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3D bioprinting for vascularized tissue fabrication

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Abstract

3D bioprinting holds remarkable promise for rapid fabrication of 3D tissue engineering constructs. Given its scalability, reproducibility, and precise multi-dimensional control that traditional fabrication methods do not provide, 3D bioprinting provides a powerful means to address one of the major challenges in tissue engineering: vascularization. Moderate success of current tissue engineering strategies have been attributed to the current inability to fabricate thick tissue engineering constructs that contain endogenous, engineered vasculature or nutrient channels that can integrate with the host tissue. Successful fabrication of a vascularized tissue construct requires synergy between high throughput, high-resolution bioprinting of larger perfusable channels and instructive bioink that promotes angiogenic sprouting and neovascularization. This review aims to cover the recent progress in the field of 3D bioprinting of vascularized tissues. It will cover the methods of bioprinting vascularized constructs, bioink for vascularization, and perspectives on recent innovations in 3D printing and biomaterials for the next generation of 3D bioprinting for vascularized tissue fabrication.

Keywords

Bioprinting; bioink; 3D printing; tissue engineering; vascularization

INTRODUCTION

The advancement of 3D bioprinting technology has provided an enhanced feasibility and precision to tissue engineered construct fabrication.[53, 69, 73, 76, 128] Compared with the traditional tissue fabrication methods, 3D bioprinting offers a reproducible, scalable fabrication methodology with precise 3D control. It allows for the fabrication of mechanically supportive 3D structures with bioactive/cellular components for a variety of biomedical applications, including regenerative medicine, in vitro disease models, and the exploration of fundamental cell and tissue-level mechanisms.[11, 20, 23, 26, 45, 70, 96, 126] In particular, computer-aided 3D bioprinting enables the direct translation of medical images into tissue construct design for patient-specific organ repair.[1, 35, 117]

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One of the fundamental design criterion and major challenges for 3D tissue fabrication is the ability to maintain high cell viability and normal function throughout the construct, which is largely determined by the access to nutrients and oxygen.[68, 84, 99] The current inability to fabricate thick tissues has been attributed to insufficient integration of the implanted tissue construct to the host vasculature and/or the lack of endogenous, engineered vasculature or nutrient channels in the engineered tissues.[49] Without vascular integration with host tissue or an engineered vasculature, the size of a tissue-engineered construct is limited to the diffusional limit of oxygen required for cellular metabolism (100-200 μm).[94] Engineering approaches to extend beyond the diffusional limit of nutrients/oxygen have explored a variety of strategies, including improving tissue/scaffold perfusion and culture (e.g., scaffold porosity, bioreactor)[7, 18, 33, 34, 41, 67, 75, 88, 90, 118], incorporating oxygen delivery mechanisms[59, 85, 95, 97, 109], and constructing biomimetic vessel structures, with or without cells.[5, 24, 95, 107] In addition, advances in the biomaterials (e.g., hydrogel) and vascular cell biology have been leveraged to recreate the natural vasculogenic (i.e., de novo vessel formation) and angiogenic (i.e., new vessel forms from pre-existing vessel) environment to form organized vessel sizes from micron to millimeter dimensions.[21, 25, 36, 38, 43, 110, 114] The overarching goal of the vascularization of tissue-engineered constructs (in vitro or after transplantation) is to provide a cell-based, long-term (i.e., stable) solution for supply of oxygen and nutrients to the tissue. To address this goal, a multi-disciplinary understanding and a multidimensional approach are required to advance the field of 3D vascularized tissue fabrication.[2, 65, 84, 93, 100]

Naturally, vascularized tissues in the body includes a range of branching vessels from millimeter-sized, small diameter vessels to micron-sized capillary networks supporting tissue-specific functions. As a combination of technological and biological advances, 3D bioprinting provides a powerful means to replicate this architecture in vitro. Bioprinting of larger nutrient channels creates a perfusable vessel that can be surgically connected to host blood vessel and/or in vitro seeded with vascular cell types for angiogenic sprouting. In addition, the use of bioactive bioink (i.e., materials used for bioprinting) in the bioprinting process can provide instructive signals to direct cell behavior to promote the formation of microvasculature throughout tissue constructs. Bioprinting of biomimetic vascularized tissue constructs may also involve design and fabrication of supporting structures for the complex vascular networks that consistent of branching vessels in 3D space. To adequately control the fabrication process, the bioprinter and printing method must have sufficient resolution (i.e., within or below nutrient diffusional limit) for the desired structure and maintain cell viability throughout the printing process when using a cell-loaded bioink.[69, 76] Bioinks must also maintain printing resolution (e.g., not deform after printing), cell viability, and support neovascularization.[40, 69, 101] To achieve these multi-faceted design considerations, research in 3D biofabrication methods has led to advances in the bioprinting technologies and bioink design. This review aims to cover the recent progress in the field and will cover the methods of bioprinting, the bioink, and perspectives on recent innovations in vascularized 3D tissue fabrication.

