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Harnessing synthetic biology to engineer organoids and tissues

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SUMMARY

The development of an organism depends on intrinsic genetic programs of progenitor cells and their spatiotemporally complex extrinsic environment. *Ex vivo* generation of organoids from progenitor cells provides a platform for recapitulating and exploring development. Current approaches rely largely on soluble morphogens or engineered biomaterials to manipulate the physical environment, but the emerging field of synthetic biology provides a powerful toolbox to genetically manipulate cell communication, adhesion, and even cell fate. Applying these modular tools to organoids should lead to a deeper understanding of developmental principles, improved organoid models, and an enhanced capability to design tissues for regenerative purposes.

RECONSTITUTING DEVELOPMENTAL PROCESSES *IN VITRO*

Understanding the development of an organism or a complex organ is not feasible without breaking the process down into smaller, approachable parts-stages, lineages, tissues, pathways-and then reconstructing these parts to understand the process as a whole. In addition to the progenitor cells that give rise to a tissue, recapitulation of developmental processes should also involve the complex and dynamic environment that surrounds these cells. Within this environment, neighboring cells and molecular matrices present a host of chemical and mechanical signals in a spatially and temporally defined manner (Figure 1A).

Over the past few years, a fundamental advance in developmental biology has been the use of organoid systems. Organoids are 3D structures typically derived from one or multiple types of progenitor cells and their progeny, which display the capacity to self-organize into structures that recapitulate the *in vivo* properties of a chosen tissue or organ (Figure 1B). Organoids can be applied to explore or mimic diverse developmental systems and have expanded our understanding of cell differentiation and the interactions between tissues

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DECLARATION OF INTERESTS

W.A.L. holds equity in Gilead and Intellia and is a scientific advisor for Allogene. He is on the board of directors for the Burroughs Wellcome Foundation. O.D.K. is a founder of and holds equity in Stemodontics, Inc.

and their environment. Beyond this, they also provide a promising clinical tool for disease modeling and regenerative medicine.[1] Organoids can be generated from adult mammalian tissues or through differentiation of pluripotent stem cells. As an example, human intestinal organoids are derived from induced pluripotent stem cells by treating with a sequence of growth factors that drive their differentiation into interdependent lineages, including gut epithelium and mesenchyme.[2] Other well-established examples include optic cup organoids, kidney organoids, mammary gland organoids, and pulmonary organoids.[1] More recently, exciting organoid models of early embryogenesis (sometimes called embryoids or gastruloids) have been generated from embryonic stem cells (ESCs).[3–6]

Several approaches have been used to drive organoid formation (Figure 1C). First, "soluble morphogens" can be added to the culture media to mimic the signals the progenitor cells would receive *in vivo.* Most organoid protocols have at their core a sequence of morphogen stimulation steps that drive a substantial fraction of progenitor cells into the relevant tissue lineages.[1] Simple addition of morphogens to the media, however, does not mirror the complex spatial geometry in which these signals are normally presented *in vivo.* Thus, resulting organoids can lack the complex asymmetric structure of native tissue. One approach to address this limitation is to use engineered materials and devices to provide some of the mechanical and spatial environmental cues present *in vivo* (see Brassard and Lutolf[7,8] for review). Approaches like 3D bioprinting[9] or microstampin,[10,11] for example, allow the recreation of native-like tissue topologies *in vitro.* However, these approaches are often technically challenging and limited in their degree of control.

Despite the progress of the past few years, organoids still lack the complex structures of their functional counterparts *in vivo.* The process of organoid development is notoriously heterogeneous, often showing low reproducibility among individual organoids. Generating organoids is also expensive in terms of resources and time. A key challenge in the field remains to improve the robustness, precision, and efficiency of organoid development.

To address these challenges, new approaches must be established to better mimic an *in vivo* developmental milieu. For instance, morphogen signals would ideally be delivered in spatially asymmetric and dynamic ways. In this perspective article, we propose that synthetic biology could be harnessed to addresss these issues. We first define synthetic biology and how it applies to developmental systems. We then review emerging synthetic biology tools and discuss how each of these could be applied to organoid systems to better recapitulate, control, or rewire development.

APPLYING SYNTHETIC BIOLOGY TOOLS TO DEVELOPMENTAL BIOLOGY

The emerging field of synthetic biology has much to contribute to developmental biology, especially in the effort to recapitulate development *in vitro* through organoid growth. Synthetic biology uses genetically encoded modules to engineer cellular behaviors. A growing number of synthetic biology tools allow researchers to modulate user-defined cell-cell communication, including juxtacrine and paracrine signaling, cell adhesion, and cell fate. We can envision at least two approaches to engineering organoids. On the one hand, direct engineering of progenitor cells using optogenetic or chemogenetic tools can be used to

introduce asymmetric, user-controlled induction signals.[12,13] Alternatively, non-progenitor cells could be engineered as organizer cells that act as a "living" extrinsic environment.[12] An engineered synthetic cellular environment could dynamically communicate with and instruct progenitor cells, thus recapitulating more of the complexity of the *in vivo* environment. Presenting signals via engineered cells might therefore provide a way to improve the precision and reproducibility of organoid generation.

