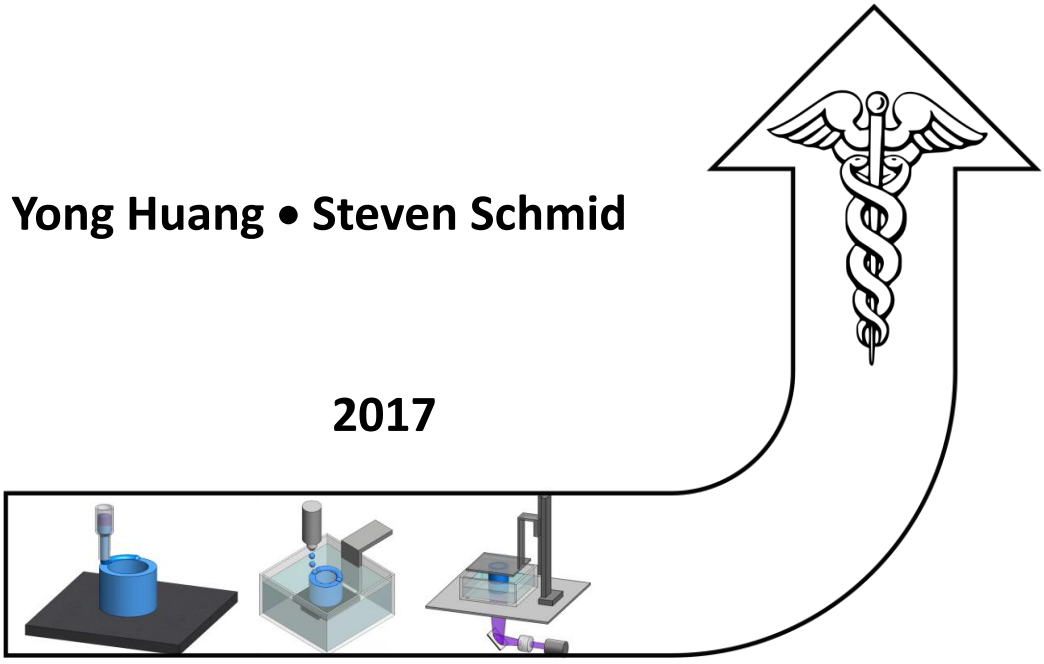
Report of NSF Workshop on Additive Manufacturing for Health

Yong Huang • Steven Schmid

2017



Report of NSF Workshop on Additive Manufacturing for Health

NSF Additive Manufacturing for Health Workshop
March 17 and 18, 2016

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Published by

University of Florida
Center for Manufacturing Innovation
PO Box 116250
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United States of America

2017

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EXECUTIVE SUMMARY

The additive manufacturing (AM) process is distinctly different from traditional manufacturing techniques (such as cutting or milling) that involve removal of material. AM is a suite of computer-automated technologies to fabricate 3D structural and functional parts. To date, AM technology has been used in a number of diverse industries including automotive, aerospace, biomedical, energy, consumer goods and many others. In particular, AM for health has received significant attention, and ample AM application opportunities exist in the health field, including the fabrication of custom shaped orthopedic prostheses and implants, medical devices, biological chips, tissue scaffolds, living constructs, drug-screening models, precision medicine, and surgical planning and training apparatuses, to name a few. However, basic research directions and emerging scientific topics, which are needed to enable the full-scale adoption of AM for health, have yet to be formulated and identified by AM stakeholders, and there is no clear vision for future research directions for AM in health.

At the 2016 National Science Foundation (NSF) AM for Health workshop held in Arlington, Virginia on March 17 and 18, 2016, stakeholders from industry, academia, and federal agencies reviewed the status and current applications of AM for health, identified gaps and needs facing AM for health for both existing products and new AM-enabled technologies, and formulated recommendations for basic research initiatives. This report summarizes the current state, gaps and needs, and recommendations related to AM for health based on the workshop discussions. In this workshop report, AM for health applications have been classified based on additively manufactured parts: soft constructs/structures and hard structures. While soft structures, usually deformable, mainly provide biological and chemical functions ranging from muscular contraction to metabolism to neural processing, hard constructs generally provide mechanical stability as load-bearing components. Soft structure fabrication is further divided into direct and indirect bioprinting (Figure 1); direct bioprinting utilizes build materials containing living cells, while indirect bioprinting build materials are acellular. Once fabricated, both soft and hard structures can be seeded with living cells as needed, although it is difficult to control the spatial distribution or heterogeneity of such seeded cells. It is noted that some structures providing mechanical as well as biological/chemical functions are classified as hard structures in this report for convenience.

Overarching manufacturing-related knowledge gaps mainly fall into the materials, design, process innovation, part characterization, and policy and education categories. 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 standardized bioink formulations or post-fabrication procedures. 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 multi-material designs, and criteria for choosing printing over other non-printing fabrication techniques. In terms of process innovation, gaps and needs are related to scale-up printing, multi-material multi-functional products, customization, generation of multiscale feature sizes ranging from micron or sub-micron to centimeters, optimal planning to balance speed and resolution, real-time monitoring of fabrication processes and feedback for online correction of defects, and control of part quality and process reproducibility. 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 along with patient health. In addition, questions remain regarding processing-property relationships as well as how the resulting properties affect biological responses. The relationship between properties (surface finish, mechanical properties, porosity, pore size, etc.) and biological responses (such as stem cell differentiation, tissue integration, and vascular anastomosis) is a major gap in the current understanding of how AM constructs affect and respond to biological systems. 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.

For improved implementation and sustainable application of AM for health, general recommendations are summarized below for materials, design, process innovation, modeling, characterization, and policy and education:

- Materials: Development and standardization of a broad range of economic, 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 (CAD) tools to design and printers to implement multimaterial constructs; and design of soft-hard tissue interfaces for heterogeneous constructs.
- Process innovation: Development of versatile 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 post-printing 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 products as implanted and degradation of products over time to improve tissue integration and minimize the risk of 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 non-expert 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.

