BIOPRINTING

3D-bioprinted human tissue and the path toward clinical translation

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Three-dimensional (3D) bioprinting is a transformative technology for engineering tissues for disease modeling and drug screening and building tissues and organs for repair, regeneration, and replacement. In this Viewpoint, we discuss technological advances in 3D bioprinting, key remaining challenges, and essential milestones toward clinical translation.

INTRODUCTION

Since its inception, tissue engineering has held the promise of creating tissues for a range of applications from in vitro disease modeling to in vivo regeneration, organ repair, and replacement. However, to date, few technologies have reproduced the complex tissue architecture and cell spatial heterogeneity required to recreate physiologic function. Over the past decade, three-dimensional (3D) bioprinting has rapidly grown as a biofabrication approach to enable spatially controlled deposition of biomaterials and cells in 3D with unprecedented precision and control. 3D bioprinting is unique in using computer-aided design (CAD) software and multiaxis robotic hardware to create 3D structures with unrivaled complexity when compared to traditional tissue engineering approaches. Equally important is the ability to use medical imaging data, such as computed tomography (CT) or magnetic resonance imaging (MRI), to create patient-specific anatomic models, offering a tailored approach to tissue and organ engineering. In this Viewpoint, we focus on recent 3D bioprinting innovations with the potential to build volumetric human tissue and the proof-of-concept applications that address long-standing challenges on the path toward clinical translation.

ADVANCING THE STRUCTURE AND FUNCTION OF 3D-BIOPRINTED TISSUES

3D bioprinting approaches

As the field has advanced, 3D bioprinting has undergone technological diversification in terms of printing approaches, materials design, and new applications. This includes a range of techniques to create scaffolds and tissues using extrusion, photocrosslinking, inkjetting, laser sintering, laser-assisted transfer, and related 3D bioprinting methods (*1*, *2*). Here, the focus is on understanding the subset of extrusion-, embedded-, light-, and laser sintering–based 3D bioprinting techniques that have enabled important advances toward building large volumetric human tissues of ≥ 1 cm^3 (Fig. 1).

Briefly, extrusion-based printing, also known as direct ink writing, relies on the deposition of material through a nozzle to create a defined 3D structure (*3*). This has worked well for 3D printing thermoplastics and mineralized scaffolds for hard tissues where the materials are relatively rigid. However, because many of the biomaterials that make up our body, such as cells and extracellular matrix (ECM), are soft and deformable, it is difficult to form multiple layers and maintain shape. To address this, researchers have developed ways to support cells and soft biomaterials during the printing process. A common approach is to modify the rheology of hydrogel bioinks to impart a yield stress so that the material can be extruded under an applied load but then rapidly resolidify afterward and hold its shape. This can be achieved in multiple ways, including using weak cross-linking, adding fillers such as nanosilicates, or forming the bioink into a microparticle slurry (*4*–*6*). One challenge of the approach with cell-laden bioinks is that there is often a limit to the maximum cell density to maintain sufficient hydrogel concentration for printability. Another widely used approach is to print a thermoplastic, lattice-like scaffold composed of polycaprolactone (PCL) or another biodegradable polymer to provide mechanical support to cell-laden bioinks extruded within the openings (*7*). A challenge with this approach is that the support scaffold limits the volume of bioink that can be used. Researchers have also either extruded or used laser sintering of thermoplastics like PCL or surfactants such as Pluronic F-127 to create a rigid network of filaments, cast a cell-laden hydrogel around these filaments, and then dissolve the filaments to create vascular-like channels within the cellularized hydrogel (*8*, *9*). This has been effective for engineering perfusable constructs, but it can be challenging to create multiscale, hierarchical vascular networks that obey Murray's law (*10*).

More recently, embedded 3D bioprinting techniques (*11*, *12*), such as free-form reversible embedding of suspended hydrogels (FRESH), have been developed whereby cells and hydrogel-based bioinks are deposited within a support bath that holds the soft materials in place during the polymerization or curing process (*1*). The support bath is then removed to nondestructively release the 3D-bioprinted construct. Advantages of embedded printing include customization of the aqueous chemical environment within the support bath to expand the usable bioinks and enabling printing of large tissue at organ scale without deformation due to gravity (*13*, *14*). Further, there has been rapid growth in variations of embedded 3D bioprinting, such as sacrificial writing into functional tissue (SWIFT) developed by Skylar-Scott *et al.* (*15*), that uses a support bath composed of cell spheroids that form the bulk tissue into which sacrificial Pluronics filaments are printed to create a vascular-like network for perfusion.

