April
2018

Bioprinting Landscape: Technology and Trends Report

BROWN UNIVERSITY – SENIOR THESIS INQUIRY

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Executive Summary

The primary objective of this report is to provide the bioprinting community with an educational tool that can be used to navigate and develop a path towards commercial readiness and therapeutic application for vascularized bioprinted tissue products. We hope this technologies and trends landscape (1) effectively illustrates the current innovations and drivers of the bioprinting arena, (2) establishes a more complete understanding of the experiences and struggles of players at every level of the field, (3) bridges the information gap between academic and industrial players, and (4) provides stakeholders insight into how to effectively coordinate and collaborate to drive the field forward.

This report focuses on the current-state-of-the-science for developing vascularized tissue constructs, and includes a literature review, market analysis and primary research effort that characterize and analyze impactful players and innovations in the bioprinting pipeline, with conclusions that elucidate (a) what is driving the industry forward, and (b) what is holding it back. To produce this landscape, we employed a multi-tiered research approach that incorporates a comprehensive range of stakeholder perspectives. Our secondary research effort involved reviewing hundreds of specific sources, including preeminent bioprinting studies, market reports and industry websites, to establish an understanding for the leading bioprinting approaches and players in the field. We subsequently engaged a primary research effort by interviewing 18 industry experts who provided 'on the ground' insights into stakeholder developments, industrial trends, and innovative bioprinting technologies that supplemented and validated our secondary findings.

Our research revealed that the bioprinting field has advanced rapidly in the past two decades and is close to creating tissue products with high commercial and therapeutic value. We found that the commercial adoption of bioprinted products will likely occur for *in vitro* tissue models first, within 5-10 years, for simple tissue grafts like skin and bone second, in 10-15 years, followed by vascularized patches and functional organ regeneration implants third, in 20-25 years, and organ transplants last, 3-4 decades in the future.

For these developments to occur, an intentional and coordinated effort to build the basic technical, application and value chain ecosystems is needed. The development of this infrastructure – collectively the 'innovative infrastructure' that supports innovation – is critical because early-stage bioprinting technologies, developers and companies face a potential 'valley of death'. This valley of death is substantiated by a disconnect between stalling innovation and insufficient revenue streams preventing the development of clinically proven and commercially viable product-offerings. Early-stage bioprinting players are attempting to evolve basic-demonstrative technologies through higher levels of proof and technical readiness but are challenged by an absence of established commercial revenue. The bioprinting field must overcome this disconnect by focusing on building its infrastructure, which will require sustained funding, time, and commitment, before the ambitious goal of procuring truly therapeutic offerings can be broadly realized.

In this report we have identified opportunities and outlined suggestions at selected levels of the bioprinting pathway to help the bioprinting community advance on this agenda through the development of new and impactful innovations, the amelioration of a wide range

of prohibitive technical and administrative challenges, and the solidification of a growing and thriving industry. Our research has shown that each stage of the bioprinting value-chain pathway present an array of unique technical and administrative challenges. In an attempt to help the bioprinting community overcome these wide-ranging limitations, we have developed best-effort suggestions, informed by our primary and secondary research, that identify and set forth considerations for the future growth of the field. Below are illustrative examples of a small selection of these research-findings; a comprehensive analysis can be found in the conclusion section of this report. Notably, we found that:

1. **Adoption curve ascension strategy**: The establishment of effective and commercially viable bioprinted *in vitro* models will be essential for the ultimate adoption of bioprinting products and technologies. The short-term applicability of these products must be demonstrated by emerging-market players in the field by developing a body of supporting scientific and clinical data that educates and reassures stakeholders about these novel products and technologies, thus allowing bioprinting technologies to ascend the end-user adoption curve.
2. **Open market opportunities**: Given the bioprinting market's segmented nature, high growth rate and vacant target market-areas, there is enormous opportunity for (1) new market entrants to construct and navigate their own industrial niche while avoiding direct competition with existing players, (2) established players to expand their current capabilities into new arenas, likely with the help of additional investment and research capabilities, and (3) watchers of the industry to take investment action, given the high likelihood that producers will productize commercially viable products in the next 5 years.
3. **Characterized bioprinting guidelines**: Research labs must establish shared community guidelines dictating how various printed materials, bioprinting systems and environmental factors combine to produce tissue products with defined characteristics; this includes understanding how tissue products perform given differing printing pressures and bioink compatibilities. This guideline will allow researchers to easily predict how bioprinted tissue products behave without having to fully characterize and reassess the effects that every component of their printing system has on the functionality and behavior of an engineered tissue.
4. **Bioprinter optimization**: Bioprinter technologies are reaching a cost and performance pivot-point that will increase the accessibility of complex and highly functional bioprinting systems for the bioprinting community. Our findings revealed that (1) the development of a diverse toolbox of bioprinting approaches will be needed in order to advance research-efforts towards solving the vascularization issue, but that (2) the creation of a vascularized tissue product with high commercial potential will require the selection and focused development of a bioprinting technique that can be incorporated with other tissue-engineering approaches.
5. **Synergistic collaborations**: Increasing collaborative exchanges across multiple tiers of the bioprinting field will enhance the industrial capabilities of contributing players while helping them identify viable market opportunities. To this end, a platform that encourages and facilitates the development of collaborative efforts must be developed

and implemented, because collaborations benefit players at every level of the bioprinting space.

6. **Regulatory considerations**: To establish an efficient and productive regulatory discourse, a 'divide of regulatory concern' must be established and maintained between academic and industrial players. This divide must ensure that university researchers stay separated from regulatory factors and concentrated on moving basic experimental research forward, while industrial players address regulatory issues by working and communicating with regulators directly.

These types of findings help to identify, address and overcome limitations and opportunity growth-areas that can be targeted by the bioprinting community in order to set a foundational infrastructure that sustains bioprinting innovations past the valley of death, and into the future.

Introduction

The fields of tissue engineering (TE) and regenerative medicine (RM) have experienced exceptional growth in recent decades, driven by the prospect of constructing 3D living tissues for therapeutic and pharmacokinetic purposes. TE involves creating tissue structures with, "physical, chemical, biological and engineering processes that control and direct the aggregate behavior of cells." These processes are increasingly being used in RM, which is defined as, "the application of tissue science, TE, and related biological and engineering principles that restore the structure and function of damaged tissues and organs."[1]

In both TE and RM fields, great strides have been made engineering highly biomimetic tissues with unprecedented levels of complexity, function and clinical merit. Critical to this rapid development has been the field of bioprinting, which is defined as, "the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization in order to produce bioengineered structures with RM applications, pharmacokinetic and basic cell biology studies[1,2]." This technology has emerged as a promising biomanufacturing technique that allows for unparalleled spatial control of cells and biomaterials in a highly scalable and modular fashion in the production of three-dimensional (3D) tissue constructs. TE, RM and biomedical professionals are adopting this technology to engineer scalable tissue models for *in vitro* pharmaceutical testing and autologous tissues with regenerative applications. This convergence of technical sophistication and commercial value has opened a window of opportunity for the bioprinting field at large. To effectively navigate and revolutionize this novel, resource intensive, interdisciplinary and largely unregulated space, professionals and allied stakeholders must align and develop an understanding of the bioprinting development pipeline together[3].

Value Proposition

The primary intent of this review is to educate the bioprinting community, from university researchers to commercial start-ups to large pharmaceutical companies, about the current state of the field by characterizing major technologies and market trends[4]. This examination incorporates a spectrum of stakeholder outlooks that span the administrative, technical and regulatory arenas and maintains both a university- and industry-based perspective.

It is widely acknowledged that literature reviews are effective at outlining current academic publications and innovations, while market reports provide valuable industrial summaries of macro-trends and key players. Unfortunately, these examinations are limited in scope – literature reviews are written exclusively from a university-research perspective and market analyses primarily focus upon financial and business metrics. We have worked to bridge this knowledge gap by providing the bioprinting community a trends and technologies landscape that includes the following:

1. An enhanced and expanded bioprinting knowledgebase backed by primary and secondary research that includes a diverse range of stakeholders and disciplines.

2. A landscape that outlines major trends, limitations and players in the bioprinting space that will allow for subsequent road mapping and accelerated growth of the bioprinting, TE and RM fields.
3. Insight into vascular and vascularized tissue bioprinting research and development (R+D) efforts, that includes straightforward explanations of contributing companies, technical innovations and bioprinting modalities with highlighted research publications that establishes specifications for a viable vascularized bioprinted product.
4. A common terminology framework for addressing bioprinting issues and innovations in the future.
5. Illustrative examples, including a market-based opportunity area, literature highlights and company profiles that illuminate the real-world application and trends of bioprinting developments.
6. Considerations to be taken into account for the bioprinting community in the coming years, with compiled visionary outlooks from a range of stakeholders and a future product commercialization timeline.

These topics are organized into four sections. The 'Fundamental Information' section provides a background knowledge bank that outlines the fundamental approaches, terminology, history and goals of bioprinting technologies. The second section describes 'Vascularized Tissues' by defining the vascularization issue, establishing a minimum viable vascular product and outlining the anatomical organization of human vascular systems. The third section, 'Bioprinting Approaches and Modalities', comparatively reviews bioprinting innovations and technologies by highlighting significant bioprinting studies. In the, 'Bioprinting Industry' section, the bioprinting industry, market, and supply chain are analyzed, with a focus on vascularized tissue production. To inform this inquiry, a multi-tiered and systematic research effort was employed.

Research Approach

The nature and structure of this landscape report, which is a combination of a literature review, market analysis and primary research effort focused on producing bioprinted vascularized tissue products, was directly informed by the information we gathered during our preliminary examination of bioprinting technologies. Section 1 of this report is a literature review that relies primarily on secondary research from academic papers and publications. During this research process, we noticed that academics unanimously agreed that vascularization was the leading challenge prohibiting the TE field from producing engineered tissues with high clinical functionality that mimic human anatomy. In a vasculature study published in 2013, Hoying et al. noted that, "vascularization is increasingly being viewed as one of the primary challenges in tissue engineering of large 3-dimensional (3D) scaffolds[5]." This challenge, called the 'vasculature issue', is critical to the bioprinting field because if extensive vascular networks are not incorporated within biofabricated tissues, their long-term cellular viability and thickness will be severely limited, and wide-scale adoption of these products will be impossible. The identification of this issue through academic research, and our subsequent realization that it was intimately connected to severe limitations at the industrial level, inspired us to (1) focus on the vasculature issue throughout our inquiry, and (2) expand our literature

review to include industrial players using primary research methods. We noticed that there were many impactful and progressive developments being made in the industrial space, but our literature review did not fully capture the implications and trends driving these industrial efforts forward.

To reconcile our literature review with an analysis of the bioprinting industry, we engaged a primary research effort that involved contacting key opinion leaders (KOLs), industry experts and researchers leading the field to gain insight into how the field was developing, what trends the field is experiencing, and how the field will best be able to overcome the vasculature issue. This combination of a primary and secondary research endeavor allowed us to create a landscape that incorporates both an academic and an industrial perspective that addresses limitations across the bioprinting value chain pathway.

Secondary Research: Multiple Search Sources

Hundreds of specific secondary research sources were reviewed to establish an understanding for bioprinting technologies, approaches and applications. Resources included academic publications, research journals, conference proceedings, university databases and white-page write-ups. Selections of the most significant academic publications have been summarized, documented and annotated in an accessible spreadsheet.

In the framing of the industrial bioprinting landscape, dozens of public company reports, Form-10K financial documents (10Ks), websites, databases and publications were accessed, as well as technology roadmaps, market research studies and third-party press articles. All secondary research sources have been comprehensively documented, cited and included in the bibliography below.

Primary Research: Expert Interviews

For a contemporary and comprehensive research effort that addresses the length of the bioprinting value chain, a primary research effort was employed. This interview process supplied 'on the ground' perspectives that supplemented and cross-validated findings from secondary analyses. The following is a summation of our methodologies:

1. Responses – Key players at stratified levels of the bioprinting pipeline were identified through (a) academic literature sources and (b) exposure at the Advanced Regenerative Manufacturing Institutes (ARMI) BioFab Symposium in Manchester, NH, in January of 2018. Interviewees were then contacted by email or in-person.
2. Demographics – Interviews with 18 key stakeholders were conducted either in-person or over the phone, and included a representative sample of six industry professionals, nine university researchers, two observers closely associated with the field, and one vascular surgeon.
3. Approach – Interviewees were questioned in a non-systematized fashion about their current involvement and work, their outlook and concerns for future growth, and their opinion on critical challenges and considerations for moving bioprinting up the adoption curve.

4. Data Capture – Notes capturing direct quotes from interviewees were taken during the conversations and are used as references throughout this report. Annotated transcriptions of discussions, that include interviewees affiliations, research methodologies, and publication information, are included in the appendices. Interview content was synthesized and analyzed to identify recurring themes and perspectives unique to different parts of the development value chain.

Contributing interviewees names, organization, logistical information regarding the interviews and major publications are tabulated in Table 11 of the *Appendix*. References to organizations and companies in figures throughout this report coincide with the interviewees shown in Table 11 and should be referenced as corresponding primary sources for direct quotes.

Section 1: Fundamental Bioprinting Information

Bioprinting Technology Overview

Bioprinting integrates components of a wide range of technical fields, including cancer research, pharmaceutics, fabrication, imaging, computer-aided robotics, biomaterials science, cell biology, biophysics and medicine[2,6,7]. Bioprinting is now being used to develop 3D living tissues that can be used in a range of scenarios that include drug-testing, disease modelling, therapeutic tissue replacements and transplantation, which are visualized in Image 1 below.

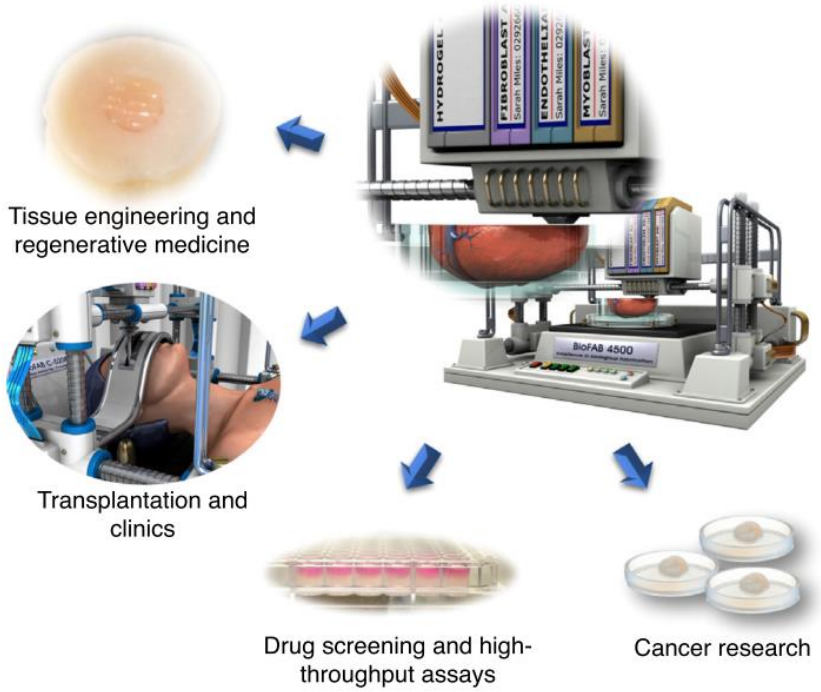

Image 1: Application Areas of Bioprinting Technologies
Reproduced from ref. #[8]

Given its precise spatial control, modular platform functionality and high manufacturing potential, bioprinting technologies have been used to manipulate biomaterials[9], cells[10], synthetics[8] and organ-specific microtissues[11]. Of these products, a wide variety of tissues types have been successfully engineered, including skin[12], nerve[13], blood vessel[14], vasculature[15], cartilage[16], ear[17], bone[18], cardiac[11], osteochondral interface[19], ovarian[20], thyroid[21], liver[22] and kidney[23]. Several of these products have been implanted in animals to evaluate functionality, vascular integration and anastomosis, but studies in humans have been limited. The basic technical considerations of bioprinting approaches can be divided into six fundamental elements:[24,25]

1. **Imaging and 3D modeling software**: Patient imaging data is gathered and subsequently modified and translated into 3D engineering images using computer-aided design (CAD) software. Next, path-planning software uses these models to guide the printer head's

speed, direction and orientation as it builds the tissue model layer-by-layer, in an additive fashion.
    - *Types*: X-ray, CT scans, MRI imaging, tomography, 3D engineering software, CAD models, automated path-planning software.
2. **Tissue construction**: Bioprinters can build tissues using a variety of modalities that can be used exclusively or alongside alternative TE technologies.
    - *Approaches*: biomimicry, self-assembly, bioassembly.
3. **Synthetic material selection**: Scaffolds and synthetic biomaterials can help dispense and structurally support living components in a biofabricated system.
    - *Considerations*: synthetic vs natural polymers, extracellular matrix (ECM) characteristics, material and chemical properties, printability.
4. **Living material selection**: The behavior and overall function of cellular components are influenced directly by their environment and must be incorporated holistically into a bioprinting process that considers bioprinting modality, approach, and material selection.
    - *Considerations*: cell type, target tissue type, heterogeneity, differentiated, pluri- vs. multi-potent stem cells, growth and differentiation factors.
5. **Bioprinting modality**: Different modalities utilize divergent methods to process, dispense and position living and material components, with pertinent advantages and disadvantages embodied by each technique that must be considered.
    - *Modalities*: scaffold-based (SB) or scaffold-free (SFB), microextrusion based bioprinting (MBB), droplet (DBB), laser (LBB) or stereolithography (SBB).
6. **Application**: Throughout the printing process, from pre-printing to end-user, products must be kept in a cell-friendly, sterile and supportive environment that maintains the viability, functionality and structural integrity of a tissue.
    - *Considerations*: product type, culture, incubation, cell viability, maturation, implantation, scale-up, testing *in vitro*, and transportability.

Image 2 is a prototypical visualization of the 6 steps involved in the bioprinting process, from imaging to application. Each step of this process is examined in detail throughout this report.

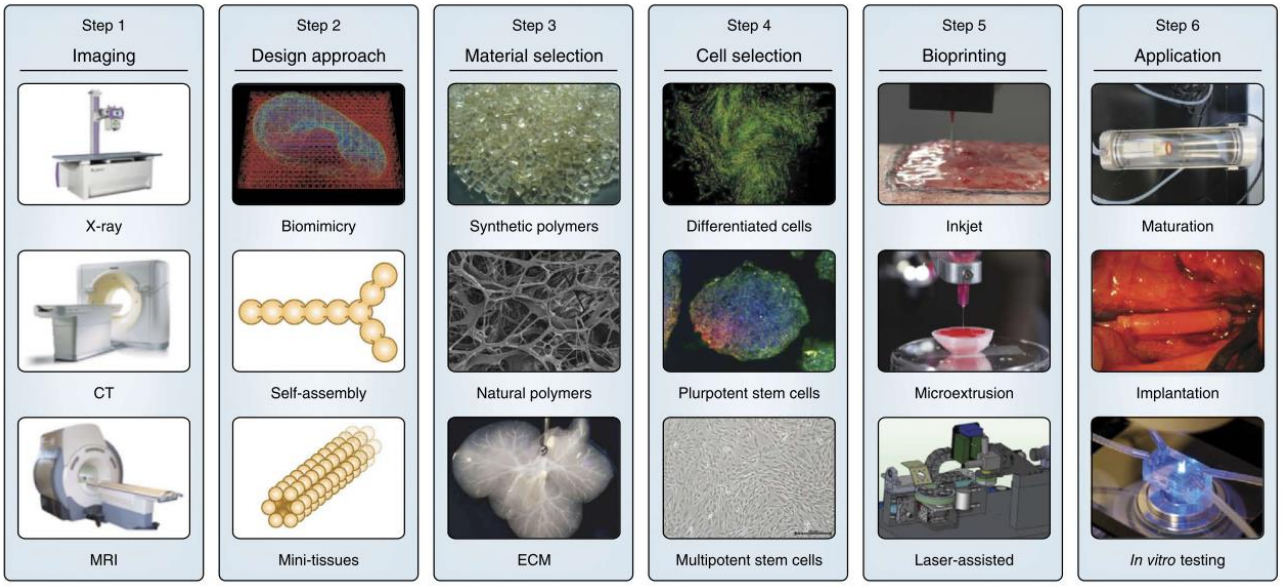

Image 2: A Typical Process for Bioprinting 3D Tissues
Image reproduced from ref. #[24]

Bioprinting History

To fully appreciate the current state of bioprinting it is essential to understand its origins, which date back to basic printing processes and the emergence of additive manufacturing in medical applications. Printing technologies and developments have increased our ability to reproduce and disseminate knowledge across the world and have moved human communication from two to three dimensions. Before the advent of additive printing processes like press, inkjet and 3D printers, written communication was entirely subtractive; stones and chisels were used to mark and inscribe woods and minerals to create records like Hammurabi's Code, Mayan trade-ledgers and the Tablet of the Ten Commandments. Now, additive printing processes allow users to rapidly and uniquely build in 3D. This innovation has modest roots, originating from the first large-scale printing system: the Gutenberg Printing Press.[26]

Both bioprinters, which are 3D-printers that deposit cells and materials in bioprinting processes, and the Gutenberg Printing Press, replicate printed content in a precise mechanical fashion. Bioprinters are defined as automated instruments that are able to additively manufacture 3D scaffolds that have the ability to, "instruct or induce the cells to develop into a tissue mimetic or tissue analog structure, for example, through distinctive cell interaction, hierarchical induction of differentiation, or functional evolution of the manufactured scaffolds falls within bioprinting[1,2]."

Before the printing press, which was invented by Johannes Gutenberg in 1440, only priests, scribes and royalty were literate, and books had to be painstakingly copied by hand. Using the press, books and papers were widely distributed, and the public had access to written knowledge for the first time in history. This meant that oral dialogue was no longer the primary method of knowledge dissipation, and texts like the Bible could be replicated for the world to read, providing an essential platform for the exchange of ideas and philosophies. The increase in literacy across Europe and the printing of the bible in various languages was nothing short of radical - it initiated the European Renaissance and the Protestant Revolution. These

movements, and the ensuing exchange of ideas, were inspired by the power of printing which led to the development of modern science and education, the basis for the society we have created today.[26,27]

This printing press eventually gave way to more advanced modes of printing; before the 1960s, writing and typing were done mostly on typewriters, but after the invention of the printing calculator it was clear that a more direct modality was needed. Calculator printing in the 1960s and 70s was principally done with dot-matrix printers – automated impact printers that were large, slow and loud. The 80s saw the extensive adoption of home computer systems, but these personal computers (PCs) needed an accompanying printer. Hewlett-Packard (HP), Epson and Canon all began research on inkjet printing technology, and in 1980, John Vaught, an HP engineer in Palo Alto, demonstrated a mechanism that used thermal heat to eject ink droplets from a print-head. Vaught was the first to create a scalable printer that could translate PC document data into printed text.[6,28]

In the late 80s and 90s, printing went 3D. Stereolithography (SLA), which uses light to solidify photopolymers, was invented in 1986 by Churles Hull, who later commercialized SLA printers started 3D Systems. After SLA came fused deposition modelling printers (FDM), which are extrusion-based systems that heat plastics, metals and ceramics and dispense them layer-by-layer in 3D. The first FDM printer was patented by S. Crump of Stratasys in 1992[7]. SLA, FDM and even laser-sintering systems, which use lasers to solidify solids, can now print at rapid speeds with resolutions close to the micron level.[24,28]

Over time, 3D printing also became more accessible. In 2008, Dr. Adrian Bowyer's 'Replication Rapid-Prototype' project, called RepRap for short, was completed. This project was an open-source initiative for developing a 3D modelling, testing, and printing system that could print parts to duplicate itself. RepRap set the precedent for future crowd-source 3D printing initiatives that not only increased the number of 3D printers entering the market, but drove up the technological capabilities of 3D printers while driving down their price.[29,30]

In industry, high-resolution 3D printers started to be used for rapid-prototyping and modelling through the 1990s, and in the early 2000s on-demand printing of industrial parts became a market standard. Now, 3D printers are being used to create incredible inventions: In April of 2017, engineers at the University of Southampton released the world's first 3D-printed unmanned aircrafts into the skies, and ecologic researchers at Kor manufacturing have been driving Urbee, a lightweight 3D-printed car that purrs along at 200 miles per gallon.[28]

As the precision and speed of 3D printers advanced, they were adopted for use in the medical world for prosthetics. At first, they were used to create crude instruments for surgeons, but now they're being used for personalized therapies. These therapies include patient-specific hip and knee replacements, miniature synthetic blood vessel stents and plastic segments of human skull which are now being produced large-scale by startups. The fetchingly titled Limbitless Solutions Company sells $300 3D-printed prosthetic forearms which can be controlled by the patient's bicep muscles, CSIRO prints metal sternums and ribs for patients with chest sarcomas, and orthodontists are producing 3D plaster models and aligners as non-invasive dental solutions.[31,32] Similarly, one of the most advanced products to date is the Fortilink-C IBF/VBR System, marketed by RTI Surgical and produced with Tetrafuse 3D printing technologies, which is a nano-rough porous surface plastic used for spine implants that increases fusion with nearby bone segments, has high antibacterial characteristics and bone-

like mechanical properties. This implant improves upon the current gold standard for cervical discectomy and fusion surgeries, like titanium-coated substrates and other plastic implants with minimal cellular fusion potential.[33]

What's more, surgeons are now using MRIs of a patient's unique organ or internal tissue environment to 3D print anatomically precise replicas of their anatomy. Surgeons can use this model, which could be a heart, brain or liver, to practice procedures stress-free, or visualize a diseased area pre-surgery. This preparation has resulted in dramatically improved outcomes during complex surgeries. These medical applications showed that it was possible to use 3D printing technologies for therapeutic purposes.[6,34]

As 3D printing was adopted in curative settings, researchers began to wonder if in addition to metals and plastics, living cells could also be used as printing material. The first instance of bioprinting was in 1988, when Klebe positioned living human cells, taken from differentiated or cultured laboratory cell lines, using a modified HP inkjet printer to build 2D synthetic tissues. This experiment sparked the intensity of innovation and development in bioprinting technologies the field has experienced over the past two decades. Soon after, in the late 1990s, Thomas Boland's Lab at Clemson University applied this technique to build 3D structures with living components by replacing ink cartridges with cellular components, and the paper with an electronically controlled receiving substrate mobile in the x, y and z directions. This approach crystallized the possibility of printing live cells with a 3D orientation.[3,35] An example of a bioprinting process that converts medical images into 3D tissue structures can be observed in Image 3. Klebe's first experiments with cytoscribing laid the groundwork for the development of these types of bioprinting techniques, which are now utilized in bioprinting labs across the world.

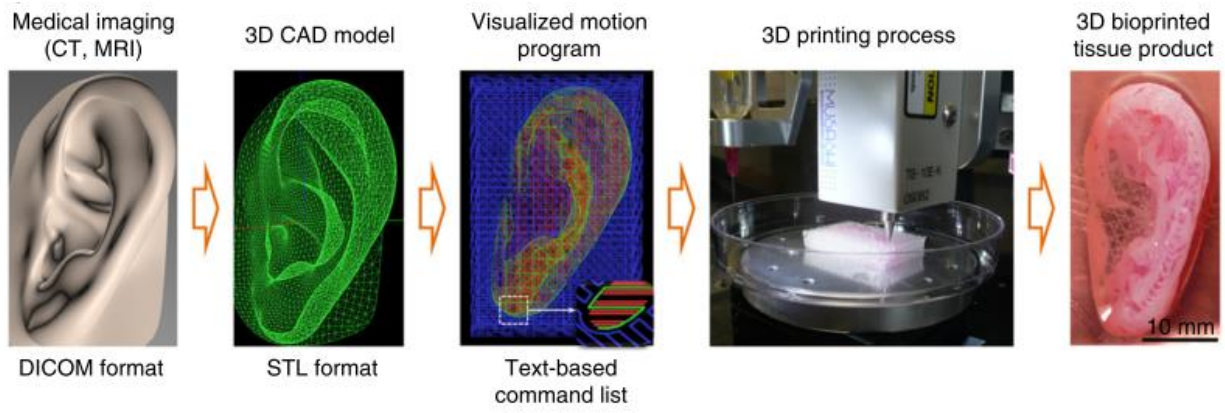

Image 3: Example Bioprinting Process
Reproduced from ref. #[17]

Capitalizing on Klebe's foundational idea, a research team at Wake Forest developed a bioprinter capable of printing tissue products with clinical application[8]. In 2004, the team printed biomaterial scaffolds to grow autologous bladder cells into pouches, which were implanted around the bladders of seven children with end-stage bladder disease who required cytoplasty[8,36]. The team printed biodegradable bladder-shaped collagen scaffolds, seeded them with autologous bladder cells, cultured the constructs for seven weeks, and implanted them around patients' bladders. Urodynamic studies five years post-implantation showed improved bladder compliance and capacity, increased structural and functional integrity, and no reported adverse side-effects.[37] This pioneering study demonstrated the therapeutic potential for bioprinting tissues that can be transplanted in humans. Since 2004, further *in vivo* experimentation has been limited, but the excitement and intensity of bioprinting research has grown and led to the production of increasingly innovative and impactful TE aproaches.[8,15]

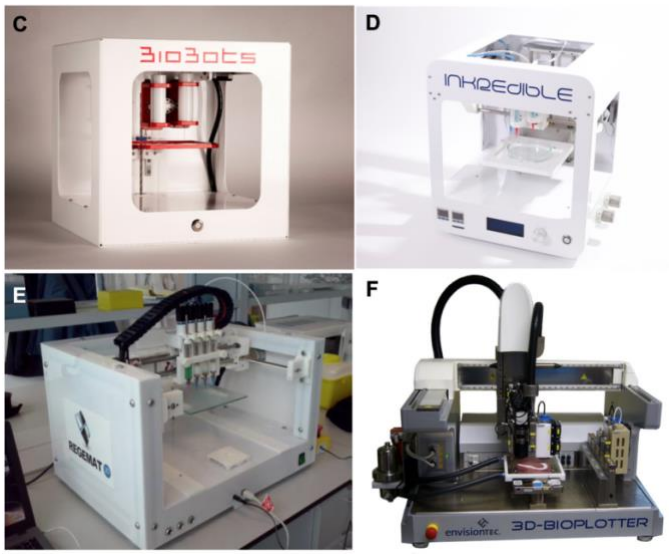

Image 4: Examples of Modern Bioprinting Systems
Reproduced from ref. #[38]

In summation, first came the printing press, followed by inkjet and 3D printers, and now we have bioprinting. Each of these printing platforms has been developed alongside the most impactful technologies of their generation and served to revolutionize how these innovations were replicated and communicated. The printing press initiated a golden age of ingenuity and research through the propagation of scientific knowledge, the inkjet printer allowed computing systems to translate digital code and text to paper, 3D printers created a new way to accurately build individualized industrial and medical products, and bioprinters have established a groundbreaking methodology for manipulating living materials with real-world therapeutic application. Now, bioprinting is moving the TE field towards the ultimate goal of RM: the production of a renewable source of human anatomy.

Terminology Clarification: Biofabrication, Bioprinting and Bioassembly

The discussion of bioassembly and its classification as a bioprinting technique raises critical terminology issues, as the current literature does not clearly differentiate between and define the terms bioassembly, bioprinting and biofabrication[2]. In this report, we use the term 'bioprinting' as an all-encompassing term that refers to both 'bioprinting' and 'bioassembly' strategies, which are all referred to as 'biofabrication' approaches. This is because biofabrication is intimately intertwined with the interdisciplinary fields of TE and RM and includes both bioprinting and bioassembly processes. Biofabrication is defined as, "the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, or hybrid cell-material constructs, through *bioprinting* or *bioassembly* and subsequent tissue maturation processes[1]." This definition refers to bioprinting and bioassembly as disparate approaches, largely derived from antiquated differentiations between their functional building unit.

Differentiations between the dimensions and scale of the functional building unit used in bioprinting and bioassembly approaches has traditionally distinguished these terms; bioprinters print at the cellular (sometimes referred to as the 'molecular') level, while bioassembly techniques manipulate larger microtissues at the organoid level. Cellular-level bioprinting involves directly printing cells onto a substrate or encapsulating cells in bioinks, and organoid-level bioassembly aligns microtissues in a designated structure.[1] Nonetheless, both methodologies seek to manipulate cellular components in the aim of constructing 3D living tissue architectures with complex geometries through an automated process with the ultimate goal of replacing and regenerating human anatomy using manipulated cellular components. Given this combined ideological and functional biofabrication approach, future biofabrication techniques will likely be contingent on both bioprinting and bioassembly techniques used in conjuncture to form engineered constructs with micro- and macro-constituents (using both cellular and organoid building blocks). Therefore, it is appropriate to include 'bioassembly' within the umbrella-term of 'bioprinting'.

Why Bioprinting?

Bioprinting is being embraced throughout the TE field because it boasts seven differentiating advantages. These abilities can be observed across a variety of technical areas:[24,39,40]

1. **Deposition**: The alignment of biomaterials, growth factors, hydrogels, genes, living matter, and neo-tissues in complex 3D frameworks.
2. **Accuracy**: The deposition of biologics and soft materials in a layer-by-layer fashion with precise control of material and chemical properties, such as degradability, pore distribution, and polymerization mechanisms.
3. **Scaffolds**: The fabrication of structurally sound, porous and biologically supportive scaffolds, or the precise manipulation of microtissues in scaffold-free approaches.
4. **Software**: The application of 3D imaging and computer-aided patient data in custom deposition pathway planning.

5. **Heterogeneity**: The simultaneous manipulation of multiple biomaterials and cell types, and consequent co-culture of heterocellular constructs.
6. **Automation**: The robotic control of reproducibly patterned tissues in a high-throughput manner.
7. **Accessibility**: The creation of tissues either on-site for on-demand application or off-site manufactured products (and consequent preservation and shipment)

Compared to bioprinting, the manufacturing capabilities of conventional TE approaches, like solvent-casting, molding, electrospinning, gas foaming, particulate leaching, freeze-drying, and emulsification are limited, and have only been able to produce 2D tissue sheets 1-3 cells thick[41]. These thin, low-density cultures are easily constructed, perfused and studied, yet do not mimic native cellular behaviors and interactions, given that much of the living components are in contact with a synthetic substrate. This results in poor histochemical and biological functionality and limited therapeutic applicability. Non-bioprinting TE approaches also exhibit limited control of 3D structural and physiochemical properties like porosity, scaffolding architecture, bioactivity and material distribution, and have a restricted range of amenable materials.[6]

In the construction of a report that informs researchers and contributors about the most impactful trends and technologies shaping the bioprinting landscape, primary and secondary research tools were recruited to answer the question of, 'why bioprinting?'. It is essential to develop a nuanced answer to this question, so that the benefits of bioprinting and the justifications for using these technologies over alternative TE methods can be completely understood.

During primary interviews, we developed an answer to this question by asking bioprinting researchers, developers and experts how and why they employ bioprinting in their research labs and biofabrication facilities. Interviewees were asked, "Why have you decided to use bioprinting as opposed to alternative TE techniques, and what are the advantages and disadvantages of bioprinting techniques?". The representative response samples shown in Figure 1 illustrate macro-themes and patterns observed during all 18 interviews, which were correspondingly identified during literature searches and analyses. Selected responses were compiled, and these direct quotes from interviewees were organized based on the respondent's level of development, which spanned from 'research' focused institutions to 'established' commercial entities:

Figure 1: 'Why Bioprinting?' Responses

It is evident, given the information compiled in Figure 1, that established players prefer bioprinting because of its large-scale manufacturing capabilities, while university and academic players are attracted by the high geometric control and unique multi-material processing power of bioprinting approaches. This finding is logical, given players with industrial mindsets must consider GMP and scalable metrics in order to create a commercial product, while university researchers are less concerned with wide-reaching product distribution and more apt to utilize a technique with superior technical printing qualities. Given these advantages, bioprinting will be a critical component in the greater TE manufacturing arena, but this technology must be supported by scale-up processes and inputs to ensure future growth. These supporting factors must be compatible and synergistic with bioprinting technologies in moving tissue products from print-bed to the end-user; they include a variety of material, technical and administrative products, which can be found in Table 1.

Table 1: Manufacturing Considerations for Bioprinting

Inputs	Cells, biomaterials, reagents, hydrogels, scaffolds, living components, synthetics, bioinks
Equipment	Manufacturing equipment, processes and software, bioprinters, alternative TE systems
Robotics	Automation, software
Characterization	Measurement and data management technologies, *in vitro* sensing, live 3D monitoring
Transport	Preservation, transport and storage systems, production facilities, on-site bioprinters
Regulation	Quality systems (QbD), autologous product organization and culture, GMP

Across the board, respondent groups also believed that bioprinting would be used alongside alternative TE approaches in the near future – they did not believe it was the only solution for the technical challenges facing the TE field. This universal finding agrees with the current literature, which cites that other TE methods must supplement bioprinting methodologies in order to create fully vascularized and functional tissue products. The incorporation of vascular networks into tissue structures is integral to this observation; it is clear that bioprinting alone will not be able to create hierarchical vessel structures down to the capillary level[42]. Alternative TE approaches that are thought to supplement bioprinting towards creating vascularized tissues are listed in Table 2.