1. INTRODUCTION

1.1. Background

The ASTM International Committee F42 on Additive Manufacturing (AM) Technologies defines AM as the "process of joining materials to make objects from three-dimensional (3D) model data, usually layer by layer, as opposed to subtractive manufacturing methodologies" [ASTM2009]. Thus the AM process is distinctly different from traditional manufacturing techniques (such as cutting or milling) that involve removal of material. It is a suite of computer-automated technologies to fabricate 3D structural and functional parts from metallic, plastic, ceramic, electronic, biological, and composite materials [Huang2015]. According to the ASTM F42 committee, AM processes are classified into seven categories [ASTM2009]: vat photopolymerization, material jetting, binder jetting, material extrusion, powder bed fusion, sheet lamination, and directed energy deposition; within each category, there are multiple specific implementations.

As AM transforms industry, its impact continues to grow, which can be quantified by the total number of printed products, the number of new start-up companies, the range and number of funding opportunities, and the amount of scholarly activity in terms of publications and patents. Throughout the development of AM, a series of events and reports have been produced, providing a context and historical trajectory of progress in AM. Some notable events and workshops are listed as follows, which were organized to examine AM technology, explore its potential, identify its gaps and needs, and/or formulate research, development, and educational recommendations from different perspectives. A World Technology Evaluation Center (WTEC) study on rapid prototyping in Europe and Japan was performed in 1996. Two years later, a roadmap study regarding rapid prototyping, sponsored by the National Center for Manufacturing Sciences in the United States, was completed. A second WTEC study was performed to assess the level of activity in Europe in additive/subtractive technologies in 2003. In 2009, a Roadmap for Additive Manufacturing (RAM) Workshop sponsored by the United States National Science Foundation (NSF) and Office of Naval Research (ONR) was conducted with a primary objective to define and focus research activities in the area of AM with the intent to accelerate the technology's commercial acceptance, to increase the impact and significance of AM research, and to articulate a roadmap for research in this field for the next 10-12 years. The 2013 NSF Workshop on Frontiers of Additive Manufacturing Research and Education, largely educational in nature, provided a forum for disseminating information and sharing ideas about the frontiers of AM research, education, and technology transfer. The follow-up 2014 NSF Workshop on Environmental Implications of Additive Manufacturing explored five areas related to environmental and health impacts of AM: lifecycle impacts, occupational health, energy use, waste, and cross-cutting/policy issues to identify knowledge gaps and uncertainties that can inform an agenda for future research efforts. To accelerate AM-related technology transfer, the 2015 NSF Workshop on Finding Pathways from NSF-Funded Basic Research to Department of Energy (DOE)-Funded Applied Research on Additive Manufacturing brought basic and applied researchers together to promote collaborations and develop a pipeline for AM technologies to progress through to market impact. As the AM impact in health was increasingly recognized, the U.S. Food and Drug Administration (FDA) organized a 2014 workshop, entitled "Additive Manufacturing of Medical Devices: An Interactive Discussion on the Technical Considerations of 3D Printing," to provide a forum for FDA personnel, medical device manufacturers, additive manufacturing companies, and academia to discuss technical challenges and solutions for

medical device AM. The reports and roadmaps from these events/workshops provide seminal information for future AM-related events and have helped to focus research on important topics in the field.

Currently, AM technology is used in a number of diverse industries including automotive, aerospace, biomedical, energy, consumer goods, and many others. AM has seen significant expansion of capabilities since its first demonstration in the 1980s. At first limited to stereolithography, fused deposition modeling, laminated object manufacturing, and subsequently selective laser sintering, a number of novel processes have been developed for various applications using different build materials, and AM is now being used for production, not just prototyping. Significant improvements in AM processes, hardware, process control software, and computer-aided design (CAD) 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 biology and microtechnology, where AM fabrication techniques have enabled new areas of research. In particular, AM for biomedical applications [Huang2015] 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 by AM stakeholders. Unfortunately, there was no consensus on research needs or clear vision for future research directions for AM in health, which eventually led to the organizing of the NSF AM for Health Workshop in 2016.

The 2016 NSF AM for Health workshop was uniquely distinct from all the previous AM related workshops in the objectives of the workshop – to explore AM potential in health and identify basic research to close any identified gaps to achieve full potential. Recently, AM for health had received a great deal of attention, including the aforementioned 2014 FDA workshop, however, its basic research and emerging scientific topics, which would accelerate the full-scale adoption of AM for health, were yet to be identified and formulated by AM stakeholders. 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 close gaps for wider applications of AM in health for years to come.

This workshop report introduces the workshop objectives and its overview, the AM for health current state-of-the-art, gaps, research needs, and bioprinting-related recommendations. The report does not intend to be another review article on AM for health; instead, it aims to identify manufacturing process and equipment-related gaps, research needs, and recommendations, promoting the vigorous advance of AM for health research, applications, and commercialization.

1.2. Objectives

In order to outline AM potential in health and identify basic research to close identified gaps to achieve full potential as shown in Figure 1, the 2016 NSF AM for Health Workshop had the following four specific objectives:

- Review of the state-of-the-art in basic research on AM/3D printing for health;
- Examination of future prospects of AM for health and sharing of perspectives on AM for health from funding agencies;

- Identification of gaps, needs, and challenges facing AM for health for both existing products and new enabled technologies; and
- Formulation of recommendations for basic research initiatives.