BIOPRINTING FOR VASCULARIZED TISSUE FABRICATION

The bioprinter and 3D printing method play an integral role of constructing vascularized tissue constructs. Bioink design and tunability also plays an important part in printability and will be discussed in the next section.[40, 42, 101] Bioprinting primarily utilizes two additive manufacturing concepts, independently or combined, for construct fabrication: indirect and direct printing (Figure 1). Indirect printing involves printing a "negative" sacrificial structure that provides a molded scaffold that can be later cellularized. Direct printing methods involve actively printing the structure with cell-loaded or cell-compatible bioink. This method requires quick gelation/crosslinking to maintain a stable structure. A combination of methods can also be used to fabricate a vascularized tissue construct that covers a variety of desired vasculature. For example, direct printing can be used to control the placement of cells for micron-range capillary network formation, while indirect printing can provide a molded channel for cell infiltration of millimeter-range diameter vessels. Bioprinting methods to fabricate 3D cell-laden constructs require sufficient resolution to ensure oxygen and nutrients supply to the center of the largest printed features. In addition, structural design of the tissue construct allows for the incorporation of inter-structure spaces (e.g., lattice form) to improve the diffusion of oxygen and nutrients. There are a variety of bioprinting methods in tissue engineering that can be applied to vascularized tissue engineering, which have been discussed previously.[76] Here we review recent innovations in bioprinting approaches for vascularized tissue fabrication, outlined in Table 1.

EXTRUSION BIOPRINTERS

One of the most common bioprinting approaches is the use of extrusion-based bioprinters that deposit bioink layer-by-layer through a syringe-like printer head.[10, 69, 76] This requires a structurally stable bioink or in situ crosslinking mechanism for 3D fabrication. Given its biomedical adaptability as a biomaterial, alginate has been extensively used in extrusion bioprinting.[51, 77] Extrusion bioprinting of alginate involves either submersion printing in an aqueous calcium solution or printing on calcium-containing substrates. Khalil and others established key parameters to print rat heart endothelial cells (RHECs) with alginate extrusion printing in a calcium solution.[44] Extrusion-based 3D printing of alginate was further established on a gelatin calcium substrate for indirect printing of a vascular mold and for direct printing of a cell-loaded lattice.[40, 110]

Recently, Gao and others developed a coaxial extrusion nozzle to direct print microchannels.[24] This type of printing nozzle allows for interior flow of calcium solution with exterior flow of alginate solution (i.e., bioink), creating constructs with endogenous, perfusable microchannels (Figure 2). These hollow microchannels are printed onto a stage that progressively lowers into a calcium bath solution for secondary crosslinking. The authors demonstrated the versatile 2D and 3D printing, resolution (~900 μm microchannel diameter, <200 μm wall thickness), and improved cell viability with added microchannels. As an alternative to liquid submersion printing, Hinton and others developed an extrusion method that can use a variety of hydrogels for direct structure printing supported in a sacrificial, gelatin-microparticle bath to facilitate crosslinking.[32] Though not implemented

with cell-loaded bioinks, the authors provided proof-of-concept applications of 3D imaging data for vessel (~200 μm line width), bone, whole heart, and brain model fabrication.

Although alginate provides a straight-forward, biocompatible method of extrusion bioprinting, the complexity of the desired tissue construct is limited to self-supported structures. When constructing a 3D branching vascular network, the vessel structure must be supported during printing and in vitro culture until cells can establish structural integrity. To print a 3D perfusable vascular tree, Wu and others used an indirect approach to extrude sacrificial Pluronic F127 filaments within a Pluronic F127-diacrylate gel reservoir to provide support during printing (Figure 3).[124] Pluronic F127 is a synthetic copolymer with hydrophobic poly(propylene oxide) (PPO) segments and two hydrophilic poly(ethylene oxide) (PEO) segments arranged in a PEO-PPO-PEO triblock configuration.[108] Wu and others exploited the high shear thinning behavior of Pluronic F127 solutions by using controlled applied pressures to extrude through a smaller diameter nozzle for varying microchannel sizes (200-600 μm diameter). After photocuring of surrounding acrylate-modified Pluronic F127-diacrylate, the unmodified Pluronic F127 channels can be liquefied when the temperature is reduced to below its critical micelle temperature, leaving behind perfusable channels. Using a similar indirect method of vessel formation, Lee and other deposited layers of a collagen supportive matrix around gelatin containing human umbilical vein endothelial cells (HUVECs).[54] Post-printing, the gelatin was melted, which served to "activate" the cell seeding of HUVECs onto the surrounding collagen. Lee and others further progressed this model and implemented a pro-angiogenic design.[55, 80] HUVEC-laden fibrin was printed between sacrificial gelatin channels to encourage formation of functional capillary network and validated the improved diffusional permeability.