Here, we define synthetic biology as the use of cellular engineering to purposefully redesign a cell's function or behavior. Synthetic biology serves two complementary goals. First, it provides an approach to better understand the function of individual biological components and how they hierarchically work together. Second, it allows us to design, modulate, or improve physiological functions. Inherently, synthetic biology involves manipulation at a wide range of scales. For instance, protein domains can be mutated to modulate structure at the tissue or organ scale. As genome-editing techniques become more widespread and efficient,[14,15] engineering tools also become more viable, and we can broaden their prospective application to additional cell types and tissues.

Given our improved ability to synthetically alter cells, we must establish how best to apply such approaches to development and tissue formation. What are the tools that would be most useful for building and modifying tissue structures? In the context of *in vitro* development, synthetic biology can provide unique capabilities to reconstitute spatial and dynamic signaling, modify cell-cell communication, and construct complex "niches" around tissues of interest. Below, we will review existing and emerging synthetic biology tools and how they might aid in the construction of *In vitro* models of development. Specifically, we touch on five classes of cell engineering tools: optogenetic/chemogenetic tools, short-range (juxtacrine) cell-cell communication systems, long-range cell communication systems, cell adhesion molecules, and cell-fate switches.

APPLYING EMERGING SYNTHETIC BIOLOGY TOOLS TO ORGANOIDS

Optogenetic tools

Optogenetic proteins are stimulated by light and offer user-defined control over a cell that is engineered to express the optogenetic receptor. In principle, spatially and temporally controlled activity within a tissue could be generated either by targeted illumination or by restricting the type of cells that are made optogenetically responsive (Figure 2A). Various optogenetic methods have been developed to control cells and their activity, and these have been extensively reviewed elsewhere.[16–18]

Due to the flexibility of light illumination that allows high spatial and temporal precision of stimulation, optogenetic approaches have key advantages over traditional pharmacological manipulation. For example, researchers have developed light-inducible chimeric receptors by fusing a photo-inducible protein to the cytoplasmic region of morphogen receptors. The light-oxygen-voltage domain of photoreceptors or the photosensory region of Cryptochrome2 are typically used for a photo-inducible protein. These domains oligomerize upon light stimulation, inducing the activation of the receptors. Within mammalian cells, optogenetic chimeric receptors have been developed to induce diverse morphogen signaling

pathways, including Wnt/β-catenin, Wnt/Frizzled7, transforming growth factor beta (TGFβ), bone morphogenetic protein (BMP), Nodal, fibroblast growth factor (FGF), and extracellular signal-regulated kinase (ERK).[13,20–23] Such tools have been used in proof-of-principle experiments to disrupt development or rescue genetic defects by replacing morphogen inputs with light inputs in *Drosophila* and zebrafish embryos.[21,24] Optogenetic tools are already being applied to mammalian organoid systems. One such study used optogenetics to stimulate Wnt signaling in subpopulations of ESCs cultured in 3D, thereby inducing differentiation, migration, and cell sorting reminiscent of human gastrulation.[13] The ability to mosaically induce Wnt signaling with light highlights the importance of differential Wnt activity in this early developmental event. Likewise, optogenetic tools have been used to locally induce Sonic hedgehog (SHH) signaling in neuronal organoids, creating spatial differentiation domains within the 3D structure.[25] Optogenetic induction of signaling cues can therefore create tissue asymmetries in a more controllable way than adding a drug in the culture media. However, optogenetic tools can control more than morphogen signaling. An example of this is OptoShroom3, a light-inducible version of the protein Shroom3, which regulates apical constriction of epithelia.[26,27] When OptoShroom3 is introduced into mouse and human neuronal organoids, it drives morphogenic events like epithelial thickening and lumen reduction. These early studies open the door to a wide range of possibilities to use light to manipulate patterning in organoids. The precise control of optogenetic activity in a reproducible and high-throughput manner in 3D, however, remains a challenge.

Cell communication through short-range (juxtacrine) signaling

During development, cells communicate with one another to make decisions about differentiation and morphogenesis. Thus, tools that control user-defined juxtacrine communication would be powerful. Synthetic biologists have engineered various types of receptors that mediate customized sense-and-response programs and link extracellular target recognition and intracellular signaling to user-defined responses. Other reviews more comprehensively discuss synthetic receptors.[28] Here, we focus on synthetic receptors relevant for controlling organogenesis. For example, the synthetic Notch-based system (synNotch) is a powerful tool for manipulating cell-cell interactions. In the synNotch system, a sender cell presenting user-defined antigen is recognized by a receiver cell expressing synNotch. synNotch is composed of an extracellular antigen-recognition domain (typically a single-chain variable fragment, scFv or nanobody), a Notch core transmembrane domain containing proteolytic cleavage sites, and an intracellular transcription factor. When synNotch receptors recognize their target antigen, they induce custom transcriptional regulation through release of an engineered transcription factor (Figure 2B). A number of other synNotch variants have been developed, including ones with reduced noise and tunable functions.[29,30]

We envision several applications of engineered juxtracrine signaling in organoids. SynNotch can help monitor cell interactions in 3D tissues by serving as a reporter of direct cell-cell contact. In 2D, activated receiver cell rings that surround a clone of sender cells can be created.[31] This ability to use synNotch as a neighbor-labeling system was demonstrated in 3D in early mouse embryo aggregate cultures.[32] Alternatively, the down-stream response to cell-cell contact can be adapted to stimulate cell-fate decisions. This strategy was applied

to mouse ESCs engineered to activate the neuronal differentiation factor Neurogenin1 with synNotch.[32] When these receiver ESCs were put in contact with sender cells, the boundary that formed between the two induced neuronal differentiation. Similarly, synNotch could be used to control other differentiation pathways, induce the production of morphogens or receptors, or induce expression of adhesion molecules that lead to cellular rearrangements.

