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Advanced Cell and Tissue Biomanufacturing

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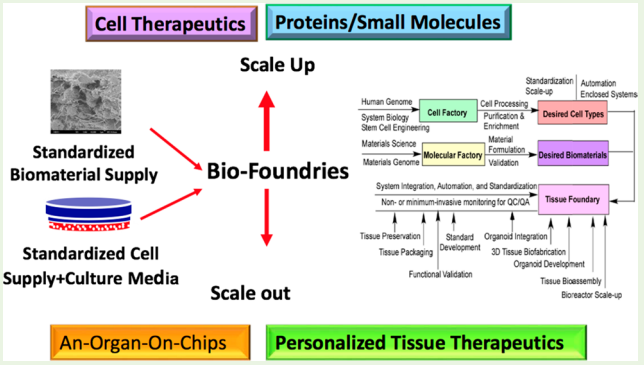
ABSTRACT: This position paper assesses state-of-the-art advanced biomanufacturing and identifies paths forward to advance this emerging field in biotechnology and biomedical engineering, including new research opportunities and translational and corporate activities. The vision for the field is to see advanced biomanufacturing emerge as a discipline in academic and industrial communities as well as a technological opportunity to spur research and industry growth. To navigate this vision, the paths to move forward and to identify major barriers were a focal point of discussions at a National Science Foundation-sponsored workshop focused on the topic. Some of the major needs include but are not limited to the integration of specific scientific and engineering disciplines and guidance from regulatory agencies, infrastructure requirements, and strategies for reliable systems integration. Some of the recommendations, major targets, and opportunities were also outlined, including some "grand challenges" to spur interest and progress in the field based on the participants at the workshop. Many of these recommendations have been expanded, materialized, and adopted by the field. For instance, the formation of an initial collaboration network in the community was established. This report provides suggestions for the opportunities and challenges to help move the field of advanced biomanufacturing forward. The field is in the early stages of effecting science and technology in biomanufacturing with a bright and important future impact evident based on the rapid scientific advances in recent years and industry progress.



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1. INTRODUCTION

Advanced biomanufacturing is an emerging discipline that focuses on the use of biological systems or the products of

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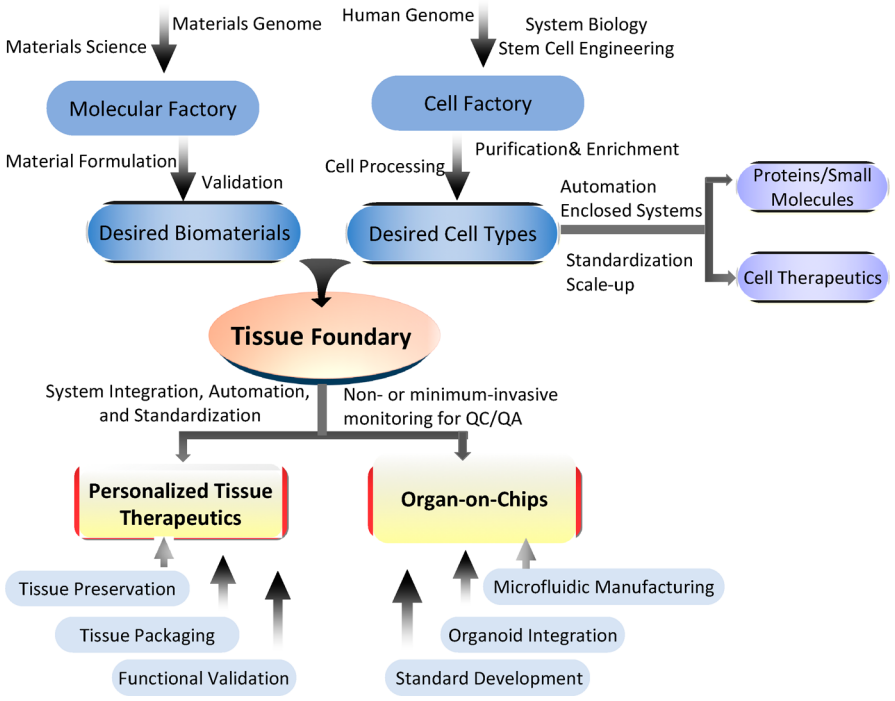

Figure 1. Emerging technologies for cell and tissue biomanufacturing.

biological systems to generate new materials and new therapeutic products with a view toward scalability, standardization, and industrialization. These processes include the use of technology to generate biologically relevant materials and systems wherein biological components and/or processes are included. The key is to utilize building blocks, materials, or synthesis systems such as cells or related components to exploit control that biology can provide over materials, such as from a structural hierarchy and complex systems/tissue assembly perspectives.

The anticipation is that the development of advanced biomanufacturing technologies will lead to new modes of generating components/building blocks, biomaterials, tissue organoids, automated bioreactors, and systems for a range of needs, from medical devices, biosensors, therapeutic cell and tissue products, to new ways to alter the supply chain, manufacturing environment, and environmental compatibility, as shown in Figure 1. To navigate this vision, this position paper assesses where the state-of-the-art is, what the paths are to move forward to reach the vision, and to identify the major barriers to success. These needs encompass the science and engineering involved, the regulatory and infrastructure needs, and the systems integration required. The idea is to disrupt and transform, not to take a small step. However, there are many examples of activities upon which to build, where small successes and opportunities can serve as a guide to the larger impact, eventually leading to a new sector of science discipline.

The scientific tools to support the vision for advanced biomanufacturing have been emerging over the past 10−15 years, empowered by advances in genomics and proteomics, cell biology, regenerative medicine, 3D bioprinting, process engineering, and design and systems integration. These advances, originally focused more on the human genome and needs toward personalized medicine, can now be targeted toward materials, cell manufacturing, and tissue biofabrication in ways not even feasible ten years ago. Importantly, these opportunities are now driven by the many confounding healthcare challenges presented to society. Historically, biomanufacturing has focused mostly on the pharmaceutical industry, referring to fermentation, purification and formulation needs, including upstream and downstream aspects of the process. This industry continues to thrive, and many of the insights and advances from the production of pharmaceuticals can be used as a guide to develop new technologies for advanced biomanufacturing.

Important Lessons from Biology. We approached the challenges in advanced biomanufacturing from a hierarchical perspective. This approach was selected in part because this is the model from biology, and some emulation of this approach was hypothesized to inform and guide our plans in a positive way. As such, the design and control of building blocks,[1−3] the ability to program and generate polymers,[4,5] the concept of self-organizing cells,[6−9] and the ability to print complex tissues and organs[10,11] provide the scaling to be explored. While this helps identify the tools, paths, and systems, it also establishes some self-imposed limitations that need to be recognized. Perhaps the largest of these is that in nature, these individual scales are not segregated but intimately connected, providing seamless integration to permit efficient and productive systems to function.

Building Blocks, Molecular Recognition, and Hierarchy. The ability to design biomanufacturing processes by encoding information content from the building block stage is one of the remarkable and empowering features in biology and is also very distinct from current manufacturing technologies adopted in industry. These rules of control (e.g., stereochemistry/chirality, self-assembly, self-sorting, etc.) provide the core of many systems discussed. Further, encoding information content at the building block level facilitates the scaling and assembly required to achieve more complex structures and functions in systems. This is not a trivial issue to embrace, as the subtle rules that guide structural hierarchy remain

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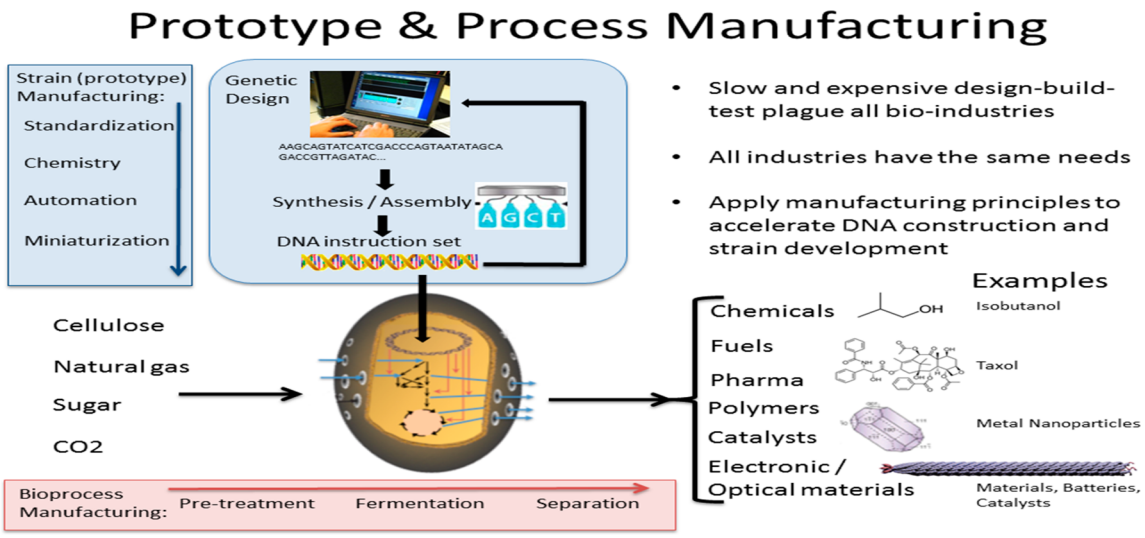