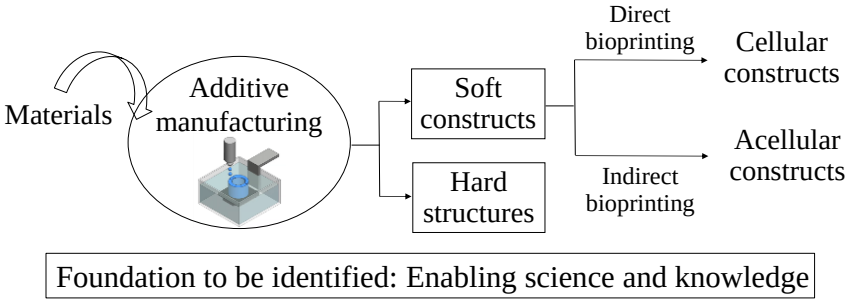


Figure 1. Illustration of the workshop scope.

1.3. Workshop overview

The one-and-half day NSF AM for Health Workshop was held in Arlington, VA on March 17 and 18, 2016. The workshop covered two main themes: status, applications, vision, gaps, and research needs (Theme 1) and federal agency perspectives (Theme 2). In particular, the first theme had sixteen presentations from scholars in industry, academia, and research organizations, and the second theme included five presentations from representatives of federal agencies. These presentations, as detailed in Figure 2, reviewed the state-of-the-art in AM for health, examined future prospects of AM for health, shared perspectives on AM for health from various funding agencies, and/or identified related needs, gaps and challenges facing AM for health, forming a solid background for future basic research initiatives.

The intellectual merit of this workshop includes identification of research efforts and funding perspectives that will be needed to close the gaps between the future potential and the current state-of-the-art in AM technology for health. In particular, the identified challenges and needs are summarized in the following sections. The AM for health research challenges and research needs identified in this workshop fall into three general categories: those applicable to the field as a whole, those specific to soft tissues, and those specific to hard structures. The field as a whole requires more materials, better design tools, improved regulatory pathways, and a better understanding of how design affects outcomes. For soft tissues, the identified challenges and research needs relate to the inclusion of living cells: maintaining viability, spatially resolved characterization, and integration with host tissue. Hard structures are generally acellular and often non-degradable, so challenges are related to design, functional integration, corrosion, and build material sources and recycling. In general, addressing these issues is necessary to enable clinical translation; they represent gaps in the understanding of how AM products actually function *in vitro* and *in vivo* and the resulting inability to adequately standardize and regulate their use *in vitro* and *in vivo*. It should be noted that many of the identified challenges and research needs also apply to non-AM products; these are due to gaps in current biomedical knowledge, not just in the AM field.

In terms of broader impacts, the workshop helps realize the full potential of AM technology for various health-related applications and products. Firstly, the research thrusts initiated based on

workshop recommendations and a better understanding of AM for health are expected to increase the practical adoption of AM in various medical research and development efforts. These are critical for competitive manufacturing in the field of healthcare, leading to the creation of many high-tech health-related jobs in the United States. Secondly, health advances enabled by AM positively impact society by offering novel treatment products and approaches and providing new medical research tools as well as regulatory guidelines. Finally, the ideas coming from the workshop participants are helpful to decision makers in setting coordinated priorities and strategies for AM research for health at government agencies, academic institutions, and industrial companies.

