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Engineering and modeling of multicellular morphologies and patterns

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Synthetic multicellular (MC) systems have the capacity to increase our understanding of biofilms and higher organisms, and to serve as engineering platforms for developing complex products in the areas of medicine, biosynthesis and smart materials. Here we provide an interdisciplinary perspective and review on emerging approaches to engineer and model MC systems. We lay out definitions for key terms in the field and identify toolboxes of standardized parts which can be combined into various MC algorithms to achieve specific outcomes. Many essential parts and algorithms have been demonstrated in some form. As key next milestones for the field, we foresee the improvement of these parts and their adaptation to more biological systems, the demonstration of more complex algorithms, the advancement of quantitative modeling approaches and compilers to support rational MC engineering, and implementation of MC engineering for practical applications.

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Motivation

How large numbers of cells spatially organize to collectively perform complex functions is a fundamental question in biology and engineering (Figure 1). Deeper insight into this question can lead to practical biotechnological applications because multicellular systems can perform tasks that single cells cannot. We define multicellular (MC) systems as collections of cells that are physically adhered to one another and that perform a cooperative task. This includes, for example, MC organisms replicating through a germ line [1], biofilm ecologies [2•], or artificial organoids [3]. It is also instructive to consider systems outside of this strict definition that can be viewed as 'precursors' of MC systems, such as self-organized [4] or externally driven cell swarms [5]. Engineering MC systems has many potential applications, such as artificial tissues [6], modularizable biosynthesis pathways [7], programmable smart materials [8], and as a build-to-understand methodology complementing traditional MC research [9].

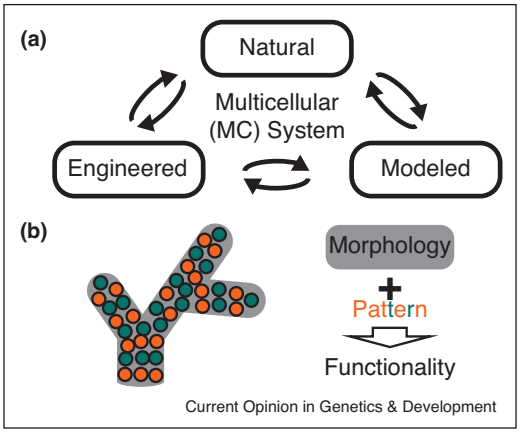
Here we review current concepts in rationally engineering and modeling spatial order and physical properties of MC systems (Figure 1a). We then review recent advances from the past two years in developing such systems and in modeling approaches that directly address or have the potential to significantly advance MC engineering. Finally, we provide suggestions on future milestones for the field.

Concepts

Much of current MC engineering is concerned with achieving a desired MC morphology or MC pattern, which are defined as the macroscopic arrangement of cells in 3D space and the ordered identity of cells within this arrangement, respectively (Figure 1b). Cell identity in this context may refer to attributes ranging from expression of a simple fluorescent reporter to complex visco-elastic or biosynthetic properties, ultimately determining the appearance, behavior, and functionality of a MC system.

Engineering MC systems benefits from a toolbox of standardized parts [10] (Figure 2). Cell–cell adhesion, cell–cell signaling, differentiation, and cooperation (in various combinations) (Figure 2) are generally considered to be the key elements of a minimal MC toolbox that enabled the transition from unicellular to MC life [1]. An extended MC toolbox may enable greater control of cell features like movement, apoptosis, or shape changes. A part can be, for example, a molecule, a gene, a circuit of many genes cross-regulating their expression, or even a specific cell type, but can also be combined into higher order parts. The key purpose in having defined parts is to have them serve as components that simplify MC-scale engineering by combining such parts, similar to how electronic transistors are combined into logic gates and

