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Perspective

Synthetic living machines: A new window on life

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SUMMARY

Increased control of biological growth and form is an essential gateway to transformative medical advances. Repairing of birth defects, restoring lost or damaged organs, normalizing tumors, all depend on understanding how cells cooperate to make specific, functional large-scale structures. Despite advances in molecular genetics, significant gaps remain in our understanding of the meso-scale rules of morphogenesis. An engineering approach to this problem is the creation of novel synthetic living forms, greatly extending available model systems beyond evolved plant and animal lineages. Here, we review recent advances in the emerging field of synthetic morphogenesis, the bioengineering of novel multicellular living bodies. Emphasizing emergent self-organization, tissue-level guided self-assembly, and active functionality, this work is the essential next generation of synthetic biology. Aside from useful living machines for specific functions, the rational design and analysis of new, coherent anatomies will greatly increase our understanding of foundational questions in evolutionary developmental and cell biology.

INTRODUCTION

As observational knowledge grows, and models of the underlying patterns are inferred, it becomes possible to begin constructing systems that exploit natural laws in new ways. The trajectories of computer science and physics in the last two centuries clearly demonstrate the value of the engineering approach—efforts to build specific novel devices led to fundamental advances in thermodynamics and theory of computation. Biology is embarking on its own exciting journey via the emerging field of synthetic morphology (expansion of cell-level synthetic biology tools toward reprogramming the anatomy and behavior of multicellular collectives from tissue to organism levels). Moving beyond the reverse engineering of natural systems such as embryogenesis (Beloussov and Grabovsky, 2006; Levin and Martinez Arias, 2019) to the construction of novel ones provides useful technologies with broad applications for society and improves the fundamental understanding of basic mechanisms (Figure 1). A key concept in this framework is that of guided self-assembly: understanding the system-level properties of cellular collectives and using this knowledge to design material and informational inputs to control the emergent form and function.

Continuous feedback between analysis and modeling of surprising biological phenomena, and the rational engineering of living systems with novel structure and function, is a powerful driver of exponential progress (Kamm et al., 2018; Sample et al., 2019). Below, we highlight recent advances in the bioengineering of synthetic morphology and discuss ways in which the resulting new examples of form and function are advancing biological science. These novel living systems constitute a pivotal emerging technology at the intersection of several fields that provides new "model species" in which to develop and test meso-scale morphogenetic theories. This is a quintessentially multidisciplinary undertaking, integrating input of techniques and deep ideas from cell and developmental biology, physics, computer science, and neuroscience.

Design, known as a specification or process for the construction of a system in engineering disciplines, has been less often practiced in generating biological models. Recent approaches in biological engineering and synthetic biology have tried to implement such processes at different scales from synthesis of gene circuits (Brophy and Voigt, 2014) to artificial cell-cell communication (Toda et al., 2020). Standard model system organisms developed as a result of evolutionary processes produced through tinkering, rather

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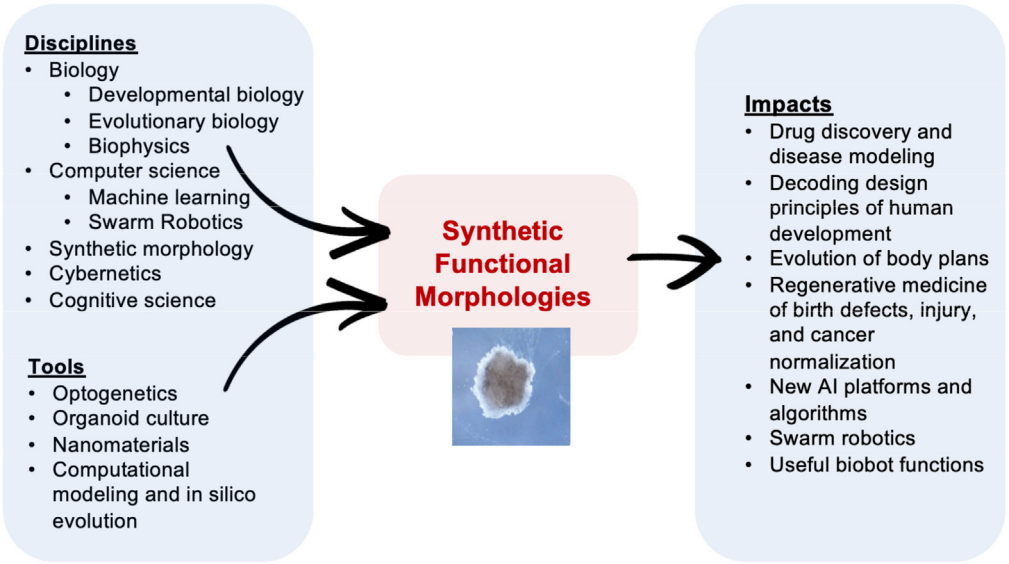

Figure 1. Disciplines and tools that are enabling for development of synthetic functional morphologies and their related impacts on life

than rational design. The promise of building living constructs with pre-defined topology and functionality enables an implementation of rational design approaches to control developmental processes or manufacture systems with close proximity to *in vivo* counterparts. It also can result in novel systems (not designed before) with new set of specifications and objectives with medical and environmental applications.

Like in other areas of engineering, biology makes significant use of modular decomposition and analysis of parts. The recombination of sophisticated components into novel systems can occur at multiple levels. For example, organ transplants and parabiosis experiments have a long history as tools to understand and control body-level physiology by constructing novel hybrid bodies at the large scale. At the other end of the size range, genomic editing, transgenics, and nanomaterials are extensively used to produce novel biology by modulating the lowest level components. Here, we focus on a critical new direction at the meso-scale: taking synthetic biology to the next level beyond reprogramming single-cell phenotypes to the rational control of growth and form—employing genetic circuits, growth factors, and materials, together with top-down external stimuli to implement multicellular morphogenesis to desired structural and functional specifications. An important aspect of this developing field is gaining predictive control over system-level outcomes; inference of the required starting configurations and real-time stimuli is facilitated by computational modeling approaches and *in silico* experiments in virtual embryogeny (Delile et al., 2017; Faure et al., 2016; Pascalie et al., 2016; Varenne et al., 2015; Villoutreix et al., 2016). Such computational efforts are an essential part of a quantitative, rigorous understanding of the rules of morphogenesis and how they relate to the rules governing cell behavior, deriving, for the first time, the equivalent of, for example, Boyle's Law, to enable the guidance of the activity of organized cellular *collectives* toward specific anatomical outcomes.

STATE OF THE ART

One feature of bioengineering at the meso-scale that is unique (with notable exceptions [Brambilla et al., 2013]) is the fact that bioengineers build out of parts that are themselves highly competent (Levin, 2019), for example, cells that have their own internal homeostatic and signaling systems. Thus, the experiments that are done with biological parts have the potential to help understand how swarm intelligence (Couzin, 2007; Valentini et al., 2018) plays out at the tissue level to solve morphogenetic problems. Such advances not only contribute to improved control of anatomy (the holy grail of regenerative medicine) (Pezzulo and Levin, 2015) and its disorders such as injury and cancer (Deisboeck and Couzin, 2009) but also act as an inspiration for novel architectures in machine learning, artificial intelligence, and resilient autonomous swarm robotics (Slavkov et al., 2018). The integrated focus on controlling the emergent self-assembly of artificial protobodies (Doursat, 2013; Doursat and Sanchez, 2014; Doursat et al., 2012, 2013; Fernandez et al., 2012;

Olle-Vila et al., 2016), as well as of their computational and behavioral capacities (Macia et al., 2012; Sole et al., 2016; Sole and Macia, 2013, 2014; Urrios et al., 2016), parallels the biological study of natural model systems and forms an important, embodied branch of the field of Artificial Life (Bedau, 2005).

SIGNIFICANT ADVANCES SO FAR

Here, we focus on efforts aiming toward developing new living systems using cells as starting building blocks. This "build-to-understand" approach enables decoding of novel design principles as well as generation of useful technological platforms (Elowitz and Lim, 2010; Raspopovic et al., 2014; Schaerli et al., 2014; Slavkov et al., 2018). We loosely catalog two sets of studies. In one set, the main objective is to reconstitute or emulate developmental mechanism *in vitro* either in cell lines with no developmental potentials or in stem cells with unique self-organization properties. For instance, genetic circuits and artificial cell-cell communication were engineered to induce cell sorting and pattern formation in cell lines (Davies and Glykofrydis, 2020). Exploiting the innate self-organization properties of stem cells could also generate mimetic of early developmental tissues or later human organogenesis *in vitro*. As a result, these efforts have produced a myriad of living models to study and understand the principle of human development.