Table 2: Comparison of Vascularization Approaches

Strategy	Materials/Instruments Required	Advantages	Disadvantages
Cell-Cell Interaction	Proangiogenic cells, supportive culture techniques	Effective vasculogenesis at the capillary level, controlled composition	Lack of precision/resolution in network architecture
Functionalized Scaffolds	Angiogenic growth factors, biodegradable polymers	Controlled release of growth factors	Lack of precision/resolution in network architecture; control of factor gradients through space and time
Microfluidics	Active flow source (i.e. pump), soft-lithographized polymer, supportive scaffolding	Ability to administer and study defined flow rates, generate growth factor gradients, and control ECM	Lack of precision/resolution in network architecture
Sacrificial Materials	Dissolvable polymers or removable molds	Increased precision for defined network architecture compared to other approaches; ability to integrate with alternative strategies	Possible cytotoxicity with undissolved material; multiple processing steps
Spatial Micro-patterning	Soft-lithographized or photo-lithographized polymers	High geometrical precision, controlled cellular and growth factor patterning/ presentation	Size-scale is limited; Background blocking can change substrate properties
Decellularized Scaffolds	Donor tissue/organ, chemical detergents	Native vascular network is largely preserved	Chemical detergents can destroy integrity of tissue; requires donor tissue/organ; antigenicity from xenogenic tissues
3D Bioprinting (scaffold-based)	3D bioprinter, compatible inputs (biomaterials, cells, inks, gels)	High precision in 3D structure	Technology still in infancy; Uncharacterized bioprinting processes; resolution restricted to macro-vessel level
3D Bioprinting (scaffold-free)	3D bioprinter, proangiogenic cellular building blocks	Entirely biological; harnesses de novo vasculogenic processes	Lack of self-assembly control; manipulation impacts on cell viability unclear

Table adapted from ref. #[42]

Microtissue Construction Processes: Biomimicry, Self-assembly and Bioassembly

Three central processes driving tissue creation are utilized in 3D bioprinting: biomimicry, self-assembly and bioassembly[24]. Biomimicry precisely replicates the biological and anatomical features of a tissue or organ space, which requires high-precision imaging and spatial control. In order to inclusively position the required intra- and extra-cellular components in an anatomically-correct fashion, a complete functional understanding of the native extracellular matrix (ECM) must be developed. The accurate recreation of the ECM is critical for TE and bioprinting approaches because the mammalian ECM supports cellular adhesion, communication and differentiation through a combination of fluids and molecules that provide essential signaling and nutrient pathways between cells. The ECM can be found within all tissues of the human body and includes both the interstitial matrix (which composes the interstitium) and the basement membrane of cellular structures. Correspondingly, the form and function of these biological specifications must be accurately recreated in four-dimensions (4D) by controlling factor gradients, ECM interactions, and cell-cell positions using a bioprinter[43]. To a limited extent, current bioprinting technologies can control these components and recreate foundational aspects of native biology *in vitro*, but a TE method that can produce tissue structures that fully mimic anatomical parameters is absent. For this reason, many TE approaches are now using the body's own remodeling tendencies to complement direct biomimicry.[16,24,36]

The body naturally reacts to injuries by repairing and rebuilding itself through a coordinated wound-healing response, which includes inflammation, ECM remodeling, granulation and new tissue formation. By harnessing key drivers of this process, the natural embryonic tendency of cells to self-organize, proliferate, fuse and heal can be used to mimic developing tissues in a process called autonomous self-assembly. Intricately complex chemical and biological gradients, cues and signals control self-assembly to construct vascularized tissues replete with micro-architecture like capillary beds, nephron tubules and neurons. It is difficult to conceptualize an imaging and printing process capable of recreating these high-resolution anatomical structures, but it is possible to engineer cellular environments that support self-assembly and guide histogenesis in a controlled manner. This can be observed in the creation of embedded microvasculature tumor-model networks[44] and in the fusion of cellular components to establish macro-tissue nerve structures[45]. Additionally, self-assembly is used alongside biomimicry approaches to fill in high-resolution chemical and biological details, and in parallel with bioassembly in the construction of self-aggregated organoids.[16,24,25,36]

Bioassembly involves the manipulation of mini-tissues, the smallest structural and functional unit of an organ, which could be a miniature neuronal network, cellular spheroid or liver islet. These units are created through a combination of both precise cellular positioning (biomimicry) and self-aggregation (self-assembly).[1,2] One bioassembly approach involves culturing microtissue building blocks, which are small-scale components of a larger tissues, like sheets[46], spheroids[47], honeycombs[48] or toroids[49]. These blocks are self-assembled by allowing cells to naturally conglomerate as they attempt to reduce their collective surface area, increase cellular interactions and consolidate their ECM[16]. In another approach, miniature cellular environments can be created on microfluidic chips that provide supportive architectures for cells to self-assemble. This technique is most prominent in the development of organ-on-a-chip

models[44], where microtissues of various shapes, sizes and cell types can be co-cultured in a matrix alongside supportive guiding factors and centralized flow[50]. These two bioassembly strategies are both considered scaffold-free approaches, as they minimize the interaction of cells with scaffolds and extraneous biomaterials during the printing process.

Short- and Long-Term Goals

Outline:

1. Short-Term Goals
    a. *In vitro* models
        i. Need: replacement for animal models, 2D culture platforms and early clinical trials
    b. Simple avascular tissue implants
        i. Need: wound healing, basic replacements
2. Long-Term Goals
    a. Vascularized tissue implants
        i. Need: organ regeneration and function reconstitution
    b. Organ transplants
        i. Need: organ transplantations

Short-Term Goals

There are two short-term goals of the bioprinting field. The first is to produce scalable, reliable and reproducible *in vitro* platforms for pharmacokinetic testing, and the second is to create simple anatomical structures for therapeutic purposes that supplant current gold standards of treatment.

3D *in vitro* tissue models have the potential to supplement (or replace) animals and early human clinical trials in a range of scenarios, including drug-testing, toxicology and cosmetic evaluations. This need is driven by shortcomings of current testing procedures, which represents a substantial and largely untouched market potential. Animal models do not accurately mimic human biology and carry enormous ethical and social insinuations, and PETA cites that 100 million animals are killed in US laboratories each year[51]. Furthermore, early human trials are exploratory, expensive and hazardous. With proper alignment and conditioning, 3D architectures more closely resemble *in vivo* drug responses, measures of toxicity, tumors and diseases than 2D cultures. This is because 3D tissues have heterogeneous microarchitectures that mimic the cell-cell and cell-matrix interactions of native tissue environments[52].

One application of this translatable functionality of *in vitro* tissue models is in renal pharmaceutical drug testing, which is already being adopted and commercialized by industrial players. To construct renal tissue platforms, iPSCs from a human kidney are sequestered, differentiated and bioprinted onto a simple 3D chip which accurately recreates renal physiological responses to mechanical and chemical stresses. For safety, efficacy and dosage testing, the chip is integrated into a microfluidic bioreactor, pharmaceutical drugs are administered into the system and cellular responses are measured using a host of sensors and monitors.[50] Similarly, in pathological studies, infected or malfunctioning human cells can be cultured *in vitro* as a biomimetic recreation of internal disease in a dynamic 3D environment. This is especially beneficial for modelling individualized diseases like HIV or cancer which can be isolated, examined and treated with a personalized therapy. *Ex vivo* pathological analysis allows

for a sensitive and acute understanding of an individual's specific cellular disease state through non-invasive testing.[53] 3D bioprinted models are in the early stages of pharmaceutical adoption, but further product characterization and the development of reliable, scalable and economically advantageous biomanufacturing platforms must occur before they are adopted wide-scale.[22,54]

The second short-term goal of bioprinting is to create simple tissues that can be used in clinical scenarios to aid in the body's healing and regeneration process. To date, epidermal layers, bladder pouches, cartilage, aorta, and trachea have been bioprinted and tested in humans[24]. These products are predominantly avascular and do not accurately replicate the cell density, microvasculature, or overall function of anatomical features. Many companies are also working to produce bioprinted tissues that can regenerate modular portions of human anatomy, like soft-tissue liver patches[55], mandible bones, ears, heart valves and macro-vasculature structures, but these products have yet to be successfully tested in humans, and are still being tested in proof-of-concept studies.[9,17]

Recently, increases in funding allocations, sponsorships and investments of government agencies like the Department of Defense (DOD) and National Aeronautics and Space Administration (NASA) towards biofabrication and bioprinting technologies have spurred further developments. The DOD is hoping to eventually use bioprinted tissues to help regenerate and heal soldiers on the battlefield, and to improve prosthetics technologies for veterans. NASA hopes that simple tissue grafts can be used to aid healing processes of astronauts in outer space, where the boy's natural regenerative processes are stymied by extreme conditions[56–58].

Long-Term Goals

The long-term goal of bioprinting is to provide an on-demand source of transplantable organs for patients with damaged or non-functional organs and tissues, which is primarily driven by the massive worldwide need for organ and tissue transplants. Rising demand for tissue replacements is due to a rising prevalence of diseases like Chronic Kidney Disease and a growing geriatric population demographic[59]. In 2017, there were over 120,000 people on the American organ waiting list, yet only 26,034 transplants were performed between January and September[60].

Fully transplantable bioprinted organs will not be ready for therapeutic application for another 3-4 decades, given the high number of unknown variables and prohibiting limitations that must be overcome. In the meantime, vascularized tissues that can be implanted to reconstitute organ functionality, such as liver patches to heal IEM defects, can be applied. From the technical perspective, engineered tissues must mimic the complex biologic anatomy, diverse heterocellular composition, and extensive vascularization of native tissues in order to regenerate or replace their basic functionality. From the regulatory side, manufacturing and biofabrication processes must align with governing agencies and standards of good manufacturing practices (GMPs), sterility, and Food and Drug Agency (FDA) regulations.

Section 2: Vascularized Tissues

Our primary and secondary research showed that the vascularization issue is a significant limitation not only for the bioprinting community, but for the TE and RM fields in their entirety. These findings are described in 'Section 4', where we examine how acutely restrictive the vascularization issue is to the growth of these fields and the goal of producing functional tissue products, as vascular networks must be established in tissue products if they are to reach their full functional potential and be adopted wide-scale[52,61]. Thick tissues found in the human body require dense capillarization to maintain cellular viability and functionality, which must be recreated in order to produce bioprinted tissues that accurately mimic *in vivo* tissue environments. In this section, we describe the vasculature issue and outline critical characteristics, anatomical components and growth processes of vascular networks. All provided information was sourced from the current state-of-the-art vasculature literature and medical studies.

The Vasculature Issue

The vasculature issue is largely technical in nature; the creation of vascular networks within bioprinted products will be contingent upon both the technical approaches and technologies of the bioprinting field and in the establishment of enhanced biological and chemical understandings of cellular environments. Here, we examine the physiochemical, biological and anatomical factors that govern human vascular networks. We do so because tissues for *in vitro* drug testing, patch replacement therapies, vascular prostheses and integration into engineered tissues must be provided a sufficient exchange of nutrients and oxygen through diffusion with vasculature in order to maintain cell viability[5]. On the acutely biological level, a vascular network must reach proximally to all cells in a living tissue construct to overcome the diffusion limit, which is between 100µm and 200µm at native cell-densities.[6] If engineered or bioprinted vessel networks are to overcome this diffusion limit, they must be:

1. Capable of perfusing all cells in a tissue by providing adequate oxygenation and nutrients while simultaneously removing cellular waste products[43]
2. Organized in a hierarchical branching structure that includes arteries, veins, and microcapillary beds from the micro- to macro-level[62]
3. Able to selectively control diffusion across vascular membranes between the lumen flow-source and surrounding tissue to maintain hemostatic fluid levels: too much outflow results in cellular edema, and too little results in hypoxia, necrosis, and intra-vessel aggregation of cellular waste products[5]
4. Easily connected to an external vasculature, either within a patient (*in vivo*) or another tissue structure (*in vitro*), which can be accomplished via microsurgery of large vessel sutures or proximal alignment of capillary beds with rapid inosculation[63,64]
5. Dynamic in its reaction to and control of biological and mechanical elements (like pulsatile flow, shear stress, hypoxia and endemic pressures) using a variety of cellular and chemical pathways (like VEGF secretion, mural cell recruitment, and SMC vessel integration)[65]

The minimum viable vascular network integrated in a tissue construct must embody these characteristics, and in doing so will have to incorporate and mimic many aspects of the human vascular system anatomy. An in-depth discussion of the human vascular system's form and function is provided below.

Human Vascular Anatomy Overview

If dynamic and personalized macro-network vascular tissues are to be constructed using bioprinting, an intimate understanding of the hierarchical and organ-specific native vascular tree must be cultivated. The modular form and function of these vessel types must be considered and incorporated into any biomimetic vascular bioprinting endeavor so that vessel cell types, anatomical structures, supporting components and geometric characteristics can be recreated accurately to produce a functional bioprinted vascular product.[66]

Vessels are categorized as arteries, capillaries, veins or lymphatics; larger vasculatures like veins and arteries are distinct anatomical entities, while micro-vessels like capillaries are comprised in the tissue where they are located. The organization of these vessels, moving away from the heart, into the body's tissues and back to the heart once again, is outlined below. Lymphatics are included in this overview but do not comprise the systemic or pulmonary circulation.

At a macro-level, the circulatory system pumps oxygenated, nutrient-rich blood to the body's tissues and then removes and transports used fluids. The pulmonary circulation is a loop that oxygenates blood through a gas-interface in the lungs and returns plasma to the heart, where freshly oxygenated blood is driven into the systemic circulation. This system forces blood through arteries towards capillaries, which are embedded in the body's tissues. After capillary diffusion, oxygen-depleted blood enters veins, which provide passive transport back to the heart and the pulmonary circulation once again. These circulatory systems are organized based on the anatomy of their functional components:

1. **Arteries**: carry oxygenated blood away from the heart to the body
    a. Arterioles: small, branching arteries
    b. Metarterioles: connect arterioles to the capillary network
2. **Capillaries**: diffusion site within the body's tissues
    a. Continuous: small-molecule exchange
    b. Fenestrated: dynamic, fluid-controlled exchange
    c. Sinusoid: large-molecule exchange
    d. Post-capillaries: connect capillary bed to venules
3. **Veins**: carry recycled blood and metabolic waste to the heart from the body's tissues
    a. Venules: drain capillary beds, connect to veins
4. **Lymphatics**: filter and transport lymph fluids
    a. Vessel types:
        i. Prelymphatics: microscopic vessels within tissues
        ii. Lymphatic capillaries: drain prelymphatics
        iii. Collecting ducts: vein-like vessels that drain capillaries
        iv. Lymphatic trunks: thick vessels that move lymph to duct drainage regions

b. Lymph Nodes and Organs: filter and clean lymph fluids

These functional components can be observed in Image 5, which identifies the major arteries and veins of the human vascular network.

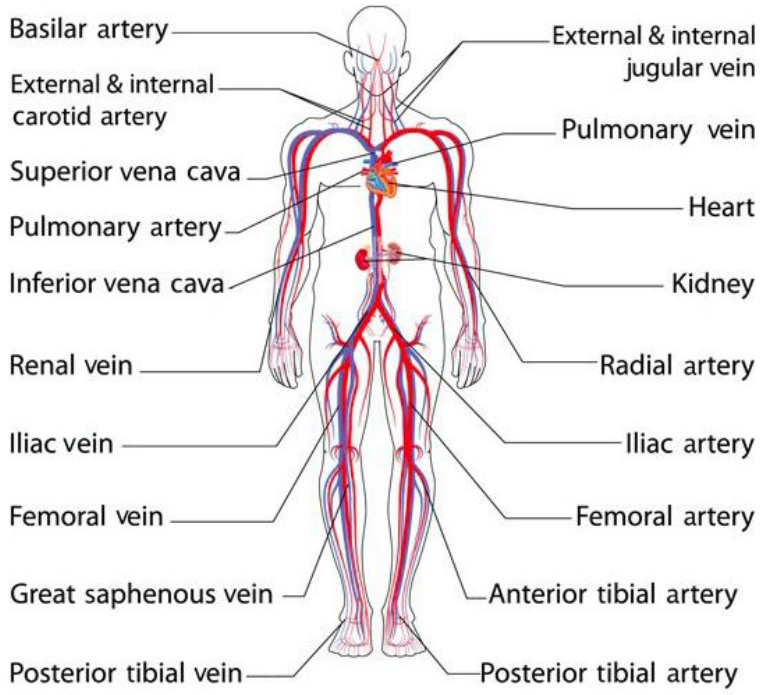

Image 5: Macro-components of the Human Vascular Network
Reproduced from ref. #[67]

While the form and function of vessels throughout the native hierarchical vascular tree varies immensely depending on localized form and function, the system maintains several fundamental characteristics. The human vasculature system carries 9% of total body fluid volume (5.4 liters) through vessels with inner diameters (IDs) that range from 8µm capillaries to the ~3cm aorta[66,68]. Most vessels have three anatomical components, including a single flat inner lining of endothelial cells (ECs), an elastic layer of collagen fibers, and smooth muscle cells (SMCs) controlling constriction and dilation, which are all divided into the intima, medial and adventitia layers[8,69].

1. **The intima layer** is a thin EC barrier between the lumen and wall of a vessel that reduces thrombogenicity and infection rates[66]. The basement membrane is the 2nd innermost lining of this layer, is composed of Type IV collagen and laminin and is surrounded by a protective internal elastic lamina.
2. **The medial layer** is composed of Type I and III collagen and SMCs that control vessel contractions and is surrounded by an external elastic lamina. This layer is thick in large arteries because of the need for contractile properties and extensive central nervous system (CNS) innervations.

3. **The adventitia layer** is composed of fibroblasts secured in a loose collagen matrix and stabilizes blood vessels by anchoring them to surrounding tissues and organs.

These anatomical components are largely consistent throughout human vessels, as displayed in Image 6, but the four categories of vasculature exhibit a wide range of anatomical characteristics depending on their location and function in the body.[66,70,71]

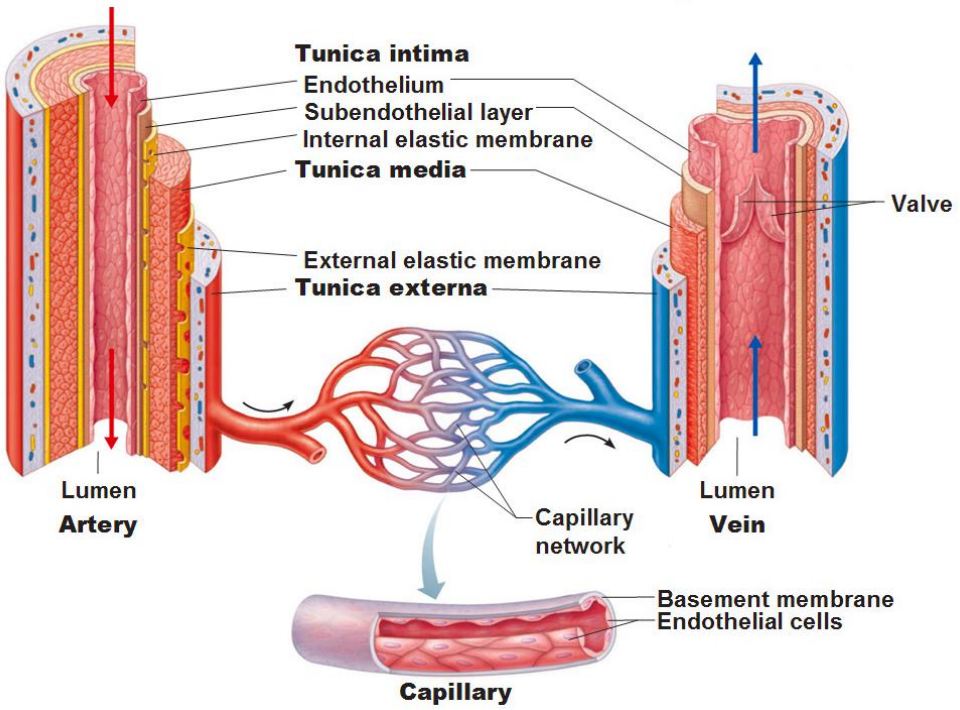

Image 6: Blood Vessel Anatomy
Image reproduced from ref. #[72]

Arteries, Capillaries and Veins

Arteries and arterioles carry blood, full of oxygen and nutrients, from the heart to every living tissue in the body. Arteries have an average diameter of 20-30µm, and have thick muscular walls composed of many layers of SMCs. This high percentage of SMCs makes capillaries highly elastic, distensible, and capable of withstanding shear stresses imposed by high-pressure blood flow. These macro-vessels dictate blood pressure using contractile muscular forces that are mediated by a variety of factors, including extracellular metabolic wastes, sympathetic innervations, and hormones like noradrenaline. Arterioles are smaller, thinner arteries 10-200µm long with an average ID of 30µm. Arterioles help convert cardiac ejections to vascular pressure, and contain high numbers of sympathetic neurons so they can rapidly expand and contract when required.[5,66,70,71,73]

Pressurized blood flows from arterioles into capillary networks, where solute is exchanged between blood and tissues. Capillaries are minute in size, with an average ID of 8µm and a minimum viable ID of ~4µm, yet they comprise the bulk surface area of the vascular system. The majority are composed of one EC layer and a basement membrane of structurally

supportive pericytes, but no SMCs. Endothelial capillary walls have micro-spaces between cells ranging from 10-25nm, which differ in size depending on the surrounding tissue type. Capillaries are categorized by these intra-membrane distances as continuous, fenestrated and sinusoid:

1. **Continuous capillaries** have an uninterrupted basement membrane with tight EC junctions, and are found in areas with primarily small-molecule exchanges in muscle, skin, lung, CNS and post-capillary venules.
2. **Fenestrated capillaries** are more porous, with ~1000 pores/micron^2 that are 25nm thick and 100nm in diameter. These can be found in dynamic exchange areas like exocrine and endocrine glands, the renal medulla, intestinal mucosa, or structures producing and controlling fluids like the renal glomerulus and cerebral choroid plexus.
3. **Sinusoid capillaries** have incomplete basement membranes with large gaps between ECs that allow for whole-cell and large-molecule exchanges at locations like the liver, spleen and bone marrow.[66,70,74,75]

The capillary semipermeable membrane allows oxygen, salts, glucose and other nutrients to flow out of the low-pressure blood and into surrounding cells, while waste, metabolites and CO_2 can flow back in. The differential solute permeability created by cellular gaps prevent larger molecules, like proteins, to pass back and forth. Macro-molecules in capillaries create fluid-attracting osmotic gradients that oppose hydrostatic pressures working to expel liquids. As molecules diffuse out of capillaries, a dynamic equilibrium is established that increases inter-vessel osmotic blood pressure closer to veins. Imbalances during this hydrostatic pressure exchange lead to edema, which occurs when water collects in cavities and causes tissue-swelling.[5,66,70]

Varying metabolic requirements in tissue types requires differing capillary volumes; in the brain, kidneys and liver capillary density is roughly 2500-3000/mm^3, while in skeletal muscles it is 300-400/mm^3 with 8-10 capillaries per metarteriole. Metarterioles are where capillaries connect to arteries through pre-capillary sphincters, which are the only contractile element in the capillary system. Metarterioles are 10-20µm in ID and derive from terminal arterioles that have IDs of ~10-50µm. Post-metarteriole transport, capillaries merge with post-capillaries (ID 8-30µm) and finally collecting venules (ID 10-50µm).[66]

After diffusion, venules, which are small veins with an ID of 8-100µm, drain capillaries arising from metarterioles and return this low-pressure deoxygenated blood to veins. Veins and venules are highly distensible, experience low shear-stresses, contain unidirectional valves to prevent fluid backflow, and carry close to 70% of a human's blood volume. They rely primarily on skeletal muscle contractions and organ gyrations to involuntarily propel blood flow towards the heart but do have small numbers of minimally constrictive SMCs. Their walls have a thin EC lining reinforced with elastic fibers encompassed by an outer tunica externa composed of connective tissue.[65,66,70]

Lymphatic System

The lymphatic system filters blood and transports uncollected plasma back to the central circulatory system. Similar to veins, lymphatic vessels are low-pressure and low shear-

stress, rely on involuntary skeletal muscle and organ movement for fluid locomotion and contain unidirectional valves for backflow prevention. These vessels provide an alternate route for lymph to return to the arteriovenous circulation. Lymph is an alkaline ultrafiltrate of blood plasma that has seeped through capillary walls and has a similar composition to interstitial fluid. Lymph organs produce lymphocytes and act as phagocytic filters of RBCs, bacteria and particulates in lymph fluid. Lymph nodes, which are an abundant lymph organ, filter plasma for bacteria and pathogens, and are connected to 6-10 lymph vessels each.[66]

Lymph vessels have similar structures to veins but have thinner walls, more valves, and contain interspersed lymph nodes. The hierarchical organization of the lymphatic vessels, from smallest to largest, includes the prelymphatics, lymphatic capillaries, collecting ducts and lymphatic trunks:[66]

1. **Prelymphatics** are non-EC tissue channels with IDs of .1-.2µm that penetrate tissues of the body. These microscopic passages are drained by lymphatic capillaries, which are porous EC tubes 15-75µm in ID.
2. **Lymphatic capillaries** are more porous than blood capillaries, with endothelial cell-cell gaps ranging from .1-3µm.
3. **Collecting ducts** are larger vein-like vessels that drain the capillaries and move lymph to the lymphatic trunks.
4. **Lymphatic trunks** are thick-walled macro-vessels that drain lymph from the body's right-duct and thoracic-duct drainage regions.

Micro-network Processes: Vasculogenesis and Angiogenesis

Vasculogenesis and angiogenesis are natural processes that develop micro-networks at the capillary level; vasculogenesis is the generation of blood vessels during embryonic development and angiogenesis is the growth of new vessels from pre-existing vasculature structures[70]. Throughout both processes, biochemical and biophysical cues control cellular differentiation into arteries, veins and connecting capillaries[18,65].

Both vasculogenesis and angiogenesis must be precisely controlled to properly integrate micro-networks within bioprinted tissues using both biomimicry and self-assembly. Strategies include progenitor cell recruitment, environmental controls (like introducing fluid flow), or stimulation of vascular remodeling agents[5]. To this end, various bioprinting strategies, including spatial micro-vessel deposition within hydrogels[5,76], printing of growth factor gradients[77] and alignment of parallel microfluidic vascular tubes inside cellular beds[78] have been investigated. These approaches, and all micro-network approaches yet to come, are impingent upon an extensive appreciation and understanding of neovascularization processes, as direct spatial construction of micro-networks at the sub-micron level is far beyond the capabilities of current bioprinting and TE technologies.

Vasculogenesis

Vasculogenesis progresses *de novo* alongside hematopoiesis and contributes to adult tissue regeneration processes. During embryonic vasculogenesis, EC precursors, called angioblasts, differentiate into ECs to form a primitive capillary network. In adults, bone-marrow

derived EPCs or circulating ECs are mobilized, enter the circulation, travel to a damaged tissue or tumor site, and differentiate into mature ECs.

The process of embryonic vasculogenesis begins at the yolk sac, where EPCs and HSCs unite to form 'blood islands' within the ECM. ECs rise from blood islands and fuse to form tubular lumen structures, from which the primitive vascular plexus develops. Plexus-derived angiogenic sprouts continue to advance vessel formation and remodel the plexus ECM by creating an interconnected micro-network with limited organization. This establishes a foundational structure for the hierarchical arteriovenous tree for the remainder of embryonic development.[18,65,70,74,79]

Vasculogenesis can be controlled to create disorganized vascular networks within bioprinted building blocks, like organoids or hydrogel scaffolds, through the direct seeding of vasculogenic factors. For example, EPC recruitment is stimulated by molecules like SDF1 and GCSF, which can establish networks that can be expanded *in vitro* and implanted *in vivo* to enhance neovascularization[9,80]. The precise stimulation and control of vasculogenesis during the bioprinting process will be an essential contribution to the creation of vascularized tissues.

Angiogenesis

Angiogenesis is the sprouting of new vessels from pre-existing capillaries and is the primary driver of neo-vessel growth. New vascular networks are controlled, supported and stabilized by both cell-cell and cell-ECM interactions, which are chiefly regulated by local tissue oxygen levels; hypoxic tissues secrete growth factors and chemokines which activate angiogenic inosculation and intussusception.[70,74,81]

Cell-cell interactions recruit vascular support cells to stabilize vessels during angiogenesis. For example, the secretion of PDGF and VEGF by ECs recruits mural cells, like pericytes, to micro-vessels for growth and sprouting support during development, and triggers SMCs to mobilize towards macro-vessels for structural reinforcement. VEGF controls the proliferation, migration, differentiation and survival of ECs via Notch signaling crosstalk, which guides EC sprout-tips and regulates their matured shape and orientation during vessel sprouting.[65,82]

There are a variety of other chemical factors that influence angiogenesis, like bFGF, which controls EC maturation and induces macro-vessel growth. Additionally, Ang1 reinforces EC survival mechanisms and intercellular junction tightening. This factor is inhibited by Ang2, which competes for the same binding site, and is produced by ECs during remodeling. The function of Ang2 is sensitive to VEGF: in the presence of VEGF, Ang2 sensitizes ECs to proliferation signals and proangiogenic factors to stimulate EC migration; in the absence of VEGF, Ang2 triggers EC apoptosis and regression.[74,82]

Cell-ECM interactions are also controlled through signaling cues in dynamic chemical environments. Angiogenesis starts with the disruption of the EC membrane and the subsequent inflow of plasma-derived factors like fibrinogen, vitronectin, and fibronectin, which establish the angiogenic ECM environment. These structured and complex factors require an intimate biological and chemical understanding of their underlying interactions, function and character in order to control them in an accurate and precise manner that triggers angiogenesis.[65]

Intussusception is controlled by these cell-cell and cell-ECM factor interfaces and includes both sprouting and splitting network-expansion mechanisms. Sprouting involves the

formation and elongation of new vessels, while splitting is the creation of neo-vessels through the division of a pre-existing vessel. During sprouting, a vessel's EC basement membrane is resorbed while infiltrating ECs proliferate within the lumen space to create an extended tube. Tube growth is directed by tip cells as pericytes reinforce the recently developed blood vessel. Splitting is the internal bifurcation of an existing lumen, in which the two halves are guided and sealed to create new vessels through cell-cell and cell-ECM angiogenic pathways.[65,70]

Sprouting and splitting are observed during the transplantation of vascularized tissues as they connect to a host's vasculature in a process called inosculation. Inosculation involves neo-sprouts migrating towards existing capillaries and splitting to form multiple connection points between networks. Arteriogenesis, which is the maturation of neovessels, occurs post-inosculation. During arteriogenesis, which is thought to be triggered by fluid flow shear stress, supporting pericytes, SMCs and mural cells migrate to new vessels and stabilize neo-structures by increasing wall diameter and thickness.[62,65,70,74]

Angiogenesis has been harnessed in the development of vascularized tissue models and pre-vascularized tissue implants using bioprinting. *In vitro*, it is essential to integrate micro-networks within 3D models for pharmaceutical testing for long-term viability and the accurate recreation of internal vascularized anatomy. *In vivo,* a pre-vascularized construct is integrated with the host to establish blood flow post-implantation.[78]

The functional components of the native vasculature structural anatomy and neo-vascular processes are essential to consider in the development of TE and bioprinting approaches that seek to overcome the vasculature issue. In this aim, the development of bioprinting systems has evolved around a wide variety of approaches and modalities specifically designed to mimic and replicate the human vasculature. Section 3 provides an in-depth overview of these seminal approaches.

Section 3: Bioprinting Approaches and Modalities

Bioprinting approaches used to engineer macro- and micro-networks must (1) be in accordance with the anatomical structure of veins and capillaries and (2) properly account for angiogenic and vasculogenic manipulations. Developing technologies and recent technical breakthroughs in the bioprinting field are reviewed below, with a focus on techniques employed to create vascularized tissues. First, micro- and macro-network developments are defined, and scaffold-based and scaffold-free approaches are outlined. Next, the four primary bioprinting approaches are described according to an organizational hierarchy that classifies methods based on their deposition modality. All four types are described in detail and include descriptions of contemporary published studies that are selected based on their innovative technical impact.

1. **Macro-networks**
    a. *Size*: ID >100µm, macro-vessel level
    b. *Types*:
        i. Perfusable matrices: vascular channels embedded within gels
        ii. Vascular tissues: free-standing vascular conduits
    c. *Bioprinting Processes*:
        i. Direct patterning
        ii. Sacrificial patterning
2. **Micro-networks**
    a. *Size*: ID <100µm, capillary level
    b. *Bioprinting Processes*:
        i. Guided organization: manipulation of angiogenesis and vasculogenesis
        i. *In vivo* integration: host remodeling and inosculation

Macro-network Definition

The macro-network approach involves creating either perfusable matrices or vascular tissues that have central vessel IDs greater than 100µm.

Perfusable matrices are created by directly patterning either (a) single large-diameter channels or (b) interconnected lumen networks within a tissue scaffold. Perfusable matrices are usually created via sacrificial molding to create channels that can be lined with ECs pre-implantation. These channels provide perfusion at a distance of 200µm to surrounding cells embedded in the printed tissue scaffold to maintain adequate cellular density, viability and functionality[5]. Integrated luminal macro-networks can induce microvessel sprouting from a central channel, as evidenced by lab-on-a-chip[47] and slab-gel[83] studies. These approaches are contingent upon optimizing scaffold and tissue microfluidic properties so that tissues can be perfused in bioreactor systems over extended periods of time, or before connection with a host vasculature *in vivo*. During transplantation, macro-techniques can be advantageous as they provide clear channel locations for anastomosis[84].

Vascular tissues are a macro-network that involves the construction of free-standing tubular structures with an ID between 1-10mm (or more)[70]. Short term, these macro-

vasculature constructs will have clinical benefit as biologic small- or large- diameter vascular grafts to replace damaged or missing vasculature and long-term they will be fundamental structures in the development of vascularized tissues; many TE approaches that are currently dedicated to creating vascular tubes also directly contribute to developing vascularized tissue constructs.[85]

Functional vascular macro-tissue bioprinting will likely require both direct-printing and organoid manipulation approaches, which inherently incorporate both SB and SFB techniques[29]. Despite the simple geometry of tubular vascular tissues, during construction it is difficult to recreate layered and heterocellular vessel components while building biomimetic structures that maintain their structural and biologic integrity throughout the printing, incubation, and implantation periods. Pre-implantation, perfusion bioreactors are used to perfuse engineered tissues using a variety of microfluidic and macro-channel strategies that maintain flow and cell viability of thick tissues *in vitro*. The physical and chemical structure of vascular anatomy *in vivo* must be understood in order to recreate similar architectures outside the body.[70]

Micro-network Definition

Micro-networks, which are vessel with an ID less than 100µm, complete the hierarchical branching structure of vascular networks down to the capillary level, and can be created in two ways: through guided organization, or *in vivo* integration. Guided organization is the patterning of biochemical factors to induce angiogenesis and vasculogenesis within a matrix. This can be accomplished by arranging vascular fragments, like ECs and support cells, alongside chemical and growth-factor gradients using a bioprinting system that deposits biomaterials in a prescribed fashion within a hydrogel. These arrangements inluence local angiogenic microenvironments within a tissue to initiate the self-assembly of interconnected vessel systems.[70,74,86]

In vivo integration, on the other hand, uses the body's natural propensity to regenerate and form vessels *de novo.* Once a healthy, biocompatible and functional tissue is implanted, a host's vascular system will invade and remodel the transplanted tissue, much akin to wound-healing granulation and tissue-formation processes. Currently, the majority of *in vivo* integration bioprinting approaches rely on extensive engineering of tissues pre-implantation in order to prepare transplanted tissues for host-integration, including induced or direct pre-vascularization, scaffold growth-factor seeding and microfluidic incubation. Ultimately, a widespread combination of TE approaches are required to prepare bioprinted tissues for successful implantation and *in vivo* vascularization.

Bioprinting Technologies and Approaches Classifications

This section's organizational schema is deliberate, and we hope it provides a clear and natural differentiation between modalities and approach-types. Leading bioprinting studies are first divided by macro- and micro-network approaches, then by scaffold-based and scaffold-free methodologies, and finally by modality type.

