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3-D bioprinting technologies in tissue engineering and regenerative medicine: Current and future trends

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Abstract

Advances in three-dimensional (3D) printing have increased feasibility towards the synthesis of living tissues. Known as 3D bioprinting, this technology involves the precise layering of cells, biologic scaffolds, and growth factors with the goal of creating bioidentical tissue for a variety of uses. Early successes have demonstrated distinct advantages over conventional tissue engineering strategies. Not surprisingly, there are current challenges to address before 3D bioprinting becomes clinically relevant. Here we provide an overview of 3D bioprinting technology and discuss key advances, clinical applications, and current limitations. While 3D bioprinting is a relatively novel tissue engineering strategy, it holds great potential to play a key role in personalized medicine.

Keywords

Additive manufacturing; Bioprinting; CAD/CAM; 3D printing; Tissue engineering

Introduction

Advances in computer-aided design (CAD) and fabrication technologies have brought rapid progress to the field of three-dimensional (3D) printing in recent years. Also known as additive manufacturing (AM), rapid prototyping (RP), and free form fabrication (FFF), 3D

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Conflict of interest

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printing was initially conceived by Charles Hull in 1986.[1,2] Hull's concept was based on the idea that successive layers of a base material could be applied on top of each other to 'print' objects. Since its inception, 3D printing has impacted several fields including engineering, manufacturing, and medicine. In recent years, the development of biocompatible systems for 3D printing have been especially promising for tissue engineering applications. The field of tissue engineering has conventionally involved culturing cells, seeding them into biocompatible scaffolds, and allowing growth and maturation (in vitro or via bioreactor) to form the desired tissues.[3] We use the term 3D bioprinting to describe the precise layering of cells, biologic scaffolds, and biologic factors with the goal of recapitulating a biologic tissue. Compared to traditional tissue engineering methods, the technologies utilized by 3D bioprinting systems allow for greater precision in the spatial relationship between the individual elements of the desired tissue. 3D bioprinting holds great promise for regenerative medicine applications (see Fig. 1).

General approaches

Three central approaches to bioprinting are biomimicry, autonomous self-assembly, and a microtissue-based method. These general strategies are not exclusive to bioprinting and are broadly applied to many investigational areas within the larger scope of regenerative medicine. In many cases, these are used for tissue engineering applications unrelated to bioprinting. However, a discussion of these fundamental strategies is necessary when considering the optimal approach to bioprinting objectives. Each of these may applied to specific bioprinting applications to varying degrees based on factors such as target tissue type, user experience, and printing method. It is not uncommon to combine strategies for more complex tissue types.[1] We discuss each of these in detail below.

Biomimicry

With the understanding that function will follow form, a biomimetic approach attempts to engineer each individual component of native tissue. While it is the most conceptually straightforward approach, it is extremely difficult to reproduce all elements that make up the milieu of a given target tissue. Even for relatively simple tissue types, the sheer volume and dynamic nature of cellular interactions that occur reach staggering complexity. In addition to the numerous cell types, signaling molecules, and structural elements within the tissue itself, all environmental factors including pressure, temperature, and electrical forces must be considered.[4,5] As tissues become more complex, the 3D structure and resultant mechanical forces add yet more complexity.

There are several ways that these complexities are minimized when utilizing a biomimetic approach to bioprinting project design. The selection of an appropriate scaffold material is crucial. An optimal scaffold can closely approximate many of the structural and mechanical requirements of a target tissue. Scaffold choice also heavily influences signaling through cellular interactions with the extracellular matrix component (ECM).[5] The use of bioreactors to regulate environmental parameters is also critical to successful biomimicry. Bioreactors essentially create an environment or a set of microenvironments that mimic that of the target tissue.[3] Depending on specific needs, a bioreactor can regulate any combination of chemical,

mechanical, and electrical variables.[5] These variables may also change over time to create an appropriately dynamic environment that allows for sequential maturation.[6] For many tissue types, the environment does not simply support its development. Instead, these external forces provide vital signaling cues that stimulate normal development of a tissue. An ideal bioreactor precisely facilitates these dynamic interactions that occur between tissue and environment. In most cases, the bioprinting process does not end following tissue printing and some period of maturation is typically required. It is at this stage that a bioreactor may be utilized to influence bioprinted tissue through biomimicry.