Figure 2. Aspects of biomanufacturing processes.

somewhat elusive. However, the general knowledge that small forces (e.g., van der Waals, electrostatic, hydrogen bonds, etc.), a role for water in the process of controlling interactions among components, self-assembly in terms of molecular recognition and interfaces, and scaling driven by sequence chemistry encoded in the building blocks (e.g., sugars, amino acids, DNA, etc.), can all be exploited in advanced biomanufacturing. Further, at the lower scale, modes to design and synthesize designer building blocks are in hand, and the modes to encode patterning and spatial features are somewhat in hand but need further robustness and insight. Subtle forces such as membrane potential and associated biophysical factors are only just emerging as important themes to be exploited in the field.

Bottom-Up vs Top-Down. A top-down approach reflecting a more traditional manufacturing approach can be contrasted with the bottom-up approach using building blocks organized with hierarchical structure and function. For instance, building or assembling tissues from the top-down remains early stage, yet advances are emerging,[3,12,13] from inkjet printing to self-assembling gels to generate complex tissue assemblies.[14−17] These advances inform our enthusiasm for the future potential of the approach and suggest that the tools and the basic insights are emerging to drive this approach in the field. Thus, the timing is right to build on these starting points to examine a path forward for advanced biomanufacturing and empower growth of this field.

Tools: Molecular Biology. Major advances in genomics, proteomics, and synthetic biology, with the associated databases and tools, have pushed forward the ability to design and implement new genetic approaches to encode pathways, control loops, compounds, building blocks, and polymer designs. These tools empower biological design, control, and production processes to a level not previously achievable. While the bulk of this focus has been on *Escherichia coli*, Chinese Hamster Ovary (CHO) cells, and a few other systems, the opportunities to expand the repertoire of tools to other host systems with scalable options and robust features is now available.

Tools: Polymers. Major advances in polymer synthesis have been driven by insights from bacterially derived polyhydroxyalkanoates and efforts to generate bioplastics like this over the past 25 years.[18−20] Additional insights have come from understanding the cellular machinery required to optimize polymer yield. Understanding upstream and downstream design needs, tailoring polymer composition, and generating useful products from biological systems have all expanded significantly. Specific insight and studies into the synthesis of polymers such as alginates, xanthans, hyaluronic acids, amylose/amylopectin, tropoelastin, collagens, and others have also helped to push these technologies ahead. Issues of purification, processing, and control of polymer features all feed into these topics. Additional topics such as purification related to endotoxin removal has been addressed related to the medical utility for the systems.

Tools: Patterning and Control. Major advances in cell patterning and control would empower a next generation of tissues, devices, and systems. These features emerge from developmental biology and require subtle insight and control into stem cell biology, matrix interactions, mechanical forces, electrical forces, and many other factors. Self-sorting based on cell receptors and programmed cell functions are endemic to these features. When these aspects of cell pattern control are then integrated with external manipulation of such features, such as by novel processing tools and deposition processes (e.g., inkjet and 3D printing), new generations of complex systems can be envisioned. For example, tissue and organ printing, pattern control of cell biology and many related themes have emerged in recent years.

Tools: Systems Integration. The associated manufacturing needs to support building functional devices are critical. Without these processes fully integrated into upstream synthesis and formation of components, the more complex, functional systems will not emerge. Thus, it is critical to consider how we can exploit current manufacturing processes in new ways, how biology solves these processes in a supply/demand way and at scales matched to system requirements, and how energy conservation and recycling/reuse are the routine and not the exception.

2. MOLECULAR APPROACHES AND BUILDING BLOCKS

2.1. Molecular Building Blocks: The Role for Synthetic Biology in Advanced Biomanufacturing. At the molecular

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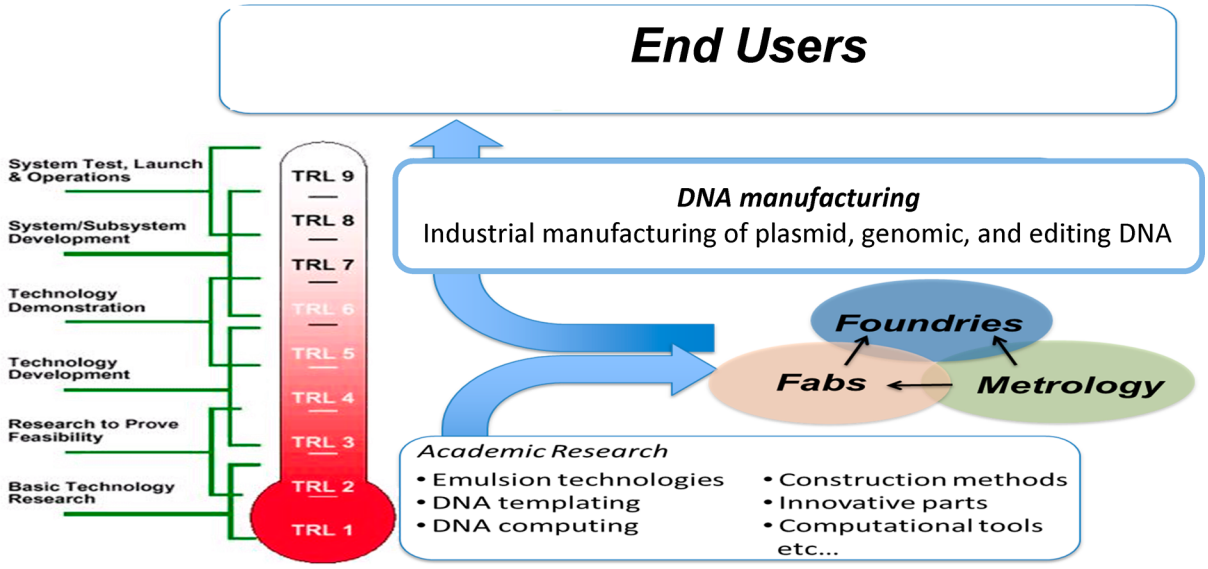

Figure 3. Illustration of proposed flow of molecular biomanufacturing technologies necessary to facilitate transition from proof-of-principle through DNA manufacturing to end-users in industry. There is a critical infrastructure need for Fabs to populate the space of biological parts, for Foundries to design these parts into large systems, and metrology to establish common measurements and standards for the exchange and dissemination of parts and designs.

level, rapidly advancing tools have empowered the ability to design and implement new cell capabilities, including cellular rewiring,[21,22] the introduction of new metabolic pathways,[23,24] the synthesis of biological building blocks or components for more complex materials, and expanding the toolkit from nature to synthetic options such as nonnative amino acids and modified sugars. These capabilities permit the generation of useful components and pathways toward new monomers for building polymers or for functionalizing devices or systems.

A substantial fraction of these capabilities is accessed by programming cells with DNA. Cells are provided synthetic DNA that encodes for collections of genes and other genetic elements that work together to accomplish a desired function. To this end, DNA manufacturers already have established production pipelines for relatively small DNA constructs (around 1000 DNA base pairs) and are continually improving these processes. This DNA manufacturing capacity for building polymers or for functionalizing devices or systems (Figure 2) has already provided the basis for consumer biomanufactured products in the chemical, fuel, and pharmaceutical industries.

Although there are already commercial applications, the difficulties intrinsic to transitioning complex systems through development stages have hindered molecular biomanufacturing from realizing its full potential (Figure 3). Systems containing dozens or hundreds of interrelated DNA elements have the potential to provide a tremendous variety of valuable new functions, chemicals, and materials. However, the development of such systems typically stalls at the proof-of-principle stage due to the complexity. To address this barrier, dedicated efforts are necessary to establish a reliable bridge for complex engineered systems to transition from proof-of-principle to production readiness, thereby providing a route for these designer systems to enter into existing mainstream biomanufacturing. To achieve such a bridge, three distinct efforts are necessary:

First, there is a continuing need for efforts dedicated to the discovery, characterization, and dissemination of useful DNA sequences. For instance, NSF funded a BioFab in 2009. The BioFab had a specific mission of creating repositories of DNA sequences that can be easily accessed and reused for multiple classes of engineering projects. More generally, facilities following this model (referred to here as Fabs) are needed to build and test large sets of genetic parts emerging from academic research, catalogue their behavior, and centralize their distribution, thereby removing barriers of access to sequences and information.