1. **Approach: Macro-network**
    a. *Scaffold Based (SB)*

i. Droplet Based Bioprinting (DBB)
    1. *Direct*
    2. *Sacrificial*
ii. Microextrusion Based Bioprinting (MBB)
    1. *Direct*
    2. *Sacrificial*
iii. Laser Based Bioprinting (LBB)
iv. Stereolithography Based Bioprinting (SBB)

b. Scaffold Free (SFB)

2. Approach: Micro-network

a. Guided organization
b. *In vivo* integration

Macro-network Approaches

In the current bioprinting literature, two categorical approaches for bioprinting vascular constructs for TE and RM purposes are employed; the first is SB, traditionally referred to as 'bioprinting', and the second is SFB, typically classified as 'bioassembly'. Here, 'bioprinting' refers to both SB and SFB approaches.[1,2,70] SB utilizes bioinks, which are hydrogels, soft-materials or decellularized ECMs embedded with living cells. These bioinks are used as an additive print material that can be combined with other supporting materials to form architectural scaffolds and sacrificial components of a 3D structure.[1,8] SFB is contingent upon the self-aggregation of large tissue building blocks using cell-sorting and cell-fusion processes. These blocks can be stacked, picked, placed, or otherwise manipulated into desired geometries.[1,87]

Scaffold-Based (SB)

There are four main types of free-form SB: DBB, MBB, LBB and SBB. Descriptions, and the structural, physical, biological and economical characteristics for each modality type are outlined and compared in Table 3, and visuals of these systems are observed in Image 7. Characteristics in Table 3 are colored based on their competitive advantages compared to other modalities; for example, MBB and LBB have superior droplet-size control than most DBB and SBB approaches.[2,3,8,70]

1. **DBB**, which is also known as 'inkjet bioprinting', uses thermal, piezo, or acoustic pressure-pulses to drive droplets from a print head.
2. **MBB** uses mechanical or pneumatic mechanisms to push a continuous filament through a nozzle.
3. **LBB** uses laser-energy to heat an absorbent substrate lined with cellular material, which detaches cells from the heated slide so they fall onto the collecting substrate.
4. **SBB** uses the irradiation of photo-polymerizable materials to vertically cross-link solid products.[2,35]

Table 3: Bioprinter Modality Comparison

		DBB	MBB	LBB	SBB
Structural	Droplet Size	<1 to >300 pL drops (50-300µm wide)	5µm to >1mm	>20 to 80µm	>50µm
	Spatial Resolution	medium	low to high	medium to high	medium to high
	Vertical Structure	poor	good	medium	good
Physical	Material Viscosity	3.5 to 12 mPa·s	30 mPa·s to >600 kPa·s	1 to 300 mPa·s	<5 Pa·s
	Gelation Method	ionic, enzymatic, photo-crosslinking, thermal, chemical	ionic, enzymatic, photo-crosslinking, thermal, chemical, shear thinning, pH	ionic, chemical, photo	photo
	Gelation Speed	high	medium	high	high
	Multi-bioink Delivery	low-medium	high	medium	low
Biological	Cell Viability	>85%	40 to 95%	>95%	25-85%
	Cell Density	low	high, cell spheroids	medium, 10^8 cell mL^{-1}	medium, 10^7 cell mL^{-1}
	Encapsulation Control	low	medium	medium to high	low
Economical	Preparation Time	low	low to medium	medium to high	low to medium
	Print Speed	fast (1-10,000 droplets^{-1})	slow (10-50µm s^{-1})	medium to fast (200-1600 mm s^{-1})	fast
	Throughput	high	medium	low to medium	low to medium
	Printer Cost	low	medium	high	low to medium

Quality Comparison: **Superior** **Moderate** **Limited**

Table adapted from ref. #[7]

DBB and MBB scaffold-based modalities can further be categorized into 'direct' and 'sacrificial' approaches. Direct printing involves precisely depositing materials in a pre-defined pattern in 3-dimensions to create a desired shape from the bottom-up. Intra- or post-printing crosslinking mechanisms are used to solidify the final structural geometry and, to a large extent, affect the cellular viability of printed products, along with shear-thinning stresses and the bio-physiological chemical environment in extruded materials.[70,88,89] Sacrificial approaches employ a sacrificial hydrogel that can be flushed from the final product to create lumens and channels within the base material. The sacrificial hydrogels, like alginate, agarose, gelatin, or pluronic, are removed post-printing using thermal and chemical means. For example, in the construction of an open channel embedded in a hydrogel, the sacrificial material is initially printed in vertical or horizontal cylindrical geometry, cell-laden hydrogels are printed surrounding the sacrificial cylinders, the construct is heated, and cylinders melt away to reveal open lumens that can be lined with ECs.[65,70,90]

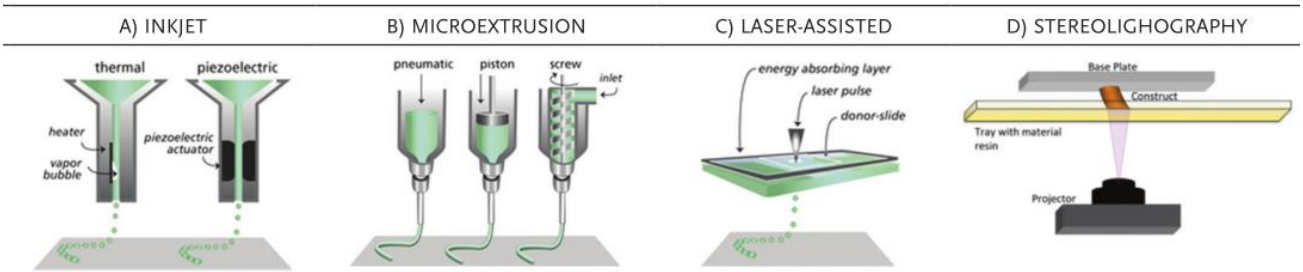

Image 7: The Four Primary Bioprinting Modalities
Reproduced from ref. #[7]

Droplet and Inkjet (DBB)

In DBB, cells are encapsulated in hydrogel carriers that are dispensed in a non-contact manner by a nozzle positioned over a print-bed. The nozzle releases cellular drops one-by-one in a pre-determined 3D geometry that can be controlled via thermal or piezoelectric actuators. 3D bone[7], vascular[91], cartilage[92], cardiac[93], skin and nervous[94] tissues have all been constructed using droplet printers.

The most common DBB approaches use thermal or electric print-head droplet systems, while a few acoustic and micro-valve approaches have been developed. Thermal printers locally heat a print head with short duration (~2µs) electrical bursts at 200-300°C to instantaneously raise the internal print-head temperature by 4-10°C. This small heat current, concentrated in a brief interval of time, produces a bubble of material that will expand and collapse to produce a pressure pulse, pushing the loaded bioink from the chamber and into the nozzle. Control of pulse frequency, temperature gradient and bioink viscosity can alter droplet size, usually between 10-150pL.[24,95,96] Piezoelectric printers use an acoustic wave to move materials into the nozzle and separate bioinks into the required droplet formations. This wave is created by a piezoelectric material, usually polycrystalline ceramic, that is distorted in response to voltage and vibrates or alters shape to convert electric energy into mechanical pressure that ejects the bioink from the print-head.[14,35,65,70] Acoustic systems eject droplets of controllable size and rate using an ultrasound field with alterable pulse, duration and amplitude[24,65]. Micro-valve printers contain a pressurized fluid chamber with a droplet release valve that is opened and closed using a magnetic field. These systems are intrinsically faced with a trade-off between resolution, cell viability and print speed.[2,24]

DBB Advantages and Disadvantages

The primary advantages of DBB is the creation of high-resolution droplets whose deposition position can be controlled at a spatial resolution of 50-300µm. DBB is also advantageous because it can introduce gradients of cells and chemical factors throughout a construct[24]. Commercial DBB printers are low-cost, and many labs economically build home-made systems by modifying industrial inkjet printers with living bioink cartridges and xyz-controlled stages.[7]

The disadvantage of inkjet printers arises when building large-scale constructs, as print-speed and wall-thickness decrease exponentially as a function of increasing product volume; the average droplet printing processes are 20% the speed of MBB printers[2,14,65]. The DBB print-

head design also restricts structural integrity and reproducibility as a result of cellular aggregation and settling in the ink reservoir which can clog the nozzle mid-print. Suitably, small nozzle-tip diameters are used for high-resolution print specifications, but small tips induce higher shear stresses, mechanical forces and thermal pressures within the print-head that negatively affect bioink cell viability. To ensure printability, cell densities in DBB bioinks are constrained to a maximum 10^6 cells/mL, which is less than human tissues[7]. Furthermore, biomaterials must be printed in liquid droplet form and subsequently converted into a 3D product with solid structural features, which limits the maximum liquid viscosity close to 0.1Pl, as highly viscous hydrogels can lead to clogging[24]. Deposited cell-carriers can be polymerized post-deposition with ultraviolet (UV), chemical, ionic, or enzymatic cross-linking to secure architectural and structural integrity, thus DBB material must be compatible with crosslinking factors and have printable material viscosities between 3.5-12mPa·s[95]. Concordantly, the majority of studies resort to sodium alginate, PEGDMA, fibrin, calcium phosphate or hydroxyapatite as bioink carriers[7,97].

DBB Highlighted Studies

The recent work of Xu and his coworkers provides a case-study exhibiting how meticulously bioprinting systems must be characterized when employed with a range of cell types, materials and end-structures. The team characterized a piezo-DBB system by altering breakup time, droplet size, velocity and bioink cellular concentration on quantifiable print parameters.[98,99] The team subsequently used their in-house MicroFab pneumatic DBB to print tubular alginate structures within a $CaCl_2$ crosslinking bath that simultaneously provided buoyancy support and chemical polymerization during printing. They printed both vertical and horizontal structures with an overhang to assess the advantages and disadvantages of various printing configurations. This printing process can be observed in Image 8.[91] Subsequently, in 2015, Xu's team exhibited the capabilities of their highly-controllable printing process by building conduits with branching and hemi-branching components using mouse-fibroblast laden alginate bioinks. The structures showed minimal deformation, closely recapitulated the computer-modelled branching structures and maintained 90.8% cell viability 24 hours post-printing. This work demonstrates the feasibility of using DBB within an aqueous environment to produce structurally complex vascular geometries, but future efforts to incorporate living components in heterogeneous layers with varying wall-thicknesses is necessary.[96] Xu's lab worked to quantify the structural, geometric and biomaterial characteristics of droplet systems while developing a novel printing technique – a much needed examination of bioprinting inputs and pioneering application that substantiates a future of progressively functional bioprinted products.

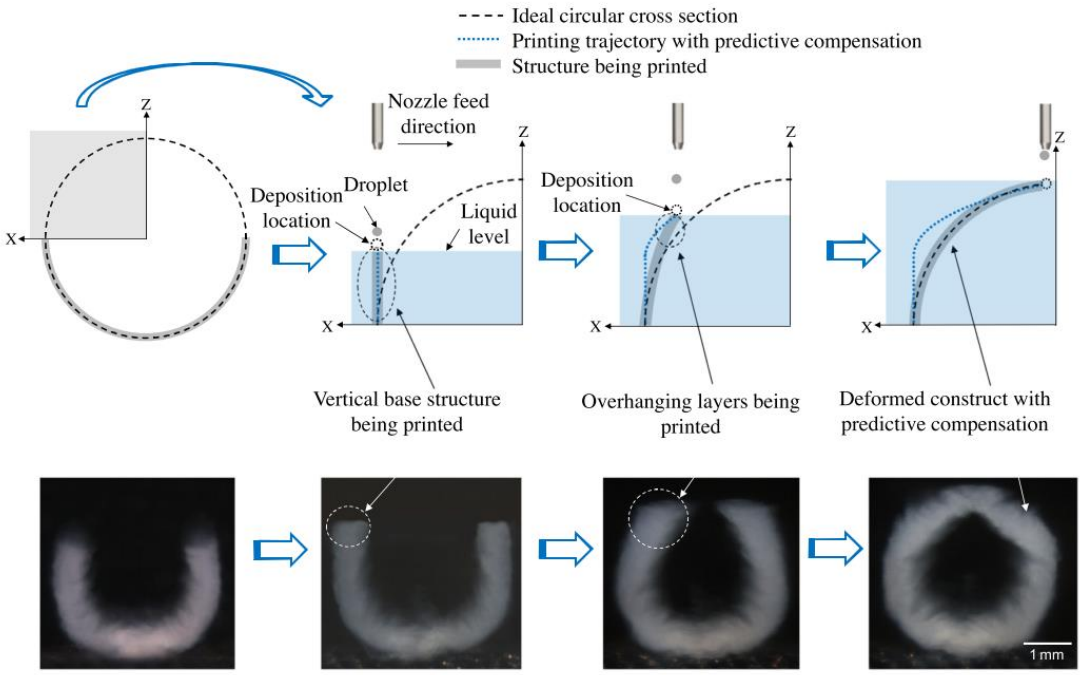

Image 8: Horizontal Printing with Predictive Compensation
Reproduced from ref. #[98]

Researchers based in RPI, led by Vivian Lee and Guohao Dai, showed the practicality of using sacrificial materials to form and reinforce vascular channels – an approach quite distinct from Xu's slurry bath support. Lee's team built a straightforward perfused open lumen using a homemade micro-valve DBB by surrounding a cylindrical gelatin construct composed of a 1:1 combination of gelatin and HUVECs with collagen rods that formed a concentric circle around the center gelatin layer, as shown in Image 9. The construct was incubated at 37°C to melt the gelatin, inverted, perfused with media-flow, and lined with HUVECs to form a confluent cellular layer around the channel. Under gentle flow conditions HUVECs did not exhibit angiogenic sprouting but maintained viability up to 5mm from the channel at a density of 5M cells/ml. Unexpectedly, under static flow, active sprouting was observed at the vessel surface but viability was reduced.[100]

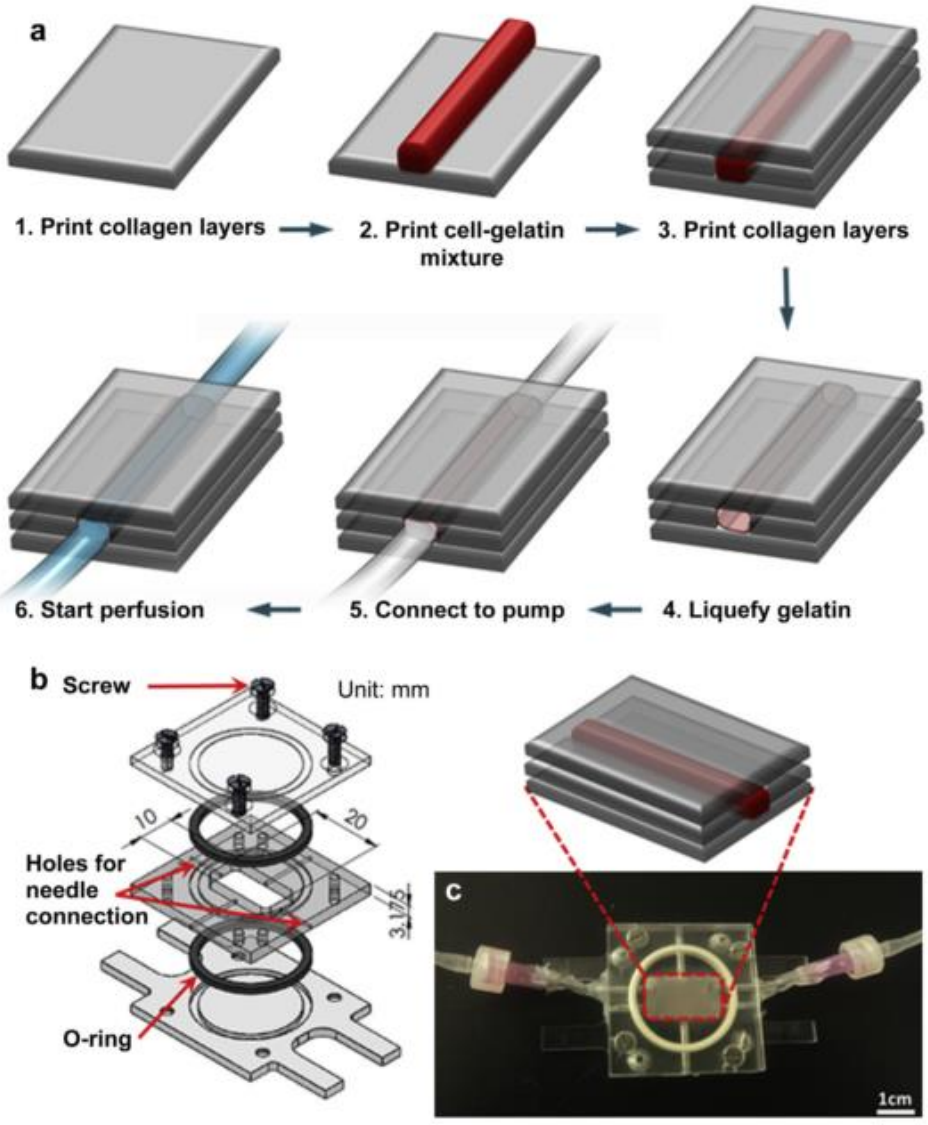

Image 9: Vascular Channel Construction Procedure and Integrated Flow Chamber

(a) Schematics of the vascular channel construction procedure using cellegelatin mixture. (b) Custom-designed flow chamber consists of three transparent polycarbonate pieces, two o-rings for sealing, and screws. (c) Actual picture of flow chamber. The chamber is connected to the perfusion system through needles installed on the side of chamber; Reproduced from ref. #[100]

In continuation of their work, Lee's team applied the same approach to construct two parallel open channels 0.5-1mm in diameter, separated by fibrin gel embedded with HUVECs and NHLFs. The NHLFs proliferated rapidly and HUVECs sprouted from channels under low flow conditions, growing up to 400µm after one week of culture. Lee found that increasing media flow in an incremental step-wise fashion allowed for the identification of a flow speed that balanced tissue perfusion and EC angiogenesis. This study combined the construction of macrovasculature lumens with a manipulation of chemical flow-factors and channel distributions in space in order to initiate microvasculature sprouting and angiogenesis. This pursuit successfully increased the overall permeability of the pericyte-loaded fibrin construct in

a dramatic way, and showed how direct and sacrificial DBB can be used to harness angiogenic micro-network processes.[101]

Microextrusion (MBB)

MBB printers deposit continuous steams of material using a robotic print-head to direct liquid flows in a layer-by-layer fashion[102]. These continuous beads of material can be controlled by altering flow rate, temperature, applied pressure or nozzle diameter of the print-head to print from the 5µm up to the millimeter range[81]. Multiple print-heads can work concurrently to extrude a wide range of synthetic and natural polymers by moving the dispensing system or substrate stage in the x, y and z dimensions[7,17,24]. To maintain structural integrity, materials are crosslinked either before or after extrusion; post-printing this process is similar to the thermal, enzymatic, ionic, or photo-crosslinking mechanisms that are utilized in DBB, but *in situ* or intra-print crosslinking is a process called 'microfluidic spinning'[3]. In microfluidic spinning, a co-axial print head exudes an inner flow of cell-laden biomaterial, typically sodium alginate, simultaneously with an outer sheath of crosslinking material, usually $CaCl_2$, which initiates polymerization[88,96,103,104]. This mechanism allows for materials with lower viscosities and shear rates to be used in comparison to alternative modalities, like DBB[87,105].

The most common MBB approaches use either an air-pressure controller to produce pneumatic pressure, or a piston- and screw-assisted mechanism to create mechanical pressure that ejects materials from the print-head. Mechanical systems provide instantaneous control over material flow and have complex structural components, which increases spatial control and ejection response times but doesn't limit print-head force production. Pneumatic systems are simple in design but have slightly reduced force production potential.[39,70,104,106] Thus far, tumor models[53], organ-on-a-chip bioreactor systems[22,107], vascularized tissues[15,108–110], skin tissue composites[111,112], aortic valves[9,113] and liver tissue models[114,115] have all been produced using MBB systems[39].

MBB Advantages and Disadvantages

The primary advantage of MBB is the ability to deposits bioinks with high cell densities. Highly viscous materials over 600 kpa-s can also be used through continuous droplet ejection, which is an advantage over DBB approaches[7]. Thus, dense and highly viscous products can be produced and solidified using a variety of polymerization mechanisms, which lends itself to the creation of vertical, large-geometry products at high aspect ratios[116]. Even though vertical structures require thick supporting walls that could increase build-times, MBB approaches enable high-throughput fabrication processes of thick tissues with an increased potential for manufacturing applications when multiple print-heads are used in conjecture[15,106,117].

The disadvantage of MBB is that cell viability, which ranges between 40-86% in most MBB approaches, is affected by altering many system characteristics that control print quality[7]. Increased print-head reservoir pressure increases shear stresses on cells encapsulated in viscous materials, which decreases cellular viability. Resolution also affects viability, as higher-resolution deposition requires smaller nozzle diameters and increased pressure to discharge materials[118]. Lower printing resolutions make it difficult to directly print small features or establish cellular and chemical gradient patterns, like in the promotion of angiogenesis, where the generation of micro-capillary gradients requires close proximal positioning of ECs to induce

neo-vessel growth[70]. Printing with smaller nozzles also negatively affects fabrication time; printing at the micron level, it would take upwards of 9 months to directly print an entire human kidney, based on calculations performed at the Lewis Lab at Harvard University[119]. Thus, increasing speed and resolution while controlling cell viability will be a major challenge for the future development of MBB systems.[24]

The overall difficulty in scaffold-based MBB is that it requires extensive knowledge of the printing, bioink, material and cellular characteristics at each level of production because the interaction between living and non-living components is affected by particular conditions like temperature, pressure or rheology[120]. MBB must be biocompatible, crosslinkable, possess shear-thinning and high-flow properties, maintain high cell viability at print-level temperatures and stability throughout the printing process[39]. Typically, soft hydrogels like fibrin, sodium alginate and GELMA are used in conjecture with printing additives like gelatin or HA that improve printability, binding capacity and shear thinning properties[39,118]. Microfluidic spinning is also used to overcome difficulties with printing viscous materials but can increase the tendency for intra-print-head clogging to occur[39].

MBB Highlighted Studies

In 2015, Hinton et al. created high-resolution constructs with mechanical integrity within a fugitive support bath using a unique bioplotting technique. The method, called FRESH, uses a customized MBB MakerBot Replicator to build branched coronary arteries and complex hollow anatomical structures using a single extrusion head to deposit layers of biomaterials within a supporting gelatin slurry. The bath is composed of gelatin micro-particles which act as a Bingham plastic at controlled temperatures and can be melted away after crosslinking. At low shear stress, when the print-head is not pushing through the aqueous medium, the support bath maintains its high structural integrity. Under high shear stress, during low-friction freeform needle movement, the bath behaves as a viscous fluid. The bath is sterile and supports printing with cells like myoblasts and fibroblasts, as well as hydrogels like alginate, collagen and fibrin. Hinton's research team used open-source software and hardware as well as a modified $400 3D printer to print hollow, bifurcated, mechanically robust vessel structures with wall thickness under 1mm that formed cellular networks and maintained high cell viability during one week of culture. This study demonstrates the increasing accessibility and applicability of bioprinting technologies, but the FRESH method's precision and material functionality are limited by the low-cost materials and printing system. The approach is hindered by its requirement for a liquid platform maintained at below-native temperatures (under 37°C) that decreases cell viability and limits the range of compatible cells and materials. Furthermore, Hinton's team utilizes a single-nozzle syringe which prevents the deposition of multiple biomaterials simultaneously. Further *in vitro* testing and biological characterization of printed products are needed in order to confirm the therapeutic potential of this printing method, but it is a pioneering step forward in the incorporation of MBB with numerous crosslinking mechanisms and a dynamic support bath.[102]

MBB printers can be used to deposit multiple materials at once using coaxial nozzle print-heads. This microfluidic spinning process was demonstrated impressively by Gao et al. in 2015, when his team showed that alginate and $CaCl_2$ can be concurrently deposited for intra-print crosslinking. The team printed hollow tubular alginate filaments, with an average ID and

OD of 892mm and 1192mm respectively, by controlling flow rate, material concentration and nozzle diameter, as shown in Image 10. Hollow cylinder, grid, cuboid and hemispheroid structures were bioprinted on a z-controlled stage that was lowered into a $CaCl_2$ bath during printing to initiate filament fusion. This system allowed the top two filament layers to settle and partially polymerize with printed $CaCl_2$ while the following layers solidified in the gelation bath. Perfusion was established through the structures via the open-filament lumens, mechanical analyses showed maintained structural integrity over a one-week period and cell viability assays revealed microchannel structures besting control groups 70% to 50% after seven days of incubation. This study exhibited the functionality of coaxial nozzle systems and microfluidic spinning as a method to produce perfusable thick tissue structures.[104]

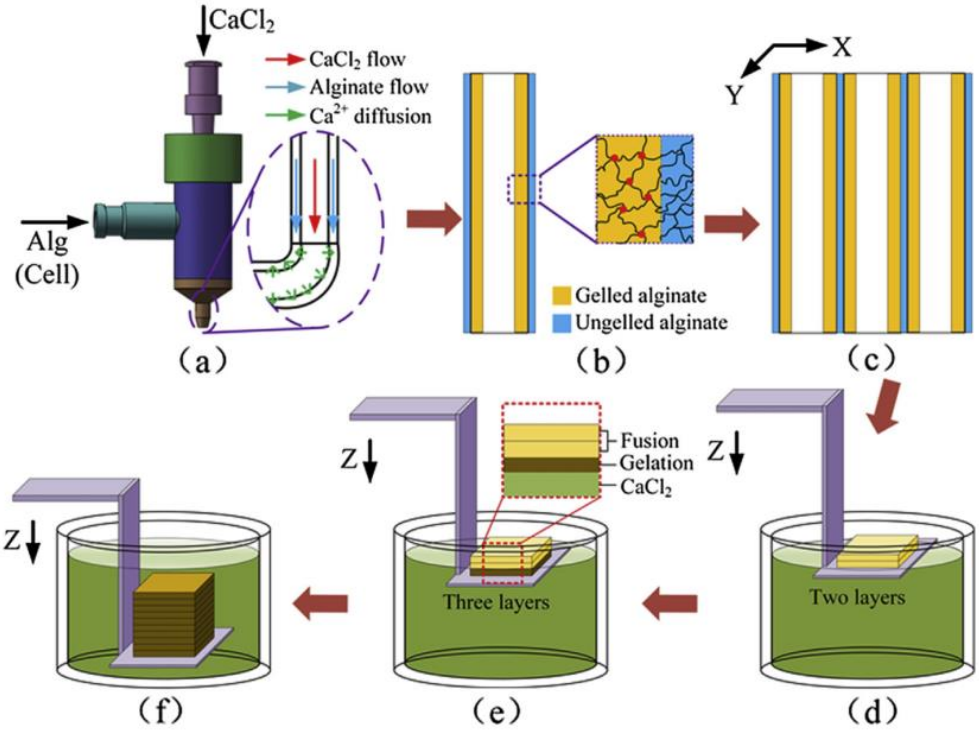

Image 10: Fabrication of a 3D Alginate Structure with Built-in Microchannels
Reproduced from ref. #[104]

One of the most advanced bioprinting systems to date is the four-nozzled ITOP that was developed in 2016 and advanced both the FRESH and coaxial methods. Kang et al. reported the fabrication of human-scale tissue constructs in the form of mandible-bone, ear cartilage, and skeletal muscle fiber constructs with high levels of mechanical strength, nutrient diffusion, and cell viability. Clinical radiological data from CT and MRI scans provided imaging data, which mapped the motion control pattern of the xyz-controlled microextrusion printer. First, a structural framework of polycaprolactone (PCL) was printed to provide mechanical support and form horizontal and vertical open micro-channels with an area of either $500x300um^2$ or $650x450um^2$. PCL addition was followed by the extrusion of a sacrificial outer layer of Pluronic F-127 hydrogel, and finally a composite hydrogel bioink consisting of gelatin, fibrinogen, HA, glycerol and DMEM (high glucose) was mixed with respective encapsulated cell types. This

process can be observed in Image 11. After printing, crosslinking and sacrificial material removal, printed constructs were implanted into rat models, where they showed zero necrosis, native tissue ingrowth, and evidence of vascular integration with EC markers according to vWF immunostaining. This was the first time bioprinted living tissues have been implanted in animal models and shown negligible side-effects, maturation, and vascularization up to 5 months post-implantation. In future studies, the ITOP system must incorporate multiple cell types at near-organ level cell densities so that *in vivo* therapeutic assessments can be performed, and additional testing for vascularization and integration into animal-models must be established before tissues with therapeutic human application can be produced.[17]

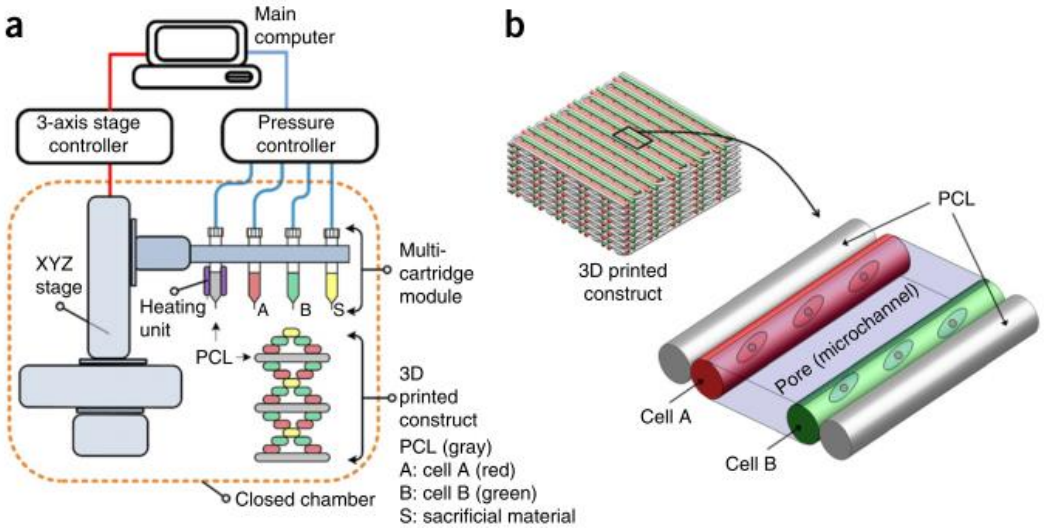

Image 11: The ITOP Bioprinting System

(a) three major units: (i) 3-axis stage/controller, (ii) dispensing module including multi-cartridge and pneumatic pressure controller and (iii) a closed acrylic chamber with temperature controller and humidifier. (b) Illustration of basic patterning of 3D architecture including multiple cell-laden hydrogels and supporting PCL polymer; Reproduced from ref. [17]

In another approach using a multi-nozzle bioprinter, Kolesky et al. built heterocellular tissues on a perfusion chip with embedded microvasculature using Pluronic F-127 sacrificial ink and a customized 4-nozzle microextrusion bioprinter. Parenchyma, stroma and ECs were printed within a customized ECM at a thickness >1cm and volume of 10cm^3. The team aligned biomaterials with a homemade air-controlled multi-material printer with a minimum resolution of 100µm and a maximum print speed of 1mm/sec. First, silicone ink was printed on a glass slide and cured to create the perfusion chips. Next, the cell-laden bioink composed of fibrinogen, gelatin, and the desired cell type was deposited in accordance with the pluronic-thrombin fugitive ink. The chip was then encapsulated in an ECM composite hydrogel containing gelatin, fibrinogen, thrombin, and transglutaminase. Finally, the sacrificial Pluronic, which formed embedded vascular networks and internal lumens, was cooled and melted away, and the resulting cavities were lined with ECs and perfused. Printed HUVEC, hNDF and hMSCs maintained high levels of proliferation and viability within the tissue product after integration of media flow in a customized perfusion system and differentiated toward an osteogenic lineage with the introduction of growth factors over a 6-week period. Endothelial network integration and permeability results are shown in Image 12. Vascularized constructs with post-

printing cell densities ranging from 0.1 million/ml to 10 million/ml were produced, which is less than tissue-level cell densities. Further work needs to be done to scale-up this approach, characterize the vascularization capacity and increase end-product cell-density, either through incorporation of microtissues or by harnessing the proliferation potential of cells during the incubation period.[85]

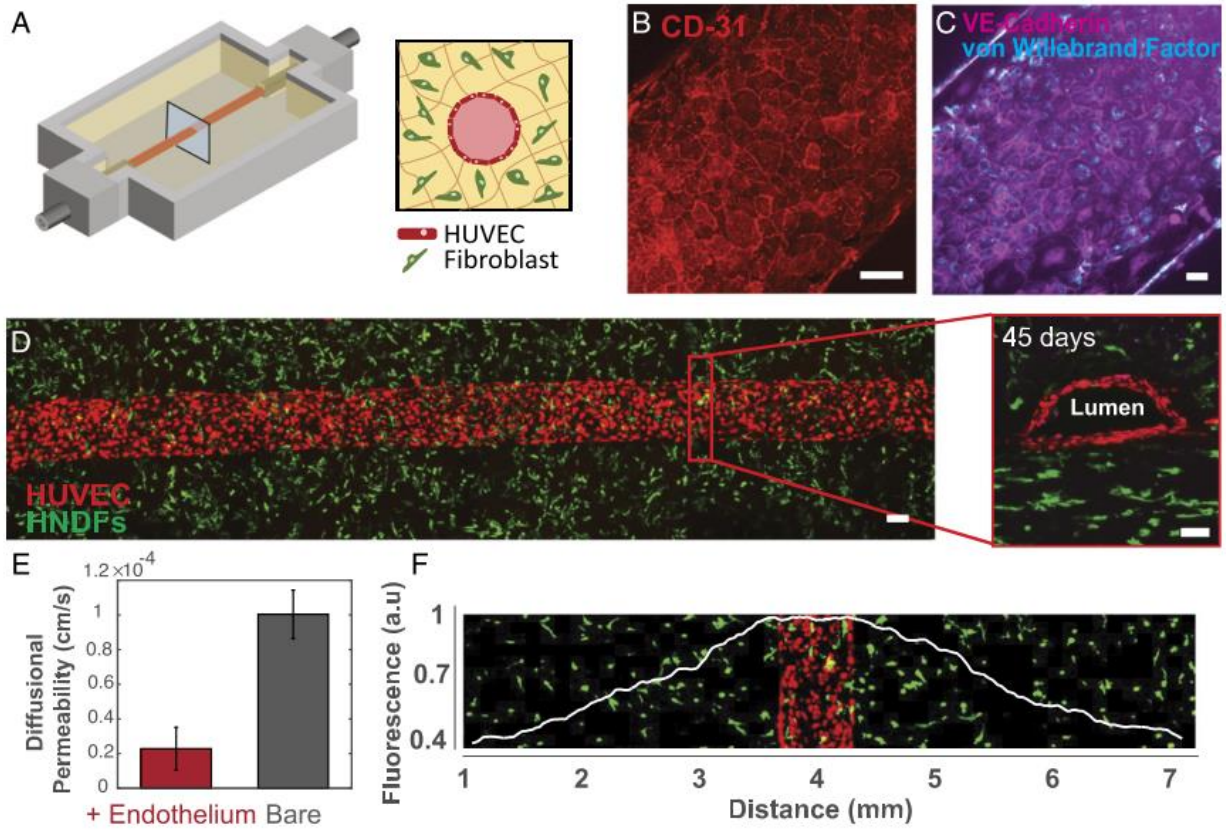

Image 12: 3D Vascularized Tissues Remain Stable During Long-term Perfusion

(A) Schematic illustration of the tissue manufacturing process. (i) Fugitive (vascular) ink, which contains pluronic and thrombin, and cell-laden inks, which contain gelatin, fibrinogen, and cells, are printed within a 3D perfusion chip. (ii) ECM material, which contains gelatin, fibrinogen, cells, thrombin, and TG, is then cast over the printed inks. After casting, thrombin induces fibrinogen cleavage and rapid polymerization into fibrin in both the cast matrix, and through diffusion, in the printed cell ink. Similarly, TG diffuses from the molten casting matrix and slowly cross-links the gelatin and fibrin. (iii) Upon cooling, the fugitive ink liquefies and is evacuated, leaving behind a pervasive vascular network, which is (iv) endothelialized and perfused via an external pump. (B) HUVECs growing on top of the matrix in 2D, (C) HNDFs growing inside the matrix in 3D, and (D) hMSCs growing on top of the matrix in 2D. (Scale bar: 50µm.) (E and F) Images of printed hMSC-laden ink prepared using gelatin preprocessed at 95 °C before ink formation (E) as printed and (F)after 3 d in the 3Dprinted filament where actin (green) and nuclei (blue) are stained. (G) Gelatin preprocessing temperature affects the plateau modulus and cell viability after printing. Higher temperatures lead to lower modulus and higher HNDF viability post-printing. (H) Photographs of interpenetrated sacrificial (red) and cell inks (green) as printed on chip. (Scale bar: 2mm.) (I) Top-down bright-field image of sacrificial and cell inks. (Scale bar: 50µm.). (J–L) Photograph of a printed tissue construct housed within a perfusion chamber (J) and corresponding cross-sections (K and L). (Scale bars: 5 mm.); Reproduced from ref. #[78]

Laser (LBB)

The most common form of LBB is called laser-induced forward transfer (LIFT) and uses a focused laser beam to selectively stack and pattern cells[121], peptides[122] and DNA[123] to create pre-defined tissue structures. LIFT has 3 major components: a high-frequency laser source, a target 'ribbon' that holds biomaterials, and a receiving substrate to catch falling cells and materials. The ribbon is conventionally made of glass and is comprised of a top and bottom layer; the superior metal layer (gold or titanium) faces the laser, and the posterior coating of aqueous print materials (hydrogels, cells or media) faces the receiving substrate. Focused energy is targeted at the ribbon and the pulsed laser beam excites the top receiving metal to a higher vibrational energy. This energy generates localized heat in a targeted location on the ribbon, which vaporizes the concomitantly layered bioink, thus generating micro-bubbles that eject the printed materials from the bottom of the ribbon. This focused displacement of biomaterials causes them to fall onto the receiving substrate. The size of the jet and the amount of released bioink is determined by a variety of factors, including laser pulse energy, distances between the receiving substrate and donor ribbon, bioink cell density and physical properties of the bioink that includes surface tension, thickness, viscosity and wettability. The use of LBB has not been extensive given its limited accessibility and highly technical nature. Nonetheless, skin tissues at human-level cell densities[124], bone depositions[125] and nervous system components have all been created using LIFT.

LBB Advantages and Disadvantages

The low-stress deposition method of LBB is devoid of nozzles and pressurized extruders, which allows LIFT printers to maintain a high cell viability over 95%, removes the potential for clogging, and allows the deposition of high cell densities up to 10^8 cells/ml. This approach is compatible with low viscosity materials ranging from 1-300mpa·s, like collagen and nano-hydroxyapatite, which have been used extensively in LBB studies[125]. Droplets can be tuned down to the microscale resolution, between 20-80µm with a single cell per droplet. This increase in resolution institutes an inherent tradeoff in print speed, which tends to be between 200 and 1600mm/sec.

The elevated cost and mechanical complexity of LIFT systems is a major drawback that prohibits LBB adoption for the vast majority of researchers. From a technical standpoint, laser printers are restricted to the construction of thin structures because of their narrow range of printable viscosities that principally limit compatible materials to matrigels, alginates, and gelatins. Rapid crosslinking must occur in order to assure structural integrity post-printing, which confines LBB to chemical- and photo-crosslinking strategies. Each ribbon loaded into a laser printer must be individually coated with a single cell or material type, which increases preparation time exponentially when building heterogeneous structures. Metallic residues can also evaporate from the ribbon during the printing process and collect in the final product, introducing unwanted and extraneous materials in a living tissue; the negative effects of this laser irradiation remain unclear.[3]

LBB Highlighted Studies

In an early LIFT approach that established laser-printed cellular functionality, Gaebel et al. engineered cardiac tissue patches and transplanted them into rats. Patches were made of

PEUU and HUVECs and hMSCs were patterned in a mesh-like structure on top of the PEUU. HUVECs formed square grids and hMSCs filled in the squares outlined by HUVECs, while the control patch was seeded randomly with no cellular organization. Using LIFT, an aluminum garnet laser was directed at the donor substrate and ablated the gold receiving metal, displaced cells from the opposing side of the donor slide and caused them to fall onto the PEUU patch. Post LAD-ligation, patches were transplanted to infarcted cardiac zones of rats, and after eight weeks cardiac performance was measured with left ventricular catheterization. Patterned patches showed increased vascularization and regenerative cardiac functionality compared to controls. This study not only illustrates the superiority of LBB in the creation of high-viability tissues, but shows how the spatial organization of cellular patterns effects long-term tissue functionality and is an exhibition of direct regenerative application of bioprinted tissues in living models.