Autonomous self-assembly

The self-assembly approach attempts to replicate embryonic environmental and structural elements with the end goal of creating correct embryologic anatomy. Bioprinting may be utilized to produce these discrete units that can self-organize for tissue development.[1] This strategy depends on the understanding that with the correct embryonic elements in place, development will recapitulate that seen in nature, with cells and supporting structures self-organizing and interacting to create any other raw materials needed during development to mature tissue.[1,7] This is distinct from the biomimetic approach in which an attempt is made to externally influence the maturation of a tissue at all stages of development. The autonomous self-assembly approach does not adhere to the traditional tissue engineering approach of seeding cells onto a scaffold but relies on the conceptual reasoning that tissues and organs have inherent mechanisms for development and often do not require a template or scaffold.[8] With this approach, limitations due to scaffold degradation and scaffold biocompatibility are addressed. Studies have demonstrated that utilizing an autonomous self-assembly method can result in high cellular density, improved cellular interactions, accelerated growth, and improved long-term function (see Table 1).[9–12]

Autonomous self-assembly has successfully been utilized to create many different tissue types. L'Heureux and colleagues utilized this approach to create the first biologic-based engineered blood vessel.[13] In this study, human smooth muscle cells and fibroblasts were engineered to form functional blood vessels that displayed advanced organization, sound mechanical properties, and an abundant ECM. In another example, self-assembly has been used to construct cardiac muscle tissue. Shimizu and colleagues utilized rat cardiomyocytes that could self-assemble to form pulsatile, multilayered sheets of cardiac tissue that exhibited appropriate electrical coupling.[14]

Microtissues

The concept of a microtissue approach to bioprinting relies on the fact that a typical complex *in vivo* tissue is composed of many simpler units whose combined structure and function contribute to the overall whole. In utilizing this strategy, tissue engineering techniques are used to form the smallest structural and functional units which can be combined into the final target tissue.[1] The term *minitissues* is often used interchangeably with the term *microtissues* by some investigators. When a microtissue-based approach is used, the term *macrotissue* is typically used to distinguish fully developed tissue from its smaller constituent units. Microtissues are incorporated into bioink and consolidation to macrotissue

occurs after printing.[8] Self-assembly or biomimetic strategies may be used to facilitate this consolidation.

There is a multifactorial effect on the speed and efficiency of the bioprinting process when using a microtissue-based approach. First, the smaller size of microtissues are more easily incorporated into bioinks for bioprinting, a process that greatly increases the overall efficiency of the bioprinting process.[8] With the use of larger discrete printing units, also known as droplets, the efficiency of the printing process is decreased due to frequent clogging and decreased flow through the bioprinter.[15] Speed of production is also generally increased due to the standardized size of microtissue units, which allows for a degree of automation and scalable production.[8] Several investigators have also demonstrated accelerated tissue maturation when using a microtissue-based approach.[8,16] As with an autonomous self-assembly approach, microtissues can often be used in bioprinting without scaffolds. Elimination of the scaffold formation step is yet another way that a microtissue-based approach can increase speed and efficiency.[13,16]

The advantages of a microtissue-based approach to bioprinting have been shown in several studies across many investigational areas. In one prominent example, Kelm and colleagues utilized myofibroblasts and endothelial cells to engineer microtissue building blocks which they were able to successfully assemble into mature blood vessels.[16] In part due to their use of microtissues, they obtained accelerated rates of ECM production, maturation, and differentiation of vascular tissue.[16] In another example, Yu and colleagues were able to engineer mature cartilage tissue strands up to 8 cm in length from 400 μm microtissue units.[9]

Bioprinting process

The bioprinting process occurs in three distinct phases. First, the *pre-processing phase* includes all the planning details that precede production of bioprinted tissue. This phase includes imaging (CT, MRI, etc.) to analyze the anatomical structure of the target tissue and subsequent CAD to translate the imaging data into a blueprint for bioprinting. [17] Specialized software programs (e.g. AutoCAD, SOLIDWORKS, and CATIA) transform imaging data into cross-sectional layers of appropriate scale such that the bioprinting device will be able to add them in a layer-by-layer fashion.[17,18] The *processing* phase occurs next and involves all steps involved in the actual construction and manufacturing of the bioprinted tissue. Complexity at this stage arises in choosing a specific printing method and formulating a combination of materials (bioink, scaffold, and other additives). Each selection has the potential to alter the interaction of the individual components and to affect the final tissue product as a result. Each variable of the processing phase, printing method, bioinks, and stem cell utilization, will be discussed in detail in later sections. Finally, the *post-processing* phase involves all steps that must occur before bioprinted tissue is fully mature and ready for *in vivo* usage. For most 3D bioprinting applications, this usually takes places within a bioreactor. While bioreactors have certainly played a pivotal role in bioprinting, more refinement of the bioreactor technology is needed. Current bioreactors are not able to appropriately recreate the *in vivo* environment for many tissue types which often results in loss of tissue viability during the maturation period.[8,19]