In comparison, light-based 3D bioprinting selectively photocrosslinks bioinks to create 3D constructs through either extrusion or vat photo polymerization using stereolithography (SLA) or digital light processing (DLP). Photocrosslinking bioinks during or

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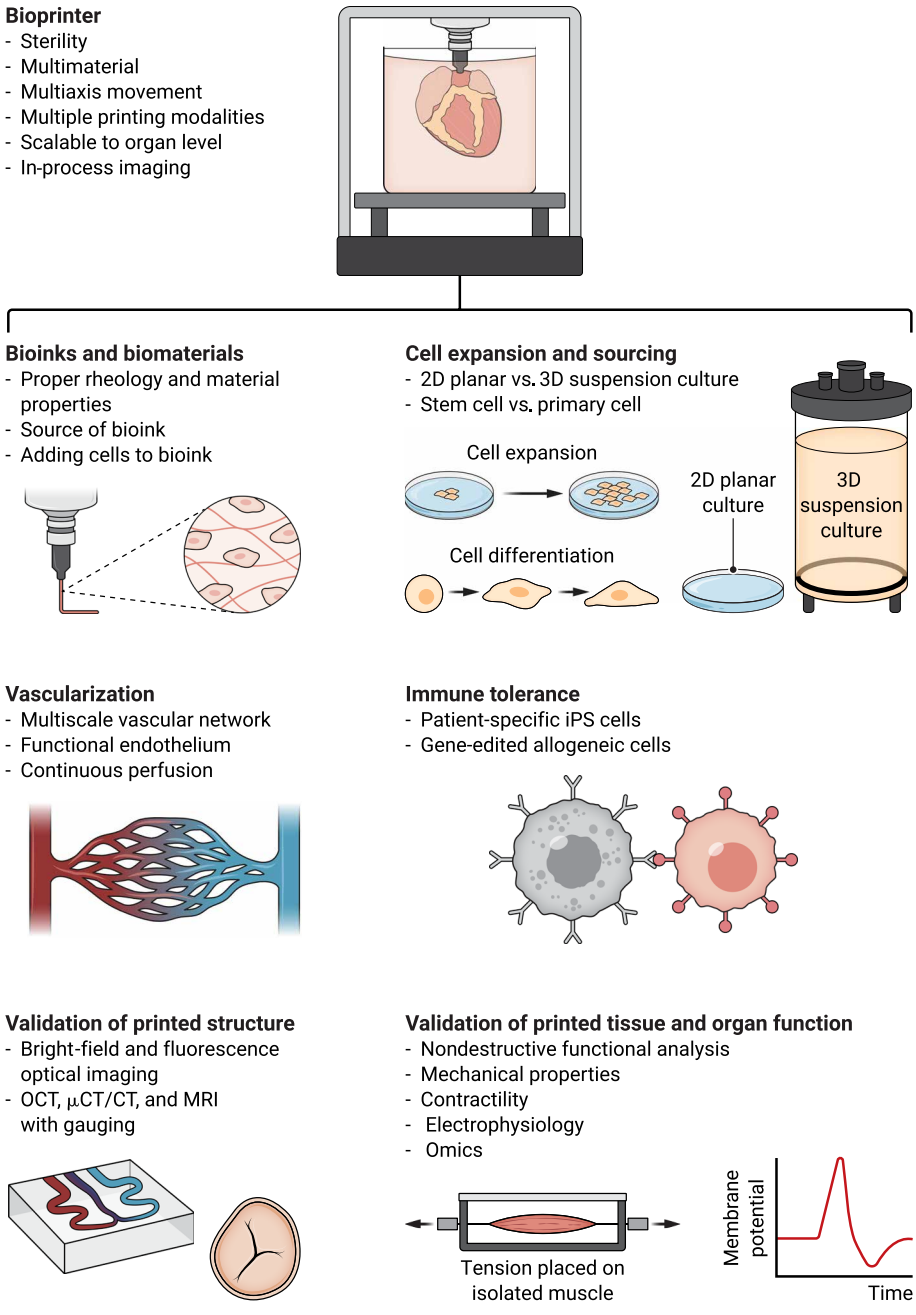