In 2015, Xiong et al. showed that branching lumen tubes with significant overhangs can be created using LIFT. The team created straight and Y-shaped alginate tubes with two different bioinks: an 8% and a 2% alginate-solution, both seeded with mouse-fibroblasts. All tubes were printed in a 2% $CaCl_2$ bath using predictive compensation to lower printed tubes into the crosslinking bath. It took the team 180 minutes to print branching tubes 9.5mm tall with a wall thickness of 2.5mm. Immediately after printing, cell viability was measured at 64% and after 24 hours of cellular proliferation in an incubator viability reached 68%. This study shows how LIFT technologies can be employed to create branching vascular structures in an aqueous environment that support cellular proliferation within printed scaffolds over short periods of time, and provides a novel orientation methodology for constructing tubular structures.[126]

The most impressive LIFT application to date was in the printing of mesenchymal stromal cells for *in situ* bone regeneration in a calvaria defect model in mice, put forth by Keriquel et al. in 2017. This work established a platform for integrating vascularized approaches with direct implantation treatments of bioprinted tissues, as shown in Image 13. The team first printed an nHa-collagen disc 2mm in diameter and 100µm in thickness directly onto the defect, patterned D1s either in a ring or disc formation on top of the collagen base, then printed another layer of collagen on top of the cellular droplets. Three groups of calvaria mice were studied: one with a cellular ring pattern between collagen, one with a disc pattern, and a final acellular control group. Over the course of two months, cellular proliferation was measured with luciferase assays and bone repair was tracked with micro-CT histological analyses. The disc pattern showed a higher ratio of bone volume repair to total bone volume, increased differentiation towards an osteoblastic lineage and superior overall regenerative function compared to the other two groups. The ring pattern maintained high levels of cellular proliferation around the periphery but failed to fully differentiate into an osteogenic lineage. These tomographic results, including bone volume measurements and bone repair assays findings, are shown in Image 14. Future studies are needed to study the integration, remodeling and vascularization potential for this type of *in situ* treatment and to further characterize printed cellular behavior within the calvaria models, but overall, Keriquel at al. showed the potential for using direct LIFT technologies in the RM space and reported the first *in situ* printing of mesenchymal stromal cells on a bone defect using LIFT.[127]

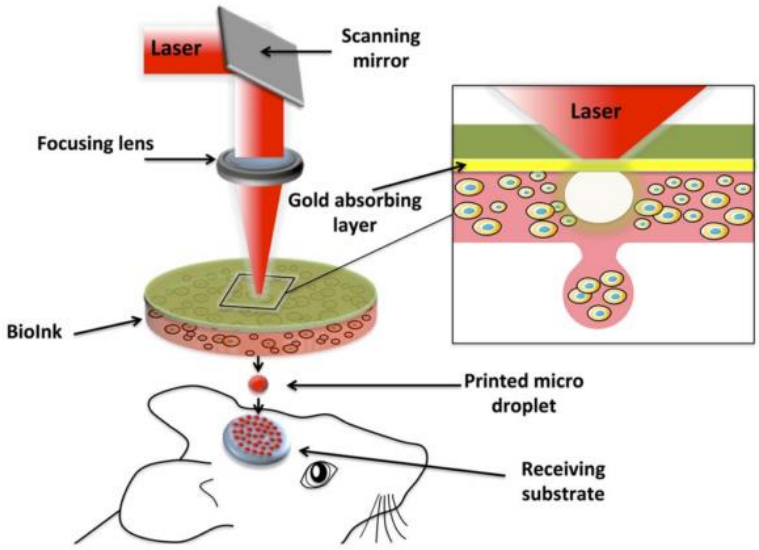

Image 13: Schematic Representation of the LBB Approach

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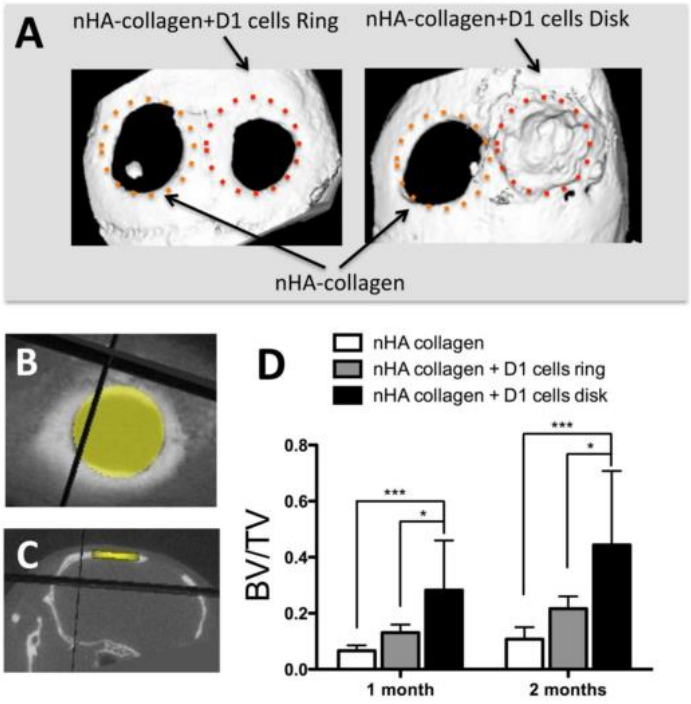

Image 14: Calvaria Defect LBB Results

*(A) Representative X-ray micro tomography (µCT) reconstruction images of nHA collagen and D1 cells printed in a ring or disk geometry (calvaria defect in the right side), or nHA collagen alone (calvaria defect in the left side), at 2 months post printing in a mice calvaria model. Horizontal (B) and coronal (C) µCT projection and regions of interest (3.3 mm diameter and 0.5 mm thick disk) for the evaluation of bone repair, in a calvaria defect in mice at 2 months post impression. (D) Quantitative assessment of bone volume/total volume (BV/TV) by µCT evaluation of nHA collagen and D1 cells, printed in a ring or disk geometry, or nHA collagen alone at 1 and 2 months post printing (Average ± SD, n = 9, *and ***denote $p < 0.05$ and $p < 0.001$, respectively); Reproduced from ref. #[127]*

Stereolithography (SBB)

The term 'stereolithography' is used to describe vat photo-polymerization methods that rely on light or laser energy to cure a photosensitive resin bath layer-by-layer[2]. Other vat polymerization TE methods that fall within the SBB classification include DLP and CDLP[6]. In SBB, a macromere solution is polymerized on the surface of a vertically controlled build-stage tub that is sequentially lowered following energy-beam exposure to expose new layers of material[128]. The penetration depth must solidify both the superior material-layer, which is uncured, and reach the inferior layer, which was previously solidified, in order to fuse the two substituent layers together. Crosslinking depth is dependent on the material's physical properties and the strength of the polymerizing beam[7]. SBB approaches typically use singular beam paths that can be controlled to solidify specific areas of material at a minimum resolution of 50µm, or use 'masks' to cover parts of a resin bath during uniform light exposure[128]. DLP is similar to SBB but uses a strong diffuse light source from the bottom of a shallow resin bath that solidifies the entire bath simultaneously. CDLP is a modified version of DLP in which the bath moves towards the light source slowly so that the entire bath doesn't have to be cured in conjunction.[6] Photo-crosslinkable polymers, like PEGDMA, PPF, TMC and CL that support cellular encapsulation must be used in SBB. SBB is not widely used but has shown promise producing bone[129], pre-vascularized[128], skin and neural[130] tissue structures.

SBB Advantages and Disadvantages

SBB is the fastest bioprinting modality, especially when producing large and thick tissue products, as thick vertical structures benefit from strong interlayer bonding during the printing process. Unfortunately, SBB suffers from limited cell viability, which lies in the 25-85% range, because it is sensitive to biological and chemical encapsulation characteristics within curable bioinks, which also require embedded toxic photo-initiators. These qualities prevent SBB from exceeding printable cell densities of 10^7 cells/ml, which is less than native tissue specifications. Furthermore, the harmful effects of UV radiation and laser light on cell viability are unknown.[7,70]

SBB Highlighted Studies

In 2013, Huang et al. exhibited a novel SBB technique by examining the effect of numerable printing patterns on HeLa and murine noncancerous 10T1/2 fibroblast behavior. The method, called DMD-PP, projects images onto a photosensitive bioink using an array of digitally controlled micro-mirrors. This SBB system is shown in Image 15, where Huang's team took an optical pattern from a CAD model, created a micro-mirror design and projected UV light onto the photopolymerizable pre-polymer, which was sequentially lowered during the solidifying light-exposure printing process to reveal uncured material layers. Branched honeycomb micro-vessel structures were created within PEGDA hydrogel that had channel ID widths of 25µm, 24µm and 120µm in accordance with the IDs of anatomical vessel lumens. Using these channels, cell morphology and migration speed were compared between cancerous and noncancerous cells. The results revealed that channel size had minimal effect on HeLa cells, but fibroblasts increased in size with increasing vessel diameter. Furthermore, fibroblasts maintained the same migration speed in all groups, but HeLa cell migration rate increased as a function of decreasing channel diameter. These results show that cancerous cell lines migration

speed and metastasis rate are affected by their environmental channel size *in vitro,* which may have implications for how cancers behave, move, and proliferate within the body.[131]

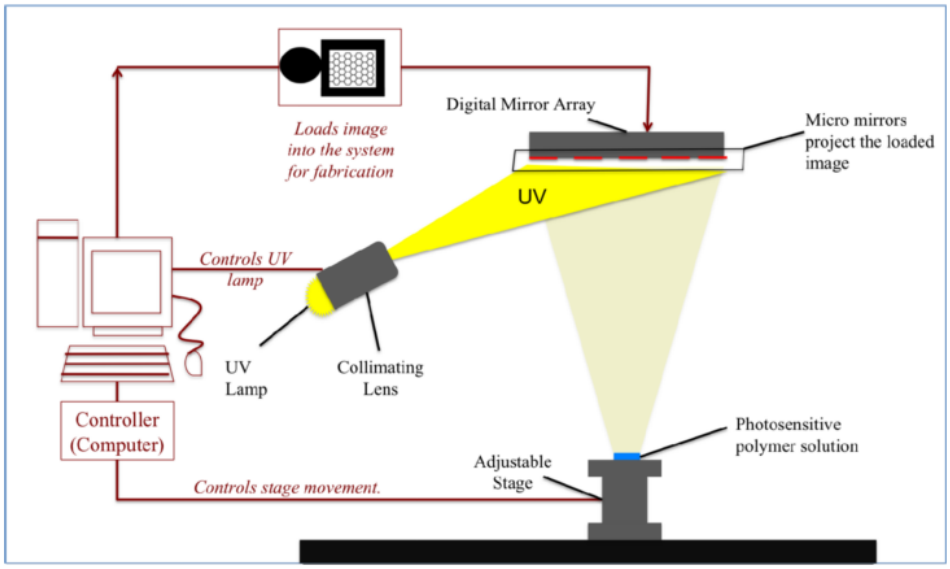

Image 15: Schematic Diagram of the DMD-PP System

The UV light illuminates a programmable digital micromirror array and is reflected down onto the photosensitive monomer solution. The result is a selectively polymerized structure based on a user-defined design; Reproduced from ref. #[131]

Scaffold-Free (SFB)

SFB approaches rely on autonomous self-assembly and bioassembly mechanisms to construct tissue structures in the absence of scaffolds, biomaterials, bioinks or other non-living architectures[24]. Neotissues, like high-density cell suspensions[47], tissue building blocks[132] and bio-fibers[87], are cultured via autonomous self-assembly and subsequently assembled into tissue structures, followed by post-printing cell sorting and tissue fusion in controlled incubation and microfluidic environments[133]. When support structures are required, agarose[45] and alginate[134] molds are typically used because they are resistant to cell-adhesion and provide temporary cell-maturation environments while controlling the structural geometry of aligned microtissues[69]. To date, vascularized thyroid gland constructs[21], liver tissues with maintained function over time[75], sciatic nerve grafts[45,133], tubular aorta grafts[135], myocardial tissue sheet implants[46] and 3D tumor models[44,136] have all been generated using SFB technologies.

SFB Advantages and Disadvantages

The advantages of SFB are clear: foreign scaffolds and materials are minimized, tissues at organ-level cell densities can be fabricated, and high-resolution micro-architectures within tissue products can be self-assembled. The absence of scaffolds and bioinks in the printing process minimizes cellular contact with synthetic and non-native polymers and maximizes cell-cell interactions and ECM cohesion[134]. After decades of experimentation and characterization of innumerable biomaterials across the TE field, an ideal biomaterial that is entirely compatible with living structures is yet to be identified; the optimal material must provide mechanical strength and structural integrity in the construction of large tissue products in accordance with

associated cellular biology[137]. Once printed and fixed, biomaterials must mitigate residual polymer components that hinder cell growth, provide a healthy living environment for cellular proliferation and ingrowth, and degrade over time[70]. Degradation is necessary because once implanted, there must be space for integration of vasculature and tissue between the host – the degradation rate must match the ongoing neo-tissue formation rate[9]. Additionally, degrading materials can produce toxic components that tend to trigger immunological and inflammatory reactions *in vivo*[70].

SFB techniques are currently hampered by challenges associated with cell behavior and culture. The majority of scaffold-free products are thick tissues made entirely of cellular components, which requires large numbers of cells to be cultured[35,135]. This process can take anywhere from days to months per trial, is highly expensive, and is prone to complications in batch-to-batch variability associated with cellular viability, differentiation and overall functionality[16,119]. After printing, it takes spheroids 5-7 days to fuse completely, further augmenting production time[138]. Most SB approaches use fewer numbers of cells and can experiment using primarily biomaterials, but SFB trials require the use of entirely cellular components in order to appropriately model cellular behavior. Additionally, thick avascular tissue building blocks like spheroids can develop a necrotic core unless perfusion throughout the entire microtissue is established[35]. Inherently, revoking the use of scaffolds also comes at a price; scaffolds provide structural support both *in vitro* and *in vivo*, can be used as a template for early cell growth culture and expansion, supply a platform for nutrient-transport and can deliver ongoing mechanical and physiochemical cues for supporting cell growth and differentiation[8,39].

SFB Highlighted Studies

In an early SFB study, Norotte et al. created tubular tissues in controllable shapes and diameters situated in a hierarchical tree formation using spheroid tissue strands. HUVSMC, PASMC and HSF pellets were self-assembled into spheroids in capillary micropipettes 300µm or 500µm in diameter and subsequently fused to create cylindrical bioinks. This bioink was dispensed using an automated ram-driven MBB system with two heads: one for bioinks, and one for agarose. Agarose rods were cast layer-by-layer along with cellular cylinders to provide structural support during printing and spheroid fusion. After printing, spheroids took 2-4 days to fuse, agarose rods were removed, and single- and double-layered vascular tubes with an OD between 0.9mm and 2.5mm were revealed. This study showed the serviceability of creating fully biological vascular structures with controllable geometry and multiple cellular layers in an automated and reproducible fashion. Efforts to improve upon this work, including the characterization of spheroid fusion, modifying the removal of agarose support rods and the creation of thinner-walled constructs to reduce internal cellular hypoxia are ongoing.[69]

An alternative SFB approach utilizing agarose and MEF pellets has been proposed by Kucukgul et al. The team developed fine-tuned pathway-control algorithms to direct their Novogen MMX 3D Bioprinter to construct a human-sized aorta. The team took structural CT scans of a human aorta, converted them into 3D CAD, transferred these images to their homemade path-planning program which modelled the deposition of the fully-cellular bioink and exterior hydrogel supports, and printed the aortic structure and surrounding scaffold according to their modelling software. Print time, layer placement and support minimization

were all optimized using the computerized algorithms. The team used cylindrical cell aggregates as a living bioink (composed entirely of cells) by fusing together a long string of spheroids to create tissue strands that could be extruded by the bioprinter. An aorta-shaped construct made entirely of cells was produced, yet the research team was unsuccessful assimilating vascular networks or the heterogeneous composition of the aortic wall; this shortcoming resulted in rapid apoptosis within a necrotic core that led to complete tissue degradation over the course of four days. This study commandeered novel approaches to developing print algorithms, imaging techniques, cylindrical bioink deposition, and human-scale printing, but failed to address more complex biological considerations needed to create a long-term functioning tissue product. Bioprinting will need collaborations between engineering-focused teams, like the one shown here, and research teams that include biologists, chemists and mathematicians to ensure future success.[138]

One of the main advantages of SFB is the capability to create highly dense tissue constructs, as demonstrated by Blakely et al., who developed a novel microtissue manipulation system called the Bio-P3 to create thick, perfused tubular tissues. The homemade bioprinter, which is controlled by an x-y-z manual controller, picks up tissue building blocks using peristaltic-driven fluid suction, moves the microtissue to the desired location and releases the tissue-part, all with negligible effects on structure or viability. This printing process, and constructed vertical toroid-based tissue stacks, are shown in Image 16. The print-bed for the Bio-P3 is a porous perfusion surface in an aqueous media tub that maintains flow across assembled structures intra- and post-printing. Using a specialized agarose-based culture technique, tissue building blocks made entirely of living cells were made in the shape of toroids, honeycombs and spheroids. Non-adhesive micro-molds made by Microtissues Inc. provide a platform for self-assembly to proceed into pre-defined shapes. Using the Bio-P3, the research team 'picked and placed' toroids comprised of 300,000 KGN cells around a capillary tube to create a vertical conduit 16 toroids high, which fused for 72 hours under perfusion. This method demonstrates a novel SFB approach for creating high-density tissues; the Bio-P3 team is now working to refine the system by automating the construction process, constructing more complex designs, incorporating multiple cell types and creating tissues with quantifiable levels of functionality.[49]

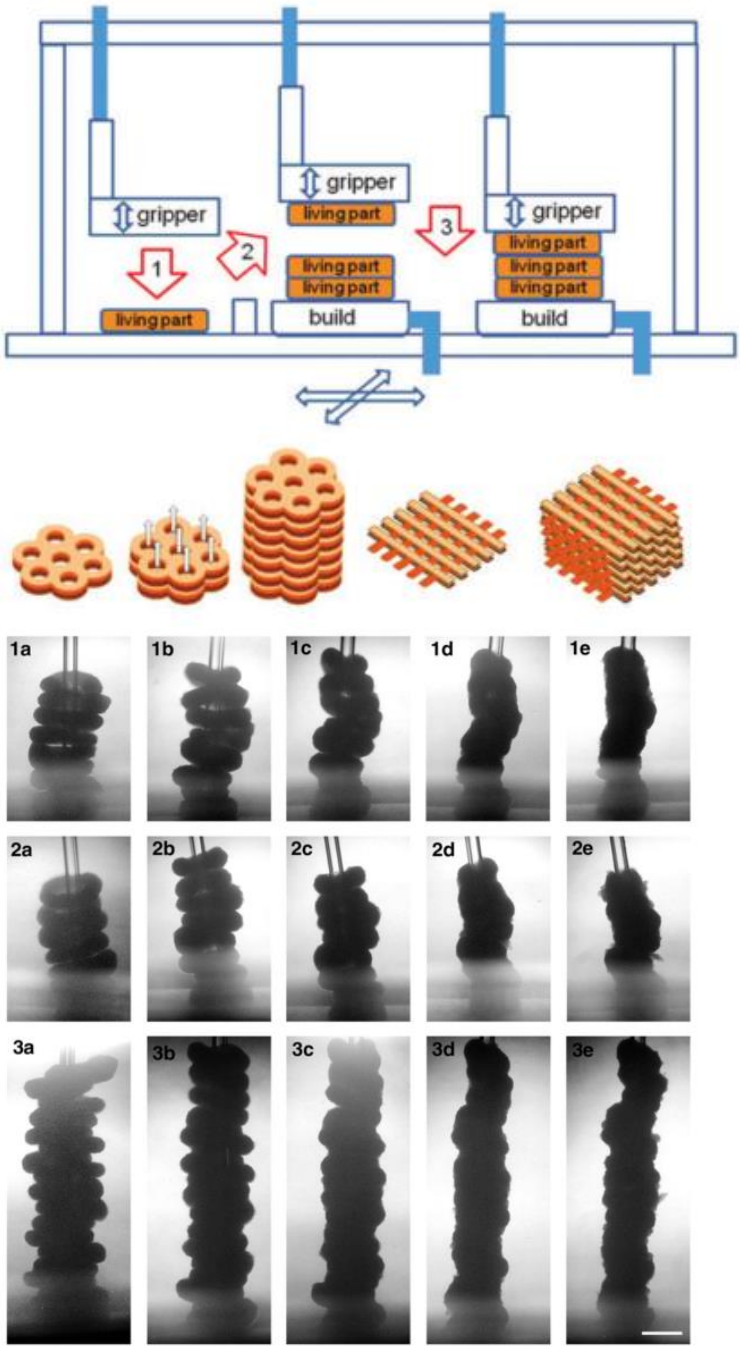

Image 16: Bio-Pick, Place, and Perfuse Instrument and Vertical Toroid Stacking

Top: Schematic of Bio-Pick, Place, and Perfuse instrument; Bottom: Toroids that are gripped and stacked will fuse into a single tissue. The Bio-P3 was used to pick and place toroids comprised of 30,000 KGN cells on a capillary tube (170mm OD and 100mm ID). Side view images of stacks of 8 toroids (top row 1), 8 toroids (middle row 2), and 16 toroids (bottom row 3) at 0 (a), 12 (b), 24 (c), 48 (d), and 72 h (e) after stacking showing fusion into single tissues. Scale bar 500mm; Reproduced from ref. #[139]

Micro-Network Approaches

Both guided organization and *in vivo* integration approaches employ the full spectrum of SB and SFB bioprinting modalities outlined above, with an attenuated emphasis on direct-patterning biomimicry approaches to produce vascular structures. Micro-network studies tend to focus their attention on harnessing cellular *de novo* remodeling capabilities of anatomical systems in order to produce tissues that develop their own micro-networks *in vitro,* or through integration with a host's vasculature.

Guided Organization

The fields of TE and RM have yet to procure a method for directly engineering capillary networks at the sub-micron level, thus, current bioprinting approaches are concentrated on guided vascular growth to establish hierarchical micro-network structures. Compared to direct channel patterning, micro-vascular approaches are straightforward, but there are a wide variety of factors at the chemical, biological and physical level that control angiogenesis through space and time; these considerations have been outlined succinctly by Rouwkema et al., and are summarized briefly here.[74]

The optimization of chemical microenvironments is essential in the establishment of capillary networks *in vitro*. VEGF controls EC growth and branching and responds preeminently to irregular gradient distributions; it has been shown that VEGF uniformly distributed throughout an ECM, scaffold or hydrogel fails to initiate EC proliferation.[18] Other chemical factors controlling vascular development, like PDGF[140] and Ang1[141], must also be patterned in defined gradients to control vessel maturation following VEGF-guided vessel growth. Furthermore, ECM mechanobiology affects the maturation and proliferation of embedded vessels and is a crucial consideration in material selection. For example, collagen stiffness influences HUVEC growth[142], hydrogel density alters angiogenic sprouting in spheroids[143], and tunable collagen material properties impact EC responses to VEGF and other growth factors that affect sprouting neo-vessels' direction, abundance, and length.[144] Finally, the physio-mechanical properties of a micro-network environment must be considered. Localized fluid flow guides EC organization and maturation in response to shear stress, while interstitial flow concurrently induces shear stress and diffuses 4D chemical factor gradients through EC sites to trigger angiogenesis.[145,146] Given these considerations, micro-network approaches are typically employed alongside macro-network strategies, as vascular networks will autonomously grow through a tissue if the proper cell types, flow conditions, growth factors, ECMs and chemokine gradients are present through tissue-space and time[65].

Guided Organization Highlighted Studies

In 2012, Culver et al. arranged chemical enzyme factors within a hydrogel to control cellular ingrowth and establish organized capillary networks. Using a high-resolution double-photon laser, Culver's team spatially patterned PEG-RGDS, an EC-derived enzyme that degrades ECM during the EC invasion process, in PEG-PQ hydrogels by selectively curing the biomolecule in the anatomical shape of human microvasculature according to 3D images derived from cerebral cortex tissue samples. HUVEC and 10T1/2 fibroblasts were homogenously

encapsulated in the hydrogel before the PEG-RGDS was immobilized via SLA crosslinking. Results showed that HUVECs and mural precursors organized themselves into microvascular networks within the hydrogel in accordance with outlined patterns. This shows how SBB can be used to accurately guide cell migration into the shape of micro-vasculature features within a scaffold, but little analysis of cell viability, vessel characteristics or overall tissue functionality is provided. Future studies could employ this type of approach within larger tissue constructs in order to guide capillary-growth between macro-networks.[147,148]

In the creation of a tissue model for liver studies, Lee et al. manipulated the cellular environment of a hydrogel to form dense capillary networks. The team used an in-house pneumatic MBB equipped with a dual print-head system for bioinks and thermo-plastic biomaterials. Previous mechanical testing showed that collagen was prone to collapse when printed in porous architectures, so PCL frames were used to support embedded collagen. Three different collagen bioink groups were tested, with varying concentrations of cells: group-1 was exclusively HCs, group-2 included HCs and NHLFs, group-3 combined HCs, HLFs and HUVECs, and the control group had no cells. After 14 days, group-3 HUVECs had formed a dense 3D capillary network throughout 40% of the hydrogel, and group-3 also showed drastically increased levels of urea synthesis and albumin secretion compared to other groups. It is likely that this superior functionality is a result of increased vascularization and capillary integration in the hydrogel after a long incubation period. As 3D tissue models develop, it will be essential to characterize these systems and understand how multiple cell types, materials and structure-designs affect relevant functional outcomes.[106]

An extensive study examining capillary follicle formation within a thyroid implant was reported by Bulanova et al. in 2017, in collaboration with 3Dbio[149]. The team used SFB spheroids as micro-vascular thyroid tissues to surround ECs with dense capillary follicles. Characterization of a novel printing approach, spheroid fusion, vascularization and thyroid transplant functionality in mice was detailed. The team used a Fabion 3D turnstile bioprinter with a customized temperature-control system to position embryonic TS's next to AS's so they would self-assemble into thyroid tissues. The AS invaded and vascularized the TS, as AS contain ECs and TS's produce high levels of VEGF-A, resulting in increased vascularization when cultured in close proximity. Both spheroid types developed extensive vascular networks and ECs from the TS also formed follicles after four days in culture. The thyroid constructs, which were composed of six AS surrounding three TS, were grafted under the kidney capsule of hypothyroid mice to evaluate functionality *in vivo*. Histological analyses three and five weeks post-transplantation showed maturation, integration and vascularization of the tissues, with new layers of capillaries forming throughout the structures. Furthermore, the implants restored circulating T4 levels and normalized body temperature homeostasis. This proof-of-concept study incorporated MBB and self-assembly to set forth a strategy for creating functional tissue patches with high levels of vascularization and functionality, and shows how simple vascularized tissue-patches may be used in clinical scenarios in the near future.[21,149]

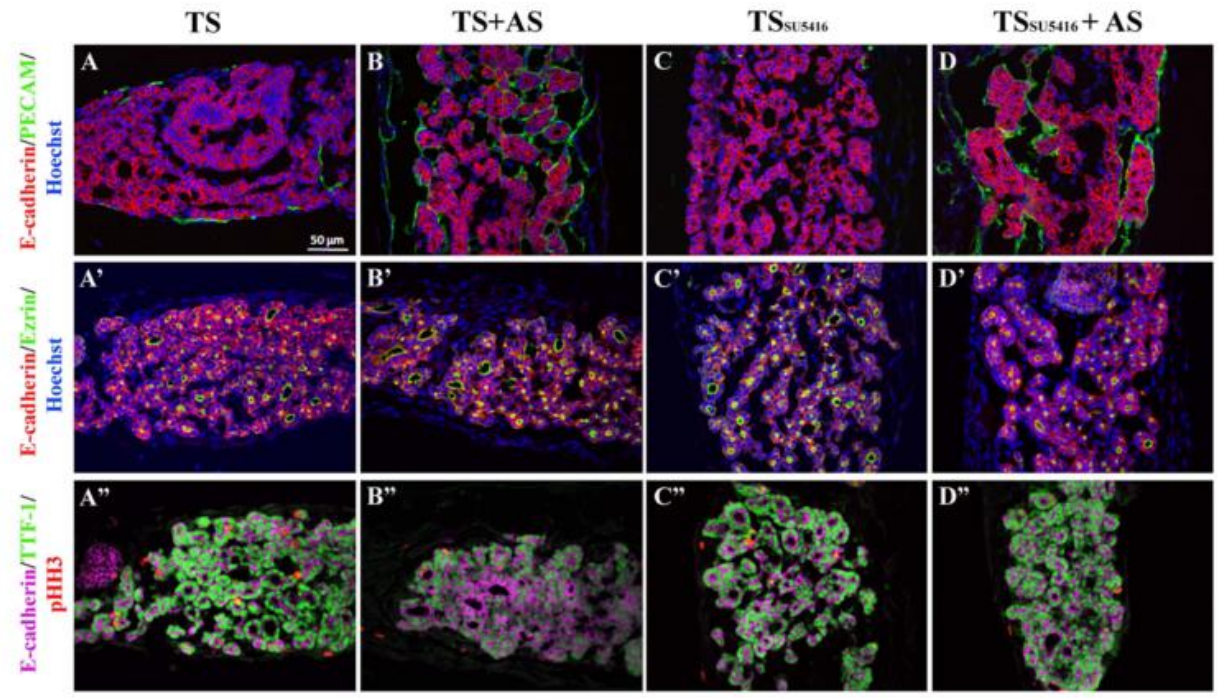

Image 17: Vascularization of Bioprinted Mouse Thyroid Constructs

(A) Bioprinted thyroid spheroids (TS) display endothelial cells (EC) only at the periphery of the tissue. (B) TS bioprinted together with allantoic spheroids (AS) reveal intense PECAM labeling around and inside the TS. (C) TS pretreated with SU5416 (TSSU5416) during hanging drop culture do not show endothelial labeling. (D) In the presence of AS, TS depleted of EC(TSSU5416) are invaded b yEC from AS. Thyroid follicle formation (A')–(D') as well as cell proliferation (A")–(D") was comparable in all the cultured bioprinted constructs; Reproduced from ref. #[21]

In vivo Integration

Vascularized tissues can generate microcirculatory networks at diseased regions or encourage vessel ingrowth from host to implant *in vivo*, both of which can repair damaged tissue areas or reconstitute vascular functionality at local sites[150]. This integration is difficult to control because it introduces challenges innately intertwined with complications associated with transplant procedures. Time is of the essence during the implantation process because a tissue must have near-constant access to blood (or media) flow during in-growth, which can take days to weeks to establish[79]. Successful integration is accomplished in two ways[73]. First, if a transplanted tissue has macro-channels, microsurgery or vessel-alignment techniques can be used to directly connect channels[84]. Second, natural in-growth and inosculation can be encouraged. Ideally, both integration methods are employed to establish micro-networks within implanted tissues.[62,151]

In vivo Integration Highlighted Studies

One pre-vascularization strategy involves patterning angiogenic microvascular precursors within hydrogel pre-implantation, as demonstrated by Chang et al. in 2013. Using an MBB BioAssembly Tool configured with two independently controlled dispensing systems, Chang's team bioprinted collagen beds embedded with RFMFs, which are neovessels isolated from rat adipose microvessel segments. The fragments were patterned in unidirectional parallel

arrays through the collagen scaffold, which was implanted into mice. The form and topology of vascularized tissues within the implant was monitored for 30 days post-implantation, during which the frame was extensively integrated with a dense capillary network that exhibited high levels of vascular functionality. Over these four weeks, the pre-patterned microvessels lost their alignment and structure, likely due to extensive remodeling during host-integration in response to vessel caliber, density and flow pathway position alterations over time. This study was successful in implanting bioprinted structures *in vivo* but failed to control the morphological form and direction of endovascular networks with their RFMF patterning technique; clearly, further work is needed to quantify and characterize contributing parameters that affect neovessel growth and topology within bioprinted scaffolds.[152]

In a recent microvessel patterning approach published in 2016, Shen et al. illustrated the effectiveness of using stem cell seeded scaffolds to improve the wound healing rate of type-1 diabetic ulcers. HA hydrogels were engineered as implantable grafts, seeded with either ECFCs, EVCs, or EVCs differentiated into EVC-BC1s or EVC-T1Ds from hiPSC cell lines, and cultured for three days to establish microvascular networks throughout the HA gels scaffolds. Next, tissues were implanted into burn wounds of diabetic mice and monitored *in vivo* for wound closure rates, healing effectiveness, blood flow profile, macrophage infiltration and vascular integration. In all of the cellular scaffolds extensive vascular remodeling, angiogenesis, macrophage infiltration, advanced wound closure and vascular integration was observed, and is shown in Image 18. Notably, blood perfusion was established rapidly with the host and was measurable three days post-implantation with EC progenitors facilitating the earliest wound closure rate. Even though bioprinting was not directly employed to create the tissue scaffolds outlined in this study, it is apparent that the incorporation of bioprinting technologies would be able to increase the efficiency, preparation time, cellular encapsulation and manufacturing capabilities of this engineering approach. Utilizing a bioprinter to position and develop morphologically advanced scaffolds could enhance this therapy's efficacy using pre-vascularized constructs to treat wounds and initiate *in vivo* integration.[153]

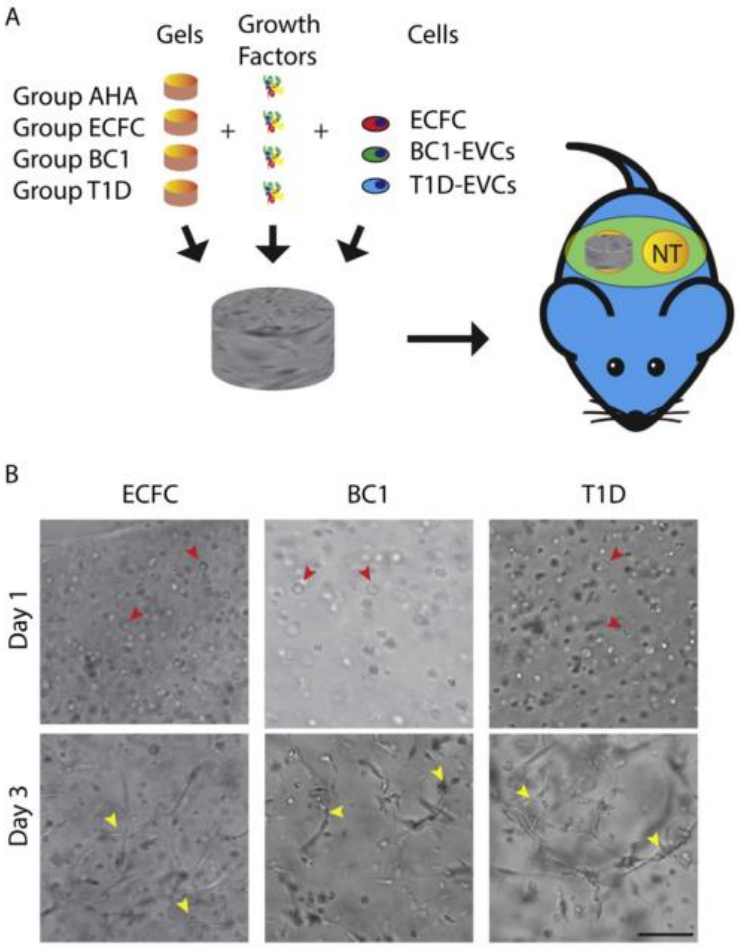

Image 18: Experimental Groups and In Vitro Engineered Human Vascular Constructs

*(A) Schematic drawing of the timeline of immunodeficient diabetic mellitus wound model in mice (i) 5 daily consecutive 50 mg/kg STZ injection. (ii) Glucose levels exceeded 300 mg/dL on 2 consecutive readings 48 h apartwas selected (N ¼ 6). (iii) At day 0, two 6mmfull thickness punch wounds were created. Treated vs non-treated served as control and dressed with Tegaderm. (B) Blood glucose measurements during the experimental period. 300 mg/dL is considered diabetic (C) Photos showing (i) wound after bandage using betadine and tegaderm and (ii) planetary measurement of the wound (shown at day 0 & 7). Shaded area is calculated as wound area (in this example, 100% on day 0 and 47% on day 7). Scale bar is 1 cm. Two way ANOVA post test for significance *p < 0.05; Reproduced from ref. #*[153]

In 2016, Sooppan et al. described a novel surgical technique for connecting a bioprinted vascular tissue graft *in vivo* in a rodent model. To create the grafts, a modified MBB printer deposited PDMS with embedded sacrificial sugar-glass lattices. Post-printing, lattices were removed to form a ladder-shaped macro-vessel structure consisting of two primary vessels with 1mm ID connected by four vessels with 300µm ID. This graft was implanted at the right femoral artery of mice using an experimental direct anastomosis technique. Pulsatile flow was detected in the implanted tissue for three hours post-implantation using Doppler imaging to monitor hind limb ischemia. Past three hours, grafts collapsed as a result of their small ID and lack of incorporated cellular components, though small amounts of vascular host-integration were observed. *In vitro,* these macro-channel grafts showed HUVEC sprouting and remodeling when seeded with ECs. They also exhibited encouraging levels of anti-thrombogenicity, which unfortunately failed to translate *in vivo.* This is a viable method for overcoming temporal

hypoxia and nutrient deficiencies within implanted tissues immediately post-implantation, but future work is needed to incorporate ECs into transplanted gels to increase host remodeling and neovascularization and to reduce blood clotting long-term.[154]

Another study examining *in vivo* anastomosis of vascular conduits was presented by Gao et al. in 2017. The team bioprinted biodegradable PCL grafts resembling rabbit tracheas and soaked them in chondrocyte suspensions for either two or four weeks. The seeded PCL grafts were subcutaneously implanted into nude mice and rabbits, and developed mature cartilage tissue surrounded by extensive capillary regions as well as invasive cellular inflammatory areas over the course of ten weeks. Animals with four-week scaffolds had a significantly longer survival rate, though the majority of the models died within ten weeks due to granuloma formation around the implant. This study is a simple exhibition of implanting a tissue graft that initiates neo-vascularization from the host and maintains functionality over multiple months. To increase the integration potential, raise graft efficacy and reduce the risk of thrombogenic occurrences, bio-prostheses directly seeded with tracheal cell-types, including ECs and mural support cells, should be developed and compared to the cultured scaffolds used here.[155]

Section 4: The Bioprinting Industry

The basic research and bioprinting approaches outlined above are essential to the overall progression of the bioprinting field. These advancements are driving the growth of the industry, which is centered around providing lab materials and bioprinting equipment to researchers, and the production of manufactured tissue products with therapeutic and pharmacokinetic applications. To supplement this literature review of the current state-of-the-art approaches for bioprinting vascularized tissues, a primary research effort was engaged. Interviews were used to garner an enhanced knowledge-base of the current technologies, to verify and validate the current state of the art, and to provide outlooks on how these technologies are being applied in industrial settings. It is the objective of this report to provide, perhaps uniquely, a review that intimately connects a bioprinting literature review with industry and value chain trends, drivers and KOL opinions. Influential industry trends and supports, including developments, players, and pathways, are outlined in this section in the following order:

1. **Market Overview** – Our market inspection orients and frames the field by drawing upon secondary source materials, like market reports and technology roadmaps, to procure relevant industry data, growth metrics and macro-drivers.
2. **Archetypes** – We have segmented industrial players, including bioprinting companies and material suppliers, into defined archetypes based on conglomerated 'zoomed out' observations and defining characteristics identified during our primary and secondary research efforts. The current inclinations and characteristics of these archetypes are displayed as a 'value chain' that shows the progression of the bioprinting pipeline, from developmental to established bioprinted products, which includes critical administrative limitations at each step of the value chain.
3. **Bioprinting Pathway** – We illuminated the 'bioprinting pathway' using both primary and secondary research results, which is a surface exploration of critical parameters, inputs and considerations that are relevant to bioprinted products along their production path from basic input procurement to the final tissue construction. This pathway includes overviews of material inputs, collaboration structures and regulatory outlooks of key players.
4. **Product Timeline** – Synthesized stakeholder interview feedback extensively supplemented primary research efforts and were specifically used to create a product commercialization timeline. This timeline displays the pace of development and time to commercialization of for *in vitro* and therapeutic tissue products.
5. **Potential Opportunity Areas** – To provide an exemplification for the potential marketability, real-world impact and functionality of bioprinted tissues, a case study examining large- and small-diameter vascular grafts is featured. This opportunity area includes an interview with a vascular surgeon to ascertain sensitivities surrounding the adoption of bio-prosthetics in a range of clinical scenarios.