The other approach has been less driven by naturally developed human tissues and developmental events, but rather follows designs that are mainly based on a set of specifications for applications of interest. Such synthetic living systems can represent a new form of artificial life that is heavily built on morphology and function by design, examples of which are the artificial stingray and Xenobots. All these efforts are paving a path toward development of complex biological living machines with adaptive and autonomous operation, sensory inputs, signal processing, and actuation in the form of organismal behaviors. Additionally, both sets of studies teach us key lessons for a better practice in engineering form and function.

Synthetic multicellularity

When it comes to engineering synthetic multicellularity, lessons learned from past evolutionary and ecological processes of multicellular development on earth will provide important principles for a successful outcome (Sole et al., 2018). The transition from single cells to multicellular entities has been a key invention in life that has fascinated scientists from diverse fields. Likewise, how a single zygote can robustly produce a whole organism with diverse cell types, function, and controlled organization has been a central question. Seminal works across distinct scientific disciplines, from evolutionary biology to bioengineering, have focused to reconstruct these processes *in silico* or at bench (Sole et al., 2018).

After million years of evolution in existing animals, the organismal forms and anatomies show certain degree of prevalence in stereotypical morphological motifs such as folds, segments, and cavities. To explain the conserved nature of such structures, the presence of physiogenetic processes to control pattern, shape, or structures was suggested (Newman, 2012). The generic physical properties (e.g., viscoelasticity), active in both living and non-living systems, promote generation of such morphological motifs (Newman, 2012, 2014, 2019). The GRNs governing cellular interaction, initiation, and development of multicellular entities operate within this framework, by fine-tuning, stabilizing, and mobilizing the core generic properties (Newman, 2012).

Cell-cell communication and pattern formation are one of the early events during development of multicellular systems. Hence, understanding and engineering different modes of communication and patterning in prokaryotic and eukaryotic cells have been a quest for developmental and synthetic biologists (Basu et al., 2005; Davies and Glykofrydis, 2020; Green and Sharpe, 2015; Levin and Martinez Arias, 2019; Matsuda et al., 2012; Weber et al., 2007; You et al., 2004). Inspired by pioneering works by Steinberg (1963), seminal studies used cadherins to probe differential adhesion, cell sorting, and pattern formations (Cachat et al., 2016; Davies and Glykofrydis, 2020). Combining cadherin-mediated cell sorting with artificial cell-cell communication also produced multi-layer self-organization and multicellular patterning (Toda et al., 2018, 2020). Instead Skine et al., showed synthetic mammalian reaction-diffusion patterns via reconstituting Nodal-Lefty network in mammalian cells (Sekine et al., 2018). Future efforts will focus on engineering other morphological motifs such as controlled tube formation and folding (Cachat et al., 2014). It is not clear how far one can go in reconstituting fully new forms and whether our ability to invent is limited by inherent constraints that are imposed by natural physical properties within biological systems (Sole et al., 2018).

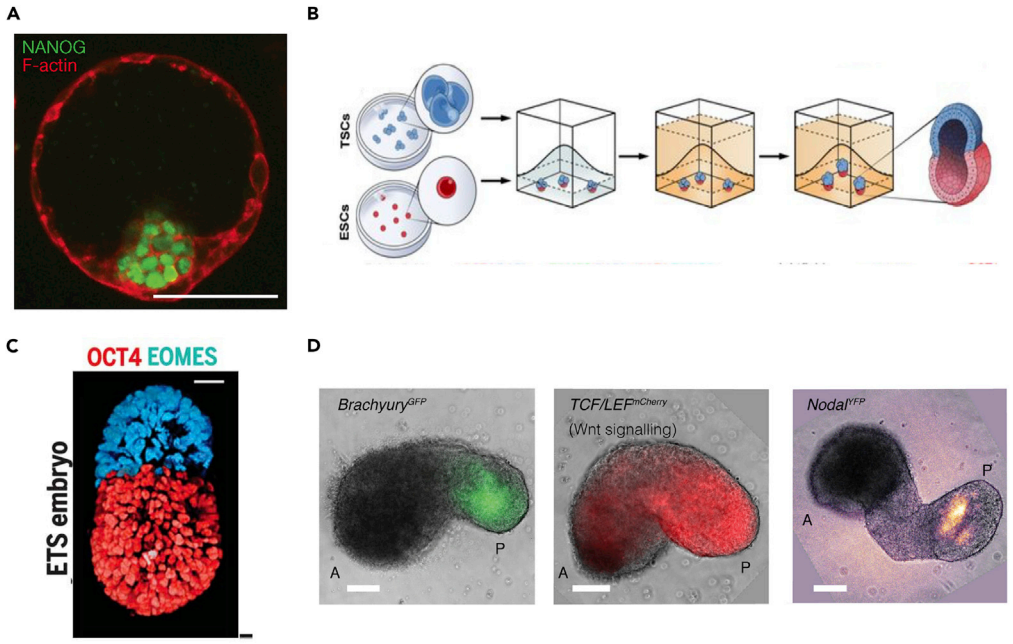

Figure 2. Synthetic embryo-like entities

(A) Blastoids co-stained with F-actin (red) and Nonog (green). Nanog shows ESC identity (taken with permission from Rivron et al., 2018).

(B) Schematic to show strategy for generation of mouse embryonic-like structures via combining mouse ESC (red) and TSCs (blue). Cells are suspended in 3D extracellular matrices and allowed to self-organize (taken with permission from Harrison et al., 2017).

(C) ETS-embryo structure. Red shows Oct4 expression from ESCs and cyan is Eomes in TSCs (taken with permission from Harrison et al., 2017).

(D) Gastruloids at 120 h after aggregation showing features of mouse embryo at embryonic day 1 and spatially confined presence of signaling cues and markers associated with axis formation such as Brachyury-GFP, WNT signaling activity (TCF/LEF-mCherry), and Nodal-YFP (taken with permission from [Beccari et al., 2018]).

Synthetic embryo-like entities for early developmental engineering

The study of human embryos *in vitro* is constrained by broad ethical consensus restricting their study to maximum period of 14 days after their creation. This consensus has resulted in a broad gap in knowledge regarding the genetic and morphological changes that are involved in gastrulation, the specification of human germ layers, and the subsequent early stages of body plan segregation and organ specification. Models of early development encompassing living systems, entitled "embryoids," "blastoids," and "gastruloids," produced from both human and murine stem cells, have become an important means to gain insights on the processes underlying this "black box" of human development (Figures 2A–2D) (Fu et al., 2020).

Pioneering studies used human embryonic stem cells subjected to geometric confinement in circular micropatterns. In response to key developmental cues, the cells self-organize and pattern into fates similar to the three germ layers (Warmflash et al., 2014). These three cell types arrange themselves in circular layers that are ordered in an equivalent arrangement to the embryo, with a core-to-edge arrangement of concentric rings of ectoderm, mesoderm, endoderm, and extraembryonic ectoderm. This phenomenon has been used to study the patterns of signal diffusion in a quantitatively reproducible way, and even showed how chirality of single cells leads to a large-scale asymmetry of tissue-level structure (Chen et al., 2012; Wan et al., 2011, 2016; Wan and Vunjak-Novakovic, 2011).

Subsequent to these efforts, major 3D embryonic stem cell models emerged that focused on the integration of early embryonic and extraembryonic mouse stem cells to drive the formation of self-organizing embryo-like structures. The combination of these cells in tightly defined ratios resulted in the formation of structures that surprisingly resembled the early pre-implantation embryo, including a compacted epiblast

surrounded by a blastocyst cavity created by trophoblast cells (Figure 2A) (Rivron et al., 2018). These blastocyst-like constructs (blastoids) are capable of supporting the development and decidualization of trophoblast stem cells when implanted into the mouse uterus; however, they fail to achieve later developmental milestones (Rivron et al., 2018). These blastoids offer a high-throughput way to investigate the morphological and genetic features of early embryo-like structures.