Kolesky and others implemented a similar strategy to co-print channel structures of Pluronic F127 and cell-loaded gelatin-methacrylate (GelMA) in a pre-designated sequential process. The printed constructs were encapsulated in a cell-free GelMA bioink for photopolymerization to cross-link the matrix.[47] The Pluronic F127 channels were then liquefied at low temperatures and channels were seeded with HUVECs to create a heterogeneous vascularized tissue construct. Recently, they further progressed this strategy by 3D printing a centimeter-sized construct with perfusable vasculature without photopolymerization.[46] A cell-loaded gelatin-fibrinogen "cell ink" was used to print cellular structures with human neonatal dermal fibroblast and mesenchymal stem cells. Pluronic F127 mixed with thrombin was designated as a "vascular ink" for indirect printing of sacrificial channels, allowing for quick gelation of surrounding fibrinogen bioink before evacuation of Pluronic F127. The printed structures were then surrounded by a cell-free gelatin-fibrinogen bioink containing thrombin and transglutaminase to crosslink the remaining gelatin and fibrinogen. After evacuation of the Pluronic F127, channels were seeded with HUVECs for a perfusable, vascularized construct that facilitated osteogenic differentiation of mesenchymal stem cells over 6 weeks. This work demonstrates a foundation for the use of 3D bioprinting for fabrication of vascularized thick tissue constructs.

Whereas many bioink formulations are in liquid phase, rod extrusion printing involves a direct depositing of semi-solid macrofilaments around non-adhesive sacrificial supporting

structures (i.e., agarose).[105, 106] The resulting structure dimensions are then dependent on the diameter of the extrusion head (250-1000 μm vessel inner diameter) and cellular self-assembly post-printing when agarose rods are physically removed. Additionally, this concept has been executed using 100% scaffold-free vascular cell rods (i.e., smooth muscle cell and fibroblast) or cell-loaded surrounding material with physical removal of the bioprinted agarose rods.[4, 5]

INKJET BIOPRINTERS

The innovative application of a standard inkjet printer to print cell solutions has quickly evolved to become a viable method for direct bioprinting (Figure 4).[13] The general mechanism of printing has been well described.[13] Generally, inkjet methods rely on generation of small air bubbles (e.g., heat-induced) that burst to provide a pulse of pressure to eject bioink droplets (with cells) onto the surface. Although the first generation of inkjet bioprinters demonstrated viable bioprinting, they were limited to 2D applications with low resolution (~400 μm line width) using a saline/cell suspension.[14, 127] To extend its application to 3D bioprinting, Nakamura and others developed an alginate-based bioink with immediate chelation when inkjet printed into a liquid $CaCl_2$ solution.[78] The chelated alginate creates microgel droplets as building blocks (~25-40 μm diameter). The resulting higher resolution allowed for fabrication of a cell-compatible alginate vessel with a 200 μm diameter and demonstrated feasible printing of computer-designed images.[1, 78, 82, 125] On the other hand, Pataky and others combined alginate bioink with a sacrificial calcium-containing substrate (e.g., gelatin) to 3D bioprint an acellular ~200 μm diameter bifurcated vessel.[89] In addition to alginate-based bioink, a thrombin/calcium based bioink has been printed onto a fibrinogen surface to replicate the natural polymerization process in wound healing.[12, 50] This method allows for a dry inkjet printing method with in situ gelation of bioink. The inkjet-printed fibrin fibers demonstrated relevant burst pressure strength, allowed for 2D cellular microvascular patterning (<30 μm line width), and stable channel structure formation (~350 μm diameter) over 21 days.[12]

OTHER PRINTING METHODS FOR VASCULARIZATION

Though less common than inkjet and extrusion bioprinters, alternative methods for bioprinting have recently been explored to provide novel methods for vessel construction. Laser-assisted bioprinters offers a precise, resolute fabrication method to deliver a controlled amount of cells. The technology consists of a pulsed laser beam that is focused onto a "ribbon," made of an energy absorbing metal (e.g., gold or titanium) layer and a bioink layer, to produce a high-pressure bubble that propels the bioink to the surface.[27] For vascularization efforts, laser bioprinting has shown the feasibility of printing endothelial cells in a 2D configuration in branching vascular structures and validated sequential printing of HUVECs and smooth muscle cell to improve stability of the vascular structure.[123] Overall, laser bioprinters have been limited to 2D applications but may be useful in combinatorial methods for 3D bioprinting by offering high cell density printing and layer-by-layer microscale organizational control.[3, 28, 29]

Using the indirect vessel formation strategy, rapid casting of carbohydrate glass fibers into sacrificial 3D patterned networks has been demonstrated to be perfusable and adaptable for vascularization in tissue engineering.[72, 107] Miller and others extruded a dextran-incorporated sucrose-glucose solution to form a 3D carbohydrate glass lattice skeleton. This lattice skeleton was then embedded in cell-loaded matrices (e.g., fibrin, Matrigel, alginate). Following matrix-embedding, the construct was put into media to dissolve the lattice skeleton to create a connected network of channels from 150-750 μm vessel diameters for subsequent endothelial cell seeding (Figure 5).[72] They further demonstrated that a construct of hepatocytes with channels had sustained metabolism than one without channels, as indicated by increased albumin and urea production per day. The benefits of each of the discussed techniques provide a foundation for the development of a combinatorial approach that may best address vascularization in tissue engineering using 3D bioprinting.