Second, rapid design and prototyping facilities are needed to determine how to most effectively assemble the parts produced by Fabs into systems that produce desired behaviors. Such facilities, referred to here as Foundries, address the intrinsic contextual complexity of large biological systems via high-throughput design-build-test-learn cycles.[25,26] To do this, Foundries design and build large sets of combinations of genetic parts, test the combinations, and then apply learning algorithms to extract assembly rules to enhance function. By doing so, Foundries can leverage knowledge from Fabs to shepherd complex systems from the proof-of-concept stage to one suitable for production. Moreover, Foundries will provide a critical role in technology dissemination of techniques for design and prototyping by industrializing early stage DNA-manipulation techniques from academia. They also can provide associated training to scientists in manufacturing industries and generate demand for such techniques in manufacturing settings by establishing manufacturing viability for complex, previously inaccessible systems.

Third, the exchange of information between Fabs, Foundries, and manufacturers that utilize their output will rely critically on metrology. Metrology refers to the development of standards for the measurement of the behavior of biological components (including "parts"), the descriptions of components (such as sequence, necessary context, metadata, etc.), ontologies, methods, models, quality metrics, and software specifications such as for data interchange.

Although they will fulfill a critical niche to enable molecular biomanufacturing, the establishment of Fabs, Foundries, and metrology are all at nascent stages. It is important to note that

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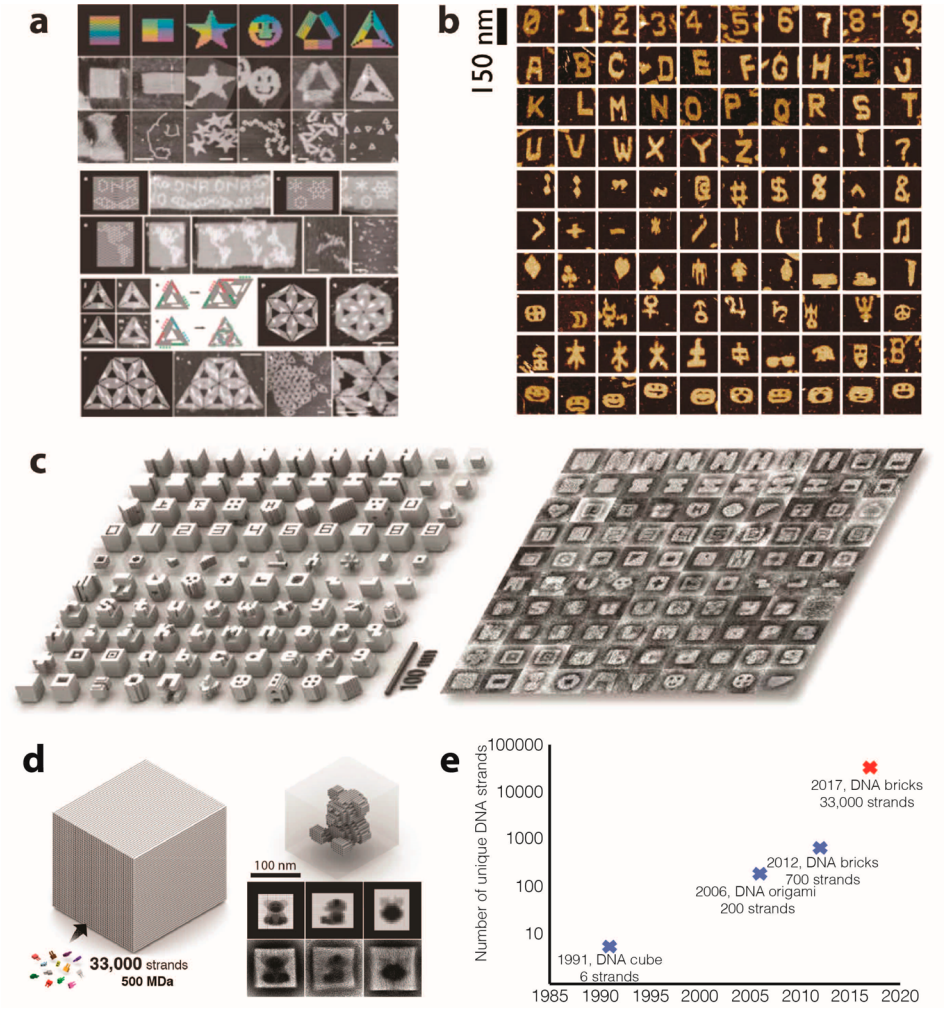

Figure 4. Digital self-assembly of (a) 2D DNA origami structures, reproduced with permission from ref 27, Copyright 2006 Springer Nature; (b) 2D DNA-brick structures, reproduced with permission from ref 28, Copyright 2012 Springer Nature; (c) 3D DNA-brick structures, reproduced with permission from ref 29, Copyright 2012 AAAS; (d) gigadalton DNA-brick structures, reproduced with permission from ref 30, Copyright 2017 Spring Nature. (e) Complexity of digital DNA self-assembly has increased exponentially.

initiatives such as these generally fall outside of the conventions of traditional academic research or industrial research and development (R&D). However, there is strong precedent for investment in such infrastructure. The establishment of factory-scale DNA sequencing centers has revolutionized biomedical and pharmaceutical R&D. Similarly, continued investment in transitional infrastructure will enable more complex, next-generation molecular approaches to fully leverage already-established biomanufacturing infrastructure.

2.2. Structural DNA Nanotechnology. *State-of-the-Art.* Digital fabrication, in comparison to analog fabrication, is more powerful and versatile due to its modularity and high accuracy (Figure 4). Nucleic acids, especially DNA, have been used by nature as digital molecules for programming cellular behaviors in biological systems. In contrast, structural DNA nanotechnology instead tries to harness the power of this versatile biomolecule for digital self-assembly and fabrication. The field has grown rapidly and become an effective approach for constructing sophisticated synthetic molecular structures and devices.

Diverse synthetic nucleic acid structures such as lattices, ribbons, tubes, finite 2D and 3D objects with defined shapes, and macroscopic crystals have been created. Many dynamic devices have been constructed in parallel, including tweezers, switches, and circuits. Recently, the field has also made a number of breakthroughs in digital self-assembly of nanoscale 2D, 3D, and microscale crystals using "DNA bricks" as modular building blocks. In all of these cases, the resolution approaches 2 nm. Additionally, as DNA and RNA can be interfaced with other functional molecules in a technologically relevant fashion, synthetic nucleic acid structures promise diverse applications; researchers are using DNA/RNA structures and devices to direct functional material arrangements, facilitate nuclear magnetic resonance (NMR) protein structure determination, develop bioimaging probes, and organize and regulate molecular pathways in living cells.

The structural size and complexity of digital self-assembly by using DNA as building blocks has grown exponentially (Figure 3e). One of the first 3D objects, a DNA cube, contained 6 DNA strands. Rothemund's DNA origami in 2006 was considered as a "quantum leap" that increased the number of DNA components to ~200 per structure.[27] The DNA bricks further increased the number of DNA components to ~700 in 2012[28,29] and then to ~33 000 in 2017,[30] a more than 5000-fold increase in comparison to the DNA cube.

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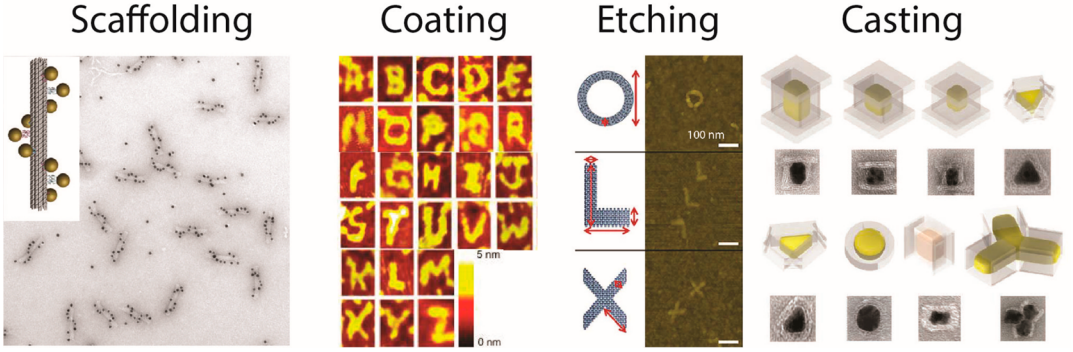

Figure 5. Digital nanofabrication of inorganic material via DNA-directed scaffolding of gold particles, coating with silicon dioxides, etching of graphene patterns, and casted growth of custom-shaped gold particles, reproduced with permission from refs 31−34, Copyright 2012 Spring Nature, 2013 ACS Publication, 2013 Springer Nature, and 2014 AAAS.