Other methodologies also resulted in novel synthetic embryo-like structures using combination of embryonic and extraembryonic stem cells in 3D culture (Figure 2B) (Harrison et al., 2017; Sozen et al., 2018, 2019; Zhang et al., 2019). These studies have demonstrated the capability to spatially organize into a structure that sorts into polarized embryonic compartments and form lumens in a manner that replicates the organization of a post-implantation embryo (Sozen et al., 2018). These "ESC + TSC + XEN" embryos (ETX-embryo) (Figure 2C) develop anterior and posterior structures, recapitulating the spatial opposition of anterior visceral endoderm and primitive streak (Sozen et al., 2018; Zhang et al., 2019). Similar to the murine blastoids described previously, these embryoids can implant and decidualize *in utero*, although they also exhibit limited ability to develop into more advanced embryonic structures (Sozen et al., 2019). Additionally, gastruloids mimicking the posterior portion of embryo were developed using aggregates of defined size for mouse embryonic stem cell that are pulsed with a Wnt agonist (Figure 2D) (Beccari et al., 2018). The cell aggregates elongate and polarize, forming structures similar to the post-implantation embryonic somites (Beccari et al., 2018). In addition to the formation of T-brachyury+ and Gata6+ poles, these structures show spatial segregation of Hox genes. These 3D models of embryogenesis provide a set of models to build and understand tissue development *in vitro*.

In a parallel effort, microfluidic devices were used to recapitulate more controlled signaling environments that could effectively mimic the signaling roles of extraembryonic tissues. For instance, by exposing pluripotent cells to a polar signaling environment, these cells organize and differentiate in a manner that mimics Epiblast-Amnion morphogenetic events (Zheng et al., 2019). Human pluripotent stem cells seeded into a pocketed two-channel microfluidic device creating a directional BMP4 signal show the development of a lumen and segregation of amnion from epiblast (Zheng et al., 2019). The epiblast-like cells subsequently differentiate into primitive streak-like and primordial germ cell-like cells. Development of these channeled microfluidic devices could allow the recapitulation of more complex signaling environments, up to and including the post-implantation uterine environment.

Organoids and synthetic tissues to mimic human organ-like structures

A class of synthetic living systems dubbed organoids represent 3D partial mini replicas of human organs, which were made by combining stem cell and developmental biology with bioengineering (Sasai, 2013). Most of the studies focused mainly on recapitulating the interaction of the stem cells and the niche in laminin-rich 3D extracellular matrices using defined developmental cues. Such interaction controls the processes of self-renewal, differentiation, and generation of diverse cell types with a variety of states that self-organize to functional forms mimicking organogenesis (Zinner et al., 2020). Seminal studies by Sasai and colleagues showed self-organized formation of apico-basally polarized cortical tissues from human pluripotent stem cells (Eiraku et al., 2008) or self-formation of optic cup using designed 3D conditions with tightly controlled medium and cell number (Figure 3A) (Nakano et al., 2012). Parallel studies by Sato and by Clevers and colleagues showed formation of mini-gut organoids using adult stem cells in Matrigel with ever-expanding potential (Clevers, 2013; Sato et al., 2009). A landmark study in 2013 presented the generation of cerebral organoids from human pluripotent stem cells that showed features of cortical development, brain region identities, and organization with a subset of cells maturing to form functional cortical neurons with calcium oscillation (Figure 3B) (Lancaster et al., 2013). Within a short period, these tissue replicas rapidly emerged as tools to decode principles of organogenesis and model human disease with a long-term perspective for regenerative therapies. For a comprehensive history of organoids, we refer readers to several excellent reviews (Del Dosso et al., 2020; Dutta et al., 2017; Kim et al., 2020; Lancaster and Huch, 2019; Lancaster and Knoblich, 2014; Simian and Bissell, 2017; Takebe and Wells, 2019). Endoderm- (e.g., intestine, liver, lung), mesoderm- (e.g., kidney, heart), and ectoderm- (e.g., brain, optic cup, mammary gland) derived organoids have been generated (Kim et al., 2020; Lancaster and Huch, 2019). More complex behavior was also successfully modeled; for example, traveling wave-like expression patterns representative of the segmentation clock, which is central to rhythmic emergence of somites and the axial skeleton, was shown (Diaz-Cuadros et al., 2020; Matsuda et al., 2020b). Each one of these models provides a window through which to study the black box of human development, enabling us to understand broad questions

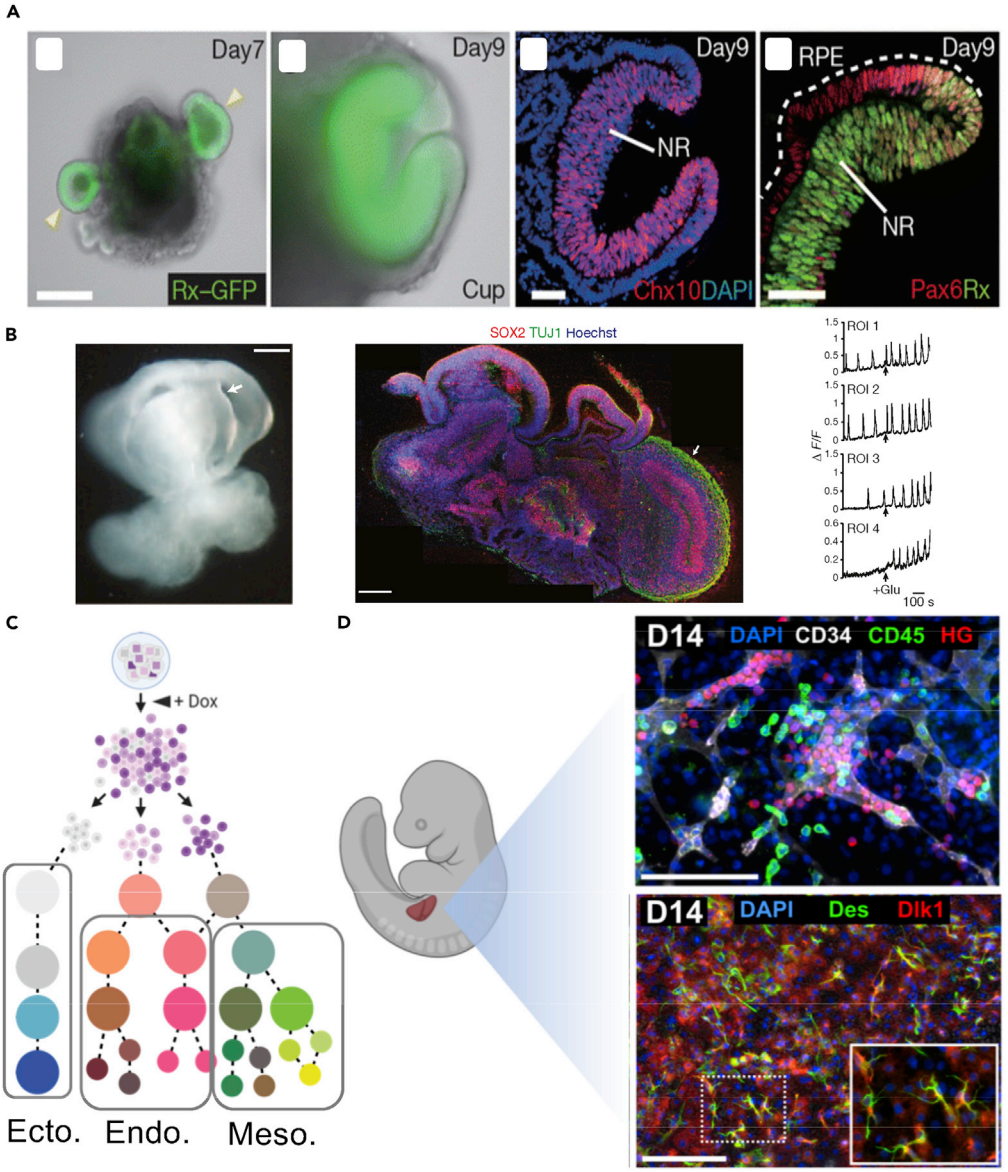

Figure 3. Examples of organoids and multicellular tissues

(A) Self-organization of an optic-cup-like structure with differentiating epithelial cells from ES cell aggregates in 3D. Rx-GFP shows retinal analge (taken with permission from Nakano et al., 2012).

(B) Left: Bright-field view of cerebral organoid with cavities (arrow) representing ventricle-like structures of brain. Middle: Immunostained cerebral organoid shows tissue morphology with neural progenitors (SOX2, red) and neurons (TUJ1, green). Right: Recorded neural activity using calcium dye (taken with permission from Lancaster et al., 2013).

(C) Schematic for genetically guided engineering by using stem cells engineered with inducible genetic circuit to pre-program cell fates associated with different germ layers. Doxycycline (Dox) turns on the expression.