Fig. 1. Key challenges and requirements for 3D-bioprinted tissues and organs. Translation of 3D-bioprinted tissues and organs requires advances that span the engineering, biological, and medical sciences. This includes (i) faster 3D bioprinters that create larger and more complex tissues, (ii) optimized bioinks and biomaterials, (iii) the ability to expand large numbers of cells and differentiate them to target cell types, (iv) vascularization and perfusion of volumetric tissue, (v) immune tolerance to ensure long-term viability in patients, (vi) nondestructive validation of tissue structure, and (vii) validation of the tissue and organ function that will be required for successful clinical translation. OCT, optical coherence tomography; CT, computed tomography; MRI, magnetic resonance imaging.

immediately after extrusion has been widely demonstrated using gelatin methacryloyl (GelMA) and other polymers (*16*); however, because these bioinks remain relatively soft after printing, some form of support is required for large constructs. SLA leverages a raster-based scanning laser, and DLP uses image projection to cross-link an entire layer at once, which substantially increases the speed at which the 3D print can be obtained. However, vat photopolymerization is challenging to use with multiple materials, and there is also a limit on the maximum cell density to maintain sufficient hydrogel concentration for printability.

Bioinks and biomaterials

The end goal for these bioprinting approaches is to print cells and biomaterials into complex 3D structures to recreate physiologic tissue function. Key to this is understanding that tissues and organs consist of cells embedded in an ECM of collagen, elastin, glycoproteins, proteoglycans, growth factors, and much more. To recreate this environment, bioinks are typically designed to recapitulate the structural and functional components of the tissue and organ that they are intended to repair or replace. Hydrogel bioinks are synthetic or naturally derived, may contain cells, and must be printable, which requires shear thinning fluid properties amenable to gelation/cross-linking after printing. In addition, bioinks are typically designed with specific biomechanical properties and cell-ECM interactions to control biological function. For example, Kupfer *et al.* (*16*) screened bioink formulations for printability, viability, attachment, and subsequent differentiation of human-induced pluripotent stem cells (hiPSCs) into cardiomyocytes and identified a unique formulation of GelMA, collagen methacrylate, fibronectin, and laminin-III. This bioink was FRESH 3D bioprinted into chambered heart muscle pumps that generated pressure-volume loops, a step toward rebuilding functional hearts. Another promising approach inspired by tissue decellularization (*17*) is the decellularization and solubilization of ECM into bioinks. Noor *et al.* (*18*) formulated a bioink from omental tissue to create a vascularized heart muscle patch using an embedded printing approach, highlighting the potential for both the cells and bioink to be patient- and tissue-specific.

Printing multiple bioinks into the same integrated tissue can present additional challenges. Hull *et al.* (*19*) addressed this using bioorthogonal functional groups grafted onto different polymers combined with a universal click-chemistry cross-linker dissolved within a support bath. This enabled gelatin, hyaluronic acid, polyethylene glycol, and an elastin-like protein to be printed into one cohesive structure with high cell viability. There have also been advances using microfluidics to achieve multimaterial printing using DLP; however, the confined build volume limits this to small constructs (*20*). Another challenge is the trade-off between increased

print time or a loss of build volume due to mechanical switching between nozzles. Skylar-Scott *et al.* (*21*) demonstrated the deposition of up to eight materials from a custom microfluidic print head extruder using solenoid valve control. Overall, these studies and others demonstrate that both bioink composition and bioprinting hardware can be used to improve tissue biofabrication.

Cell sourcing, expansion, and printing

Cells are a key component of bioprinted tissues and must be sourced appropriately to maximize viability and provide the required function for the engineered tissue (*22*). Autologous cells have the advantage of minimal immunogenicity; however, the time frame (i.e., weeks to months) required to expand these cells and the associated cost of culturing on a patient-specific basis limit translational potential. This is particularly true for hiPSC-derived cells where differentiation and maturation remain work in progress for many tissue types. Alternatively, allogeneic cell sources could be considered through either cell banking, haplotype matching, and/or the creation of universal donor cell lines (*23*). Indeed, recent advances in xenogeneic transplants from humanized pigs suggest that gene editing of human allogeneic cells to reduce immunogenicity may be a clinically viable strategy (*24*). Other considerations on cell sourcing have been reviewed extensively elsewhere (*25*, *26*).

