., Ehrbar, M., and Lutolf, M.P. (2013). In situ cell manipulation through enzymatic hydrogel photopatterning. Nat. Mater. *12*, 1072–1078.

Murphy, W.L., McDevitt, T.C., and Engler, A.J. (2014). Materials as stem cell regulators. Nat. Mater. *13*, 547–557.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., and Sasai, Y. (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell *10*, 771–785.

Navis, M., Martins Garcia, T., Renes, I.B., Vermeulen, J.L., Meisner, S., Wildenberg, M.E., van den Brink, G.R., van Elburg, R.M., and Muncan, V. (2019). Mouse fetal intestinal organoids: new model to study epithelial maturation from suckling to weaning. EMBO Rep. *20*. Published online February 2019. https://doi.org/10.15252/embr.201846221.

Nelson, C.M., Vanduijn, M.M., Inman, J.L., Fletcher, D.A., and Bissell, M.J. (2006). Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. Science *314*, 298–300.

Noguchi, T.K., Ninomiya, N., Sekine, M., Komazaki, S., Wang, P.C., Asashima, M., and Kurisaki, A. (2015). Generation of stomach tissue from mouse embryonic stem cells. Nat. Cell Biol. *17*, 984–993.

Norotte, C., Marga, F.S., Niklason, L.E., and Forgacs, G. (2009). Scaffold-free vascular tissue engineering using bioprinting. Biomaterials *30*, 5910–5917.

Nunes, S.S., Miklas, J.W., Liu, J., Aschar-Sobbi, R., Xiao, Y., Zhang, B., Jiang, J., Massé, S., Gagliardi, M., Hsieh, A., et al. (2013). Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat. Methods *10*, 781–787.

O'Brien, L.E., Zegers, M.M.P., and Mostov, K.E. (2002). Opinion: Building epithelial architecture: insights from three-dimensional culture models. Nat. Rev. Mol. Cell Biol. *3*, 531–537.

O'Brien, P.J., Luo, W., Rogozhnikov, D., Chen, J., and Yousaf, M.N. (2015). Spheroid and Tissue Assembly via Click Chemistry in Microfluidic Flow. Bioconjug. Chem. *26*, 1939–1949.

Ootani, A., Li, X., Sangiorgi, E., Ho, Q.T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I.L., Capecchi, M.R., and Kuo, C.J. (2009). Sustained *in vitro* intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat. Med. *15*, 701–706.

Poling, H.M., Wu, D., Brown, N., Baker, M., Hausfeld, T.A., Huynh, N., Chaffron, S., Dunn, J.C.Y., Hogan, S.P., Wells, J.M., et al. (2018). Mechanically induced development and maturation of human intestinal organoids in vivo. Nat. Biomed. Eng. *2*, 429–442.

Ranga, A., Gobaa, S., Okawa, Y., Mosiewicz, K., Negro, A., and Lutolf, M.P. (2014). 3D niche microarrays for systems-level analyses of cell fate. Nat. Commun. *5*, 4324.

Ranga, A., Girgin, M., Meinhardt, A., Eberle, D., Caiazzo, M., Tanaka, E.M., and Lutolf, M.P. (2016). Neural tube morphogenesis in synthetic 3D microenvironments. Proc. Natl. Acad. Sci. USA *113*, E6831–E6839.

Recho, P., Hallou, A., and Hannezo, E. (2019). Theory of mechanochemical patterning in biphasic biological tissues. Proc. Natl. Acad. Sci. USA *116*, 5344–5349.

Ribes, V., and Briscoe, J. (2009). Establishing and interpreting graded Sonic Hedgehog signaling during vertebrate neural tube patterning: the role of negative feedback. Cold Spring Harb. Perspect. Biol. *1*, a002014.

Rivron, N.C., Frias-Aldeguer, J., Vrij, E.J., Boisset, J.C., Korving, J., Vivié, J., Truckenmüller, R.K., van Oudenaarden, A., van Blitterswijk, C.A., and Geijsen, N. (2018). Blastocyst-like structures generated solely from stem cells. Nature *557*, 106–111.

Ronaldson-Bouchard, K., Ma, S.P., Yeager, K., Chen, T., Song, L., Sirabella, D., Morikawa, K., Teles, D., Yazawa, M., and Vunjak-Novakovic, G. (2018). Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature *556*, 239–243.

Rossi, G., Manfrin, A., and Lutolf, M.P. (2018). Progress and potential in organoid research. Nat. Rev. Genet. *19*, 671–687.

Ruiz, S.A., and Chen, C.S. (2008). Emergence of patterned stem cell differentiation within multicellular structures. Stem Cells *26*, 2921–2927.

Sachs, N., Tsukamoto, Y., Kujala, P., Peters, P.J., and Clevers, H. (2017). Intestinal epithelial organoids fuse to form self-organizing tubes in floating collagen gels. Development *144*, 1107–1112.

Sagner, A., and Briscoe, J. (2017). Morphogen interpretation: concentration, time, competence, and signaling dynamics. Wiley Interdiscip. Rev. Dev. Biol. *6*. Published online July 2017. https://doi.org/10.1002/wdev.271.

