The ability to synthesize biological constructs on the scale of the organisms we observe unaided is probably one of the more outlandish, yet recurring, dreams humans have had since they began to modify genes. This review brings together recent developments in synthetic biology, cell and developmental biology, computation, and technological development to provide context and direction for the engineering of rudimentary, autonomous multicellular ensembles.

What is multicellularity?
Macroscopic organisms generally comprise numerous cells, usually from a common genetic parent, differentiated through environmentally sensitive genetic programs. That is, they are multicellular. But what is multicellularity? How could we approach the multifaceted challenge of engineering autonomous multicellular ensembles? What is missing today from the synthetic biological repertoire? Are there approaches that veer away from the paths taken by nature? Lastly, is there a good reason to do this at all?

A discussion of the definition of multicellularity – particularly when appended to the word organism or when discussed in the context of complex bacterial communities – is beyond the scope of this short paper and excellent reviews already exist [1–4]. Needless to say, any student contemplating these issues should take a developmental biology course to lose themselves in the myriad routes to multicellularity in the natural record; classic textbooks include Wolpert [5] and Gilbert [6]. The seminal synthesis by D.W. Thomson, *On Growth and Form* [7], and the many highly readable monographs by J.T. Bonner [8–10], although in many ways dated, issue forth inspiration from almost any page. Lastly, the elegant little book *Vehicles: Experiments in Synthetic Psychology* [11] provides a conceptual roadmap toward engineered complexity that is well worth the read.

I will avoid in this paper the notion of coopting existing mammalian cell lines to form synthetic multicellular ensembles; that is primarily the province of tissue engineering. This is not to say that is not a useful endeavor, of course; but these lines already contain within them complexes – and poorly understood – systems for producing specific metazoan forms. They harbor millions of years of evolutionary baggage, so to speak. To form a working definition of multicellularity (to which we can later add the label synthetic), we would like to start much closer to the beginning.

**Multicellularity and emergent behavior**
At its most fundamental level, multicellularity arises when cells come together and find means to couple their internal states in such a way that the connections result in emergent behavior – generally with improved fitness for a set of problems – that arises from the collective of cells. This is a somewhat weak definition of multicellularity, because it allows for numerous collective ensembles (e.g., bacterial biofilms or intestinal microflora) that are themselves not considered organisms, but for the engineer it allows a certain flexibility when approaching the problem. There is now a well-developed literature in applied mathematics, computer science, and the biology of social insects that deals with the analysis and design of similar multi-agent systems. It has long been known, for instance, that the complex functions carried out by social insect communities (foraging and detection of environmental signals, construction of complex structures [12], and even specification of insect phenotype [13]) emerge from a beautiful interplay between environmental factors, ‘simple’ behaviors encoded in the individual insects, and the exchange of information between individuals. This cooperation results in a type of ‘swarm intelligence’ that is robust to insult and environmental change and can carry out optimization tasks [14–16]. Numerous engineering efforts, notably in computer science and applied mathematics, have taken inspiration from these phenomena to develop software packages for routing and optimization of many real-world problems [17], to explore the theoretical aspects of emergent systems as computational engines [18,19], and to build computation into physical structures [20,21] (an active endeavor that is fundamentally similar to the topic that concerns us here). That many of these ‘social’ behaviors bear striking resemblance to functions and properties of multicellular organisms motivates a closer inspection. The point here is that before we delve into how multicellular ensembles might be constructed, we should spend some time learning the rich computational background that now exists for designing and analyzing systems with emergent properties. In this regard, the *Dictyostelium discoideum* community has perhaps some of the best demonstrations of the remarkably complex, yet computationally tractable, emergent behaviors arising from cells executing relatively simple processes. From Bonner [10,22] through Nakagaki’s maze-finding slime molds [23] to the so-called Tokyo subway experiment [24], *Dictyostelium* has become one of the models for studying emergence and multicellularity [25]. The broader point is that, as with almost all engineering efforts initially inspired by nature, once we computationally understand emergence, we are likely to find solutions not available from
the observable natural catalog (e.g., consider the robot Rhex [26] or synthetic nacre [27], both inspired by but very different from natural systems). Armed with this conviction, we can now ask what ‘parts’ are needed to synthesize collective behavior in cells.