3-D printing technologies

Inkjet 3D bioprinting

The first attempts at bioprinting utilized a commercial 2D inkjet printer modified to print biological ink in layers.[20,21] Inkjet bioprinters, also known as drop-on-demand printers, use a non-contact technique that may use thermal, piezoelectric, or electromagnetic forces to expel successive drops of bioink onto a substrate, replicating a CAD design with printed tissue.[22,23] Although there were worries that the inkjet printing approach would harm cell viability due to the very high local temperatures in the nozzle of up to 300 °C, the duration of localized heating is an extremely short period of time (~2 μs) and has been shown to increase the bioink temperature only 4–10 °C.[24] Studies have demonstrated that these temperature increases alone do not significantly impact the stability or viability of mammalian cells.[25,26] Key benefits of inkjet bioprinting include high speed, availability, and relatively low costs. Disadvantages include lack of precision with regards to droplet size and droplet placement compared to other bioprinting methods. There is also a requirement for low viscosity bioink, which eliminates several effective bioinks from being used with this method. Users have also reported frequent nozzle clogging and cellular distortion with the use of inkjet bioprinters (see Table 2).[15]

Microextrusion 3D bioprinting

Extrusion based models utilize mechanical or pneumatic forces to dispense bioink through a nozzle that follows a computer-generated pattern.[27,28] Microextrusion bioprinters produce continuous streams of material in contrast to the discrete droplets of inkjet bioprinters. These extremely small streams are directed by CAD software connected to the bioprinter. Microextrusion is the most common bioprinting method in use today.[15] Unlike inkjet bioprinters, microextrusion bioprinters can successfully print high viscosity bioinks such as complex polymers, cell spheroids, and clay-based substrates.[28,29] Another major advantage of this method is the ability to print very high cell densities for tissue formation.[30] One of the major disadvantages of microextrusion bioprinting is the distortion of cellular structure and loss of cellular viability that results from the pressure used to expel the bioink. One study demonstrated an inverse relationship between extrusion pressure and cellular viability, which was found to be as low as 40% following especially high extrusion pressures.[31]

Laser-assisted 3D bioprinting

Laser-assisted 3D bioprinting (LAB) is a non-contact, nozzle free printing process initially developed for high-resolution patterning of metals, such as the process often used for computer chip fabrication.[32,33] The technology directs laser pulses through a “ribbon” containing bioink. The ribbon is supported by a titanium or gold layer capable of absorbing and subsequently transferring energy to the ribbon. The bioink and cells are suspended on the bottom of the ribbon and when vaporized by the laser pulse, create a high-pressure bubble that eventually propels discrete droplets to the receiving substrate that lies just beyond the ribbon.[33–35] This step is repeatedly performed to functionally create the 3D structures. The main advantage of laser bioprinting is the high degree of precision and resolution possible for the printed structures. This has made possible bioprinting of micropatterned peptides, DNA, and cell arrays.[33] Resolution as high as one cell per droplet

has been achieved.[36] Like extrusion microprinting, laser bioprinting is able to print a very high density of cells. Guillotin and colleagues were able to show that laser bioprinting could successfully utilize a bioink concentration of 10^8 cells/ml to print discrete droplets containing at least one cell.[36] Additionally, the lack of a nozzle creates more options for potential materials that may be used with no concern for viscosity limitations or clogging.[33] LAB has demonstrated high retention of phenotype and cell viability after printing.[37] The major limitation of laser bioprinting is a lower cellular viability compared to other bioprinting methods.[38] Another drawback is the time consuming process of ribbon preparation. Following the ribbon preparation, however, the fabrication process is relatively fast (see Fig. 2).[33]