The self-assembly of DNA bricks was extended for constructing microscale crystalline structures.[29] This capability is especially important to scale up the sizes of DNA digital fabrication. It is the first general strategy for construction of complex DNA crystalline structures with precisely controlled depth and prescribed intricate nanoscale 3D features. These crystals can grow to micrometer size with prescribed depth up to 80 nm with a resolution around 3 nm.

Digital Fabrication of Inorganic Materials. Could we combine the power of digital self-assembly with functionality of many other functional materials that are widely used in industry, especially inorganic materials such as semiconductors, metals, and carbon-based materials? In a way, this is a similar challenge that the top-down technology has to address: how to rapidly prototype a wide range of materials to achieve desired functions. Recently, a few publications have demonstrated pioneering work of transferring structural information on digital DNA self-assembly to functional materials, including metallic nanoparticles, metal oxides, and graphene, through a variety of processes.[31−34] The typical resolution of the final structures is around 10−20 nm (Figure 5).

The semiconductor industry and other emerging applications such as nanophotonics and nanoelectronics are in constant pursuit of low-cost, high throughput manufacturing of materials/devices at smaller and smaller scale. Digital DNA self-assembly offers an alternative, promising route to conventional top-down lithography. . First, it can potentially assemble materials at sub-5 nm resolution. Second, self-assembly is a parallel process. Millions or even billions of products of the same shape and function can be produced simultaneously. Third, 3D materials and devices can be assembled in a single-step, unlike the conventional lithography 3D manufacturing, which typically requires multistep, layer-by-layer processes.

Despite the rapid progress, large-scale manufacture of nanoscale materials via DNA-directed digital fabrication has yet come to fruition. What needs to be done to capitalize on its potential? Three imminent challenges have to be overcome: (1) develop either chemical or enzymatic methods for high-quality, low-cost, large-scale production of DNA or RNA, (2) reduce the loss of resolution during the fabrication down to a few nanometers or even angstroms, (3) interface with a wider range of materials and develop multicomponent fabrication approaches (molecules and materials that can currently be controlled include proteins, nucleic acids, some small molecules, nanoparticles, graphene, and semiconductors), and (4) improve device performance by using highly ordered precursor or post-treatment to improve crystallinity under extreme conditions.

Gaps and Barriers. Some of the gaps and barriers include the creation of self-assembled DNA structures with high complexity, resolution, and precision, transferring the spatial information to more diverse technologically relevant functional materials with high accuracy and resolution, moving from simple prototype (e.g., etching a simple grapheme ribbon and producing a single field effect transistor) to integrated functional structures and devices (e.g., etching wafer size integrated circuits), large-scale production of DNA and RNA at low cost and high quality, developing effective computational tools for structure design and simulation, and improving stability of self-assembled structures under extreme conditions.

Art of the Possible. We envision that discrete, uniquely addressable structures over 1 μm size can be possibly created. Extended crystal structures with complex geometrical features at 5−10 nm resolution can potentially cover millimeter to centimeter surface area. Transferring of the spatial features of the synthetic DNA/RNA structures to diverse technologically relevant materials with complex features with nanometer resolution and over micrometer to millimeter area is also possible. Another technological advance that we envisioned is the creation of tunable thickness and composition of DNA crystals to enhance the resistance at extreme conditions. The combination of DNA/RNA digital manufacturing with the semiconductor industry, electronics, photonics, and spatially organized protein nanofactories could lead to the production of programmable molecular instruments for molecular diagnostics and therapeutics.

Modeling and Simulation. Existing design and analysis tools such as CAD can be used to design user-friendly interfaces for DNA designs. Sequence design tools that use experimentally attained information and simple elastic model of DNA duplex can be used to simulate twisting and bending of DNA origami structures. Computational models can be used to predict deformed DNA shapes based on symmetry minimization or thermodynamics of DNA molecules. The challenge is to include the need for more sophisticated and powerful design and analysis software tools that have much-needed functions such as rapidly simulating lowest-energy state of large structures and designing complex dynamic self-assembly of structures. Computer tools that fully automate the integrated design, construction, and test cycles are highly desired. The advancement of the field also calls for developing more design and analysis tools for materials beyond DNA

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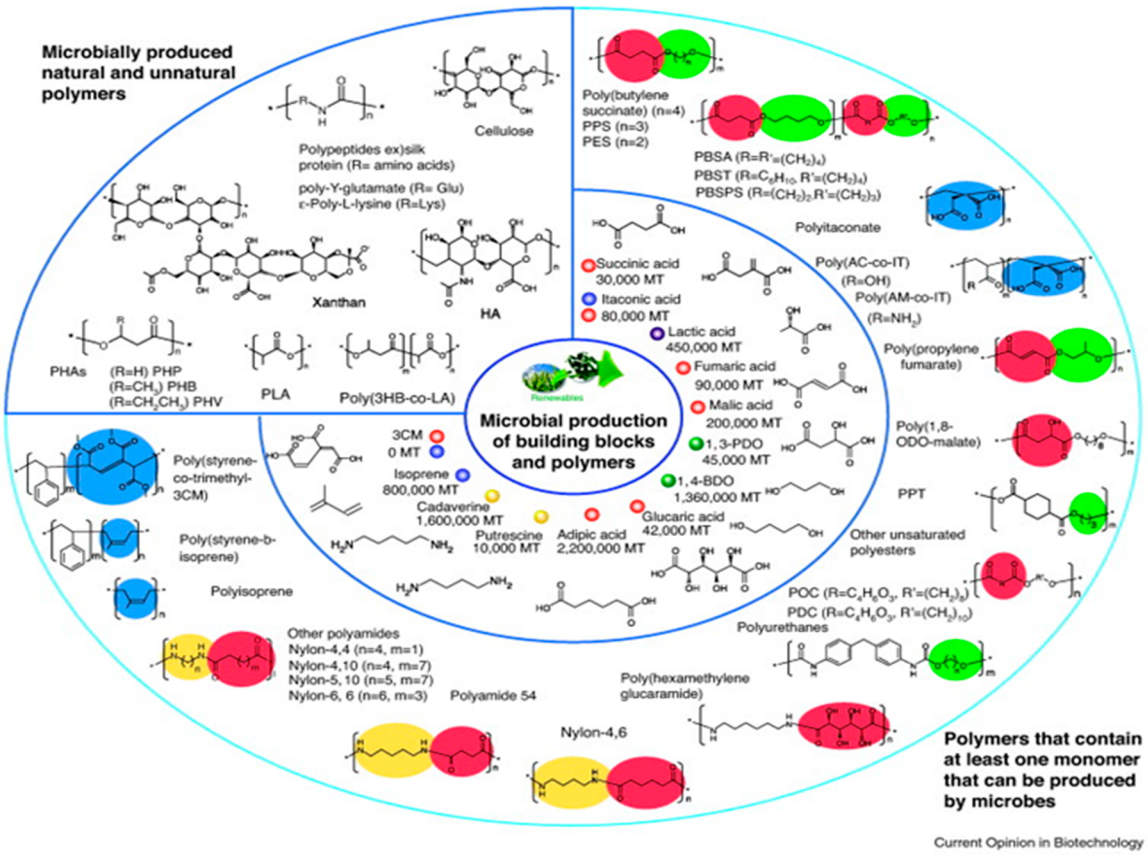

Figure 6. Microbially produced natural or unnatural building block chemicals used for polymer synthesis as well as polymers that can be directly produced in vivo, reproduced with permission from ref 40, Copyright 2011 Elsevier. Numbers below each chemical name in the inner circle designate the amount of total annual production where MT represents metric ton. Colored balls across layers indicate specific functional group(s) within chemical structures, which are specified by red for dicarboxylic acids, yellow for diamines, blue for alkenes or dienes, purple for carboxylic acids, and green for diols. It should be noted that colored regions of each polymer in the outer layer specifically indicate building block chemicals having specific functional groups indicated by the aforementioned colors. Abbreviations are 3CM, 3-carboxymuconic acid; 3HB, 3-hydroxybutyrate; AC, acrylate; AM, acrylamide; BDO, butanediol; HA, hyaluronic acid; IT, itaconate, LA, lactate; ODO, octanediol; PBSA, poly(butylene succinate-*co*-butylene adipate); PBSPS, poly(butylene succinate-*co*-propylene succinate); PBST, poly(butylene succinate-*co*-butylene terephthalate); PDC, poly(1,10-decanediol citrate); PDO, propanediol; PES, poly(ethylene succinate); PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; PHP, polyhydroxypropionate; PHV, polyhydroxyvalerate; PLA, polylactate; POC, poly(1,8-octanediol citrate); PPS, poly(propylene succinate); PPT, poly(propylene terephthalate).

structures, e.g. for DNA-templated inorganic structures and devices.