The transition to multicellularity
Where might we turn for a first look at what genetic and epigenetic modules are needed to engineer multicellularity? The biomolecular complexity of the classic model systems in developmental biology (e.g., Drosophila melanogaster, Caenorhabditis elegans) can be daunting [5]; even Hydra [28] and the simple flatworm [29] are already far down the path into multicellularity. Luckily, the past decade has seen an explosion of work on species bordering the transition from the unicellular to multicellular lifestyle [30–34]. It is here that, stripped of all but the barest essentials, we may begin to identify the specific components we must engineer. Sometime around 600 million years ago, colonial and truly multicellular ensembles began to emerge from unicellular flagellates [34]. Today, some of the closest relatives to metazoa are the choano-flagellates (Figure 1) – a collection of small (3–10 μm), flagellated eukaryotes, some of which are unicellular, some multicellular, and some that can adopt either phenotype [35]. What is so intriguing about these creatures is not only that certain species exhibit both unicellular and multicellular phenotypes – which makes it possible to observe in detail the genetic and structural changes that allow multicellularity [36,37] – but that spectra of closely related species exist that together capture the heritable transition to multicellular behavior. Moreover, genomic and proteomic analysis has shown that, remarkably, components of many of the genetic systems once thought specific to metazoa and bilaterians (cadherins, receptor tyrosine kinases [RTKs], bilaterian miRNAs, Piwi-interacting RNAs [piRNAs]) and thought to be crucial in the development and maintenance of complex forms are present in choanoflagellates [30,32,33,38]. Some of these pathways (e.g., cadherins) appear to have arisen before multicellularity, were involved in environmental and prey–predator detection, and were coopted during the transition [39].

Numerous studies [1,31,36,40–44] point to the handful of basic ‘enabling technologies’ we must consider engineering: the excretion of an extracellular matrix (ECM), the presence of cytoplasmic bridges, cell–cell adhesion, and molecular differentiation (or inherited functional specialization). A fifth observation, likely to be emergent, is that choanoflagellate colonies appear to form not due to aggregation, but due to non-separation after division (with the concomitant production of a matrix and cell junctions). Let us consider these enabling technologies one at a time.

A recent review [45] provides an overview of ECM domains as well as the known phylogeny in the catalog; Özbek et al. provides an evolutionary viewpoint [46]. Choanoflagellates can secrete multiple types of ECM [36], although apparently none with the complexity of true metazoan ECM [45]. The type prevalent in colonial phenotypes of Salpingoeca rosetta appears ‘amorphous, loose and space-fitting’, whereas unicellular phenotypes of the same species are capable of producing denser, more precisely shaped ECM as well (the so-called theca ‘goblet’) [36]. Cells of the multicellular alga Volvox embed themselves in a complex ECM and convert part of their cell walls to ECM; the massive amount of ECM secreted by these cells allows for very large colonies (in proportion to their cell number) [30]. In more complex eukaryotes, the ECM constitutes not only a mechanical support layer, but is a fundamental communication channel between cells, allowing chemical and mechanical signals to be exchanged by cells attached to it [46,47]. The fact that ECM is usually laminar with respect to some axis of the ensemble provides a ready reference to establish polarity [6,47]. Bacteria are known to also secrete some sort of matrix, particularly when forming biofilms, but they seem to lack the same richness of communication modes as eukaryotes capable of more complex multicellularity despite the fact that they can form differentiated spatial structures with different structural ECM for different functional components [45].

