Additive Manufacturing for Health: State of the Art, Gaps and Needs, and Recommendations

Yong Huang[1]
Department of Mechanical and Aerospace Engineering,
University of Florida,
P.O. Box 116250,
Gainesville, FL 32611;
Department of Materials Science and Engineering,
University of Florida,
Gainesville, FL 32611;
Department of Biomedical Engineering,
University of Florida,
Gainesville, FL 32611

Steven R. Schmid
Department of Mechanical and Aerospace Engineering,
University of Notre Dame,
Notre Dame, IN 46556

Additive manufacturing (AM) involves using computer-controlled machines to fabricate three-dimensional (3D) structural and functional parts layer by layer. To date, ample AM application opportunities exist in the health field. Based on the outcomes at the 2016 National Science Foundation AM for Health workshop, this paper summarizes the current state, gaps and research needs, and recommendations related to AM for health, in particular, hard structure and medical product printing and soft construct bioprinting. Manufacturing-related knowledge gaps and needs mainly fall into the materials, design, process innovation, part characterization, and policy and education categories. Hard structures and medical products can be designed to integrate with tissues, and their gaps and needs are typically related to the material-process-property-functionality relationship. Bioprinting-specific gaps and needs include build material selection and construct design, printed construct preservation, process selection, scalability and modeling, bioprinting-induced cell injury management, postprinting tissue fusion and maturation, and printed construct evaluation. Research recommendations encompass aspects ranging from fundamental research support to development of suitable standards for clinical use of AM products and are summarized in terms of materials, design, process innovation, modeling, characterization, and policy and education. Hard structure and medical product-specific recommendations are mainly related to build materials and structure design. For bioprinting, recommendations are summarized based on preparation, bioprinting process, and postbioprinting treatment. Furthermore, a biomedical manufacturing landscape is proposed, the potential of bioprinting as transformative research is introduced, and manufacturing-related scientific challenges are listed. [DOI: 10.1115/1.4040430]

1 Introduction

Additive manufacturing (AM) has become pervasive in the past decades, and applied to fabricate three-dimensional (3D) structural and functional parts from metallic, plastic, ceramic, electronic, biological, and composite materials [1–5]. While all AM processes produce 3D objects from model data, usually layer by layer, they can be classified into seven categories [6]: vat photopolymerization, material jetting, binder jetting, material extrusion, powder bed fusion, sheet lamination, and directed energy deposition; within each category, multiple specific implementations are available.

The impact of AM continues to grow, and it has been used in a wide variety of industries including automotive, aerospace, defense, clothing, energy, consumer goods, biomedical, and many others. AM has seen significant expansion of capabilities since its first demonstration in the 1980s. At first limited to stereolithography, laminated object manufacturing, selective laser sintering, and fused deposition modeling (FDM), a number of novel processes have been developed for various applications using different build materials. AM is now being used for production, not just prototyping. Significant improvements in AM processes, hardware, process control software, and computer-aided design modeling software, as well as the proliferation of inexpensive machines, have occurred in recent years, leading to the pervasiveness of this technology. Advances in research have led to more robust materials, rapid tooling, and extension to new areas, notably in architecture, biology, and microtechnology where AM capabilities have enabled new areas of research. In particular, AM for biomedical applications [4] has received significant attention; however, basic research questions and emerging scientific topics, which would enable the full-scale adoption of AM for health, have yet to be formulated and identified. The lack of a clear vision for future research directions for AM in health eventually resulted in the National Science Foundation (NSF) AM for Health Workshop in 2016. Rather than discussing particular development problems for AM in health, the workshop aimed to identify fundamental research needs and topics, which would help realize AM potential for health and accelerate innovations as illustrated in Fig. 1.

Since several notable AM for health review papers [7–16] and books [17–19] already exist, this paper focuses on printing of hard structures/medical products and bioprinting rather than duplicating their efforts by reviewing the process-related technological details. Instead, this paper summarizes the current state-of-the-art, gaps, research needs, and recommendations to promote the vigorous advance of research, applications, and commercialization in AM for health.

Herein, the classification of AM for health applications is based on whether or not printed structures are hard and stiff or soft and compliant. Hard structures, usually as various medical products, generally provide mechanical load-bearing. Soft constructs/structures mainly provide biological and chemical functions ranging from muscular contraction to metabolism to neural processing. Soft construct fabrication is further divided into direct and indirect bioprinting; direct bioprinting utilizes build materials containing living cells, while build materials during indirect bioprinting are acellular. Once fabricated, both hard and soft structures can be seeded with living cells as needed. This paper summarizes AM applications in terms of hard structure and medical product printing and soft construct/structure bioprinting. In particular, bioprinting-related topics are highlighted since it has emerged as an interdisciplinary, transformative technology with significant broader impacts to healthcare.

2 Current State

2.1 General Description. The use of AM in healthcare applications has attracted considerable interest over the past decade for its potential to reduce healthcare costs and increase healthcare quality. In particular, AM is uniquely suitable for medical device customization with a short lead time. The area of AM for health has been identified as a promising direction at the 2009 Roadmap for Additive Manufacturing Workshop sponsored by NSF and the Office of Naval Research (ONR) [20] and highlighted by the 2013 NSF Workshop on Frontiers of Additive Manufacturing Research and Education [21]. AM in health is also a focus area of the recently established manufacturing institute in the U.S.: Advanced Regenerative Manufacturing Institute [22].

[1]Corresponding author.

Manuscript received February 28, 2018; final manuscript received May 22, 2018; published online July 3, 2018. Assoc. Editor: Zhijian J. Pei.

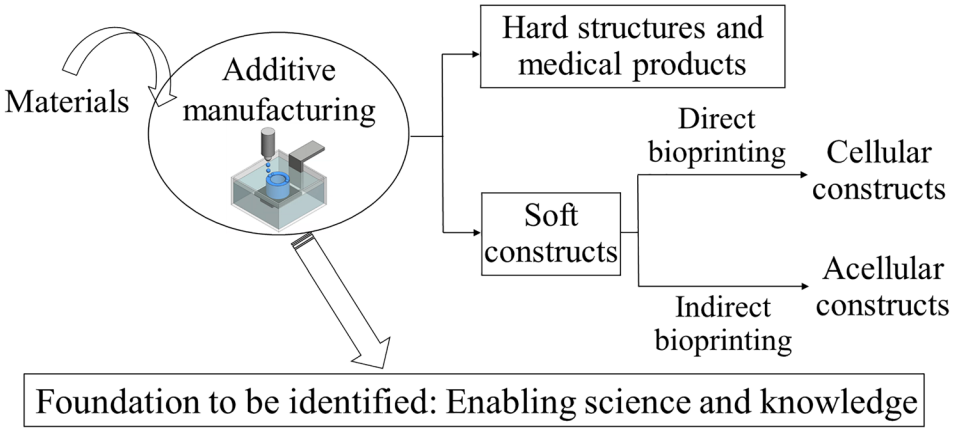


Fig. 1 Illustration of the workshop scope

Ample AM applications have been identified in the health field, including the fabrication of custom shaped orthopedic prostheses and implants, medical devices, surgical planning and training equipment, precision medicine, tissue scaffolds, biological chips, and living constructs, among others. Moreover, living constructs can be used for tissue implantation, robust pharmaceutical drug screening investigations, and high-fidelity developmental biology studies.

Specifically, some notable hard structure and medical product examples are illustrated in Fig. 2. Customized orthopedic implants, in which a bone ongrowth or ingrowth surface as well as designed flexibility to avoid stress shielding can be seamlessly incorporated, may be fabricated using selective laser sintering of titanium alloy (Ti-6Al-4V). Cranial reconstruction implants of titanium, stainless steel, or polyether ether ketone can be readily customized and fabricated on demand for individual patients; dental implants, seamlessly incorporating rough threads, are small and can be produced effectively in batches using AM. Similarly, orthodontic aligners can be produced directly from AM instead of being thermoformed on AM-produced molds. Furthermore, integrated active systems can be printed with sensors and actuators for prosthetic applications.

