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# Creating Living Cellular Machines

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**Abstract**—Development of increasingly complex integrated cellular systems will be a major challenge for the next decade and beyond, as we apply the knowledge gained from the subdisciplines of regenerative medicine, synthetic biology, microfabrication and nanotechnology, systems biology, and developmental biology. In this prospective, we describe the current state-of-the-art in the assembly of source cells, derived from pluripotent cells, into populations of a single cell type to produce the components or building blocks of higher order systems and finally, combining multiple cell types, possibly in combination with scaffolds possessing specific physical or chemical properties, to produce higher level functionality. We also introduce the issue, questions and ample research opportunities to be explored by others in the field. As these "living machines" increase in capabilities, exhibit emergent behavior and potentially reveal the ability for self-assembly, self-repair, and even self-replication, questions arise regarding the ethical implications of this work. Future prospects as well as ways of addressing these complex ethical questions will be discussed.

**Keywords**—Tissue engineering, Systems biology, Synthetic biology, Biobots, Vascular networks, Neuromuscular junctions, Biological machines.

## INTRODUCTION

The past century has witnessed tremendous advances in science and technology, but these are especially evident in the biological sciences. New discoveries in synthetic biology, tissue engineering, systems biology, and developmental biology, which have accelerated in the past 10–20 years, are nothing short of phenomenal. This new appreciation of fundamental biological processes is changing our lives in numerous ways ranging from new healthcare technologies to alternative energy sources to environmental protection.

But the potential for even greater and more significant advances lies ahead. Until recently, biological research has led primarily to a detailed yet largely qualitative understanding of fundamental phenomena at the molecular and cellular scales. These qualitative concepts are being increasingly cast in quantitative form through new programs at the intersection of engineering and biology. The synthesis of ideas and approaches from these diverse disciplines has been termed *convergence* in a recent study from the *National Research Council* that lays out a visionary plan for future biological research.[44] While many of the specifics remain ill defined, it is becoming increasingly evident that the advances in molecular and cellular biology of the 20th century are certain to translate into entirely new technologies in the 21st.

One of the greatest opportunities lies in the potential to understand and control populations of multiple cell types and their interactions.[33] To a large degree, this has been one of the driving forces behind developmental biology, tissue engineering, and systems biology. Yet, it can be argued that a tremendous gap exists between understanding processes at the level of a single cell and the behavior of large-scale tissues, i.e., how the local rules of interaction result in global functionalities and diverse phenotypes. This is an issue involving complex systems of multiple interacting components that could fruitfully draw upon considerable advances in the engineering realms of forward engineering, forward design, and manufacturing of large, complex systems.

## THE FOUNDATIONAL DISCIPLINES

Creation of living machines is critically dependent upon developments drawn from a range of existing disciplines, a unique and thoughtful fusion of which

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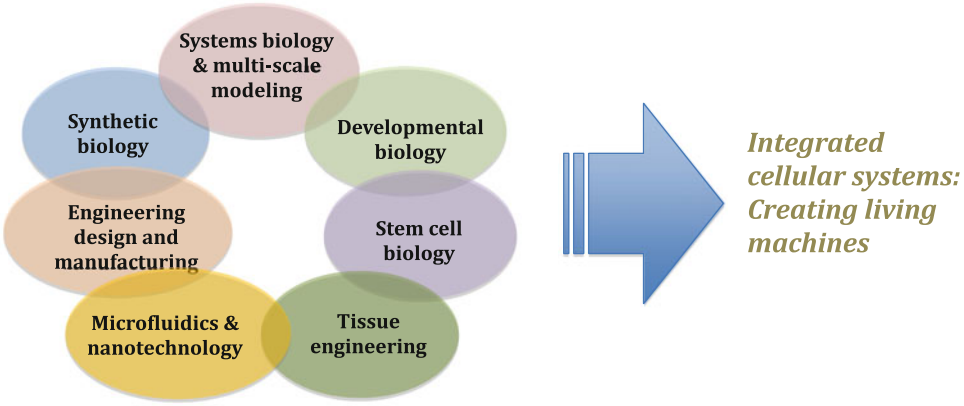


**FIGURE 1. The fusion of different disciplines and specialties needed to develop living machines.**

can result in a new discipline where engineers are designing machines and systems with biological components and cells at various length scales (Fig. 1). We first review some of the significant advances, and discuss them in the context of the present state-of-the-art (see Fig. 2 for a collection of representative research nuggets). Progress in creating living machines relies on the fusion and convergence of these different fields that have to be seamlessly integrated to result in the pedagogical foundations of a new discipline dealing with design and realization of engineered biological machines from cells.

Synthetic biology closely parallels what we propose. Among the major advances that have been made is a detailed and systematic process for mathematically modeling gene regulatory networks.[2,13] For this purpose, a systems objective has been adopted that is capable of describing and analyzing how changes in the genetic code of the cell affect cell behavior. More than that, it also provides the framework for engineering desired modifications in cell behavior in order to confer new and useful functionality. A similar framework might be used for engineering cell–cell interactions operating on larger length scales and with a variety of interacting cell types.

The basic idea of design and realization of living machines mimics that of forward engineering design, where precise models and specifications exist for the components. Using these, the design of more complex machines can be achieved. This is similar to the goals of synthetic biology where libraries and specifications for parts are used to create newer functions. Similarly, systems design and top-down decomposition of a complex system such as a biological machines and the design specification of the sub components will be critical for the design of different machines using those sub-components.

As significant as these advances are, much of the work thus far has been directed toward engineering individual strains of bacteria or yeast systems.[45] This is a logical approach for many reasons, but places constraints on the types of functionality that cells might ultimately achieve by interactions with multiple different cell types. Mammalian cells, in particular, offer unique capabilities, developed over millions of years of evolutionary pressure, and exhibiting a wide range of coordinated behavior. It is not coincidental that mammals are considered the highest form of life, since the collective behavior of an organism created from a large variety of different cell types has far more potential to attain high-level function, which would, in fact, be impossible with a single phenotype/genotype. Therefore, although not all biological machines will be based on mammalian cells, the ability to apply the lessons learned from synthetic biology to all types of cells, acting in a coordinated manner, will be essential in creating machines that are both multifunctional, and ecologically stable in the sense that no one cell type overtakes the others.