Stereolithography

The stereolithography (SLA) method of bioprinting utilizes photopolymerization, a process in which a UV light or laser is directed in a pattern over a path of photopolymerizable liquid polymer, cross-linking the polymers into a hardened layer.[28] As each layer is polymerized, the printing platform can be lowered further into the polymer solution allowing for multiple cycles to form a 3D structure.[28] This technique is particularly useful when curable acrylics and epoxies are used as the photopolymerizable material. These substances result in a high degree of fabrication accuracy compared to other techniques.[39] In one example, stereolithography has also been used to create CT-based molds for generating artificial heart valves.[40] The main drawback to using SLA for biomedical purposes is the necessity for intense ultraviolet radiation needed for the cross-linking process. Other limitations are the lengthy post-processing time requirement and the relative few materials compatible for use with SLA.[28]

Bioink

Simply stated, bioink is the material that is printed in layer-by-layer fashion during the process of bioprinting. Typically, bioink is made up of cellular material, additives (growth factors, signaling molecules, etc.), and a supportive scaffold. However, extensive diversity in bioink composition exists across investigational areas. For example, it is possible to omit the supportive scaffold from bioink with certain bioprinting strategies. Microtissue and autonomous self-assembly based approaches, as previously discussed, often allow for a scaffold-free bioink.[8]

The specific properties required of a given bioink highly depend on the printing modality and target tissue type. Inkjet printing requires low viscosities and low thermal conductivity to avoid nozzle clogging and heat damage respectively.[41] Alternatively, extrusion bioprinting can tolerate much higher viscosities but other properties such as shear thinning become more important with increased potential for mechanical damage to cells.[41–43] The concentration for a given bioink is another important consideration. A fine balance must be achieved between providing the required structural properties without negatively affecting cell viability. Beyond an optimal bioink concentration range, further concentration increases can negatively affect cellular viability by preventing cell migration and diffusion.[27]

Bioink scaffolds

Compared to the other main bioink components, cellular materials and additive factors, scaffold choice tends to deviate more from the materials used in traditional tissue engineering strategies. This is largely because scaffold properties are the most crucial in supporting the physical demands of the printing process. Bioink scaffolds must provide cells with secure attachment and protection from the mechanical and thermal stresses of printing. They also must support cellular growth and proliferation without affecting the cell phenotype.[41,44] Adequate biocompatibility is the greatest limiting factor for potential scaffolds as they must be cytocompatible without causing immune response, inflammation response, or premature stem cell differentiation.[39] The diversity of specific bioink scaffolds used by investigators is extensive. Below, we discuss properties of the most common categories of bioink scaffolds (see Table 3).

Hydrogel scaffolds

Hydrogels are arguably the most important bioink. Hydrogels are moldable polymers engineered to mimic the extracellular environment of the body's tissues and can absorb thousands of times their dry weight in water.[45,46] Hydrogels have been produced from a wide range of components including collagen,[47,48] fibrin,[49,50] alginate,[28,51,52] and several other materials.[45] Examples of current hydrogel applications include soft contact lenses[53] and biological adhesives such as polyethylene glycol (PEG) polymers that are often used to prevent postoperative air leaks following lung resection.[54,55] A tissue engineering example is the use of hydrogels to form aortic valve conduits.[51] Hydrogels can be printed alone for subsequent cell seeding or may be bioprinted with cells already suspended in the hydrogel matrix. In one study, an anatomically accurate bionic ear construct was bioprinted by pre-seeding an alginate hydrogel matrix with viable chondrocytes around an inductive coil antenna, which could successfully receive electromagnetic signals over an expansive frequency range.[56]

Synthetic scaffolds

Bioinks can be composed of synthetic materials or naturally derived sources. Common examples of synthetic hydrogels include polyethylene glycol (PEG)-based materials such as PEG diacrylate (PEGDA) and polyacrylamide (PAAm)-based gels. The primary advantage of synthetic bioinks over naturally derived sources is the ability to manipulate their chemical and physical properties as necessary. The molecular weight, functional groups, crosslinking rates, and other mechanical properties of synthetic bioinks can be optimized for a specific bioprinting method or target tissue.[33] The main disadvantage in using synthetic bioinks is a more limited opportunity for cellular interactions. Synthetic bioinks do not typically contain natural cellular attachment sites and do not effectively mimic the environment of a biological ECM.[33]