Cell communication through long-range (diffusible) signaling

Diffusible morphogens and their gradients are central to developmental biology. Various tools are emerging that allow generation of user-defined long-range cell-cell communication. A growing number of synthetic receptors can recognize soluble factors and activate user-defined gene-regulation programs, thereby flexibly rewiring input and output. Some are based on endogenous transduction receptors, including the synthetic receptors TANGO,[33] CHA-CHA,[34] MESA,[19] and GEMS.[35] These can be used as biosensors that report on the activity of a specific pathway. For example, TANGO receptors report on G-protein-coupled receptor activity in HEK cells,[33] and FRET-based biosensors indicate ERK activity in patient-derived intestinal organoids.[36] Alternatively, the ligand-binding domain can be rewired, as can downstream effectors to drive recombination[34] or trans-gene expression.[35] Although the application of these synthetic receptors in developmental settings has been limited so far, they transmit spatial information about the local environment and can be used to build programmable multicellular systems.

Another approach to building synthetic long-range communication systems is to engineer an orthogonal morphogen. Orthogonal morphogens enable us to explore signaling circuits and positional information without interference from complex endogenous circuits. An arbitrary molecule, such as GFP, can be converted into a morphogen. Soluble GFP secreted by a sender cell can be detected by a receiver cell in multiple ways, such as by designing an artificial receptor with a GFP binding nanobody at its extracellular domain. Upon binding to GFP, the artificial receptor would mediate signaling or transcriptional responses. Taking this approach, a diffusible version of the synNotch cell-cell communication system has been created.[37] Although, in this setting, soluble GFP did not activate synNotch because it requires the mechanical force of a membrane-tethered ligand, this limitation was overcome by tethering diffusible GFP to the cell surface with an anchor protein (Figure 2C). This strategy imitates native morphogens that interact with the extracellular matrix (ECM) or cell surfaces to achieve a more stable gradient.

Importantly, synthetic morphogens allow greater control over the production and interpretation of a diffusible signal.[37,38] Using single or multiple morphogens, circuits can be programmed to induce *de novo* multidomain tissue patterns *in vitro.* These patterns include spatially distinct expression domains, such as those resembling Wolpert's classic French Flag pattern.[37] Some important technical aspects to consider when building synthetic diffusible signals include signaling range, signal capture in the microenvironment, receptor affinity, and interactions or feedback loops between morphogen pairs.

A similar synthetic diffusible morphogen network has been reported *in vivo* in *Drosophila.*[38] In this instance, a synthetic GFP gradient was used to substitute for a natural morphogen to organize growth and patterning of the fly wing.[38] To rewire the endogenous signaling

pathway, researchers made chimeric receptors by fusing an anti-GFP nanobody to the receptors of BMP homolog Decapentaplegic (Dpp). As endogenous Dpp dimers bind to two pairs of their receptors, GFP dimers were created to activate the same signaling cascade. To mimic the Dpp expression pattern, those GFP dimers were secreted from the patched locus, a genomic region that is activated along the anterior-posterior boundary. In such a synthetic GFP gradient system, GFP could replace Dpp to organize patterning and growth *in vivo.* One limitation of this system is leakage, possibly due to the free diffusion property of GFP. Of note, co-expression of non-signaling receptors reduced this leakageby capturing extra GFP and expanded the gradient length scale to wild-type level. Such proof-of-concept studies open the door for a wide spectrum of applications in organoids.

Engineered cell adhesion and multicellular architectures

The differential adhesion hypothesis, dating back to the 1960s, stipulates that, much like liquids, tissues segregate based on differences in surface tension that result from differences in intercellular adhesion. Varying the *expression level* of endogenous adhesion molecules, namely cadherins, or varying the *type* of cadherin expressed (E–, N-, or P- cadherin), results in autonomous sorting of fibroblasts.[39–43] Weakly adherent cells will form a loose "shell" around more strongly adherent tissues, which tend to cluster together. Similarly, populations of cells expressing different types of cadherins that have strong homotypic but weak heterotypic binding strength will segregate into different domains. These simple principles can be extended beyond fibroblasts to tightly regulate autonomous rearrangements of different cell types *in vitro.*

In addition to natural adhesion molecules, orthogonal cell-cell adhesion toolkits are being developed (Figure 2D).[44,45] In synthetic adhesion systems, cell-cell interaction selectivity is provided by a membrane-displayed nanobody and its target antigen. *E. coli* expressing these types of synthetic adhesion molecules showed multicellular self-assembly, such as phase separation, coaggregation bridging, and sequential layering.[45] Recent work from our lab shows that it is possible to engineer a set of highly flexible synthetic adhesion molecules for mammalian cells.[46] How such synthetic adhesion molecules engage the intracellular cytoskeleton is a major determinant of the type of morphological interface that is formed and its strength. By tuning the relative strength of homotypic and heterotypic interactions in synthetic adhesion systems, and by developing a broader set of cell-adhesion tools, we created precisely defined, highly reproducible self-organizing multicellular assemblies.