Regenerative medicine including tissue engineering also has much to contribute to the creation of living machines. Notably, tissue engineering has achieved some major technological advances over the years. Artificial skin was the first engineered tissue to reach the market. In 1982, the first composite living skin was produced which led to a commercial product.[70] Other tissues such as bladder and cartilage are either available commercially or soon to come onto the market (see Table 1 in Berthiaume *et al.*[6]). Still others have been the subject of intense investigation, and the technologies are rapidly improving. Products are at various stages of development for liver,[1,15] bladder,[3] pancreas,[36] heart tissue,[22] and others,[6] but fundamental barriers persist, such as the need for a vascular

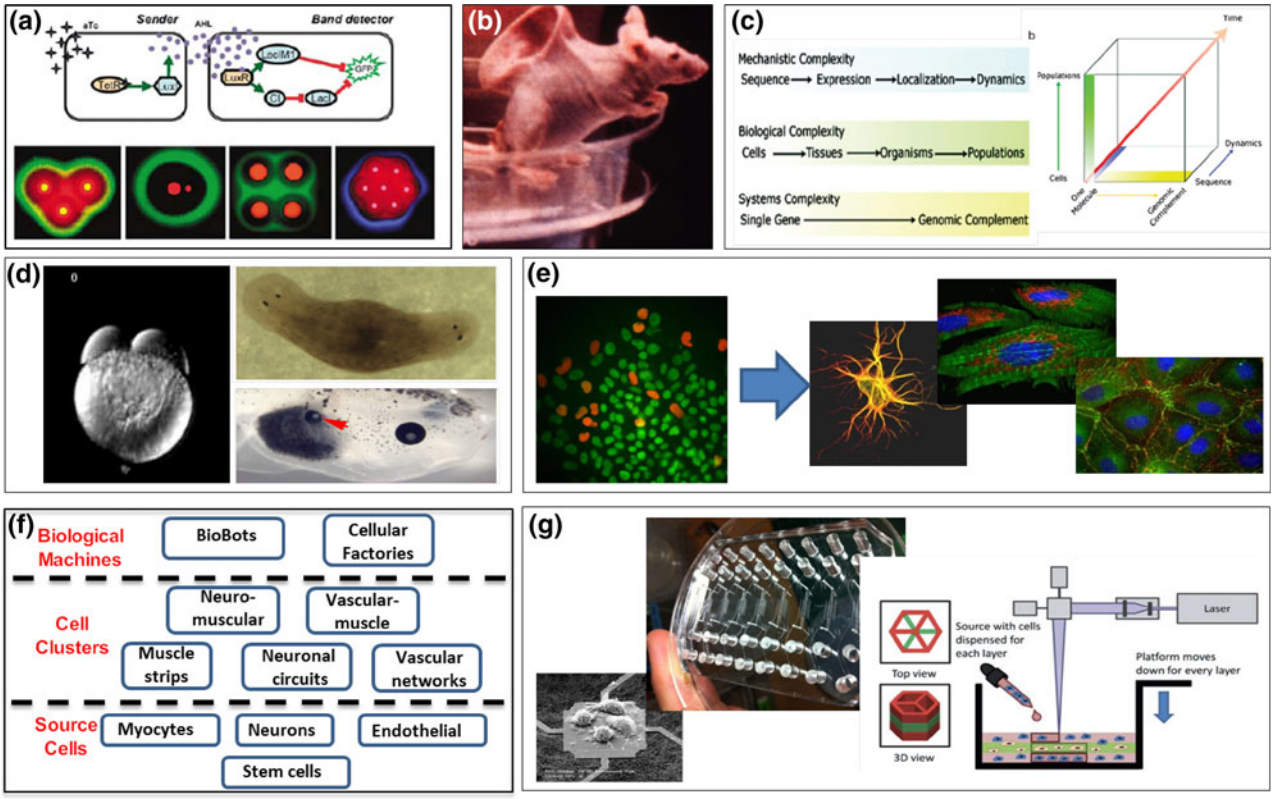


**FIGURE 2. Synthesis and examples of the different disciplines: (a) gene networks in bacteria can be programmed to result in different patterns, reproduced with permission from Basu *et al.*,[4] (b) the "Vacanti mouse" as an example of tissue engineering and control of biological phenotype, reproduced with permission from Vacanti *et al.*,[61] (c) systems biology depicted in terms of increasing complexity of mechanistic, biological, and systems understanding, reproduced with permission from Lauffenburger,[32] (d) developmental biology examples such as growth of a zebrafish embryo (left) and examples of phenotypic control by altering the electrical polarization of adult stem cells to generate a 2nd head on a planarian, or to develop a second working eye induced on tadpole gut, adapted from Basu *et al.*,[4] (e) stem cell differentiation to produce neurons, muscle or endothelial cells, (f) systems design and top-down decomposition of example biological machines consisting of cell clusters and specific cell types, and (g) examples in microfluidics, lab on chip, and 3-D fabrication using stereolithographic printing of cells and polymers for tissue engineering and 3-D soft systems, Adapted from Chan *et al.*[9] and Park *et al.*[46]**

system to meet the metabolic needs of the tissue construct.

It might be argued that one of the factors that has prevented tissue engineering from having attained broader success is the lack of a fundamental understanding of the complex processes that lead to tissue formation, complexity that comes in many forms such as mechanistic, biological, or systems. Systems biology has been building the tools that will enable this deeper understanding, through the development of computational approaches to simulate biological processes at multiple length scales.[12,32] These range from models based on first principles to those that are purely data-driven, and all have been fruitfully employed. But whatever their approach, they collectively offer the potential to serve as a receptacle of the knowledge gained about a particular system or process, and allow us to capture and simulate the complex interactions between systems and between scales that might otherwise be incomprehensible.

Much of our fundamental understanding of biological systems and the methods that might be used to grow them comes from developmental biology. Since the study of developmental biology most closely mimics the 'bottom-up' or emergence driven behavior of cellular clusters, foundational principles from this very broad and widely researched field will play a critical role in creation of the proposed biological machines. Since these studies are challenging to do in mammalian based systems due to the longer time spans and the ethical considerations involved, model systems such as zebra fish are being studied in great detail.[20] Genetic manipulation and engineering is an important tool to vary the genetic programming in these model systems to examine the resultant phenotypes and correlate genes to phenotype and function. Many of the morphogens that enable cell–cell signaling during development have been identified and the process of development can be manipulated by controlling morphogen gradients or localized cell depolarization. For

example, introduction of ion channels that alter the local transmembrane potential are also being used to study the resulting development of model organisms such as planarians or tadpoles to form organisms with two heads or multiple organs with stably formed and reproducing organisms.[5]

Cells used in biological machines ultimately need to be compatible with each other in terms of inter-cellular signaling and maintaining long-term stability, and this argues for the use of cells from the same species if not the same organism (although we also recognize that there are numerous examples in nature where cells from different species interact in a synergistic manner, for example in the human gut where bacteria enjoy an symbiotic relationship with human cells). And since it is likely that some of the cells will need to be genetically modified, one logical solution would be to derive cells from a pluripotent source. Therefore, stem cell biology is another pillar in the discipline of biological machines, and here again, we are able to draw upon a wealth of prior and ongoing work in support of these efforts. While there are still many challenges in producing functional and stable differentiated cells, numerous protocols already exist for the differentiation of many of the cell types from stem cells or other precursors needed for the various functionalities required by these machines (see e.g., http://www.nature.com/nprot/series/stemcells/index.html#archive). Not every cell type can be derived, but the methods developed thus far and the currently identified morphogenic agents serve as a useful starting point. Similarly, differential of different cell types from the same cluster of stems cells, for example, is quite challenges as the media requirements might be different for the two target cell types.

