

Synthetic Tissue Biology: Tissue Engineering Meets Synthetic Biology

Samuel K. Sia,* Brian M. Gillette, and Genevieve J. Yang

We propose the term “synthetic tissue biology” to describe the use of engineered tissues to form biological systems with metazoan-like complexity. The increasing maturity of tissue engineering is beginning to render this goal attainable. As in other synthetic biology approaches, the perspective is bottom-up; here, the premise is that complex functional phenotypes (on par with those in whole metazoan organisms) can be effected by engineering biology at the tissue level. To be successful, current efforts to understand and engineer multicellular systems must continue, and new efforts to integrate different tissues into a coherent structure will need to emerge. The fruits of this research may include improved understanding of how tissue systems can be integrated, as well as useful biomedical technologies not traditionally considered in tissue engineering, such as autonomous devices, sensors, and manufacturing. **Birth Defects Research (Part C) 81:354–361, 2007. © 2008 Wiley-Liss, Inc.**

INTRODUCTION

If one sure approach to decipher a complex device is to take apart its components and reassemble them into a functional unit, does the same concept apply to understanding complex metazoan organisms?

Through reverse engineering, synthetic biology promises to increase our understanding of the essential working elements of life, and at the same time, provide useful biomedical technologies. The term “synthetic biology,” following recent developments in whole-genome sequencing and synthesis, has been defined as the design and construction of new biological parts, devices, and systems, and the redesign of existing, natural biological systems for useful purposes (Hartwell et al., 1999; Endy, 2005; Dueber et al.,

2007). Despite these broad aims, most current discussions and research efforts have focused on imposing *genetic* changes in unicellular organisms to effect desired phenotypes and functions. The capabilities of unicellular organisms, even in large populations, are limited. By contrast, metazoan organisms, consisting of multiple tissue systems, are endowed with complex capabilities that cannot be attained by concerted action of unicellular organisms. Thus, to engineer systems with metazoan-like complexity, it may be advantageous and perhaps necessary to engineer *tissue systems* rather than simply DNA sequences.

We propose the term “synthetic tissue biology” to describe the engineering and interfacing of tissue systems to produce biological machinery endowed with complex

capabilities and emergent properties possessed by metazoans. Although synthetic tissue biology has not yet formed a focused effort, the increasingly mature field of tissue engineering is perfecting many of the necessary techniques (but for the purpose of regenerative medicine). In this review, we highlight recent successes in synthetic biology (mostly in unicellular organisms) (“Synthetic Biology: From Genes to Unicellular Organisms”), review recent innovations for engineering synthetic tissue systems (“Synthetic Biology for Multicellular Systems: Tissue Engineering as Foundation” and “Examples of Engineered Tissues”), identify special considerations for integrating multiple engineered tissues into complex *ex vivo* biological systems, and discuss potential applications outside of regenerative medicine (“Integrating Tissue Systems Into Organismal-Level Control”).

SYNTHETIC BIOLOGY: FROM GENES TO UNICELLULAR ORGANISMS

When the term first appeared, “synthetic biology” referred to aims ranging from genetically engineered bacteria to the construction of unnatural organic molecules (Hobom, 1980; Rawls, 2000; Benner and Sismour,