BIOINK FOR VASCULATURIZED TISSUE FABRICATION

The discussed bioprinting methods provide a variety of reproducible approaches for vascularized tissue engineering. Each method depends on the bioprinter method (e.g., deposition mechanism, resolution) and chosen bioink properties (e.g., printability, bioactivity) for successful vascularized tissue fabrication. Current technology offers a limited cell printing resolution (~150-200 μm) that makes it unfeasible to print capillary-like structures in the 10-20 μm scale.[87] Extending beyond the bioprinter-directed construction, bioinks can facilitate endothelial cell organization during and after printing.[47, 54, 65, 80] This can be achieved by depositing cell-adhesive channel structures for subsequent cellular integration or by co-delivery of cells and supporting material around perfusable channels for nutrient-directed migration.[5, 54, 55, 123] In conjunction with the development of bioprinting technologies, bioinks have advanced to support multi-scaled vessel fabrication past the bioprinting resolution. For the purposes of this review, bioink refers to the use of three printable ink formulations: cell-free bioink, cell-loaded bioink, and cell-only bioink.

CELL-FREE BIOINK

As a means to support cellular infiltration, cell-free bioink offers a cell-adhesive, structurally supportive bioink for vessel fabrication. Particularly to create 3D branching of complex vascular designs, cell-free bioink can be used with extrusion bioprinters for indirect vessel printing followed by cellular integration via bioprinted cell-based bioink or cell suspension perfusion.[47, 56] For cell-free bioink, the materials' physical properties play an important role to support the construct structure during printing or subsequent cellular interaction. Strategies to ensure sufficient structure support and resolution during and after printing include use of photocurable (e.g., acrylate-modified hydrogels) and/or natural polymerizing materials (e.g., collagen, fibrin) for in situ crosslinking.[47, 56, 57, 77, 101, 104, 106, 130] Notably, a cell-free bioink with tunable (adjustable) physical properties and high biocompatibility is most desirable.[40, 42, 101, 124] Kolesky and others demonstrated the use of a cell-free, photocurable collagen bioink, methacrylated gelatin (GelMA), as a means to encapsulate printed sacrificial Pluronic F127 structures.[47] Photopolymerization and cooling after-printing served to crosslink the GelMA and liquefy the Pluronic F127, leaving a perfusable channel for HUVEC endothelialization. Photocurable GelMA allows for control (e.g.,

polymer concentration, UV exposure time) over hydrogel physical properties.[113] This makes it a versatile bioink for tissues with specific mechanical requirements, as demonstrated with cartilage.[103]

The use of ECM-derived materials in cell-free bioink aims to provide not only the physical matrix but also the endogenous, cell-supportive cues seen in the environment of natural vascularization.[86, 102, 120, 121] An ECM-derived cell-free bioink can facilitate host cell integration once transplanted into the body.[49, 87] One of the widely used cell-free bioinks is collagen-derived products.[57, 81, 130, 131] Lee and others bioprinted collagen around a HUVEC-containing gelatin channel (Figure 6A).[54] The HUVECs sank and attached to the surrounding collagen channel after the gelatin was liquefied (Figure 6B). The collagen channel supported endothelialization for increased vessel wall integrity (i.e., lower diffusional permeability) and validated the role of collagen to support angiogenesis (Figure 6C).[80] Fibrin is used as a bioink for cell-loaded applications to support vascularization, but its bioactivity to support endothelial cell growth and proliferation may suggest its use for cell-free bioink formulations.[31, 39, 98] Because cell-free bioink depends on separate delivery of materials and cells, the use of this cell-free bioink may provide fabrication of "off-the-shelf" channeled scaffolds for later in vitro cell seeding or host tissue cellular integration.