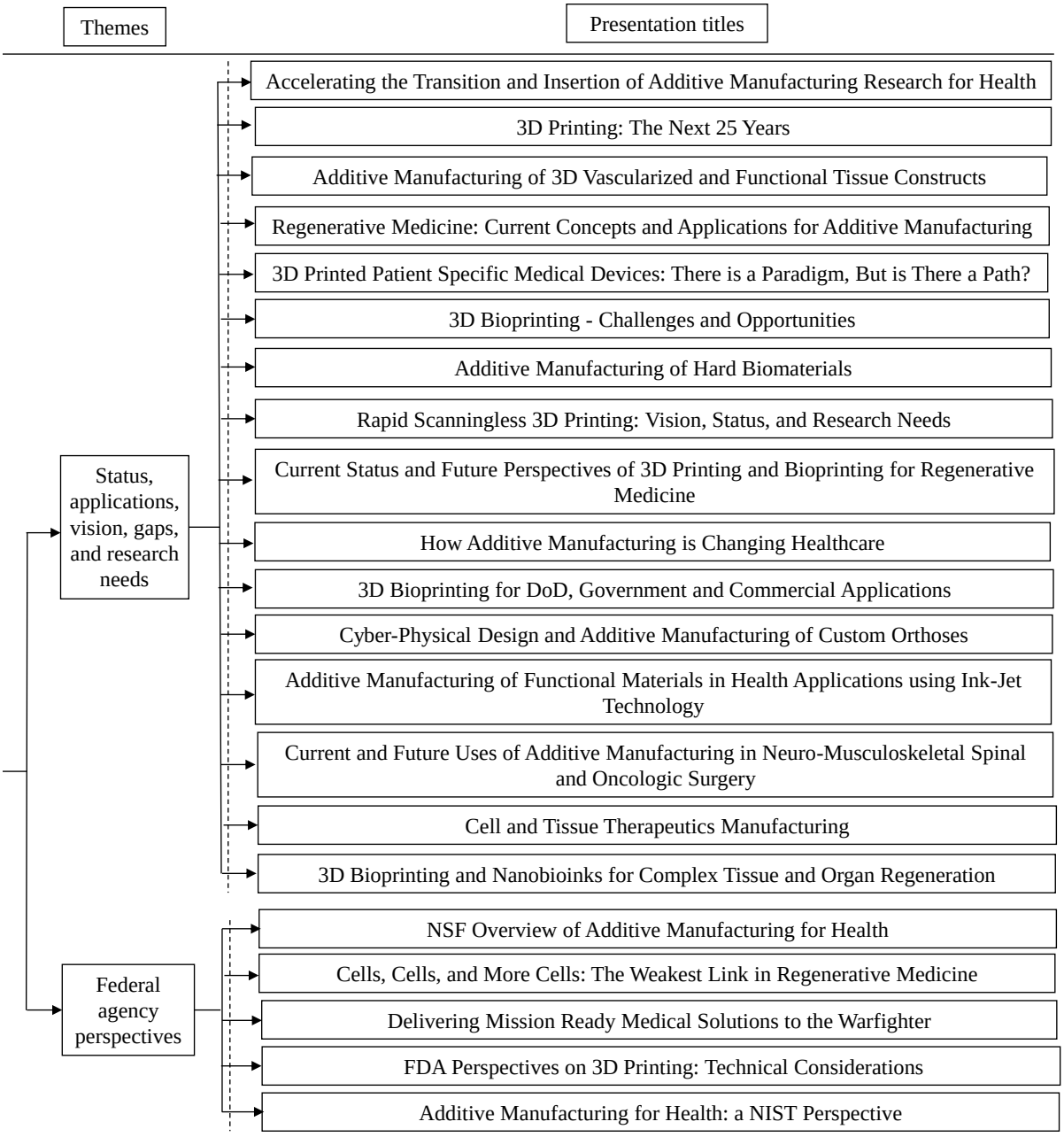


Figure 2. Workshop themes and presentation topics.

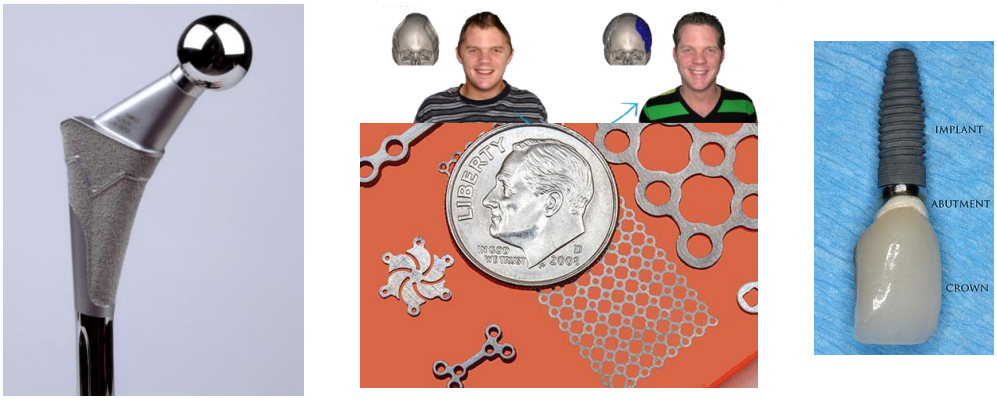
The workshop had 178 officially registered participants from industry, academia, and federal agencies. Appendix A lists the workshop organizers, moderators, and invited speakers and participants; Appendix B lists the sponsoring NSF program. This report serves to capture the main points presented by the speakers and the input provided by the participants during the discussion sessions.

2. CURRENT STATE

2.1. General description

The use of AM in health applications has attracted considerable interest over the past decade for its unique benefits in reducing healthcare costs and increasing 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 (RAM) Workshop sponsored by NSF and ONR and highlighted by the 2013 NSF Workshop on Frontiers of Additive Manufacturing Research and Education.

Ample AM application opportunities exist in the health field as illustrated in Figures 3 and 4, including the fabrication of custom shaped orthopedic prostheses and implants, medical devices, surgical planning and training apparatuses, precision medicine, tissue scaffolds, biological chips, and living constructs, to name a few. Living constructs can be used for implantation, drug-screening and pharmaceutical investigations, and developmental biology studies. Printed thick tissues with vascularized networks bring a promising solution to the current challenge of organ donor shortage.



(a) (b) (c)

Figure 3. Selected examples of AM in current medical devices and areas where AM is expected to have a major influence. (a) Orthopedic implants, where a bone ongrowth or ingrowth surface as well as design flexibility to avoid stress shielding can be inherently incorporated. Source: Courtesy Zimmer Biomet, Inc. (b) Cranial reconstruction implants using titanium, stainless steel or PEEK. Source: Courtesy Johnson & Johnson, Inc. (c) Dental implants, incorporating rough threads. Source: Courtesy Zimmer Biomet, Inc.

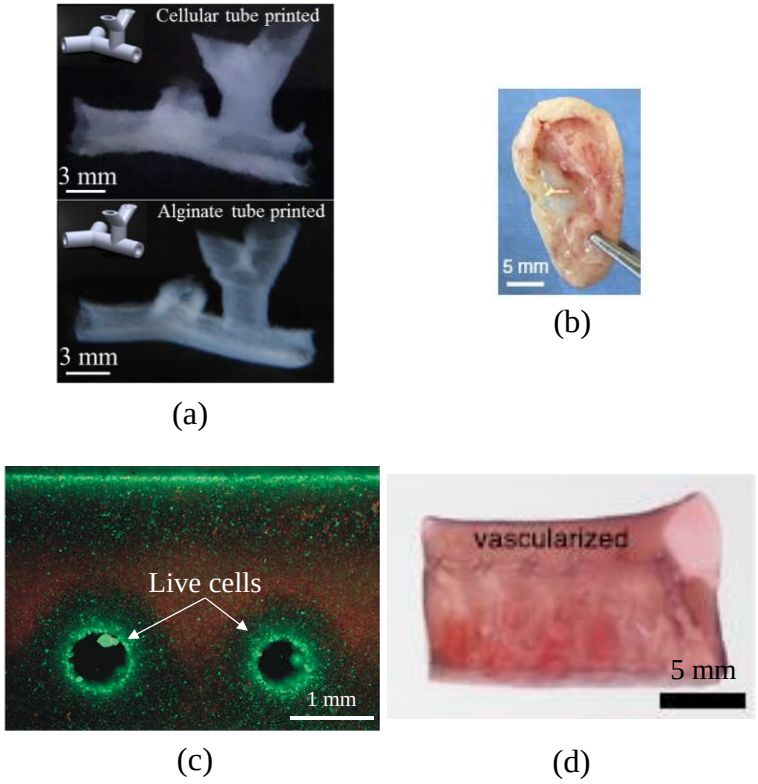


Figure 4. Bioprinting-related advances. (a) Multidirectional branching vascular-like structures printed using inkjetting materials [Christensen2015]. (b) Gross appearance of a printed human ear at 1 month after implantation [Kang2016]. Thick vascularized tissues fabricated using bioprinting and casting: (c) primary rat hepatocytes and stabilizing stromal fibroblasts in agarose gel after 8 days of culture [Miller2012] 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 [Kolesky2016].