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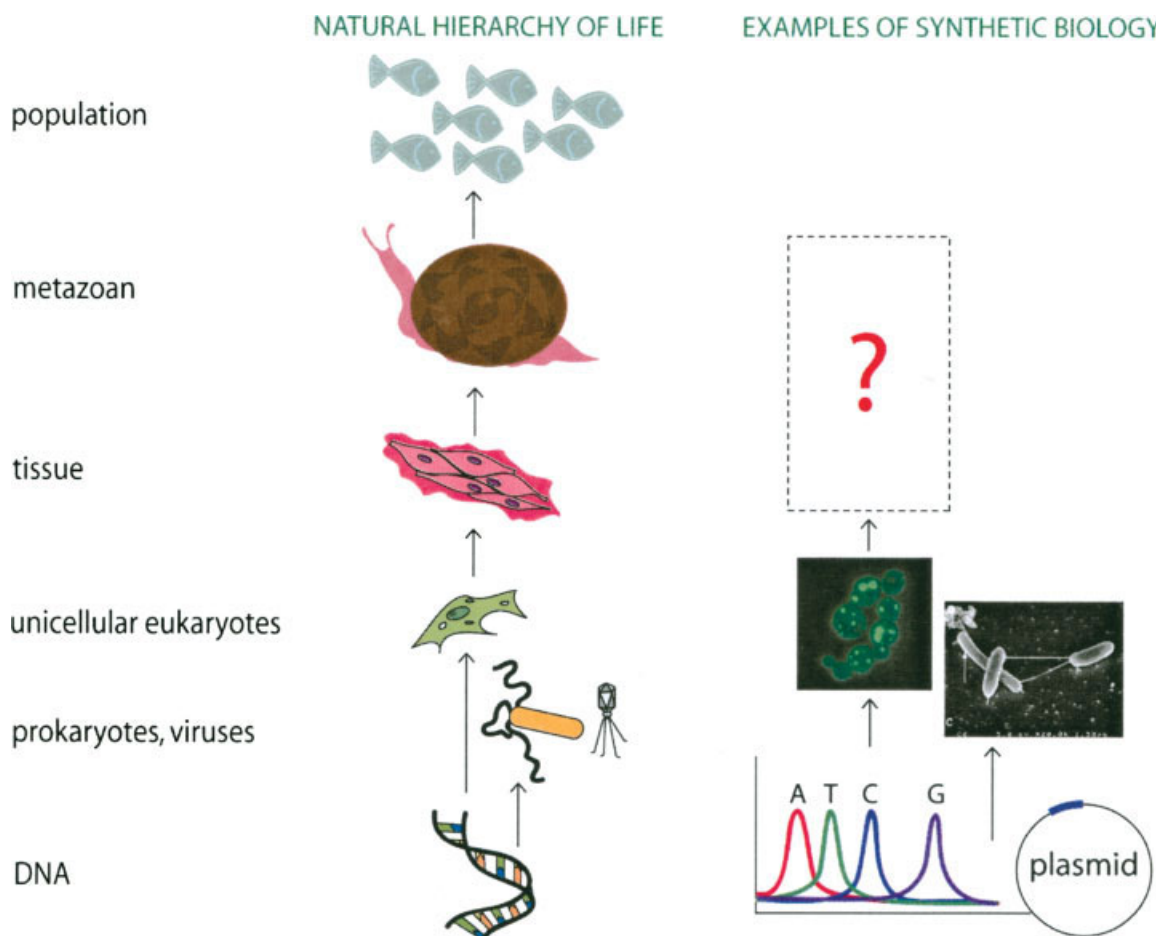


Figure 1. Hierarchical forms of biology. Left: The natural hierarchy from molecules to populations of complex organisms. Right: Examples of synthetic biology. Advanced molecular biological tools such as DNA sequencing and synthesis are used to engineer bacteria and other unicellular systems (for example, yeast engineered to produce therapeutic proteins investigated for treatment of Parkinson's disease (Cooper et al., 2006), and bacteria producing electrically conductive "nanowire" proteins (Cooper et al., 2006)). This review discusses concepts and examples of synthetic biology at the level of tissues and metazoan whole organisms, as indicated by the question mark.

2005). Recently, most efforts have focused on engineering and manipulating DNA, RNA, and proteins in unicellular organisms to achieve design goals (Fig. 1). For example, using advanced methods in recombinant DNA, genes encoding biosynthetic enzymes can be transformed into bacteria to form photosensitive biological films that act as high-definition, two-dimensional images (Levskaia et al., 2005), entire metabolic pathways in bacteria can be manipulated to produce useful biosynthetic products (such as polyhydroxyalkanoates as biodegradable plastics (Reddy et al., 2003) or artemisinin, an antimalarial drug (Ro et al., 2006)), or in an extreme form, the entire genome of a

poliovirus (Cello et al., 2002) or a mycobacterium (Lartigue et al., 2007) can be synthesized de novo. Moreover, new protein functionality can be engineered through rational design and directed evolution (Cramer et al., 1997; Liu et al., 2007), genetic circuits can be both transcriptionally and translationally regulated through modification of genes encoding regulatory elements (reviewed in Andrianantoandro et al., 2006; Marguet et al., 2007), rational protein design using computational modeling allowed construction of surface receptors that responded to unnatural ligands (Looger et al., 2003), and engineering of genetic circuits that responded to airborne

molecules allowed the construction of a "synthetic ecosystem" (Weber et al., 2007).