CELL-LOADED BIOINK

To facilitate direct printing of vascularized constructs, cell-loaded bioink serves as a means to co-deliver vascular cell types and supporting scaffold material (e.g., hydrogels). Similar to cell-free bioink, biocompatible candidates that possess tunable physical properties and endogenous cellular cues for high printability are most applicable, such as alginate[44, 129] and GelMA.[4, 5, 47] Jia and others addressed this tunability for cell-loaded alginate bioinks and established criterion for high printability with extrusion bioprinters.[40] Notably, they determined that homogeneous cell suspension for consistent cell distribution and high cell viability during printing become additional design requirements for cell-loaded bioink in contrast with cell-free bioink. For cell-loaded bioink applications, Khalil and others helped to establish the bioprintable range of alginate bioink in a $CaCl_2$ solution.[44] With the proper extrusion printing parameters and alginate/calcium concentrations, they were able to print RHECs in a lattice pattern (~200 μm line width) and maintain viability for two weeks. 3D bioprinting capabilities of inkjet printers have also been demonstrated using cell-loaded alginate in calcium solution to produce image-designed 3D structures.[1, 82]

To address the natural bioinert properties of alginate, Jia and others created a library of cell-adhesive alginate bioinks (i.e., RGDSP-modified) with a range of degradability.[40, 51] The authors then demonstrated its use as a cell-loaded bioink with a custom extrusion printer to promote cellular network formation.[19, 40] Kolesky and others applied their cell-adhesive GelMA bioink to dispense vascular supporting cells (i.e., fibroblasts) around printed channels (Figure 7).[47] This application validated the use of a photocurable bioink for cell-free and cell-loaded bioink with high viability. Cell-loaded bioinks in combination with indirect channel formation have also been shown to induce capillary network formation. Lee and others bioprinted HUVEC-loaded fibrin between endothelialized perfusable channels and observed capillary network formation between channels and angiogenic extensions from

the channels after two weeks (Figure 8).[55] Co-delivery of a bioactive matrix and vascular cells in a cell-loaded bioink provides both a supportive environment for angiogenic sprouting and necessary cues for endogenous vessel formation to functionalize the bioprinted structures.

CELL-ONLY BIOINK

While cell-loaded bioinks offer simultaneous delivery of cells and supporting scaffold, increasing attention has been given towards scaffold-free approaches in 3D bioprinting.[38, 70, 73, 74, 114] Cell-only bioink offers biomimetic vessel formation through cell-directed self-assembly mechanisms and production of endogenous ECM.[36, 38] Also, in contrast with the physical requirements (e.g., high viscosity) for cell-free and cell-loaded bioinks, cell-only bioinks based on an aqueous cell suspension solution with low viscosity can be used with a variety of bioprinter systems, including inkjet,[12, 127] extrusion,[83, 110] and laser-assisted bioprinters.[28, 29, 123] Importantly, cell-only bioink requires sufficient biological signals (e.g., cell-adhesive matrix, supporting cell types) after printing to stabilize cell-cell interactions. Wu and Ringeisen demonstrated this fundamental concept after laser-assisted bioprinting HUVECs onto a glass surface that formed a tubule-like network one day post-printing.[123] The structure began to lose organization after five days, which was prevented with layered printing of smooth muscle cells. Providing sufficient cell support post-printing, whether additional cells or matrix, is a critical design criterion when using cell-only bioink. [12, 29, 50]

An alternative approach to cell suspension bioinks is the use of cell aggregates as building blocks.[37, 70] In this way, endogenous ECM production within the cellular aggregate can stabilize cell function during printing and serve as a foundation for tissue assembly. In addition, bioprinting multi-cellular aggregates can deliver tissue-specific cell types in an organized structure for subsequent tissue assembly (Figure 9A). The use of spheroids (i.e., spherical aggregates) as building blocks in 3D extrusion bioprinting has been shown to be effective for 3D vessel fabrication in the conjunction with indirect printing methods (Figure 9B).[74, 83, 110] Norotte and others extruded cellular aggregates (i.e., spheroids or rods) onto sacrificial agarose support rods to fabricate a perfusable vessel with high cell density and multi-cell composition.[83]

The use of single cells or cellular aggregates requires an understanding of cell-specific levels of cohesion to orchestrate self-assembly after printing and a bioprinter capable of dispensing bioinks with high cell density.[29, 38] After printing, single cells follow a differential cell-adhesion model of tissue self-assembly.[22] Conversely, the use of cellular aggregates for modular assembly requires disassembly and reassembly of the preformed extracellular matrix within the microtissue.[15] The mechanisms of tissue assembly must be considered when using cell-only bioink for complex vascularized tissue fabrication. Due to these challenges, cell-only bioink strategies may be best used in combination with other material-based bioinks that provide physical support and direct cell behavior. Nevertheless, cell-only bioinks offer accurate cell patterning strategies and encourages natural processes through increased cell-cell interactions and cellular matrix production for long term support.