3. CELLULAR APPROACHES, ASSEMBLIES, AND POLYMERS

3.1. State-of-the-Art. An ability to use cells, organisms, or communities of organisms to generate polymers and structurally active materials is one of the hallmarks of biological systems. In particular, cells can generate polymeric materials with diverse properties for biomanufacturing devices or cell–material assemblies. These biologically derived materials have advantages in enhanced chemical uniformity, defined molecular weight and monodispersity, and controllable physicochemical properties. Furthermore, they provide scaffolding in which cellular assemblies can develop into organized tissues and form structured chemical factories for biomedical applications and beyond.

Existing biomanufactured materials are already successful. For instance, elastin-based materials provide resilience and elasticity to biological tissues.[35−37] Silk-based materials have high strength.[38,39] Other types of natural materials such as polysaccharides and collagen also provide unique and important properties. These types of materials can be derived either from biological sources or prepared by recombinant techniques (Figure 6). When cells secrete or interact with these biomaterials, they have been coaxed to form functional human and animal tissues. Structured microbial biofilms that are protected against material degradation or form self-healing materials or highly organized viral assemblies that can act as flexible piezoelectric materials are additional examples of assemblies. The design and manufacturing processes for the assemblies of these biomaterials and active cellular and viral components are currently heterogeneous, primitive relative to other manufacturing disciplines, and not standardized. The processes span molecular design, biological pathway design, materials design, and overall assembly of the complex mixture of cells and materials. There is an opportunity to integrate theory, computation, characterization, and physical manufacture to improve the scalability and reliability of these biomanufacturing processes.

Gaps. Some of the gaps in the field include interrelationships, moving from lab protocols to assembly lines, ex vivo storage/preservation, and functional heterogeneity of multiscale biomolecule or tissue assemblies. The interrelationships

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among the biomechanical/biochemical/bioelectrical factors, cell/tissue microenvironment, biology/physiology, and implant/tissue integration must be better elucidated. It is unclear how microenvironmental cues (such as soluble chemical factors, ions, local stiffness, microarchitecture, topography, porosity, diffusivity, and their gradients) influence cell machinery to produce proteins and polymers, or to remodel the environment resulting in new biomaterials. As cells enter a given microenvironment, their interaction with that environment leads to self-assembly, organization, patterning, and cell−cell signaling. Despite significant advances in biology, much of the underlying principles are largely unknown. Understanding these principles will also require significant efforts directed at understanding the ion-flux dependence of relevant processes such as secretion, proliferation, differentiation, migration, apoptosis, etc. These gaps in knowledge limit our ability to fully exploit these processes to develop the predictive models that are essential for the manufacturing of these polymers. Once these dependencies have been better described, the door will be open to important advances such as live-cell mediated delivery systems capable of targeting specific sites with reagents for both enhancing (e.g., inducing tissue generation, etc.) or inhibiting (e.g., drugs for cancer, cardiovascular disease, diabetes−related diseases, etc.), as well as improved implant biocompatibility and tissue integration. Cell generated polymeric biomaterials may be passive or they can serve as live, active, hybrid biopolymer with specific functionalities. These hybrids may self-generate, self-heal, and continue to emerge and evolve refined functionalities, depending on their usage.

Moving from Lab Protocols to Assembly Lines. The success of various types of biomanufacturing processes is still out of reach due in part to the lack of understanding some key elements of these processes. For example, in tissue engineering, in vivo scaffold degradation is often predicted from the outcomes of in vitro degradation studies, and these often do not correlate. However, direct quantitative determination of degradation in vivo has been problematic due to the difficulty in separating the infiltrated/regenerated tissues from their porous scaffolds; thus, many predictions remain untested and still require in vivo quantitative validation. It is imperative that we find in situ real-time methods to facilitate tracking or monitoring dynamic changes in tissue regeneration and scaffold degradation processes without sacrificing animals. This issue has been addressed somewhat; however, the field of tissue engineering still remains a trial-and-error process, to some degree. New biomaterial tools, engineering methods, design principles, noninvasive, and real-time assays are urgently needed to move the field of tissue engineering forward.

Ex Vivo Storage/Preservation. Technologies are lacking for off-the-shelf tissue grafts that incorporate live cells. Measuring tools and methods of quality assurance for biomanufactured tissue products in storage must also be developed before the biomanufacturing process can be used to develop tissues in a clinically relevant scale.

Functional Heterogeneous Multiscale Biomolecule or Tissue Assemblies. Manufacturing heterogeneous and multiscale structures that achieve desired compositions, architecture, functionality, and chemical and physical properties is not currently possible, largely because of a lack of studies on how different manufactured assemblies interact when combined and how structural stability and viability are maintained. Use of these mixed biomaterials is necessary to support a full spectrum of cell types and behaviors required to meet the promise of advanced biomanufacturing.

3.2. Modeling and Simulation. We need to consider key functions of biopolymers by including their working circumstance instead of isolated systems. We also need to take the effect of chemical environment (pH, temperature, ionic conditions, etc.) on the material functions into consideration. Indeed, these facts make the material functions such as strength and degradation rate no longer an intrinsic property of the building blocks. This method enables us to consider the interplay between the biomaterial and the environment in a dynamic way, and the result will be helpful for the life cycle design of biomaterials. There are few frameworks for transferring the knowledge from modeling and simulations to manufacturing of functional cellular and viral assemblies.[41] Development of integrated, scalable, open, computer-aided design and manufacture and high-throughput screening technologies backed by the proper information systems to learn from failures and successes is essential. The key is rapid prototyping and screening infrastructure to support this. Another idea is to connect experiments with bottom-up modeling and simulation to optimize sequence of proteins, assembly conditions, and processes of their assembly.[42] Such knowledge is difficult to obtain from top-down studies but critical for advanced biomanufacturing.

Barriers. We envision needs for developing computational models and scale-up biomanufacturing technologies. Computer-aided theoretical and computational models, computer-aided design of integrated systems, and modeling of biopolymer materials and cellular assemblies will play a critical role in generating testable hypotheses based on realistic principles. However, neither computational nor mathematical theories currently exist in sufficient detail to actively contribute to the experimental process. Multiscaling, multiphysical, and mixed-abstraction modeling, with uncertainty and big data management, remain a challenge. More interdisciplinary studies comprising mathematical modelers, biologists, and bioengineers should be encouraged. These groups can address pressing issues such as appropriate choices of assumptions, biological correctness, and applicability to bioengineering issues such as the role of bioelectrical signaling, interrelationships, scaling challenges, and tissue heterogeneity. Multiscale modeling has the potential to reach the goal and connect these discrete areas but needs to be developed in a standard and well-documented way so that it can be used by people without in-depth backgrounds.

There are also needs for developing methods for designing, identifying, characterizing, storing, and assuring the quality of advanced biomanufacturing processes for diverse applications. Ideally, one would have a top-down design for a final assembly of biomaterials, including geometry, specific interaction among cells, and input/output behavior of cells and entire aggregates. Ultimately, the methods employed will need to specify the three-dimensional spatial organization and help understand how it develops over time in terms of mechanical, chemical, and electrical properties of the system. When compiled, such language would specify a number of physical interactions and processes necessary to achieve the goal. This would be processed further into a series of abstract physical implementations with known manufacturing processes for biomaterials scaffolds and cellular surface properties and cellular processes. Finally, this would be transformed into a series of molecular, genetic, cellular, and material manipulations that could be

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carried out by a manufacturing process. The manufacturing process would be based on a series of standard primitives for these processes, including additive manufacture, self-assembly methods, etc. with more predictable functions. Predictable engineering includes the tools for directing and predicting the manufacturing process, allowing for in situ real-time assessing progression and failures of the process.

While there are emerging examples of modular materials and biological components, more diverse functional systems for operation in more environments with more actuation modalities better designed for interoperation are necessary. The creation of a computationally accessible knowledgebase of these primitives and their characterization is necessary to support a scalable computer-aided design and manufacturing framework. This leads to many areas of need:

- Biosynthetic systems for cellular production and controlled secretion of structured biopolymers that form external structures and organize interactions at a high level;
- Modular molecular elements of biopolymers that predictably form self-organized supermolecular structures with known compatibilities in different environments;
- Cellular sensors of electrical, mechanical, and chemical signals that can affect cell and aggregate behavior; and
- Precision manufacturing for protein designs, genetic encoding of functions in cells, biomaterial design, and cellular printing into microniches that support aggregate development and preservation.