6. **Company Profiles** – Examinations of preeminent bioprinting companies are provided, which describe company attributes, relevant published data, and tissue-product qualities in detail.

Market Overview and Macro-Trends

The bioprinting industry is a seminal sub-sector of the 3D-printing and additive manufacturing space that is beginning to resemble qualities of a formative sector poised for massive growth. Across this maturing sector, niche commercialization strategies are beginning to specialize and segment, and key stakeholders continue to merge and collaborate to expedite advancements in fundamental bioprinting technologies. While competition across the industry is currently low, it is increasing as new players enter the market, and bioprinted products edge closer to poignant commercial application. According to market reports, this competition will soon reach a critical threshold that will begin to dissuade collaborations and initiate industry consolidation.[59,156]

The market size of 3D printing, which includes all non-biological and bioprinting fields, was approximately $2.2B in 2012, and is expected to total $10.8B by 2022 at a compound annual growth rate (CAGR) of 17.3%. The sub-sector industry of bioprinting, which includes the sale of bioprinters and living bioprinted products, has grown rapidly in recent years. According to a report from Grand View Research Inc., the global bioprinting market was valued at $487M in 2014 and is expected to reach $1.82B by 2022 at a CAGR of 17.9%. During this time, the number of bioprinters shipped to medical facilities is expected to double.[59,157] Startups and investors entering the market are optimistic about the field's technical advantages, recent growth, and procured short-term products. As a result of rising numbers of organizations and laboratories printing with living materials, competition between research facilities has increased, and the price and accessibility of bioprinting materials and printers has declined dramatically over the last decade[39].

This growth is expected to be driven by a variety of factors, including emerging medical applications in pharmaceutical testing, drug discovery, disease modelling, TE and RM. Central to these uses will be the increased demand for organ transplantations, motivated by the rising prevalence of chronic diseases like Chronic Kidney Disease (CKD), geriatric populations, and life-style oriented diseases. The elderly demographic is especially prone to age-related organ diseasesand , is expected to increase in size from 12.3% (in 2015) to 16.5% by 2030, a direct result of extended life-expectancy worldwide.[59] In 2017 there were over 120,000 people on the American organ waiting list, yet only 26,034 transplants were performed between January and September. During this time period, an average of 22 people died each day waiting for a transplant. The need for a technology that can provide functional transplantable organs has never been higher.[60]

Despite rapid growth, the market for bioprinted tissue products is currently at an impasse. Initial basic research and proof-of-concept technologies have demonstrated enormous potential, but there are few commercial tissue products that have been adopted wide-scale. This state is considered the 'valley of death' in the R+D pipeline, as emerging technologies that fail to exhibit marketable and applicable characteristics, ranging from manufacturability to transportability to cost, tend to lose interest and funding if expedient advancements are not substantiated. A range of technical and market issues must be solves, and a substantive body of

science supporting tissue-product functionality must be exhibited in order to move the bioprinting field out of the valley of death and towards industrialization[151].

Industry Player Archetypes: Producers, End-Users and Watchers

The bioprinting industry consists of 3 major archetypes: watchers, end-users and producers. Watchers are the 'big fish' industrial players and investors interested in the field, end-users are entities purchasing and utilizing bioprinted products, and producers are companies working to research, develop and market translatable products. Producers can be categorized as suppliers, which are companies producing bioprinters, bioinks and printing materials, or builders, who build bioprinted products like tissues or vessel-grafts. Industry commercialization archetypes are described in the following order:

1. **Producers**: suppliers and builders
    a. *Suppliers*: bioprinters, hydrogel- and cell-based bioinks, bioprinting software
    b. *Builders*: producing bioprinted tissue products
2. **End-Users**: buyers of bioprinting products
    a. *Tissue-Users*: buyers of tissue products
    b. *Research-Users*: buyers of research products and inputs
3. **Watchers**: industry watchers and investors

Industry Archetype: Producers

Producers are directly invested in the research, materials, and products driving the growth of the bioprinting field, and are categorized as suppliers or builders. Suppliers are developing and marketing the infrastructure technology and raw material inputs for bioprinting, like bioprinters, print materials, hydrogel- and cell-based bioinks and bioprinting software. Many suppliers are larger additive manufacturing and 3D printing companies that have adapted their technologies to print living materials like Nscrypt and Seraph Robotics, while others are exclusively developing bioprinters, like CellInk, Aether Bioprinting, Cellenion, Biobots and RegenHU.

The bargaining power of input suppliers is currently limited, given the scarcity of demand and openness to collaboration between suppliers and builders. Examples of these collaborations include Cyfuse, who has a direct and exclusive pipeline for cell sourcing from Cell Applications, a worldwide producer of cells and cell culture products[158]. Another example is Collplant, in the development of a universal bioink, who joined Israeli company ReMDO's advanced biomanufacturing initiative in late 2017, which seeks to develop a highly modular bioink that can be used in a wide range of clinical applications[159].

Builders, on the other hand, are bioprinting companies marketing living bioprinted products, like drug testing platforms, ligament replacements and vascular grafts. Some builders, like Regenovo, TeVido Biosciences and Poeitis, are working to make non-vascularized constructs, while others are focused predominantly in the vascularized tissue space. Advanced Solutions Life Sciences (ASLS), Organovo, Cyfuse, 3Dynamic Systems (3DS), 3D Bioprinting Solutions (3Dbio) and Sichuan Revotek (SR) are leading the bioprinting field in the pursuit of creating a marketable vasculature product with clinical application. Rivalries between builders

is limited, as these companies are more apt to join efforts or target different end-applications rather than engage in head-to-head competitive behavior[156].

Industry Archetype: End-Users

End-users are the buyers of bioprinted products who are driving the commercial value of living 3D tissue product and applying them in the real world; they are classified as 'tissue-users' or 'research-users'. Tissue-users include surgeons, who may be transplanting small diameter vascular tissue grafts in a vessel replacement surgery, or pharmaceutical companies purchasing organ-on-a-chip products for *in vitro* drug testing or disease modelling. These users are differentiated from the research-users purchasing bioprinting materials and bioprinters, like university research labs and industrial development facilities who are using bioprinters in R+D.

Hospitals are illustrative end-users busy preparing themselves for the adoption of bioprinted tissues through collaborations with clinically-minded bioprinting companies. In early 2017, Organovo announced a partnership with the Murdoch Children's Research Institute at the Royal Children's Hospital in Melbourne, Australia, for the development and application of Organovo's therapeutic kidney patch[160]. Surgeons are also open to the idea of incorporating bioprinted tissues and bio-prostheses into their surgical suite, according to Dr. William Tanski, a leading vascular surgeon specializing in peripheral surgeries at Dartmouth-Hitchcock in NH. Dr. Tanski believes that vascular surgeons are exceptionally open to experimenting with bioprinted vascular grafts. He noted that he would experiment in an academic environment at Dartmouth before applying a bioprinted vascular conduit in surgery, and would have to consider, "logistics, sterility, approval and functionality within patients," but confirms that, "introducing something like a biologic graft could be an important clinical tool for our practice."[161]

Industry Archetype: Watchers

Watchers are large companies 'watching' the bioprinting industry in anticipation of commercial promise, technological breakthroughs and improved tissue-product functionality. These companies include biomedical and pharmaceutical organizations, like Johnson & Johnson (J&J), Merck, Proctor and Gamble (P+G), and Astra Zeneca (AZ), large software developers, like Microsoft, Hewlett Packard (HP) and Autodesk, and large companies specializing in 3D printing, like 3D Systems, Materialase NV, Stratasys and ExOne[162]. These companies will commit to a large-scale investment once bioprinting technologies pass a critical developmental tipping point, which is determined by the scalability, applicability and marketability of the bioprinting industry.[58] Venture capital firms and companies not directly involved with the technical R+D side of bioprinting are also considered 'watchers'; these companies are already investing in the field.

Watchers have expressed interest in expanding their TE and bioprinting capabilities, and many are collaborating with bioprinting companies by providing funding or market guidance. For example, AZ has established a 'BioVentureHub' which provides biotech companies lab-space and access to world-class technical and scientific professionals. The goal of the Hub is to bring together a wide range of biotech companies who can collaborate and benefit from each other; AZ provides facilities for companies to research and develop healthcare solutions together, while contending no claim on any products or intellectual property (IP) produced in

the Hub.[163] CellInk has already moved four researchers into the lab space, who are working to produce kidney, heart, liver and lung tissue-models for use in pharmaceutical development[164]. Likewise, J&J announced in early 2018 the funding and building of a bioprinting research laboratory in collaboration with the Irish materials science institute AMBER at the J&J 3D Printing Center of Excellence in Dublin, which will provide space for supportive and collaborative bioprinting research[165].

The Bioprinting Pathway

This pathway provides a road forward for the bioprinting industry by outlining and analyzing critical market areas along the bioprinting market development roadmap and providing essential factors and considerations for the future development of this evolving field. These market areas were identified as key commercialization, industrialization and manufacturing bottlenecks by our interviewees and through multiple-search-source secondary research methods, moving from basic material inputs to a final vascularized tissue product. While not an all-encompassing overview, this storyline sets the groundwork for aligning and sensitizing stakeholders to pertinent shifts and themes in the bioprinting space.

Industry-Wide Technical Limitations

For the bioprinting field, the development of technologies that will produce vascularized products is contingent upon the amelioration of a wide range of limitations affecting the field at the industrial, academic, technical and administrative levels. Common administrative challenges are addressed below in the exploration of the commercial pipeline value-chain, while technical limitations, relevant to the research and technologies driving bioprinting growth, are examined here.[58]

Many technical limitations are preventing the procurement of a method for building characterized and predictable products, including live-sensing, quality material supply chains, and 3D data-capture capabilities. We have adapted, with supporting literature metrics, a current-future table outlining basic research, GMP, scale-up and standardization challenges the bioprinting and biofabrication fields currently face. Table 4 data is sourced from leading TE reports and translational research efforts, as well as from interview responses from bioprinting KOLs. Identified limitations directly inform the supplemental analysis of the characterization and vascularization issues, which are addressed in detail in Table 4, and establish a comprehensive overview of the technical challenged facing the field.

Table 4: Industry-Wide Technical Challenges

	Current	Future
Basic Research	Effect of processing/printing modality and environmental characteristics on cell phenotype and viability is not understood	Systematic understanding of both physiochemical environment and bioprocessing forces on cell phenotype and function
	Very little data capture, live monitoring, or subsequent management and analysis	Live-dynamic and inline sensor technologies at appropriate stages of process and product lifecycle
	Incomplete understanding of basic biology, cellular complexities and self-aggregation	Complete characterization of biological processes
	Cumbersome and time-consuming culture and incubation technologies	Functional and expedient culture techniques
	Minimal control and integration of vascular networks at both the micro- and macro-level	Fully vascularized tissue products
	Printed tissues do not fully recapitulate *in vivo* environments	Biomimetic tissue products closely recreate anatomical structures, including high cell densities and multi-layered heterogeneous compositions
	Post-printing characterization of scaffold structural integrity, material behavior, and tissue-ECM over time is poorly understood	Predictable long-term scaffold and ECM architectures post-printing
	Optimal bioprinting modality unidentified	Integration of multiple TE approaches that ensure product quality, scalability and cost-effectiveness
GMP	Aseptic technique and clean room required	Operations conducted in CNC spaces
	Conducted in ambient air	Closed systems
	Reliance on a static process-control strategy	Understanding and allowing process variability based on FDA QdB principles
	Unreliable and low-quality inputs: biomaterials, bioinks, cells, media printing systems	Standardized, characterized and improved inputs
Scale-up	Labor intensive with high workforce demands	Fully automated production and printing
	Dozens of manual unit operations	Process simplifications and synergies
	Organization and segregation of autologous patient grafts during processing and incubation is difficult to implement	Segregation and identity of patient samples is automated
Standardization	Relationship between product quality and bioprinting process are not understood	Optimized bioprinting process based on QbD principles
	Highly variable raw materials and inputs	Control and management of raw materials
	Complex and tenuous supply chain	Efficient and superfluous supply chain

Table adapted from ref. #[166]: "ARMI Investment Roadmap" in Tom Bollenbach's keynote address at the ARMI BioFab Symposium in Manchester NH on January 4th 2018;

To gain enhanced insights into specific areas from Table 4 that are the most critical to the development of the bioprinting field, interviewees during our primary interview process were asked the question, "What technical limitations do you see facing the bioprinting field at large in the construction of a process that can construct commercially viable and applicable tissue products?" and were subsequently questioned at length about their opinions on the issues of highest importance. While answers were not quantitative, two themes became apparent across our body of responses: the vascularization issue, and the characterization issue.

Technical Limitation – Vascularization Issue

Academics cited the 'vascularization issue' as being the largest technical challenge facing the bioprinting community. This finding supports published literature findings; Datta et al. provides a representative explanation, "Since the last two decades, it has been agreed that it is essential to recreate the hierarchical vascular network which can ensure steady perfusion of implant-injury sites for successful engineering of most complex tissues; however, this issue has persisted as a major limitation over years on generating engineered tissues of clinically-relevant volumes and complexities[70]." This issue is significant because thick tissues at organ-level cell densities require integrated vascular networks to overcome the diffusion limit and maintain an efficient fluidic exchange.

Our interviewees confirmed the critical important of the vascularization issue; Matt Pavlovich, the editor of CellPress Journal, stated that, "Vascularization is the most obvious technical issue for the bioprinting field[61]," and Dr. Datta from IIEST concurred, saying, "We don't have a fabrication technique in which you can establish channels inside of a gel - there is no method that can do this - that's the most important challenge.[167]" In agreement, Dr. Homan and Ms. Kroll from the Lewis Lab at Harvard University noted that, "Broadly, establishing function of vascular networks is the biggest challenge and the next frontier for TE… We want the physiological act of perfusion because that allows us to study things we haven't studied before[119]." Given these aggregated outlooks, it is evident that if adequately perfused tissues cannot be constructed, the adoption of engineered tissues will be severely limited, and the construction of replacement human anatomy will be unattainable. This 'holy grail' of TE concerns is discussed at length in Section 2 of this report, and technical approaches leading the field in developing a solution to this challenge are discussed at length in Section 3.[6,35,79,101,168]

Technical Limitation – Characterization Issue

Second only to vascularization is the characterization issue – stakeholders are universally worried about the characterization of bioprinting inputs and products. 'Uncharacterized biomaterials, scaffolds and ECM' was the top industry-cited challenge, and 'poor cell biology characterization' was the second-most frequently mentioned limitations for all respondents. These issues are largely prominent because it is impossible to reliably recreate tissue products with enormous numbers of structural, chemical and biological variables without a complete understanding for the materials, cells, and processes that are being combined. These components must be controlled and understood so that the outcomes of future products

can be predictable and reliable. Our secondary research distinguished trends and themes across the bioprinting pipeline, which are displayed in Figure 2.

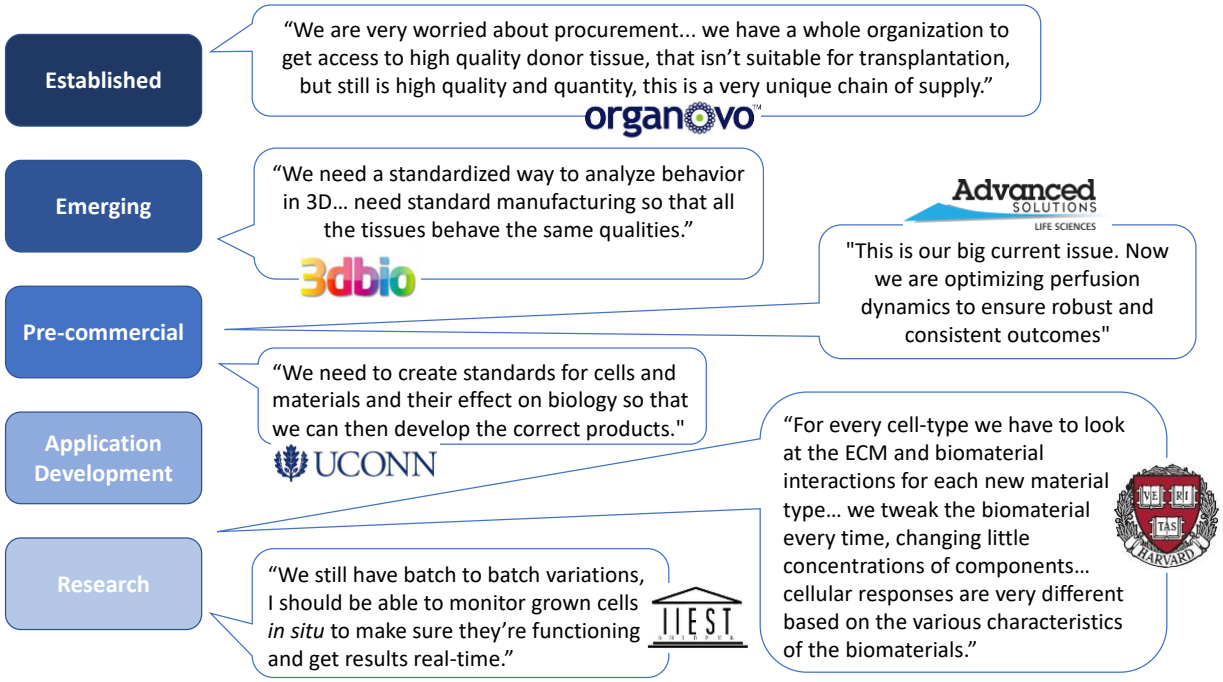

Figure 2: Input and Product Characterization Responses

Figure 2 shows that industrial players like Organovo are working to establish robust sources for characterized products and reliable inputs so that they can maintain high levels of quality and reliability in their engineering processes. This is critical in the industrial space because there is little room for functional error. Minute changes in the quality of materials and products can affect operative outcomes of tissue products, like *in vitro* models, which are being systematically compared to existing gold standards for drug testing for specific qualities; slight variabilities in a bioprinted tissue's performance could lead to sub-optimal comparative test results, which could halt R+D for that product line entirely. Companies are especially sensitive to consistency control because bioprinting companies are still in a start-up phase of development and are working adamantly to establish convincing data that will facilitate their movement up the adoption curve. Setbacks, like intermittent product performance lapses due to manufacturing inconsistencies or input quality lapses, could be a life-or-death situation for developing companies like Organovo, 3Dbio and ASLS.

Academic players are less worried about slight variations in product performance and are more concerned about developing processes that substantially move forward the TE body of science by developing novel techniques and approaches. University labs, like the Lewis Lab at Harvard, are hampered in this objective because for each cell type, printing modality and biomaterial they employ they must go back to the drawing board and work to control these dynamic interactions in a methodical way. It is difficult for these labs to determine the potential effectiveness of new bioprinting approaches and TE strategies when there is no standardized resource that establishes predictable outcomes. Illustrating this need is Vivian Lee, who we

asked, “What would be helpful in bringing your lab’s product to market?”, to which she responded, “Every-time we print a new material, we have a hard time finding printing parameters for this type of printed material… we need an outlined guide for characteristics like print speed and pressure… that would save a lot of time and effort because there are so many different printing modalities and so many compatible bioinks.” Research labs need a guide that provides printing specifications for each material type, and in extension, for different cell types and printing modalities as well.

Value Chain and Administrative Considerations

The following value chain shows the progression of product development in the bioprinting field from basic research to end-user, and includes contributing players at each pipeline progression, as informed by our archetypal market organization. Feedback from our interview process and an extensive survey of published market data backed the current administrative limitations, challenges and market-related properties that are described. These administrative characteristics and market themes were frequently cited during interviews and in academic research as prominent challenges for each archetypal group and are meant to educate the bioprinting community on major industry trends while also providing example-areas that can be targeted, addressed and resolved in order to move the field as a whole forward. These findings are shown in Figure 3, which displays contributors at each level of the bioprinting value chain with corresponding administrative challenges and characteristics below.

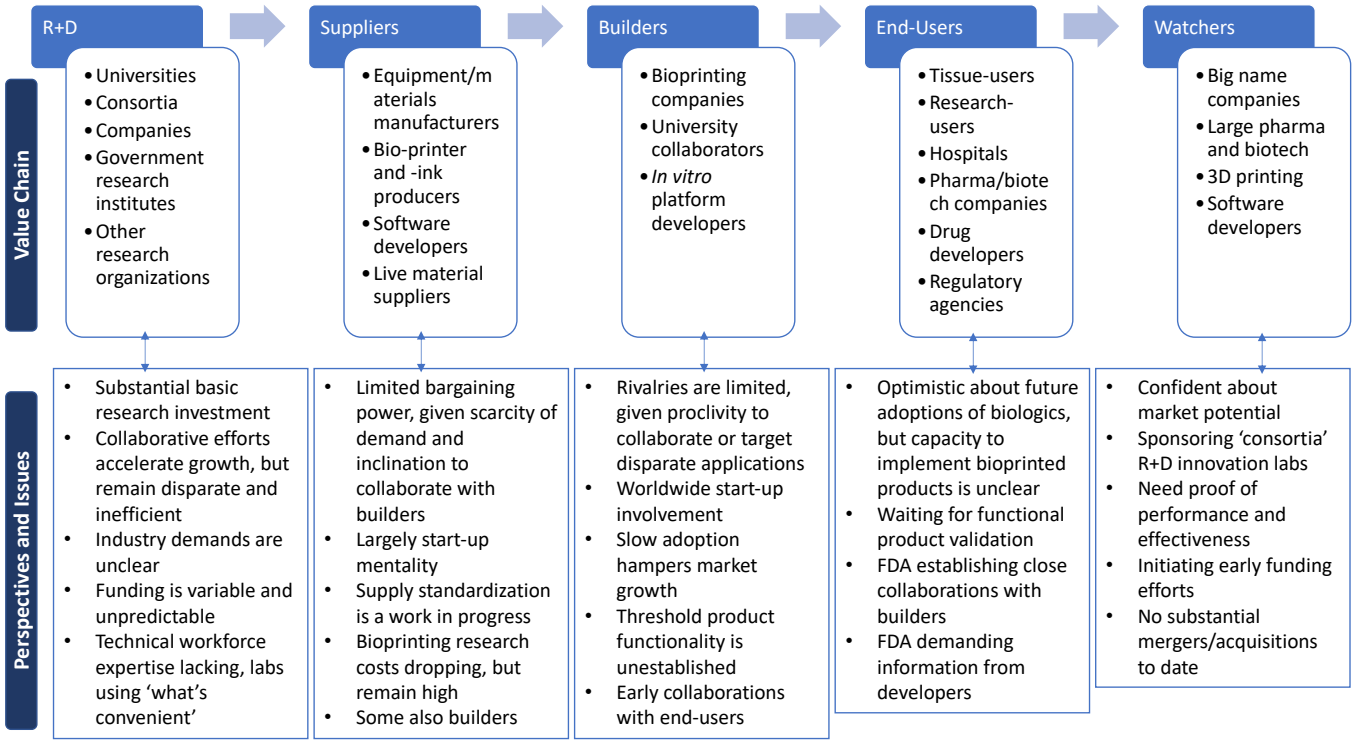

Figure 3: Current Archetype Value Chain

This value chain begins with the production of raw materials and inputs. Bioprinting requires many material inputs that include high-precision bioprinters, imaging technology and hydrogels for scaffolds and culture. Currently, many of these products are produced by disparate and specialized companies, but the market is already collaborating to increase the efficiency of input production, distribution and application. Cooperation (and even mergers) between industrial producers, whether they are builders or producers, will be essential, so that the highest quality goods and services can be utilized by research and development institutions in a maximally economical and productive manner.

Bioinks and Material Inputs

Material selection is a fundamental consideration for bioprinting facilities and is an integral part of the bioprinting pathway. Materials must not only be compatible with a given bioprinting process, they must also appropriately integrate with living components and support the mechanical and biological properties of sprinted tissues. The composition, polymer source, printability, biocompatibility, degradation kinetics and byproducts, biomimicry, structural and mechanical properties all must be controlled and accounted for when choosing a suitable bioink or scaffold material.[24] The material characteristics and specific advantages and disadvantages of various materials used in bioprinting are not analyzed here – considerations for choosing various synthetic and natural materials are outlined in detail elsewhere:

1. ***Ref. #*[169]**: An in-depth, material-by-material analysis of commonly used natural and synthetic bioinks with details examinations of their properties and applications.
2. ***Ref. #*[14]**: An overview of clinically promising natural hydrogels, including alginate, collagen, gelatin and fibrin, with advantages and disadvantages of each.
3. ***Ref. #*[24]**: A discussion of basic biomaterial features and their compatibility with traditional bioprinting approaches that includes material biomimicry, degradation and structural properties.

During interviews, ‘unreliable inputs’ was cited as one of the top technical limitations facing the bioprinting field by both university researchers and industry players. Hannah Strobel from Worcester Polytechnic Institute (WPI) explained that the field, “must understand available materials and their interactions before incorporating bioprinting… extraneous synthetic materials can negatively impact cellular behavior.” Lakshmi Nair from the University of Connecticut (UConn) concurred, noting that the field, “needs to create standards for cells and materials and their effect on biology so that we can develop the correct products". In order to ensure reliable and high-quality material sources in the future, expectations must be aligned across the industry. This cooperation has already begun: the bioprinting TE lab at Northeastern, led by Guohao Dai is collaborating with CellInk to characterize and improve their bioinks[170]. CellInk is also installing bioprinters and bioink systems at the University of Minnesota for use in undergraduate workforce education[171]. Stakeholders must continue to gain an understanding of the ‘suppliers’ that are producing and distributing bioprinting-compatible materials. Table 5 is an outline of the dominant bioink producers in the bioprinting space.

Table 5: Bioink Suppliers

Company	Country	BioInk	Base Material	Website
CellInk	Sweden	10 bioink types	10 material types	https://cellink.com
Advanced Biomatrix	USA	Lifelink 100 Lifelink 200 Lifelink 300 Lifelink 400	Methacrylated collagen Concentrated type-1 collagen Methacrylated gelatin Methacrylated HA	https://www.advancedbiomatrix.com
Allevi	USA	7 bioink types	7 material types	https://allevi3d.com
RegenHU	Switz.	ECM-Bioinks (5 types) Osteoink	Peptide:water hydrogel Calcium phosphate paste	https://www.regenhu.com
BioInk Solutions / 3DS	South Korea / UK	Gel4Cell (-BMP, -VEGF, -TGF) PolyInks-PCL PolyInks-PLA PolyInks-PLCLs	Gelatin PCL PLA PCLS	http://www.bioinksolutions.com http://www.bioprintingsystems.com/index.html
Collplant	Israel	rhCollagen	Recombinant collagen	https://www.collplant.com

The leading bioink development and supply companies identified in Table 5 must work with research organizations to fully characterize bioinks and material inputs for the bioprinting industry so that reliable manufactured products with predictable architectures and tissue functionality can be produced. Vivian Lee elucidated this trigger-point, saying that there is a, "printability problem with bio-carriers… if I had to prioritize our technical limitations, then I would say that biomaterials are first – I need tissue-specific ECM that can actually develop the desired tissue. This is highly dependent on the application, because different materials and structures are needed for different tissue-types and applications[172]."

Bioinks must be understood in context of their biologic function, but other biofabrication considerations are also critical in their application, including the effects of extrusion and deposition, printed geometric configurations, curing mechanism compatibility, incubation procedures, and integration with other materials on bioinks' physical and biochemical integrity. Hannah Strobel from WPI observed that, "cell settling, even suspension maintenance, and printing heat and shear forces can damage tissues; these issues haven't really been worked out yet… we will need to understand materials and their interactions before incorporating bioprinting[173]." Soon, these issues will be addressed, and preferable manufacturing, distribution and engineering methods for bioinks will be identified. This will allow the field to develop a select few bioinks that are extensively understood, rather than employ a range of poorly characterized materials. Ideally, these bioinks will have decreased variability, increased applicability and fully quantified biofabrication characteristics.

Bioprinters and Equipment Considerations

Bioinks are essential for developing supportive and functional scaffolds and bio-carriers, but they are only as effective as the printers that control them. Five years ago, a basic bioprinter could easily cost as much as $750K, there were almost no companies producing bioprinters on the market, and even fewer bioprinters being sold. In its infancy, the 3D-printing space was similarly inaccessible[40]. Since the expiration of major 3D-printing patents from 2009 to 2015 and the emergence of open-source 3D-printing platforms like RepRap, 3D-printers can be easily purchased or assembled for a low cost. Before the major inkjet patent expired in 2009,

inkjet 3D-printers cost $10K; now, they can be purchased for a tenth of the cost, with cheaper models, like do-it-yourself kits, priced as low as $500. A similar trend is already occurring in the bioprinting industry, as affordable companies providing bioprinters with advanced specifications are entering the market, increasing competition, and decreasing exclusive barriers to entry.[3,6,33]

This trend was verbalized by Pallab Datta from the Indian Institute for Engineering Science and Technology (IIEST), when he explained that, "In 2015 we developed our own bioprinter which is simpler than what is commercially available… now we are planning on purchasing a CellInk or EnvisionTEC printer… we have seen a lot of publications using those and have heard they're the best on the market[167]." Vivian Lee also developed her own printer but has since converted to a commercial model: "In '07 and '08 we had to build our own printer, now there are so many out there that can just purchase their own… I like the BioX printer from CellInk, it works well and comes at a good price[172]." CellInk has been able to differentiate itself from other bioprinter producers on the market by emphasizing intimate collaborations with universities – a trend that is likely to continue as bioprinting systems become more advanced and accessible.

To get a sense for the price and quality of current marketed bioprinters, we aggregated publicly available data from industry reports and literature reviews. This information is summarized in Table 6 and 7; Table 6 shows average price ranges for different modalities of bioprinting systems, and Table 7 displays a selection of the most affordable, widely-used and functional bioprinters available commercially. Predictably, the price of a bioprinting system depends on the desired specifications and modality. The most accessible systems are microextrusion-based, which are the cheapest on the market. Dual-head MBBs with photo and thermal cross-linking capabilities cost between $5-10K, but have reduced cell viability, print resolution and automation compared to high-end systems, which range from $150-200K. The most affordable dual-extrusion system on the market is the INKREDIBLE 3D Bioprinter by CellInk, which costs $5K, and is an economic motivator for university research facilities to begin in-house bioprinting operations. High-end printers manufactured by 3DS, CellInk and BioBots range between $10-23K, systems from 3Dbio and ASLS are between $100-160K, and top of the line systems made by RegenHu, EnvisionTEC and Cyfuse cost more than $200K.[156]

Low-cost DBB systems can be assembled in lab using modified inkjet printers, but these have small nozzle openings which have trouble printing large viable mammalian cells in the 20-25µm range and lack 3D x-y-z control. More expensive DBB printers are available between $20-70K, with price increasing as more print-heads are installed and resolution increases. Commercial LBBs are nonexistent but can be assembled for approximately $200K. The highest quality bioprinter on the market is currently priced around $1M; only five have been produced in the US as they far out of the budget range of most academic research institutions. Scaffold-free approaches necessitate a tissue manipulation system that can be built inexpensively, however, the process of cell-culture and self-aggregation to produce macrotissues demands close to 100M cells for a medium-sized tissue sample. The price of acquiring and culturing large cell quantities can vary considerably depending on the cell type used and the target tissue being produced.[3,6]

Table 6: Bioprinter System Cost Ranges

Modality	Basic	Intermediate	Advanced
Microextrusion (MBB)	$5-10K	$10-23K	$150-200K
Droplet (DBB)	$500-$1000	$20-70K	$100K
Laser (LBB)	NA	$200K	$1M

Table 6 shows generalized price ranges for various levels of complexity across bioprinting modalities, but for a more detailed and comprehensive analysis of all printers on the market and their specific capabilities, we turn our readers to *ref. #*[3]. For an additional summary of a selection of the most common and applicable commercially available bioprinters and suppliers on the market, Table 7 is shown below. This table displays companies leading the market developing bioprinters, printer-system names and reference websites. Companies were selected based on secondary market research, consumer reports and from interview feedback from researchers who are currently using (or considering adopting) these products in their labs.

Table 7: Commercially Available Bioprinters

Company	Country	Bioprinter	Website
3Dbio	Russia	FABION	http://www.bioprinting.ru/en/
3DS	UK	3DS Alpha, Omega, Delta	http://www.bioprintingsystems.com/index.html
ASLS	USA	BioBot; BioassemblyBot	https://lifesciences.solutions/vipm/
Aether Bioprinting	USA	Aether 1	http://bioprinting.aether1.com
Allevi	USA	Allevi 1, 2, 6	https://allevi3d.com
Aspect Biosystems	Canada	RX1 Bioprinter	https://www.aspectbiosystems.com
Bio3D Technologies	Singapore	Explorer; Life-Printer 'X' BioScaffolder	https://3dprint.com/11136/life-print-x-bio3d/
BioDan Printing	Spain	BioDan PrintSkin	http://www.biodangroup.com/print.html
CellInk	Sweden	Inkredible; Bio X Bioprinter	https://cellink.com
Cyfuse Biomedical	Japan	Regenovo Bioprinter	https://www.cyfusebio.com/en/
EnvisionTec	USA	3D Bioplotter	http://www.envisiontec.de
GeSiM	Germany	uContact; Bioscaffolder	https://gesim-bioinstruments-microfluidics.com/category/home/?lang=en
nScrypt	USA	3Dn 300 TE Series (300, 500, tabletop)	https://www.nscrypt.com/solutions/bioprinting/
Ourobiotics	Ireland	Revolution 3D Bioprinter	https://www.weare3dbioprintinghumans.org
Poietis	France	LBB NGB 17.03	http://www.poietis.com/en/index.php
Regemat 3D	USA	Regemat 3D	http://www.regemat3d.com
RegenHu	Switz.	3D Biofactory; 3D Discovery	https://www.regenhu.com
Regenovo	China	Regenovo Bioprinter	http://www.regenovo.com/English/index.aspx
SE3D	USA	Rebel X Bioprinter suite	https://www.se3d.com
Seraph Robotics	USA	Fab@Home Model 3	http://www.scientist3d.com
The Technology Partnership	UK	Vista 3D	https://www.ttp.com

In the future, combinations of developed versions of the bioprinting systems identified in Table 6 will be required to support the translation and manufacturing of bioprinted products to meet a range of clinical needs, but more basic research is needed to identify which approach is appropriate for which applications. "Right now," Matt Pavlovich explained during an interview, "the paradigm seems to be mostly, 'let's use whatever we have convenient in our lab,' which I think will be replaced by more rational planning as the field matures… the field hasn't caught up to which technique is best for which purpose." Increased basic research and proof-of-concept innovation will illuminate what technologies and methodologies are most

advantageous, and the field will stop using a multitude of 'convenient' approaches and will begin to use 'what's maximally effective'. The importance of basic university research and creative bioprinting experimentation cannot be overlooked.