This, of course, brings us to cell–cell adhesion molecules, principally the cadherins. Choanoflagellates possess cadherins [39] and integrin domains (but no true integrins) [45], putatively allowing for cell–cell and cell–ECM adhesion. This enables the coupling of external mechanical events and information to internal changes in cell state. This is one of the functions most sorely lacking in the bacterial synthetic biology repertoire. Although ongoing work has demonstrated that the Mscl family of channels in bacteria are sensitive to membrane strain and affect transmembrane potential [48,49], there are, at present, no well-characterized modules for coupling strain or adhesion to genetic expression. Cadherins are crucial enablers not only of cell polarity, but also of emergent and coordinated relative motion and reorganization within a cell ensemble, a topic of recent interest [50–53]. Cytoplasmic bridges, which in choanoflagellates seem to arise from incomplete cytokinesis [36], also seem to play a role, both in maintaining physical connections among colonial members and in the constrained exchange of signaling molecules.

Figure 1. A spherical colony of Sphaeroeca choanoflagellates. Reproduced, with permission, from http://commons.wikimedia.org/wiki/File:Sphaeroeca-colony.jpg.
Lastly, we come to the topic of differentiation (or heritable cell state). In eukaryotic systems, this is a vast field of inquiry, well outside the scope of this review. Within microbial synthetic biology, switches and feedback loops that allow an inducible, switchable state and that can pass this state to daughter cells have been demonstrated; I will return to this below in the context of circuits and systems already developed by synthetic biologists. It is worth noting that, in flagellates, there is an inherent trade-off between motility and division, because both processes make use of microtubule-organizing centers (MTOCs). King [34], Buss [54], and Michod [55] have pointed out that differentiation between flagellated cells on the surface of a multicell colony and non-flagellated cells in its center arose as a solution to the MTOC trade-off, because colonies containing flagellated and non-flagellated cells would be under strong environmental pressure to expose as many motile cells on the surface of the colony as possible. This, of course, is what one sees in many species of colonial flagellates. More importantly, it points to the idea that directed evolution [56] in the presence of some of the basic components described above might allow us to recapitulate something as powerful as gastrulation [5,6] and spatial organization [55]. In fact, almost 30 years ago, Edelman suggested in his ‘regulator hypothesis’ that selection acting on just cell–cell adhesion genes and differentiation gates could account for stable and varied body plans ‘within relatively short periods of evolutionary time’ [57].

Moving forward – synthetic engineering beyond the genetic circuit

Efforts over the past decade have yielded a catalog of basic cell state circuit motifs [58,59] that include bistable switches and memory elements [60–63], oscillators [64–66], simple logic gates [67,68], and amplifiers [69,70]. The complexity of genetic circuitry will no doubt increase in the coming years, enabling more sophisticated programming of cell state and, thus, at least some degree of synthetic differentiation in microbial models. What else then should we focus on in the quest for multicellularity?