Engineering cell adhesion has already been used to increase the efficiency of self-organization in so-called "ETX" embryoids. In this model, ESCs are recombined with trophoblast stem cells and extraembryonic endoderm. When these cells self-organize, the resulting structures form a lumen and induce a gastrulation-like epithelial-to-mesenchymal transition in the ESCs, resulting in mesoderm and definitive endoderm specification—similar to embryos *in vivo.*[47,48] However, this three-component assembly has limited efficiency, with only about 15% of structures rearranging into the proper conformation. Engineering each of these layers to express an optimal cadherin code improved this efficiency almost 3-fold,[49] highlighting how synthetic biology can be used to overcome the limitations of organoid models. Beyond improving native structure, we can imagine

using engineered cell adhesion to create multicellular structures or induce rearrangements over time in organoids. To this end, orthogonal adhesion molecules, which are less likely to interfere with endogenous cell-cell interactions, will be particularly useful.

Engineered fate control

Proliferation, cell death, and differentiation are basic processes that shape the transition from a small number of stem cells to a larger, stable, functional tissue. The ability to grow while controlling the number of cells and ratio of cell types is a classic challenge in developmental and synthetic biology. Early attempts to regulate cell growth rely on inducing apoptosis. For instance, one study reports a suicide switch circuit that includes modified caspase-9 fused to FKBP to allow conditional dimerization using a small molecule, AP1903.[50] When the cells receive the small compounds, caspase-9 activates downstream effector caspases, such as caspase-3, and induces apoptosis. Such a suicide strategy can control the potent activity of an engineered T cell therapy.[51,52] Using a drug-controlled switch, however, requires external human operation and is not amenable to autonomous population control. How can we build a multicellular system that autonomously senses and controls the size of a cell population?

Another study borrowed ideas from the principles of bacterial quorum sensing and built synthetic mammalian quorum-sensing circuits based on the plant hormone auxin.[53] An important issue in engineering population control is evolutionary robustness. When a cell's growth is limited, critical mutations that escape the population control circuit are evolutionarily favored. To overcome this issue, the authors constructed a paradoxical control that both stimulates and inhibits cell proliferation at different concentrations and cell death with different sensitivities to regulate population size. Although we have not seen these types of circuits applied to organoids to date, they could be harnessed to control the growth of organoids, regulate their size, or eliminate synthetic cells once they have performed a desired organizer role.

During development, cells increase not only their population size but also the diversity of cell types. Classical strategies to genetically drive differentiation are to artificially induce master regulators in mammalian cells. These approaches often have been limited to two-state systems and are challenging to expand to control multi-state systems. Gene circuits for multi-state control were inspired by endogenous fate-decision mechanisms.[54] In pluripotent cells, fate-decision factors, such as SOX2, OCT4, and SOX17, are connected in positive autoregulatory feedback loops, but heterodimers of these factors often have opposite effects on their target genes. Based on this observation, an orthogonal gene circuit (MultiFate) was developed that controls multiple stable mammalian states over generations (Figure 2E). MultiFate uses engineered zinc finger transcription factors that self-activate their own transcription as homodimers and mutually inhibit one another when they form heterodimers. Their homo- or heterodimerization can be controlled by small molecules. Thus, by expressing only three types of such synthetic transcription factors, the cell lines can generate seven distinct cell states. Of note, this MultiFate system can, in principle, be combined with other synthetic circuits mentioned above, which enables cells to make a series of fate choices in multicellular systems as in normal tissue formation. When applying

these tools to organoids, it is important to consider their tunability. How much signal is required to induce a response, and what will be the intensity or duration of the response?

COMBINING MODULAR TOOLS TO CREATE MORE COMPLEX DEVELOPMENTAL SYSTEMS

To date, most of these emerging tools have only been applied to simple cellular systems as proof of concept. The greater potential of applying synthetic biology to developmental models has yet to be realized. We imagine a spectrum of potential goals for synthetic development. On one end of this spectrum, these approaches could be used to improve the process of generating native tissue structures. On the other end of the spectrum, it may be possible to build non-natural tissue structures that, for example, are better suited for selected functions. To alter tissue structure, we could directly engineer and refine the intrinsic developmental programs within progenitor cells. Alternatively, we could engineer synthetic “niche” or “organizer” cells that change the guiding instructions provided to native progenitor cells. Even small modifications in how we approach *in vitro* development could overcome some of the current limitations of organoid models. Below, we discuss how synthetic biology tools might improve reproducibility, refine the structure and function of organoid systems, and allow spatiotemporally defined delivery of signals to the tissue.