PERSPECTIVE AND CONCLUSIONS

The natural process of vascularization to supply nutrients to tissue has inspired the incorporation of vasculature-like system in tissue-engineered constructs. To achieve the complex architecture of vascularized tissues, 3D bioprinting has provided a scalable approach for the assembly of cells and biomaterials into 3D vascularized constructs. Bioprinting methods can fabricate >150 μm diameter blood vessels/nutrient channels. Microvasculature in the constructs can be introduced by using bioactive bioink that is pro-vasculogenic to support self-assembly into capillary-like networks. The ideal bioprinting approach maximizes the bioprinter capabilities (e.g., resolution, printing speed) and bioink design (e.g., cell-directing bioactivity). Recent innovations on each side can be combined to further advance the field of 3D bioprinting for vascularized tissue fabrication with improved functionality.[92]

For accelerated or large tissue fabrication, the future of bioprinter technology must address the balance between resolution and time for fabrication. Classical methods in 3D bioprinting involve layer-by-layer freeform fabrication. To address the time-limiting layered method for 3D printing, Tumbleston and others demonstrated a continuous (i.e., layer-less) fabrication process with high resolution (50 μm stem diameter) using continuous liquid interface production with a controllable oxygen-dependent polymerizing zone.[112] Accelerating the printing process would allow for higher throughput and decreased time for patient-specific 3D tissue constructs. For tissues that require a range of physical properties, a combinatorial printing approach can be used to maximize benefits of different printing methods and materials.[122] Visser and others reinforced GelMA constructs using melt electrospinning writing to 3D print a high porosity, microfiber poly(ε-caprolactone) scaffold.[116] This method showed a range of mechanical properties that can complement the cell compatible GelMA bioink for musculoskeletal applications. Electrospinning has been mainly applied to the demand for vascular grafts in contrast to vascularized tissue constructs.[6, 30] Although this review mainly focuses on the progress made in vascularized tissue engineering, 3D bioprinting may also be adapted for singular vascular graft fabrication. Future innovations of combined methods for 3D tissue fabrication may take advantage of the fiber orientation control of electrospinning to make a matrix like structure for vascular cell seeding.[8, 52]

To efficiently direct multi-scaled vascularized tissue construction past the current limitation of bioprinter resolution, materials strategies have demonstrated the ability to direct cellular behavior beyond cell-adhesion.[111] In comparison to the single polymer approach, a biomimetic combination of ECM proteins using solubilized, decellularized ECM as a bioink offers a tissue-specific combination of cellular cues for improved cellular function and organization.[91, 115] In order to further tune the cell-directing capabilities, functional segments of larger proteins (i.e., peptides) and growth factors (GFs) have been increasingly studied and can be applied to 3D bioprinting strategies. Particularly, RGD peptides have been incorporated in bioink design to improve cell attachment and proliferation.[16] The role of other ECM- and GF-derived peptides for directing cell behavior for vascularization are still being defined, including the use of a peptide that mimics vascular endothelial growth factor to direct angiogenesis.[9, 58, 60, 119] Also, peptide-driven self-assembly/gelation has been proposed as an improved bioink for organotypic culture and shown to be effective for

in vivo angiogenesis.[17, 61-64, 79] Based on current trends, peptide modified polymers can be envisioned as a future direction for programming cell-specific bioink in 3D constructs to direct cell behavior for vascular patterning.

In summary, the ideal 3D bioprinting solution for vascularized tissue fabrication would require a high throughput, high resolution bioprinter capable of dispensing pro-vasculogenic bioinks to fabricate functional vasculature, ranging from capillaries to larger vessels, within a tissue construct. Figure 10 was constructed to visualize our proposed 3D bioprinting strategy that combines extrusion printing for cell-specific spatial patterning with cell-directing biomaterials for cell-loaded bioinks. This strategy incorporates both larger fabricated channels and self-assembled microvasculature, each facilitated by bioactive bioinks. The 3D printing strategies discussed here have contributed to the use of bioprinting for vascularized tissue fabrications and the recent innovations in 3D printing and biomaterial design can be envisioned to better address the anatomical and functional levels for next-generation organ biofabrication. Notably, as tissue fabrication advances, parallel progress in maintenance and culture of tissues must be addressed for accurate in vitro modeling or pre-implantation conditioning, with special attention to bioreactor design, mass transport of nutrients, and industrial cell expansion.[48, 66, 67, 71, 90] The advancement of 3D bioprinting in combination with novel technologies would lead to tissue engineering solutions for repairing or replacing diseased tissue and organs.

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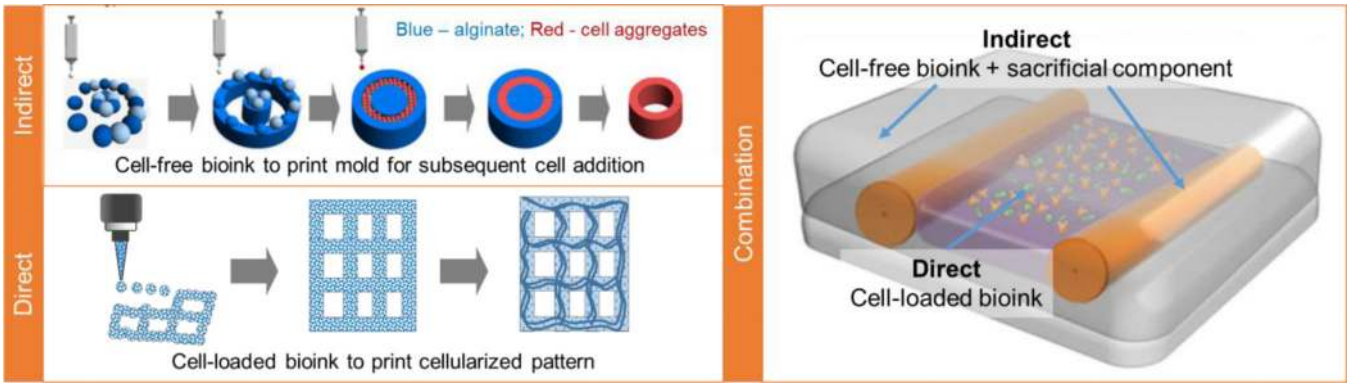