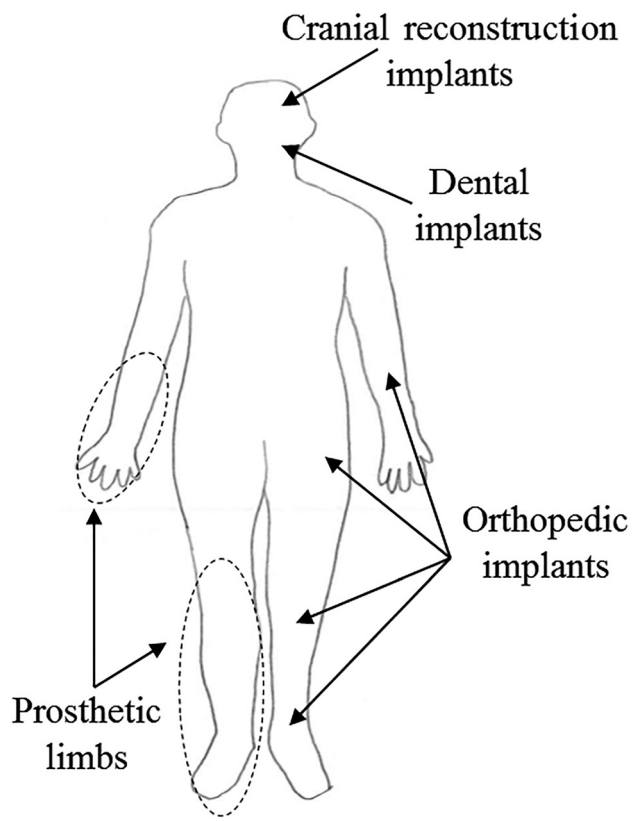


Fig. 2 Select examples of AM in current medical products and areas where AM is expected to have a major influence

Another notable AM process innovation is in the area of bioprinting, also known as cell or organ printing [4,8–10,12] as illustrated using a vascular tree construct fabrication process in Fig. 3, which is a developmental biology-inspired scaffold-less biofabrication approach. 2013 marked the 15th year of bioprinting, an ambitious vision to create developmental biology-enabled, scaffold-less living tissue constructs and organs by printing living cells, which will eventually help mitigate the challenge of organ donor shortage [8,23]. In particular, fabrication of thick tissues with vascularized structures is also a NASA Centennial Challenge [24], which was announced to the public at the June 2016 "Saving Lives and Giving Hope by Reducing the Organ Waiting List" White House event. In addition, bioprinting should be expanded to space and microgravity conditions [25], supporting deep space exploration activities. These bioprinting topics are further detailed later when commenting on the gaps and needs and recommendations.

Thus far, various tissue constructs have been successfully fabricated such as an inkjet printed fibroblast tubular construct (Fig. 4(*a*)) [26] and an extrusion printed human ear (Fig. 4(*b*)) [27]. In addition, bioprinting has been successfully integrated with casting to fabricate various thick vascularized tissues as shown in Figs. 4(*c*) [28] and 4(*d*) [29].

2.2 Hard Structure and Medical Product Printing. Hard structures for biomedical applications, typically used as medical products such as implants, are usually made from engineering materials including metals, ceramics, solid polymers, hydrogels, and composites. Suitable hard structure materials include biocompatible metals such as tantalum, titanium, stainless steel, and cobalt alloys; ceramics including bioglass and hydroxyapatite; solid polymers such as poly(caprolactone), poly(lactic-co-glycolic acid), and poly(propylene fumarate); tough hydrogels including collagen, alginate, poly(ethylene glycol), silk fibroin, and various blends; and composites. Composite materials are usually polymeric matrices filled with ceramic particles, which mimic the mineralized extracellular matrix (ECM) of native bone. They are typically processed much like the unfilled matrix, although the ceramic filler may increase stiffness and make the composite more brittle than the pure matrix. Another type of composite consists of a pure bulk material with a coating of a different material to improve its performance in vivo; this strategy is often

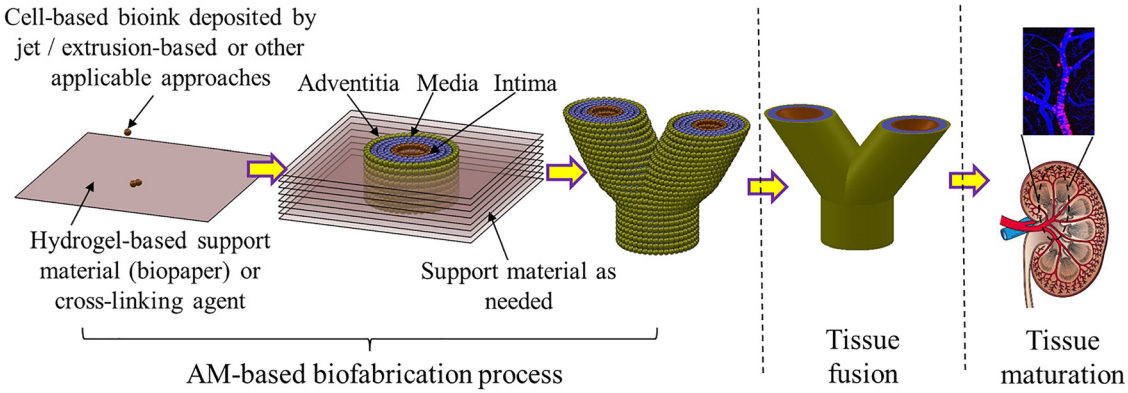
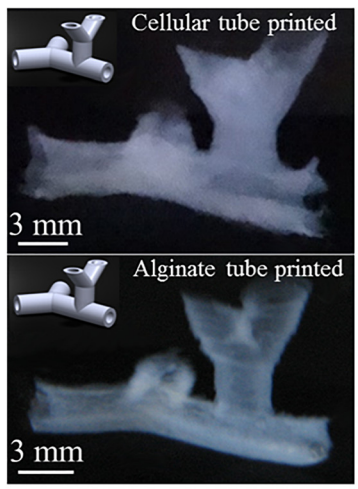


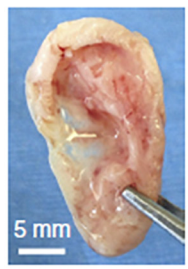
Fig. 3 Schematic of a vascular tree bioprinting process

employed to improve the biological response to permanent, non-degradable implants.

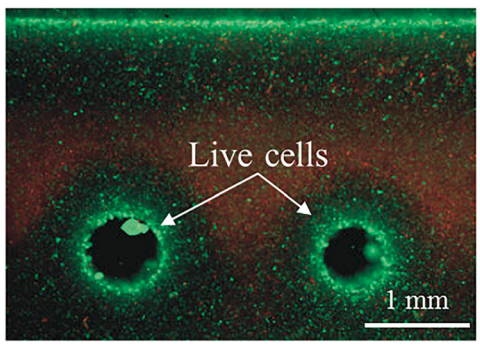
Since their mechanical and processing characteristics are similar to engineering materials, hard tissue structures can be fabricated using many traditional techniques as well as advanced manufacturing tools. In addition to casting/molding and subtractive techniques such as milling and turning, AM is currently one of the most popular methods for fabricating hard constructs for biomedical applications, offering unmatched control over shape, size, internal features, surface quality, and material heterogeneity



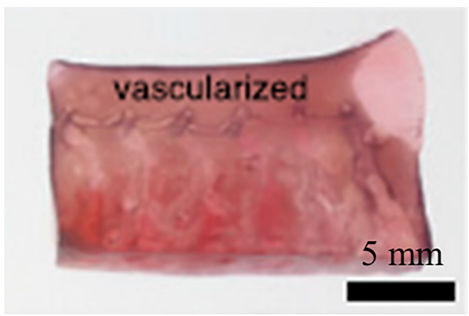
(*a*)



(*b*)



(*c*)



(*d*)

Fig. 4 Bioprinting-related advances: (*a*) multidirectional branching vascular-like structures printed using inkjetting materials [26] and (*b*) gross appearance of a printed human ear at 1 month after implantation (Reprinted with permission from Kang et al. [27]. Copyright 2016 by Springer Nature). Thick vascularized tissues fabricated using bioprinting and casting: (*c*) primary rat hepatocytes and stabilizing stromal fibroblasts in agarose gel after 8 days of culture (Reprinted with permission from Miller et al. [28]. Copyright 2018 by Macmillan Publishers), and (*d*) human mesenchymal stem cell and human neonatal dermal fibroblast tissue after 30 days of osteogenic media perfusion with alizarin red stain showing location of calcium phosphate [29].

as well as the potential for rapid customization [27,30–36]. While popular, it should be noted that AM is not the only approach having unmatched control of product features. For example, tantalum is deposited using a proprietary chemical deposition process to create a nano- and microtextured surface topography and build the Trabecular Metal® material [37]. Figure 5 illustrates the four most commonly adopted AM techniques [38] for hard structure printing: FDM, a material extrusion process; selective laser sintering, a powder bed fusion process; stereolithography, a vat photopolymerization process; and 3D printing (3DP), a binder jetting process.