Sasai, Y. (2013a). Cytosystems dynamics in self-organization of tissue architecture. Nature *493*, 318–326.

Sasai, Y. (2013b). Next-generation regenerative medicine: organogenesis from stem cells in 3D culture. Cell Stem Cell *12*, 520–530.

Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., and Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature *459*, 262–265.

Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M., and Clevers, H. (2011a). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature *469*, 415–418.

Sato, T., Stange, D.E., Ferrante, M., Vries, R.G.J., Van Es, J.H., Van den Brink, S., Van Houdt, W.J., Pronk, A., Van Gorp, J., Siersema, P.D., and Clevers, H. (2011b). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology *141*, 1762–1772.

Serra, D., Mayr, U., Boni, A., Lukonin, I., Rempfler, M., Challet Meylan, L., Stadler, M.B., Strnad, P., Papasaikas, P., Vischi, D., et al. (2019). Self-organization and symmetry breaking in intestinal organoid development. Nature *569*, 66–72.

Shao, Y., Taniguchi, K., Gurdziel, K., Townshend, R.F., Xue, X., Yong, K.M.A., Sang, J., Spence, J.R., Gumucio, D.L., and Fu, J. (2017). Self-organized amniogenesis by human pluripotent stem cells in a biomimetic implantation-like niche. Nat. Mater. *16*, 419–425.

Shin, H.S., Hong, H.J., Koh, W.G., and Lim, J.Y. (2018). Organotypic 3D Culture in Nanoscaffold Microwells Supports Salivary Gland Stem-Cell-Based Organization. ACS Biomater. Sci. Eng. *4*, 4311–4320.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature *470*, 105–109.

Stevens, K.R., Ungrin, M.D., Schwartz, R.E., Ng, S., Carvalho, B., Christine, K.S., Chaturvedi, R.R., Li, C.Y., Zandstra, P.W., Chen, C.S., and Bhatia, S.N. (2013). InVERT molding for scalable control of tissue microarchitecture. Nat. Commun. *4*, 1847.

Stooke-Vaughan, G.A., and Campàs, O. (2018). Physical control of tissue morphogenesis across scales. Curr. Opin. Genet. Dev. *51*, 111–119.

Suga, H., Kadoshima, T., Minaguchi, M., Ohgushi, M., Soen, M., Nakano, T., Takata, N., Wataya, T., Muguruma, K., Miyoshi, H., et al. (2011). Self-formation of functional adenohypophysis in three-dimensional culture. Nature *480*, 57–62.

Tabata, Y., and Lutolf, M.P. (2017). Multiscale microenvironmental perturbation of pluripotent stem cell fate and self-organization. Sci. Rep. *7*, 44711.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature *499*, 481–484.

Takebe, T., Enomura, M., Yoshizawa, E., Kimura, M., Koike, H., Ueno, Y., Matsuzaki, T., Yamazaki, T., Toyohara, T., Osafune, K., et al. (2015). Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell-Driven Condensation. Cell Stem Cell *16*, 556–565.

Tewary, M., Ostblom, J., Prochazka, L., Zulueta-Coarasa, T., Shakiba, N., Fernandez-Gonzalez, R., and Zandstra, P.W. (2017). A stepwise model of reaction-diffusion and positional information governs self-organized human peri-gastrulation-like patterning. Development *144*, 4298–4312.

Toda, S., Blauch, L.R., Tang, S.K.Y., Morsut, L., and Lim, W.A. (2018). Programming self-organizing multicellular structures with synthetic cell-cell signaling. Science *361*, 156–162, https://doi.org/10.1126/science.aat0271.

Todhunter, M.E., Jee, N.Y., Hughes, A.J., Coyle, M.C., Cerchiari, A., Farlow, J., Garbe, J.C., LaBarge, M.A., Desai, T.A., and Gartner, Z.J. (2015). Programmed synthesis of three-dimensional tissues. Nat. Methods *12*, 975–981.

Toyoshima, K.E., Asakawa, K., Ishibashi, N., Toki, H., Ogawa, M., Hasegawa, T., Irié, T., Tachikawa, T., Sato, A., Takeda, A., and Tsuji, T. (2012). Fully functional hair follicle regeneration through the rearrangement of stem cells and their niches. Nat. Commun. *3*, 784.

Tsuda, Y., Shimizu, T., Yamato, M., Kikuchi, A., Sasagawa, T., Sekiya, S., Kobayashi, J., Chen, G., and Okano, T. (2007). Cellular control of tissue architectures using a three-dimensional tissue fabrication technique. Biomaterials *28*, 4939–4946.

Tsurkan, M.V., Wetzel, R., Pérez-Hernández, H.R., Chwalek, K., Kozlova, A., Freudenberg, U., Kempermann, G., Zhang, Y., Lasagni, A.F., and Werner, C. (2015). Photopatterning of multifunctional hydrogels to direct adult neural precursor cells. Adv. Healthc. Mater. *4*, 516–521.

van den Brink, S.C., Baillie-Johnson, P., Balayo, T., Hadjantonakis, A.K., Nowotschin, S., Turner, D.A., and Martinez Arias, A. (2014). Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells. Development *141*, 4231–4242.