Figure 1



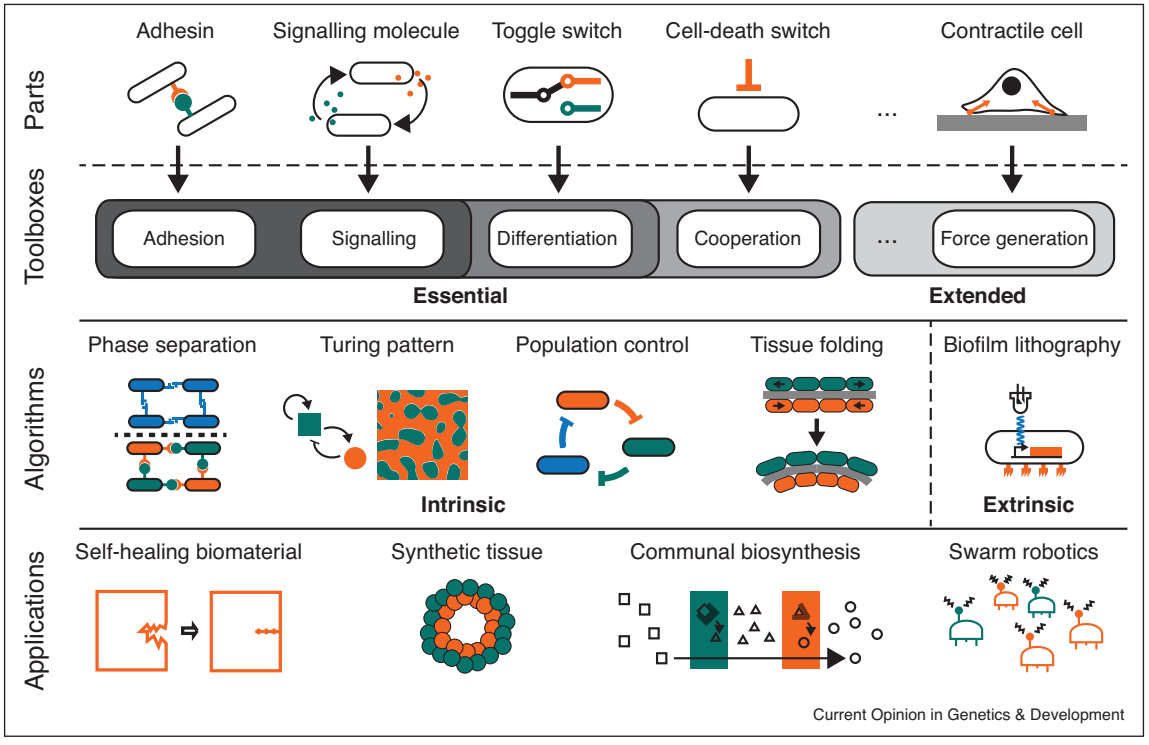
Characterization of multicellular (MC) systems. **(a)** Studying natural MC systems, engineering synthetic MC systems, and modeling MC systems all inform one another. **(b)** The morphology and pattern of a MC system determine its appearance and functionality.

further into CPUs. Such parts can be natural [11••], modifications of natural parts [12••], or designed completely de novo [13]. When developing or using standard parts multiple properties should be considered depending on the specific application needs [14] (Table 1).

These parts are then combined to constitute the hardware (or 'bioware') which runs morphogenesis and patterning algorithms, that is, a set of basic instructions that define a sequence of operations yielding a MC structure (Figure 2). Algorithms can rely on intrinsic manipulation (e.g. synthetic gene circuits), or on extrinsic manipulation (such as light guiding input or deposition of cells during tissue printing [5,15•,16]). Different algorithms can lead to the same outcome, and each algorithm comes with trade-offs. Algorithms are agnostic to the specific parts, that is, the same algorithm can be implemented through very different molecules and at different time and length scales via different physical parameters, for example in plants versus animals. Some of these algorithms initiate the production of other parts, which in turn may run additional algorithms.

Understanding which algorithm and parts to use is imperative for rational and effective MC engineering. Modeling aids design and interpretation of these choices and the resulting dynamics. We distinguish between discrete models, where cells (or subcomponents or clusters of cells) are individually represented, and continuum models, where cellular discreteness is neglected [17]. While

Figure 2



Engineering MC systems requires physical parts that can be conceptually sorted into toolboxes based on functionality. Parts can be combined to execute intrinsic and extrinsic MC algorithms that generate MC morphologies and patterns, which promise various applications.