(D) Generation of liver bud-like tissue using genetic programming. Immunostained tissue exhibits the presence of subset of cells similar to fetal liver. CD34 marks vasculature, CD45 and HG mark blood cell lineages, DLK1 and Des mark hepatocyte progenitor and pericyte populations, respectively (taken with permission from [Guye et al., 2016]). Part of this figure is created using BioRender (*BioRender.com*).

in the contexts of evolution, aging, and organismal differences. For instance, human laboratory-grown brain organoids taught us about the temporal cell atlas of great ape forebrain and shed light on molecular pathways that are unique to humans (Kanton et al., 2019). Ebisuya and colleagues also used a human segmentation clock model to understand species-specific differences in developmental timing via kinetics of biochemical reactions (Matsuda et al., 2020a).

Although quite promising, one key challenge with multicellular morphogenesis *in vitro* centers on the ability to control the process of morphogenesis and self-organization (Brassard and Lutolf, 2019; Ebrahimkhani and Ebisuya, 2019; Garreta et al., 2020; Johnson et al., 2017). Additionally, tools for quantitative and universal assessment of tissue function, identity, and cellular composition across studies is missing. The ability to both control and quantitate cell fate and tissue morphogenesis allows improved robustness and reproducibility in manufacturing (Velazquez et al., 2018). Many studies have added a cocktail of growth factors to cell culture medium to reconstitute niche-mediated signaling cues. However, the application of growth factors in media can encounter certain challenges such as supraphysiological concentration. It is also challenging to identify and reconstitute spatiotemporal dynamic and combinatorial cues displayed in native developing tissues. Microfluidic approaches as well as engineered matrices offer a cell-extrinsic based control for signaling cues (Brassard and Lutolf, 2019). For instance, shape-morphing materials dubbed kinomorphs can rationally control the shape and size of multicellular networks (Viola et al., 2020). Additionally, self-organization of intestinal organoids-on-a-chip using scaffold was shown to enable regulation of morphological specifications. Subsequently, externally guided forms can improve maturation and composition of cell types, providing mini-intestines with physiological hallmarks of the *in vivo* organ (Nikolaev et al., 2020).

Engineering synthetic genetic circuits offers a cell intrinsic-based approach that can be used to enable control over cell fate, communication, and self-organization properties (Santorelli et al., 2019; Velazquez et al., 2018). Employing cells as the niche-associated signaling centers is a powerful method to reconstitute developmental cues and re-create the niche-stem cell cross talk. For instance, genetically engineered Sonic Hedgehog (SHH)-expressing sender cells were used to establish a gradient of the morphogen and improved cell fate and self-organization along dorsoventral and anteroposterior axes in forebrain organoids (Cederquist et al., 2019). Genetically embedded gene circuits can be also used to drive multilineage co-differentiation to produce genetically pre-programmed tissues and organoids (Figure 3C). For instance, inducible and transient expression of GATA6 transcription factor was used to trigger symmetry breaking, co-development of endoderm and mesoderm germ layers, and formation of human fetal liver bud (Figure 3D) (Guye et al., 2016). In a follow-up study, multilineage maturation was also achieved through the synthetic control of GRNs (Velazquez et al., 2020). Recent studies have also used orthogonal differentiation of genetically engineered stem cells to produce multicellular tissues such as vascularized brain organoids (Skylar-Scott et al., 2020). The use of transcription factor-based cell fate programming offers less dependence on signaling cues present in the medium, alleviating the challenge of finding a supporting medium for generation of multiple cell types. Additionally, synthetic genetic programs can interface with cell state (McDonald et al., 2020; Xie et al., 2011) as well as mechanical cues (Nims et al., 2021), light (Johnson et al., 2020), and heat (Corbett et al., 2020). How the engineering of genetic circuits might alter mechanical and electrical characteristics of cells to modulate morphogenetic events is an attractive area that warrants future studies.

Medusoids and optogenetic stingrays: the swimming biobots

There have been several efforts to understand and apply the design principles governing biomechanics of marine organisms, to generate novel technologies such as bio-inspired autonomous underwater vehicles (Raj and Thakur, 2016; Russo et al., 2015). A group of studies combined living and non-living systems to achieve this objective. For instance, medusa jelly fish were reverse-engineered to identify key specifications and design in the context of structure-function, stroke kinematics, and animal-fluid interactions (Nawroth et al., 2012). Jellyfish stroke is composed of a fast contraction and slow elastic recoil. To build in this feature, a bilayer of muscle tissue and soft synthetic elastomer material from silicone polymer was engineered to be controlled by an electrical field and named a medusoid. The muscle provided the force to contract, whereas the elastomer could restore the original shape. Further examination of jellyfish muscle contraction showed importance of highly ordered myofibril organization paired with spatiotemporal coordination to function. The authors found that rat cardiac tissue, evolved to deliver ventricular heart function, can generate a self-organized sheet of tissue with well-defined axes of force generation and cell-to-cell electromechanical coupling with spatiotemporal coordinated contraction. When medusoids with the cardiac tissue are stimulated by an external electric field, their muscles contract in a way that follows the pattern of jellyfish's power stroke and is followed by a pullback of the elastic silicone to the initial flat shape. This contraction and relaxation give the medusoid biobot the ability to move very similarly to a jellyfish, while still missing several features of jellyfish movement such as full control over navigating turns, directions, or sense-response features. Design and computational simulations with experiments were used to find optimum parameters at each step. Medusoids show a successful generation of macroscale functional

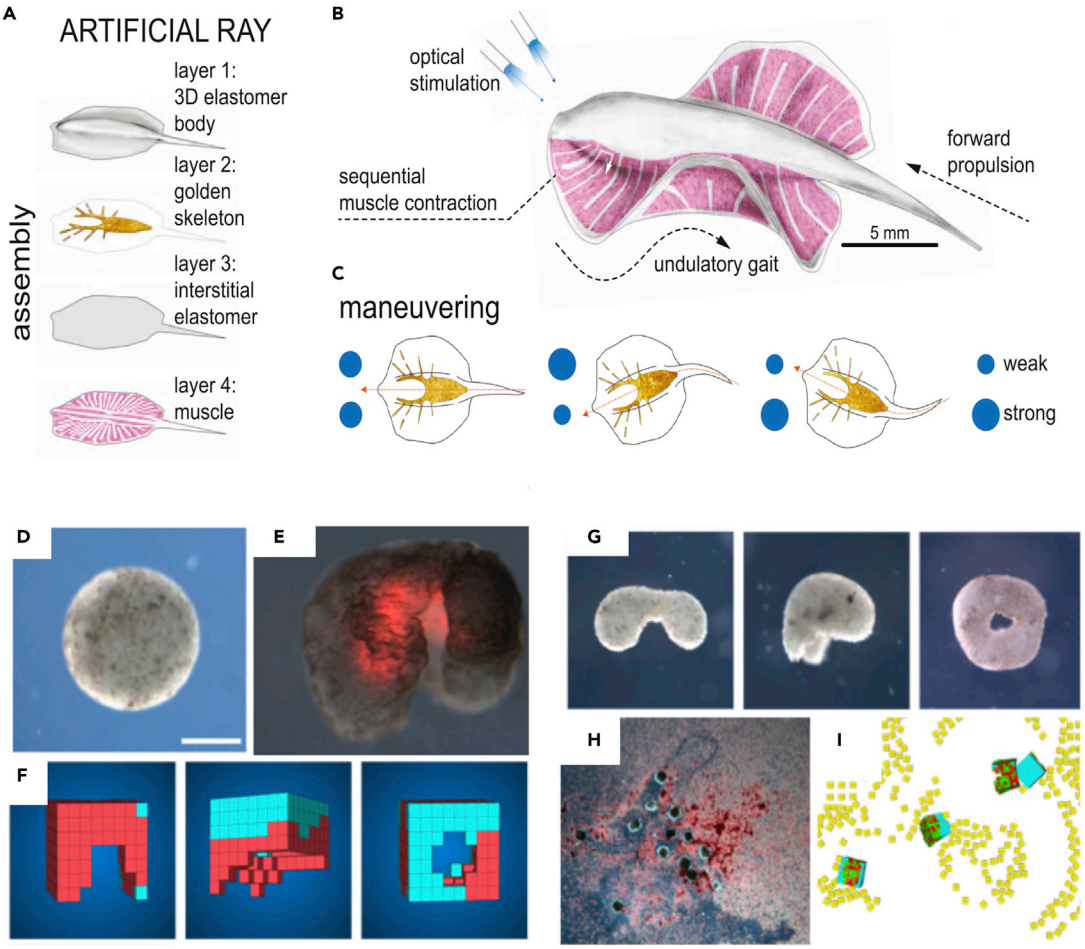

Figure 4. Biobots and Xenobots

(A) Four layers of body architecture for engineered artificial ray as an example of a biobot.