Specifically, some notable examples are listed as follows. 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) (Figure 3(a)). Cranial reconstruction implants of titanium, stainless steel, or polyether ether ketone (PEEK) can be readily customized and fabricated on demand for individual patients (Figure 3(b)). Dental implants, seamlessly incorporating rough threads, are small and can be produced effectively in batches using AM (Figure 3(c)).

One notable AM process innovation is in the area of bioprinting, also known as cell or organ printing [Ringeisen2013] [Huang2015] as illustrated using a vascular tree construct fabrication process in Figure 5, which is a developmental biology-inspired scaffold-less biofabrication approach. Around 118,000 people are on the waiting list for different organ transplants in the US alone [UNOS2017], and some of them die every day due to organ donor shortage; the year 2013 marked the 15th year of bioprinting, an ambitious vision to create a developmental biology-enabled, scaffold-less technique to fabricate living tissues and organs by printing living cells, which will eventually help mitigate the challenge of organ donor shortage [Ringeisen2013]

[Mironov2014]. Thus far, various tissue constructs have been already successfully fabricated such as the fibroblast tubular construct printed using inkjetting (Figure 4(a)) [Christensen2015] and the human ear printed using extrusion (Figure 4(b)) [Kang2016], to name a few. In addition, bioprinting has been successfully integrated with casting to fabricate various thick vascularized tissues as shown in Figure 4(c) [Miller2012] and 4(d) [Kolesky2016].

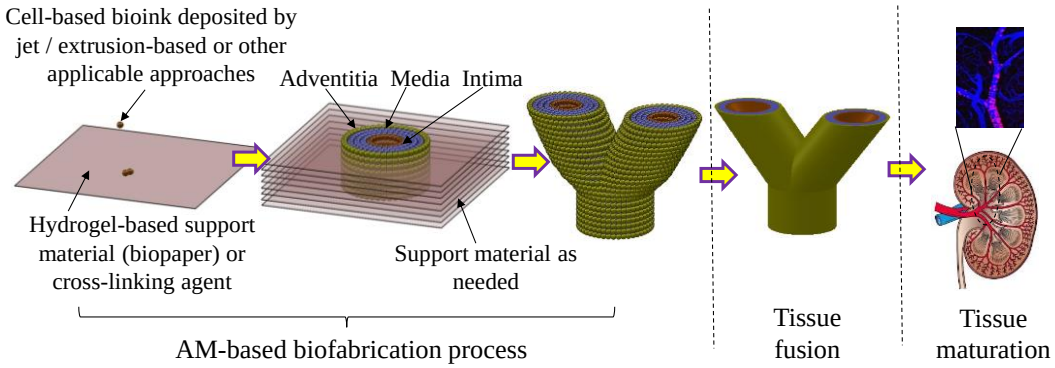


Figure 5. Schematic of a vascular tree bioprinting process.

In this workshop report, AM for health applications have been classified based on additively manufactured parts: soft constructs/structures and hard structures. While soft structures, usually soft and deformable, mainly provide biological and chemical functions ranging from muscular contraction to metabolism to neural processing, hard constructs generally provide mechanical stability as load-bearing components. Soft structure fabrication is further divided into direct and indirect bioprinting (Figure 1); direct bioprinting utilizes build materials containing living cells, while indirect bioprinting build materials are acellular. Once fabricated, both soft and hard structures can be seeded with living cells as needed, although it is difficult to control the spatial distribution or heterogeneity of such seeded cells. It is noted that there may be some structures providing mechanical as well as biological/chemical functions and they are classified as hard structures in this report for convenience.

2.2. Soft construct printing

Soft constructs, usually with embedded living cells via direct bioprinting, may have a wide range of mechanical and chemical properties, from nearly rigid cartilage to spongy brain tissue, and they perform a correspondingly wide range of functions, from drug evaluation *in vitro* to organ replacement *in vivo*. In general, soft constructs have very little mineral content and their functions rely on cellular activity rather than mechanical properties.

Typically, soft construct printing research focuses on better control of construct material properties and cell interactions to direct the behavior of the increasingly sophisticated cell populations embedded in each construct. Research spans the entire fabrication, maturation, implantation, and degradation process, ranging from material development to cell isolation and manipulation to process innovations to bioreactor design to 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 [Huang2015] [Kang2016] [Kolesky2016].

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 rates and mechanisms vary widely depending on applications [Forgacs2013]. 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 extracellular matrix (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 rapidly degrading solid scaffolds of poly(caprolactone) (PCL), poly(lactic-co-glycolic acid) (PLGA), 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) (PEG), provide a hydrated matrix analogous to native ECM [Forgacs2013]. 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 [Kang2016].

For soft tissue constructs, processing is limited by their weak 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 (Figure 6(a)) [Jin2016], a type of material extrusion process, and droplet-based techniques such as inkjet printing (Figure 6(b)) [Christensen2015] and laser-induced forward transfer (Figure 6(c)) [Schiele2010] [Xiong2015], types of material jetting processes. Layers built of these filaments (Figure 6(a)) or droplets (Figure 6(b) and (c)) form 3D constructs which can be designed to resemble native tissues including material and cell type heterogeneity. It is noted that vat polymerization of hydrogel precursors [Ma2016] (such as stereolithography, a type of vat photopolymerization process as shown in Figure 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 3D printing and the intrinsic higher stiffness of materials suitable for these processes.