Table 1

Properties of parts—whether desired or not depends on application

Property	Description
Genetically encoded	Instructions contained within cell's genome; can be part of a genetic circuit
Predictable	Quantitative understanding of part functionality that enables rational design
Tunable	Easy adjustment of part parameters, for example, input sensitivity or response strength
Programmable	External control (e.g. through optogenetics) in real-time or prior
Orthogonal	No crosstalk with/isolation from other parts and native biological functionality
Compatible	Interfacing with other synthetic or natural biological parts or standards [10]
Extensible	Easy adaptation of part to an arbitrary library of related parts
Modular	Divisible into largely self-contained subparts with defined functions
Composable	Ability to combine modular parts into new parts with predictable behavior [14]
Noise tolerant	Either robustness to or utilization of inevitable biological noise
Evolvable	Compatible with optimization through (directed) evolution
Functionalizable	Modification with non-biological parts, for example, chemical surface additions

discrete models are often more computationally expensive, significant progress in the underlying computational frameworks [18,19] have empowered greater adoption of discrete modeling either as software packages, for example, CellModeler [19], PhysiCell [20], Chaste [21], or bespoke implementations. Analytical treatments of biophysical models, while not always feasible, can also provide deep insights [2•,22,23].

Recent advances in MC parts and toolboxes

Cell–cell adhesion proteins are critical to establishing and maintaining MC morphology and pattern. Glass and Riedel-Kruse [12••] developed a synthetic cell–cell adhesion toolbox in *Escherichia coli* containing a composable library of nanobody-antigen based proteins enabling homophilic and heterophilic binding between cells (Figure 3a).

Cell signaling is essential for cells to coordinate behavior within MC systems. Billerbeck *et al.* [24] used G protein-coupled receptors to build a modular and scalable interdependent signalling network where individual yeast strains signalled their neighbours to produce an essential gene (Figure 3a). Scheller *et al.* [25] also demonstrated a generalized extracellular-molecule sensor platform in mammalian cells, employing a pair of antigen binding proteins that bind different epitopes of a given signal. This caused the transmembrane domains to dimerize and activate one of several natural intracellular signalling pathways (Figure 3a).

Symmetry breaking and differentiation is key to establishing complex MC systems. Molinari *et al.* [26•] developed a synthetic genetic circuit that achieves asymmetric cell division in *E. coli*. Colocalized plasmids were retained by only one daughter cell after cell division, creating an irreversibly differentiated cell (Figure 3a).

Logical operations at the MC level enable a few signalling pathways to accomplish a large number of functionalities. Guiziou *et al.* [27] developed the Composable Asynchronous Logic using Integrase Networks (CALIN), which uses recombinases to achieve MC logic with 4 distinct inputs [28]. The authors also demonstrated a key benefit of multicellularity by distributing computation load between multiple strains (Figure 3a).