Local relationships lead to global organization

Numerous synthetic intercellular signaling systems have been demonstrated in the past few years [59,71], with quorum sensing-derived circuits gaining widespread use [72]. Quorum sensing in bacteria relies on the well-known acyl homoserine lactone (AHL) family of freely diffusible and membrane-permeable compounds. The ability to both induce the production of AHLs via the LuxI gene product homologs and detect AHL concentration via AHL–LuxR complexes acting on inducible promoters allows for the introduction of diffusible intercellular signaling modules; an early seminal result was the coupling of an AHL signaling circuit to a cell ‘killer’ gene to synthetically regulate bacterial culture growth curves [73]. The number of truly orthogonal AHL communication channels is somewhat limited, but that catalog may grow. One of the outstanding challenges in the field is the demonstration of a robust framework for coupling intercellular signaling and circuits encoding cell state to generate programmable patterns in cell state across an initially homogeneous multicellular population. Early seminal efforts demonstrated that groups of ‘receiver’ cells sensitive to the presence of AHL could be made to respond in a tunable fashion to the presence of ‘sender’ cells that produced diffusible AHL [74]. This work was subsequently extended to demonstrate consensus cooperation in microbial communities [75], synthetic predator–prey systems [76], and intercellular communication enabling edge detection across light/dark areas [77], among others. A series of papers from the Hasty laboratory have since demonstrated the use of AHL-coupled oscillators for entraining and synchronizing populations [66,78,79]. More recently, AHL-based signaling was used to provide chemical ‘wires’ between cell colonies, each carrying out distinct computations to enable more complex functions than those possible within single cells [80]. Much more remains to be done in this area. True symmetry-breaking systems such as the classic Turing [81] and Meinhardt–Gierer [82] diffusible signal pattern generators have not yet been robustly demonstrated, despite recent experimental [Ting, L. et al. (2008) Pattern formation in a synthetic multicellular system. 2008 APS March Meeting. 53 (http://meetings.aps.org/link/BAPS.2008.MAR.J17.7)] and theoretical results [83]. Despite the early work on the subject, little has been done to create systems in bacteria with multiple, simple, orthogonal cell–cell communication motifs operating to produce complex emergent behavior in communities of cells. As cited above, there is a wide literature in computer science and applied mathematics concerning cellular automata and emergent systems, many of which have been shown to be capable of propulsion, reproduction and size control and to be Turing complete [19,84–86]. As additional orthogonal communication channels become available (see below), this type of spatial programming should become accessible. Moreover, emergent behavior need not arise exclusively via chemical dynamics. A recent example elegantly demonstrates how motility and density can be coupled via a synthetic AHL-mediated circuit to produce stripe formation in Escherichia coli [87]. Decades of theoretical treatment of the mechanical underpinnings of metazoan morphogenesis have provided several testable models of how mechanical communication via cell–cell and cell–ECM coupling would result in complex shape changes and modulation of gene expression [51,88–91]. Emergent pattern formation from simple ECM-mediated processes has been documented. An old example [92] demonstrates how fibroblast traction on collagen matrix itself aligns the underlying collagen fibers to tracks toward the cells, driving further aggregation. This simple mechanical instability was capable of driving cell density-dependent Turing-like pattern formation. This result reflects a still rather unexplored facet of cellular pattern formation: the interplay between cell processes and physicochemical forces in the environment, which together drive complex emergent behavior [51].

Missing links

It is believed that several of the genetic and epigenetic processes that regulate multicellularity in eukaryotes are missing in bacteria; these include cadherins, tyrosine kinases, and complex ECM production (see above). Given that bacteria as a whole do exhibit some matrix production,
cell–cell contact-mediated signal transduction [93–95], strain sensing [49], rapid membrane potential depolarization events [96], and even direct electron transfer channels [97–100], it seems likely that analogs of eukaryotic processes can be synthetically engineered into or mined from bacteria. Three specific missing components would be invaluable to the pursuit of multicellularity and are likely to be obtainable in the near term (Figure 2): a stress/strain sensor coupling either membrane stress or stress on an extracellular process (i.e., pili, flagella, or cilia) to gene regulation; a cell–cell contact-mediated channel for either cytoplasmic contact or signal exchange; and a system for inducing and maintaining cell polarity in bacteria relative to a matrix plane. Coupled with synthetic cell–cell adhesion, a stress/strain sensor might allow bacteria to approach the level of cytomechanical control multicellular eukaryotes employ for cell sorting and multicell motion. On the issue of cytoplasmic bridging, some reports appear to indicate the presence of cytoplasmic exchange – including plasmid transfer – across ‘nanotubes’ seen between *Bacillus subtilis*, *Staphylococcus aureus*, and *E. coli* [101]. Again, choanoflagellates may be invaluable, because it is thought that metazoan adhesion proteins may be derived from proteins used by heterotrophic flagellates to recognize and bind bacterial prey [39]. A fourth, and far more difficult, need is for modular RNA- or protein-based systems capable of switching states much more rapidly than allowed via transcriptional regulation (i.e., for fast responses to the environment) [102,103]. Although components do exist, they are not yet sufficiently well understood, modular, or orthogonal to enable the flexible engineering of, for example, sensor-to-flagellar control modules in bacteria (i.e., fast synthetic navigation systems).