Once a concentrated number of favorable techniques are identified, the manufacturing and deliverability of these systems will have to be considered. Both on-site printers, for immediate individualized autologous production, and off-site printers, for large-scale manufacturing purposes, will be required to deliver personalized autologous therapeutic products. On-site printers would include portable simplified systems that could be used at hospitals for building expediently transplanted products. Brisbane, Australia an bioprinting center has been created inside of a hospital, which will hopefully be producing customized bed-side tissues in the near future. Off-site bioprinting will likely incorporate large-scale biofabrication facilities capable of producing high-throughput assays, tissue models or grafts which could be mass-produced for in-house testing or shipment to the end-user.[149]

Tissue Producer Companies

The examination of bioprinted products and their application at the end of the bioprinting pathway is only complete if the producers that are developing and marketing these products are acknowledged. In Figure 4, we provide a development timeline that organizes producers based on their commercial readiness and pipeline progress. Producers are distributed across the two macro-product categories and are shaded (using our best judgement) according to the volume, integrity and functionality of publicly available body-of-science data supporting their R+D and product portfolio.

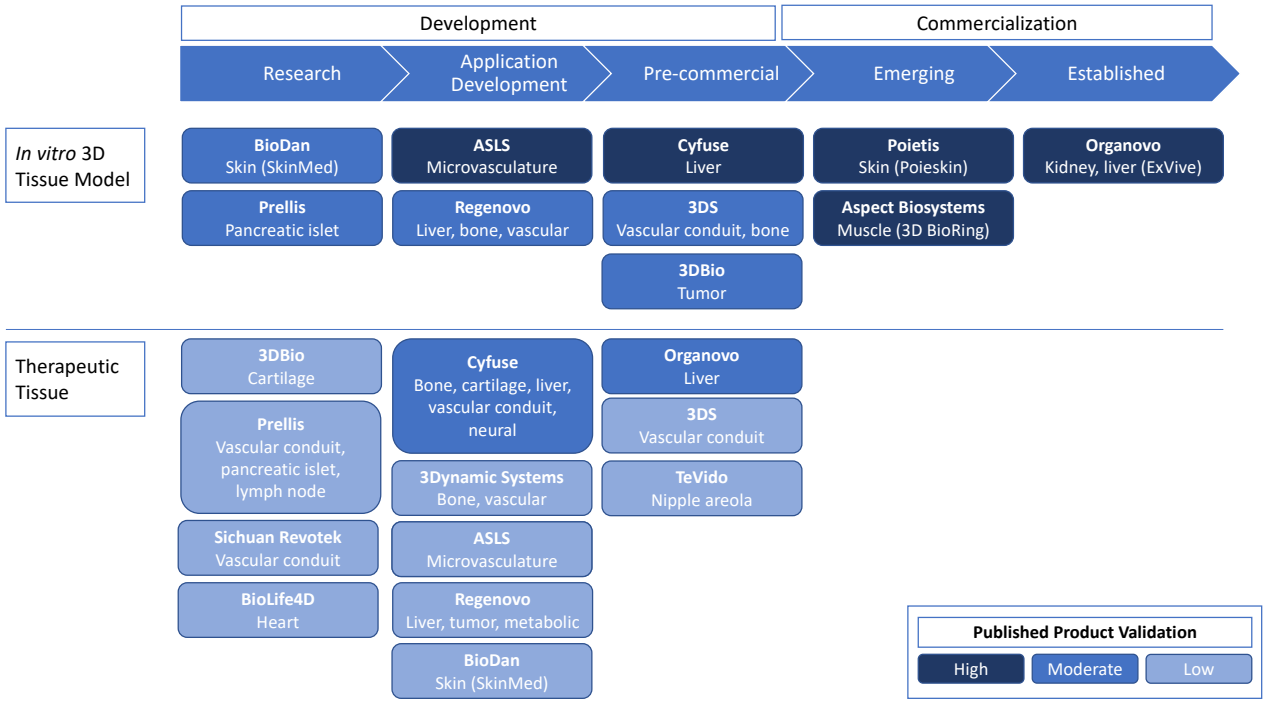

Figure 4: Producer Product Development Comparison

Figure 4 shows in what tissue-areas and product-categories there is already significant work towards commercialization by leading market players. This visual provides insights into observers of the field attempting to navigate or entre the space by outlining where translational R+D is already occurring. For example, Organovo is currently leading the *in vitro* space with its ExVive kidney and liver products, but Aspect and Poietis are close behind with skin and muscle airway models. These companies are leaders in the field, but their products are not exhaustively applicable in every clinical scenario, and only target certain types of tissues and models. Since the market is so segmented at this point in time, there is still ample opportunity for new entrants to target untouched tissue-spaces in either the *in vitro* or therapeutic tissue spaces. Competition is low between companies in each tissue area; only one or two players are operating within each tissue-space and product-area.

An overview of product types and major contributing players across products and target therapies is displayed in Table 8, so that onlookers can ascertain an understanding for where the current players are dedicating their resources. These are the leading players in their respective categories. Information in Table 8 is compiled from secondary research that includes company 10K reports, market analyses and third-party interviews and articles.

Table 8: Producer Company Information

Type	Company	Country	Founded	3D Printer: Type	Target Therapy / Tissue	Website
Vascularized Tissues	3Dbio	Russia	2013	FABION: spheroid extrusion	Cartilage, tumor	http://www.bioprinting.ru/en/
	3DS	UK	2012	3DS-Alpha, Omega, Delta: extrusion	Bone, vascular	http://www.bioprintingsystems.com/index.html
	ASLS	USA	1987	Biobot, Bioassemblybot: extrusion	Microvasculature	https://lifesciences.solutions/vipm/
	Biolife4D	USA	2017	Biolife4D Bioprinting Process	Heart transplant	https://biolife4d.com
	Cyfuse Biomedical	Japan	2010	Regenova: bioassembly	Bone, cartilage, vascular, neural, liver	https://www.cyfusebio.com/en/
	n3D Biosciences	USA	2009	Magnetic levitation: magnetic spheroid bioassembly	Vascular, aorta, skin (wound repair), tumor	http://www.n3dbio.com
	Organovo	USA	2004	Novogen MMX: extrusion	Liver (IEMs), kidney	http://www.organovo.com
	Prellis Biologics	USA	2016	Laser-based	Type 1 diabetes, lymph node, microvasculature, Zika disease models	https://www.prellisbio.com/about/
	Regenovo	China	2013	Regenovo-Lite, -Pro, -WS: extrusion, bioassembly	Liver, bone, tumor, matabolic syndrome	http://www.regenovo.com/English/index.aspx
	Sichuan Revotek	China	2014	Biosynsphere: extrusion	Vascular	https://www.linkedin.com/company/10324278/
Avascular Tissues	Aspect Biosystems	Canada	2013	RX1 Bioprinter: microextrusion	Muscle, airway, macro-tubules	https://www.aspectbiosystems.com
	BioDan	Spain	2013	BioDan Print: extrusion	Epithelial; burn, wound, mucosa regeneration	http://www.biodangroup.com/print.html
	Poeitis	France	2014	NGB17.03: laser	Epithelial	http://www.poietis.com/en/index.php
	Tevido	USA	2011	Cellatier: droplet	Vitiligo, breast cancer	http://tevidobiodevices.com
Synthetic 3D Printing	MedPrin	China	2008	Extrusion bioprinter	Post brain-surgery dural repair	http://www.medprin.cn/en/product/index.aspx
	Osteopore	Singapore	1999	Synthetic extrusion bioprinter	Post brain-surgery bone repair	http://www.osteopore.com
	RTI Surgical	USA	1969	TETRAfuse® 3D technology	Bone repair surgery	http://www.rtix.com/en_us/
	TRS	USA	2005	Selective laser sintering	CMF, bone, orthopedic, spine fusion, dental implants, growth factor delivery	http://tissuesys.com

Bioprinted Tissue Products

The bioprinting value chain culminates in the creation of the ultimate bioprinted end-product: 3D engineered tissue structures. Bioprinted products can be categorized as either *in vitro* tissue models or therapeutic tissues, as outlined in the 'short-term goals of bioprinting' section. While the adoption and market for bioprinted products is young, *in vitro* models are beginning to be commercialized and utilized by watchers. We used both primary and secondary research, combining market reports, online sources, company profiles and interview responses, to create a landscape of the current product market, which is displayed below in Figure 5 and Figure 6. Products are organized based on target application using the human body to provide a spatial sense for the tissue-types and target-applications that are being chosen by market players. All target areas are from company development pipelines and are organized within each area top-to-bottom according to a commercial readiness scale – orientation of an overall timeframe for these product categories can be observed in the 'product commercialization timeline' above. One visual, Figure 5, was produced to display *in vitro* applications and a second, Figure 6, was created for therapeutic target-areas.

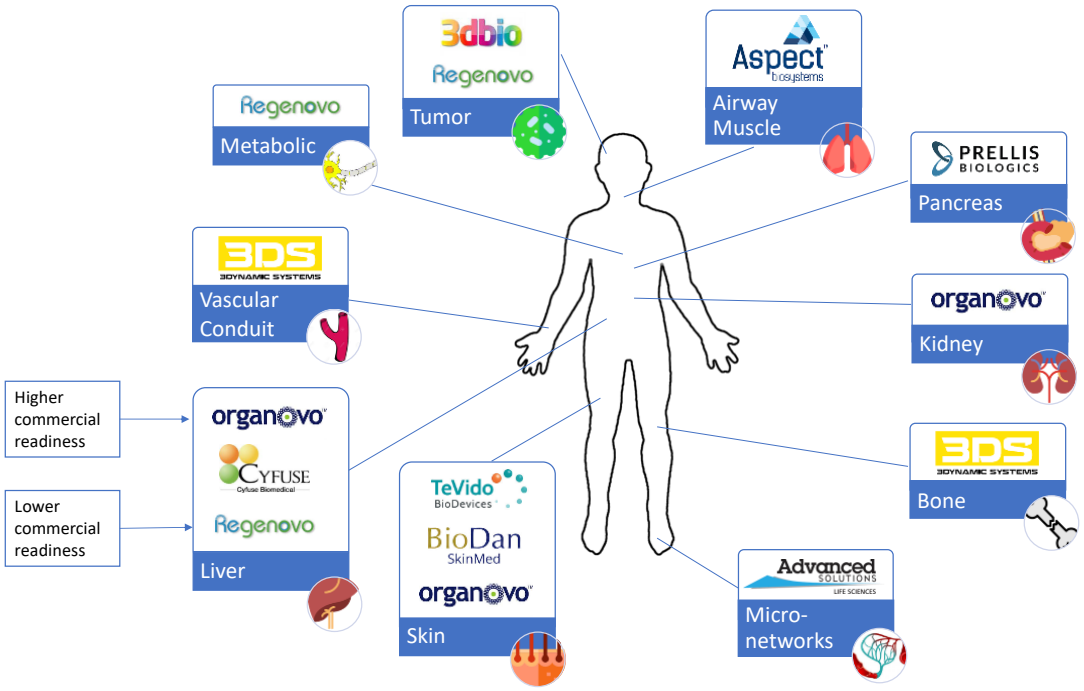

Figure 5: Commercial In Vitro Tissue Product Areas

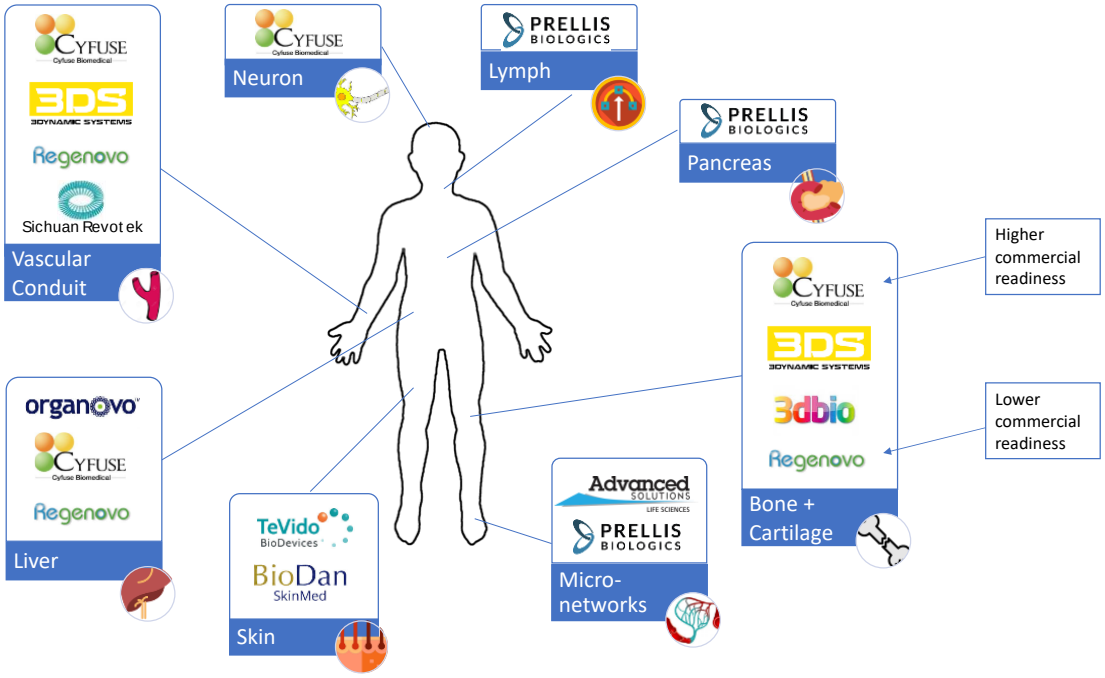

Figure 6: Commercial Therapeutic Tissue Product Areas

Figure 5 and 6 show the high levels of target-application variation across industrial players in the bioprinting field. This space is unique because it is a relatively new technology that has advanced over the last two decades, meaning it not fully characterized, but also has high potential for new entrants entering the market to establish their own commercial niche. Basic research has not yet established an 'optimal' bioprinting approach and only one company (Organovo) is close to moving through clinical trials with a bioprinted tissue or biologic. Companies are focused on developing technologies and capabilities that can produce tissue constructs in the specific tissue-areas described above, but they have yet to adopt specific approaches for wide-spread applicability. This leaves room for start-ups and watchers to target tissue areas and develop a specialty early before optimal or (closer to) idealized bioprinting and TE techniques are developed and identified. Entering this space in a timely fashion, and building infrastructure and specialization around certain tissue areas, will be advantageous in the future once these products have moved closer to clinical applicability.

Industrialized bioprinting products are also charted in Table 9 in an exhaustive fashion, according to their producer type and production company. Marketed products are delineated, and products that are still in development are marked with an '(R+D)'. The 'Type' column signifies producer categories – companies within the 'Vascularized Tissues' type are working predominantly to create vascularized or vascular tissue products, 'Avascular Tissues' type companies are focused on simpler vessel-free tissue structures, and 'Synthetic 3D Printing' companies are using 3D printers to print synthetic therapeutic products that can be integrated with biological systems. While 3D printing companies are outside of the strictly bioprinting space, like bioprinting producers, they are working to create novel implantable products with medical value using similar additive manufacturing technologies in an uncharacterized space. The bioprinting community can use the companies described in Table 9 as analogs, as the 3D

printing field is more advanced than the world of bioprinting, but both share similar goals, methodologies, and challenges.

Table 9: Industry Tissue Producers and Products

Type	Company	Country	Tissue Product	*In vitro* Product
Vascularized Tissues	3Dbio	Russia	*In situ* cartilage printing (R+D)	Cancer drug-testing model (R+D)
	3DS	UK	Vascular conduits (R+D)	Bone and vascular tissues (R+D)
	ASLS	USA	Micro-vascularized tissue (R+D)	Micro-vascularized tissue (R+D)
	Biolife4D	USA	Heart transplant (R+D)	
	Cyfuse Biomedical	Japan	Bone and cartilage, vascular, neural, liver tissues (R+D)	Liver tissue (R+D)
	n3D Biosciences	USA		Vascular toxicity "aortic ring" assay; wound healing, toxicity, oncology platforms (marketed)
	Organovo	USA	Liver tissue patch (R+D)	Ex-Vive™ kidney and liver platforms marketed; skin and gut models (R+D)
	Prellis Biologics	USA	Microvasculature scaffold; islets of langerhans; lymph node organs; (R+D)	Pancreatic platform (R+D)
	Regenovo	China	Liver, bone, vascular (R+D)	Liver, tumor, metabolic syndrome (R+D)
	Sichuan Revotek	China	Vascular graft (R+D)	
Avascular Tissues	Aspect Biosystems	Canada		3DbioRing™ airway muscle tissue (marketed)
	BioDan	Spain	Epithelial tissue (R+D)	Epithelial platform (R+D)
	Poeitis	France		Poieskin® tissue (marketed)
	Tevido	USA	Nipple areola tissue (R+D)	
Synthetic 3D Printing	MedPrin	China	Neodura™ dural repair patch; ReDura™ dural defect patch (marketed)	
	Osteopore	Singapore	Osteoplug™ synthetic cranial burr-hole bone implant; Osteomesh™ craniofacial synthetic bone implant (marketed)	
	RTI Surgical	USA	Fortilink®-C IBF/VBR System (marketed)	
	TRS	USA	PCL scaffold Affinity™ coating (marketed)	

Figures 5 and 6 (as well as Table 9) show that companies are primarily focused on developing products to address differing target areas because it is easier for companies to either address divergent target areas or collaborate between one another rather than engage in direct competition. This involves building tissue structures that mimic a specific region of the body, including relevant cell types, ECM factors and anatomical structures. Many companies are addressing disparate areas, especially in the *in vitro* space, where companies are inhabiting specific target niches rather than competing to create the same tissue-platform; examples include the metabolic, kidney and pancreas areas, as shown in Figure 5. In the therapeutic tissue space, which is displayed in Figure 6, singular target areas are receiving significant attention from a large number of companies, like in the development of conduit, bone, cartilage and liver tissues. Concentration in these areas is high because they are simpler tissue structures, or, as in the case of liver, they represent a large unfulfilled market need for replacements and functional regeneration.

In the future, it is reasonable to expect that competition will continue to increase between builders as technologies advance, commercialization intensifies, and more players enter the market. As the field matures, there will be an increasing number of companies who specialize in specific market niches, like ASLS, who is already focusing their efforts on

developing a microvasculature platform that can be used by other builders to induce vascularization within their own products; this technology is largely vasculature-specific, but has enormous synergistic potential to be used alongside other approaches in a wide range of tissue-based R+D scenarios. Similarly, Sichuan Revotek has focused its efforts on creating vascular conduits, and is not worried about simultaneously developing other tissue products as well. It will take both concentrated efforts and collaboration between industrial players that have complementary technologies in order to create clinically applicable tissue products in the future.

Product Commercialization Timeline

To provide a wholesome view of future product pipeline categories, developmental intensity, and a timeline for the expected commercialization of bioprinted products, a product commercialization timeline is shown in Figure 7. This visualization embodies a representative sample of insights derived from stakeholder interviews and intuitions drawn from an examination of the current state of the bioprinting science. It exhibits the temporal distance to wide-spread commercialization for a variety of bioprinted products and includes the pace of developmental metrics.

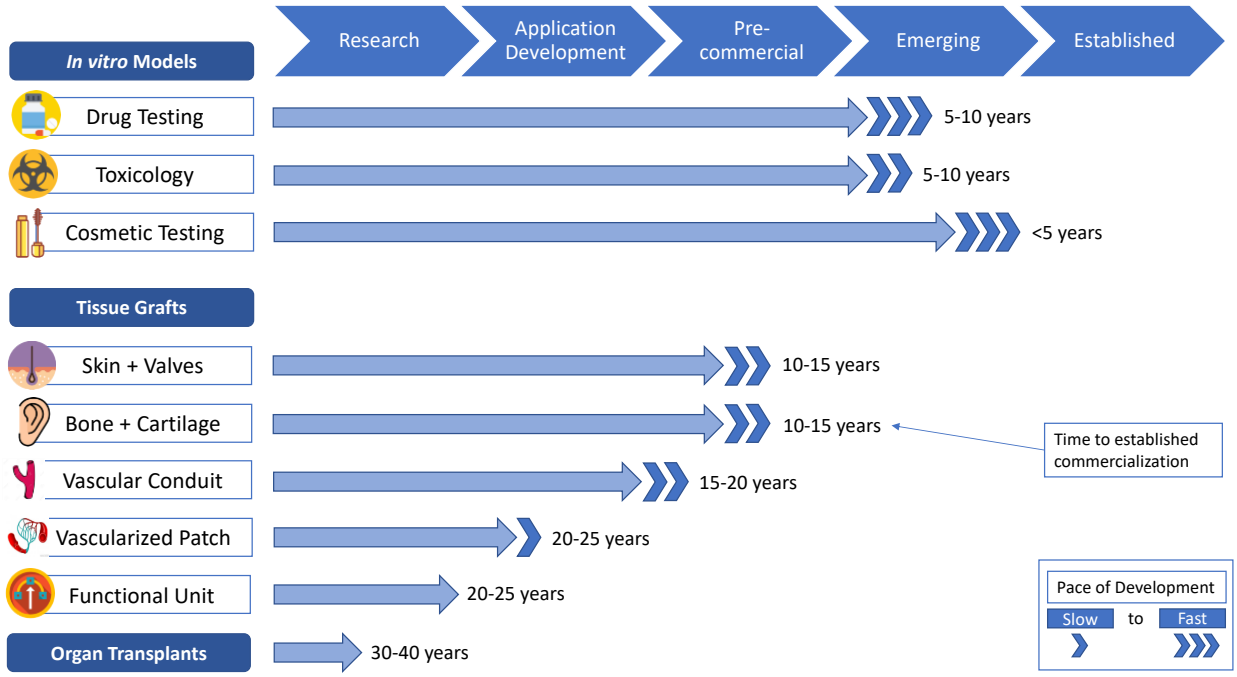

Figure 7: Product Commercialization Timeline

The commercialization of *in vitro* models is being led by Organovo, with their ExVive products, and Poietis, with their epithelial Poieskin product released in early 2018. Because of its simple nature and easy adoption, it is likely that cosmetic testing will be established wide-scale before more sensitive testing procedures like drug testing and toxicology, and because Poieskin is already being marketed and used by large companies like L'Oréal. All respondents agreed that 'drug testing' would generally be the first *in vitro* established product area, but Anthony Holmes of NC3Rs provided additional insights into why toxicology may reach

established adoption first, saying, “There will be wider uptake in the pharma industry in the next 5-10 years, with toxicology modelling being the primary focus of the early adopters…toxicity testing approaches are generally regulated procedures, so there is a degree of consistency in the way these tests are done at J&J, Eli Lily and others, so there’s more incentive for big pharma companies to work together to get these to work for toxicology testing[58]." Despite this outlook, a larger proportion of respondents agreed that there is a greater need and pipeline interest in products for disease-modelling and efficacy-testing than for toxicology models, meaning the pace of development for toxicology may be marginally slower.

The establishment of commercial *in vitro* models is also essential to the ultimate development of therapeutic tissues because simple short-term applicability of bioprinted products must be demonstrated in order to sensitize the field to these novel products and technologies. Anthony Holmes stated that we must, “Build confidence with simple models, then ramp us complexity where this is appropriate for answering the research questions… there needs to be more substantive, publicly accessible validation data for *in vitro* models to be adopted… we need technology developers to understand what is needed in R+D pipelines in product development for big companies.” Industrial builders in the bioprinting field concur; in reference to Organovo’s ExVive products, Steve Kunszabo mentioned that, “Companies want to see data, performance and proof of effectiveness to move us up the adoption curve". Once a critical mass of data is established that supports and characterizes these bioprinted tissue models, it is safe to assume that there will be a wave of newfound interest, investment and excitement in the TE field, bioprinting in particular, which would only aid in supporting the R+D of medically relevant tissue products.

There was agreement across all interviewees that simple structures, like skin and cartilage, would be the first therapeutic tissue grafts commercialized. Bill Tawil, from UCLA, mentioned that he was working with, “skin, bone and cartilage because they're the most simple and easy to use[174].” Unfortunately, many of these products are still far in the horizon and will require the successful commercialization of *in vitro* bioprinted products to pave the way for their development.

Funding and Workforce Development

In our primary research, a collection of significant market factors were highlighted by interviewees and are expanded upon below. These factors, which include funding, collaboration and regulatory considerations, are integral to overcoming limitations at the technical and administrative level and must be considered by all navigators of the bioprinting space working to translate bioprinted products.

Given the highly technical and expensive nature of the ‘emerging’ bioprinting field, funding is a critical consideration for researchers and industry players alike. To ascertain stakeholders’ outlooks on funding sources, we asked interviewees, “What limitations have you experienced in ascertaining funding for bioprinting research and development, and what type of issues have you observed across the field?”. Archetypal answers, with selected direct quotes from interviews, are tabulated in Figure 8.

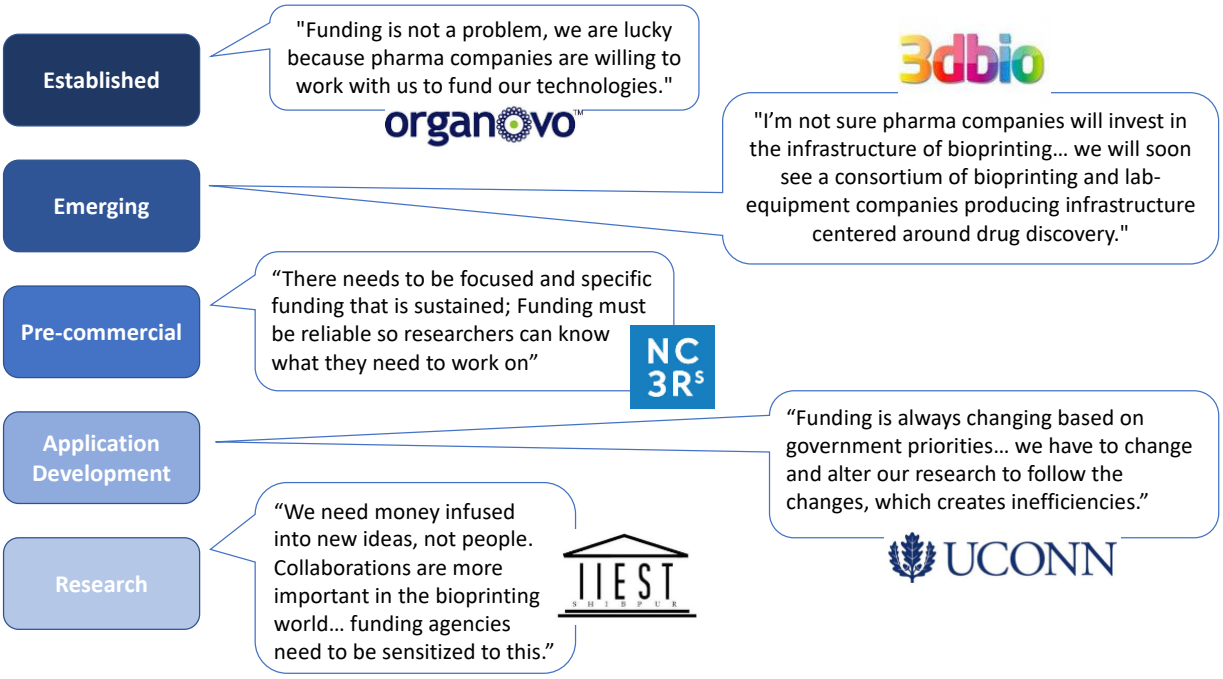

Figure 8: Funding Responses

Figure 8 shows that established players are considerably less concerned with funding limitations than research entities. Every university researcher we spoke to referenced funding coming from industry collaborators and grants as being 'variable' and 'unpredictable'. This type of erratic funding has made it difficult for researchers to focus their efforts on certain issues in the field and train a workforce that is committed to solving specific problems with acquired particular skillsets. Lakshmi Nair from UConn noted that specialized bioprinting-focused workforce development is difficult because, "Changing funding makes it difficult to train and educate researchers... they need to be flexible and can't specialize on one project long-term to gain deep knowledge on a subject when we are constantly repositioning."[175]

It's difficult for universities to develop a specialty in a certain area when they must obtain the required infrastructure and equipment, establish standards and an intimate understanding for their desired procedure, and finally train a skilled workforce capable of innovating and moving the field forward using the provided equipment. Labs that work with undergraduates are faced with diminishing returns and an uninspired workforce when their students are given a project, and suddenly that project loses its funding because sponsorship funding shifts two years after the project has begun[175]. Similarly, grad-student heavy labs struggle to maintain proficient numbers of skilled and experienced technical employees working together when funding is largely transient. This is because funding volatility alters project-demands on labs, forcing them to change target-experiments in a random and rapid manner which makes it difficult for bioprinting researchers to develop proficiencies in specified technical areas. Reliable sources for academic funding must be established in order to maintain sufficient levels of interest and expertise at the academic level required to move the field forward.

From the industrial perspective, players like Organovo, 3DS and ASLS were unconcerned about funding sources and were optimistic about their future collaborative asset requirements. Daniel Thomas, the founder of 3DS, noted that, "In the UK we have lots of funding for research, there's lots of support from the European funding agencies for us, like Horizon2020[176]." Other players, namely 3Dbio and NC3Rs, were not as optimistic; Anthony Holmes from NC3Rs pointed out that, "Some companies are very good at marketing their successes, sometimes without making the data to support this available. We need to see the actual data to support some of these claims, preferably data from an independent laboratory…. this field is very reliant on funding and in the UK we haven't had the levels of critical capacity-building funding which I would like to see." These divergent outlooks make it difficult to point out what exactly is happening in the industrial funding space; it is possible that optimistic companies are simply putting forward a hopeful and confident demeanor to obscure funding challenges they are facing, but it is also possible that certain companies have a better developed commercial platform and direct large-money connections that enforces their genuinely confident visage. Once more concrete product data is published, intimate watcher partnerships are established, and the adoption curve is ascended, funding challenges will become less of a conflicting limitation for industrial players.

Academic and industrial funding consideration taken together, it is evident that there is a ripe opportunity for collaborations that ensure long-term and dedicated partnerships between research agencies and industry players, as well as other market entities. The commitment to investment in basic research will allow academics to train a productive and specialized workforce that will be inspired to contribute to the TE and bioprinting world in the future.

Collaborations

It is obvious that cooperative and synergistic collaborations are required at all levels of the bioprinting space in order to drive of this emerging technology and market domain. During our interview with Steve Kunzabo, the VP of Investor Relations and Corporate Communications at Organovo, he explained that collaborations are, "Hugely important both at the academic and industrial level… collaborations take us to other research areas, help us branch out, build a business case around a new market… we pivoted from toxicology to disease modelling because of people who came to us asking for disease platforms." Collaborations not only enhance the current capabilities of contributing players, they also help them identify viable market opportunities. Similar insights highlighting the importance of mutually beneficial collaborations were noted by players across the field, from research-based institutions to established market participants. These responses are displayed below in Figure 9, which is a representative visual indicative of the outlooks we compiled in our secondary research effort.

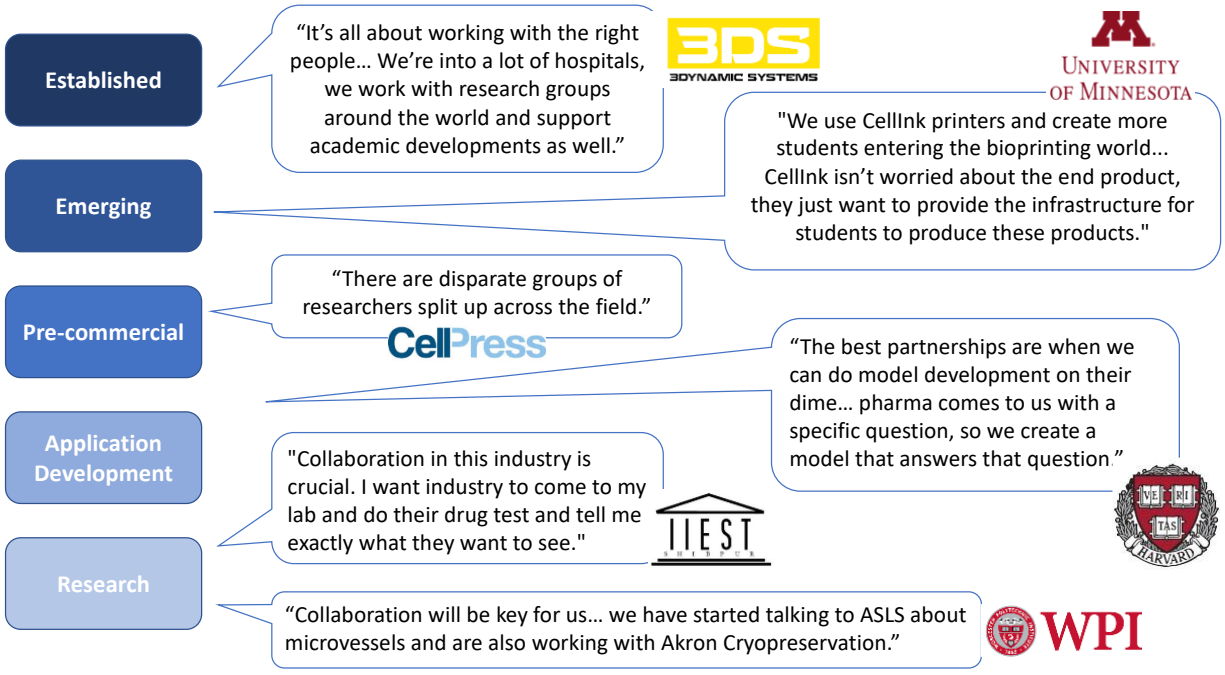

Figure 9: Collaboration Responses

Universities want watchers and builders to come to them with specific instructions, and industry players are optimistic about their involvement with universities and end-users alike. It is reasonable to assume that collaboration frequency and intensity will continue to increase and will eventually result in significant market mergers and acquisitions. Matt Pavlovich from CellPress also noticed that there are groups of researchers in the field that are performing similar work that fail to align their interests and efforts in a reciprocally productive manner. Funding, IP and credit complications can be prohibitive to these types of collaborations, but more commonly than not, concentrating efforts rather than pursuing iterative and inefficient research endeavors can occur by establishing basic knowledge-shares, licensing expectations and collaborative guidelines between institutions.

In industry, many builders and end-users are already coming together. Buyers, which are usually larger biotech or pharma companies, express their needs and demands to builders, who are usually small bioprinting start-ups eager to garner funding and develop their technologies towards a marketable application. For example, Yusef Husani from 3Dbio noted that, “Pharma companies interested in 3D tissues want to pick up bioprinting tech... there are 10-15 pharma companies working and watching this field right now[149].” Current commercial exchanges, which may soon result in more official partnerships, are provided in Table 10.

Table 10: Commercial Collaborations

Seller Company	Buyer Company	Product/Service
Aspect Biosystems	DePuy Synthes (J&J)	Bioprinted cartilage
Insphero	NCATS	Tumor model
Insphero	Physiomics	Tumor model
Organovo	Merck	Liver construct
Organovo	Janssen R+D	Liver construct
Organovo	Bristol-Myers Squibb	Liver construct
Organovo	Astellas Pharma	Liver construct
Organovo	Viscient Biosciences	Liver construct
Organovo	Ardea Biosciences (AZ)	Kidney construct
Organovo	La Jolla Pharma	Kidney construct
Organovo	L'Oreal	Bioprinted skin
Poietis	L'Oreal	Bioprinted hair
Poietis	BASF	Bioprinted skin

Table adapted from ref. #[156]

Intra-academic and intra-industry collaborations are commencing and flourishing, but cooperative efforts that transect the value chain will also be essential for the future development of the bioprinting field. For example, at WPI, Marsha Rolle's lab is having conversations with ASLS about using their BioassemblyBot to print vascular tissues tracheas[173], and in early 2017 Aether Bioprinting donated and installed multiple Aether 1 Beta Bioprinting Units in Stanford's Center for Cancer Nanotechnology Excellence and Translation for modeling research[177]. Additionally, ASLS is moving their bioprinting labs to Manchester in April so they can access a wider variety of expertise, input sources and perspectives through ARMI's BioFab Initiative[76]. More collaborations like these are needed to simultaneously capitalize on the focused market-outlook, funding sources and translational knowledge of industry players, and the innovative, experimental and (usually) economical qualities of academic research organizations.

Regulatory and Intellectual Property Concerns

Right now, the bioprinting industry is an unregulated space. In the USA, under the FDA, any living organism or natural matter cannot be patented. Under these conditions, the closer bioprinted products resemble anatomical tissues, the more difficult it is to protect them. Short-term *in vitro* models can be used alongside gold-standard testing models, like animals and early human clinical trials, but regulatory intervention will be needed if they will ever displace these standards entirely. Similarly, therapeutic tissues will not be commercially viable until full regulatory participation is accounted for. Iwona Cicha, noted that, "We need *in vivo* proof-of-concept at the regulatory level… the key issue is to make sure tissues remain patent and don't introduce thrombogenicity… *in vitro* studies don't need regulatory oversight, but in the clinic you need them to be produced and compliant with regulatory GMP standards."[178]

Despite the lack of current regulatory mediation, producers have already started protecting their IP. The number of publicly filed 3D bioprinting patents increased by 36% from 2016 to 2017; companies holding the most patents, either directly or through corporate exchanges, include Organovo, Koninklijke Philips, HP, Medprin Regenerative Medical Technologies and Corning. The patent turnaround time for patents is crucial in the bioprinting industry because of its fast-paced and novel character. The time between filing and publishing of patents in the US typically takes 18 months. If competitive bioprinting companies are filing for similar patents, or developing analogous products and technologies, these 18 months could be detrimental to a new and developing company that learns it is infringing on a patent and realizes all of its R+D investment has been nullified. This is especially applicable for small start-ups that have concentrated their research efforts in a specific area without knowing that a competitor has applied for a patent in the same space a year prior.[156]

The regulation of biologics is currently limited, but it will be important for the bioprinting community to understand contributors' sensitivities and outlooks on regulatory oversight moving into the future. We compiled direct feedback, in quote format, answering the question, "How important are regulatory considerations for your institution, and what does the current bioprinting regulatory landscape look like?" and displayed our findings in Figure 10.