Each of these AM techniques is distinct, although some share common features. Vat photopolymerization relies on projected light to solidify defined regions in each layer of resin, while powder bed fusion utilizes an energy beam to fuse selected regions of a thin layer of loose powder to form each layer. It should be noted that photopolymerization can also be implemented in different configurations such as two-photon induced polymerization [31], MultiJet or PolyJet modeling, and digital light processing with or without oxygen permeable optics (to establish an inhibition layer). Like powder bed fusion, binder jetting builds objects using thin layers of powder; however, instead of supplying energy to melt or sinter the powder in a defined pattern, a binder material is delivered in the form of droplets to form a solid particle composite. With metal powder, a further sintering step in a furnace is generally necessary. Both binder jetting and material jetting involve deposition of droplets, but in material jetting, the entire structure is built solely of jetted build materials deposited in layers on a solid surface. Material extrusion, with FDM as the most common implementation, fabricates objects by depositing fluid material in the form of thin lines/filaments, which rapidly solidify in response to ambient conditions or applied stimuli. In addition to FDM, material extrusion can be implemented alternatively such as freeze-form extrusion [33].

Materials for AM are diverse, and many engineering materials are also suitable for hard tissue applications. Polymers (including hydrogels) and composites may be processed to produce build material for vat photopolymerization, powder bed fusion, binder jetting, material jetting, or material extrusion. Ceramics are suitable for powder bed fusion, binder jetting, and directed energy deposition; they may also be fabricated using special preceramic polymers, which are suitable for vat photopolymerization. Metals may be processed using powder bed fusion, binder jetting, and directed energy deposition. In addition to flexibility in material and process selection, these are freeform processes so custom constructs can be generated rapidly and efficiently to match patient-specific needs and design constraints.

Additive manufacturing enables the fabrication of sophisticated hard tissue structures for medical use. Historically, bone tissue scaffolds have been simple solid or porous hard constructs, either mass-produced or custom-made for a specific defect. Although hard structures once suffered from limited cell retention and tissue integration [39,40], most orthopedic companies now offer implant materials that promote bone tissue ingrowth. More recent

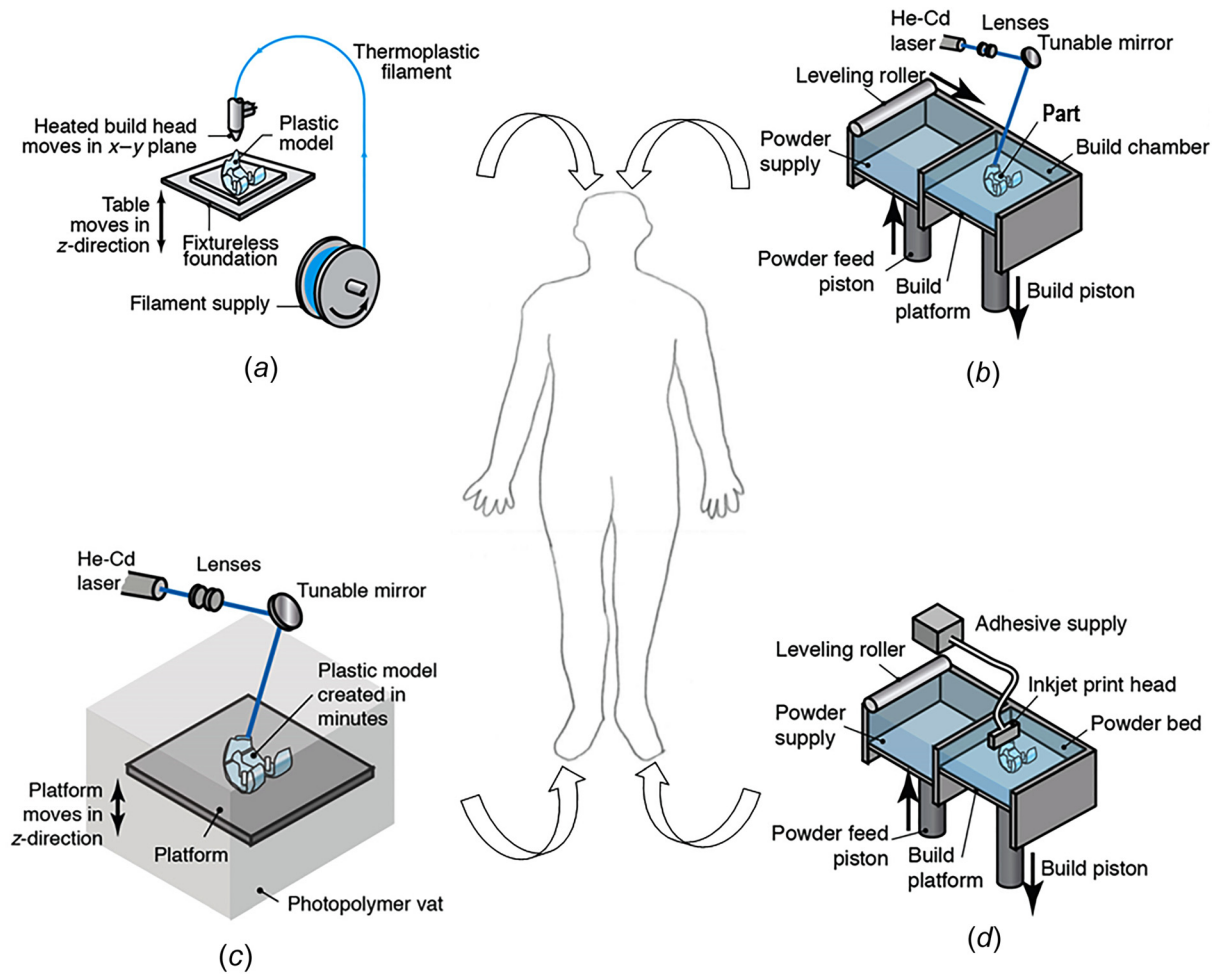


Fig. 5 Schematic illustrations of popular AM processes relevant to hard structure/medical product manufacturing: (*a*) FDM, (*b*) selective laser sintering, (*c*) stereolithography, and (*d*) 3DP

strategies focus on designing biomimetic environments which retain cells, resemble native tissues, and degrade controllably. Recent work has focused on composite constructs consisting of a hard continuous scaffold (made of metal, polymer, and/or ceramic materials) and an infiltrated or codeposited soft cell-laden gel, providing a balance of mechanical support and regenerative stimuli [27,40]. In addition, plasma spray and chemical deposition of growth factors and/or hydroxyapatite have been adopted for bone tissue engineering. Current research directions include optimization of architectures, characterization of the effects of surface finishes, and coprinting of soft, cell-laden components to promote rapid regeneration within rigid lattices [4,27].

2.3 Soft Construct Bioprinting. Direct bioprinting of soft constructs incorporating living cells yields structures with a wide spectrum of mechanical and chemical properties, ranging from nearly rigid cartilage to spongy brain tissues. These printed soft constructs can perform a correspondingly wide range of functions, from drug evaluation in vitro to organ replacement in vivo. In general, soft constructs have minimal mineral content with their biological functions primarily governed by their cellular activity rather than mechanical properties.

Typically, soft construct bioprinting research focuses on better control of material properties and cell interactions to direct the behavior of the increasingly sophisticated cell populations (in terms of cell types as well as cell concentrations) embedded in each construct. Research spans the entire fabrication, maturation, implantation, and degradation process, including material development; cell isolation and manipulation; process innovations; bioreactor design; characterization tools; and performance metrics to evaluate constructs in vitro and in vivo. Current research focuses on integrating multiple cell types and controlled channels in thick tissue constructs, characterizing and controlling cell responses, improving similarity to native tissue, and optimizing material properties, handling, and degradation [4,27,29].

Cell selection and handling is a critical aspect of soft tissue construct biofabrication; isolating, expanding, and maintaining functional cells for construct fabrication is currently the subject of much research. In addition, appropriate mechanical and chemical properties are crucial in fabricating functional soft tissue constructs since cells rely on such cues to perform their functions properly. Materials for soft tissue constructs are almost always biodegradable, though degradation rates and mechanisms vary widely depending on applications [17]. Controlled and predictable degradation rates are important for soft tissue since the scaffold should remain only long enough for the embedded cells to secrete their own ECM. The ECM of each tissue is unique with a complex hierarchical architecture to support and direct the functions of embedded cells. Once fabricated, one of the most difficult challenges in soft tissue regeneration is successfully integrating the implanted construct with native vascular, neural, lymphatic, and other systems to ensure adequate nutrition, circulation, communication, and functionality in vivo. Often, soft tissue constructs are cultured in vitro for some time before implantation to ensure adequate functionality and develop networks suitable for anastomosis in vivo.