**Putting it all together**

Paralleling the increasing complexity of the constructs in Valentino Braitenberg’s seminal book *Vehicles: Experiments in Synthetic Psychology*, it is tempting to entertain the notion of a series of grand challenges. Who can be the first to demonstrate a synthetic, motile colony? With programmable sensor-to-motility response? With programmable form? With cell lineage differentiation? With reproduction? With memory? Here arises the issue of assembly. The classical position of the genetic engineer is to look for the gene or network of genes, however mythical, that will generate a desired phenotype when introduced into a cell. This is, in essence, a self-assembly approach: we genetically engineer desired behaviors in cells and then allow them to grow and form multicellular systems. A crucial area of work in the context of engineering multicellularity via self-assembly is the so-called ‘decomposition problem’ [104–106]. That is, given a final form and a palette of possible operations or gene circuits, can I compute the sequence of such operations that form the final form? Can I determine the minimal set of ‘functions’ (that is, cellular behaviors such as adhesion, force production, migration, and signal production) required to arrive at a certain multicellular form?

Alternatively, we can impose external constraints on the cells that direct the formation of multicellularity. This is an example of constrained assembly, common to classic, industrial age manufacturing wherein form or function is engineered via a sequence of top-down steps (as in the assembly of a mechanical watch). Biology, in fact, makes extensive use of constrained assembly. The *Drosophila* egg chamber, for example, is in part an apparatus for symmetry breaking and driving the constrained assembly of the early fly embryo. Developmental biology courses usually focus on the beautiful sequence of events that unfold in the fertilized embryo starting with the Bicoid gradient (specifying, for example, segments and assembling organs) and ending with an adult fly. What is relevant to this discussion is that the initial anterior–posterior and dorsal–ventral axes of polarity are determined by sequestered mRNA laid down in unfertilized eggs by maternal nurse cells in the egg chamber; thus, symmetry breaking is accomplished in the embryo by the mother [5,107]. Many examples of these maternal effects can be documented phenomenologically, but detailed mechanistic understanding appears to be emerging [108].

When considering synthetic multicellular engineering, it is likely that abiotic ‘maternal effect’ technologies – that is, machines that break symmetry for the developing system or otherwise impose boundary conditions on the multicellular ensemble to guide development – could play a role. A vast repertoire of non-biological devices can be
brought to bear. Whether or not natural colonial and multicellular systems arise through self-assembly, human engineers need not build them that way. Modern advances have resulted in a wide variety of devices for specifying the chemical, mechanical, optical, and electrical milieu both intra- and extracellularly. An exhaustive review is outside the scope of this piece, but some examples are provided below. Given the medical utility of many of these methods (including drug delivery, tissue engineering, and clinically relevant sensing [109]), they continue to advance rapidly. Simply put, having established an engineering goal (e.g., a 1-mm diameter, 1024-cell motile, flagellated colony that swims up a specified concentration gradient and lyses at a programmed threshold), we should explore technologies beyond those available in natural systems to engineer it.