Improving efficiency and reproducibility of current organoid models

We have discussed how cell-adhesion tools can make self-organization of multi-layered ETX embryoids more efficient,[49] and sophisticated culture approaches have enabled embryos to complete critical events including gastrulation *in vitro*.[6,55,56] Likewise, engineering the cell fate of ESCs by inducing overexpression of GATA4 turns ESCs into extraembryonic cell types.[57] These cells arrange around each other to form gastrulating structures that faithfully recapitulate features of *in vivo* development up to day 8.5 post fertilization. Typically, *in vitro* differentiation schemes involve treating the tissue with a precise sequence of signals at specific timepoints. Instead, juxtacrine communication tools could be adapted to create ligand-responsive signals and networks that make spatiotemporal regulation more autonomous. For instance, as new cell types emerge from differentiation, their unique ligands could be sensed to induce dynamic signals, which drive a new phase of differentiation. Such an ambitious combination of synthetic biology tools could eventually serve to recreate the scheme of how different organizers emerge over the course of *in vivo* development.

Refining structure and function of organoids

Cell-cell adhesion tools provide an important avenue to define organoid structure. In one example of self-organizing tissues, our group took fibroblasts and engineered them with circuits in which sender cells activate cadherin expression in receiver cells using synNotch. Variants of such multicellular circuits resulted in highly reproducible, multi-layered self-organizing patterns where each cell acquires its adhesive/sorting properties by a network of self-reinforced communication with its neighbors[41] (Figure 3A). One could harness this approach to enhance the reproducibility of self-organizing, multi-tissue organoids, such as the ETX embryoids.[47,49,58] Alternatively, synthetic self-organizing cells can be used to

"mold" cells of interest into the desired conformation. For example, one group established a circuit where synthetic adhesion is used to pattern two cell populations in monolayer culture.[43] Next, a drug-inducible apoptosis signal is activated, leaving only one cell type as a reticulum-like network.[43] Applying such an approach to 3D organoids in a way that mimics existing developmental events could improve the morphological patterning of existing organoid structures. Organoids of tissues with distinct geometric organization of cell types (e.g., liver, kidney) could benefit from these approaches.

Driving spatiotemporally defined delivery of signals

We have touched on using optogenetic tools to induce spatially or temporally restricted signals. Several groups have used alternative cell engineering approaches to generate initial morphogen asymmetry within a progenitor cell cluster to drive more native-like development. One study used a pre-plated "signaling center" that produces the morphogen SHH to specify positional identity in forebrain organoids plated over this node[59] (Figure 3B). A similar approach using a Wnt/Nodal-producing signaling center has also been used to drive differentiation of embryoids from mouse ESCs (mESCs).[60] To keep the signaling center localized in relation to the tissue, Glykofrydis et al. produced WNT from a self-organizing cluster of cells expressing high levels of P-cadherin.[12] Thus, combining tools to control cell adhesion and morphogen signaling, they have created a self-sorting signaling center that is sufficient to drive symmetry breaking in mESCs cultured in 3D. Using these tools, one could, in principle, engineer signaling centers that adopt a variety of conformations and provide a range of signals for any tissue organoid. Or, instead of endogenous morphogens, synthetic morphogens could be adapted to pattern 3D tissues in a tightly controlled manner (Figure 3C).[37,38] Synthetic "niche" cells could remove the need for expensive morphogens in the media by continuously producing the necessary signals. They would act like feeder cells, which have been used in the early days of developmental biology, but with greater control over where and when the signals are produced and over how the signals are presented.

A VISION OF PREDICTIVE SYNTHETIC DEVELOPMENT

Synthetic development is still in its early phases, but it has vast potential. This can be illustrated by comparing it to how molecular engineering has matured as a field: today, one can computationally design the formation of large complex molecular structures (10–100 nm scale) composed of either synthetic proteins or nucleic acids (e.g., DNA origami structures). This more recent capability was built upon our deep understanding of the interactions, architecture, and driving forces of molecular structures. In a similar way, synthetic biology might allow us to predict and design more complex tissue morphologies and functions in the future (Figure 4). Designing a molecular structure, however, is arguably a simpler, more constrained problem. It is focused on identifying a sequence whose free-energy minimum matches the target structure. In contrast, designing a developing tissue involves engineering a dynamic circuit that only manifests itself when the relevant cells properly communicate with one another through sequential steps. Attempting to hierarchically program synthetic organoids will undoubtedly force us to gain a much deeper understanding of cellular self-organization first. This line of investigation opens up important questions. For example, how

can developmental pathways be robustly designed to avoid getting trapped in the wrong, off-pathway structures?

As the field of predictive tissue programming matures, so should our ability to generate more native-like and functional organoids with higher complexity and reproducibility. These may be superior disease models, allowing for better drug testing *in vitro.* Improved organoids might also provide substrates for transplantations. Alternatively, we might be able to design modified organoids with improved or purposely altered functionalities. Such organoids could potentially carry out therapeutic roles, while others might execute combined functions. Advanced organoids might be easier to transplant and/or help avoid rejection. It may even be possible to engineer *in situ* regeneration at sites of injury.