At cellular and subcellular scales, synthetic systems with useful design goals were constructed by using tools and knowledge appropriate for the biological system (e.g., advanced tools in recombinant DNA and knowledge of genetic and biochemical pathways). With the emergence of this exciting research, the fertile field of synthetic biology is poised to embrace the next step in the natural hierarchy of life (Fig. 1), to move from unicellular designs based on the engineering of DNA sequences to metazoan-level designs based on the engineering of tissue systems. We hypothesize

		Artificial Tissues					
		skin	cardiac muscle	arteries	capillaries	skeletal muscle	liver
Components	ECM	collagen					
		Matrigel					
		PGA					
		fibrin					
		PEG-DA					
	cells	hepatocytes					
		myoblasts					
		keratinocytes					
		cardiomyocytes					
		smooth muscle					
		fibroblasts					
	growth factors	endothelial cells					
		VEGF					
		FGF					
		serum					

Figure 2. Assembly of components into tissue systems in “synthetic tissue biology.” To develop tissues in vitro, engineers combine standard components (i.e., cells, ECM, and molecules) using available engineering tools to construct a cellular microenvironment and to apply an appropriate stimulus regimen. Knowledge from basic science fields, especially developmental biology and physiology, are important for selecting the proper components, geometry, and method for assembly, and stimuli given an intended tissue type and design goals. Black boxes indicate components commonly used in assembly of the indicated tissue type (note that many examples simply use animal serum from various species, which contains uncharacterized concentrations and types of growth factors). The use of serum-free defined media presents challenges for cell culture but may improve reliability and understanding of culture conditions giving rise to differentiated tissues.

that this transition is where tissue engineering and developmental biology could make an important impact.

SYNTHETIC BIOLOGY FOR MULTICELLULAR SYSTEMS: TISSUE ENGINEERING AS FOUNDATION

Tissues feature a complex biochemical and mechanical interplay of cells, extracellular matrix (ECM), and signaling factors. To synthesize tissues de novo, molecular and genetic tools are undoubtedly valuable; the nature of tissues, however, demands attention not just to mechanisms within cells, but also to the complex extracellular environment responsible for directing morphogenesis of cells into tissues.

Over the last two decades, the field of tissue engineering has developed a vast toolkit for both engineering and analyzing complex tissues. A traditional approach is to assemble cells, ECM precursors, and growth factors into functional tissue systems (Fig. 2) (Ingber et al., 2006), just as synthetic biologists propose to select and assemble biological components (such as genes or proteins (Endy, 2005)) into func-

tional biological systems. Despite a number of successes in regenerative medicine, the choice of components and method of assembly so far in tissue engineering have often been performed in an ad hoc rather than a systematic manner (Ingber et al., 2006; Vunjak-Novakovic and Kaplan, 2006). A rational and systematic approach to tissue engineering would be appealing for applications in regenerative medicine as well as for synthetic tissue biology, such that the best suited components and method of assembly—along with specified spatial, temporal, or chemical constraints—can be determined in advance for a set of given design goals (akin to designing integrated circuits to perform given computational tasks). Such a determinate approach encounters numerous obstacles: individual cellular responses depend on coupled intracellular signaling pathways (for example, cellular response to a growth factor may depend on which integrin-ECM interactions are engaged), multicellular dynamics are complex (featuring many higher-order interactions), and the temporal evolution of a tissue system is difficult to predict (as a complete description of morphogenic events in vivo is lacking). Nevertheless,

new methods are emerging. For example, high-throughput screening approaches may become increasingly important in systematic approaches to tissue engineering (Underhill and Bhatia, 2007). Also, the ability to actively direct tissue morphogenesis may be enhanced by new methods that allow dynamic spatiotemporal control of microenvironment parameters (Hui and Bhatia, 2007) (for example, by using microvalves; S.K. Sia, J. Lii, W. Hsu, and R. Rouse, unpublished results) as well as leveraging knowledge gleaned from developmental biology (Kaplan et al., 2005).