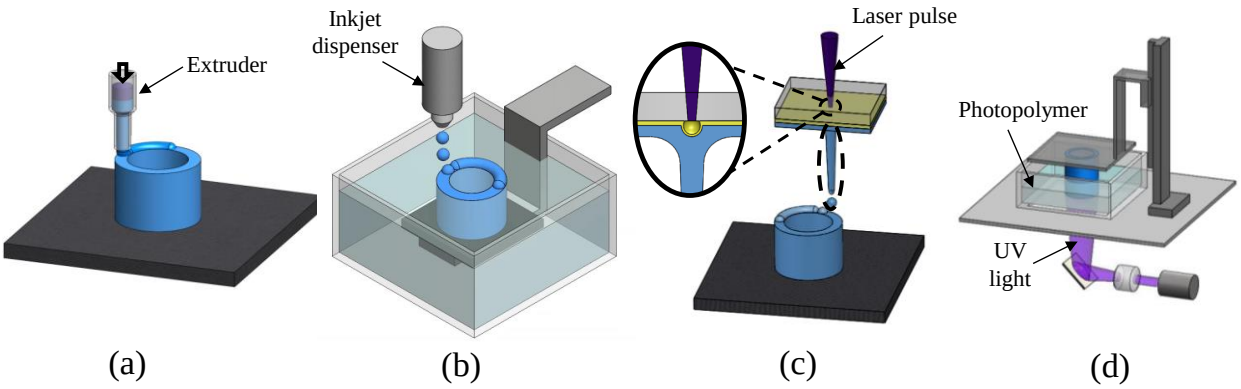


Figure 6. Representative soft construct fabrication techniques: (a) filament deposition, (b) inkjet printing, (c) laser-induced forward transfer, and (d) stereolithography.

2.3. Hard structure printing

Hard structures for biomedical applications are usually made from engineering materials including metals, ceramics, solid polymers, hydrogels, and composites. Suitable hard structure materials include biocompatible metals such as titanium, stainless steel, and cobalt alloys; ceramics including bioglass and hydroxyapatite; solid polymers such as PCL, PLGA, and poly(propylene fumarate) (PPF); tough hydrogels including collagen, alginate, PEG, silk fibroin, and various blends; and composites. Composite materials are usually polymeric matrices filled with ceramic particles, which mimic the mineralized 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 employed to improve the biological response to permanent, non-degradable implants.

Since their mechanical and processing characteristics are similar to engineering materials, hard tissue constructs 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 [Huang2015] [Kang2016] as well as the potential for rapid customization. Figure 7 illustrates the four most commonly adopted AM techniques [Schmid2014] for hard structure printing: fused deposition modeling (FDM), a material extrusion process; selective laser sintering (SLS), a powder bed fusion process; stereolithography, a vat photopolymerization process; and three dimensional printing (3DP), a binder jetting process.

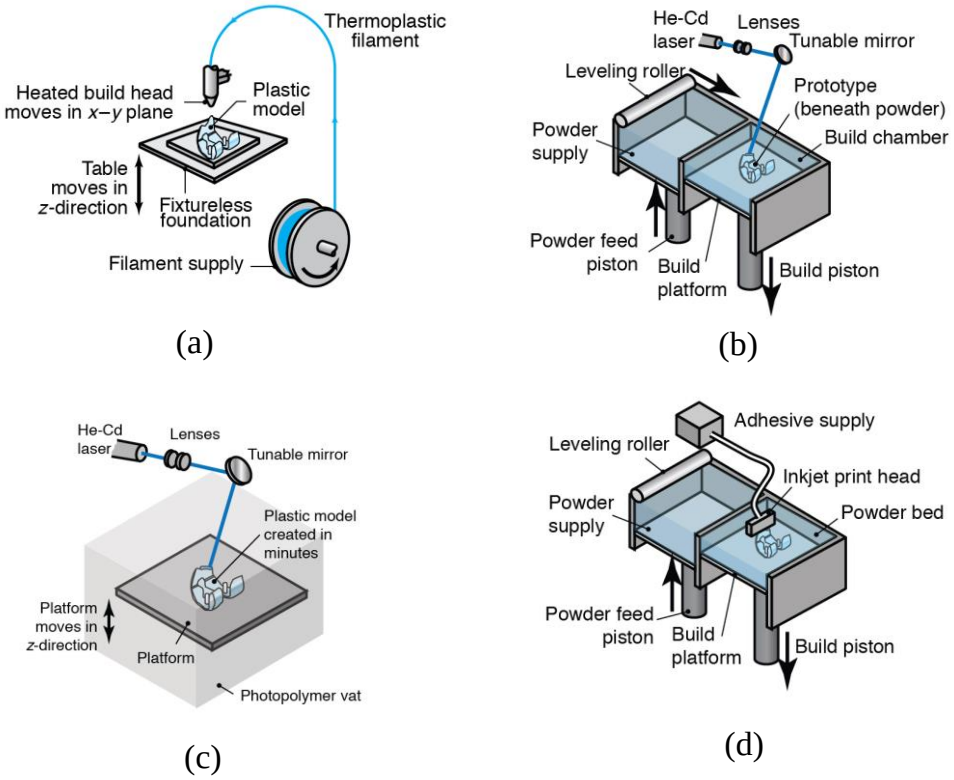


Figure 7. Schematic illustrations of popular AM processes relevant to hard structure/medical device manufacturing [Schmid2014].

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. 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. Both binder jetting and material jetting involve deposition of droplets, but in material jetting, the entire structure is built solely of jetted build material 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.

Materials for AM are diverse, and many engineering materials are also suitable for hard tissue applications. Polymers, 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 pre-ceramic 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.