In either case, a major challenge is generating sufficient cells to create tissues with physiologic cell density, which can range from millions to tens of billions of cells (*27*). One option is to have cell proliferation occur after printing, like that demonstrated by Kupfer *et al.* (*16*)—printing hiPSCs within an optimized bioink to promote proliferation followed by in situ differentiation into cardiomyocytes, although differentiation efficiency was diffusion limited. Alternatively, Skylar-Scott *et al.* (*15*) printed sacrificial Pluronics filaments within a support matrix of cardiac cell spheroids to form vascular-like channels, achieving cell density comparable to human myocardium (~10^8 cells/ml). Similarly, as demonstrated by Lee *et al.* (*14*), high-density hiPSC-derived cardiomyocyte and fibroblast bioink (~2.5 × 10^8 cells/ml) together with a collagen bioink were FRESH printed to engineer beating ventricle–like tissues. These examples demonstrate the capability to 3D bioprint with high cell density, which is a requirement to achieve physiologic function for many tissues.

Clinical translation of bioprinted tissues faces challenges similar to those of other cell-based therapies (*28*). The choice of cell type and source is crucial because these factors not only influence biological performance but also may affect regulatory considerations, such as requirements for autologous versus allogeneic cells. Following appropriate industry and regulatory standards such as good laboratory practice (GLP), good cell culture practice (GCCP), and good in vitro methods practices (GIVIMP) is necessary. This also relates to manufacturing approaches needed for cell scale-up, such as 3D bioreactors for suspension culture and the good manufacturing practice (GMP) guidelines that must be met to ensure consistency across batches (*29*). Last, end-to-end quality control and standard operating procedures (SOPs) are necessary to create clinical 3D-bioprinted tissue products, and these are generally absent at the research stage.

Fabrication of vascular networks

Vascularization is required to support the viability of dense, volumetric tissues and is an application where 3D bioprinting has made multiple advances. Examples include printed interconnected networks from fugitive materials to form vascular-like channels within cell-laden hydrogels (*9*, *30*), endothelialized channels to improve viability and function in liver (*31*) and bone constructs (*9*), and directly printing multiscale vascular-like networks in collagen and gelatin scaffolds (*14*, *32*, *33*). When implanted in vivo, these vascular-like channels have also shown the ability to anastomose and functionally integrate with the host vasculature, perfusing and oxygenating the integrated cells (*31*, *33*). Although many approaches can fabricate patent vascular-like channels, there are several outstanding challenges, including but not limited to creating bioprinted blood vessels that have similar architecture to native vessels with spatial distributions of distinct cell types and ECMs and maturation and maintenance of the vasculature using bioreactor perfusion. Specific challenges faced in developing multiscale vasculature have been reviewed extensively elsewhere (*27*, *34*–*37*).

BUILDING AND VALIDATING BIOPRINTED TISSUES AT CLINICALLY RELEVANT SCALES

Tissue and organ design

Successfully bioprinting full-scale tissues requires multiple considerations including creating the 3D anatomic model and selecting the bioprinting method(s) and bioinks to use. As 3D imaging and computational capabilities improve, fully segmented datasets will soon follow; however, to create a complete tissue, both geometric and compositional requirements must be met. Geometric constraints can be derived from MRI, CT, and other imaging modalities, but there are trade-offs that lead to incomplete datasets that focus on either micro or macro tissue architecture. Deidentified medical imaging data from MRI or CT have been used to generate 3D CAD models for bioprinting the heart and ear (*7*, *13*, *14*). However, these models often lack necessary internal details that are difficult to generate using automated segmentation approaches. Building a comprehensive 3D model will likely require combining data from multiple imaging modalities into a fully segmented 3D structure consisting of tissue geometry, vasculature, spatially defined mechanical properties, and region-specific cell types.

Matching cell and ECM properties

From a compositional standpoint, advances in single-cell sequencing and tissue decellularization are revealing the heterogeneity of human tissues. For example, the adult heart has more than 10 major ECM proteins, 11 cell types, varying elastic moduli, and a multiscale vascular network ranging from 5 μm to 5 mm in diameter (*38*, *39*). Bioprinting functional cardiac tissue requires recapitulating some of this complexity, but matching all of it would require multimaterial deposition (>21 bioinks), a way to alter elastic moduli (e.g., ECM concentration, stiffness, and cross-linking), and feature resolution spanning more than three orders of magnitude (micrometers to centimeters). Other tissue types will have similar needs, and addressing these may require integration of multiple bioprinting methods into a single system that uses multi-extruder tool changing for extrusion- and embedded-based approaches combined with the speed and versatility of light-based DLP and SLA.