Current technologies (reviewed in Tsang and Bhatia, 2004; Stevens and George, 2005; Khademhosseini et al., 2006; Khetani and Bhatia, 2006; Nelson and Tien, 2006; Borenstein et al., 2007; Fisher and Peattie, 2007) relevant for engineering cellular microenvironments include two- and three-dimensional patterning of cells and biomaterials, tunable synthetic extracellular matrices, controlled release systems, bulk and microscale loading systems, and bioreactors. By changing the composition, concentration, and geometry of ECMs along with encapsulated cells and growth factors (Khademhosseini et al.,

2006), these techniques manipulate important drivers of tissue morphogenesis, such as intercellular communication (via cell-cell contacts, paracrine interactions, or mechanical signaling across ECM components), cellular interactions with ECM components and soluble chemical factors, and applied mechanical or electrical stimulation. Ultimately, these techniques allow high-resolution control over cellular phenotypes such as migration, networking, proliferation, and differentiation, to achieve directed assembly of tissue structures from the ground up.

EXAMPLES OF ENGINEERED TISSUES

The following examples demonstrate a variety of techniques that can be used to generate different tissue types *in vitro* (Fig. 3). Since different methods for engineering a tissue type can yield comparable results, it is often unclear which method is best for engineering a particular tissue type.

Skin

Compartmentalization is a key element of life. In metazoans, the skin provides a protective barrier critical for survival, and treatments for conditions such as severe burns and chronic wounds such as diabetic foot ulcers have improved through the use of a variety of tissue engineered skin products. For example, Apligraf (Organogenesis Inc., Canton, MA) (Supp and Boyce, 2005) is a structurally complex skin substitute that is commercially available, incorporating a dermal layer consisting of live fibroblast-seeded bovine collagen with an epidermal layer of keratinocytes on top. Although such grafts have shown considerable clinical success, much work has been done to further incorporate natural features such as sweat glands, hair follicles, pigmentation, and vasculature, as well as improving healing response by incorporation of anti-inflammatory cytokines (Supp and Boyce, 2005; MacNeil, 2007).

Additionally, an important aspect of mediating proper healing response is regulating infiltration of host cells into grafted skin. Microstructured ECMs may offer the potential to regulate cell migration into engineered skin grafts. As an initial step towards this goal, we have recently developed a microfabrication-based approach to engineering artificial skin with high spatial and chemical control (S.K. Sia, C. Chin, and K. Khanna, unpublished results).

Heart

The cardiovascular system is critical for supplying nutrients across different tissues and for maintaining homeostasis in the whole organism. As heart disease is a leading cause of death, and with a lack of donor hearts for patients with severe heart failure, engineered cardiac tissue grafts may be used to treat heart failure (Wu et al., 2006). Despite exciting progress by several groups toward a functional engineered myocardial patch for surgical repair of the heart (Shimizu et al., 2006; Zimmermann et al., 2006a, 2006b), a number of technical and regulatory hurdles must yet be overcome before tissue engineers can realize the long-term goal of creating living myocardial replacements for surgical implantation in humans (Kaplan et al., 2005). Thus, engineered cardiac tissues also may find important short-term applications as high-throughput *in vitro* models for biomedical research (Wakatsuki et al., 2004). Toward this goal, Costa and coworkers recently reported the development of living, fully biological, engineered cardiac organoids, or simplified heart chambers, that demonstrated several hallmark characteristics of natural cardiac pump function (Lee et al., 2005). Adapting earlier tissue engineering techniques (Costa et al., 2003), organoids were created from primary neonatal rat cardiac myocytes and fibroblasts suspended in collagen and Matrigel (BD, Franklin Lakes, NJ); after seven to 10 days of culture, the resulting organoids had a simplified thin-walled spherical geometry exhibiting diameter and contraction rate similar to the neonatal rat hearts from which the cells were harvested. Fluorescence microscopy revealed a relatively homogeneous cell distribution with randomly oriented myocytes, which more closely resembled the avascular embryonic (day 10.5) rat heart (Nakagawa et al., 1997) than the highly aligned and vascularized structure of mature myocardium (McDevitt et al., 2002).

Radisic et al. (2004) formed functional contractile sheets of cardiomyocytes using electrical stimulation mimicking native myocardial electrical signaling. The electrical stimulus regimen in the study was critical in enhancing physiologically relevant cell organization, coupling, and synchronous contraction of the cardiomyocyte sheet. Establishing proper cellular organization and contractile properties will be essential for developing cardiac tissues for integration into humans. Because complex coordinated movements of cardiac tissues are involved in establishing proper contractility of the heart, successful implementation of cardiac tissue grafts *in vivo* requires careful consideration of tissue biomechanics and interfacing of engineered grafts with healthy tissue.