(B and C) (B) Concept of operation and (C) phototactic control via optical stimulation that triggers muscle activation and produces undulatory locomotion and swimming. Asymmetric stimulation controls the direction (taken with permission from Park et al., 2016).

(D) Ectodermal and muscle cells from *Xenopus* embryos are extracted, allowed to re-associate, and then micro-sculpted to remove some cells.

(E–H) (E) The remaining cells self-organize a large-scale pattern (with muscle inside, shown by red fluorescent protein signal) to enable forward locomotion of the Xenobot using the available features of the structure. The sculpting is done in accordance with a simulated Xenobot evolved in a virtual computer environment (F), and many different shapes with diverse emergent movement profiles are possible (G). The movement of these synthetic organisms alters their environment by moving materials (H and I) in ways exactly as predicted by the computational model of the swarm behavior.

morphologies via careful division of complex behavior into mechanistic components, exploiting self-organization ability of cells, with the engineering design algorithm and quantitative benchmarking of performance. The similarity of mechanical behaviors between these biobots with other synthetic muscular pumps such as human heart enable the application of this framework to model and build therapeutically valuable muscular pumps at macroscale size.

The swimming behavior in medusoids is limited in control with no ability to execute turning or maneuvering. A follow-up study aimed to address this limitation. Bathoid fish such as skate and ray show a unique anatomy such as flattened body, extended pectoral fin, swimming pattern characterized by spanwise bending deformations, and chordwise front-to-rear undulatory motion. These design parameters collectively enable forward movement, swim stability, and high-energy-efficiency maneuvers. Additionally, asymmetrical actuation of pectoral fins provides turning capacity during the swim. To this end, a system-level design and engineering was applied for bottom-up composition of a synthetic ray. The developed artificial ray is within the size of a nickel, a 10-fold reduction in size when compared with live animal (Figures 4A–4C) (Park et al., 2016). It integrates optogenetic technology, rat muscle cells, and three-dimensional elastomer

(polydimethylsiloxane) body with a rudimentary skeleton made of gold (Figure 4A). This biobot shows front-to-rear undulating motion that is controlled via light stimulation and displays the same rhythmic motion as a real stingray (Figure 4B). The muscle cells reacted to optical stimuli using a genetically engineered light-sensitive ion channel. These cells were electrically coupled via gap junctions, which enabled propagation of action potential and contractility. The speed and direction of movement is controlled by altering light frequency and the synchrony of stimulation between right and left fins. For example, stimulation of both sides results in forward navigation and asynchronous light-mediated stimulation produces counterclockwise and clockwise turns (Figure 4C). In this way, the synthetic ray can navigate a curved obstacle course with a speed of about 1.5 mm/s over a distance of roughly 15 times longer than its length (250 mm). However, the biobot moves and turns much slower than its naturally evolved counterpart and requires a laminar flow regime, in contrast to the living ray that can navigate turbulent settings. Integration of multiple cell types with novel materials with sense-process-response circuits can equip the synthetic rays with diverse behavior appropriate for each environmental condition. An example of this design is a recent study that combined computational design and optogenetic and neurovascular unit for neuron-enabled biohybrid machines (Aydin et al., 2019). Overall, the synthetic ray couples sensory information to coordinated motor function and behavior; this sets the stage for adaptive living machines with increasingly sophisticated primitive cognition, as additional processing circuits (either bioelectrical or biochemical) are added in the future between the sensory and effector components.

Walking biological machines

Another type of locomotive biobots was made by exploiting various designs (Chan et al., 2012; Feinberg et al., 2007; Xi et al., 2005) that combined contractility of muscle cells as actuators with materials to bestow the moving functions. A silicon-based microdevice was used to grow and self-assemble cardiac muscle bundles on appropriate substrate (Au films) in poly-N-isopropylacrylamide (PNIPAAm), a thermally responsive polymer. After being cooled to ~32°C, PNIPAAm undergoes a solid-liquid phase transition, and the hybrid structures (about 0.5 mm size) can then be controllably released (Xi et al., 2005). This approach allowed assembly, growth, and maturation within the device to form functional structures, enable control of tight bindings in key location and joints, and then release the structure for operation. The manufactured hybrid (biotic/abiotic) structure operates as a result of collective cooperative motion of muscle bundles. The contraction of muscle bundles governs the device movement, with a force that must overcome opposing forces such as friction. The speed of this biobot was about 38 μm per second, a function of step size and contraction frequency. Living machines with more efficient locomotion were recently developed with velocities in the range of 150–500 μm per second (Chan et al., 2012; Feinberg et al., 2007; Pagan-Diaz et al., 2018). These studies demonstrated the integration of 3D printing, hydrogel scaffold, and living muscle tissue with computational design and engineering (Chan et al., 2012; Pagan-Diaz et al., 2018).

Xenobots

Metazoan cells with a standard genome normally undergo embryogenesis and regeneration that reliably builds or repairs a body with a species-specific morphology and behavior. However, it has been argued that the genetically specified cellular components can implement physiological networks that provide considerable morphological and functional flexibility and plasticity (Levin, 2014; Sullivan et al., 2016). An engineering approach asks: what else are normal cells able to build besides their genomic default outcomes? Can cellular collectives cooperate to form novel target morphologies in the absence of genetic change? The study of artificial embryogenesis (Metzger et al., 2018; Ouyang and Chen, 2010; Rosado-Olivieri and Brivanlou, 2021; Shao and Fu, 2020; Tomoda and Kime, 2021) provides a large option space of novel model systems where the relationship between evolutionary forces, genomic information, and structural/behavioral outcomes can be studied. Examples include two recent types of novel living constructs called "Xenobots" (a portmanteau word joining "biobot" and "Xenopus," as the cells were sourced from embryos of the frog *Xenopus laevis*) (Kriegman et al., 2020) (Figure 4D-4I).

The first type consists of skin and muscle cells. A computational process (an evolutionary search over possible architectures) identified specific patterns in which these cells can be layered together and then subtractively sculpted (Kriegman et al., 2020). Such Xenobots exploit the "legs" revealed by the removal of some of the cellular mass to exhibit forward movement (a kind of "walking"). These constructs require no external stimulation, the muscle cells contract in synchrony on their own and the skin cells conduct electrical signals and calcium waves that are synchronized to produce a coherent forward motion (Figure 4D-4I). The *in silico* evolution was used to identify patterns of cell removal that was predicted to result in forward

locomotion, whereas the rest of the cellular signaling, force transmission, and pacing occurred as emergent features.

The second type of Xenobot is even more self-organized. These result from prospective ectoderm cells isolated from embryos (related, but not identical to, the classic animal cap technique of developmental biologists [Sive et al., 2007]). Dissociated cells, liberated from the rest of the embryo, reboot their multicellularity along a novel path: they coalesce and assemble into a spherical proto-body that has a morphology, behavior, and developmental sequence different from typical *Xenopus* embryos (Blackiston et al., 2021). These exhibit a wide range of diverse swimming behavior driven by ciliary motion (including traversal of several different kinds of mazes and dynamic interactions with features in their environment), ability to repair after mechanical damage (to their new Xenobot forms), and collective behavior that rearranges particles in their milieu into patterns that was effectively predicted by a multi-scale computational model.

One way to view these constructs, which blur traditional definitions attempting to cleanly demarcate categories of living beings, robots, and machines (Bongard and Levin, 2021), is as a platform for bio-robotics. For example, the current implementation demonstrated a simple photo-memory functionality, where a synthetic protein was used to implement a long-term, externally readable engram of fluorescent light exposure experience, as well as movement and collective behavior that was predictably derived from a computational approach. Clearly, there is an opportunity for much future work to program the morphogenesis and behavior of these constructs into biobots with highly diverse outcomes by including existing synthetic biology circuits (Nandagopal et al., 2018; Slusarczyk and Weiss, 2012; Toda et al., 2018) in the tractable *Xenopus* cells. However, another aspect of this model highlighted by the first two Xenobot studies is their *endogenous* plasticity, because these functions were obtained from genetically wild-type cells—their shape and function would not be guessable from inspection of the genome alone, which is 100% *Xenopus laevis*.