AM enables the fabrication of sophisticated hard tissue constructs. Historically, bone tissue scaffolds have been simple solid or porous hard constructs, either mass-produced or custom-made for a specific defect. However, such constructs suffer from limited cell retention and tissue integration [O'Brien2011] [Shrivats2014]. More recent 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 co-deposited soft cell-laden gel, providing a balance of mechanical support and regenerative stimuli [Shrivats2014] [Kang2016]. Current research directions include optimization of architectures, characterization of the effects of surface finishes, and co-printing of soft, cell-laden components to promote rapid regeneration within rigid lattices [Huang2015] [Kang2016].

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 [Huang2015], 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 inter-connectivity; 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. Firstly, 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 standardized bioink formulations or post-fabrication procedures. Secondly, 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 multi-material designs, and criteria for choosing printing over other non-printing fabrication techniques. Thirdly, in terms of process innovation, gaps and needs are related to scale-up production, multi-material 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. Fourthly, 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. Finally, 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. Soft construct-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 Figure 8, there are a few gaps and needs to be addressed:

- What to print in terms of build materials 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 also needs to be scalable, be cost effective, and comply with good manufacturing practice requirements. Typical starting materials in conventional manufacturing are non-living 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 unprecedented constraints, metrics, and outcomes. Considering living cells as a special type of heterogeneous composite living material, process development, modeling, monitoring, and control as well as quality control and supply chain management for cell manufacturing and biofabrication must be adapted by the manufacturing community collectively to account for unique challenges associated with living materials. In addition, tissues containing living cells currently suffer from a limited shelf life, which reduces their clinical potential and needs research attention. Furthermore, bioink formulations for printed constructs are to be standardized for key tissue constructs, and a better understanding of how construct design affects functionality is needed to maximize functionality as well as production efficiency.
- Where to print in terms of support medium selection: Support bath or medium, as needed, is to be standardized for key printing techniques.
- Which printing technology in terms of the understanding of each available bioprinting technique: Each AM technique has strengths and weaknesses for soft construct printing, so scientific criteria are needed for process selection. Regardless of AM technique(s) selected, printing dynamics of a variety of complex fluids including viscoelastic polymer solutions and soft cell-laden suspensions 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 post-printing has been of concern [Gudapati2014] [Zhang2017]. Generally, there are two types of cell injury and death: apoptosis and necrosis. 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.

- How printed tissue fuses and matures: Post-printing tissue fusion and maturation and their associated microenvironment are to be understood for better developmental engineering of soft tissue constructs.
- How to evaluate a printed construct: 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.

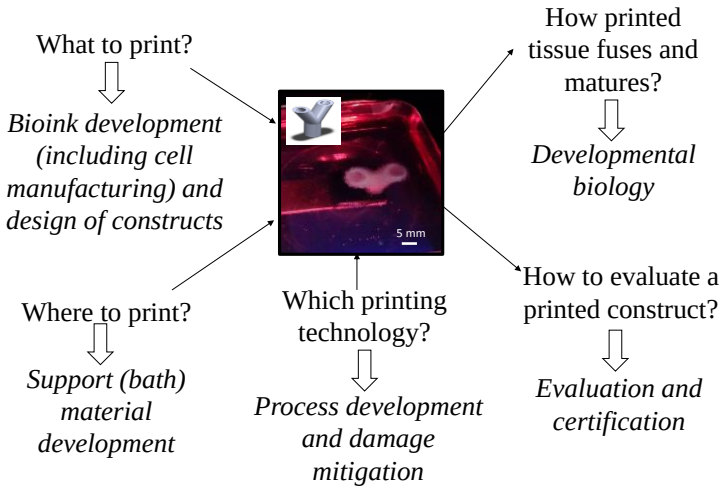


Figure 8. Illustration of soft construct-specific gaps and needs.

3.3. Hard structure-specific gaps and needs

While they rarely incorporate living cells, hard structures have their own unique gaps and research needs, in particular related to the material-process-property-functionality relationship as discussed below. Firstly, the build material properties such as purity, powder size, molecular weight, 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. Secondly, 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. Thirdly, 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. Finally, 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.

4. RECOMMENDATIONS

4.1. General recommendations

Manufacturing-related research recommendations identified at this workshop encompass aspects of AM ranging from fundamental research support to development of suitable regulations for clinical use of AM products. For more effective adoption and sustainable application of AM for health, general recommendations are summarized for materials, design, process innovation, modeling, characterization, and policy and education:

- Materials: Development and standardization of a broad range of economic, 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 (CAD) tools to design and printers to implement multimaterial constructs; and design of soft-hard tissue interfaces for heterogeneous constructs.
- Process innovation: Development of versatile 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 post-printing 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 products as implanted and degradation of 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 non-expert 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.

4.2. Soft construct-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 post-bioprinting treatment.

4.2.1. 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: 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. 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.

4.2.2. 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 vascularized structures.
- 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.

4.2.3. Post-bioprinting treatment

- Design of bioreactors: Bioreactors should be designed and manufactured and operating conditions should be optimized to promote the 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.

4.3. Hard structure-specific recommendations

Hard structure-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.

5. BIOMEDICAL MANUFACTURING LANDSCAPE AND GRAND CHALLENGES OF FUNCTIONAL TISSUE BIOPRINTING

5.1. Biomedical manufacturing landscape

While AM for health has been the frontier of the manufacturing research community, part of advanced manufacturing research has also been directed towards the grand landscape of biomedical manufacturing by seamlessly blending biomedical and manufacturing engineering. Figure 9 specifically illustrates a roadmap for the evolving discipline of biomedical manufacturing.