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Vasculature

The enormous clinical need for functional arteries (including small-diameter vascular grafts for cardiac bypass surgeries) has driven efforts to engineer functional arteries *in vitro* (Isenberg et al., 2006). In an early example, Niklason et al. (1999) developed functional arteries by positioning endothelial and smooth muscle cells within a bioreactor that allowed physiologic pulsatile flow of media inside the lumen. The arteries functioned in animal models for at least a month. Other cell-based artery grafts have been developed *in vitro* (L'Heureux et al., 1998, 2006), however, none have yet been able to completely establish all the parameters

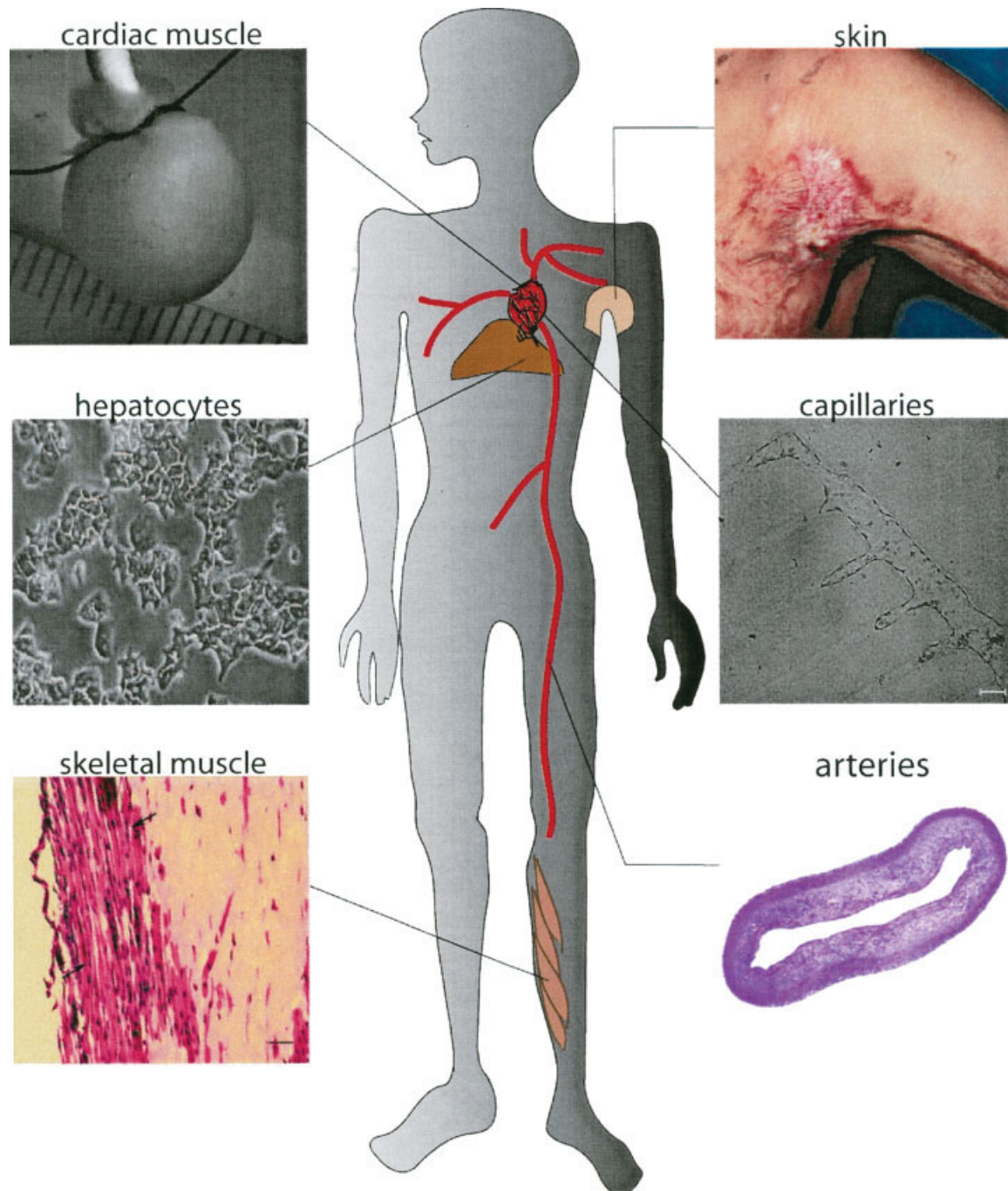


Figure 3. Tissue engineered components designed to mimic in vivo counterparts for synthetic tissue biology. Examples shown are a cardiomyocyte organoid developed for in vitro modeling (left, top; Courtesy of K.D. Costa and E.J. Lee), cultured hepatocytes exhibiting physiologic function (left, middle; from Allen et al. (2006)), differentiated skeletal muscle developed in vitro (left, bottom; from Rhim et al. (2007)), artificial skin developed in vitro and grafted to a patient with a serious burn (right, top; from Sahota et al. (2003)), capillary structures grown in microfabricated hydrogels (right, middle; B.M.G. and S.K.S. unpublished results), and a functional artery graft grown in vitro (right, bottom; from Niklason et al. (1999)).

required for broad clinical application (Isenberg et al., 2006).

Whereas arteries and veins distribute large volumes of blood over long distances, the microvasculature is responsible for the

exchange of nutrients and waste products at the tissue level. Incorporation of functional capillary networks into engineered tissues is a vital step towards the grafting of thick tissue replacements (e.g.,

over a millimeter) without necrosis. A useful approach may be to form microvascular structures within a tissue graft prior to implantation (Yang et al., 2001; Levenberg et al., 2005); in these

studies, host vessels quickly anastomosed with implanted vasculature to result in high tissue viability. Still, these methods typically lacked control over spatial distribution, density, and geometry of capillary networks, which are critical parameters for proper distribution of blood and tissue function for many tissue types. Although microfabrication-based strategies (for example, by seeding walls of microchannels with endothelial cells (Shin et al., 2004; Fidkowski et al., 2005; Golden and Tien, 2007)) can control the geometry of the microvasculature, these approaches have yet to show in vivo success.