Many advances in microfluidics and nanotechnology over the past decade have now allowed the development of this enabling field of technology with applications in biology, medicine, electronics, materials, energy and other areas. The technologies for fabrication of soft polymers such as PDMS, hydrogels, and biomaterials can especially have major implications in the development of biological machines. BioMEMS (bio-Micro Electro Mechanical Systems) and Microfluidic devices are being used a point of care devices,[67] *in vitro* cell culture devices for drug screening, and implantable hybrid devices for innovative solutions to organic–inorganic interfaces.[29] 3-D fabrication and printing of cells and polymer scaffolds can be used to place cells with spatial control on or in scaffolds to realize new physical designs for biological machines. Techniques such as photo-polymerization using stereolithography, 3-D rapid prototyping, and 3-D printing can be especially useful in this context.[11,73]

Many examples exist of developments in each of these important foundational areas where the fusion and synthesis of these concepts can contribute to the development of cellular and biological machines (Fig. 2). This 'forward-engineering' of biological components is certainly an important element of what is called the 'New Biology', which envisions the integration of multiple disciplines, leading ultimately to enhanced understanding of fundamental principles in biology, and ultimately novel approaches to the problems that face society (Fig. 3). Yet another perspective emerged from discussions at a recent *Keck Futures Initiative* that focused on the extension of synthetic biology into more advanced cell types, such as mammalian cells, and moving toward poly-culture systems, potentially using cells from multiple species to generate new and unique behaviors (see http://www.keckfutures.org/conferences/synthetic-biology.html). Thus, what we present here is an idea that is increasingly being explored, accepted, and embraced by the research community. Ours is one perspective on the future, in particular, a future that envisions living machines that are engineered to perform specific tasks that differ from or perhaps combine or enhance the capabilities of existing organisms. We summarize here the current state-of-the-art in the critical foundational disciplines, recent work that makes the case that many of the needed elements are already existent, and attempt to identify future needs in terms of where efforts are needed in order to achieve these ambitious goals and develop a systematic process for creating living machines.

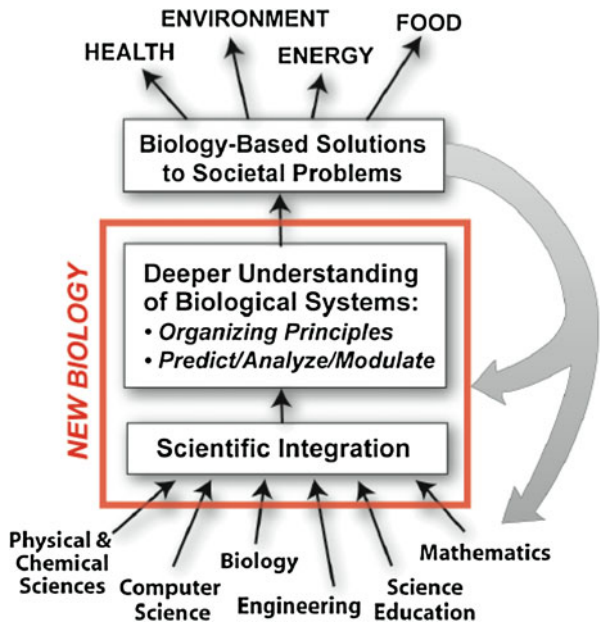


**FIGURE 3. Conceptual schematic of the interdisciplinary foundations of the New Biology with implications for many solutions to societal problems. Reproduced from *A New Biology for the 21st Century*.[44]**

## BIOLOGICAL MACHINE AND ESSENTIAL ELEMENTS

Can we truly "engineer" or "orchestrate" the growth of entire biological systems? To do so will require that we overcome a number of significant barriers. Biological systems are exquisitely complex, even at the scale of a single cell, and the level of complexity escalates precipitously when multi-cell interactions need to be considered. One embodiment of the conceptual framework leading to a biological machine is shown in Fig. 4; an increasing level of complexity is expected as one progresses through cells, modules, and eventually a machine. Take, for example, the case of simple interactions between neural-controlled muscle of a scale that requires a vascular supply, as needed for a biological robot ("biobot") of millimeter or greater scale. Certainly the individual cell types needed—neuronal, muscular, vascular—can be isolated or even derived from pluripotent cells, and these can be co-cultured in systems that enable some level of interaction. Inducing these cells to combine into a functional, stable muscle actuator for use in a biobot is an enormous step, however, and will require significant advances on many fronts.

Given the enormous complexity of living systems, it seems implausible that one could engineer and control the position of each cell and its interactive functionality with its neighbors. So, although we can and must specify the design parameters of the machine, we can only hope to assemble clusters of cells into the approximately correct arrangement, and rely on natural processes to establish the functional complexity needed for its operation. For example, one might seed motor neurons in close proximity to an engineered muscle strip in a system of the type described below, where the formation of neuromuscular junctions would follow axonal outgrowth guided by chemotactic factors secreted by the muscle cells and spontaneous pre- and post-synaptic differentiation.[31,41] In a sense, the cells must participate in the fabrication process of the machine, and in doing so, we must rely on biological processes (e.g., those that occur during development, regeneration or wound repair) to help. We consider this "emergent behavior" to be an intrinsic competence of the cells, and must at some level rely on it to proceed naturally. Here, the concepts associated with developmental biology, as mentioned earlier, come into play. So we view the manufacture of a living machine as requiring both top-down design specification and a process for "assembling the parts", as well as a reliance on emergent processes that are programmed into the genetic code of the cells, and involve various forms of cell–cell and cell–matrix interactions.

## WHAT IS POSSIBLE TODAY?

While this all may seem futuristic and to some, unrealistic, an argument can be made that the creation of living machines is not only possible, but that some simple machines could even be on the near-term horizon. We construct this argument by working up in complexity. We first consider homotypic cell clusters; that is, collections of cells of a single type that function in some unified manner. Examples considered here include muscle, neuronal networks, and vascular networks. Then we consider initial efforts to combine

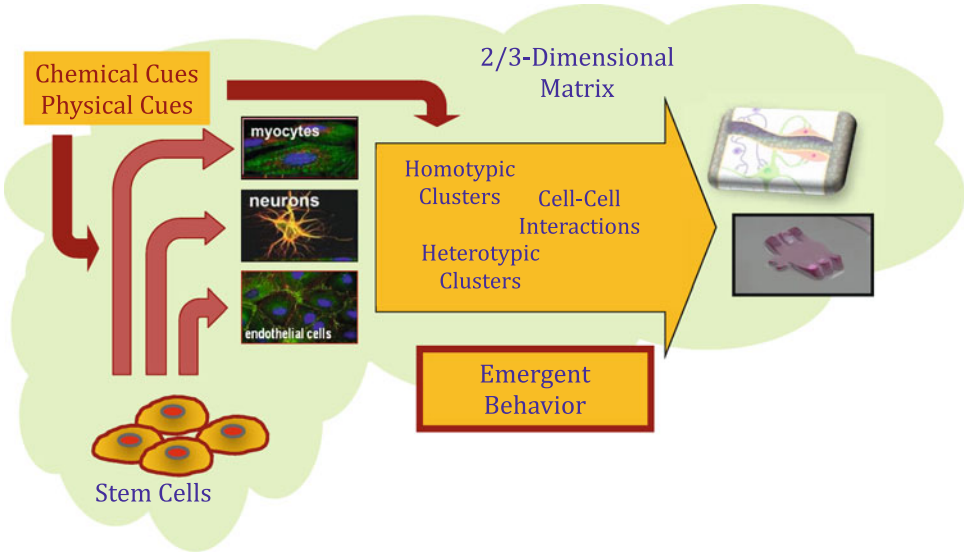


**FIGURE 4. Conceptual schematic of a Biological Machine with cells, scaffolds and physical or chemical cues to result in machines that exhibit specific functionalities.**

multiple cells types (heterotypic cell clusters) into a more complex functional unit.