The individual cells of *Xenopus* of course evolved in the Earth's biosphere, but the skin cells' genome was selected for their ability to sit on the outer surface of the embryo and keep out the pathogens. Skin cells, in a novel configuration, can self-organize structure and function toward that of a coherent proto-organism in hours—without the benefit of evolutionary timescales that provided specific selection pressure for these kinds of organism-scale anatomy and behaviors in the normal life cycle of the frog. Instead, their evolution literally took place in a virtual world inside the computers at the University of Vermont. This raises fascinating questions about the real-time plasticity of cellular collectives and their ability to reach areas of morphospace that differ from those reached by normal embryogenesis. Although the study of Xenobot behaviors (and the ability of cells from diverse species to self-organize novel functional anatomies) is just beginning, it is clear that a rich field of discoveries awaits engineering approaches that merge novel inputs with endogenous plasticity of swarms in cooperating toward diverse specific anatomical outcomes despite their wild-type genomes.

DEVELOPMENTAL MODULES TO BUILD AND CONTROL SYNTHETIC MORPHOGENESIS

Synthetic Morphogenesis field aims to systematically understand and engineer developmental process through application of engineering principles to developmental biology (Basu et al., 2005; Davies, 2008; Isalan et al., 2005; Teague et al., 2016). To study existing design principles in organismal developments, it will be useful to perform top-down decomposition of the process to operational subunits or modules, collections of signaling and morphogenetic events that can be triggered in diverse circumstances by simple trigger signals (Jimenez et al., 2017; Lowell and Pollack, 2016; Verd et al., 2019; Wagner et al., 2007). These modules work both independently and interconnected at different scales to sculpt our tissues and organs through generation of morphological motifs. They can be classified as chemical, mechanical, electrical, as well as gene regulatory networks (GRN) modules. Based on the need of organism, they gradually trigger the production of cell types, trigger cellular self-organization, and advance functional maturities. These modules also participate in physiological and pathological processes during aging, degenerative disease, and various tissue responses to injuries. Each one of the modules can be further divided to sense-process-actuation elements that direct morphogenetic processes. Cells use GRN module to differentiate and create distinct cell types with a set of soluble and insoluble signaling cues (Davidson et al., 2002). These cells then form collectives, employing multilineage communication through chemical signals (Sagner and Briscoe, 2019; Zagorski et al., 2017) as well as bioelectrical (Levin and Martyniuk, 2018; Levin et al., 2017) and biomechanical (Davidson, 2012; Miller and Davidson, 2013) mechanisms to coordinate long-range outcomes. The activation of these modules underlies the phenomena of symmetry

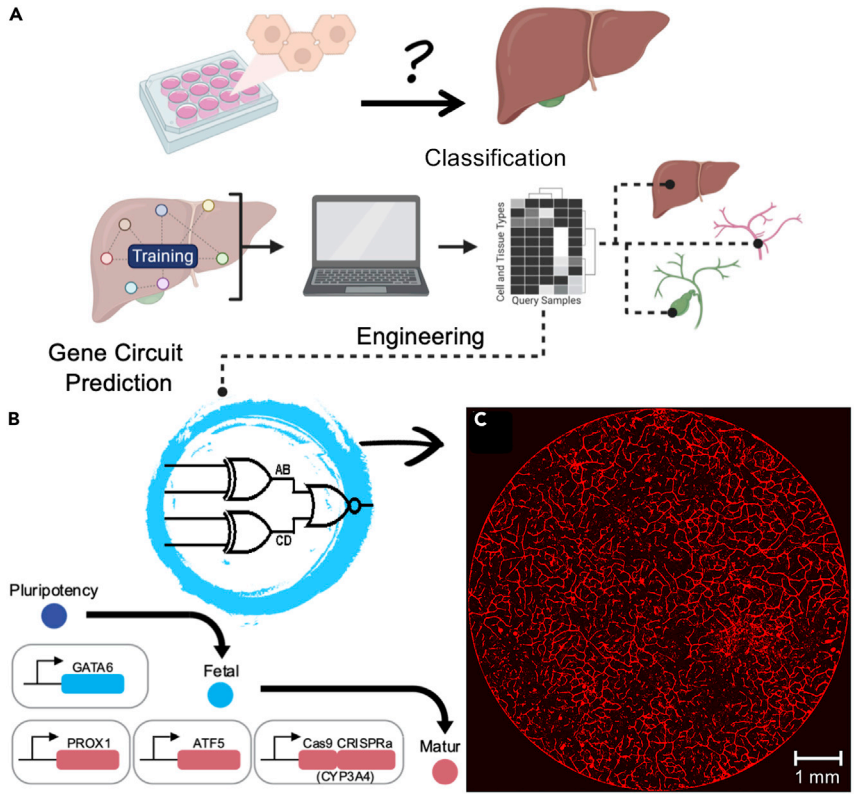

Figure 5. Integrative analysis and engineering of gene regulatory network

(A) Comparison of synthetic tissues with native tissues via quantitative computational analysis and classification algorithms. Genetic circuits to program cell and tissue fates are predicted, engineered, and delivered to synthetic tissue to program developmental trajectories and cellular interactions.

(B and C) (B) Example of genetic circuits enabled directing development of liver organoids from pluripotent stem cells; (C) self-vascularized human liver organoids generated via genetic design and engineering (taken with permission from Velazquez et al., 2020). Part of this figure is created using BioRender (*BioRender.com*).

breaking, growth, and morphogenesis. Recent evidence shows the presence of feedback regulatory controls, within and between the modules, that ensures robustness and resilience during organismal development (Chan et al., 2017; Hannezo and Heisenberg, 2019; Kunche et al., 2016). However, the details of how the distinct modules are linked, and the details of how the hierarchical directionality of connections is shaped, are the subject of ongoing studies. For instance, from the early steps of embryogenesis, cellular GRNs are controlled by signals originating in the cell itself, its neighbors, or distant tissues (Chan et al., 2017; Smith et al., 2018). This enables continuous top-down feedback controls and the establishment of setpoints to operate within the target morphology specs. Examples include the ability of some species (and Xenobots) to regenerate to the same shape after significant damage (Pezzulo and Levin, 2016), and the ability of salamander kidney tubules to form the same overall shape and size, out of cells with radically different sizes, by mechanisms that depend on cell:cell communication or single-cell cytoskeletal shape control, as needed (Fankhauser, 1945). Large-scale anatomical homeostasis also involves bioelectric signaling across all (not just neural) tissues, as recent work has shown that the setpoints of self-assembly (the overall target morphology toward which cell swarms cooperate) can be permanently changed by transiently modifying the bioelectrical information stored in ion channel-driven pattern memory circuits, rather than by rewiring (or modifying) the cells themselves (Durant et al., 2017).

In the context of synthetic living machines, bioengineers are not limited to using only those modules that we know function during the embryogenesis of naturally evolved living systems. There are other means of sensing and communication present in nature that have not had a role in traditional tissue morphogenesis, but that potentially can be utilized in our rational design of new functionality. For instance, optogenetic control of cellular behavior (a light control module) has been widely used to steer cellular functions and

Box 1. Integrated analysis and engineering of GRN modules to program living systems

A gene regulatory network is a complex dynamic system that evolved to direct cellular processes, fate, and function. From the control standpoint it would be efficient to shape GRNs in ways that can be controlled with master regulators and driver nodes. The reprogramming of somatic cells to a pluripotent state with a small set of transcription factors is an example of such control implemented in naturally designed cellular systems and used by scientists for cell fate conversion.