In terms of build materials, most soft tissue constructs are composed of hydrogels and cells, although some may include nanofibers or solid scaffolds of poly(caprolactone), poly(lactic-co-glycolic acid), or other polymers. Hydrogels, including natural biopolymers such as collagen, alginate, silk fibroin, hyaluronic acid, and fibrin, as well as synthetic polymers such as poly(ethylene glycol), provide a hydrated matrix analogous to native ECM [17]. For some applications, hard scaffolds may be incorporated in the initial construct to stabilize the desired shape and allowed to partially or completely degrade during maturation in vitro so that a soft construct remains for implantation [27].

For soft tissue constructs, processing is limited by their mechanical properties. Typically, they are formed by casting/molding, fiber spinning, or AM, which has emerged as the most popular technique for fabricating 3D soft tissue constructs. For direct bioprinting, because maintaining cell viability and compatibility during fabrication is crucial, such living constructs are typically formed using direct deposition of cell-laden hydrogel precursors in the form of droplets (material jetting) or filaments (material extrusion). Figure 6 depicts some common direct bioprinting techniques: filament-based extrusion [14,41–46] (Fig. 6(*a*)), a type of material extrusion process, and droplet-based techniques such as inkjet printing [26,47–50] (Fig. 6(*b*)) and laser-induced forward transfer [7,51–54] (Fig. 6(*c*)), two types of material jetting processes. Layers built of these filaments (Fig. 6(*a*)) or droplets (Figs. 6(*b*) and 6(*c*)) form 3D constructs, which can be designed to resemble native tissues marked by complex heterogeneous materials and cell type composition. It is noted that vat polymerization of hydrogel precursors [55] (such as stereolithography, a type of vat photopolymerization process as shown in Fig. 6(*d*)) and binder jetting to form composites may also be used to generate soft constructs but they are less popular due to the difficulty in incorporating living cells during printing and the processing requirement of intrinsically high stiffness materials.

3 Gaps and Needs

3.1 General Gaps and Needs. While the benefits of AM in health have been significant, a true transformation in its healthcare applications is promised only through basic research to enable widespread, predictable, and valuable applications. As reported at the 2013 NSF Workshop on Frontiers of Additive Manufacturing Research and Education [4], some challenges and gaps have been identified regarding the printing of 3D acellular tissue scaffolds and cellular constructs. Specifically, the challenges and gaps related to printing 3D acellular tissue scaffolds include: (1) biophysical requirements related to the scaffold's structural integrity, mechanical stability and degradation, as well as tissue-specific pore shape, size, and interconnectivity; (2) biological requirements related to cell loading and spatial distribution, as well as cell attachment, growth, and new tissue formation; (3) mass transport considerations related to pore topology and interconnectivity; (4) anatomical requirements related to anatomical compatibility and geometric fitting; and (5) manufacturability requirements related to printability and process effects. The printing of in vitro biological constructs requires: (1) the development of a new generation of biomaterials designed to formulate bioinks for dispensing with cells, growing with cells, and functioning with cells; (2) developmental research to fill the biological knowledge gap; (3) the commercialization of bioprinting tools to make 3D heterogeneous structures in a viable, reliable, and reproducible manner; and (4) predictive four-dimensional (4D) bioprinting models which include stem cell differentiation and controlled release of biochemical molecules over time for complex tissues, organs, cellular machines, and human-on-a-chip devices. As the field of AM for health advances, related fundamental gaps and research needs are to be identified and rectified for the full realization of AM potential in the healthcare field in the future.

Overarching manufacturing-related knowledge gaps mainly fall into the materials, design, process innovation, part characterization, and policy and education categories, including:

(1) In terms of materials, printable materials are still very limited: there are relatively few available materials and many reported ink formulations are prohibitively expensive for commercial production. Also, there are no reported/standardized bioink formulations or postfabrication procedures.
(2) In terms of design, gaps and needs include the difficulty of designing appropriate constructs based on specific clinical requirements, the inadequacy of current technology to handle multimaterial designs, and criteria for choosing printing over other non-printing fabrication techniques.

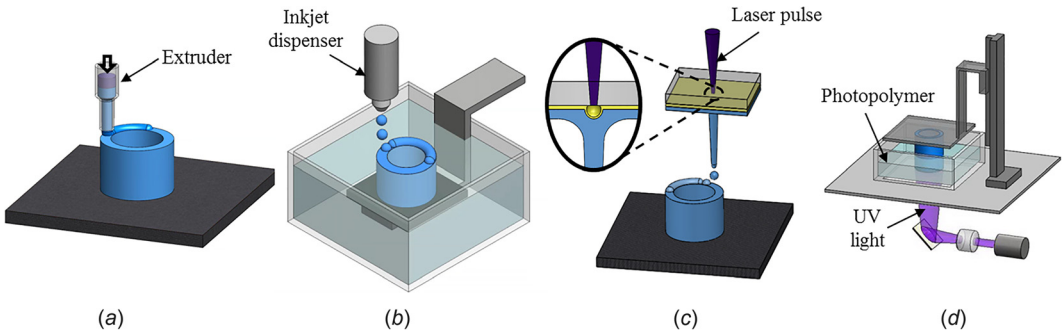


Fig. 6 Representative soft construct fabrication techniques: (*a*) filament extrusion, (*b*) inkjet printing, (*c*) laser-induced forward transfer, and (*d*) stereolithography

(3) In terms of process innovation, gaps and needs are related to scale-up production, multimaterial multi-functional products, customization, generation of multiscale feature sizes ranging from micron or sub-micron to centimeters, optimal planning by balancing speed and resolution, real-time monitoring of fabrication processes and feedback for online correction of defects, and control of part quality and process reproducibility.
(4) In terms of part characterization, there is a need for the spatially resolved characterization of AM products as fabricated as well as physical and biological characterization and monitoring in vitro and in vivo to evaluate construct/implant functionality as well as patient health. In addition, questions remain regarding processing-property relationships as well as how the resulting properties affect biological responses. The relationships between properties (surface finish, mechanical properties, porosity, pore size, etc.) and biological responses (such as stem cell differentiation, tissue integration, and vascular anastomosis) are a major gap in the current understanding of how AM constructs affect and respond to biological systems.
(5) In terms of policy and education, there is a need to develop suitable standards and regulations to govern clinical usage of additively manufactured products and promote the education of the next generation of AM innovators for health.

3.2 Hard Structure and Medical Product-Specific Gaps and Needs. While hard structures and medical products rarely incorporate living cells during printing, they have their own unique gaps and research needs, in particular related to the material-process-property-functionality relationship as discussed below:

(1) The build material properties such as purity, powder size, molecular weight (for polymers), etc. may affect final part properties; residual build materials such as residual precursors, powder, and uncured resin in/on final products can also impact biofunctionality.
(2) Dimensional accuracy is much more important for hard structures than for soft constructs, and may be affected by build materials, fabrication parameters, and post-processing steps such as autoclaving, which may result in distortion due to the release of residual process-induced stresses.
(3) Voids and porosity may or may not be desirable in certain applications; in either case, controlling their distribution or eliminating them requires a better understanding of how, where, and why they form.
(4) For prosthetic applications, monitoring the fit and functionality is also important to prevent injury and maximize patient comfort; development and selection of appropriate models and sensors remains challenging.