### *Homotypic Cell Clusters*

#### *Muscle Strips*

Many of the machines one might envision, require actuation or motility, and for this, will need to incorporate muscle or some form of contractile cell. Due to their intrinsic tendency to rhythmically contract, cardiomyocytes have been the cell of choice in many of the early motile machines. One method that garnered considerable attention was the use of temperature-responsive polymers functionalized with cell-adhesive ligands to culture cell sheets of cardiomyocytes. Uniformly grafted sheets of poly(*N*-isopropylacrylamide) (PIPAAm) were formed on polystyrene culture dishes by irradiation with an electron beam to form the cell sheets.[53] Kitamori and colleagues first demonstrated a micropump powered by a cell sheet of cardiomyocytes achieving flow rates of 2 nL $min^{-1}$.[58] The same group recently improved on their method by wrapping a sheet of functional primary cardiomyocytes around a hollow PDMS sphere with inlet and outlet capillary tubes to engineer a bio-artificial hybrid pump.[59] The fluid oscillating frequency measured at 37 °C was 0.4 Hz and the maximum observed linear displacement of tracking particles was 70 $\mu$m. The expected flow rate was 47 nL $min^{-1}$, which was an improvement over the previous design.

Cardiac muscle cells will undergo rhythmic contraction in culture,[50,51,66] either as isolated cells or in a coordinated contraction mediated by gap junction (connexion43) and cell–cell adhesion (*N*-cadherin) proteins.[7,64] Skeletal muscle can also spontaneously contract, can be paced by cardiomyocytes,[48] but it can also undergo sustained contraction (tetanus) by, for example, increasing the rate of external activation.[25,30] Skeletal muscle is less prone to ischemia than cardiac muscle.[16] And because *N*-cadherin and connexion43 are down-regulated in mature skeletal muscles,[37] their activation can be controlled such that individual cells can be induced to contract (e.g., by motor neurons) without causing contraction in neighboring cells[49] (loss of electromechanical coupling). These attributes make skeletal muscle cells an attractive alternative to cardiomyocytes in situations that require high levels of temporal and spatial control over contraction.

Recently, methods have been developed to produce contractile skeletal muscle strips, tethered to compliant posts (Fig. 5), both to support the tension needed for proper myotube formation and to allow for direct inference of the contractile stress.[62] These methods produce highly stratified muscle, with clearly delineated sarcomeric structure, however, the levels of stress generated, even taking into account the amount of matrix material incorporated into the muscle construct, (~200 Pa) are more than 100-fold lower than what an be generated *in vivo* ($>10^5$ Pa).[49] Although the reasons for this considerable difference in contractile strength are unclear, it could be due to a variety of factors, among them the lack of capillary blood flow to the muscle, differences in the mode of activation, which is often by electrical stimulation [ref] rather than synaptic activation by motor neurons, or factors associated with the *in vitro* systems used to construct them.

#### *Neuronal Networks*

Neurons are a natural source of cells that can gather and process data, and then direct another cell population to perform a desired function. For example, sensory cells detecting a toxin might transmit a signal to a neuronal network that would direct secretory cells to synthesize and release a neutralizing agent, or muscle cells to move in the direction of the stimulus to initiate other actions. In short, neuronal clusters could be called upon to receive a signal and direct a coordinated response from multiple other cell types. It should be recognized that for some applications, the sensing and responding cells may be one in the same; beta cells, for example, sense glucose levels and secrete appropriate levels of insulin in response. Often, however, complex sensing/response problems involve some degree of processing by the central nervous system.

Neuronal networks are also capable of learning through structural and functional plasticity mediated by the strengthening of selected synaptic connections, opening up entirely new possibilities. Cultured neurons can exhibit learning in various ways, but in one experiment changes in the timing and patterns of spontaneous burst discharges was observed in response to repeated external "training" through electrical excitation.[47,52] There have also been reports of learning or memory in non-neuronal systems, ranging from cardiac muscle[72] to slim mold,[43] to plants,[65] but using a variety of mechanisms.

Progress in developing functional neuronal networks has progressed, but slowly. An essential feature of such networks is their ability to communicate, and in the case of neurons, communication occurs *via* signal transmission, either bi-directionally *via* electrical signals passed through gap junctions, or in a uni-directional fashion mediated by neurotransmitters released at a synapse. Synaptic connections have been extensively studied, and can readily be established between cultured neurons of various types derived from embryonic stem cells.[14] Moreover, functional networks produced with such neurons have been demonstrated,[24] although additional reports and detailed characterization are still lacking. Creation of neuronal networks that perform specific