Natural scaffolds

Natural polymers include materials such as gelatin, collagen, fibrin, alginate, and multiple other polymers that naturally exist in nature.[57] These materials are advantageous because of their higher biocompatibility and increased potential for supporting cell viability and growth

compared to synthetic bioinks.[27] A limitation, however, is that the mechanical properties of natural bioinks do not support remodeling and resilience to the degree of synthetic bioinks. Gelatin and alginate derived bioinks, for example, suffer from poor shape fidelity, poor printing resolution, and form very soft gels at physiologic temperatures.[27] Efforts to improve these limitations have been moderately effective. Strategies such as introducing new functional groups via crosslinking and forming composites of natural and synthetic bioinks have been able to improve print fidelity, resolution, and mechanical integrity while maintaining the advantageous biocompatible properties of natural bioinks.[27,58,59]

Stem cell differentiation in bioprinting

Looking ahead to potential applications of 3D-bioprinting, the promise of tissue replacement is one of the most exciting prospects on the horizon. The ability to optimize stem cell engineering through the bioprinting process will be extremely important in this endeavor. One of the major advantages of bioprinting compared to conventional tissue engineering strategies is the ability to influence stem cell differentiation at multiple stages of the process. The choice of stem cell source, bioprinting method, scaffold selection, additives used, and mechanical forces applied can influence stem cell differentiation towards a specific target tissue. Additional benefits in using stem cells in bioprinting include their ability to induce immunotolerance and expand once incorporated into target tissue.[2]

Stem cell source

Of the three main stem cell sources most often utilized in tissue engineering applications, embryonic stem cells, mesenchymal stem cells, and induced pluripotent stem cells, each has their advantages and disadvantages when used in bioprinting. Embryonic stem cells have the highest degree of multipotency but drawbacks include more difficult procurement, ethical issues, and issues with immunogenicity. [60] Mesenchymal stem cells are more obtainable, have been shown to stimulate immunotolerance in target tissue, but do not possess the same degree of multipotency as embryonic stem cells.[61] Induced pluripotent stem cells have increased multipotency but have been shown to promote tumorigenesis in some studies.[62]

Bioprinting method

As discussed previously, each bioprinting method has its own unique effect on the bioink during the bioprinting process. The stresses inherent to each bioprinting technique can influence stem cell differentiation. Mechanical pressure, as seen to a high degree in inkjet printing, has been shown to influence mesenchymal stem cell differentiation towards cartilage and bone.[63] In another example, the shear forces seen in extrusion printing have been shown to influence differentiation towards endothelial tissue and bone.[64] Alternatively, one could utilize a more force neutral bioprinting method, such as laser assisted printing, in order to preserve multipotency and ultimately utilize other methods to stimulate differentiation as discussed below.

Scaffolds preserving multipotency

To preserve multipotency, a scaffold that allows for minimal cellular interaction is more favorable. The presence of many cellular interactions, with side groups of a given scaffold

for instance, may closely mimic a target tissue such that differentiation occurs.[2] Some bioink scaffolds, such as alginate, successfully preserve multipotency due to their lack of cellular interactions or "bioinertness."[2] The major drawback of utilizing alginate and other bioinert scaffolds is the potential to cause stem cell death due to their extreme variance from a true physiologic ECM. The lack of cellular attachment can prevent normal stem cell mobility and proliferation in the scaffold, triggering apoptosis.[65]

Another way to preserve multipotency through bioink scaffold selection utilizes the process of decellularization. As a strategy to promote bioinertness through reduction of cellular interactions, decellularization is a laboratory process utilizing trypsin and subsequent washing to convert various scaffold sources to a more differentiation neutral state.[66] The decellularized scaffold is then combined with other materials with reduced concern that the scaffold would promote premature stem cell differentiation. The drawback to utilizing this process for bioprinting applications is due to the unnatural use of toxic chemicals that must be removed and the time required for processing.

Some scaffolds, such as hyaluronic acid, do not preserve multipotency through bioinert properties, but instead by mimicking the ECM of stem cells in their native state. Hyaluronic acid does contain limited sites for cellular attachment, thus preserving stem cell function and preventing apoptosis seen with the use of alginate. The key differentiating factor is that these cellular interactions, through receptors such as CD44, mimic the physiologic interactions of native stem cells and multipotency is preserved.[67]

Scaffolds promoting differentiation

In general, the natural ECM in a given tissue contains cellular material, growth factors, and other components to influence differentiation of stem cells towards its own tissue type.[68] In bioprinting applications that require scaffold promotion of differentiation, mimicking the ECM of native target tissue is a commonly employed strategy. This mimicry can extend to structural, chemical, and mechanical features.[69] Among the most important ECM structural characteristics influencing stem cell fate are the type and arrangement of integrin binding proteins.[70] Experimental models have demonstrated that stem cell differentiation can be controlled through the type and arrangement of integrins included within a scaffold.[70]