There remain many challenges and pitfalls on the way to achieving this vision. As stated before, designing developmental programs is inherently a far more multi-scale problem than designing molecular structures. Moreover, there are facets of development that we have few tools to incorporate into synthetic circuits. For example, the ECM is an important participant in development. It is produced and modified by sender cells, and it presents signals to receiver cells via its molecular components and mechanical properties. Yet, because of their large size and complex assembly, we still have limited ways to program the production, modification, and signal transduction of ECM proteins. Dynamically incorporating ECM generation and modulation into developmental models will be essential, but, at present, this is far more difficult than engineering a cell to produce or respond to a morphogen. Combining synthetic biology solutions with those emerging from other rapidly advancing fields such as engineered biomaterials (i.e., using “designer” matrices[61]) could synergistically improve the complexity of organoid models. As another example, technologies allowing spatial positioning of cells (e.g., bioprinting,[9] microstamping,[10,11] or DNA-programmed assembly of cells[62]) can serve to lay out a static starting point for engineered organoids to model development.

Finally, new ethical issues will undoubtedly arise as the capability to generate tissues *in vitro* or to custom design them increases. The field will have to move forward in a cautious and transparent way, considering carefully what goals we should take on in this exciting but still very young enterprise.

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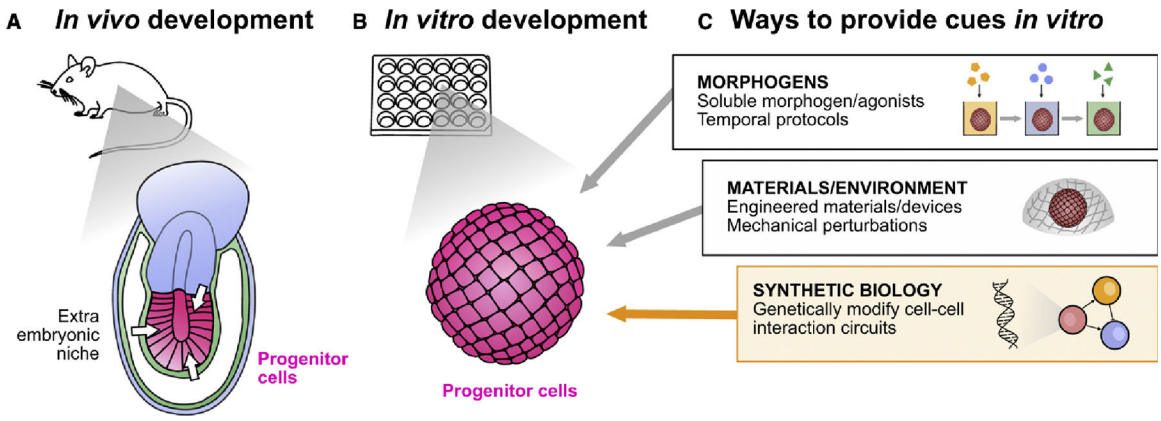

Figure 1. Reconstituting spatiotemporal complexity of development *in vitro*

(A) Schematic of *in vivo* development of a gastrulating embryo with the epiblast (progenitor cells, in pink) receiving chemical and mechanical cues from the extraembryonic tissues surrounding it.

(B) In contrast, classic *in vitro* models of development consist of isolated progenitor cells and lack the environmental cues that drive native development.

(C) These cues are supplemented *in vitro* by adding soluble morphogens to the organoid media to favor specific cell fates. Organoids can also be embedded hydrogels or materials that can provide desired mechanical cues.