3.3 Soft Construct Bioprinting-Specific Gaps and Needs. Specific challenges related to soft tissue construct printing arise from the incorporation of living cells and the use of applicable AM technologies. As shown in Fig. 7, there are a few gaps and research needs to be addressed:

- Build material selection and construct design: The development of bioinks and scale-up production of living cells for printing are significant challenges. Bioprinting demands scalable production of living cells, presenting a myriad of manufacturing research and development opportunities. To be commercially viable, cell production needs to be scalable, be cost-effective, and comply with good manufacturing practice requirements. Typical starting materials in conventional manufacturing are nonliving engineering materials. However, starting materials for cell manufacturing and biofabrication are living cells, and this requires the manufacturing community to understand, design, and control processes and systems with unique constraints, metrics, and outcomes. Considering living cells as a special type of heterogeneous composite living materials, process development, modeling, monitoring, and control as well as quality control and supply chain management for cell manufacturing and biofabrication must considered to account for unique challenges associated with living materials. Tissues containing living cells currently suffer from a limited shelf life, which reduces their clinical potential and calls for practical preservation technologies. Specifically, vascularized thick tissues are expected as self-sustainable living systems. Furthermore, bioink formulations for printed constructs need standardization for key tissue constructs, and a better understanding of how construct design affects functionality is needed to maximize functionality as well as production efficiency.
- Various support baths and media have been utilized to enable some unique printing processes such as the printing-then-solidification approach [45]. The selection of support bath or medium, as needed, is to be optimized for key printing techniques.
- Each AM technique has strengths and weaknesses for soft construct bioprinting, so criteria are needed for process selection. As with all AM technologies, there is an ongoing need to reduce cost, increase speed, and improve robustness and quality. Process validation remains an issue.
- Regardless of AM technique(s) selected, printing dynamics of a variety of complex fluids including viscoelastic polymer solutions and soft cell-laden suspensions [56–58] are to be elucidated; the droplet formation dynamics during drop-on-demand printing are of particular importance. Excessive process-induced damage has been found to cause cell injury and even death during direct bioprinting, and the cell viability and cell injury of cells postprinting has been of concern [59–61]. Generally, there are two types of cell injury and

death: apoptosis (programmed cell death) and necrosis (accidental cell death). While necrotic cells can be identified using dye inclusion/exclusion assays to assess membrane integrity, apoptotic cells cannot be detected by routine inclusion/exclusion cell viability assays and have been largely ignored in studies to date. There is a need for further research to understand, model, and mitigate bioprinting-induced cell injury.
- Mechanisms of postprinting tissue fusion and maturation, and associated microenvironment need to be better understood for the development of soft tissue constructs.
- Evaluation of printed constructs: Spatially resolved characterization is a major challenge; with the incorporation of living cells, a wide array of additional functionalities come into play and are important for determining tissue functionality over time. Heterogeneous cell populations, tissue properties, and cell responses require advanced characterization tools to observe and direct outcomes.

4 Recommendations

4.1 General Recommendations. Manufacturing-related research recommendations identified in this paper encompass aspects of AM ranging from fundamental research support to development of suitable standards for clinical use of AM products and are summarized in terms of materials, design, process innovation, modeling, characterization, and policy and education:

- Materials: Development and standardization of a broad range of economically viable, printable materials for health applications; synthesis of new materials, especially biocompatible polymers that enable new kinds of medical devices and biological constructs; and development of a standard material or set of standard materials that can be used across fabrication systems and laboratories as a baseline for comparison with other materials in order to accurately compare fabrication methods and new materials, thereby unifying data across the field and potentially facilitating regulatory approval.
- Design: Conversion of clinical needs to construct designs, allowing integration of living tissue with medical devices; development of computer-aided design tools to design and printers to implement multimaterial constructs; and design of soft-hard tissue interfaces for heterogeneous constructs.
- Process innovation: Development of versatile and scalable printing techniques for direct production of implantable/wearable devices and systems, from custom orthopedic implants, stents, heart valves and dental devices to integrated wearable systems with built-in sensors that would log and/or transmit an individual's health conditions such as respiration, temperature, body position, and data to diagnose sleep apnea, to name a few; on-line monitoring tools to detect and correct defects during fabrication; and robust techniques for the printing of difficult-to-print biomaterials and biological materials.
- Modeling: Development of predictive models of both the printing process and postprinting product properties (including developmental biological processes such as tissue fusion and maturation) is necessary to inform technological improvements and to determine what level of complexity is necessary for optimal clinical outcomes; and understanding of cellular and tissue responses to both AM implants and degradation products over time to improve tissue integration and minimize the risk of chronic inflammation and infection.
- Characterization: Nondestructive testing and quality standards for printed soft constructs and hard structures, and quantitative assessment of product/process variability with associated metrics for regulatory compliance.
- Policy and education: Development of standards and regulatory pathways, requiring new or updated metrics and standards for build materials, manufacturing facilities, process/product reproducibility, biocompatibility, and product performance; preparation of educational materials and establishment of service centers for healthcare workforces, in particular nonexpert clinicians, to design and realize custom AM products for specific patients; establishment of research networks for collaboration and knowledge dissemination; and formulation of ethical guidance for soft tissue constructs. In addition, similar to the development of the Nanoengineering educational program, a new Biofabrication and Cell Manufacturing educational program is envisioned to prepare the workforce to meet the unique demands of the maturing cell manufacturing and biofabrication industries.

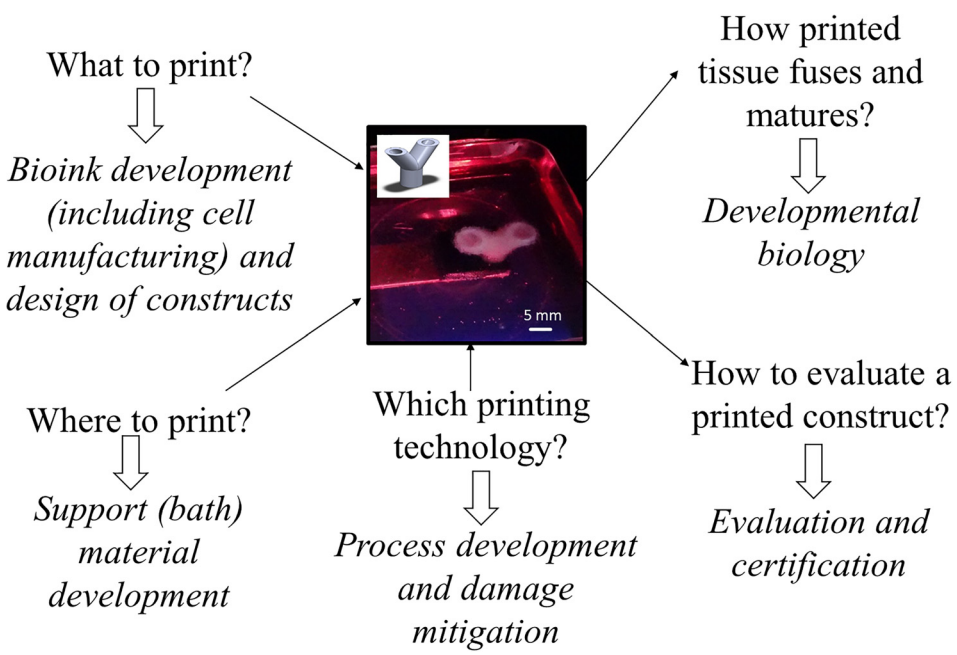


Fig. 7 Illustration of soft construct bioprinting-specific gaps and needs

- Process validation has been difficult with AM, but is required by regulatory agencies for hard structures and medical products. Methodologies that simplify process validation are sorely needed.

4.2 Hard Structure and Medical Product-Specific Recommendations. Hard structure and medical product-specific recommendations are mainly related to build materials and structure design:

- Build materials: Development of new materials including composites and alloys with tunable properties; understanding of corrosion behavior and how it is affected by material selection and fabrication parameters; understanding of how virgin and recycled build material properties affect structure properties; development of versatile technologies which support metal, ceramic, and polymer build materials; development of quality standards for build materials to facilitate regulatory approval of AM structures; and development of better in vitro and in vivo tools to assess performance and degradation.
- Structure design: Understanding of process–property relationships; understanding of influence of structural and compositional gradients on biological responses in vitro and in vivo; integrated sensors for monitoring performance after implantation; and lifelike appearance for external prosthetic structures.
- Integration with medical procedure process chain: Unlike soft constructs, hard structures and medical implants have the fit issue. For medical procedures with time gaps for healing or long lead times, there is time for fully customized AM implants or prosthetic/orthodontic parts to be printed. For some hard tissue implants, scanning/imaging technologies need to be integrated to AM fabricators, and the build time needs to be accelerated by one to two orders of magnitude. In addition, applicable AM fabricators need to be integrated into the operating room environment as part of the medical procedure process chain.

4.3 Soft Construct Bioprinting-Specific Recommendations. Recommendations specifically for soft tissues and cell-encapsulating constructs are generally related to processing (direct and indirect bioprinting) and cell behaviors, and they are summarized based on the three phases during bioprinting: preparation, bioprinting, and postbioprinting treatment.