Similar to the mechanical effect of different bioprinting techniques, the mechanical properties of bioink scaffolds also affect differentiation, typically towards a tissue type compatible with the mechanical properties of a given scaffold.[2] Scaffold density and elasticity are two properties that can influence stem cell fate. In general, soft and elastic scaffolds (0.1–5 kPa) promote differentiation towards neuronal and adipose tissue, while firm and stiff scaffolds (8–30 kPa) promote differentiation towards muscle, cartilage, and bone tissue.[2,71–74] Considering the *in vivo* stresses for these various tissue types, this represents another example of how re-creating the native environment of a given tissue type can promote stem cell differentiation towards that lineage.

Additive factors

Compared to previously discussed methods, influencing stem cells towards a specific lineage by the addition of growth factors, chemicals, and other substances to the bioink is the most

straightforward approach. It most resembles the traditional tissue engineering strategies for manipulating stem cell fate. These substances may be included as a component of bioink before printing or may be added to the printed tissue prior to an additional maturation period.[75] These additives may directly influence stem cell differentiation or more indirectly affect differentiation by altering functions such as such as transport, cell movement, and attachment. Examples of growth factors used in bioprinting include FGF, PDGF, bone morphogenetic proteins, and others that have been well investigated in traditional tissue engineering applications. [76] Other substances that have been utilized to induce stem cell differentiation in bioprinting include dexamethasone, ascorbic acid, and rosiglitazone.[77,78]

Microcarriers, a separate class of additives, have recently shown promise for affecting stem cell differentiation in bioprinting applications. These are small polymer spheres that have been shown to promote differentiation when added to bioink by providing a source of adhesion and attachment.[79] Available in several different sizes, typically under 300 μm diameter, size can be varied for different target tissues.[80] Additional benefits include the ability to stiffen the scaffold by choice of microcarrier as an additional influence on stem cell differentiation.[79,80]

Limitations

Materials and manufacturing

A critical aspect of successfully bioprinting clinically useful tissue will be the selection of optimal biomaterials. Many polymers in conventional 3D printing and traditional tissue engineering have been studied in bioprinting due to availability and previous experience with these materials. However, these materials are not the most biologically appropriate in bioprinting applications.[81] As discussed, many of these are too biologically active, causing unwanted cellular interactions and premature or undesired stem cell differentiation.[82] The focus is now turning to novel biopolymers and hydrogels which more suitably mimic the nanostructural features and responsiveness of ECM and other components of the microenvironment of native tissue. [81,83] However, these novel, more biocompatible hydrogels and biopolymers are not always suitable with conventional bioprinting methods. Many of these do not have the structural integrity needed for optimal bioprinting and can collapse if they are too soft.[81,84] Optimizing the microarchitecture of these biopolymers is an area of active research. One promising approach involves combining substances to maximize the utility of each, the mechanical properties of a firm substance with the proliferative and cytocompatible effects of a softer substance.[2] Atala and colleagues, for example, are utilizing an “integrated tissue-organ printer” to include firm polymers into a soft hydrogel scaffold containing progenitor cells.[2,38,85] They were able to successfully incorporate tricalcium phosphate into a gelatin and hyaluronic acid bioink for bone bioprinting.

In general, the efficiency of the bioprinting process needs to be improved. The current bioprinting process is time-consuming and does not currently have the capability of consistently delivering the number of cells needed for many tissue types.[2] As previously discussed, the mechanical forces imposed by the printing process often result in altered cell geometry, altered signaling pathways, and even cell death.[86,87] Given the tremendous efforts

that go into each bioprinting project, greater efficiency with regards to cell destruction and loss is required. Part of the solution will include improvement in the methods used to monitor and assess the degree of cell destruction.