In the context of multicellular self-organization, it is useful to ask whether GRN-associated driving nodes are present in cells or can be engineered rationally (i.e., as control switches) to initiate not only the cell state but also multicellular interactions and self-organization. In support of this idea, transcription factor-triggered tissue formation has been shown in a number of studies to generate tissues such as the brain, thyroid, or liver (Antonica et al., 2012; Ebrahimkhani and Ebisuya, 2019; Skylar-Scott et al., 2020; Velazquez et al., 2018). A transient and heterogeneous expression of GATA6 transcription factor in human iPSCs was sufficient to generate different germ layers, co-development of progenitors, and self-organization to a fetal liver-like tissue in 14 days (Guye et al., 2016). A systematic approach to exploit GRNs for directing tissue morphogenesis can be instrumental for rational engineering strategies. To implement this approach in multilineage liver tissue development, Velazquez et al. have recently investigated whether a set of drivers can be identified and engineered to direct fetal liver organoids across developmental trajectory and toward adult human livers (Figure 5) (Velazquez et al., 2020). They used a machine learning-based algorithms (Cahan et al., 2014; Roost et al., 2015) to quantitatively compare starting and target GRNs to predict a set of genetic targets (Figure 5A). Subsequently they built and tested genetic circuits to advance tissue development (Figure 5B). This approach enabled synthetic maturation of liver tissue and generation of organoids by a genetic design. Interestingly, the set of identified genetic circuits not only improved epithelial identity but also induced co-maturation of different cell types as well as morphogenesis of the vascular network (Figure 5C). Upon introduction of each circuit, GRN score was improved that resulted in development of tissues that are phenotypically one step closer to native human livers. In summary, by using four genetic circuits, human liver organoids with vascular networks and pericytes were generated. This study suggests the potential presence of key signaling nodes embedded in cellular GRN networks that, if triggered properly, can direct and control multicellular formation, cell-cell communication, and self-organization. It also offers a framework for iterative GRN assessment and engineering to navigate the development of *in vitro* tissues.

morphogenesis in development, regeneration, and cancer (Adams et al., 2014; Baaske et al., 2018; Bonzanni et al., 2020; Chernet et al., 2016; Izquierdo et al., 2018; Jewhurst et al., 2014), and is expected to play a major role in cybernetic control of living machines. A recent study has demonstrated the use of automated optogenetic feedback control for precise and robust regulation of cell growth (Milias-Argeitis et al., 2016). Magnetoreception is another modality used by organisms such as homing pigeons (Wiltschko and Wiltschko, 2019) and fish (Formicki et al., 2019), and new tools for magneto-sensitivity have already been employed for neural modulation (Long et al., 2015).

FUTURE: A RATIONAL INTEGRATIVE FRAMEWORK FOR ENGINEERING LIVING SYSTEMS *IN VITRO*

The design principles of organogenesis and regeneration are complex and are still not well understood. Our understanding of how tissue-level computation is executed in controlling cell fate, tissue composition, coordinated cell functions, tissue patterns, and large-scale anatomical morphologies is limited. Our comprehension of how robustness and resilience are incorporated into this process, and of how they can be engineered within synthetic tissue morphologies, is also limited. Reading and writing of the process of morphogenesis, combined with iterative design-build-test cycles, will not only help us understand biology, but also will provide a path for the generation of more advanced human tissues.

Traditional efforts in the past usually have tried to mimic developmental events by exploiting chemical modules in the form of cocktails of growth factors that are sequentially added to cells in culture. This approach, informed by data from model organisms (i.e., mouse, *Xenopus*) usually exercises a trial-and-error process to determine the proper dosage, timing, and combination of growth factors for generating self-organizing tissues. However, this method encounters key bottlenecks such as limited control of sense and response events in the culture, or challenges in finding media conditions that can initiate and steer development of cell types in multilineage tissues. Therefore, strategies that enable control over the electrical, chemical, and mechanical properties of tissue, transcriptional signatures and cellular states, cell proliferation, and communication will be fundamental to the next generation of morphogenetic engineering. For a successful operation, design strategies should combine computational modeling, quantitative high-resolution analysis, and iterative engineering. Here we provided Box 1 and 2 as examples of how exploring developmental modules can enable advancement in the tissue fates and morphologies.

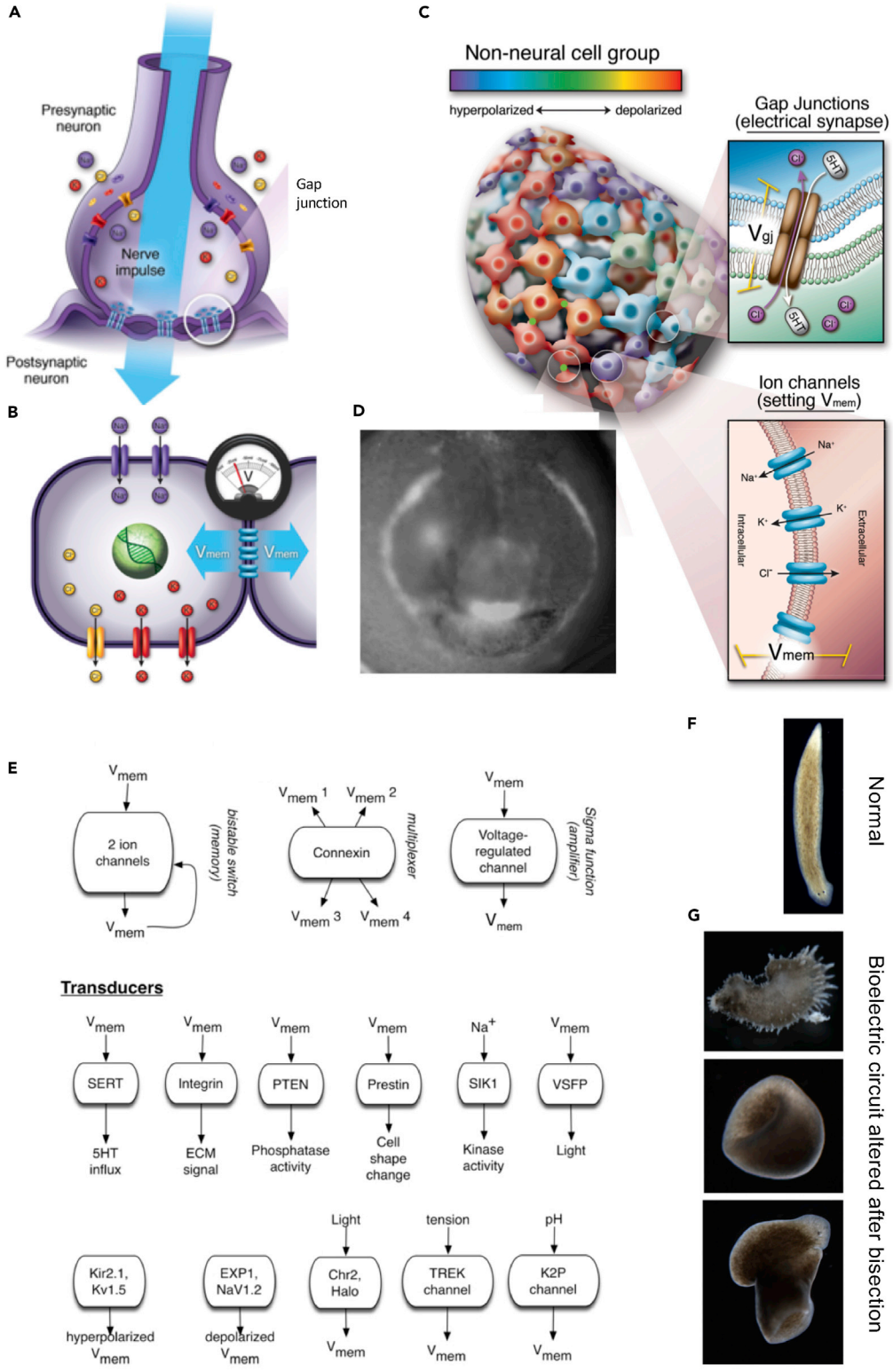

Figure 6. Developmental bioelectricity

(A) Neurons maintain a resting potential via ion channels in their membranes and propagate their electric state to neighbors via gap junctions (electrical synapses).

Figure 6. *Continued*
(B and C) (B) Bioelectric signaling using these same molecular components is a property of all cells, which join together into tissues forming networks (C) that enable large-scale electrically mediated computations to regulate distributions of morphogens and control gene expression.
(D) Fluorescent voltage dye image showing an example of an instructive bioelectric prepattern—the frog embryo face, showing the future locations of the eye, mouth, and other organs (taken with permission from(Vandenberg et al., 2011)).
(E–G) (E) A variety of channel, connexin, and neurotransmitter machinery proteins are available as a parts library, complementing canonical transcriptional modules, which enables synthetic biologists to build bioelectric circuits for control of tissue-level morphogenesis. An example of the plasticity of self-assembly beyond genetic default outcomes are shown in planaria, where normal cells can build wild-type forms (F) or highly altered morphologies (G) if the bioelectric circuit states are modified.

CONCEPTUAL IMPLICATIONS

One of the important contributions of this field to basic science is that it has highlighted problems that have previously been the province of philosophy alone, for the first time allowing empirical approaches to deep questions and pushing us to refine terms that are often used but that have no clear definition. We now have the ability to construct entirely novel functional proto-bodies, by processes that are part rational design and part simulated evolutionary algorithms, using chimerism between natural components at one level (genomes, whole cells) to make synthetic outcomes at another level (novel morphologies beyond the tissue level). This raises profound questions about how to update concepts like organism, animal, body, evolved/designed, and self (Levin, 2019; Rosen, 1972). One example is the notion of "machine."