4.3.1 Preparation

- Cell manufacturing: For effective and efficient cell expansion and manufacturing, research studies on process development, modeling, monitoring, and control as well as quality control and supply chain management for cell manufacturing and biofabrication are needed. In addition, the manufacturing community should integrate other recent advances such as data analytics for optimization of a well-defined manufacturing environment and the Internet of Things for online monitoring of tissue construct fabrication and maturation.
- Bioink formulation and characterization: Continuing research in materials for direct and indirect bioprinting should focus on identifying and standardizing printable materials which may include stimuli-responsive constituents to enable further manipulation of tissue properties after fabrication. Bioinks that depart from Newtonian behavior and protect the cells they carry are needed, and need to be carefully characterized to allow proper system design. It is important to have standardized bioinks and media for each type of tissue construct so the printing process can be consistent and predictable to enable reproducible and distributed mass production.
- Design for bioprinting: For cell-laden tissue design, studies should seek to develop: an understanding of how overall construct size affects cell survival and functionality; heterogeneous/compartmentalized constructs to mimic systemic effects of stimuli; computational models to predict the behavior and inform the design of cellular constructs; and better methods to quantify and track the fate of implanted cells as well as integration with host tissue.

4.3.2 Bioprinting

- Process innovation: A deep understanding of droplet/filament formation and deposition dynamics and the resulting printing resolution enables printing of a wider range of build materials. Effective, reproducible printing of difficult-to-print materials as well as multimaterial constructs should receive significant attention as well. The printing hardware and process control should also be improved to maximize achievable structural complexity and physiological relevance, in particular, thick tissues with vascular structures. In addition, biofabrication under space and microgravity conditions should be explored.
- Process-induced cell injury: In order to mitigate the printing-induced cell injury, a better understanding of cellular responses is critical to the success of printing processes by differentiating between post-printing apoptotic and necrotic cells. Understanding of process-induced cell injury during bioprinting will lead to its safe and efficient implementation, thus enabling its wide application for organ printing and rapid prototyping of cell-based products.

4.3.3 Postbioprinting treatment

- Design of improved, controllable, and scalable bioreactors: Bioreactors should be designed and manufactured and operating conditions should be optimized to promote tissue fusion and maturation of printed constructs. AM techniques are also valuable for fabrication of bioreactor components for both prototyping and production.
- Modeling of cell behavior and tissue fusion/maturation: In addition to experiments, theoretical approaches, either analytical or computational, should be explored to describe the cell-driven morphogenesis which dictates tissue morphology based on selected cells and incubation conditions as well as quantify the processes involved in tissue integration in vivo including anastomosis and innervation.
- Monitoring of printed constructs: Metabolic and functional properties of engineered tissues and organ structures should be monitored in situ by developing applicable sensing and signal acquisition approaches.

5 Biomedical Manufacturing Landscape and Grand Challenges of Functional Tissue Bioprinting

5.1 Biomedical Manufacturing Landscape. While AM for health has been at the frontier of the manufacturing research community, part of advanced manufacturing research has also been directed toward the grand landscape of biomedical manufacturing by seamlessly blending biomedical and manufacturing engineering for the evolving discipline of biomedical manufacturing, or biomanufacturing. For the bioprinting of human tissues, the biological research needs include the study of bioinks for bioprinting applications, strategies for vascularization and innervation, mass production of cells from stem cells, and in situ cell deposition technologies while the manufacturing research needs encompass proof-of-concept production and its scale-up, advanced bioprocess models and controls for large-scale bioreactors, biological metrology, and virtual validation. For advanced tissue fabrication, the biological research needs include instrumentation and improved

bioreactors while the manufacturing research needs encompass the development of regulatory pathways, improved and standardized raw materials, and quality control and assurance approaches. For cell/gene therapies, the biological research needs may vary, but in general, require derisking of laboratory scale research while the manufacturing research needs call for scale up and out production, lowered regulatory hurdles, and real-time release/testing. For energy efficiency, in particular for the pharmaceutical industry, reducing energy demand during biomedical manufacturing and conversion to continuous processing for process efficiency are important. From the industrial perspective (materials, protein therapies and vaccines), the biological research needs vary by application and can be related to antibiotic materials, treatments for illness, and resorbable materials while the manufacturing research needs also vary by application and may cover the scaling of efforts (up and out), greener and less energy-intensive production, quality control and metrology, and reduced cost.

5.2 Bioprinting as Transformative Research. In summary, there are many clear indications for bioprinting to be a transformative research area, perhaps the most transformative of the upcoming century, including:

- The ability to produce functional organs could potentially eliminate the organ waiting list, thereby preventing unnecessary deaths and emotional trauma while improving quality of life.
- The ability to produce nonfunctional organs could allow lesion studies in realistic substitutes.
- Lab-on-a-chip technology could be greatly advanced, leading to effective detection of biological weapons, new viruses, poisons, etc.
- Drug development could be made faster and more reliable, and animal models may be replaced by functional human tissue constructs.
- The ability to print food (meat), combined with increased clean/renewable energy sources, could decouple food production from carbon emissions. This should not be neglected—around one-third of carbon emissions are related to food production. Some cannot be avoided—fertilizer, tractor fuel, etc. This could reduce carbon emissions even if the world population increases.

5.3 Grand Challenges of Functional Tissue Bioprinting. While many knowledge gaps in bioprinting are biology-, chemistry-, and materials-related, some notable manufacturing-related grand scientific challenges articulated at the workshop and beyond are specifically summarized; most parallel well with the bioprinting-related recommendations.

- Examination of potential applications: In addition to organ transplantation/implantation and pharmaceutical needs, printed cellular constructs should be examined for applications for food production to decouple food from carbon emission as well as applications for laboratory-grown animal products such as leather, to name a few.
- Bioprinting philosophy: Since living cells including stem cells may differentiate and proliferate after printing, future implementations of bioprinting should integrate developmental biology principles. A printed tissue construct may be the meta-phase of a final construct, which will undergo morphogenesis and eventually grow into a functional tissue during incubation. This development and maturation process may introduce some unprintable features, such as capillaries formed around a printed vascular tree, into tissue constructs. For example, this may be achieved by printing adipose stromal vascular fraction (SVF) cells to promote angiogenesis since SVF cells are able to form a functional microcirculation via vascular assembly and inosculation with the host vasculature [62].
- Bioink dispensing: The understanding of printability of bioinks, which are cell-laden viscoelastic complex fluids, in the context of different AM techniques is still lacking.
- Printing of vascularized constructs. Since the angiogenesis process itself needs time (typically, 1 mm/day), effective vascularization of thick tissues has been a great challenge. While thick tissues with vascular networks can be tissue engineered by seeding cells in scaffolds with preformed channels, printing process innovations are needed to enable direct bioprinting of vascularized thick tissues with full control of cellular heterogeneity which effectively supply oxygen and nutrients to the entire construct volume while downregulating the metabolic activity of thick tissues.
- Innervation of printed tissues: The distribution or supply of nerves to a printed thick tissue cannot be ignored. Processes to promote innervation during and after bioprinting must be studied for organ printing to be a reality.
- Use of tissue precursors and/or reduction of noncellular intermediates: Most of current bioprinting research has utilized bioinks prepared from living cells, cell medium, and provisional hydrogels. It is of concern that noncellular intermediates, used to suspend cells, may impose challenges in forming the heterogeneous and anisotropic cellular organization with specific cell–cell interactions since most space of deposited constructs is initially occupied by noncellular intermediates after printing. To address this issue, tissue precursors or cell dense bioinks such as cellular spheroids/rods should be investigated for 3D bioprinting.
- Process-induced cell injury: It is of great importance to understand cell injury and death under bioprinting conditions using a cellular and molecular signaling pathway approach.
- Scale-up cell manufacturing and bioprinting: Quantitative metrics of process and product uncertainties are to be developed; although each living cell is unique, populations with reproducible and predictable characteristics are achievable and essential for clinical relevance.
- Real-time process analytics and control: It is imperative to develop sensor selection and placement and real-time data analytics-related strategies for effective bioprinting process monitoring and quality control.
- Environmental implications of bioprinting: As AM brings its unique opportunities and challenges to sustainable manufacturing [63], environmental implications of bioprinting should also be examined and carefully regulated.

Acknowledgment

The paper is mainly based on the final report of the 2016 NSF Workshop on Additive Manufacturing for Health and some follow-up discussions in the biomedical manufacturing community. The authors would like to thank all the workshop attendees for their participation and inspiring discussion. In particular, we would like to thank the invited workshop speakers (Hod Lipson, Jennifer Lewis, Anthony Atala, Scott Hollister, Wei Sun, Amit Bandyopadhyay, Shaochen Chen, John P. Fisher, Ola Harrysson, Bradley R. Ringeisen, Albert Shih, David Wallace, Michael J. Yaszemski, Kaiming Ye, Lijie Grace Zhang, Deborah J. Goodings. Rosemarie Hunziker, Carrie Laurencot, James Coburn, and Anne Plant) and invited participants. The editorial assistance from Ashley Compaan is also highly appreciated.