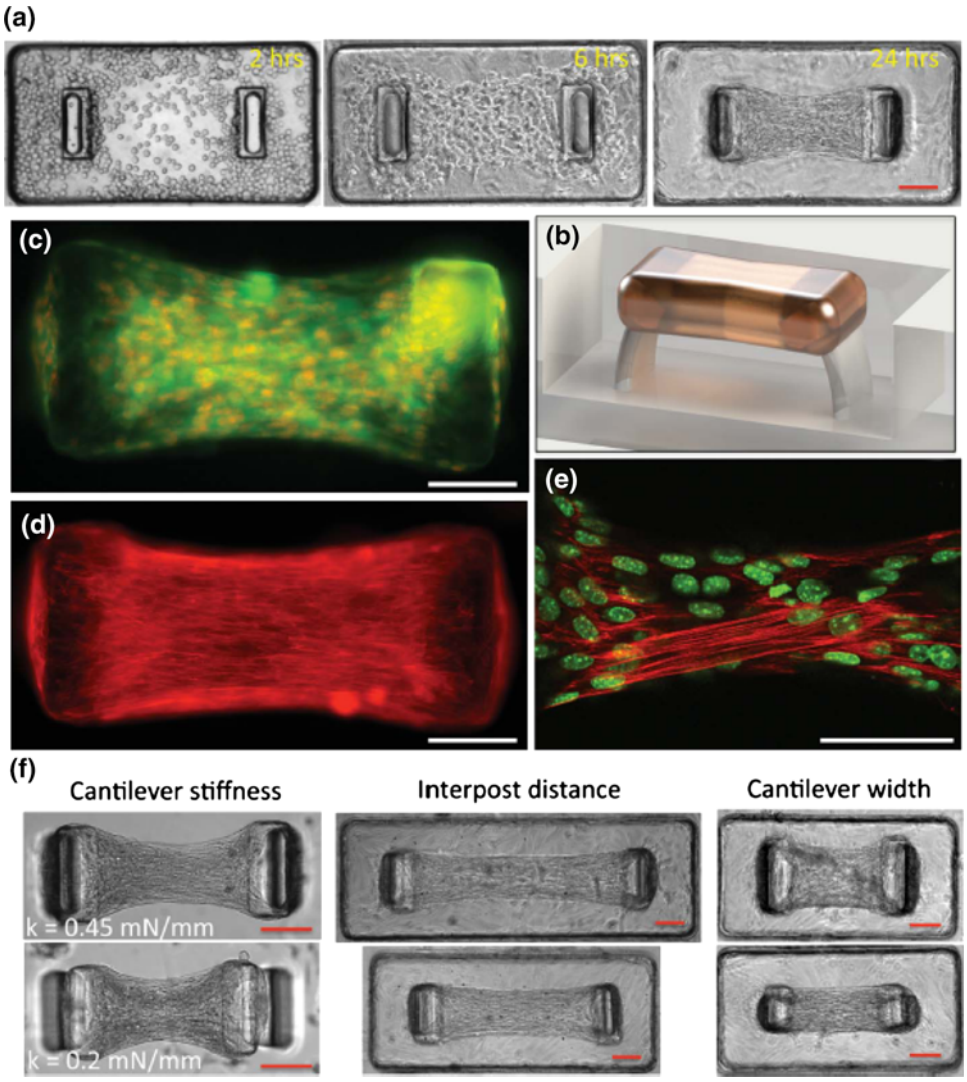


**FIGURE 5. Formation of muscle strips anchored to flexible posts. (a) Sequential images of myoblasts in a collagen/Matrigel solution after seeding into a rectangular well with two compliant posts. (b) Schematic of the resulting muscle strip formed around the two compliant posts. (c) Fluorescent image showing cell membranes (green) and nuclei (red). (d) Muscle strip as in (c) stained for actin showing cell alignment at 3 days post seeding. (e) Striated actin (red) and multi-nucleated (green) cells. (f) Effects on muscle strip morphology of changing cantilever stiffness, interpost distance, and cantilever width. Adapted from Sakar *et al.*[49]**

functions, however, has been proceeding at a slow pace, and relies generally on the ability to place the neurons in a particular geometrical arrangement and precisely control connections (Fig. 6). These constructs are often referred to as a "brain on a chip".[68] And while these experimental advances coupled with computational models of function are significant, many challenges lie ahead before these constructs can be designed and constructed to perform a specific processing function.

### *Vascular Networks*

For years, one of the barriers to progress in tissue-engineered organs was the inability to generate a perfusable microvascular network that could provide adequate gas and nutrient exchange to the regenerated tissue. Recent work in several labs has produced several methods that now overcome this constraint. The earliest work demonstrated that microvascular networks could be patterned onto a PDMS substrate, lined with endothelial cells, and perfused.[18] Others[35] demonstrated that straight channels could be cast in gel (collagen in this instance), lined with endothelial cells, and perfused, showing excellent cell viability and wall permeabilities that are approaching *in vivo* values. These later led to methods to form networks that could be cast in 3-dimensional gel matrices, the casting material removed, and endothelial cells seeded on the walls of the channels produced.[38]

One disadvantage of all these methods involving casting channels in gels, is that the smallest channels

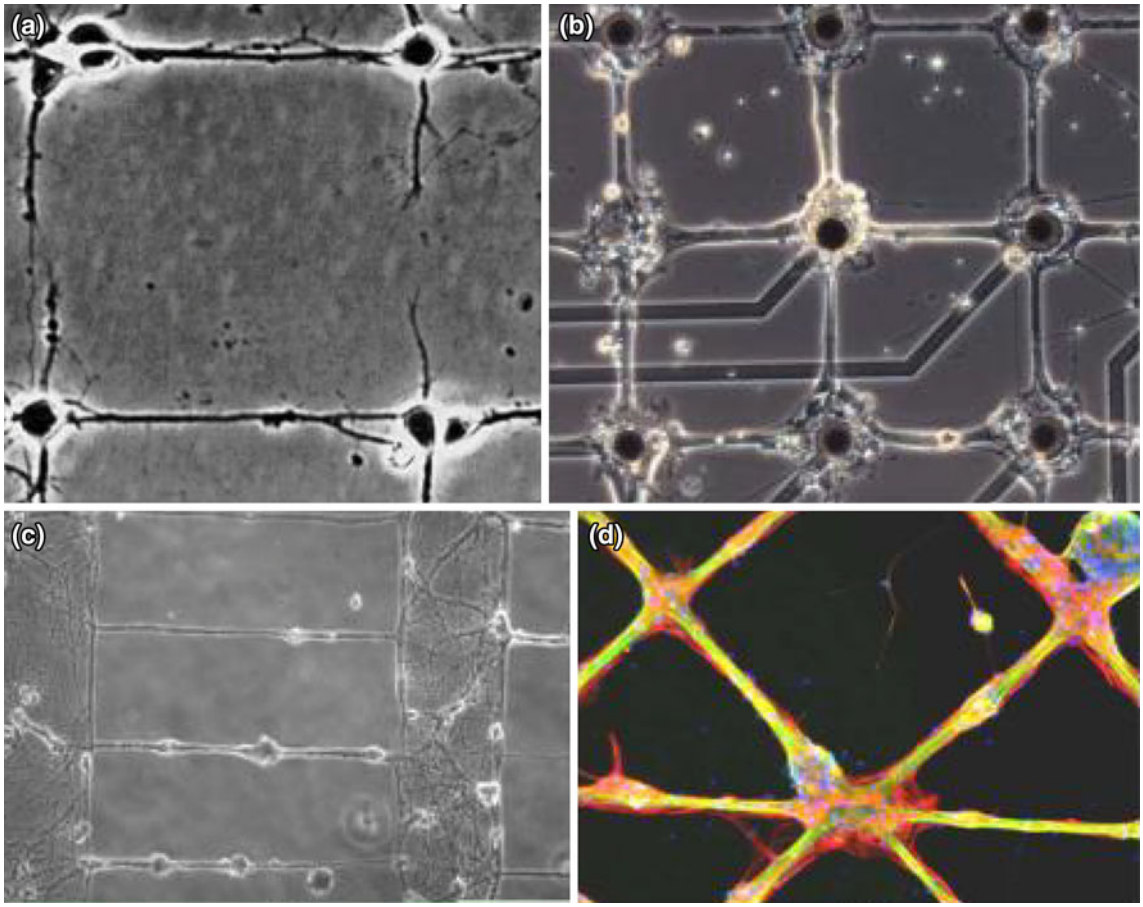


**FIGURE 6. Neuronal circuits on a chip. (a) Network of individual neurons patterned by laser, (b) 30 $\mu$m lines and 80 $\mu$m square nodes at 21 days in culture. (c) Neuropil structure separated by 500 $\mu$m with 3 $\mu$m wide lines. (d) Cross pattern of 80 $\mu$m nodes and 30 $\mu$m lines, stained for neurons (green), astroglia (red), and nuclei (blue). Adapted from Wheeler *et al.*[68]**

tend to be $>100$ $\mu$m in diameter, so still roughly $10\times$ larger than the real microvascular bed. While casting methods might yet achieve these dimensions, an alternative has been developed during the past several years that shows considerable promise. When endothelial cells are plated onto gel surfaces, they can be induced to sprout into the gel, recreating the process of angiogenesis that occurs during, for example, wound healing or cancer. These vessels form capillary-sized vessels with a more natural branching pattern,[10,42,71] and can now be grown so that they span a region of matrix of up to several mm, and can be perfused, representing an important step toward *in vitro* vascularized organs. Several methods have been demonstrated to induce network growth, including the addition of growth factors such as VEGF, either in uniform concentration or in a gradient, choice of an appropriate matrix material (fibrin or a fibrin-collagen mix appear most conducive), co-culture with various stromal cell types, and the application of physical factors such as interstitial flow across an endothelial monolayer[54,63] or through a cell-seeded 3D matrix.[42] And while many issues remain to be addressed such as functionality, control of network morphology, and long-term phenotypic stability, the recent advances bode well for ultimate success of these methods, essentially using the natural ability of endothelial cells to generate microvascular networks.