Recent advances in intrinsic MC algorithms

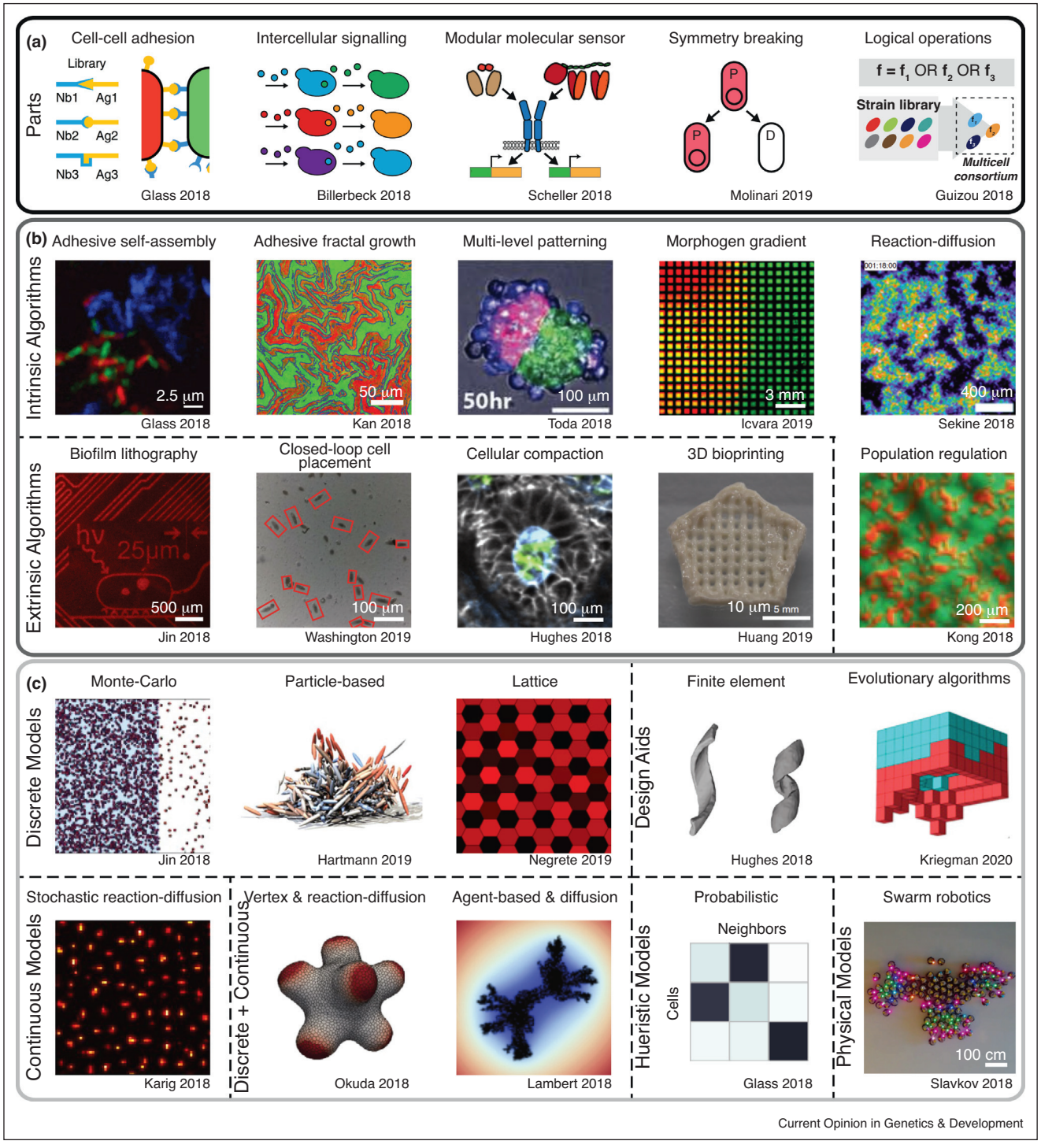
Adhesion-driven self-assembly enables MC morphology and pattern formation through the coordinated aggregation of individual cells. Glass and Riedel-Kruse [12••] developed and used a synthetic adhesin toolbox to demonstrate differential adhesion, phase separation, bridge binding, and more complex self-assembly patterns in bacteria (Figure 3b). Quantitative characterization of pairwise microscopic interactions and microscopic spatial organization enabled a heuristic probabilistic model to predict MC adhesion morphologies and patterns at the level of nearest-neighbor interactions (Figure 3c).

Cell growth combined with cell adhesion affects tissue intermixing and separation. Kan *et al.* [19] expressed the natural adhesion protein Ag43 in bacteria, and found that adhesion among growing bacteria promoted mixing of cell types that resulted in fractal tissue boundaries (Figure 3b). The authors used discrete models to simulate the biophysical processes (Figure 3c).

Multi-level patterning through signalling interlinked with adhesion-driven cell sorting enables the self-organization of complex MC structures. Toda *et al.* [11••] applied a previously developed synthetic juxtacrine signalling system to coordinate the differential expression of natural cell–cell adhesion proteins. The authors were able to create multi-level sequential MC assemblies, with the capacity for symmetry breaking, cell type divergence, and regeneration upon injury (Figure 3b).

Morphogen gradients can provide positional information, such as in the 'French Flag Model' where the gradient

Figure 3



Recent advances in MC engineering and modeling divided into the following categories: **(a)** New parts, **(b)** Morphogenesis and patterning algorithms, **(c)** Modeling and design tools.

instruct cells to differentiate at specific concentration levels. Boehm *et al.* [29] designed an AND gate by splitting T7 RNA Polymerase into two parts. Each part was induced by one of two opposing diffusible chemical signaling gradients, which led to a French-flag-like bacterial pattern. Icvara *et al.* [30] built and characterized a synthetic toggle switch responding to the concentration of a diffusible inducer. Bacteria were seeded in a grid where the inductive chemical formed a diffusion gradient. Robust patterns with sharp transitions were achieved, where features like hysteresis, position, timing, and precision of the transition region could be controlled (Figure 3b). Experiments aligned with modeling results.