Structural and functional validation

Manufactured 3D-bioprinted tissues will also be required to undergo quality control in the form of in-process and post-process characterization to validate structure and function. In-process monitoring is used to confirm that quality control metrics are met and, for medical devices, range from nondestructive optical inspection systems to

destructive mechanical testing. For 3D-bioprinted tissues, in-process monitoring has been primarily limited to optical imaging of the external surface, which cannot capture internal cellular composition and structure. Cells can be analyzed using fluorescence microscopy, but this is typically a destructive process and is limited to <1 mm of tissue penetration. For nondestructive 3D imaging, optical coherence tomography (OCT) and other imaging methods have been used to optimize bioink printing and to perform in-process error detection and 3D gauging of bioprinted scaffolds (*7*, *32*).

Assessing function is tissue-dependent and may include viability, proliferation, differentiation, electrophysiology, contractility, barrier function, protein secretion, and response to pharmacological treatments. For bioprinted cardiac or skeletal muscle, this might consist of contractile force measurements (*40*); for liver tissue, this could be albumin secretion (*41*); and for kidney tissue, this could be solute reabsorption (*42*). Some of these functions can be assessed before bioprinting or at various stages of the biofabrication process, but a final functional validation will be needed before implantation.

TOWARD CLINICAL TRANSLATION OF 3D-BIOPRINTED TISSUES

Understanding the regulatory landscape

Although the regulatory requirements for clinical translation of 3D-bioprinted tissues and organs vary across different countries, the U.S. Food and Drug Administration (FDA) guidance documents provide an example of the current landscape. The first consideration is whether the bioprinted tissue is a medical device or minimally manipulated tissue, with an example of the latter being decellularized tissue grafts. It is unlikely that a 3D-bioprinted tissue will fall under the minimally manipulated classification given the extensive processing involved to print tissue-derived bioinks. However, several tissue-engineered products have successfully obtained 510(k) approval, suggesting that there may be precedence for some 3D-bioprinted tissues to achieve approval through this process (*43*, *44*). The FDA 510(k) is a premarket submission demonstrating that a new device is "substantially equivalent" to a legally marketed device.

Acellular 3D-bioprinted scaffolds are likely to be considered class II medical devices requiring performance standards and/or special controls such as biocompatibility, sterility, and shelf life; nonclinical performance evaluations; and in vivo performance evaluation. Cellularized 3D-bioprinted tissues are likely to be considered class III medical devices requiring complete premarket approval (PMA), and, if determined to be a new product distinct from those currently on the market, would automatically be classified as such. The FDA has guidelines for 3D-printed medical devices to help with regulatory approval, with several relevant to bioprinting including the following: (i) quality assurance with in-process monitoring, post-processing evaluation, and final device testing both within and between batches; (ii) validation of geometric and material specifications for patient-specific devices; (iii) validation of data quality and feature resolution from digital design through physical device fabrication; and (iv) device evaluation within manufacturing runs to identify defective products without compromising the entire batch.

Projecting the timeline and major milestones to clinical translation

The regulatory landscape and current state of 3D bioprinting provide a general framework of the next steps for clinical translation (Fig. 2) and can be organized into three main categories. First are acellular scaffolds designed to be implanted in vivo: If composed of decellularized ECM bioinks, these scaffolds are similar to current decellularized ECM scaffolds manufactured through other processes. Thus, these acellular bioprinted scaffolds could potentially proceed through a 510(k) pathway and be translated into the clinic within 5 years. This would be a major milestone for the 3D bioprinting field because it would establish viability as a biomanufacturing platform.

Second are simple tissues composed of only a few cell types and bioinks, which could be similar to combination devices containing cells that are FDA-approved for therapeutic use, such as tissue-engineered skin-like Epicel (cultured epidermal autografts), TransCyte (fibroblast-derived temporary skin substitute on collagen-coated nylon mesh and silicone membrane), and tissue-engineered cartilage like MACI (autologous cultured chondrocytes on porcine collagen membrane). Bioprinted skin and cartilage could pursue a similar strategy to obtain 510(k) clearance. This could result in clinical translation within 5 years; although to be commercially viable, the 3D-bioprinted tissues need some advantage over existing medical devices, such as reduced cost or improved outcomes.