Vascularity

Perhaps the greatest challenge to translating bioprinting in the lab to functional tissue creation involves the creation of vascular networks. Without appropriate conduits for nutrient delivery and waste disposal, tissues of even minor complexity will not be able to survive. *In vivo*, a vascular network is required for tissues to grow beyond 100–200 μm, as this is the diffusion limit of oxygen.[88] Without a vascular network, engineered tissues will have nutrient limitations resulting in incomplete tissue formation or necrosis.[89]

To adequately perfuse bioprinted tissues, a vascular network must be present at an early enough developmental stage to prevent tissue death and to allow attachment and growth of endothelium. As development ensues, the vascular structures must take on all the roles that occur in normal development including maintaining a selective barrier for waste and nutrients, as well as participating in inflammatory reactions, coagulation, and other homeostatic functions.[90]

Current challenges to bioprinting vasculature are largely due to limitations in printing resolution and speed. Capillaries, for example, may be as small as 3 μm in diameter,[91] while the highest resolution laser-based bioprinters currently utilize a droplet size of 20 μm.[92] Even if printing resolution is improved to the degree that a complex network of capillaries could be printed, the time required with currently available technology would be prohibitive. If the printing could not be completed in a timely fashion, cell viability may be compromised.[1]

Given these challenges, several solutions have been considered. One of the most promising involves attempts to engineer i*n vivo* vascularization by incorporating angiogenic growth factors into bioinks to encourage host vasculature growth following implantation of the bioprinted tissue.[93,94] Despite encouraging results, this strategy needs refinement. Alternatively, attempts have been made to form vascular networks of synthetic origin.[95] While there has been some success in bioprinting larger diameter vessels, small-caliber synthetically engineered vascular grafts of less than 5 mm have demonstrated poor patency rates and are currently not a realistic option.[96] Unfortunately, the fundamental problem of developing mature, functional vasculature in a timely manner to prevent tissue death and support the desired development of tissue has yet to be overcome.

Conclusion

Since its inception, 3D bioprinting has made great progress towards the goal of functional tissue printing. Despite challenges, this early period of investigation has clearly proven bioprinting to be worthy of ongoing investigation. More time, effort, and multidisciplinary expertise will be needed to fulfill the clinical potential for this technology, but the future is bright. Bioprinting is poised to play a key role in personalized regenerative medicine.

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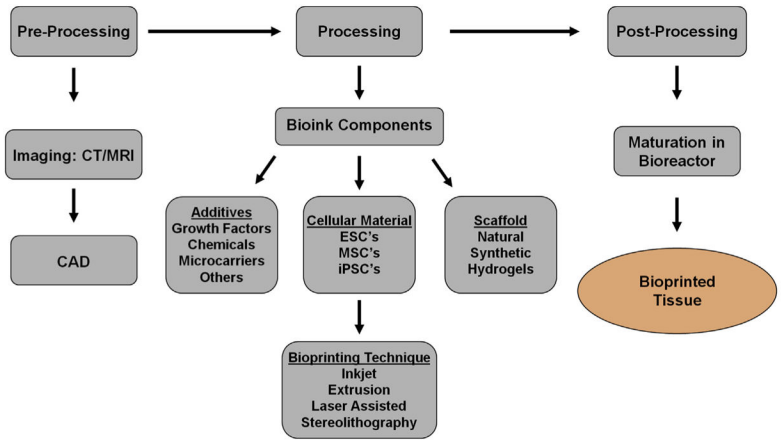

Figure 1.
Bioprinting overview schematic.