Funding Data

- Directorate for Engineering National Science Foundation (NSF CMMI-1555271).

References

[1] Kruth, J. P., Leu, M. C., and Nakagawa, T., 1998, "Progress in Additive Manufacturing and Rapid Prototyping," CIRP Ann.- Manuf. Technol., **47**(2), pp. 525–540.

[2] Guo, N., and Leu, M. C., 2013, "Additive Manufacturing: Technology, Applications and Research Needs," Front. Mech. Eng., **8**(3), pp. 215–243.

[3] Lipson, H., and Kurman, M., 2013, *Fabricated: The New World of 3D Printing*, Wiley, Indianapolis, IN.

[4] Huang, Y., Leu, M. C., Mazumder, J., and Donmez, A., 2015, "Additive Manufacturing: Current State, Future Potential, Gaps and Needs, and Recommendations," ASME J. Manuf. Sci. Eng., **137**(1), p. 014001.

[5] Kalpakjian, S., and Schmid, S. R., 2017, *Manufacturing Processes for Engineering Materials*, 6th ed., Pearson, Hoboken, NJ.

[6] ISO and ASTM International, 2018, "Additive Manufacturing—General Principles—Terminology," ISO and ASTM International, Geneva, Switzerland, Standard No. ISO/ASTM 52900:2015 (ASTM F2792).

[7] Schiele, N. R., Corr, D. T., Huang, Y., Raof, N. A., Xie, Y., and Chrisey, D. B., 2010, "Laser-Based Direct-Write Techniques for Cell Printing," Biofabrication, **2**(3), p. 032001.

[8] Ringeisen, B. R., Pirlo, R. K., Wu, P. K., Boland, T., Huang, Y., Sun, W., Hamid, Q., and Chrisey, D. B., 2013, "Cell and Organ Printing Turns 15: Diverse Research to Commercial Transitions," MRS Bull., **38**(10), pp. 834–843.

[9] Dababneh, A. B., and Ozbolat, I. T., 2014, "Bioprinting Technology: A Current State-of-the-Art Review," ASME J. Manuf. Sci. Eng., **136**(6), p. 061016.

[10] Murphy, S. V., and Atala, A., 2014, "3D Bioprinting of Tissues and Organs," Nat. Biotechnol., **32**(8), pp. 773–785.

[11] Bandyopadhyay, A., Bose, S., and Das, S., 2015, "3D Printing of Biomaterials," MRS Bull., **40**(2), pp. 108–115.

[12] Hunsberger, J., Harrysson, O., Shirwaiker, R., Starly, B., Wysk, R., Cohen, P., Allickson, J., Yoo, J., and Atala, A., 2015, "Manufacturing Road Map for Tissue Engineering and Regenerative Medicine Technologies," Stem Cells Transl. Med., **4**(2), pp. 130–135.

[13] Lee, J. M., and Yeong, W. Y., 2016, "Design and Printing Strategies in 3D Bioprinting of Cell Hydrogels: A Review," Adv. Healthcare Mater., **5**(22), pp. 2856–2865.

[14] Ozbolat, I. T., and Hospodiuk, M., 2016, "Current Advances and Future Perspectives in Extrusion-Based Bioprinting," Biomaterials, **76**, pp. 321–343.

[15] Cui, H., Nowicki, M., Fisher, J. P., and Zhang, L. G., 2017, "3D Bioprinting for Organ Regeneration," Adv. Healthcare Mater., **6**(1), p. 1601118.

[16] Lee, V. K., and Dai, G., 2017, "Printing of Three-Dimensional Tissue Analogs for Regenerative Medicine," Ann. Biomed. Eng., **45**(1), pp. 115–131.

[17] Forgacs, G., and Sun, W. eds., 2013, *Biofabrication: Micro-and Nano-Fabrication, Printing, Patterning and Assemblies*, 1st ed., William Andrew, Oxford, UK.

[18] Chua, C. K., and Yeong, W. Y., 2015, *Bioprinting: Principles and Applications*, 1st ed., World Scientific Publishing, Singapore.

[19] Zhang, L. G., Fisher, J. P., and Leong, K. W., 2015, *3D Bioprinting and Nanotechnology in Tissue Engineering*, 1st ed., Academic Press, London.

[20] Bourell, D. L., Leu, M., and Rosen, D., 2009, *Roadmap for Additive Manufacturing - Identifying the Future of Freeform Processing*, University of Texas, Austin, TX.

[21] Huang, Y., and Leu, M., 2014, *Frontiers of Additive Manufacturing Research and Education*, University of Florida, Gainesville, FL.

[22] BioFabUSA, 2018, "Manufacturing the Future of Biofabrication," Advanced Regenerative Manufacturing Institute, Manchester, NH, accessed Feb. 28, 2018, https://www.armiusa.org/

[23] Mironov, V., Viconti, R. P., Kasyanov, V., Forgacs, G., Drake, C. J., and Markwald, R. R., 2009, "Organ Printing: Tissue Spheroids as Building Blocks," Biomaterials, **30**(12), pp. 2164–2174.

[24] Harbaugh, J., 2017, "NASA's Centennial Challenges: Vascular Tissue Challenge," National Aeronautics and Space Administration, Huntsville, AL, accessed Feb. 28, 2018, https://www.nasa.gov/directorates/spacetech/centennial_challenges/vascular_tissue.html

[25] Boling, R., 2016, "First Heart Structure 3D Printed in Zero Gravity with Adult Human Stem Cells," Techshot Inc., Greenville, IN, accessed Feb. 28, 2018, http://www.techshot.com/news.php#Media

[26] Christensen, K., Xu, C., Chai, W., Zhang, Z., Fu, J., and Huang, Y., 2015, "Freeform Inkjet Printing of Cellular Structures With Bifurcations," Biotechnol. Bioeng., **112**(5), pp. 1047–1055.

[27] Kang, H. W., Lee, S. J., Ko, I. K., Kengla, C., Yoo, J. J., and Atala, A., 2016, "A 3D Bioprinting System to Produce Human-Scale Tissue Constructs With Structural Integrity," Nat. Biotechnol., **34**(3), pp. 312–319.

[28] Miller, J. S., Stevens, K. R., Yang, M. T., Baker, B. M., Nguyen, D. T., Cohen, D. M., Toro, E., Chen, A. A., Galie, P. A., Yu, X., Chaturvedi, R., Bhatia, S. N., and Chen, C. S., 2012, "Rapid Casting of Patterned Vascular Networks for Perfusable Engineered Three-Dimensional Tissues," Nat. Mater., **11**(9), pp. 768–774.

[29] Kolesky, D. B., Homan, K. A., Skylar-Scott, M. A., and Lewis, J. A., 2016, "Three-Dimensional Bioprinting of Thick Vascularized Tissues," Proc. Natl. Acad. Sci. U.S.A., **113**(12), pp. 3179–3184.

[30] Williams, J. M., Adewunmi, A., Schek, R. M., Flanagan, C. L., Krebsbach, P. H., Feinberg, S. E., Hollister, S. J., and Das, S., 2005, "Bone Tissue Engineering Using Polycaprolactone Scaffolds Fabricated Via Selective Laser Sintering," Biomaterials, **26**(23), pp. 4817–4827.

[31] Doraiswamy, A., Jin, C., Narayan, R. J., Mageswaran, P., Mente, P., Modi, R., Auyeung, R., Chrisey, D. B., Ovsianikov, A., and Chichkov, B., 2006, "Two Photon Induced Polymerization of Organic-Inorganic Hybrid Biomaterials for Microstructured Medical Devices," Acta Biomater., **2**(3), pp. 267–275.

[32] Shor, L., Güçeri, S., Chang, R., Gordon, J., Kang, Q., Hartsock, L., An, Y., and Sun, W., 2009, "Precision Extruding Deposition (PED) Fabrication of Polycaprolactone (PCL) Scaffolds for Bone Tissue Engineering," Biofabrication, **1**(1), p. 015003.

[33] Doiphode, N. D., Huang, T., Leu, M. C., Rahaman, M. N., and Day, D. E., 2011, "Freeze-Form Extrusion Fabrication of 13-93 Bioactive Glass Scaffolds for Bone Repair," J. Mater. Sci.: Mater. Med., **22**(3), pp. 932–950.