### *Heterotypic Cell Clusters and Living Machines*

#### *Neuromuscular Junctions*

Among the most basic cell–cell communication systems is one that allows neurons to communicate with other neurons, or motoneurons to activate skeletal muscle. As an example of heterotypic systems, we consider here the latter case. In the preceding section, we discussed recent work that has led to the growth *in vitro* of muscle strips that are on the scale of 100 $\mu$m in diameter and mms in length. Many of these systems have been grown from cardiomyocytes, so possess the

capability of cyclic activation, ideal for first generation biobots. In order to develop the ability to control the motion, however, will likely require skeletal muscle along with a means of activation. One promising approach has been to express in the muscle cells a light sensitive calcium channel (e.g., channelrhodopsin[49]). But the more natural means would be to use motoneurons that activate the myotubes by direct synaptic excitation. And while significant advances have been made in the generation of skeletal muscle strips *in vitro*, there has been very limited success in the formation of functional synapses.[60]

### *Co-cultures of Vascular Cells*

Vascular networks, by themselves, are of little value, since their purpose is to provide nutrients and gas exchange for other cells in a tissue. Some recent experiments attest to the viability of doing so in an *in vitro* setting. For example, Yeon *et al.*[71] have used fibroblasts as a "feeder cell" in 3D culture to help create a vascular network (Fig. 7a), and Chan *et al.*[10] have created networks through gels in the presence of agarose beads (Fig. 7b) which they show could contain tumor cells or various types of stromal cells. Other co-culture systems with fibroblasts have also been demonstrated in which the vascular network is cast into a hydrogel, which can also be seeded with other cell types.[35,38] Note that most of these publications have appeared during the past 1 or 2 years, so this area of research is likely to grow considerably.

### *Biobots*

Among the various living machines envisioned,[23] biological robots, or "biobots" are perhaps the most advanced. These are relatively simple systems capable of moving under the rhythmic contraction of cardiac myocytes appropriately seeded onto a flexible substrate. Montemagno and colleagues developed a microdevice using a silicon backbone with self-assembled cardiomyocytes grown on a chromium/gold layer.[69] They used photolithographic techniques to fabricate a micron-sized, self-assembled, and self-actuated walking bio-microactuator powered by cardiomyocyte muscular tissue, achieving a maximum speed of 38 $\mu$m/s. Park and colleagues established a swimming microrobot by micromolding PDMS.[27] Parker and colleagues assembled cardiomyocytes on various PDMS thin films with proteins to create muscular thin films (MTFs)[17] to reverse-engineer jellyfish-like constructs, dubbed "medusoids"[45] The Medusoid propulsion was like that of a jellyfish and was externally-driven by electrically-paced power and was able to replicate the momentum transport and body lengths traveled per swimming stroke of the natural system (Fig. 8a). Most recently, a 3D printer has also been used for the assembly of "bio-bots" with poly(ethylene glycol) (PEG) hydrogels and neonatal rat cardiomyocytes (Fig. 8b).[9] The bio-bots consisted of a 'biological bimorph' cantilever structure as the actuator to power the bio-bot, and the elastic properties of the bio-bots were tuned similar to that of neonatal rat cardiomyocytes to maximize their contractile force

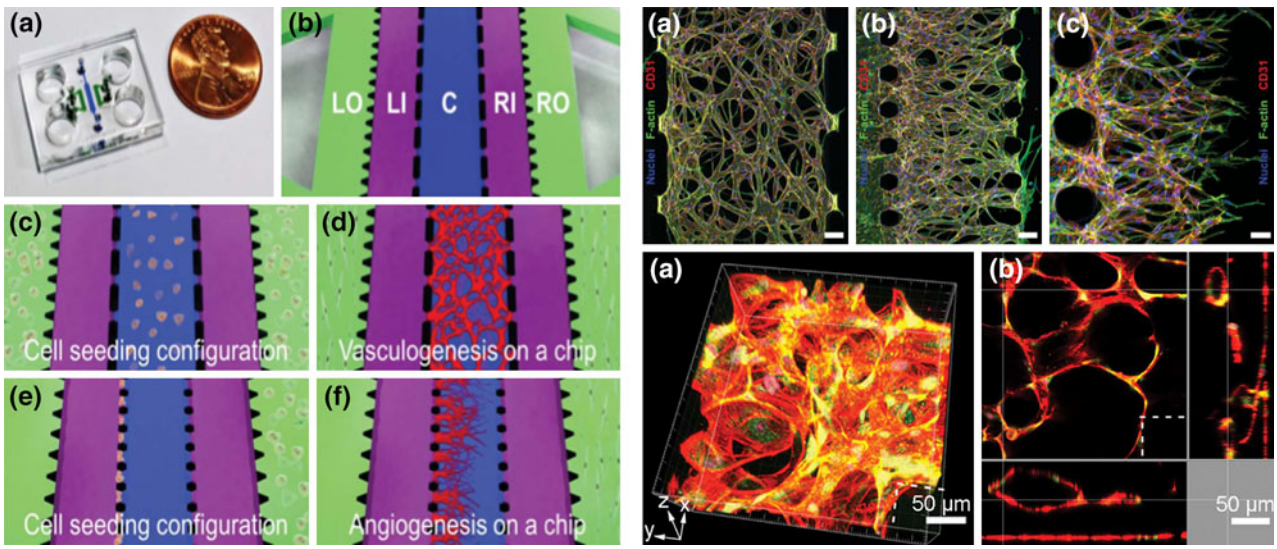


**FIGURE 7. Vascular networks formed in microfluidic platforms. Left: (a) Microfluidic system. (b) Schematic showing four parallel channels of the device with two outside gel regions (LO, RO), two media channels (LI, LO) and a central gel region. (c)–(f) Different seeding conditions for forming vascular networks. Reproduced from Kim *et al.*[28] Right top: (a)–(c) Vascular networks formed in the central gel region as in Left (a). Reproduced from Kim *et al.*[28] Right bottom: (a) Confocal image of a perfusible vascular network grown in a microfluidic gel system. (b) Slices of the network in (a) showing lumens and the 3D nature of the formed vessels. Reproduced from Chan *et al.*[10]**