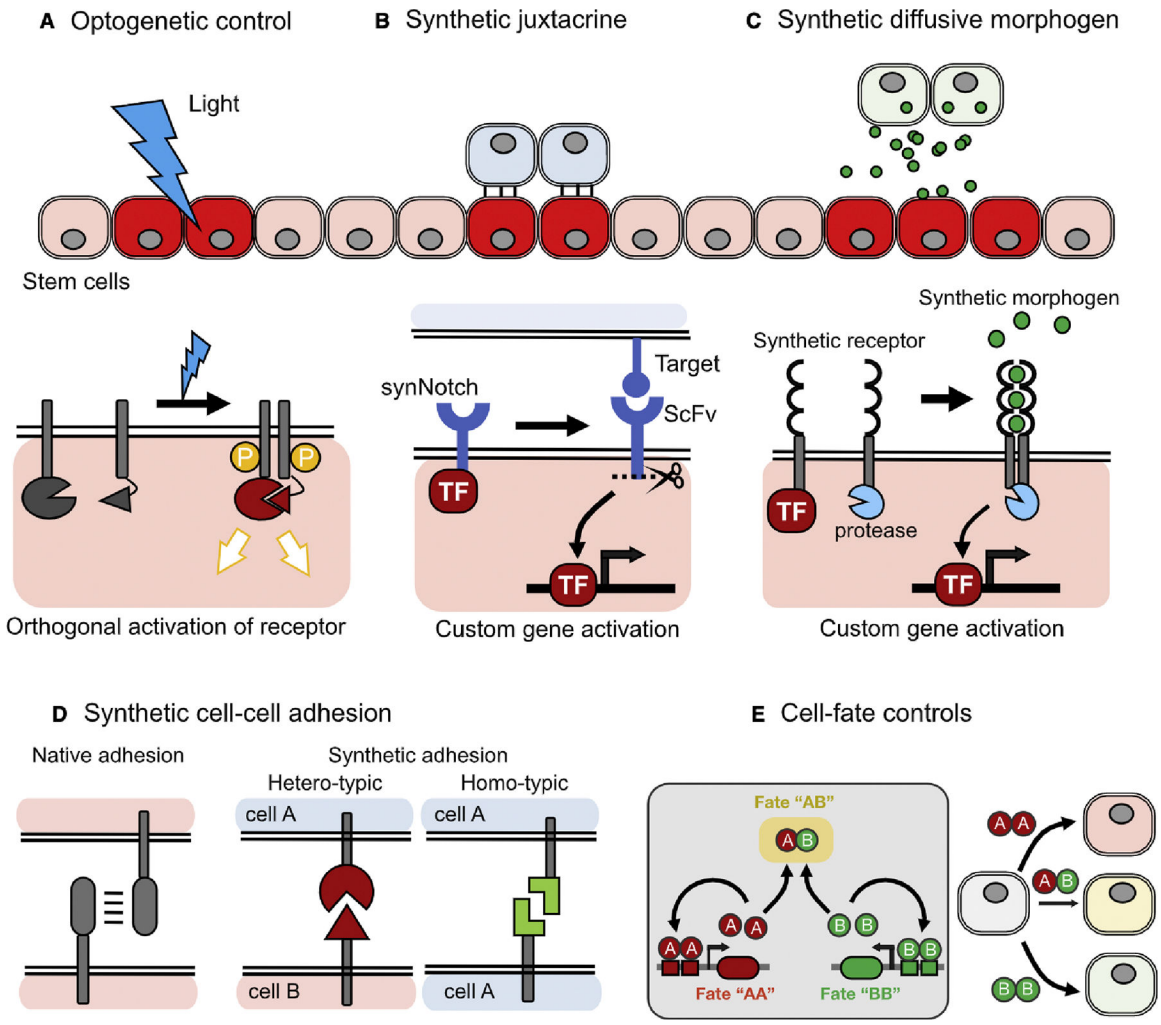

Figure 2. Emerging toolbox to create regulatory cell-cell interactions

(A) Schematic illustration of optogenetic chimeric receptors. When cells express these receptors, they respond to light and activate the downstream signaling without a morphogen. Optogenetic chimeric receptors are typically designed as the cytoplasmic regions of morphogen receptors fused with a light-responsible element, such as a light-oxygen-voltage domain. Light triggers hetero- or homodimerization of the receptors, which activates the downstream signaling.

(B) Diagram of synNotch receptor system. synNotch is composed of an extracellular antigen recognition domain, such as scFv, a central regulatory domain in transmembrane domain, and an orthogonal transcription factor (TF). When synNotch detects the antigen on the sender cells (blue), the TF s released by cleavage.

(C) Illustration of synthetic diffusive morphogen system using a synthetic receptor, such as MESA.[19] In this system, synthetic receptor dimerization occurs upon synthetic ligand binding, and such dimerization causes cleavage of the intracellular domain of the receptor, releasing the TF to activate its target gene. In all figures, activated cells are colored in red.

(D) Design of synthetic cell-cell adhesion molecules. Extracellular domains of native adhesion proteins are replaced by specific protein-protein interactions, such as GFP and anti-GFP nanobody.

(E) Multi Fate system is a synthetic circuit that controls multi-fates of mammalian cells for a long term. In MultiFate, TFs (“A” and “B”) homodimerize to self-activate and mutually inhibit one another by heterodimerization.

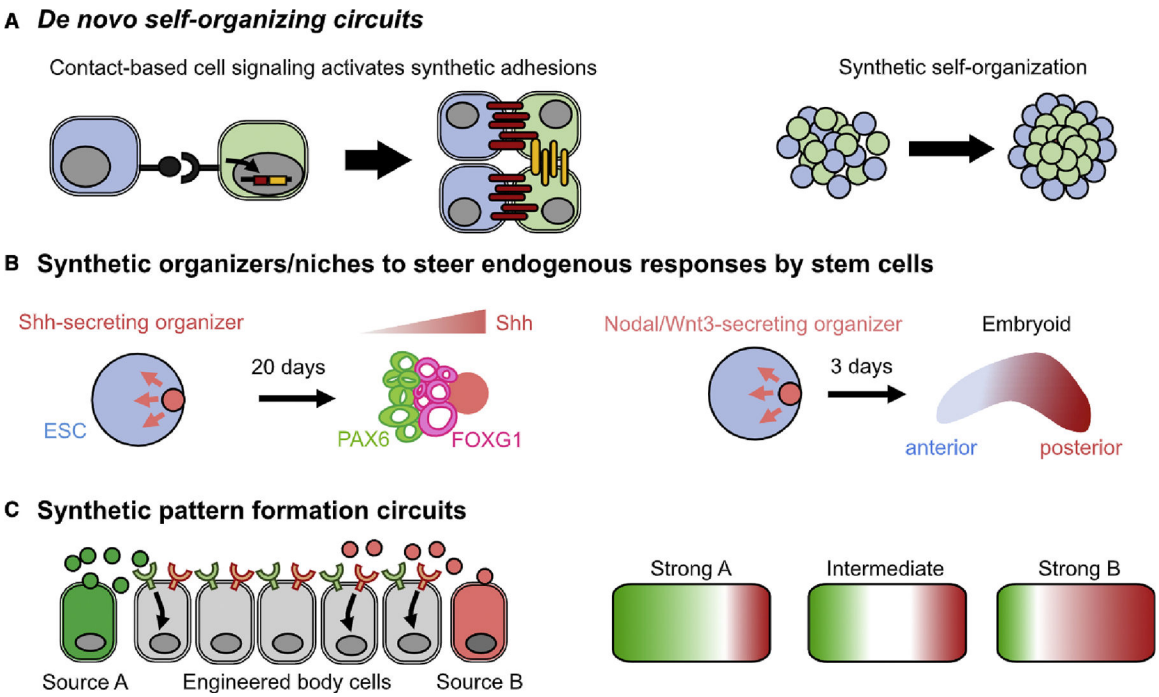

Figure 3. Constructing simple synthetic developmental networks with modular cell-cell interaction