3.3. Regulatory and Cost Issues. There are still many complex regulatory hurdles to overcome to develop cells/viruses and cell/virus aggregates into living tissues for applications to health. The issues of standards, biocompatibility, quality control, and long-term safety must be addressed. One issue that is of great relevance is the cost and scalability of cell-derived biopolymers. Due to the need to use cell bioreactors and biologically derived systems, the processes for generating these materials are inherently slow. To enable translation of such products, it is important to minimize batch-to-batch variability.[43,44] Furthermore, it is important to address long processing times, low yield, and purification limitations. Infrastructure for manufacture requires sophisticated computation, instrumentation, and automation. It is difficult for single laboratories to support such an infrastructure. Formation of biomanufacturing foundries that can be used by a range of laboratories will help advance the field. This process will aid in establishing collaborations, informational interchange, and standards as well. They would also form the basis of effective research centers. New models for handling intellectual property would also be needed.

4. TISSUE AND ORGAN APPROACHES

4.1. State-of-the-Art. The development of multicellular constructs, including tissues and organoids, is critical to advanced biomanufacturing. One of the major directions in this area is printing technologies that have been derived from various automated deposition schemes (for recent reviews on the subject, see refs 45−49), 3D organ printing technology shows promise as a viable option for creating complex, composite tissue constructs.[50,51] These printing methods can precisely place purely cellular materials or cell-encapsulating hydrogels in a layer-by-layer fashion, replicating the complex 3D structure of tissues or organs of interest.[50,52,53] New approaches such as integrated organ printing that can concurrently print synthetic biodegradable polymers and cell-laden hydrogels in a single tissue construct with applicable size, structure, and mechanical strength are necessary.[54]

Various technologies have been developed to print cells and manipulate them in small volumes. These technologies can be classified as nozzle-free and nozzle-based technologies. Some examples of nozzle-based bioprinting technologies include: inkjet, piezo-jet,[54,55] valve-based,[56] and extrusion-based printing methods.[57−60] These systems involve a droplet or a jet leaving a nozzle that encapsulates droplets and have been reported to print live cells and pattern proteins. Examples of nozzle-free technologies are laser printing[61−65]and acoustic bioprinting.[66] Laser printing involves a light beam controlling the locations of deposited cells precisely. Acoustic printing involves focusing acoustic waves to an open reservoir to generate droplets by breaking surface tension. These present techniques allow also the incorporation of DNA and proteins and other molecular entities. The resulting tissue structures have been built up to 4 mm in thickness, which have been fabricated and implanted into animal models.[50,59,67] Functional skin has been produced using extrusion bioprinting.[68−74] Bioprinted tissue arrays have been manufactured for drug testing.[52,53] Biologics can be patterned in 2D and 3D arrays with the use of lasers and cell printers. For example, patches encapsulating human mesenchymal stem cells have been implemented in animal models.[75] Additionally, 3D in vitro cancer model tissue constructs have been printed.[76,77] These printed constructs can be used as model systems to mimic the complex native microenvironment of tissues as well as cancer models.

Another approach is developing highly sophisticated and varied scaffold structures that can be implemented to develop 3D tissues.[78] This may enable complex vascularized and innervated tissues in the future. For example, to manufacture tissue-like systems with vascular conduits in 3D, fabricated sacrificial polymer layers can enable conduits to be created.[51,79,80] These fabricated polymers can be created through approaches like extruding or micromachining, which can create nonplanar features at a micrometer scale. These techniques enable the fabrication of 3D structures that are derived from more conventional manufacturing processes that have been used in the steel industry for decades.

These patterning and assembly approaches are well-positioned to be integrated with advanced manufacturing in the future to build many novel areas resulting in scientific advances.

Gaps and Barriers. Gaps exist at different levels ranging from lack of fundamental design rules to developing novel computer code to run 3D printing machinery. First, we need to incorporate developmental biology principles into tissue and organ engineering[81] and fabricate, store, and eventually deploy tissue constructs in environments different from the "natural" environment where they are intended to reside. Second, the fabrication of complex constructs and products involves combining multiple cell types in complex structures. It is more efficient and often necessary for the cells to interact with each other and develop, self-assemble, into the final structure. There is no database or comprehensive theory to guide structure building and formation.[82] A theoretical basis for general tissue/organ building does not currently exist. A database for the printing of different types of cells, interactions

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between different types of cells, culturing conditions, is a necessary prerequisite for establishing a theoretical basis and guideline for further development in the field. Moreover, there is no understanding in the field of what tolerances in building 3D structures are acceptable. For further advancing 3D tissue printing, a better understanding of the tolerances is needed in building 3D structures. While 3D fabrication of solid objects can occur with submicrometer tolerances, it may not be necessary to engineer tissues with those, as biological self-assembly is often occurring at those length scales. There is agreement that self-assembly does not occur at length scales in the millimeter to centimeter ranges within the appropriate time frame.

The definition of scale-up here also needs further clarification. "Scale-up" in production usually is perceived in terms of volume. In the case of 3D printing, "scale-up" may include an increase in the quantity produced, but biological factors are of equal importance such as an increase in number and types of cells printed. For nonplanar 3D structures, an increase in volume while decreasing surface area presents critical challenges as the inner cells lose access to the external medium. Scale-up would necessitate the development of truly large-scale cell culture techniques for the production of cells to print organ-size multicellular constructs. Such techniques do not yet exist.

The integration with host tissue has been recognized as critical but has not been studied systematically. The advantage of tissue/organ printing over other tissue engineering technologies is that vascular conduits can be constructed within the scaffold/tissue layers as they are built up. Vascularization with host tissue is seen as a critical step in assuring success. Ex vivo models of neo-vascularization have been developed and can be exploited in a systematic study of printed tissue/host vascularization.[79,80,83]

A barrier to building multicellular structures that are functional is the lack of understanding of the necessary cues that need to be provided by scaffold or matrix. Development of in vitro model systems that allow testing the effects of single and combinatorial effects of factors and/or signaling molecules on the function of tissue constructs is needed. Tools developed for cell printing and/or deposition are seen as playing an essential role in this development.

Computer control of equipment to activate manufacturing devices is limited. To achieve manufacturing, high throughputs, precision, speed, and repeatability are critical. This can only be achieved through automation. Depending on the spatial scale of the final product, the process will need to scale up from laboratory scale. The monitoring and care of products post manufacturing are also important factors for commercialization. Computerized equipment is central to all these processes, which is not available currently.

4.2. Art of the Possible. As extended bioprinted structures cannot be kept alive by relying solely on the diffusive transport of nutrients to all cells, several approaches have been suggested to overcome the major hurdle of vascularization needed for their fabrication. This can be accomplished by the active transporting of the nutrients through a network of branching conduits (the vasculature) that assures that no cell is farther than 200−300 μm from this supply mechanism. All the suggested approaches are based on sacrificial conduit networks, indicating that the solution to this problem is converging.[50,51,79,80] A sacrificial network is a blueprint of branching vasculature fabricated from a material that serves as a temporary mold. The cellular structure is constructed around this structure and, subsequently, the mold is sacrificed and removed (e.g., by variation of the temperature). The remaining network of hollow conduits is flushed with endothelial cells, which eventually seed the lumens of these conduits, providing the protective barrier akin to that in a natural vascular network. Sacrificial network molds have been fabricated either with 3D printers or micromachining tools (with linear features of 10−100 μm). Once printed multicellular structures and engineered tissues are supplied with such engineered vasculature, they will be possible to maintain in vitro until they are matured to the point that they can be used for implantation. This program will allow us to fabricate off-the-shelf tissues (and eventually organs).

From biomanufacturing prospective, biomimetic manufacturing will become a new paradigm. New materials and processes will be developed based on the capabilities of the living materials, in particular the cells. We envisage several possibilities at present and believe the range of future possibilities is wide. Cell-produced materials with unique properties such as bone could be used as construction material. Cultured leather could be used in the fashion, shoe and autoindustry (http://modernmeadow.com). Such applications will lead to considerable savings in resources (energy, water, and land) and eliminate adverse present industrial practices (e.g., toxicity in leather production). Harnessing nature's skills to fabricate tissues will allow producing food for use in constrained environments (e.g., space ships and battle ships). Learning how to employ cellular machines (e.g., molecular motors; acto-myosin contractile system) at the tissue level will lead to our ability to perform specific tasks across scales such as miniaturizing devices for medicine or performing work with engineered muscle. Further automation of the entire tissue and organ engineering process will lead to more efficient fabrication and our ability to produce these structures on-site as needed. Ultimately, we envisage that patients will walk into specialized facilities, shed their dysfunctional organs, and have ones "made to measure".