Hybrid abiotic/biotic systems are likely to be useful even beyond assembly tasks. Consider again our flagellar colony. Why not assemble such a colony on abiotic parts properly functionalized with adhesion proteins? A simple computational engine fabricated in a modern semiconductor process can be as small as 100 µm on a side. Chemical input/output [110–114], electrochemical sensors for DNA hybridization [115], nanoscale and integrated light detectors and LEDs [116], polymer strain gauges [117,118], surface energy switching [119], and even nanowire transmembrane recordings [120] can be integrated into very small systems. From my work in integrating abiotic components with live insects for flight control [121], it has become clear that hybrid systems can use the best of both worlds. Biological systems did not evolve radios or 22-nm transistors; man-made communication and computational systems have reached a staggering degree of sophistication in incredibly small, low-power packages (e.g., the off-the-shelf ATtiny10 from Atmel is a 12-MHz, 8-bit microcontroller with a 1024-byte flash memory in a package ~2 mm square; unpackaged, it is about half this size). The power requirements for the 100-µm computational engine could be <10 µW on average and powered entirely from a tiny solar cell on-chip. Conversely, man-made energy sources, motility systems, and material properties still pale in comparison with the natural arsenal. It is not feasible with modern abiotic technology to make an autonomous 1-mm swimming robot capable of locomoting indefinitely; we cannot build good synthetic chemotropics, our actuator technologies are poorly suited to that scale, and communication via radio or optics is not easy in real underwater environments. There are other, ancillary advantages of hybrid approaches. Abiotic parts would not be subject to genetic changes or selection pressure; hybrid systems would not be able to reproduce, alleviating some safety concerns, and have finite lifetimes.

**Why do this?**

Lastly is the issue of motivation: is this something worth doing? Much is still to be worked out to achieve scalable synthetic control circuits [71], not to mention fundamental technology development [122,123]. It is not clear what we do with the simple ‘colonial’ constructs I have sketched above and it is a long way to anything resembling a metazoan. The attraction of the problem, however, is threefold.

First, a grand challenge around true, synthetic multicellularity would provide an immediate test bed for the development of many other platform technologies crucial to synthetic biology. With properly formulated systems goals, we could outline specifications for the various modules needed and present multicellularity as a competitive challenge wherein groups arrive at unique solutions. Consider some medium-term applications. Multicellular, mobile colonies with silicon payloads could be deployed in rivers and lakes as environmental health monitoring devices, capable of interacting with the aquatic biology at various depths and communicating with man-made devices. Organized films of swarmer cells could clean surfaces: ingesting small particles, digesting larger ones and carrying indigestible particulates (including excess cell mass) to set locations. Cell collectives capable of taxying into and around each other in weaves (and then depositing matrix) could assemble and repair simple textiles on demand in remote places. Sophisticated biofilms could detect strain non-linearities indicative of the cracking of an underlying stratum and produce binding matrix.

Second, this endeavor may drive the development of toolsets for use in non-canonical organisms like the choanoflagellates (among others), something of great value to fundamental molecular and comparative biology.

Lastly, and most importantly, the demonstration of synthetic multicellularity with a complexity eventually rivaling that of metazoans or terrestrial plants must surely be one of the long-term goals of synthetic biology. This arises not from an argument of biomimicry – which, followed blindly, leads us down fruitless technological paths – but because we will never truly understand the limits of developmental regulation, stability, and plasticity until we have recapitulated developmental processes on our synthetic platforms. There are likely to be stable areas of the metazoan parameter space that evolution has either selected out or not explored and that possess technological value for our society. Just as the 20th century made it obvious that humans could arrive at molecules and chemistries not found in nature starting from the same constituent atoms, it is likely we will find multicellular solutions with technological value not arrived at via natural selection. That this has profound ethical and societal consequences cannot be overstated.

**Concluding remarks**

There is additionally – and perhaps controversially – an ultimately ecological rationale for this vision. For the most part, the technological base created by the industrial revolution communicates poorly with the underlying organic technology of the planet. The rapid explosion of man-made, acellular, resource-consuming, and waste-producing constructs is in large part responsible for the ecological and climatic mess we are in. Mindful of the vast ethical and societal questions raised, it is worth considering a future wherein our homes, our factories, and our consumer gadgets can ‘understand’ the language of the organic systems around them and form part of a related or hybrid framework of information and material exchange. This notion – that our societal artifacts should be mindful of their natural surroundings – has long and deep roots in many
cultures and has modern reflections in, for example, the natural building movements. It is my contention that the development of synthetic multicellular – and likely hybrid – systems is a step down this transformative road.

References
Opinion


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