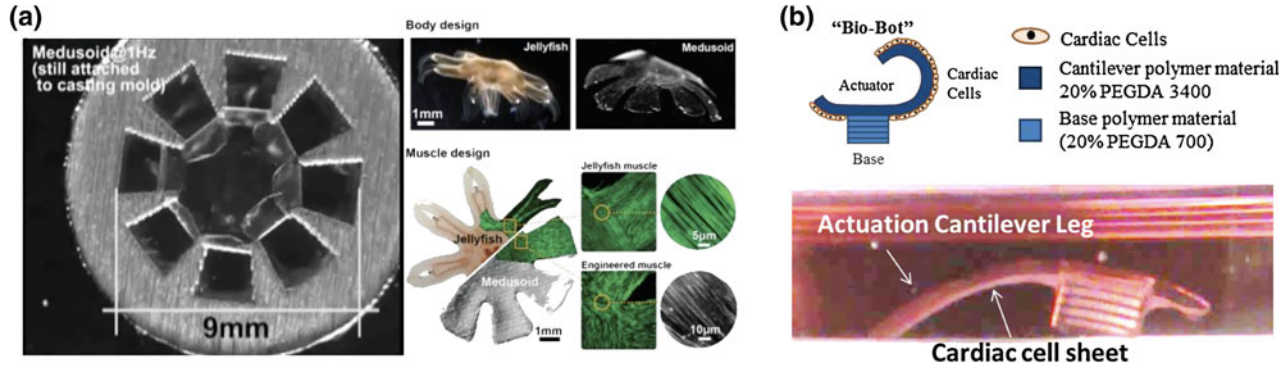


**FIGURE 8. Autonomous bio-hybrid muscle actuators capable of (a) swimming in fluid—bioengineering of an artificial jelly fish like structure capable of swimming in fluid autonomously or being pulsed by an external electric field (Adapted from Moon *et al.*[39]), (b) walking in fluid—Biological Biomorph cantilever structure actuation with the beating of primary cardiac cells resulting in a net motion with maximum velocity 236 μm/s, average displacement 354 μm/stroke and average beating frequency ~1.5 Hz. Adapted from Chan *et al.*[9]**

(~5 μN). The maximum recorded velocity of the bio-bot was ~236 μm $s^{-1}$.

While current biobots have limited capabilities (e.g., they can only function in cell culture medium and their movement is uncontrolled) they are useful in that they constitute an experimental platform on which new functionalities can be incrementally added. As mentioned above, optogenetic muscle cells can be used to gain control over the rate of contraction, hence the speed of motion. Using these same optogenetically modified cells, the direction of movement can be controlled by selectively activating muscles positioned to produce movement in multiple directions. In a further extension, motor neurons could be used to produce more efficient contraction *via* synaptic control, and these, too, could be activated by light.

### *Organs on a Chip*

Last year, the FDA, NIH and DARPA took the unprecedented step of introducing joint programs aimed at developing a disruptive change in the technologies employed by the pharmaceutical industries to discover new drugs. These programs called for the development of "organs-on-a-chip"—systems incorporating modern microfluidic technologies that replicate certain aspects of individual organ function using organotypic stromal and parenchymal cells. Extrapolating into the future, such systems could replicate human organs and, by combination on a single platform, inter-organ interactions, with sufficient fidelity to be used to screen for new therapies and their potential off-target complications that often block drugs that have reached the stage of clinical testing. Some small steps have been made in the development of microfluidic systems that can replicate aspects of organ function (Fig. 9). The grand challenge of the new programs is to further this development, and produce a "body-on-a-chip" technology that not only could model the response of a single organ to a new compound, but also anticipate the off-target effects of that same drug as it interacts with multiple other subsystems or organs. While it might be some time before such systems are capable of screening the millions of compounds in a particular library, in the nearer term, these might be useful as secondary screens, taking hits identified by the more conventional multi-well systems and refining these to a smaller number that can be moved up the ladder for further testing. At some stage, these technologies might replace animal testing, realizing significant savings in resources and time, but also providing a more realistic test bed by using human cells. Longer-term, such systems might be produced based on cells for a particular patient, providing the ultimate in identifying patient-specific treatment protocols.

## MORE COMPLEX LIVING MACHINES ON THE HORIZON

With but a little imagination, it is not difficult to envision how these nascent technologies might be developed into higher level machines with a wide spectrum of functionalities. Here we describe a few examples merely to provide stimulus for others to expand our horizons in this critical new field.

### *Smart Plants*

Society faces continuing challenges to feed our growing populations and despite dramatic improvements in food production, we continue to face shortages. In addition, problems remain in addressing the increasingly dramatic swings in the yearly cycles that periodically devastate a particular crop or food supply. What if plants could sense their surroundings, process that information, and enter into dormant or active growing periods based on that input? That is, what if

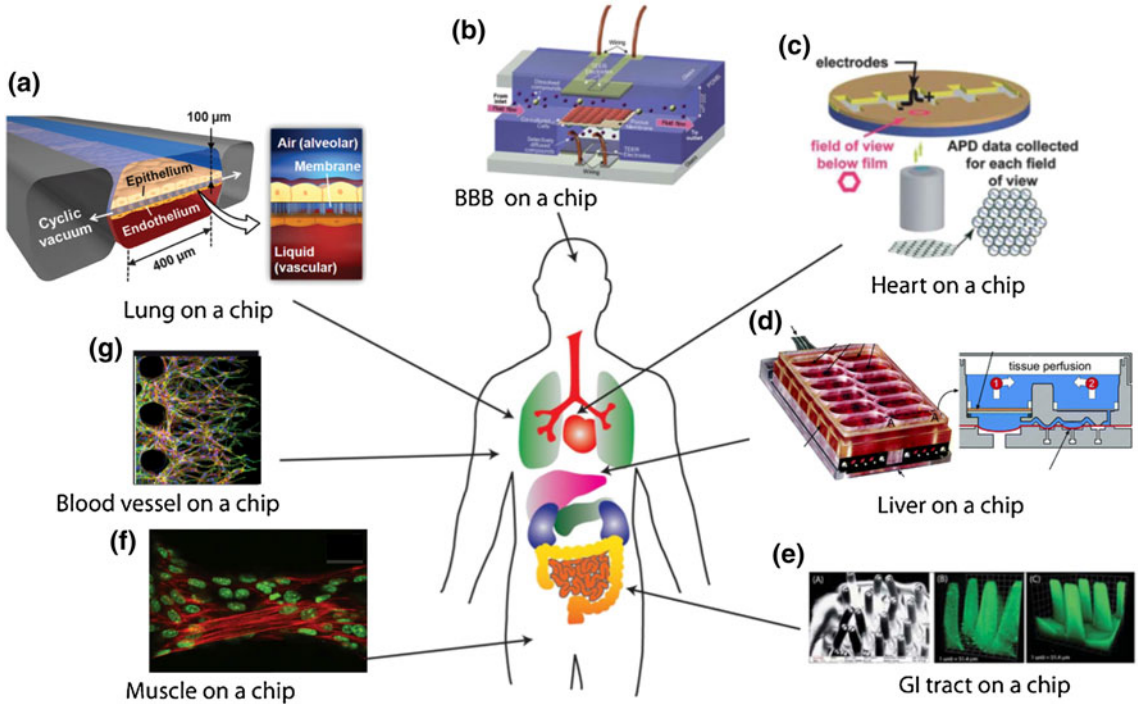


**FIGURE 9. Body-on-a-chip. Conceptual image of how the various existing organs-on-a-chip might be assembled to simulate the entire physiological system of a human for the purpose of drug screening. (a) Lung. Reproduced from Huh *et al.*[26] (b) Blood brain barrier. Reproduced from Booth and Kim.[8] (c) Heart tissue. Reproduced from Grosberg *et al.*[19] (d) Liver. Reproduced from Domansky *et al.*[15] (e) Intestinal villi. Reproduced from Sung *et al.*[57] (f) Muscle. Reproduced from Sakar *et al.*[49] (g) Blood vessels. Reproduced with permission from Kim *et al.*[28] The overall figure is adapted from Sung *et al.*[56] and sub-figures are reproduced with permission from the other references mentioned in this figure caption.**