Cell printing permits the generation of 3D in vitro tissue models for probing basic biological insights into cells and tissues as well as understanding human disease processes. Among the applications are 3D in vitro tissue analogues that mimic different cancer tissues to elicit mechanistic information. For instance, the microprinting of cancer cells patterned with fibroblasts and various angiogenesis factors can simulate some of the hallmark features of invasion and metastasis seen in cancer patients.[76,84] A microfluidic device housing 3D biofabrication tissue constructs can be developed to enable manipulation of these cells in a 3D microenvironment to help explain the fundamental biological processes of cell−cell and cell−matrix signaling and interactions as well as allowing for environmental toxin screening.

Replicating cell and microenvironment in 3D is critical in understanding the physiology and pathology of human tissue conditions. 3D tissue models permit understanding of cell and tissue behaviors in response to external stimuli. As such, use of this system may recapitulate an individual's medical condition in vitro, which would allow for the development of personalized therapy.

Cell/tissue/organ-on-a-chip technology can provide vital tools in developing disease models[85] and drug testing.[86,87] These microchip devices mimic the cell microenvironmental characteristics in vivo and also integrate the dynamic cell

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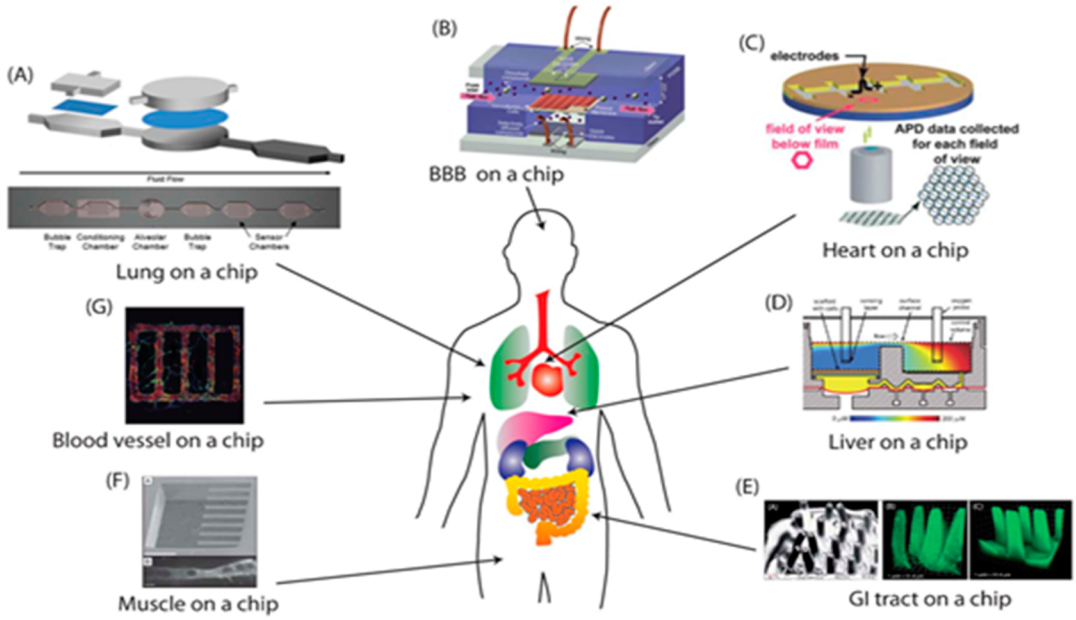

Figure 7. Body-on-a-chip. Conceptual image of how the various existing organs-on-a-chip might be assembled to simulate the entire physiological system of a human for the purpose of drug screening. (A) Lung. Reproduced with permission from ref 89, Copyright 2012 Springer Nature. (B) Blood brain barrier. Reproduced with permission from ref 90, Copyright 2012 Royal Society of Chemistry. (C) Heart tissue. Reproduced with permission from ref 91, Copyright 2011 Royal Society of Chemistry. (D) Liver. Reproduced with permission from ref 92, Copyright 2009 Royal Society of Chemistry. (E) Intestinal villi. Reproduced with permission from ref 93, Copyright 2010 Royal Society of Chemistry. (F) Muscle. Reproduced with permission from ref 94 (open access article). (G) Blood vessels. Reproduced from ref 95, Copyright 2012 National Academy of Sciences. The overall figure is adapted from ref 96, Copyright 2014 Springer Nature, and subfigures are reproduced with permission from the other references mentioned in this figure caption.

culture and high-throughput analysis together, mimicking specific organ activities, mechanics, biophysical responses, and functions in vitro. With controlled fluid properties in microchannels, one can simulate the physiological conditions for tissue/organ growth in the chip.[88]

4.4. Modeling and Simulation. To understand the complex interactions that occur in building tissue- and organ-like systems, it is critical to understand their multiscale integrated biological responses. One approach to this is to use developmental biology-based models to incorporate the naturally occurring multiscale (molecular to cellular to multicellular) behavior of living systems. Models will be built examining these systems considering a multitude of factors, including biochemical, scaffolding, mechanical, electrical, etc. These models have to cross multiple scales, yet these scales and multitude of interactions will cause challenges. Models can be used to understand these interactions and then employed to predict future integrated tissue responses. Special features such as complexity, coarse graining, multiscale character, and many others will need to be implemented and adapted to probe these biological systems.

5. ASPECTS OF SYSTEMS INTEGRATION IN BIOMANUFACTURING

5.1. State-of-the-Art. *Strategies for Comprehensive Systems Integration are Largely Product-Specific.* A key theme in systems integration is the diversity of the technical challenges. The specific challenges in systems integration are largely defined by the specific product. Other kinds of biomanufacturing strategies that are unilateral in terms of length scale and functionality can be leveraged to fabricate multiple diverse products. For example, molecular level approaches can produce designer proteins for use as structural materials such as extracellular matrix proteins, functional materials such as underwater adhesives, or bioactive therapeutics. Biomanufacturing processes at the cellular and organ levels include established and emerging technologies such as 3D-printing and cell-sheet engineering, for example. These platform technologies are often leveraged to manufacture specific articles. However, systems integration attempts to harmonize multiple articles together in a way to produce a larger multiscale system. Systems integration will ideally start with the final product, identify the relevant technology or technologies, and then integrate them into a system that can ultimately be used to fabricate the original product in mind. Systems integration deals with the seamless melding of these technologies to generate robust, scalable, and economically viable biomanufacturing systems. There are many challenges in this process, including the following:

- Identification of crossover points in which the output of one discrete technology or process can serve as the input of another process.
- Uniting stakeholders and end-users in defining appropriate metrics both within length scales (molecular, cellular, and organ) and across length scales.

Systems Integration Challenges are Numerous and Multifactorial. Systems integration approaches can be parsed out to include multiple unique thrusts. Here, we delineate a difference between process integration and systems-level design. Process integration is a key element of biomanufacturing. Process integration is defined as the ability to connect discrete unit operations of a broader process in a tractable manner. Strategies for process integration can be derived from those developed in chemical engineering. Systems-level design

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utilizes aspects of process integration for the goal of comprehensive systems design. Examples of systems-level design includes multiscale fabrication strategies for the integration of materials and cells with electronic devices including biosensors, electronic elements, and higher-level logic.

Gaps and Barriers. Many challenges and opportunities exist in the biomanufacturing of biological and cellular systems. These could be categorized as fundamental biological issues and aspects, and issues that are more technology and manufacturing related. There is very limited understanding of cell−cell interactions and communications and our abilities to manipulate these interactions. How do cells respond to physical and spatial gradients and how these cues affect the autocrine and paracrine interactions? The complexity of these interactions increases dramatically as heterotypic cellular interactions are to be considered. Imaging, sensing, and modeling approaches for examining and understanding these interactions are very much needed.

Characterizing and controlling the issues of consistency and variability of cells and biomolecules is another challenge. If cells or biomolecules are to be used for producing another product or if cells are used to make cellular systems, the control of parameters describing the physical and chemical properties of cells will be very important for biomanufacturing.

Co-differentiation of cells from embryoid bodies and cell clusters is a challenge and an opportunity to produce different cell types at the same time so their interactions could be tailored would be very useful. There exists tremendous potential and unexplored potential for using cells from different kingdoms (animal, plants, insects, microorganisms). Extracting opportunities for biomanufacturing of chemical and biological product from plant, insect and bacterial cells could be very important. Similarly, use of cells across these species for the development of cellular machines could be very useful. Could mammalian cells be reprogramed to operate at other temperatures except at 37 °C, e.g. at room temperature? Prediction and control of emergent behavior of cellular networks is a grand challenge for biomanufacturing. Technologies for characterizing and measurement of various physical and chemical properties for cell−cell communications and cell−matrix interactions need to be developed. Approaches such as imaging, chemical probes, computation, etc. will need to be integrated for measured 4D interactions. Furthermore, reliable vascularization continues to be an issue and a challenge, and every living system and exchange of nutrients and wastes will be critical to long-term operation of these systems.