Lateral inhibition and activation is a long-standing patterning paradigm most prominently associated with juxtacrine Delta-Notch signaling. Toda *et al.* [11••] and other groups before have engineered lateral inhibition circuits, but highly ordered patterns have not yet been achieved. Negrete and Oates [23] developed an analytically tractable lateral inhibition model to predict possible ordered and disordered patterns (Figure 3c).

Reaction-diffusion patterns (or 'Turing patterns') are a famous paradigm for spontaneous pattern formation, nevertheless biological examples have remained elusive. Karig *et al.* [31] have engineered bacteria that form a stochastic activator-inhibitor system with relaxed Turing conditions that generates disordered patterns (Figure 3b). Sekine *et al.* achieved Turing-like results with mammalian cells using Nodal as a short-range activator and Lefty as a long-range inhibitor [32]. Duran-Nebrada *et al.* [62] engineered synthetic activator-inhibiotor motif in a microbial consortia that produced periodic spatial patterns (note: reference was added during proof phase).

Population regulation is key for MC organisms in order to keep cell proliferation and cell death in check. Liao *et al.* [33•] engineered MC ecological interactions between *E. coli* strains through an antagonistic 'rock-paper-scissors' dynamic. Kong *et al.* and Ozgen *et al.* [34,35] designed bacterial consortia with defined social interactions through chemical or contact dependent signalling. This enabled dominance or coexistence among multiple strains, leading to multistrain patterns with characteristic feature sizes (Figure 3b). All groups supported their results through differential equation-based or agent-based modeling.

Recent advances in extrinsic MC algorithms

Optical biofilm lithography enables the controlled deposition of bacteria onto surfaces. Jin and Riedel-Kruse [15•], Huang *et al.* [8], and Moser *et al.* [36] demonstrated high-resolution spatial patterning using bacteria (Figure 3b). Jin and Riedel-Kruse implemented a discrete Monte-Carlo simulation of cellular adsorption to better understand biofilm formation dynamics [15•] (Figure 3c).

Closed-looped optical MC programming enables the dynamic positioning and differentiation of cells with real-time feedback. Washington *et al.* [37•] and Frangipane *et al.* [38] used 'swarm programming' to demonstrate spatiotemporal patterns of motile photoresponsive eukaryotic and bacterial cells (Figure 3b). Perkins *et al.* [39] demonstrated a 'cell-in-the-loop' approach to optically drive cellular gene expression that generates MC checkerboard patterns. Predictable swarm behavior also enables development of higher level programming languages, for example, 'move cells left' or 'concentrate cells within area x' [5,37•].

Cellular compaction and morphogenesis rely on physical forces generated by cells. Hughes *et al.* [40•] used substrates patterned with DNA to adhere contractile fibroblasts at specific locations and alignments in hydrogels (Figure 3B). Subsequent compaction by fibroblasts allowed for predictable folding of substrate into predetermined shapes and configurations. Finite-element modeling was used to predict the folding of the synthetic tissues over time (Figure 3c).

3D bioprinting has been implemented by several groups to engineer macroscopic MC morphologies. Huang *et al.* [16] developed a platform to 3D-print bacteria that secreted extra-cellular matrix proteins. Morley *et al.* [41] studied the bending and buckling mechanics of 3D printed scaffolds including under cellular strain enabling prediction of the final morphologies of 3D-printed scaffolds after the effects of cellular contractile forces.

Recent advances in MC modeling

While we have already discussed some modeling work in earlier sections that were incorporated within MC engineering projects, here we discuss additional recent work with a greater modeling focus.

Discrete or agent-based MC models directly address individual cells or subcomponents of cells in the simulation. Hartmann *et al.* [2•], Kan *et al.* [19], and Warren *et al.* [42] modeled bacterial biofilm growth by simulating mechanical interactions between individual cells and experimentally validated their models (Figure 3c). Similarly, Ghaffarizadeh *et al.* [20] developed the agent-based open source PhysiCell simulator to study the formation of tumor spheroids in the context of cancer. Sussman *et al.* [43] employed vertex-models, which treat cellular vertices as discrete units, to study the sharpness of tissue boundaries.