Constructs such as Xenobots and mammalian muscle-driven constructs have been called synthetic living machines. Are these constructs machines? Although the use of machine metaphors in biology has been criticized (Boudry and Pigliucci, 2013; Nicholson, 2012, 2013, 2014, 2019; Witzany and Baluska, 2012), it is important to note that many critiques are due to an outdated picture of machines as mechanical, inflexible, and driven entirely by external causes. The existence of designed biobots pushes us to ask what exactly is a machine, what features might life have that are permanently (not just temporarily) beyond the reach of engineers if they have access to the same ingredients and evolutionary methods used by natural life, and what precisely would make something *not* a machine? A modern understanding of emergence, complexity theory, chaos theory, and computation has spawned a new field studying the behavior of machines (Man and Damasio, 2019), which highlights the surprise, novelty, intelligence, self-control, and flexible behavior that can result from even today's relatively primitive autonomous agents (Braitenberg, 1984; Lehman et al., 2020; Rahwan et al., 2019). This is especially true in biology, where the boundaries between machines and living agents are being erased by progress in bioengineering.

On the one end of the continuum, biobots that are externally actuated and have a precise 3D-engineered morphology are uncontroversially machines, as they were designed in great detail and use muscle cells to generate predictable forces in the way that one might use animal power to drive a mechanical machine. In contrast, biobots with highly emergent morphologies, self-actuation, and surprising behaviors represent our journey toward the other end of the continuum, where all the key capacities of purely natural forms might be encountered. If one strips away prior centuries' assumptions about machines' simplicity and reliance on external control, one is left with a definition of a machine that focuses on the fascinating balance between surprising emergent behaviors and the possibility of rational understanding that enables design for specific shapes and functionality. Thus, we use the machine metaphor for the products of synthetic biology not to reduce or diminish the wonderous capacity of living forms, but instead, to refine and expand our currently meager conception of what a "machine" can be. As biobots and other forms of artificial life have the benefit of both design and evolutionary dynamics (Lehman et al., 2020; Miller, 2004; Stanley and Miikkulainen, 2003), there is no principled reason why future versions could not enjoy the same agency that exclusively evolved lineages do. In fact, one can conjecture that by adding design to the power of evolution, creatures will arise that have improved emergent agency and capacities over what evolution alone has done to date.

As often happens, engineering moves ahead to empirically resolve (solve or redefine) problems that have resisted pure philosophy for centuries. The creation of these constructs cuts the Gordian knot of the life versus machine philosophical debate, because they reveal the smooth continuum of every possible

Box 2. Bioelectricity: a new modality to enrich in synthetic morphology

Many biochemical tools have been created for the synthetic biology field, focused on transcriptional dynamics, protein-protein interactions, gradients, and enzymatic activity. These tools are beginning to be exploited for multicellular coordination in bioengineering (Bashor et al., 2010; Chau et al., 2012; Woodruff et al., 2017). However, there is another important aspect of endogenous morphogenesis that is poised to significantly impact this field: bioelectricity (Levin, 2021; Levin et al., 2019). All cells, not just neurons, communicate electrically by forming networks of cells that set their resting potential by means of ion channels and pumps, and then communicate those states to each other dynamically using gated electrical synapses known as gap junctions (Figures 6A–6C) (Bates, 2015; Levin et al., 2017). Evolution long ago established that bioelectric networks are an ideal way to coordinate across distance and manage complex outcomes long ago. Such dynamics can be seen in bacterial biofilms (Prindle et al., 2015; Yang et al., 2020), can be used for coordination of embryogenesis and regeneration of complex organs (Figure 6D) (McLaughlin and Levin, 2018; Silver and Nelson, 2018), and can be sped up to form specialized computational organs such as brains (Fields et al., 2020). These dynamics can now be tracked by fluorescent voltage reporter dyes (Adams and Levin, 2012a, b) and genetically encoded voltage indicator proteins (Abdelfattah et al., 2016; St-Pierre et al., 2015; Treger et al., 2015), which are especially well suited to the study of synthetic constructs that are easier to image than whole animals. Moreover, the advances in molecular developmental bioelectricity now provide bioengineers with plasmids encoding wild-type, mutant, and dominant-negative ion channels and gap junctions that enable precise control of resting potential and its spatial propagation (Figure 6E) (Levin, 2013; Mustard and Levin, 2014). In addition, techniques like optogenetics can be adapted to non-neural tissues to provide even tighter spatiotemporal control (Adams et al., 2014). Likewise, additional tools targeting transduction machinery help design circuits in which bioelectric computations are linked to transcriptional outcomes. Bioelectricity can contribute to synthetic morphology in three distinct ways. First, it provides increased control over cell-level properties, for example, resting potential is a powerful regulator of differentiation (Hinard et al., 2008; Sundelacruz et al., 2009). Second, it enables computational circuits with specific dynamic properties; non-neural bioelectricity exploits the attractive properties of neural networks (Manicka and Levin, 2019), but it is embedded in deformable media, which allows it to implement morphological computation (Cheney et al., 2014; Rieffel et al., 2010). Finally, control of the bioelectric layer provides a convenient entry point to the design of minimal cognitive agents—synthetic creations that have functional and behavioral repertoires in addition to their morphology (Baluška and Levin, 2016; Macia et al., 2017). By building simple artificial bodies that house tractable neural (and non-neural) information processing dynamics, developmental bioelectricity and synthetic morphology can be combined to better understand the evolution of the mind-body connection (Pezzulo and Levin, 2015).

combination of inorganic design and evolved species. They show that there is no sharp line between designed artifacts and the magic of "standard" life forms (those present before the rational age of biology)—every possible mix can be made, and thus we need a mature science of life, and of machines, that does justice to these empirical facts (Levin, 2020).

Several other conceptual areas are highlighted by new work in this field. First is the importance of mesoscale "laws of form" (Beloussov and Grabovsky, 2007; Briscoe and Kicheva, 2017; Graner and Riveline, 2017; Polanyi, 1968): synthetic organisms are an ideal sandbox in which to hone our understanding of system-level morphogenetic rules that are distinct from molecular-level details, akin to what thermodynamics accomplished by giving up the hope of tracking every particle. Such work is a critical complement to today's molecular profiling efforts. Similarly, this work brings up important complementarities in understanding and control of systems from bottom-up (emergentist) and top-down (representationalist) strategies, both of which have important practical techniques to offer (Pezzulo and Levin, 2016). Finally, this field brings up important issues in bioethics (Haase and Freedman, 2020; Lawrence, 2019; Levin et al., 2020): the increasing sophistication of artificial bodies, chimeric constructs with cells from various species tightly integrated with electronic interfaces/processors and engineered neural networks, is inevitably going to give rise to a wide range of creatures with completely novel behaviors and cognitive capacities, which will raise obvious but difficult questions about how we relate to truly diverse intelligences as this field matures in the coming century.

Thus, the next advances in this exciting field will occur on a number of concurrent tracks of technological and conceptual development. Computational modeling of structure and function will increasingly merge biophysically realistic simulation with machine learning of rules and inference of novel stimuli to apply to cells for desired system-level morphogenesis and behavior. At the bench, it will be crucial to develop standardized computer-controlled environments where real-time feedback during morphogenesis and behavior can be provided to synthetic morphologies. The tight integration of synthetic tissues with

optogenetic, microfluidic, and electrode interfaces to computational platforms (Hamann et al., 2015; Tsuda et al., 2009; Tsuda et al., 2007; von Mammen et al., 2016; Wahby et al., 2016) will enable real-time closed-loop controls and thus facilitate the refinement of strategies for rational *guided* self-assembly of novel living bodies and, eventually, primitive cognition. In this way, advances in synthetic morphology and biologically embodied artificial life will lead to a deep unification of several fields of basic science and applied technology, with numerous transformative impacts ranging from biomedicine to artificial intelligence.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Mo R. Ebrahimkhani (mo.ebr@pitt.edu).

Materials availability

No materials were newly generated for this paper.

Data and code availability

No data and codes were newly generated for this paper.

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AUTHOR CONTRIBUTIONS

M.L. and M.R.E. discussed together the theme and subsections, implemented the ideas, developed the figures, and wrote the manuscript.

DECLARATION OF INTERESTS

M.L. is a co-inventor on U.S. Application 63/136,564 submitted by Tufts University that covers some engineered multicellular organisms. M.R.E. is a co-inventor on US9677085B2 and Application WO2019237124 and PCT/US2020/045926, which are related to engineering multicellular systems.

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