5.2. Art of the Possible. *Technology and Manufacturing.* The spatial-temporal control of cell behavior and function would need to be controlled for developing robust biomanufacturing processes (Figure 7). The cell culture systems will need to be optimized for specific applications and specific market segment. The approaches might be modular and could be application specific versus core biomanufacturing modules that are applicable across many applications. The process control and issues of variability are more important for cellular and biological materials and cells compared to synthetic or electronic manufacturing. Similarly, the issues of biological product stability, preservation, storage, biocompatibility, and toxicity are important and would need to be considered. The concept of emergent or adaptive biomanufacturing where the assembly might be emergent or the final product might be emergent itself in the sense that it can continue to remodel in response to changing conditions is intriguing but nascent. Most biofabrication technologies cannot be considered high-throughput at present. Cell printing and placement, laser-based polymerization, etc. could be integrated with a high speed roll to roll printing, and other emerging biofabrication approaches could be integrated to realize new capabilities. This is also related to the balance between high-throughput and low-throughput processes for the appropriate applications.

Nontechnical Issues. A variety of nontechnical and regulatory issues and barriers needs to be addressed for increasing the impact and pervasiveness of the regulatory barriers. These include (i) developing standards for cell phenotypes and manufacturing of the modules, (ii) interdisciplinary language barriers, (iii) ethical issues related to biomanufacturing and self-replication, and (iv) issues related to technology adoption, ease of use, and functionality.

5.3. Modeling and Simulation. Modeling and simulation is a key aspect of systems integration. In the context of systems integration, computational models can be used to highlight some key aspects of biomanufacturing. Specifically, the following provocative questions would be of interest to the biomanufacturing community:

- Noise and error in biological systems. How much noise is too much noise? How can these definitions be addressed and modified for specific applications in systems at the different levels, including molecular, cellular, and organ scale devices?
- Signal transduction. How do we characterize noise propagation and information transfer in systems? How can figure of merits be translated to and from different aspects of the process?
- Fault tolerance and failure modes. How can we model fault tolerance in biological systems? What role can failure mode analysis play? How can we model these processes?
- Abstracting standards in molecules, cells, and organs. Can we use modeling to clearly define engineering parameters in cells? For example, in polymeric systems, complex solutions can be abstracted into practical engineering parameters such as molecular weight, viscosity, etc. Can we recapitulate these values for cells and organs? Where can modeling help in this process?
- Emergent behavior. Principles of systems integration have been successfully deployed to create complex technologies in the aerospace and microelectronic industries. A core principle that drives the commercial success of these examples is reliability and reproducibility across multiple layers of abstraction and design. Systems integration in biomanufacturing is complicated by both noise (as previously discussed) and emergent behavior, both intended and unintended. Emergent behavior is the ultimate element of complexity that compromises the efficacy of deterministic modeling. As such, efforts to apply insight from modeling and simulation must be tempered with the unpredictability and unknowns associated with emergent behavior.

5.4. Regulatory and Cost Issues. Advanced biomanufacturing can be informed by principles of product design and manufacturing from other industries with the implicit understanding that the former can be much more costly and

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complex. With respect to regulatory considerations, early engagement with regulatory authorities is almost always beneficial and advisable. On the back end, standard regulatory procedures such as good laboratory practice (GLP) and good manufacturing practice (GMP) can help inform efforts in biomanufacturing as well. Depending on the specific product, it is advisable to engage regulatory bodies such as the Food and Drug Administration (United States), the European Medicines Agency (European Union), and their respective counterparts around the world. New technologies in biomanufacturing must consider the prospective regulatory pathway and eventual adoption in the marketplace. In addition to economic consideration and regulatory requirements, scientists and engineers working in this domain have an ethical responsibility to consider to reduce the pain and suffering of animals and humans in sourcing raw materials, testing efficacy, and beyond.

As is the case with any manufactured product, costs are an important consideration. Biomanufacturing costs can be controlled by reducing the complexity of the product (if necessary), optimizing manufacturing processes to minimize the number of steps, and taking advantage of economies of scale. Best practices in biomanufacturing can benefit from insight from other industries that rely on efficient and cost-effective manufacturing principles such as quality control, quality assurance, and supply chain management. Additional considerations include independent validation of the quality of raw materials and provenance of goods. As part of the manufacturing process, integration between biological modules and nonbiological modules could incur higher cost due to compatibility, sterilization, and special packaging condition requirements. When delivering the final product to the end user, identification of how to provide the product with longer shelf life and how to make shipping and storage more cost-effective would add more value to the product.

6. CONCLUSIONS

Current biomanufacturing, where pharmaceutical production dominates the entire bioindustry, focuses on the mass production of small molecules and therapeutic agents, including proteins, vaccines, and cells. The manufacture of these products has long been dependent on the batch or continuous processing of chemicals or cells in which fermentation or batch reaction constitutes a cornerstone of the entire process. While these manufacturing processes have been and will continue to be successful, the next generation of biotherapeutics such as nanoparticle-based therapeutic and diagnostic agents, patient-tailored cell- and tissue-based therapeutic products, or molecular medicines demands new manufacturing technologies to realize their translation potential. Thus, new business models, i.e. reimbursement structures or price control models, need to be established to accommodate the aforementioned cell- and tissue-based biomanufacturing.

We are now at the crossroads where many biomanufacturing technologies developed in academia have demonstrated feasibility, but the business models for commercializing these technologies have not been established. The nascent industry of cell and tissue products has not been actively involved in these technology revolutions. The field requires an effective partnership between academic investigators and industrial R&D developers as well as strategic investment by government to promote this partnership. Co-investment by industry and government is critical for establishing the new bioindustry. Unlike chemical or other products, the bringing of bioproducts to the market requires a long and expensive development cycle, including tackling the clinical and regulatory challenges. An ecosystem built on multidisciplinary expertise involving academic researchers, industrial developers, regulatory experts, and policy makers at the early stage of development will go a long way to advance this nascent and exciting field.

Some global themes and challenges emerged from the field that can be highlighted as generic opportunities to spur innovation and ideas in the field of advanced biomanufacturing or the related topic of industrialized biology. The concepts discussed above are directed to growth in the science and industry of advanced biomanufacturing. Brief topics and descriptions are provided to help advance the field and spur the continued growth. This also should be a living list, with new ideas added regularly as appropriate. More specific needs are identified in each of the four areas including building blocks, cellular approaches and polymers, tissue and organ approaches, and systems integration. In summary, we believe that core industries that can supply building blocks for the field would be beneficial, reduce duplication of effort, provide quality control related to future FDA requirements, and provide a growth industry for jobs and infrastructure. Specific targeted products from such industries could include oligonucleotides/genes, purified recombinant proteins, engineered cells, modeling tools, and educational software/tutorials/online learning.

Capitalizing upon success with generating synthetic cells, we envision corporate entities centered on generating synthetic cells with minimal genetic requirements for basic functions. These functional biological "shells" or "containers" that will be available to laboratories to either order or add genetic machinery to produce specific building blocks of interest. This would improve efficiency of production of building blocks, avoid duplication of effort, and streamline eventual applications.

Another challenge of the field is to genetically preprogram cells to produce different components in an orchestrated approach toward the formation of complex structures. The origins are the need for generating products in resource-limited locations to reduce shipping/logistics burdens and preserve the environment (for example, in future space travel scenarios where it is not feasible to carry the supplies needed for shelter, devices, or containers). In many cases, cells are the machines driving advanced biomanufacturing, either directly or indirectly. Cells, depending on the type, function at specific metabolic rates and are generally limited by fundamental mass transfer constraints. Can cells be redesigned, or synthetically designed, to overcome some of the current limitations and improve the kinetics of production of building blocks or other products.

It is clear that we are in need of achieving "build-and-go" capability by designing biological "legos" that have all of the encoded information needed for self-assembly into complex patterns and forms, which would jump start many applications in advanced biomanufacturing. This is a hallmark of biological materials, and emulating and harnessing these features would enhance the formation of complex materials.

The ability to predict design-assembly rules, hierarchical assembly, scaling of processes, and general predictive tools remains primordial. The topic of modeling and simulation is ripe for a robust initiative applicable to every aspect of the field of advanced biomanufacturing, as outlined above. Specific

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challenges could be embedded in each of the four subthemes (e.g., develop a predictive tool to determine how a polymer sequence will self-assemble into a macroscopic material), or these initiatives could be more global in nature (e.g., develop an algorithm that can input primary structure or sequence and predetermine two orders of magnitude in scaled assembly what structure will look like, or develop a predictive tool that will guide the design of a fundamental biological building block to form a porous structure with specific performance such as mechanical compression, etc.). The answers to these challenges will spur the continuous growth of advanced biomanufacturing.

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Notes

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