Velazquez, J.J., Su, E., Cahan, P., and Ebrahimkhani, M.R. (2018). Programming Morphogenesis through Systems and Synthetic Biology. Trends Biotechnol. *36*, 415–429.

Vianello, S., and Lutolf, M.P. (2019). Understanding the Mechanobiology of Early Mammalian Development through Bioengineered Models. Dev. Cell *48*, 751–763.

Vrij, E., Rouwkema, J., LaPointe, V., van Blitterswijk, C., Truckenmüller, R., and Rivron, N. (2016a). Directed Assembly and Development of Material-Free Tissues with Complex Architectures. Adv. Mater. *28*, 4032–4039.

Vrij, E.J., Espinoza, S., Heilig, M., Kolew, A., Schneider, M., van Blitterswijk, C.A., Truckenmüller, R.K., and Rivron, N.C. (2016b). 3D high throughput screening and profiling of embryoid bodies in thermoformed microwell plates. Lab Chip *16*, 734–742.

Wang, Y., Gunasekara, D.B., Reed, M.I., DiSalvo, M., Bultman, S.J., Sims, C.E., Magness, S.T., and Allbritton, N.L. (2017a). A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium. Biomaterials *128*, 44–55.

Wang, Y., Kim, R., Gunasekara, D.B., Reed, M.I., DiSalvo, M., Nguyen, D.L., Bultman, S.J., Sims, C.E., Magness, S.T., and Allbritton, N.L. (2017b). Formation of Human Colonic Crypt Array by Application of Chemical Gradients Across a Shaped Epithelial Monolayer. Cell. Mol. Gastroenterol. Hepatol. *5*, 113–130.

Warmflash, A., Sorre, B., Etoc, F., Siggia, E.D., and Brivanlou, A.H. (2014). A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. Nat. Methods *11*, 847–854.

Wei, C., Larsen, M., Hoffman, M.P., and Yamada, K.M. (2007). Self-organization and branching morphogenesis of primary salivary epithelial cells. Tissue Eng. *13*, 721–735.

Wells, J.M., and Spence, J.R. (2014). How to make an intestine. Development *141*, 752–760.

Workman, M.J., Mahe, M.M., Trisno, S., Poling, H.M., Watson, C.L., Sundaram, N., Chang, C.F., Schiesser, J., Aubert, P., Stanley, E.G., et al. (2017). Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nat. Med. *23*, 49–59.

Wylie, R.G., Ahsan, S., Aizawa, Y., Maxwell, K.L., Morshead, C.M., and Shoichet, M.S. (2011). Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nat. Mater. *10*, 799–806.

Xiang, Y., Tanaka, Y., Patterson, B., Kang, Y.J., Govindaiah, G., Roselaar, N., Cakir, B., Kim, K.Y., Lombroso, A.P., Hwang, S.M., et al. (2017). Fusion of Regionally Specified hPSC-Derived Organoids Models Human Brain Development and Interneuron Migration. Cell Stem Cell *21*, 383–398.

Xiang, Y., Tanaka, Y., Cakir, B., Patterson, B., Kim, K.Y., Sun, P., Kang, Y.J., Zhong, M., Liu, X., Patra, P., et al. (2019). hESC-Derived Thalamic Organoids Form Reciprocal Projections When Fused with Cortical Organoids. Cell Stem Cell *24*, 487–497.

Xue, X., Sun, Y., Resto-Irizarry, A.M., Yuan, Y., Aw Yong, K.M., Zheng, Y., Weng, S., Shao, Y., Chai, Y., Studer, L., and Fu, J. (2018). Mechanics-guided embryonic patterning of neuroectoderm tissue from human pluripotent stem cells. Nat. Mater. *17*, 633–641.

Yajima, Y., Lee, C.N., Yamada, M., Utoh, R., and Seki, M. (2018). Development of a perfusable 3D liver cell cultivation system via bundling-up assembly of cell-laden microfibers. J. Biosci. Bioeng. *126*, 111–118.

Yui, S., Azzolin, L., Maimets, M., Pedersen, M.T., Fordham, R.P., Hansen, S.L., Larsen, H.L., Guiu, J., Alves, M.R.P., Rundsten, C.F., et al. (2018). YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. Cell Stem Cell *22*, 35–49.

Zagorski, M., Tabata, Y., Brandenberg, N., Lutolf, M.P., Tkačik, G., Bollenbach, T., Briscoe, J., and Kicheva, A. (2017). Decoding of position in the developing neural tube from antiparallel morphogen gradients. Science *356*, 1379–1383.

Zhao, W., Loh, W., Droujinine, I.A., Teo, W., Kumar, N., Schafer, S., Cui, C.H., Zhang, L., Sarkar, D., Karnik, R., and Karp, J.M. (2011). Mimicking the inflammatory cell adhesion cascade by nucleic acid aptamer programmed cell-cell interactions. FASEB J. *25*, 3045–3056.