(A) Schematic illustration of engineering self-organizing multi-layered spheroids. Sender cells expressing a ligand (blue) induce a synNotch-expressing receiver cell(green) to cell(green) to express homotypic adhesion molecule (yellow) and heterotypic adhesion molecule (red). When we mix these cell types together, the cell population starts to organize to form a core aggregate of receiver cells surrounded by sender cells (blue).

(B) Examples of user-defined organizers. (Left) Shh-producing hPSC aggregate is used as a local source of Shh, acting as one pole of the developing forebrain organoid. (Right) Strategy to generate a Wnt and Nodal gradient in an embryoid model. mESCs treated with BMP4 are used as an engineered morphogen signaling center. mESCs close to the signaling center differentiate into mesoderm, resembling the posterior region of a mouse embryo.

(C) The synthetic diffusible communication system generates an artificial morphogen gradient. By modulating expression levels of morphogens, we can tune the gradient patters (right).

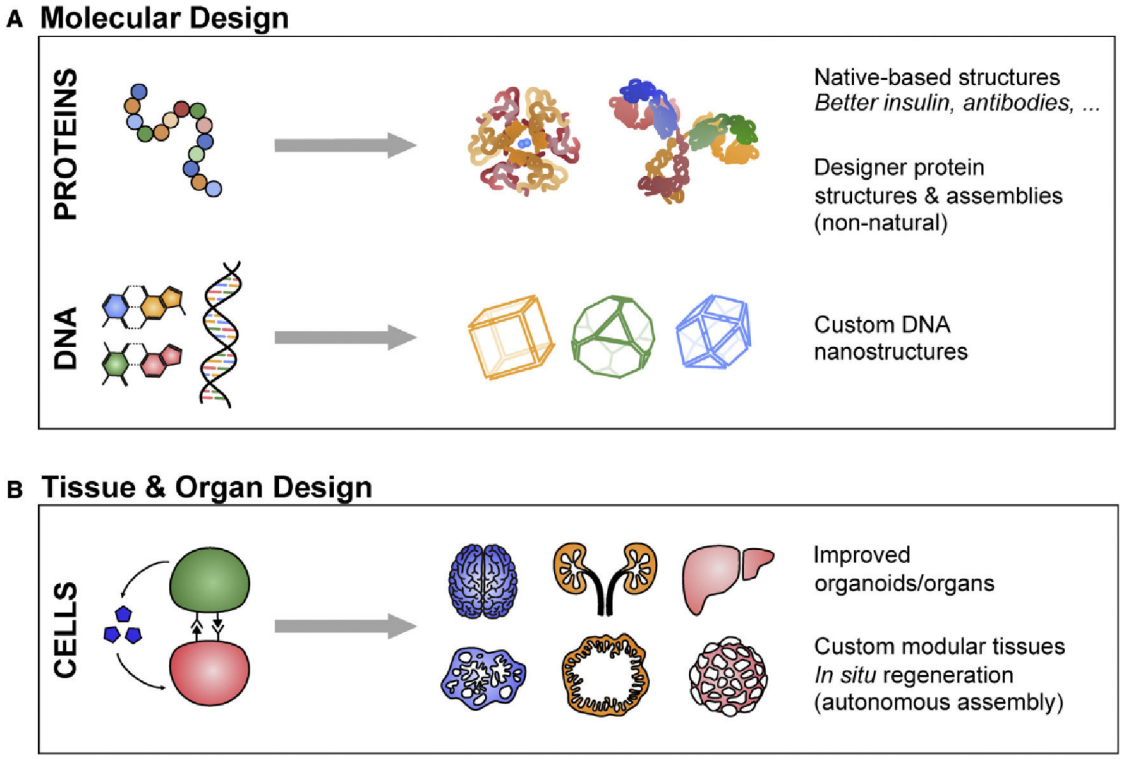

Figure 4. Tissue or organ design could follow the path of the more mature field of molecular design

(A) As the field of molecular engineering matures, we have improved our understanding of complex molecular structures composed of synthetic proteins or nucleic acids. Understanding the interactions and driving forces of molecular structure allows for the development of improved native-based structures and the design of custom protein or DNA structures that do not already exist in nature.

(B) Likewise, as the field of tissue development matures, so should our ability to predict cell fates and behaviors and to generate more native-like and functional organoids with higher complexity and reproducibility. These structures can be used to acquire a better understanding of development and design improved organoids and organs with custom modular properties. The applications of such designer tissues range from drug testing to transplantation and therapy.