Third are more complex 3D-bioprinted tissues and organs that do not have a previously approved or comparable product. Taking the heart as an example, an important initial milestone is engineering miniature organs that reproduce physiologic function for assessing drug toxicity or serving as patient-specific cardiac disease models (*14*, *40*). Given the pace of research, it is reasonable to assume that this will occur within the next 5 years. The next milestone is engineering functional organs for testing in preclinical animal models, progressing from proof of

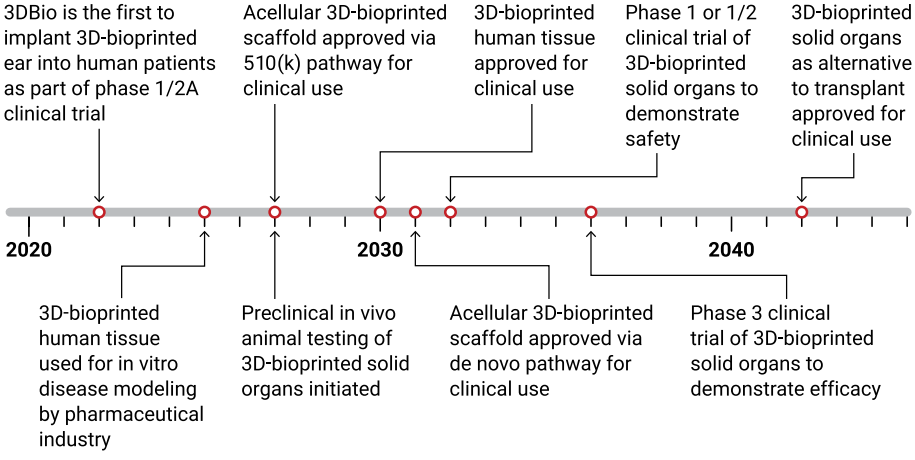

Fig. 2. A forward-looking timeline projecting the dates for major milestones in the clinical translation of 3D-bioprinted constructs. Constructs include acellular scaffolds for in vivo tissue regeneration, human tissues for in vitro disease modeling, human tissues for repair and replacement, and solid organs for transplantation.

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concept in small animals to large animal models that better recapitulate human physiology. Multiple research groups are working toward this goal, and it is expected that this can be achieved within 5 to 10 years.

The final major milestone is 3D bioprinting full-size tissues and organs and completion of human clinical trials. Although this is the ultimate goal of the field, the timeline to achieving this is hard to predict given the anticipated challenges; a reasonable estimate is that it will be at least 10 years before tissue constructs with limited function and at least 20 years before a 3D-bioprinted solid organ completes the phase 3 clinical trial that would precede regulatory approval. Companies are already pushing these technologies forward, and earlier this year, 3DBio achieved first-in-human use, implanting an ear consisting of patient-derived chondrocytes 3D bioprinted to match the size and shape of the patient's contralateral ear as a part of a phase 1/2A clinical trial (*45*). Although the ability of the 3D-bioprinted ear to retain its shape long-term remains to be evaluated and this study has not yet been shared through peer-reviewed publication, this nonetheless marks an important milestone for the field. A growing number of companies are also focused on the clinical translation of more advanced tissues and organs, including 3D Systems and Lung Bioengineering collaborating on transplant-grade lungs (*46*); Poietis developing bioengineered skin (*47*); and FluidForm manufacturing functional heart valves, contractile cardiac muscle, and regenerative ECM scaffolds (*48*).

CONCLUSION AND FUTURE OUTLOOK

3D bioprinting is rapidly advancing, and recent examples in the literature demonstrate that the size, complexity, and functionality of the human tissues that can be engineered are continually improving. Considerable challenges remain, including the integration of multiple cell types, building fully functional multiscale vasculature, and achieving adult-like tissue function. There are also developments beyond bioprinting itself that are required for clinical translation, including large-scale cell production and differentiation, bioreactor platforms for post-print tissue maturation, and a fully defined regulatory pathway for organ-scale constructs. Last, future success in clinical translation will require substantial investment to ensure a viable industry. Private-public partnerships such as the Advanced Regenerative Manufacturing Institute are working to build the technology, standards, and regulatory framework required to support biomanufacturing of tissue-engineered medical products. Academic institutions are also forming centers such as the National Institutes of Health–funded Center for Engineering Complex Tissues that partners together with the University of Maryland, Rice University, and Wake Forest University, and the Mayo Clinic and Carnegie Mellon University collaborating to transform transplantation. Last, a growing number of companies are investing in 3D-bioprinted human tissues and are working to rapidly advance through preclinical testing and initiate human clinical trials. As these efforts grow and expand, it is no longer a question of whether 3D-bioprinted human tissues are technically feasible but rather which specific applications will be the first to successfully translate to the clinic.

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