Skeletal Muscle

Skeletal muscles form a primary means of motor movement in autonomous systems. In human health, engineered skeletal muscle may be helpful for patients with traumatic muscle injury, congenital defects, or loss due to tumor ablation (Stern-Straeter et al., 2007). Rhim et al. (2007) developed mature skeletal muscle tissues from murine myoblasts cultured in mixtures of collagen and Matrigel within silicon tubes held under passive tension. Although these tissues exhibited features of fully differentiated muscle throughout most of the construct, cells in the interior tended to migrate out due to hypoxia, thereby disrupting the overall tissue structure. Molnar et al. (2007) formed differentiated myotubes on photolithographically patterned vitronectin substrates. The small scale myotubes could be used for in vitro testing or micro-robotics applications, as well as tissue engineering. Assembling large muscle tissues from small scale tissues remains a challenge, and implementation of dense vasculature will be essential to meet the high metabolic demands of skeletal muscle.

Liver

The liver is a large and complex organ responsible for more than

500 metabolic, regulatory, and immune functions (Nahmias et al., 2007). Although whole or partial liver transplantation is typically the best treatment for liver failure, the shortage of donor organs has spurred research in engineering liver tissues. The structural complexities of the liver and difficulties in maintaining hepatocyte function in vitro present important challenges. Liu Tsang et al. (2007) engineered microfabricated 3D synthetic gels (based on polyethylene glycol) containing functional hepatocytes that mimicked liver microarchitecture. The multilayered structures promoted flow of media and reduced the diffusion distance, with improved viability and function of the encapsulated hepatocytes. Although engineered whole livers developed in vitro may be a long way off, such artificial culture systems may provide means of therapy through extracorporeal bioreactors or implanted devices. Further, in vitro model liver tissues can be used as a predictive tool for drug metabolism or for determining toxic effects of drugs or environmental factors on the liver (Sivaraman et al., 2005).

INTEGRATING TISSUE SYSTEMS INTO ORGANISMAL-LEVEL CONTROL

Why Synthetic Tissue Biology?