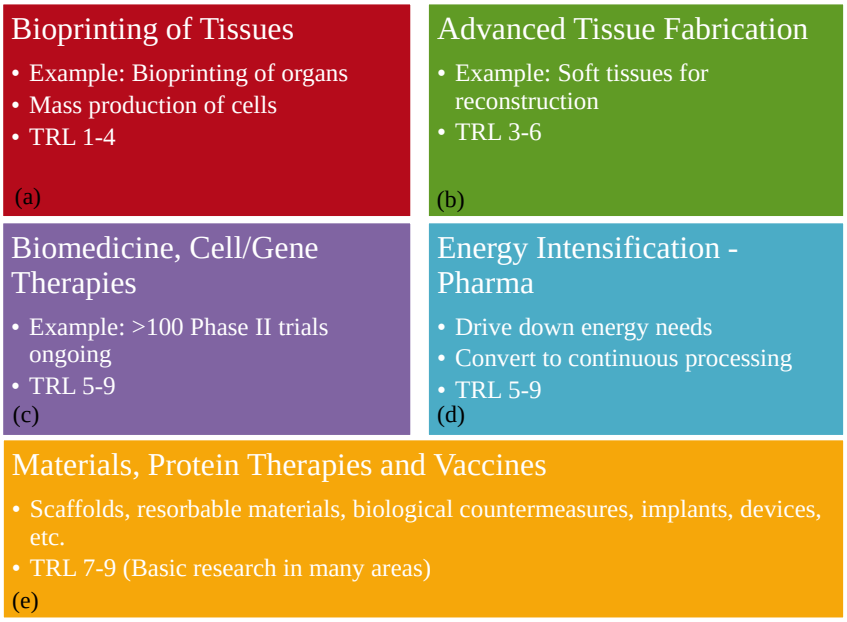


Figure 9. Roadmap for the evolving discipline of biomedical manufacturing. TRL: technology readiness level; 1 = basic research, 9 = mature and ready for implementation. Source: Steven Schmid of NSF and Kelly Rogers of National Institute of Standards and Technology (NIST).

For the bioprinting of human tissues as illustrated in Figure 9, 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 larger-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 de-risking 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 ways for bioprinting to be a transformative research area, perhaps the most transformative of the upcoming century. Some of them are enumerated as follows:

- The ability to produce functional organs could potentially eliminate the organ waiting list, and thereby eliminate unnecessary deaths and emotional trauma while improving quality of life.
- The ability to produce non-functional 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 as follows:

- 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 and engineering. 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 to tissue constructs such as capillaries formed around a printed vascular tree. 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 [Nunes2013].
- 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 pre-formed 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.

- 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.

APPENDIX A

Organizers, Discussion Moderators, Invited Speakers, and Invited Participants

Organizers

Yong Huang	University of Florida
Steven Schmid	University of Notre Dame

Discussion Moderators

Steven Schmid	University of Notre Dame
Yong Huang	University of Florida
Megan Brewster	Office of Science and Technology Policy (OSTP)

Invited Speakers (Listed based on the presentation order)

Day 1 morning

Ralph L. Resnick	Accelerating the Transition and Insertion of Additive Manufacturing Research for Health (National Center for Defense Manufacturing and Machining)
Hod Lipson	3D Printing: The Next 25 Years (Columbia University)
Jennifer Lewis	Additive Manufacturing of 3D Vascularized and Functional Tissue Constructs (Harvard University)
Anthony Atala	Regenerative Medicine: Current Concepts and Applications for Additive Manufacturing (Wake Forest University)
Scott Hollister	3D Printed Patient Specific Medical Devices: There is a Paradigm, But is There a Path? (University of Michigan)
Wei Sun	3D Bioprinting - Challenges and Opportunities (Drexel University)

Day 1 afternoon

Amit Bandyopadhyay	Additive Manufacturing of Hard Biomaterials (Washington State University)
Shaochen Chen	Rapid Scanningless 3D Printing: Vision, Status, and Research Needs (University of California at San Diego)
John P. Fisher	Current Status and Future Perspectives of 3D Printing and Bioprinting for Regenerative Medicine (University of Maryland)
Ola Harrysson	How Additive Manufacturing is Changing Healthcare (NC State University)
Bradley R. Ringeisen	3D Bioprinting for DoD, Government and Commercial Applications (Naval Research Laboratory)
Albert Shih	Cyber-Physical Design and Additive Manufacturing of Custom Orthoses (University of Michigan)
David Wallace	Additive Manufacturing of Functional Materials in Health Applications using Ink-Jet Technology (MicroFab)

Michael J. Yaszemski	Current and Future Uses of Additive Manufacturing in Neuro-Musculoskeletal Spinal and Oncologic Surgery (Mayo Clinic)
Kaiming Ye	Cell and Tissue Therapeutics Manufacturing (Binghamton University)
Lijie Grace Zhang	3D Bioprinting and Nanobioinks for Complex Tissue and Organ Regeneration (George Washington University)

Day 2 morning

Deborah J. Goodings	NSF Overview of Additive Manufacturing for Health (NSF)
Rosemarie Hunziker	Cells, Cells, and More Cells: The Weakest Link in Regenerative Medicine (NIH)
Carrie Laurencot	Delivering Mission Ready Medical Solutions to the Warfighter (US Army)
James Coburn	FDA Perspectives on 3D Printing: Technical Considerations (FDA)
Anne Plant	Additive Manufacturing for Health: a NIST Perspective (NIST)

Invited Participants:

T. Angelini of University of Florida, T. Boland of University of Texas at El Paso, D.B. Chrisey of Tulane University, A. Hughes of University of California at San Francisco, M. Kumar of Zimmer Biomet, W. Li of University of Texas at Austin, R. Narayan of University of North Carolina at Chapel Hill/NC State University, T. Render of Johnson & Johnson, B. Stanley of North Carolina State University, and M. Yost of Medical University of South Carolina

APPENDIX B

NSF Sponsoring Program

ENG Directorate:

CMMI Manufacturing Machines and Equipment (MME, ZJ Pei)

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