Figure 1.

Printing strategies applied to vascularized tissue fabrication. Indirect printing involves printing of a mold or sacrificial component for subsequent cell seeding. Direct printing is performed with cell-loaded or cell-only bioink for desired bioprinted patterning. A combination of indirect and direct bioprinting can be used for vascularized tissue constructs, such as to fabricate larger channels for cell seeding and connecting cellularized patterns for capillary network self-assembly. Adapted with permission from Tan et al 2014 [109] and Lee et al 2014 [54].

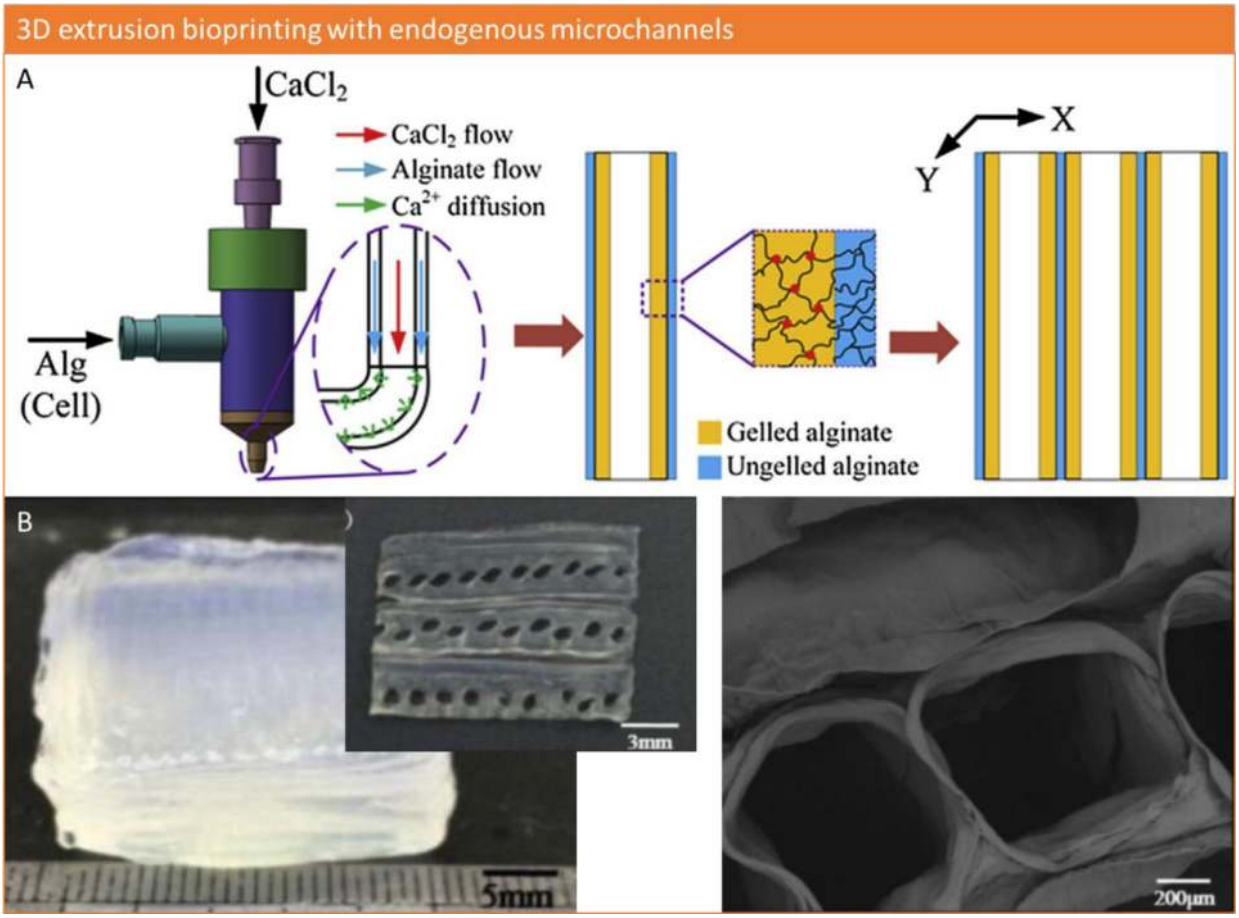

Figure 2.