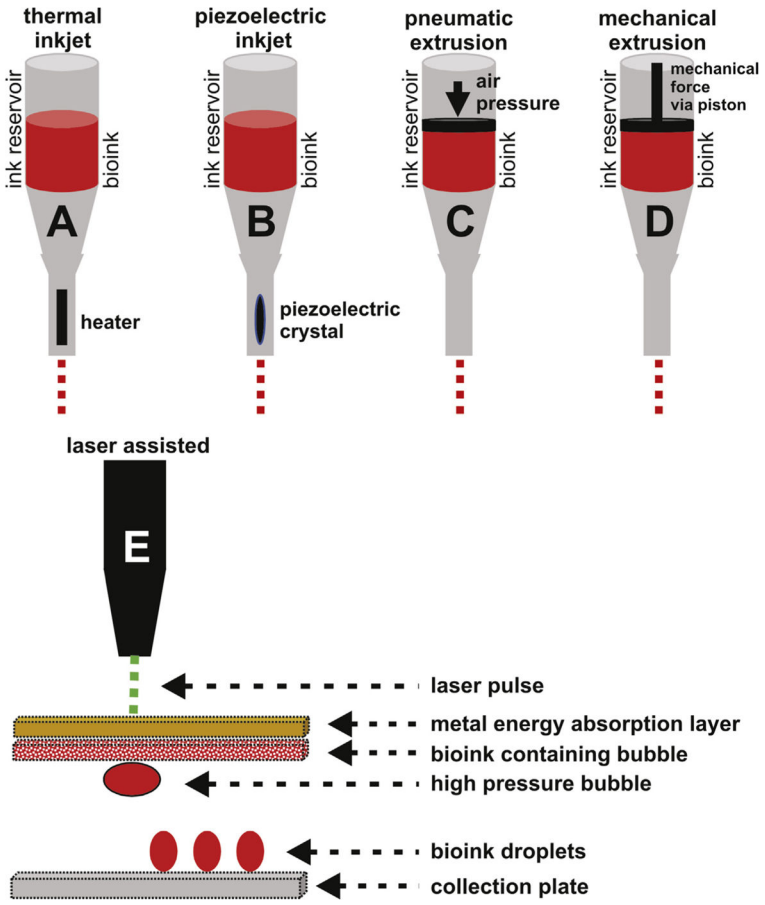

Figure 2.

Schematic depicting the most common bioprinting methods. (**A**) Thermal Inkjet Bioprinting. (**B**) Piezoelectric Inkjet Bioprinting. (**C**) Pneumatic Extrusion Bioprinting. (**D**) Mechanical Extrusion Bioprinting. (**E**) Laser-Assisted Bioprinting.

Table 1

Bioprinting strategies.

Strategy	Biomimetic	Self-assembly	Microtissues
Description	Attempts to duplicate environment and growth cues for a target tissue; Relies heavily on bioreactors	Attempts to replicate embryonic environment allowing for autoregulation and self-production of raw elements	Forms smallest possible structural and functional unites that can later be combined to form mature tissue
Advantages	Control at each step of tissue development High degree of precision in cellular positioning	Fast and efficient Scalable for automaton High cellular density	Fast and efficient Scalable for automation Potential to solve limitations in engineering vascular tissue[8]
Disadvantages	Complex given all factors that must be reproduced Slow and often inefficient	Difficult to change outcome during self-assembly process	Microtissues are difficult to create
Scaffold required	Yes	No	No

Table 2

Bioprinting methods.

Bioprinting method	Inkjet 3D bioprinting	Microextrusion 3D bioprinting	Laser-assisted 3D bioprinting (LAD)	Stereolithography (SLA)
Description	Thermal, piezoelectric, or electromagnetic forces expel successive drops of bioink onto a substrate	Mechanical or pneumatic forces dispense bioink through a nozzle	Bioink and cells are suspended on the bottom of a ribbon and when vaporized by a laser pulse, are propelled to a receiving substrate	Use digital light to cure bioink in a layer by layer fashion
Advantages	High speed, availability, low cost	Ability to use high viscosity bioink and print high cell density	High degree of precision and resolution, ability to use high viscosity bioink and print high cell density	High degree of fabrication accuracy, and low printing time
Disadvantages	Lack of precision in droplet placement and size, need for low viscosity bioink	Distortion of cell structure	Time consuming, high cost	Use of high intensity UV light, lengthy post-processing, lack of compatible materials
Effect on cells	>85% cell viability[1]	As low as 40% viability[1]	>95% cell viability[1]	>90% cell viability[2]
Cost	Low	Medium	High	Medium

Table 3

Bioprinting scaffolds.

Bioink type	Hydrogels	Synthetic	Natural
Description	Composed of hydrophilic polymers crosslinked either through covalent bonds or held together via physical intramolecular and intermolecular attractions[1]	Derived from synthetic and natural sources e.g. polyethylene glycol (PEG)-based materials such as PEG diacrylate (PEGDA) and polyacrylamide (PAAm)-based gels	Made with biological material e.g. collagen, fibrin, hyaluronic acid
Advantages	Hydrophilicity allows for easy exchange of gases and nutrients, highly biocompatible, easily modified	Easily modified e.g. Easily tailored functional groups, non-immunogenicity	Highly biocompatible
Disadvantages	Poor cell seeding, poor mechanical properties[1]	No cellular attachment sites[2]	Limited modification, shear thinning[2]
Viscosity	Adjustable[3]	PEG: low Pluronic-acid: high[2]	Gelatin and Fibrinogen: low Hyaluronic Acid: high (up to 1000 Pa s) Silk: high[2]