plants had the ability to sense, ''think'', and act in a manner that would enhance their ability to survive under adverse conditions? Higher-level organisms have developed neurons for this purpose, but plants, too, could benefit from these capabilities. Either by genetically modifying plant cells so that they can form logic circuits and process information, or by introducing a mammalian-derived neuron cell type into plants, such capabilities might be realized.

What if we were able to enable plants to not only sense their condition, but process that information and respond appropriately? One example would be if they were to shut down all vital functions—to enter into a state of dormancy—during periods of draught. Another example might be that plants experiencing inadequate water, other nurtrients or sunlight might send a signal requesting more. While much work needs to be done, methods have already been reported to introduce a synthetic ligand-responsive signal transduction system in plants.[40]

### *Hyper-Organs*

While much attention has been focused on the growth of organ systems that currently exist in order to replace a dysfunctional liver, kidney, heart,[2,4,6,15,22,33] *etc.*, one can also envision implantable systems (''organs'') that perform other functions, not currently accomplished with any existing system. For example, one might envision creating optic cells that are sensitive in the infra-red so that one could see in the dark, or a drug delivery system for chronic illnesses that senses the concentration of a desired chemical such as glucose, and then directs other cells to synthesize and secrete insulin into the circulation. Another system might consist of a simple elastic reservoir connected to the circulation *via* a sphincter controlled by cells that sense the concentration of some cytokine in the blood. Sensing drives the relaxation of sphincter contraction, allowing for the release of needed factors into the body. Additional possibilities include blood vessels that pump relieving the load on the heart, or cell based sensors to measure the increase in pressure from vascular occlusions and release anti-thrombotic factors produced by cell based factories embedded within the blood vessels.

### *Emergent Manufacturing*

One of the unique potential advantages of biological machines is their inherent capability for growth,

self-assembly, and self-repair. Living organisms require no external guidance to develop into mature systems, and one might argue that living machines should be no different. As was discussed earlier, it might be sufficient to place the machine "parts" in proper relative proximity to each other, and leave the final steps of assembly to biological processes. This can be accomplished in a number of ways. 3D printing offers the ability to assemble cell-containing microbeads with high precision, on scales as small as a single cell.[39] Microfluidic systems have also been developed that enable co-culture of multiple cell types in 3D.[55] And using gels of different shape, it has been demonstrated that various patterns of communicating cells can be fabricated in hydrogel.[18]

But it clearly is not that simple, as developmental biologist have worked for decades to understand the emergent behaviors that lead to the maturation of a living organism. At its most fundamental level, all steps of development can be reduced to the "initial condition" or pre-programming intrinsic to the embryonic stem cells of particular machine/organism, the signaling that occurs (both intracellular intercellular, and between the cells and their local extracellular matrix), and perhaps with global signals generated by the external environment. While enormously complex in its entirely, the individual steps leading to emergence can be understood and, in principle, applied. So, while emergent manufacturing may be on the distant horizon, the tools and fundamental understanding needed to make it a reality largely exist today. For this, researchers can draw upon the considerable progress in the self-assembly of non-living materials. In this context, programmable materials that are capable of changing their properties, shape or structure based on environmental cues provide useful concepts. The cues in the present case would be signals from neighboring cells or externally imposed forces, electrical excitation, or biochemical factors.

### *Bio-based Surveillance Systems*

Despite tremendous advances, living systems still outperform non-living ones in the critical areas of sensing—we still rely on dogs for detecting drugs or explosives in luggage, and voice recognition by computers continues to lag far behind human capabilities. Among these, smell is the one sense that is least dependent upon information processing by the central nervous system, and therefore, represents a logical first target for applications in surveillance or detection. Some advances have been made in the development of systems that attempt to replicate cellular sensing,[34] but the potential of using mammalian cells in a sensing device remains largely unexplored.

## POTENTIAL BENEFITS AND DANGERS OF LIVING MACHINES

The potential benefits of a fully biological machine are numerous and transformative. One might envision machines that can self-assemble, repair themselves, and even self-replicate under appropriate controls. But this raises numerous important ethical questions, in addition to the scientific/engineering barriers to progress. At what level of complexity or functionality does a biological machine become a living being? What ethical issues do researchers need to be cognizant of as they develop cellular machines with increasing capabilities? How can we balance the potential for positive impact of cell based systems on the world around us against the possibility of harmful outcomes? And how far can or should we go in engineering cellular systems that resemble in form or function an existing living entity? A machine that can sense its surroundings, process the information that it gathers, and perform some function based on that decision process possesses many of the same qualities that we often attribute exclusively to life and natural living beings. If these machines ever get to a point that they might self-replicate then the boundaries between the living and the machine become the most blurry. Obviously, the ethical issues are of tremendous importance and the time for discussion is now, not once the technologies have already been developed.

To assist us in addressing these questions, we have a considerable body of work that has emerged around synthetic biology. In a recent report by the *Presidential Commission for the Study of Bioethical Issues*,[21] a broad framework for simultaneously promoting research of this type with enormous positive potential, while at the same time providing safeguards and a process to examine ethical implications. And while they focused their discussions on the manipulation of DNA to produce new forms of life, many of their recommendations pertain equally well to the development of integrated multi-cellular systems whether or not the constituent cells have been genetically modified.

## CONCLUSIONS

Synthetic biology brought us the prospect of engineering single cells to perform entirely new functions or to exhibit characteristics different from their natural counterparts. Other disciplines have strived to create and understand through modeling and experiments the behavior of complex, multi-cellular systems. Through this emerging technology, we have already witnessed advances in mono-culture constructs (e.g., muscle

strips, microvascular networks, neuronal circuits, myocyte-driven biobots) as well as heterotypic multiculture systems (neuromuscular junctions, organs-on-a-chip, *etc*.). In this prospective, we have sought to present the case that even greater potential exists in the use of multiple cell types, each performing different functions in a coordinated manner, to produce higher-order forms of living machines. While we can only speculate regarding the future of these endeavors, the groundwork is now being laid through advances in a number of related fields. We introduce the issues, questions and ample research opportunities to be explored by others in the field. We propose here that integrated cellular systems be recognized as an emerging discipline, and that efforts be undertaken to develop the nascent ideas presented in this prospective piece to promote related research and also initiate discussions of the critical ethical questions that this research raises.

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