Figure 10: Regulatory Responses

Basic researchers were largely unconcerned with regulatory oversight, typically responding that it is simply, "too distant for consideration". This finding was predictable – basic researchers are not typically concerned with end-stage regulatory approval considerations or potential challenges they may face in the far future. On the other hand, more industry-minded interviewees noted two themes: (1) the FDA is optimistic and eager to prepare itself to oversee bioprinting, TE and biologic regulations in the near future, and understands the monumental

impact these industries will soon have on the medical world, and (2) the FDA wants producers to present data and validation in a one-way information exchange that is meant to advise and prime the FDA with data that can inform their future frameworks. These findings are indicative of a young and burgeoning field and show that the FDA is willing and able to engage with the bioprinting community to prepare themselves for a safe and efficacious supervision of bioprinting regulation.

These findings are substantiated by a recent FDA report. In late 2017, the FDA released a statement regarding a, "comprehensive technical framework to advise manufacturers creating medical products on 3D printers". In this statement, the FDA claimed that they are, "preparing for a significant wave of new technologies that are nearly certain to transform medical practice," and are, "working to provide a more comprehensive regulatory pathway that keeps pace with those advances and helps facilitate efficient access to safe and effective innovations that are based on these technologies". This statement confirms the FDA's high awareness and appreciation for the impact of 3D printing technologies. In the statement, they also address bioprinting, "The FDA also plans to review the regulatory issues related to the bioprinting of biological, cellular and tissue-based products in order to determine whether additional guidance is needed beyond the recently released regulatory framework on regenerative medicine medical products. The Center for Biologics Evaluation and Research has recently interacted with more than a half-dozen manufacturers who have expressed interest in using 3D printing in some capacity to produce their medical products."[179]

Evidently, the FDA is motivated to address the bioprinting world but will not move forward blindly. As always, collaboration and cooperation will be vital in preparing the regulatory space for biologics and novel TE products. This cooperation should primarily be derived from the industrial sector, so that the interest and focus of basic research is not distracted by prospective regulatory concerns. This maintenance of a 'divide of regulatory concern', namely the division of (a) universities who are naïve and indifferent about regulatory standards, and (b) industry players who are actively engaging with regulatory bodies, must be continued so that basic research can conserve its focus on innovation and industry can prepare itself to translate safely approved products alongside the FDA and other regulatory agencies.

Potential Opportunity Areas

The bioprinting pathway culminates with the illumination of two potential market-needs that could be met with a bioprinted tissue product. These target-market opportunities, which include the development of alternatives and advancements to vascular graft replacements of various sizes, could be capitalized on by the bioprinting community in the near future through the creation of biofabricated vascular grafts, conduits and prostheses. Findings from the vascular and cardiothoracic surgical literature were combined with conversations from bioprinting field-aggregators in order to identify these target 'vascular' markets. To garner and characterize real-world evidence of these market opportunities, we contacted Dr. William Tanski, a practicing vascular surgeon specializing in peripheral repairs, who is affiliated with both Concord Hospital and Dartmouth-Hitchcock in New Hampshire[161]. During our interview, Dr. Tanski provided insights into the types of procedures, patients, prosthetics, and disease-states in which both he and other vascular surgeons specialize. He also discussed current gold standard surgeries, devices and grafts for vascular repair and replacements, and his openness

to adopting novel bio-prosthetics and biologics. These target markets were identified by Dr. Tanski extensively, but our secondary research also illustrated the need for vascular graft improvements, given the prevalence of vascular-diseases and the inadequacy of current therapies to treat these diseases.

To frame these markets, we looked at the prevalence of cardiovascular diseases (CV), other vascular conditions, and how vascular grafts are used to treat these ailments. CV diseases are the leading cause of death worldwide for both men and women; about 610K people die of heart disease in the US every year, 370K of which are from coronary heart disease. Patients with CV disease are typically treated with autografts or synthetic grafts in bypass procedures to re-establish vascularization. Small-diameter vessel surgeries employ autografts, which have high harvesting morbidity rates and are in short supply. For large-diameter replacements, synthetic grafts are used, which have unacceptable long-term patency rates.[161]

Vascular replacements are also used to treat severe ischemia in the lower extremities, in bypass surgeries and for diabetics with angiopathy receiving dialysis. Large vascular grafts have an ID greater than 6mm, the largest being aortic grafts which are typically over 8mm in ID, and medium grafts which range from 6-8mm ID and include carotid and common femoral artery repairs. Cellular tube replacements, on the other hand, are applied in larger conduit structures like gastrointestinal and urinary organs. Tubular grafts are a potential application for extremely large-diameter conduit tissue replacements but are not discussed in detail here. It is evident that both large- and small-diameter graft replacement therapies would benefit from the incorporation of living autologous cells and controlled geometries within graft architecture, which could be accomplished using bioprinting technologies.

Potential Opportunity Area: Large-Diameter Vascular Grafts

Large vascular grafts are currently used to treat CV disorders associated with vessel blockages, deep vein thrombosis and cerebrovascular and peripheral arterial disease. The gold standard for these procedures is the suturing of synthetic vascular grafts like ePTFE prostheses, PET grafts like Dacron® or Goretex® (polytetrafluoroethylene), or biologic tube conduits that include bovine carotid artery grafts, cadaveric autologous vessels or cryopreserved veins. Principally, these grafts are indicated for vascular replacements such as coronary arterial bypasses, but they are also fairly successful for aortic, iliac and common femoral artery repairs, which are all high-flow and low-resistance environments[180]. When used below the knee for vascular and coronary bypass synthetic grafts show severely limited patency rates long-term; for a proximal popliteal artery bypass ePTFE patency is 40-50% after five years, and 20% three years after infrapopliteal bypass surgery[181]. Despite these drawbacks, current clinical synthetic-graft approaches have proved to be adequately effective. Bioprinting has the potential to modify the mechanical and biological properties of existing large-diameter synthetic grafts and engineer full-scale alternatives which are superior to the current gold standard, especially in pediatric applications.[71,73,181–183]

Current clinical trials in the large-diameter vascular space are working to improve upon ePTFE grafts by incorporating living components into the scaffolds. One such TE approach is a modified ePTFE graft called FUSION BIOLINE, which has now received European CE Mark approval[183,184]. This product was developed by Maquet Cardiovascular LLC, by adding an inner layer of bioactive coating to ePTFE grafts and surrounding this tube with an outer Dacron layer.

The bioactive material consisted of heparin sodium couple with recombinant human albumin which was intended to decrease antigenicity, promote endothelialization, and decrease recurrent thrombosis. An initial trial with 203 patients showed improved efficacy of the FUSION graft at 6 months (over ePTFE) in a femoropopliteal bypass procedure using both 6mm and 8mm grafts. However, at 12 months, primary patency was statistically equivalent. This approach leveraged a novel (but simple) layering technique to alter existing ePTFE grafts, though did not include a large proportion of cellular components.[73,183]

Biological grafts would be especially applicable in the pediatric environment, where they could integrate directly with patients and grow in accordance with their host[73]. This is a niche target-market, but the incorporation of biological grafts could have a profound clinical benefit on patient quality of life and surgical effectiveness by solving issues with delayed pediatric reconstructive surgeries (of congenital vasculature issues), implantations using oversized grafts, or repeated operations to update the size of a graft over time[9]. Various studies in large animals, using autologous cells seeded onto PGA mesh tubes, have shown increased graft volume, remodeling and endothelialization over time. Using a similar approach in a Japanese study of 25 patients (median age 5.5 years), bone-marrow derived cells were incubated on ePTFE grafts for 2-4 hours before implementation. One year later only one patient had developed thrombosis, and at late-follow up six patients showed asymptomatic stenosis, 4 of whom were treated successfully with angioplasty. The follow-up phase I safety trial in the US is currently ongoing.[62,73]

In a similar fashion to the approaches outlined above, bioprinting could alter existing graft products by incorporating living components to increase functionality. This could be accomplished using a wide variety of bioinks compatible with a multitude of biomaterials and living cells. These bioinks could be spatially controlled and directly integrated with existing synthetic grafts in order to improve host-integration, endothelialization, remodeling, biocompatibility and dynamic size requirements needed in pediatric patients.

The biggest concerns for vascular surgeons when considering tubular conduits are wall adherence, intimal hyperplasia and deliverability. Bio-prosthetics would be useful if they could improve upon the current gold standard in these areas. In aortic implants, the current standard for wall adherence depends on hook fixation to proximal arteries and sustained pulsatile flow-pressure to keep the stent in place. Bio-grafts with increased tissue in-growth and incorporation into the native wall in a requisite time period would provide a significant benefit in aneurysm repairs because better fixation and less leaking reduces the chance of aneurysms. Other vascular conditions, like peripheral arterial, iliac and infrainguinal occlusive diseases are plagued more by intimal hyperplasia than adherence issues. Long-term strength and shear stress of bioprinted conduits could be controlled to reduce hyperplasia and provide an improved treatment for these diseases. In endo-vascular surgeries, which use a catheter deployed through the groin to deliver stents and grafts, the deliverability of grafts is a major concern for surgeons. If bioprosthetics could be developed that are lower-profile, malleable and easily transported, procedures that require distal or angulated approaches to damaged areas could be improved as a result of this increased transportability.[161]

Potential Opportunity Area: Small-Diameter Vascular Grafts

Currently, there is a large market demand for engineered small-diameter tissue grafts (ID <6mm) to address a range of clinical scenarios, especially in the replacement of coronary, infrainguinal, and infragenicular arteries. The current gold standard is to either harvest autologous grafts from the internal thoracic, radial or mammalian artery, saphenous vein or arm vein[182], or employ self-expanding or balloon-expanding stents. Synthetic grafts under 4mm ID are not approved by the FDA because of standard complications like thrombosis, calcification, intimal hyperplasia, scaffold infection and enlargement incompatibilities with growing pediatric patients. These complications arise because synthetic grafts lack a confluent endothelial layer, regularly have mismatched IDs with the incised vessel and their synthetic material surface properties introduce compliance mismatch issues with native tissue.[135] Donor site quality also has a substantial effect on patency rates; peripheral graft patency is less than 25% at three years, whereas autologous vein grafts are greater than 75% in the same time period[181]. Additionally, synthetic arteriovenous grafts used for dialysis access have high failure rates and there are zero non-autologous grafts useful to replace portions of the coronary circulation. Autologous veins have lower deterioration and higher patency rates than synthetic grafts and are used predominantly for distal and nearly inaccessible areas. Synthetic PTFE or Dacron expanding stents are used in endo-vascular repairs because vessels can only be harvested for open procedures. In these scenarios, intimal hyperplasia, thrombosis and aneurysm rates are startlingly high.[70,71,73,161,180,182]

To date, only one small-caliber human-derived graft has shown clinical potential. Humacyte Inc. entered phase III of FDA trials in late 2017 with a human acellular vascular graft intended for end-stage renal disease (ESRD) patients, which is an advanced state of chronic kidney disease (CKD). The scaffold is intended to replace synthetic ePTFE grafts, the current gold standard for hemodialysis procedures[73]. It took extensive work by Niklason et al., over the course of roughly 20 years, to move this engineered product from bench to clinical trials[173]. To build the graft, SMCs from donated human organs are seeded onto PGA scaffolds, cultured for eight weeks in bioreactors, and decellularized to remove allogeneic antigens from the cultured tissue. Once implanted these arteries show signs of repopulation and integration of native cells, but are largely cell-free and non-individualized, so can be produced large-scale and delivered on-demand.[71,73]

During phase II trials grafts were implanted in 60 ESRD patients, 20 in the US and 40 in Poland. One-year post-implantation 28% of participants had primary patency, which is functional access without intervention, and 89% had secondary patency, which is functional access with or without intervention. Six months later, primary patency was 18% and secondary was 81%, with no reported aneurysms or immunologic response. In comparison, synthetic ePTFE fistula grafts typically show 18% and 81% patency (respectively) at 18 months. Biopsies at 16 months showed myofibroblast (smooth muscle heart cell) and luminal EC repopulation, with increased integration and proliferation of a variety of cell types observed in the PGA scaffold at 55 weeks. Given promising phase II results the graft is now being tested in a phase III efficacy and safety trial, which was initiated in late 2017. The ongoing trial is comparing the clinical patency and infection rates of the Humacyte graft to ePTFE fistulas for arteriovenous fistula applications.

The successful approval and translation of a human-derived TE graft product would be a valuable asset for the dialysis population and could set the stage for other small-diameter graft applications in the RM world. This type of decellularized scaffold technology could be integrated with other TE and RM approaches, like bioprinting, which could create more geometrically intricate and customizable structures through increased spatial control of PGA and living materials during the pre-incubation construction process. Ideally a combination of technologies, such as Humacyte process and bioprinting, would be used to make a dynamic and functional small-diameter product that could be used in a wider range of procedures, such as coronary artery or distal extremity arterial bypass surgeries.

This target market might be small, but this type of direct application must be identified by bioprinting companies, so that a specific market need can be met using bioprinted products and so the entire bioprinting pathway can be geared towards producing particular and focused products with measurable clinical benefit. The demonstration of niche therapeutic applications using bioprinting technologies will pave the way for future large-scale revolutions, like transplantable organ implementation, by ameliorating regulatory, technical and administrative early in the adoption process.

Conclusions and Future Directions

Bioprinting technologies were born in a convergence of additive printing approaches that have since shown enormous promise in a range of therapeutic and commercial arenas that combine technical components of biology, engineering and medicine. The field's rapid development has been nothing short of extraordinary, from the first 3D printing of living materials in the early 1990s to the complex bioprinters, engineering software and tissue-construction methodologies used today. We have found that for the continued advancement of this field there must be an intentional and coordinated effort to build the basic technical, application and value chain ecosystems – collectively the 'innovative infrastructure' – that support innovation; for any technological and commercial domain, you can only effectively innovate on the infrastructure that is in place. This field must focus on building its infrastructure before the ambitious goal of procuring truly therapeutic offerings can be broadly realized.

This development is critical because early-stage bioprinting technologies, developers and companies face a potential 'valley of death' propagated by a disconnect between stalling innovation and insufficient revenue streams preventing the development of clinically proven and commercially viable product-offerings. This chasm between university basic research efforts and industrial-scale work is visualized in Figure 11. In the bioprinting field, producers are approaching the upper-right corner of this chart via the commercialization of *in vitro* models, but vascular TE research largely remains towards the lower left corner. The bioprinting field needs to coordinate to support translational activity across the value chain to ensure the maturation of research and products across this valley of death chasm.

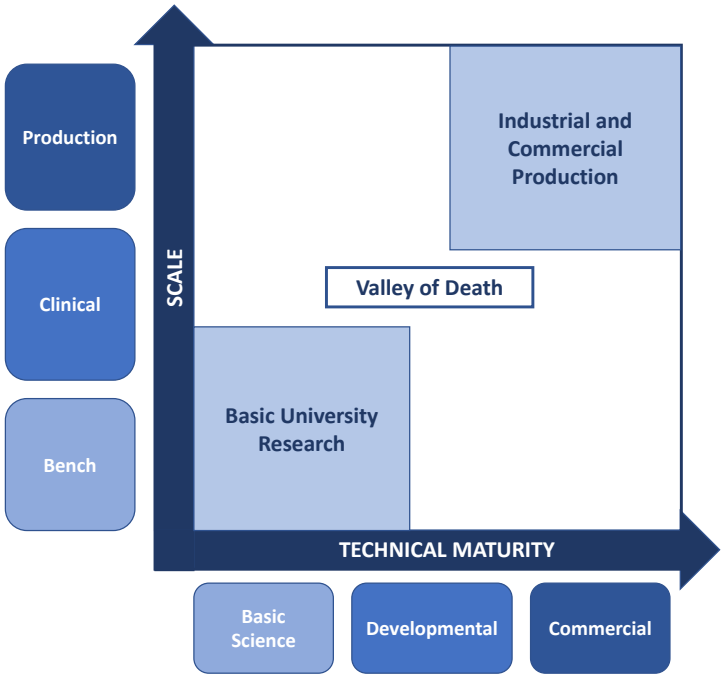

Figure 11: Valley of Death Chasm

Our research has shown that each stage of the bioprinting pathway and value chain present an array of unique challenges and considerations integral to developing a functional bioprinting infrastructure that will aid in translating bioprinted products past this gap. It will take time to fashion and update the field's support network, but this infrastructure must be developed if the bioprinting field is to continue its evolution.

In this report we have identified opportunities and outlined suggestions at selected levels of the bioprinting pathway to help the bioprinting community advance on this agenda. These target-areas are illustrative rather than comprehensive, and were selected according to our research findings, both in establishing the current state of the science and in creating a trends and technologies landscape. All areas were emphasized, validated and elaborated upon by industry experts during primary research, and corroborated with our secondary research findings. The identification of new opportunity areas where valuable and immediate work can be done is a first step in overcoming the limitations facing the bioprinting field and setting a foundational infrastructure that sustains innovation into the future. Our principal findings and conclusions are outlined below, moving through the report from beginning to end.

Section 1: Key Findings and Takeaways

As an introduction to bioprinting, the foundational terminology, history, status and goals of the field are reviewed to set the context for subsequent findings. The explication of technical terms and jargon throughout this section sets forth a terminology word-bank that will hopefully facilitate clear and rapid discussions of bioprinting technologies between members of the bioprinting community.

We also analyzed why stakeholders are adopting and utilizing bioprinting technologies in place of alternative TE approaches. We found that bioprinting researchers are doing so because they believe bioprinting systems are highly modular and customizable, scalable for manufacturing purposes, and can deposit living materials with acute precision. Our research shows that academic and industrial researchers prioritize these characteristics to varying degrees, particularly that (1) industrial players employ bioprinting systems chiefly because of their ability to manufacture at a large-scale, citing that the creation of large numbers of tissues in an automated and repeatable way is critical to their objectives, while (2) university researchers and academic labs prefer bioprinting because of its ability to create tissues with high levels of geometric complexity and its modularity in experimental scenarios; bioprinters allow researchers to customize and update various systems, inputs and techniques in-lab, which allows for unparalleled control over direct experimental tissue-product outcomes. All three of these competitive differences have been key for adopters and users of bioprinting systems as they attempt to advance the fields of TE and RM and translate functional tissue products for end-users with commercial potential.

Section 2: Key Findings and Takeaways

Section 2: 'Vascularized Tissues' establishes why the vasculature issue is critical to the bioprinting field and defines the structural and biomimetic characteristics that vessel networks bioprinted in tissue constructs will have to recreate in a functional manner. Our research shows that (1) the vasculature issue is the leading challenge facing the bioprinting and TE fields, and

that (2) bioprinting, either alone or alongside alternative TE approaches, is likely to put forth a solution to this issue in the next 5-10 years. This is because a diverse toolkit of bioprinting approaches, from MBB to LBB techniques, are being advanced across the field to target the production of vascularized tissue products that mimic human anatomy and functionality. The ensuing sections outline areas the field as a whole must address in order to advance these bioprinting technologies further in targeting this critical issue. We hope these conclusions and takeaways will address the vascularization issue while helping stakeholders of the bioprinting field to efficiently focus resources and research-efforts as they encourage positive and impactful industrial advancements towards the end-goal of bioprinting vascularized tissue products with real-world impact.[8,89,168,185]

Section 3: Key Findings and Takeaways

Establishing the current state of the bioprinting science is the primary goal of Section 3: 'Bioprinting Approaches and Modalities', which provides insight into the field's fundamental technical innovations and an understanding for the projects university research labs working towards bioprinting vascularized tissue constructs are engaging. We hope the organization of this section naturally lends itself to a logical and clear differentiation between vascular bioprinting approaches.

Attention to the vasculature issue is maintained throughout Section 3 because (1) it is critically important for the bioprinting community to develop an understanding for the work being done to overcome this issue, so that the field knows where we are and what still has to be done, and (2) because the vast majority of the most impactful and impressive bioprinting studies to date inherently address the vasculature issue and revolve around incorporating perfusable channels and vascular networks into bioprinted tissue structures. This review is not comprehensive but sets the stage for where the academic field has progressed, and what still has to be done to create bioprinted tissues with high levels of functionality.

Section 4: Key Findings and Takeaways

Section 4: 'The Bioprinting Industry' begins with a market overview and upper-level look at the current trends and metrics in the bioprinting market, while analyzing its size, grow-rate and the unmet opportunity areas the field is working to meet. Our research revealed that the bioprinting market is at a critical turning point where innovative output has surpassed the capabilities of its infrastructure to support further innovative advancements at a consistent rate, which is facing the field with a valley of death.

Considerations for Industry-Archetype Players

It will be critical to address industry-centric challenges and develop a body of supporting scientific and clinical data backing bioprinted tissue products to ensure their future translation. Our research revealed a strategy many bioprinting companies are taking to evolve through this valley of death. These companies are working to create simple tissue structures with high levels of functionality but minimal therapeutic application, like drug testing models and pharmacokinetic platforms, to generate interest and raise the medical community's sensitivity to bioprinting technologies. The functional applicability of these products can initially be

verified *in vitro* by using 3D models and tissue products alongside existing pharmaceutical gold standards of drug-testing, toxicology and disease modelling assays. After (1) a supporting body of evidence for bioprinted *in vitro* platforms is established and (2) they exhibit reliable and superior functionality over 2D models, bioprinting companies are hoping to use these platforms in place of animal models and early human clinical trials. This would reduce the prevalence of animal testing models and dangerous human testing and allow the greater scientific community to confidently accept these new technologies and products, thus moving bioprinting technologies up the end-user adoption curve. This adoption-curve ascension strategy pioneered by bioprinting companies will hopefully prepare the world for increasingly advanced biofabricated tissues, like tissue-specific implants and whole organ transplants.

In order to classify the bioprinting market, and segment the players working to move bioprinted products up the adoption curve, we divided market contributors into producer, end-user and watcher archetypes. Our archetypal research findings showed that each group has unique characteristics and challenges:

1. **Suppliers** currently have limited bargaining power. This is because there is limited demand for bioprinting research supplies and inputs as a result of a minimal number of producers and research centers working in this field, and the lack of watcher-investor engagement. Furthermore, when demand for raw material inputs, software or bioprinting equipment is demonstrated by builders, they are more likely to seek collaborations with suppliers than develop commercial exchanges.
2. **Builders** are experiencing minimal competitive pressures because they are still predominantly emergent start-up companies. We found that builders are more apt to collaborate directly or target discrete end-applications rather than engage in head-to-head competitive behavior across both tissue- and product-types.
3. **End-users** are open to the idea of adopting biologics and bioprinted products, but don't yet have enough supporting evidence or real-world data to support the incorporation of these technologies into their medical or pharmaceutical labors. Producers are employing their adoption-curve ascension strategy, outlined above, to overcome this limitation.
4. **Watchers** are hesitant to commit to the bioprinting industry until bioprinted products have established a concrete supporting body of science, and more end-users have successfully adopted these products. The failure to fully engage the bioprinting industry with funding and investment by watchers is a critical hurdle contributing towards the valley of death the field now faces. We found that watchers understand the high pharmaceutical and therapeutic promise of bioprinting technologies, but the applicability of bioprinted products must be complemented by commercial promise, open market opportunities, high levels of scalability and clear manufacturing capacities. Our research showed that the provision of sufficient and sustained funding would introduce a tipping point that would help carry producers through the valley of death. In reiteration, the establishment of effective commercial *in vitro* models will be essential to the ultimate appropriation of therapeutic tissues. The short-term applicability of bioprinted products must be demonstrated in order to educate and reassure the field about these novel products and technologies.

All four bioprinting archetypes are direct contributors to the bioprinting value chain, shown in a consolidated manner in Figure 3, which highlights each archetype's cardinal shifts and themes by identifying and analyzing critical market bottlenecks that are holding back the development of the field. The conclusions from individual pathway areas of Section 4 are detailed in the following sections.

Characterization Issue Guideline

The characterization issue is the first pathway limitation we identified. We found that industry players are especially sensitive to the reliability and characterization of material inputs because companies must maintain strict standards for manufacturing high-quality tissue products that can be consistently validated for clinical, pharmaceutical and regulatory functionality. Our research showed that product-quality lapses producers experience are typically due to unreliable and low-quality inputs. This issue is especially relevant given that start-up producers are faced with an emergent valley of death in which interest and funding in bioprinting technologies and innovators has been established, but the field has yet to produce a product with convincing supporting applicability and commercial potential. This means that companies like Poeitis, 3Dbio and 3DS have low margins of error for performance defects during the development or trial of novel bioprinting approaches and products.

Academic players, on the other hand, are more concerned with characterizing basic research protocols and products. This includes the ability to reliably predict how printed cells and biomaterials will behave given certain printing and environmental parameters. We revealed that research labs must establish shared community guidelines dictating how various printed materials, bioprinting systems and environmental factors combine to produce tissue products with defined characteristics; this includes understanding how tissue products perform given differing printing pressures and bioinks. This guideline would allow researchers to easily predict how bioprinted tissue products behave without having to fully characterize and reassess the effects that every component of their printing system has on the functionality and behavior of an engineered tissue. Research experts noted that they thought this would significantly decrease wasted research time and allow researchers to focus on developing innovative bioprinting methods rather than characterizing and working to understand techniques and products that have already been developed.

Bioink Developmental Requirements and Applicability

The optimization and characterization of the physical and biochemical integrity of bioinks is crucial to the characterization issue and will likely be solved through a combination of focused bioink development, effective communications between researchers and suppliers, and the creation of a bioprinting research guideline (discussed above). The establishment of bioinks with controllable and predictable characteristics must be established, and should developed in accordance with the biologic application, deposition method, geometric configuration, curing mechanism, incubation procedure, and influences of other incorporated biomaterials that affect how a bioink is utilized by bioprinting researchers and producers. We found that to accomplish this goal, the bioprinting field must come together to produce standards and

knowledge-shares, in the form of shared bioprinting guidelines, that dictate how bioprinting bioinks and inputs are combined in various printing scenarios, and how the resulting scaffolds and tissue products behave and function. There must be clear communication between researchers and companies using bioinks and companies supplying bioinks, so that optimal manufacturing, distribution and engineering methods can be identified with decreased variability, increased applicability and fully quantified biofabrication characteristics. This will be contingent upon creating productive and beneficial collaborations across the bioprinting value chain.

Bioprinter Selection and Optimization

As is the case for bioinks, there is still no single best bioprinting approach that has been identified, and none of the existing approaches have effectively created commercially viable bioprinted tissues. This means that a wide range bioprinting approaches must continue to be developed until optimal modalities and techniques are identified for specific applications.

Section and Table 3 outline the expansive range of approaches being applied and tested across the bioprinting field, including scaffold-based and scaffold free approaches utilizing DBB, MBB, LBB and SBB systems with a wide range of amenable materials, printing environments and platforms. Our findings revealed that right now, the development of a diverse toolbox of bioprinting approaches will be needed in order to move basic research forward, but that in the future, the creation of a vascularized tissue product with high commercial potential will require the selection and focused development of a particular bioprinting modality or approach that can be integrated with alternative TE approaches.

Our industry-centric research showed that the affordability and functionality of commercially available bioprinters entering the market is advancing rapidly, and there are already a large number of university labs converting from home-made systems to commercial printers developed by suppliers like CellInk and EnvisionTEC. This means that bioprinter technologies are reaching a cost and performance pivot point that will increase the accessibility of a range of complex and highly functional bioprinting systems, which will expand and advance research-efforts towards solving the vascularization issue.

We also discovered it is unlikely that bioprinting alone will solve the host of technical challenges facing the TE field. An illustration of this finding can be observed in work produced by the Atala Lab in 2016, which has been cited as being the most impactful demonstration of a bioprinting technology to date. Atala's system is capable of creating perfused and anatomically-accurate tissue structures using simple tissue-types, but has difficulties producing tissue structures with high cell-densities in a timely manner. It is possible that these issues could be ameliorated by incorporating scaffold-free or alternative micro-assembly approaches, like the ones developed in the Morgan Lab's 2015 Bio-P3 study, to speed up Atala's printing process by introducing thick cellular building blocks into their construction mechanism. Combining highly progressive approaches, like Atala's work, with other leading technologies from complementary labs, like the Morgan Lab, will be crucial for the field to identify idealized bioprinting processes and develop multi-faceted approaches to technical bioprinting challenges across research institutions.

Producer Market Opportunities and a Call for Action

Tissue producers are struggling to identify an optimal bioprinting technique capable of producing commercially viable bioprinted products. We found that there are a limited number of producers operating in the bioprinting arena, and that these producers are targeting a disparate set of tissues-areas and product-types. Figures 4, 5 and 6, as well as Table 8, show that there are few overlaps between producers and targeted tissue product areas, as each company is targeting disparate niche market opportunities. This is especially evident for the *in vitro* space, shown in Figure 5, where metabolic, kidney and pancreas tissue-areas are being targeted by solely a single producer. This shows that there are not yet enough companies operating in the bioprinting space to compete in targeting tissue-specific products, especially as companies are more likely to collaborate than segment and compete in this emerging industry.

In order to maintain minimal levels of competition and maximal levels of collaboration for as long as possible, producers must continue to work on their differentiated target areas and collaborate with corresponding groups with similar technologies and target outcomes; this emerging field will have a difficult time surviving and moving past the valley of death if competition between producers within target-markets rises quickly. Once simple tissue models and patches are established with viable and data-backed clinical applicability, it is likely that more entrants will move into this market and competition will begin to rise again. Our research showed that it is reasonable to expect competition to increase between builders as bioprinting technologies advance, commercialization intensifies, and more players enter the bioprinting market.

Given the market's segmented nature, high growth rate and vacant target market-areas, there is enormous opportunity for (1) new market entrants to construct and navigate their own industrial niche that isn't in direct competition with existing players, (2) established players to expand their current capabilities into new arenas, likely with the help of additional investment and R+D capabilities, and (3) watchers of the industry to take investment action, even if their commercial 'trigger-point' requirements are not met, given the high likelihood that producers will productize commercially viable products in the next five years. This field is still in an emergent stage, so early investments will have an enormously positive and impactful effect on contributors, suppliers and builders working to move this field forward. Watchers and investors entering this space to build specialized infrastructure in specified tissue areas in a timely fashion will be rewarded for their early-action as bioprinted products move closer to clinical applicability, and more producers start to crowd target-market areas.

Commercial Product Timeline Implications

The bioprinting field is incredibly close to applying its technologies in real-world applications, but while some short-term products are close to reaching commercial potential, the ultimate vision of creating engineered organs is still far in the future. According to our research, commercial adoption will occur for *in vitro* models first, within 5-10 years, for simple tissue grafts like skin and bone second, in 10-15 years, followed by vascularized patches and functional organ regeneration implants third, in 20-25 years, and organ transplants last, 3-4 decades in the future. We found that between applications of *in vitro* models, the pace of development for drug-testing will likely be faster than for toxicology. KOLs in the field are largely optimistic about the applicability and adoption of these types of products, which will

likely be in the next decade. For this vision to be reached a concerted and sustained effort in developing the infrastructural support of this industry will be needed to move innovation past the valley of death.

Reliable Funding Requirements and the Future of Workforce Development

Our research showed that the bioprinting pathway, like many emergent scientific fields, is hampered by funding-related limitations. For the bioprinting field, these issues have led to challenges in developing a skilled and highly-technical workforce. Figure 8 shows that, predictably, industrial players are considerably less concerned with funding limitations than research entities, especially companies that are more established, like Organovo, or those backed by a larger 'parent' company, like ASLS and 3DS.

We observed that universities are more sensitive to funding factors than industrial players; all university interviewees noted that shifting funding streams were a serious limitation for their lab and bioprinting research. Unreliable funding not only fosters an unpredictable and stressful laboratory environment with unidentifiable objectives, it also introduces difficulties training a skilled and dedicated workforce. Bioprinting is unique because of its highly technical nature, but when researchers are moved and stretched unforeseeably from project to project in accordance with changing funding demands, it is nearly impossible for them to develop the highly specific and focused bioprinting skillset workers hoping to advance the field past the valley of death require.

The development of consortiums and reliable streams of funding interest will be able to significantly benefit academic labs and industrial players in both the short- and long-term. In the short-term, researchers can be confident in their investment in expensive and technical bioprinting inputs like bioprinters and bioinks, and suppliers can be confident that demand for their products will continue to rise. In the long-term, a trained workforce with bioprinting-specific expertise will be able to advance the field faster than the disparate and fleeting research efforts in action today and will eventually be able to contribute to the industrial workforce.

Right now, a range of professionals with varying backgrounds in engineering, biology and the physical sciences are attempting to combine their expertise, ideas and projects in a synergistic and productive way on bioprinting research teams. We found that these bioprinting-centric collaborations are not always productive; groupings of highly skilled professionals from various fields do not always work optimally together and will tend to focus on their own individual projects rather than working cohesively as a team. Establishing clear funding sources and collaborations will allow universities to form the basic research bedrock of the field by developing a highly skilled bioprinting workforce capable of working together cohesively and productively in the development of supportive innovative infrastructure.

Collaborative Exchange Platform and Expectations

We found that increasing collaborative exchanges across multiple tiers of the bioprinting field will enhance the current industrial capabilities of contributing players while also helping them identify viable market opportunities. After speaking with academic and industrial researchers, we found that universities want watchers and builders to come to them with

specific instructions and product requests, and industry players are optimistic about their involvement with universities and end-users alike. Both groups are seeking partnerships, but complications can arise when collaborators are worried about funding and IP ownership. We found that having disparate non-collaborative research organizations working on similar projects, rather than working together, was far less productive than establishing knowledge-shares and platforms for the exchange of information, technologies and production standards, especially given the long IP approval process (18 months on average) and striated nature of the bioprinting field.

To this end, a platform that encourages and facilitates the development of collaborative efforts must be developed and implemented, because collaborations benefit players at every level of the bioprinting space. In the bioprinting field, knowledge shares between academic and industrial groups typically involve industry players requesting certain diagnostic or *in vitro* tissue products to be developed by the university player, who benefits from the funding allocations and freedom to adapt and create new bioprinting and TE methods in the production of the requested product. Deliberate and synergistic exchanges of resources, capabilities and information like these are needed to drive future growth.

The Divide of Regulatory Concern and IP Considerations

The regulatory arena is a polarizing topic for the bioprinting community. Bioprinting products are currently unregulated, but the FDA is beginning to address the TE and biofabrication arenas in light of the impending creation of engineered tissues with clinical and therapeutic application. Our research showed that basic researchers are largely unconcerned with regulatory oversight because they think the adoption of bioprinted products is too far in the future. Feedback from industrial players revealed two takeaways, namely that regulatory agencies are (1) optimistically anticipating the production and adoption of bioprinted tissues and TE products by the medical field, and are (2) relying on tissue producers to present data and proactively update them about their bioprinting developments. But, the question remains: how will these bioprinted products be regulated? The FDA is working to address this question by moving bioprinting and TE companies in-house, so they can validate the reliability of tissue products directly, and by opening communications with producers, who are expected to supply the FDA with product-data. These actions show that regulators are eager to address clinical concerns for biofabricated and biologic products.

In both the academic and industrial realm, the volume of patent applications are increasing rapidly; it typically takes an average of 18 months to process and evaluate patent proposals post-submission. We found that this elongated timeframe is uniquely detrimental for emerging bioprinting companies because it can cause inefficiencies when multiple companies inadvertently file for similar patents, or invest time and energy into a project, only to discover that a competitor has already submitted a similar patent. This can lead to over a year's worth of lost R+D time for researchers and producers, which is acutely damaging for bioprinting startups with limited R+D resources and capabilities.

To establish an efficient and productive regulatory discourse, a 'divide of regulatory concern' must be established and maintained between academic and industrial players. This divide must ensure that university researchers stay separated from regulatory factors and concentrated on moving basic experimental research forward, while industrial players address

regulatory issues by working and communicating with regulators. It will be important for companies to communicate effectively with regulators and academic institutions alike through collaborative exchanges as they navigate this novel and unregulated space and push forward their IP protections, so that objectives can be aligned through a vessel of industrially-minded producers. This will allow universities to maintain focus on basic research while industrial players worry about the real-world application of bioprinted products.

Implications of Potential Opportunity Areas

The final piece of the bioprinting pathway includes prototypical opportunity areas for which bioprinting products can be developed to target specific needs. These examples, which include both large- and small-diameter vascular grafts used in medical scenarios, show that end-users, like vascular surgeons, are open and willing to adopt and experiment with biologics. We found that end-users are positive about the future development of the bioprinting field, but they will need to see (1) established product functionality with a supporting body of science that validates the adoption of new products, and (2) an arena in which they can test novel biologics, like an academic or controlled clinical environment. These target-market illustrations show how vacant niche market opportunities in the medical field can be identified and subsequently targeted by developing bioprinted products that improve upon current gold standards of treatment and lead to productive and profitable outcomes for the bioprinting community.

Conclusion Summary

This report was our first attempt to enhance educational communication between the bioprinting ecosystem as a whole. We endeavored to help build a community of participants across the bioprinting value chain with a vested interested in enhancing the technology, value and collaborative efforts facilitating the evolution of this field. Our primary goal was to create a tool that can be used by the members of the bioprinting community to drive this field towards the creation of bioprinted vascularized tissue products that have profound applications in pharmaceutical, medical and therapeutic scenarios. We hope bioprinted products will soon be used to develop safe and effective drugs that negate the use of animal models or hazardous clinical trials, tissue patches that will help the body heal and regenerate, and organ transplants that will mitigate the extreme need for replacement organs worldwide.