Continuous MC models ignore the discreteness of cells and treat the system as a continuum. A major category of continuous models are reaction-diffusion models, such as the relaxed Turing-patterns of Karig *et al.* (Figure 3c) [31] and Sekine *et al.* discussed earlier [32]. Multiple other

groups also investigated more general and relaxed conditions for Turing-pattern formation [32,44–46], including approaches considering more realistic mechanochemical cellular environments [47]. Another class of reaction-diffusion models are the Keller-Segel models for bacterial chemotaxis, which were adapted by Cremer *et al.* [48] to explain range-expansion dynamics of swarming bacteria. Outside the category of reaction-diffusion models, Ko *et al.* [49] used a continuous approach to model the effects of adhesive forces in development, recapitulating adhesion-based cell sorting.

Combined continuous and discrete MC models allow more comprehensive simulations, for example when simulating discrete cells growing on a diffusible substrate. Ebrahimi *et al.* [50] used an agent-based model coupled to a reaction-diffusion framework to model the effects of cooperativity and structure on the degradation of organic materials by microbial communities. Undulation processes were studied using a combined Turing-vertex model by Okuda *et al.* [51] (Figure 3c). Multi-scale approaches to study branching using agent-based models were employed by Lambert *et al.* [52] to study kidney branching (Figure 3c). Martinez-Corral *et al.* [53] introduced a spatially extended mathematical model that addresses the interplay between metabolism and electrophysiology in growing biofilms.

MC computer aided design (MC-CAD) tools enable rapid testing of designs in silico before committing to the biological setting. Hughes *et al.* [40•] and Morley *et al.* [41] applied traditional engineering tools such as the finite element method (FEM) to predict morphological folding (Figure 3c). Tools have also been developed specifically for biological systems, for example, Guiziou *et al.* [27,28] developed computational tools to predict and design MC logic networks, allowing for rapid prototyping of genetic designs by automatically compiling desired outputs into DNA sequences (Figure 3c). Morsut and Lam [54] developed a computational model where contact-dependent cell–cell signaling and cellular responses recreated known morphogenic trajectories for synthetic MC spheroids (Figure 3c). Appleton *et al.* [55] developed a computer-aided design approach for recombinase-based genetic circuits that control the formation of arbitrary MC shapes (Figure 3c). Kriegman *et al.* [56•] used evolutionary algorithms to automatically design diverse candidate lifeforms in silico based on a desired functionality, which were then tested with living systems (Figure 3c).

Physical models with swarm robots provide another route for engineering and analysis of MC systems within a non-biological medium. Slavkov *et al.* [57] demonstrated self-organized morphologies and patterns in swarms of hundreds of 'kilobots' based on local interactions (Figure 3c).

Conclusions and future milestones

The biological cell provides a modular, smart building block to generate complex MC morphologies, patterns, and functionalities across scales of complexity, size, and time. We are currently witnessing a field that is increasingly capable of combining synthetic and natural components to recapitulate and more deeply understand fundamental natural MC systems. However, successful engineering of complex MC systems for practical applications is still in its infancy. Towards this goal, we propose the following future milestones:

(1) **A more advanced parts toolbox** to increase control and stability over MC systems, specifically including: homophilic adhesins [12••], contact signaling [11••], and control over cell growth, apoptosis, cell polarity, symmetry breaking [26•] along all three axes, cell shape, cell movement, and physical parameters like visco-elasticity.
(2) **A set of more versatile and complex MC algorithms**, for example demonstrating boundary formation [19,43], and segmentation clocks [58].
(3) **A self-replicating MC system** that starts from a single-celled 'germ line' and eventually produces more germ cells.
(4) **A self-sustaining MC system**, for example, which develops its own vascularization to distribute nutrients [52].
(5) **A MC system that controls its size during morphogenesis in 3D**, for example, growing a bacterial colony into a specifiable size and shape that is stable for a long time period.
(6) **A spatially organized MC biosynthesis consortium**, where individual reaction steps are distributed among multiple cell types [59], and where spatial organization further advances overall synthesis performance.
(7) **A general multi-scale MC compiler that incorporates gene regulation**, cell mechanics, and diffusion [12••,55,56•,60,61] wherein desired morphologies and patterns are specified on a computer, and the appropriate algorithms, parts, cell types, induction levels, and so on are provided automatically.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Honesty Kim: Conceptualization, Writing - original draft, Writing - review & editing. **Xiaofan Jin:** Conceptualization, Writing - original draft, Writing - review & editing. **David S Glass:** Conceptualization, Writing - original draft, Writing - review & editing. **Ingmar H Riedel-Kruse:** Conceptualization, Writing - original draft, Writing - review & editing.

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