We expect synthetic tissue biology to yield many applications outside of human health. Already, in synthetic biology at the cellular and subcellular scales, bacteria have been engineered to act as photographers (Levskaya et al., 2005), algae to carry small loads (Weibel et al., 2005), molds to control robotics (Tsuda et al., 2007), and kinesin and myosin motors to drive transport in microelectromechanical systems (MEMS) devices for on-chip assays (van den Heuvel and Dekker, 2007). These examples of synthetic biological systems take advantage of the vast capabilities of biomachinery outside health applications. With tissue engineer-

ing technologies becoming more advanced, the next generation of systems in synthetic tissue biology is emerging (such as a microfabricated pump driven by sheets of cardiomyocytes (Tanaka et al., 2007), and neuronal network cultures on microelectrode arrays engineered with adaptive goal oriented behavior for use as biologically based control systems (Bakkum et al., 2007a, 2007b)). Intriguingly, integration of physiological functions could produce robotic devices and sensors with autonomous sensing, actuation, and processing of natural inputs and energy (Mano et al., 2002). These applications would be useful for environmental sensing, military applications, and resource-poor settings, as well as potentially revealing new insights into emergent properties of autonomous biomimetic systems (Whitesides and Grzybowski, 2002).

Synthetic tissue biology, like synthetic biology at the cellular and subcellular scale, could also yield new capabilities in manufacturing. For example, just as biosynthetic pathways have been engineered to produce therapeutic agents against malaria (Ro et al., 2006), synthetic tissue biology can potentially link together new pathways of detoxification and chemical processing (liver) and biomineralization (bone and cartilage) to synthesize molecules. Taking multiple tissue integration to the extreme, a "human-on-a-chip" platform could potentially serve as a powerful in vitro drug screening tool (Khetani and Bhatia, 2006).

Regenerative medicine remains a key application of synthetic tissue biology, especially when tissue engineering (via its approach of assembling cellular, ECM, and chemical components together into a functional tissue system) is viewed through the lens of synthetic biology. An important consideration for creating durable and robust tissue systems will be to incorporate regenerative capacity: natural tissue systems are durable not as much because of the durability of component materials as their ability to replace damaged

cells, repair damage to cellular and extracellular components, and to heal wounds. Incorporation of stem cells and stem-cell niches into engineered tissues are exciting strategies for constructing tissues that will stand the test of time in both in vivo or ex vivo environments (Kolf et al., 2007).

What Are the Special Considerations for Integrating Multiple Tissue Systems?

Many recent tissue engineering approaches have recognized the challenge of interfacing multiple tissue types to form complex tissues (Mikos et al., 2006). Important challenges include the engineering of interfaces between bone and articular cartilage, ligaments, and tendons, replicating zonal inhomogeneity in articular cartilage and intervertebral disks, and integrating vascular and lymphatic systems into virtually every tissue type. A much less explored area is how these model engineered tissues could be interfaced together or interfaced with non-biological components for useful purposes in vitro. These methods will become increasingly important with new in vitro high-throughput methods to microfabricate ECM (Cheung et al., 2007).

Finally, we point out that a critical element of engineering these complex systems is to mimic useful *function*. This objective may not necessitate high-resolution mimicry of natural physiological structures and mechanisms, as long as the essential set of physiological functions of autonomous life can be approximated. For example, synthetic biomimetic systems may be useful proxies for natural tissues in some cases (Jeong et al., 2006). Whether natural or synthetic materials are used, rational design of complex biological systems—with functional equivalents of tissues—will demand new tools in multisystem computational approaches, improved understanding of biomechanics across different tissue systems, and new techniques in deep-tissue imaging.

CONCLUSION

We propose synthetic tissue biology as a long-term strategy for developing greater understanding of complex living systems and how complex biological machinery can be applied toward future medical and nonmedical technologies, which may also produce immediately applicable technologies (as the tissue engineering field already has). If successful, this research will face ethical issues like other synthetic biology research, to balance the potential harm and benefits (for example, in the current synthetic biology community, the weighing of the ease with which potential biological weapons can be constructed against the benefit of engineering bacteria with a myriad of useful functions). Practical impact of this research may include not only regenerative medicine but also applications outside of human health, including autonomous devices and sensors. This work may also help us understand the essential elements of natural biology and reveal emergent properties of systems with multiple tissue types.

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