Recognitions and Appreciations

I want to thank everyone who has supported, guided and helped me dedicate my time and energy during the research and development of this report. This culmination of my undergraduate biology education and experience would not have been possible without the intimately supportive, informative, academic, and emotional guidance I received along the way – you all completed this journey. Specifically, I want to thank:

1. **Jeffrey Morgan, Ph.D.**, my thesis advisor, for overseeing this project with essential counsel and optimistic encouragement throughout its development, especially in its early and pointedly undeveloped stages. His guidance and inclusion when I was a member of his lab, learning the ins-and-outs of tissue culture and bioassembly alongside Kali Manning and the rest of the Morgan Lab team, inspired my continued interest in the bioprinting field and pushed me to pursue this unique inquiry above and beyond what those experiences taught me.
2. **Kali Manning, Sc.M.**, for her ever-patient and continued support, teamwork, time and effort that she compassionately volunteered during our time working together in the Morgan Lab, developing techniques for stacking microtissues and pushing me to experiment and take my own initiative in a laboratory setting, as well as in helping me develop an understanding of the bioprinting field throughout the creation of this report.
3. **Tom Culver**, for spending endless hours generously, considerately and comprehensively helping me polish and review the content of this report and providing much-needed direction filling out the industrial and conclusive elements of this landscape. Tom ensured I stayed accountable striving to produce the highest possible quality of work.
4. **Edith Mathiowitz** (second reader) and **Blanche Ip** (committee member and Morgan Lab coworker) for reviewing my work and providing critical contributions and feedback for my oral defense.
5. **Dean Katherine Smith**, my undergraduate biology advisor, for her continued and valuable support throughout my academic experience and thesis work.
6. All the **friends and family** who have supported and guided me throughout this process, especially my mother for inspiring me to pursue this inquiry and my father for listening intently as I worked to synthesize and organize research findings. Both were crucially helpful as I struggled to figure out the purposeful and despairing question of, ‘what exactly am I doing?’, a question I was inevitably faced with repeatedly throughout the past year. Thanks again, one and all.

Appendix

Contents:

Producer Company Information

To supplement the discussion of the bioprinting pathway and the behaviors of producers operating within this space, companies leading the effort to produce bioprinted vascularized tissue products have been examined in detail. This examination was performed using multiple search sources across a range of market resources, with an emphasis on data taken from company websites and published studies. Relevant company metrics and publicly available data, direct interview insights, product overviews and research accomplishments are described for each producer in detail, one-by-one. The order of company profiles has been dictated by respective company's data and information accessibility; companies with large amounts of publicly available data, published studies and overall exposure are towards the top, and companies with limited product and R+D information are towards the bottom.

Organovo, Inc.

Organovo, a public company started in San-Diego in 2004, is the most well-known company operating in the bioprinting space[76]. Their business model is composed of two complimentary pieces: the first is the development of tissue models for *in vitro* testing, the second is bioprinting tissues for implantation into humans. In both arenas, Organovo designs and creates 3D human kidney and liver tissues using primary donor cells. Organovo is also working to create vascular tissues using their Novogen MMX bioprinting platform.

These products have yet to show extensive clinical applicability, yet Organovo still generated $4.2M in revenue in 2017 from increasing numbers of customer contracts for tissue research services. This was a 180% increase over 2016 revenues ($2.7M), aided by, "$0.5M due to substantial milestone achievements under collaboration agreements with multiple partners to develop custom tissue models[186]." These gains were offset by increasing R+D expenditure between 2015 and 2017, which grew from $12.9M to $19.5M over 3 years. In this time, full-time R+D staffing for product development and collaboration support increased from 68 to 80 full-time employees, resulting in an increase expense of $1.5M.[186,187] In 2012, Organovo went public through a reverse IPO merger, raising $128M through multiple rounds of offerings over the course of the next 3 years, which was the first public offering for a bioprinting company. Since then, Organovo's market cap has fluctuated between $230M and $1B, likely due to the lack of comparable industries and companies in the public market, which precipitates shareholder uncertainty and sensitivity.

The first business piece is a short-term product intended to generate revenue through *in vitro* drug testing, toxicology studies and disease modeling. Theses ExVive^{TM} tissues are fully cellular, multi-layered, scaffold-free 3D environments with integrated interstitial lumens. Organovo has been advancing the body of science supporting model functionality, particularly in disease modelling scenarios, and according to Steve Kunszabo, Organovo can now, "create a tissue that emulates NASH (fatty liver disease), which is a true competitive differentiator".[188]

In 2017, Organovo put forth substantial data supporting their models by characterizing their 3D PT ExVive Human Kidney platform. This platform is, "an *in vitro* model of the PT interstitial interface comprising renal fibroblasts, ECs, and primary human renal PT ECs[187]." The Organovo R+D team constructed the model by depositing HUVECs and PHRFs on a well-plate as a basal interstitial layer, then layering primary RPTECs as an apical monolayer, which was supported by a basement membrane. After 14 days, H+E stains showed interstitial remodeling

of HUVEC-lined networks with open lumens. Renal fibroblasts and ECs layered in this micro-architecture established an organized epithelium that supported RPTEC viability long-term and allowed for the detectable expression of PT nephrotoxicity markers like renal uptake and efflux transporters. This model closely recapitulates *in vivo* characteristics of the renal PT and establishes a supporting interstitium adequate for drug testing, but further work needs to be done to produce vascularized tissues with controllable architecture. Vascular networks in this study were thicker than native interstitium, and did not display complete biomimetic function and applicability, but the concept of spatially organizing interstitial cells with other native cell-types to induce vascularization can be applied in other TE scenarios.[23,81,189]

The second piece of Organovo's business suite is a long-term pipeline product that serves to move the ExVive *in vitro* technology towards therapeutic application. This vascularized and implantable liver tissue patch targets pediatric IEMs, which are missing genetic factors in liver patients that can cost up to $250K a year in drug therapies[55]. Steve Kunszabo hopes Organovo's patch will extend and improve the quality of life of IEM patients until they can receive transplants and expects a new IND in 2020 for this graft. This IND will be followed by years of clinical trials, meaning the patch won't be ready for clinical application until 2025. Organovo is the only company with orphan designation for a therapeutic tissue patch, which they received for IEM treatment in early 2017. This status endows Organovo with expedited approval and altered approval requirements for their bio-patch, which will likely set the precedent for bioprinting clinical tissue products into the future.

According to Ben Shepherd, who leads the Organovo therapeutic research team, liver reconstitution is possible because liver failure occurs with high functional erosion close to 90%. Shepherd is working to create small implants that reconstitutes 5-10% liver function, which would negate the need for whole-liver replacements. The Organovo research team makes the grafts by printing a bioink embedded with ECs, vascular support cells and hepatocytes into sheets 500-700µm in diameter. The sheets are then implanted and ECs remodel the perfused vascular networks *in vivo*. Currently, these implants are limited in their cell density because microvascular networks within the tissues are easily occluded.[55,188]

Organovo announced successful animal-model test results for their liver grafts in May of 2017 at the World Advanced Therapies and Regenerative Medicine Congress in London. Results showed minimal cell death and production of liver-specific molecules, like albumin and cholesterol, within liver tissue. In light of these results, Organovo believes they are close to bioprinting a cure for IEM patients, and is continuing preclinical development. Two months after the announcement, Organovo initiated a collaboration with researchers from the UCSD School of Medicine to study non-alcoholic fatty liver disease with a $1.7M grant from the NIH. Organovo is aiming to submit an application to the FDA for clinical approval of their 3D liver product in 2020.[132,190–193]

In 2009, Organovo demonstrated the scaffold-free construction of a multilayered small-diameter vascular tissue construct using multicellular spheroids. Gabor Forgacs, Organovo's primary academic collaborator, directed the study, which included researchers from Yale and the University of Missouri. The team created a fully-cellular bioink by aggregating pre-formed spheroid building blocks within a micropipette. This cylindrical bioink was extruded into a supporting structure made of stacked agarose rods through a special-purpose bioprinter attachment. Multi-layer walls were made by arranging rods of HUVSMC and HSF cylinders in

concentric circles. This rapid prototyping and automated bioprinting method allowed for the compositional and geometric control of branched macrovascular structures at organ-level cell densities, removed issues associated with scaffold-based approaches and created a minimally perfusable tissue product. The maturation process was limited by extended (2-4 days) cellular fusion during bioink construction and cylindrical bonding. Furthermore, this process relied on self-adhering cell types that led to inhomogenous fusion and could create only open-ended tubes with a minimum ID of 900µm. The incorporation of native tissue types, creation of thinner bioink cylinders and characterization of implantation viability must be assessed for future applications.[69,81]

Cyfuse Biomedical, K.K.

Founded in 2010 in Tokyo, Japan, Cyfuse Biomedical is well-known for their unique Kenzan bioprinting method and vascular tissue products. The company closed its series B private funding effort in early 2015 after raising $12.5M in capital, which brought their net invested total to $17.8M[156]. The company offers a line of scaffold-free bioprinters that have produced subchondral bone, liver, cartilage and vascular tissues that all showed positive clinical results during animal testing. Cyfuse has pioneered a novel bioprinting platform called Kenzan, a name derived from the Japanese art of Ikebana that employs spikes to secure flowers in specified locations. Using the Regenova needle-based bioprinter, pre-formed spheroids are picked up and pushed onto microscale needles. These 'kabobs' of spheroids can be proximally aligned in the shape of a blood vessel or specific tissue, allowed to fuse, then removed from the needles post-aggregation. Once the fused product is detached, the stabbed tissues heal into an unimpaired tissue product. Research has shown vascular structures formed with the Kenzan method can bear long-term blood pressure and shear stress and maintain a luminal aperture under physiological flow conditions.[75,133,158]

Cyfuse tested the functionality and feasibility of small-caliber tubular grafts as rat aorta replacements in 2015. Using the Kenzan bioprinting method, MCSs consisting of 40% HUVECs, 10% HASMCs and 50% HDFs were fashioned into tubular structures with an ID of 1.5mm and a length of 7mm. Tubes were incubated for four days under 4ml/min media flow and then anastomosed end-to-end in the abdominal aorta of 15 nude male rats. Five days post-implantation, graft patency, abundant ECM deposition, wall area thinning, lumen enlargement, and histological EC remodeling and differentiation was observed. This printing approach was scaffold-free, reproducible, could produce a product in eight days devoid of foreign materials, and exhibited control of the tubular feature geometry (size, shape and length). Additionally, the cell type, size, and count of MCSs could be controlled through the self-assembly and printing steps. The study could have been improved by implementing a PTFE graft control, examining graft performance long-term in large mammals, characterizing and identifying the underlying mechanisms controlling EC remodeling and migration and analyzing mechanical properties of the printed product (like elasticity, tension and burst pressure). This work has set the standard for bioprinting small-diameter multicellular biological conduits with clinical applications.[135,194]

Advanced Solutions Life Sciences, Inc.

ASLS' vascularizing technology is focused on isolating and printing microvessels in order to establish microcirculatory perfusion within tissues. In 2017, ASLS announced its human adipose derived microvessel product, which contains the, "requisite cell types and structure of the microcirculatory system for 3D cell culture systems[195]." Instead of digesting capillaries down to their constituent parts (cells, growth factors ECM), ASLS is leveraging the ability of their microvessels to sprout and grow via angiogenesis.

ASLS is a subsidiary of Advanced Solutions, Inc., and has over 150 employees in its Louisville, Kentucky headquarters. ASLS is both a supplier and a builder, boasting a range of products, including, "3D modeling software, the world's first 6-axis robotic 3D printers, bioinks, and in-house expertise empower researchers to make important advances in fields that span from basic to translational research." The company provides commercial services and support for all their bioprinting, bioinks and bioprinting supplies, and is moving to Manchester, NH, in March of 2018 in order to expand their collaborative horizons through the ARMI-BioFabUSA innovation center, where they hope to gain access to a wide variety of connections, expertise and perspectives across TE and biofabrication fields.[76,196]

ASLS has two technology platforms, which are (1) robotic bioprinting platforms that include the BioBot (with software) and the BioAssemblyBot, and (2) its vascularizing technology. The BioBot can print up to five materials in a single fabrication task and retails for less than $5K, and the advanced robotic-arm-based BioAssemblyBot system retails for ~$150k. Researchers at ASLS use the BioAssemblyBot platform for in-house R+D and are assembled into specific project-teams according to technical proficiencies. ASLS neo-vessels conserve basic vascular anatomy but have collapsed lumens and no central flow. Procedures like liposuction create a reliable source for adipose tissue, and once extracted, microvessels can be shipped in 10K or 50K frozen cryovials. ASLS has successfully incorporated the proper ECM and cellular components needed to build a vascularized tissue, and are now in the process of optimizing their microfluidic perfusion dynamics to ensure robust and consistent outcomes.[76,195]

When implanted in mouse models, microvessel-hydrogel scaffolds integrate, remodel and persist for months within the host. *In vitro,* recreating that *in vivo* environment is not so easy. According to the ASLS scientific advisor James Hoying, ASLS is currently using a microfluidic platform to provide fluid flow to embedded microvessels *in vitro* in order to recreate the native ECM, so that capillaries will grow throughout a hydrogel bed. They do so by encapsulating microvessels within collagen and printing them in conjecture with larger lumen structures. First, a horizontal Pluronic-F127 cylinder surrounded by a bed of microvessel-laden collagen is printed, the sacrificial Pluronic-F127 is melted to create an open lumen, and media flow is introduced through the central lumen. Hoying hopes that the flow and shear stress will trigger angiogenic responses that cause the surrounding neo-vessels to integrate with the central lumen and allow flow to enter the surrounding microvessels. Hoying is optimistic about the compatibility and viability of microvessels within hydrogel bioinks and is also working to extract vessels from neuronal tissue; he expects that a solution will be in place for the microvasculature ECM issue by the end of Q3 2018, at which point a target application can be determined. Currently, ASLS is selling the product to universities and industry players intent on incorporating these vessels in their own biofabrication experiments.[76]

3Dynamic Systems

Recently, 3DS announced the commercialization of its vascular scaffold constructs, which are made with a specialized bioprinting and incubation system. 3DS is marketing the tissues as an organ-on-a-chip technology for drug testing, which will hopefully be commercialized by 2020, but is ultimately working to approve them for operative repairs. The 3DS headquarters is on the Swansea Campus in Whales, UK, and was founded in 2012 as a basic research company by Dr. Daniel J. Thomas.[197]

The 3DS printing process involves bioprinting cell-laden bioinks into tissue plates using their dual-head 3Dynamic Omega Tissue Engineering Workstation Bioprinter. Post-printing, cells are matured for two weeks as they self-assemble in an incubator. During this time, HA is added to sustain proteoglycans, and other growth factors are incorporated to support cellular growth and development. Using this methodology, 3DS has been able to produce 3D heterogeneous tissue platforms for pharmaceutical testing and are working to increase the body of science supporting their models. Dr. Thomas believes that the vascular grafts will be used in hospitals in the next 5-10 years, and is striving to optimize the ECM, reduce infection rates and harness renewable stem cells so the grafts will be ready for clinical adoption.[198,199]

Below are images presenting the range of vascular tissues that 3DS currently produces, provided by Dr. Thomas as a follow-up to our phone conversation:

Images: 3DS Bioprinted Vascular Tissue Products[176]

The other bioprinting platform developed by 3DS is the 3Dynamic Alpha series, which has a singular print-head for extruding tailored calcium phosphate composites in 3D. The composite is seeded with PDGF, along with other factors, which stimulate self-organization of the cellular materials into bone and blood vessel structures. The Alpha series is used exclusively for bone tissue construction, but uses a similar proprietary incubation technique, used for both Alpha and Omega printers. Like Organovo, 3DS has produced a high-throughput drug testing vascularized tissue model, but further characterization and development of the 3DS product is needed. 3DS already has several bioprinters installed in German and Italian hospitals for on-site printing of vascular tissues. Dr. Thomas envisions 3DS producing customized tissues on-site for immediate surgeries and also manufacturing large numbers of drug-testing models and tissue grafts at their central bioprinting facility that can be cryopreserved and shipper to the end-user.[197–199]

3D Bioprinting Solutions

3Dbio was founded in 2013 and is largely invested by an expansive Russian technology company called Invitro. The small start-up is centered in Moscow, but has bioprinter manufacturing facilities in Austria, and marketing centers in New York at their mother company's facility. 3Dbio boasts five multi-faceted market approaches that stretch across R+D, technology and supply arenas, including:

1. **Fabion Printer**: 3Dbio's platform bioprinting technology boasts a highly modular spheroid extrusion technology with five nozzles that can alter print modalities and polymerization mechanisms depending on the desired material and cell type. This printer incorporates DBB, MBB, SFB, bioassembly, UV and ionic polymerization approaches to position spheroids in 3D.
2. **Magnetic Bioprinting**: Looking beyond current layer-by-layer printing modalities, 3Dbio is working to assemble and fuse spheroids using scaffold-free magnetic levitation in outer space. They envision an open-source platform on the Russian International Space Center that can be used by researchers from around the world.
3. **Acoustic Bioprinting**: To expand the capabilities of their Fabion printer, 3Dbio is experimenting with acoustic soundwaves to manipulate tissue spheroids in 3D.
4. ***In situ* Cartilage Tissues**: By directly printing chondrocytes, 3Dbio is working on a methodology to cure cartilage defects inside the body.
5. ***In vitro* Drug Discovery**: 3Dbio is ramping up their drug modeling services so they can join the ranks of Organovo and Poietis in 3D the testing of medications, diseases and compounds for pharmaceutical companies. They are focusing on cancer models, specifically carcinoma and melanoma tissues.

To demonstrate the functionality of their turnstile Fabion printer, researchers at 3Dbio built dense, vascular thyroid constructs that experienced minimal graft rejection or loss of cell viability when implanted in mice. In 2017, the team published a paper demonstrating the *in vivo* applicability of the thyroid tissues, in which thyrocytes and ECs were self-assembled into TSs and ASs, which were encapsulated in a collagen hydrogel and printed one-by-one using their Fabion printer. TS and AS spheroids were printed in close proximity and allowed to fuse, which resulted in the formation of a dense microvascular network and follicles throughout the tissue, a result of AS EC invasion and VEGF-A release. This vascularization was evidenced by EC stains, immuno-labelled visualizations, and maintained internal cell viability within the highly dense tissues over time. After printing, the collagen gel was melted away, the structures were placed in an incubator for four days and were then grafted onto the kidney of hypothyroid mice. After three and five weeks mice exhibited normalized thyroxine levels and body temperature measurements. There was also evidence of numerous follicular growths with mono-layered epithelium at the cortical host region and erythrocyte-filled capillary formation throughout the constructs.[21,200,201]

3Dbio is now working to scale-up their printing process and analyze their thyroid tissues in a wider range of *in vivo* settings. The company has collaborations with universities all over the world. While 3Dbio specialized in bioprinting, their collaborators contribute by providing

cell-lines, characterize biomaterials and hydrogels. Akin to other bioprinting companies improving their future technological capabilities, 3Dbio is working to:[149]

1. Optimize cell sources, as they need to procure tens of millions of cells which will likely be from iPSC line
2. Characterize and monitor their disease models and standardize a way to analyze toxicity, tumor and drug reactions within 3D tissue environments
3. Maintain standardized manufacturing quality across all products and reduce batch-to-batch variability
4. Induce vascularization at the micro-level in a reliable and reproducible way throughout large and small tissues

Poietis

On October 25th, 2017, Poietis announced a framework agreement with the cosmetics company BASF to continue the French bioprinting company's development of human skin models for evaluating cosmetic ingredients and skin care products. Only three months later, on January 23, 2018, Poietis announced the launch of their Poieskin skin testing product as the first commercialized bioprinted tissue model available to the public. Poieskin is printed with Poietis's NGB laser bioprinter and is composed of primary human fibroblasts, dermal collagen and epidermal primary human keratinocytes. The skin model is touted as a reproducible, flexible and customizable platform for cosmetic testing and will go on sale in April for European customers. Poieskin is the first product of its kind, has the potential to provide an attractive alternative to animal testing. To bring this product to market Poietis partnered with laser technology companies like Alphanov, cosmetic giants like L'Oreal, received funding from the French National Research Agency and Aquitane Region, and collaborated with the University of Bordeaux.[6,202] With this product, Poietis will join the ranks of Organovo in the commercialized bioprinting *in vitro* tissue-modelling space.

Prellis Biologics

Prellis is working to create a vascularized platform for whole-organ bioprinting by incorporating microvasculature into scaffolding structures. The company was founded in 2016 by Melanie Matheu and Noelle Mullin, and now includes two additional researchers. The team believes they are 4-6 years away from building entire kidneys, which they anticipate will take 4-6 months to print for $80K each. Until then, they will be using their proprietary technology to produce less complicated tissues, like lymph nodes and pancreatic islets, built upon their foundational microvasculature support. Excited by these prospects, Prellis has received $1.92M in seed funding from True Ventures, Civilization Ventures and 415 Ventures, which are all venture capital firms.[203,204]

The Prellis printing method involves the culture and differentiation of human cells into desired cell types, imaging of a target vascular or anatomical structure, and, according to their website, the creation of non-toxic, "layers of cell-containing extracellular matrix at almost instantaneous speeds… in accordance with 3D CAD images." The printing method is laser-based, boasts sub-micrometer printing resolution and negates the necessity of additional

seeding or curing steps. Prellis has yet to publicly disclose any studies, published work or technical specifications for their bioprinting process.[203,205]

To date, Prellis has filed three provisional patents; one for the laser-based prototyping procedure used to render 3D images of printed structures, another for their collagen-based microvasculature scaffold, and the final for the clinical development of bioprinted islet of Langerhans tissues. Prellis hopes to start clinical trials for insulin-producing islet implants in 2021, which they hope will be provide a functional therapy for Type-1 Diabetes patients that could replace the need for daily insulin injections. Additionally, Prellis has printed Lymph Node Organs (LNOs) with clinical potential that have shown immunogenic properties in animal studies. The team explained that in proof-of-concept studies they, "created 14 novel class-switched human IgG antibodies that are reactive to the Zika Virus in less than 8 weeks."[203,205,206]

Given their audacious future outlook, it is likely that the Prellis microvasculature scaffold has shown sufficient functionality in supporting the future development of simple tissue structures. It is possible that Prellis has found a way to overcoming the diffusion gradient in their bioprinted constructs, but more documented information is needed. Prellis is among a group of companies in the bioprinting space with laudable goals who have yet to procure a commercially viable TE product.

TeVido Biosciences

The US biotech start-up TeVido Biosciences is developing DBB processes to create reconstructed nipples using autlogous cells for breast replacement post-mastectomy. These nipple areola complexes (NACs) are pre-vascularized with projections of adipose grafts and will be engineered to have custom color pigmentation. Long term, the company is planning to regenerate lumpectomy spaces with tissue constructs, and short-term they are working to market their NAC product and protect their work[6]. TeVido was the recipient of a $900K NIH grant which funded their initial R+D, but was forced to crowd-fund $30K from the public using the Indiegogo investment platform to patent their inkjet bioprinting technique.[81,207,208]

Aspect Biosystems

Aspect Biosystems Ltd. is focused on developing tissue platforms for *in vitro* pharmaceutical testing. In late 2017 they partnered with the German cell-screening company InSCREENeX and the Fraunhofer ITEM, which is focused on developing novel airway therapies and is a branch of the Fraunhofer-Gesellschaft, Europe's leading organization for applied research. The partnership is intended to catalyze the development of bioprinted contractile tissue for testing airway medications, a product space that Aspect Biosystems dominates with its 3D BioRing Airway platform and Lab-on-a-printer Technology. The BioRing Airway product site claims it is, "the world's first 3D printed airway tissue that exhibits highly physiological contraction and relaxation responses in an *in vitro* setting." The bioprinted muscle expands and contracts in response to histamine and salbutamol administration, which allows researchers to study pharmaceutical treatments for disorders like asthma. Printing is performed with Aspect's RX1 bioprinter, takes less than one minute per tissue and can be customized to control cell density, ECM composition and mechanical specifications depending on the target application.

Aspect's flagship Lab-on-a-printer Technology allows for this high level of modulation and speed. This technology is unique because it uses modular microfluidic print-head cartridges to control tissue design in three dimensions. Bioinks are generated within the cartridge using the RX1 Bioprinter, which is equipped with coaxial microextrusion nozzles that can push cell-laden fibers throughout the cartridge architecture to produce heterogeneous 3D products, like the BioRing™ Airway. In a press release from September 2017, Aspect disclosed the completion of their first round of institutional private funding, saying it will, "accelerate the commercialization of our Lab-on-a-Printer™ bioprinting platform and tissue applications… which is enabling advances in understanding fundamental biology, disease research, development of novel therapeutics, and regenerative medicine."[209–211]

Regenovo

Regenovo, a Chinese company associated with researchers from Hangzhou Dianzi University, has produced its own bioprinting technologies that include scaffolds, biomaterials and the Regenovo Bio-Printer, which comes in -Lite, -Pro and -WS configurations, all with varying degrees of complexity and price. The company has marketed three bioprinted TE products that includes liver, bone and blood vessel products, and according to their website, the 3D blood vessel can be printed in single-branch or vascular-tree shapes. Regenovo also offers three drug-discovery platforms consisting of liver hepatotoxicity, tumor growth and metabolic syndrome models. Characterization and product specifics are limited, but Regenovo is making great strides commercializing a large portfolio of bioprinted products.[212]

BioDan

The Spanish company BioDan's corporate structure is composed of two distinct entities: Bio-Print, and Bio-SkinMed. The Bio-Print wing is working to scale-up a bioprinted allogeneic skin product, which has already shown successful pre-clinical results for drug, cosmetic, sanitary and chemical consumer product testing. Studies show that the 3D skin product is composed of a double-layer of keratinocytes and fibroblasts imposed on a fibrin scaffold that produces its own collagen ECM. Bio-Print is hoping to act as a third-party testing service but is also looking to partner with large companies to move their skin-printers on-site for dynamic on-demand applications.[213]

Bio-SkinMed is also developing an autologous bioprinted skin product but is focused on RM and healing applications that include burn therapy, wound healing and internal mucosa surgeries. The double-layered skin product and manufacturing process are patented, and BioDan claims they can produce, "4.4cm^2 of a patient's own skin within 22 days of a small biopsy and have already produced over 2.500m² of skin for burns, wounds and reconstruction processes." In 2014, BioDan received FDA approval for their skin therapy, which is classified under the European Parliament Regulation as an 'Advanced Cellular Therapy' for TE and claims to already save the Spanish National Health Service, "50 days of Intensive Care hospitalization for patients with corporal skin burns of between 50-60%." BioDan is one of the only companies that has marketed bioprinted products currently being used in clinical scenarios.[214]

Sichuan Revotek

Sichuan Revotek (SR) is one of the few companies extensively testing bioprinted vascular grafts in animals. The company's headquarters were built in the city of Chengdu in 2014 and has since grown to employ 150 full-time workers. The company was enticed to Chengdu, which is located in the Sichuan Province of China, by a $33M investment from a local real estate company, and curiously, by the abundance of rhesus monkeys living in local forests. In addition to its vasculature product, SR offers a one-stop-shop bioprinting package for individualized culture and printing of stem cells that includes a 3D bioprinter, cloud computing access, and Biosynsphere, a stem cell bioink technology.[215,216]

The SR vascular product is formed by bioprinting a graft outline and self-assembling the tissue *in vitro*. Kang Yujian, Chief Scientist of Sichuan Revotek, explains in a report on 3Dprint.com that, "In five days, a new layer of endodermis will be formed, meanwhile, the smooth muscle cells will grow as well. And within 28 days, all these cells will go through tissue differentiation. That means the tissues we implanted will have mingled with the original ones and grown into a regular vessel. This is unprecedented[216]." The team removed a 2cm segment of abdominal artery from 30 local rhesus monkeys and replaced it with their vascular graft. Five days later, stem cells differentiated into ECs and SMCs, and one month later vessels showed full integration with the monkeys' arteries, characterized by complete reconstitution of vessel functionality and deposition of supportive collagen-rich ECM. This trial shows the applicability of the SR vessel product to be used in clinical trials as an autologous vascular graft and demonstrates its ability to maintain high levels of patency long-term. Minimally restrictive regulatory laws in China may also allow SR to advance clinical trials before its competitors.[215,216]

Contributing Interviewees

We want to thank the following contributors for volunteering their time and energy during our interview process. All of the individuals shown in Table 11 are working to move the field of bioprinting forward in a positive and productive way, and provided invaluable, on-the-ground, first-person input for this landscape. We tried to appropriately quote as reported; any misrepresentations or misquotations from interviews are entirely our responsibility. These professionals were open, honest and straightforward in their discourse, and received no compensation for their time other than the knowledge that their work and outlook for advancing the fields of TE and bioprinting would be included in our report. This analysis would not have been complete without their insights and contributions.

Table 11: Contributing Interviewees

Name	Organization	Date	Time	Medium	Major Publications and Credentials
Dr. Kimberly A. Homan; Ms. Katherina Kroll	Lewis Lab; Harvard University	2/14/18	4:00-5:00pm	Telephone	Kolesky, D. B. et al. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv. Mater. 26, 3124–3130 (2014).
Dr. Pallab Datta	IIEST; Shibpur, India	2/6/18	11:00-11:45am	Telephone	Datta, P., Ayan, B., & Ozbolat, I. T. (2017). Bioprinting for vascular and vascularized tissue biofabrication. Acta Biomaterialia, 51, 1–20. https://doi.org/10.1016/j.actbio.2017.01.035
Dr. Guohao Dai	Northeastern University	2/15/18	5:00-6:00pm	Telephone	Lee, V. K., Kim, D. Y., Ngo, H., Lee, Y., Seo, L., Yoo, S. S., … Dai, G. (2014). Creating perfused functional vascular channels using 3D bio-printing technology. Biomaterials, 35(28), 8092–8102. https://doi.org/10.1016/j.biomaterials.2014.05.083
Dr. Iwona Cicha	University of Erlangen, Germany	2/5/18	8:30-9:30am	Telephone	Aggregator
Dr. Matt Pavlovich	Cell Press Trends in Biotechnology' journal; Editor	2/2/18	11:00-11:45am	Telephone	Aggregator
Dr. Vivian Lee	Northeastern University	2/1/18	12:15-1:00am	Telephone	Lee, V. K., Kim, D. Y., Ngo, H., Lee, Y., Seo, L., Yoo, S. S., … Dai, G. (2014). Creating perfused functional vascular channels using 3D bio-printing technology. Biomaterials, 35(28), 8092–8102. https://doi.org/10.1016/j.biomaterials.2014.05.083
Dr. Bill Tawil	UCLA Bioengineering	1/30/18	10:00-10:45am	Telephone	Aggregator
Dr. Lakshmi Nair	University of Connecticut	1/5/18	11:00-11:15am	In-person: ARMI BioFab Conference, Manchester, NH	Managing editor of RE and TM Spring Journal
Ms. Hannah Strobel	WPI; PhD Marsha Rolle's Lab	2/22/18	11:00-11:45am	Telephone	Limited bioprinting experience, but two seminal TE vascular studies: (1) Fabrication and characterization of electrospun polycaprolactone and gelatin composite cuffs for tissue engineered blood vessels; (2)A modular strategy to engineer complex tissues and organs
Dr. Angela Panoskaltsis-Mortari	University of Minnesota	1/5/18	12:00-12:15pm	In-person: ARMI BioFab Conference, Manchester, NH	Started and teaches bioprinting class at Uminn. for undergrads and grad students

Dr. Benjamin Shepherd	Organovo; Director of Therapeutics	1/5/18	1:00-1:15pm	In-person: ARMI BioFab Conference, Manchester, NH	Leads Organovo therapeutic effort
Dr. Anthony Holmes	NC3Rs; UK	2/20/18	11:00-11:45am	Phone Interview	Holmes, A. M. et al. Rising to the challenge - Applying bioprinting approaches for better drug and chemical development. Biofabrication 9, 33001 (2017).
Mr. Steve Kunszabo	Organovo; VP, Investor Relations and Corporate Communications	2/23/18	1:00-2:00pm	Phone Interview	King, S. M. et al. 3D proximal tubule tissues recapitulate key aspects of renal physiology to enable nephrotoxicity testing. Front. Physiol. 8, 1–18 (2017).
Dr. James Hoying	ASLS; Partner and Chief Scientist	2/26/18	4:30-5:30pm	Phone Interview	Chang, C. C. et al. Determinants of Microvascular Network Topologies in Implanted Neovasculatures. 32, 5–14 (2013).
Dr. Daniel Thomas	3DS; Founder and Executive	2/26/18	12:15-1:00pm	Phone Interview	Founded 3Dynamic Systems in 2009, purely focused on vasculature bioprinting
Mr. Yousef D. Hesuani	3Dbio; Cofounder and Managing Partner	3/1/18	8:30-9:30am	Phone Interview	Balakhovsky, Y., Ostrovskiy, A. & Khesuani, Y. Emerging Business Models Towards Commercialization of Bioprinting Technology. (2018).
Mr. Steve Horvath	RTI Surgical; Product Manager and Marketing	3/1/18	3:00-3:45pm	Phone Interview	Largely 3D printing-centric; had outlook on bioprinting developments; Tetrafuse 3D printing platform; Fortilink plastic spine implant in ACDF surgeries

Table 12: Terms and Abbreviations

Bioprinting Approaches	
Continuous direct light processing	CDLP
Digital light processing	DLP
Droplet-based bioprinting	DBB
Freeform reversible embedding of suspended hydrogels	FRESH
Integrated tissue-organ printer	ITOP
Laser induced forward transfer	LIFT
Laser-based bioprinting	LBB
Microextrusion-based bioprinting	MBB
Scaffold-based bioprinting	SB
Scaffold-free bioprinting	SFB
Stereolithography	SLA
Stereolithography-based bioprinting	SBB
Cells and Biologics	
Allantoic spheroids	AS
Angiopoietin-1	Ang1
Angiopoietin-2	Ang2
Cartilage progenitor cell	CPC
Chinese Hamster Ovary	CHO
Early vascular cell	EVC
Extracellular matrix	ECM
Granulocyte colony stimulating factor	GCSF
Green fluorescent protein	GFP
Human amniotic fluid stem cell	HAFSC
Human aortic smooth muscle cell	HASMC
Human coronary artery smooth muscle cell	HCASMC
Human dermal fibroblast	HDF
Human embryonic kidney cell	HEK
Human hepatocellular liver carcinoma cell line	HEPG2
human mesenchymal stem cell	HMSC
Human microvascular endothelial cells	HMVEC
Human ovarian granulosa	KGN
Human proximal tubule epithelial cell	HPTEC
Human skin fibroblast	HSF
Human umbilical vein endothelial cell	HUVEC
Human umbilical vein smooth muscle cells	HUVSMC
Human-bone-marrow-derived mesenchymal stem cell	HBMSC
Immunoglobulin	Ig
Induced pluripotent stem cell	IPSC
Mouse embryonic fibroblast	MEF
Multicellular spheroids	MCS
Multipotent mouse bone marrow stromal precursor cell	D1
Normal human dermal fibroblast	HNDF
Normal human lung fibroblast	NHLF
Platelet derived growth factor	PDGF
Porcine aortic smooth muscle cell	PASMC
Primary rat hepatocyte	HC
Rat fat microvessel fragments	RFMF
Red blood cell	RBC
Renal proximal tubule epithelial cell	RPTEC
Smooth muscle cell	SMC
Stromal-derived factor-1	SDF1
Thyrocyte spheroids	TS

Vascular endothelial growth factor	VEGF
White blood cell	WBC
Institutions, Organizations and Companies	
Astra Zeneca	AZ
3D Bioprinting Solutions	3Dbio
3Dynamic Systems	3DS
Advanced Regenerative Manufacturing Institute	ARMI
Advanced Solutions Life Sciences	ASLS
Center for Biologics Evaluation and Research	CBER
Department of Defense	DOD
Food and Drug Administration	FDA
Fraunhofer Institute for Toxicology and Experimental Medicine	ITEM
Hewlett Packard	HP
Johnson & Johnson	J&J
National Aeronautics and Space Administration	NASA
National Center for Replacement, Reduction and Refinement of Animals in Research	NC3Rs
People for the Ethical Treatment of Animals	PETA
Proctor and Gamble	P+G
Tissue Regeneration Systems	TRS
University of California Las Angeles	UCLA
University of California San Diego	UCSD
University of Connecticut	UConn
University of Minnesota	UMinn
Worcester Polytechnic Institute	WPI
Materials and Polymers	
Aqueous polyvinyl alcohol	PVA
Calcium chloride	CaCl2
E-caprolactone	CL
Expanded polyterafluoroethylene	ePTFE
Gelatin methacryloyl	GELMA
Glycidal methacrylate-hyaluronic acid	GM-HA
Hyaluronic acid	HA
Lithium phenyl-2,4,6 trimethylbenzoylphosphi-nate	LAP
Microcarbon nanotubule	MCNT
Perfluorotributylamine	PFTBA
Polycaprolactone	PCL
Polycarprolactone-co-lactides	PCLS
Polydimethylsiloxane	PDMS
PolyDL-lactide-co-glycolide	PDLGA
Polyester urethane urea	PEUU
Polyethylene glycol	PEG
Polyethylene glycol diacrylate	PEGDA
Polyethylene glycol dimethacrylate	PEGDMA
Polyethylene glycol-tetra-acrylate	PEGTA
Polyethylene terephthalate	PET
Polyglycolic Acid	PGA
Polyhydroxyethylmethacrylate	PHEMA
Polypropylene fumarate	PPF
Star polyethylene glycol-co-lactide acrylate	SPELA
Thermoplastic polyvinyl alcohol	PVA
Trimethylene carbonate	TMC
Xanthum gum	XG

Measurements	
Micrometer	µm
Microseconds	µs
Milliliter	ml
Nanometer	nm
Pascal second	Pa.s
Picoliter	pl
Poiseuille	Pl
Miscellaneous	
Cardiovascular	CV
Central nervous system	CNS
Chronic Kidney Disease	CKD
Compound annual growth rate	CAGR
Computed tomography scan	CT
Computer-aided design	CAD
End-stage renal disease	ESRD
Form 10-K annual report	10K
Four dimensional	4D
Good manufacturing practice	GMP
Inborn error of metabolism	IEM
Inner diameter	ID
Intellectual property	IP
Key opinion leader	KOL
Magnetic resonance imaging	MRI
Micron/micrometer	µm
Million	M
Outer diameter	OD
Peripheral nervous system	PNS
Proximal tubule	PT
Quality by design	QbD
Regenerative medicine	RM
Research and development	R+D
Thousand	K
Three dimensional	3D
Tissue engineering	TE
Two dimensional	2D

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