[34] Dean, D., Wallace, J., Siblani, A., Wang, M. O., Kim, K., Mikos, A. G., and Fisher, J. P., 2012, "Continuous Digital Light Processing (cDLP): Highly Accurate Additive Manufacturing of Tissue Engineered Bone Scaffolds," Virtual Phys. Prototyping, **7**(1), pp. 13–24.

[35] Bose, S., Vahabzadeh, S., and Bandyopadhyay, A., 2013, "Bone Tissue Engineering Using 3D Printing," Mater. Today, **16**(12), pp. 496–504.

[36] Gaharwar, A. K., Mukundan, S., Karaca, E., Dolatshahi-Pirouz, A., Patel, A., Rangarajan, K., and Khademhosseini, A., 2014, "Nanoclay-Enriched Poly(ε-Caprolactone) Electrospun Scaffolds for Osteogenic Differentiation of Human Mesenchymal Stem Cells," Tissue Eng. Part A, **20**(15–16), pp. 2088–2101.

[37] Zimmer Biomet Medical Affairs, 2018, "Trabecular Metal™ Technology," Zimmer Biomet, Warsaw, IN, accessed Feb. 28, 2018, http://www.zimmerbiomet.com/medical-professionals/common/our-science/trabecular-metal-technology.html

[38] Schmid, S. R., Hamrock, B. J., and Jacobson, B. O., 2014, *Fundamentals of Machine Elements*, 3rd ed., CRC Press, Boca Raton, FL.

[39] O'brien, F. J., 2011, "Biomaterials & Scaffolds for Tissue Engineering," Mater. Today, **14**(3), pp. 88–95.

[40] Shrivats, A. R., McDermott, M. C., and Hollinger, J. O., 2014, "Bone Tissue Engineering: State of the Union," Drug Discovery Today, **19**(6), pp. 781–786.

[41] Norotte, C., Marga, F. S., Niklason, L. E., and Forgacs, G., 2009, "Scaffold-Free Vascular Tissue Engineering Using Bioprinting," Biomaterials, **30**(30), pp. 5910–5917.

[42] Duan, B., Hockaday, L., Kang, K., and Butcher, J., 2012, "3D Bioprinting Heterogeneous Aortic Valve Conduits With Alginate/Gelatin Hydrogels," J. Biomed. Mater. Res. Part A, **101**(5), pp. 1255–1264.

[43] Highley, C. B., Rodell, C. B., and Burdick, J. A., 2015, "Direct 3D Printing of Shear-Thinning Hydrogels Into Self-Healing Hydrogels," Adv. Mater., **27**(34), pp. 5075–5079.

[44] Jose, R. R., Brown, J. E., Polido, K. E., Omenetto, F. G., and Kaplan, D. L., 2015, "Polyol-Silk Bioink Formulations as Two-Part Room-Temperature Curable Materials for 3D Printing," ACS Biomater. Sci. Eng., **1**(9), pp. 780–788.

[45] Jin, Y., Compaan, A., Chai, W., and Huang, Y., 2017, "Functional Nanoclay Suspension for Printing-Then-Solidification of Liquid Materials," ACS Appl. Mater. Interfaces, **9**(23), pp. 20057–20066.

[46] Jin, Y., Liu, C., Chai, W., Compaan, A., and Huang, Y., 2017, "Self-Supporting Nanoclay as Internal Scaffold Material for Direct Printing of Soft Hydrogel Composite Structures in Air," ACS Appl. Mater. Interfaces, **9**(20), pp. 17456–17465.

[47] Boland, T., Tao, X., Damon, B. J., Manley, B., Kesari, P., Jalota, S., and Bhaduri, S., 2007, "Drop-on-Demand Printing of Cells and Materials for Designer Tissue Constructs," Mat. Sci. Eng. C, **27**(3), pp. 372–376.

[48] Nishiyama, Y., Nakamura, M., Henmi, C., Yamaguchi, K., Mochizuki, S., Nakagawa, H., and Takiura, K., 2009, "Development of a Three-Dimensional Bioprinter: Construction of Cell Supporting Structures Using Hydrogel and State-of-the-Art Inkjet Technology," ASME J. Biomech. Eng., **131**(3), p. 035001.

[49] Xu, C., Chai, W., Huang, Y., and Markwald, R. R., 2012, "Scaffold-Free Inkjet Printing of Three-Dimensional Zigzag Cellular Tubes," Biotechnol. Bioeng., **109**(12), pp. 3152–3160.

[50] Christensen, K., Compaan, A., Chai, W., Xia, G., and Huang, Y., 2017, "In Situ Printing-Then-Mixing for Biological Structure Fabrication Using Intersecting Jets," ACS Biomater. Sci. Eng., **3**(12), pp. 3687–3694.

[51] Guillemot, F., Souquet, A., Catros, S., Guillotin, B., Lopez, J., Faucon, M., Pippenger, B., Bareille, R., Rémy, M., Bellance, S., Chabassier, P., Fricain, J. C., and Amédée, J., 2010, "High-Throughput Laser Printing of Cells and Biomaterials for Tissue Engineering," Acta Biomater., **6**(7), pp. 2494–2500.

[52] Riggs, B. C., Dias, A. D., Schiele, N. R., Cristescu, R., Huang, Y., Corr, D. T., and Chrisey, D. B., 2011, "Matrix-Assisted Pulsed Laser Methods for Biofabrication," MRS Bull., **36**(12), pp. 1043–1050.

[53] Koch, L., Deiwick, A., Schlie, S., Michael, S., Gruene, M., Coger, V., and Chichkov, B., 2012, "Skin Tissue Generation by Laser Cell Printing," Biotechnol. Bioeng., **109**(7), pp. 1855–1863.

[54] Xiong, R., Zhang, Z., Chai, W., Huang, Y., and Chrisey, D. B., 2015, "Freeform Drop-on-Demand Laser Printing of 3D Alginate and Cellular Constructs," Biofabrication, **7**(4), p. 045011.

[55] Ma, X., Qu, X., Zhu, W., Li, Y.-S., Yuan, S., Zhang, H., Liu, J., Wang, P., Lai, C. S., Zanella, F., Feng, G.-S., Sheikh, F., Chien, S., and Chen, S. C., 2016, "A Deterministically Patterned Biomimetic Human iPSC-Derived Hepatic Model Via Rapid 3D Bioprinting," Proc. Natl. Acad. Sci. U.S.A., **113**(8), pp. 2206–2213.

[56] Xu, C., Zhang, M., Huang, Y., Ogale, A., Fu, J., and Markwald, R., 2014, "Study of Droplet Formation Process During Drop-on-Demand Inkjetting of Living Cell-Laden Bioink," Langmuir, **30**(30), pp. 9130–9138.

[57] Xu, C., Zhang, Z., Fu, J., and Huang, Y., 2017, "Study of Pinch-Off Locations During Drop-on-Demand Inkjet Printing of Viscoelastic Alginate Solutions," Langmuir, **33**(20), pp. 5037–5045.

[58] Zhang, Z., Xu, C., Xiong, R., Chrisey, D. B., and Huang, Y., 2017, "Effects of Living Cells on the Bioink Printability During Laser Printing," Biomicrofluidics, **11**(3), p. 034120.
[59] Ringeisen, B. R., Kim, H., Barron, J. A., Krizman, D. B., Chrisey, D. B., Jackman, S., Auyeung, R. Y. C., and Spargo, B. J., 2004, "Laser Printing of Pluripotent Embryonal Carcinoma Cells," Tissue Eng., **10**(3–4), pp. 483–491.
[60] Gudapati, H., Yan, J., Huang, Y., and Chrisey, D. B., 2014, "Alginate Gelation-Induced Cell Death During Laser-Assisted Cell Printing," Biofabrication, **6**(3), p. 035022.
[61] Zhang, Z., Chai, W., Xiong, R., Zhou, L., and Huang, Y., 2017, "Printing-Induced Cell Injury Evaluation During Laser Printing of 3T3 Mouse Fibroblasts," Biofabrication, **9**(2), p. 025038.
[62] Nunes, S. S., Maijub, J. G., Krishnan, L., Ramakrishnan, V. M., Clayton, L. R., Williams, S. K., Hoying, J. B., and Boyd, N. L., 2013, "Generation of a Functional Liver Tissue Mimic Using Adipose Stromal Vascular Fraction Cell-Derived Vasculatures," Sci. Rep., **3**(1), p. 002141.
[63] Rejeski, D., Zhao, F., and Huang, Y., 2018, "Research Needs and Recommendations on Environmental Implications of Additive Manufacturing," Addit. Manuf., **19**, pp. 21–28.