3D extrusion bioprinting for microchannel filaments. (A) Coaxial extrusion bioprinting allows for direct printing of a scaffold with endogenous microchannels. (B) A multilayered construct with a cross-section inlay shows embedded microchannels with relevant dimensions. Adapted with permission from Gao et al. 2015 [24].

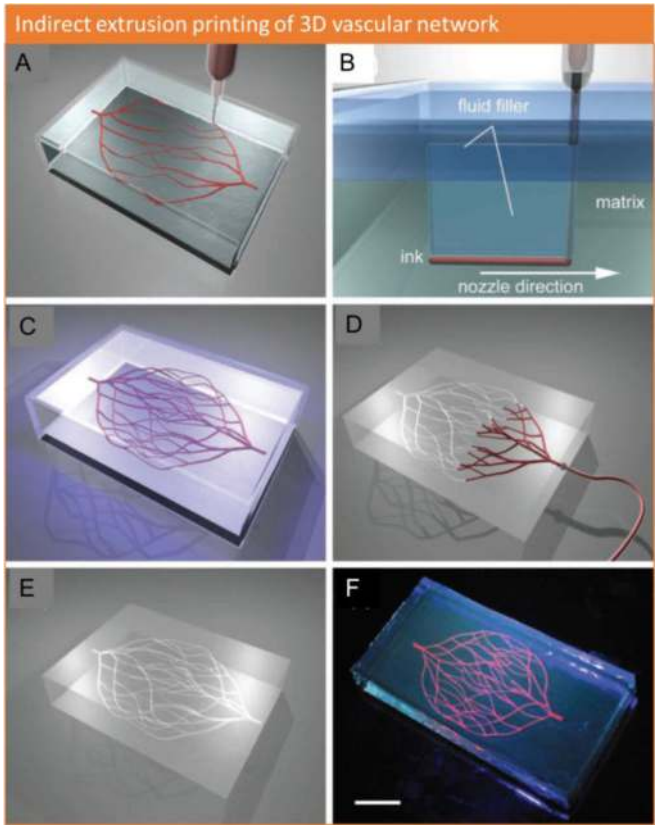

Figure 3.

Indirect printing of perfusable vascular network. (A and B) Pluronic F127 is printed into a supporting gel bath (matrix and fluid filler) of Pluronic F127-diacrylate. (C) Photopolymerization covalently crosslinks support gel. (D and E) Unmodified Pluronic F127 is liquefied at low temperatures and vacuumed out to leave a perfusable vascular network (F). Adapted with permission from Wu et al. 2011 [123].

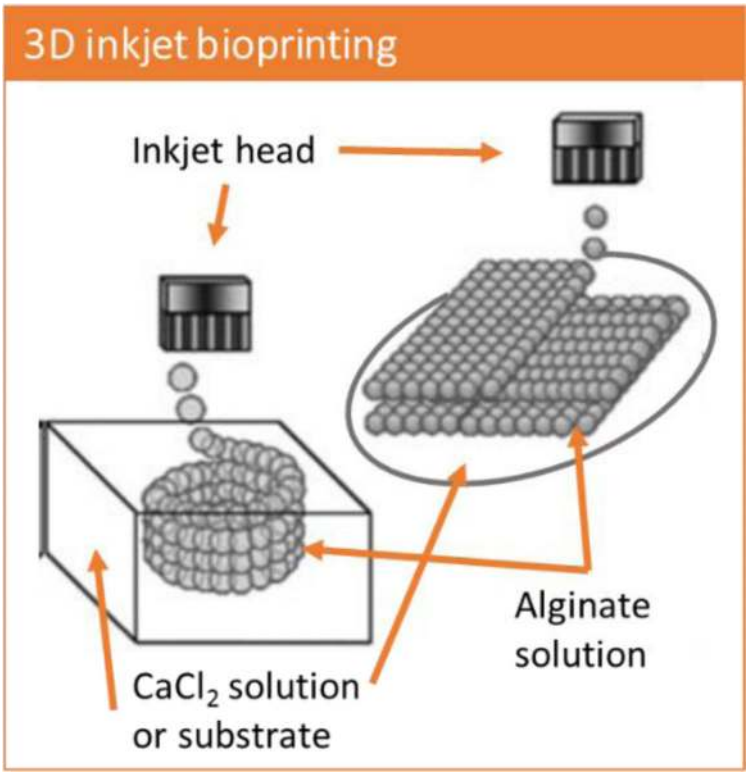

Figure 4.
Inkjet strategies for 3D bioprinting. Alginate can be printed in a calcium-containing solution or substrate for layer-by-layer fabrication. Adapted with permission from Nakamura et al. 2008 [77].

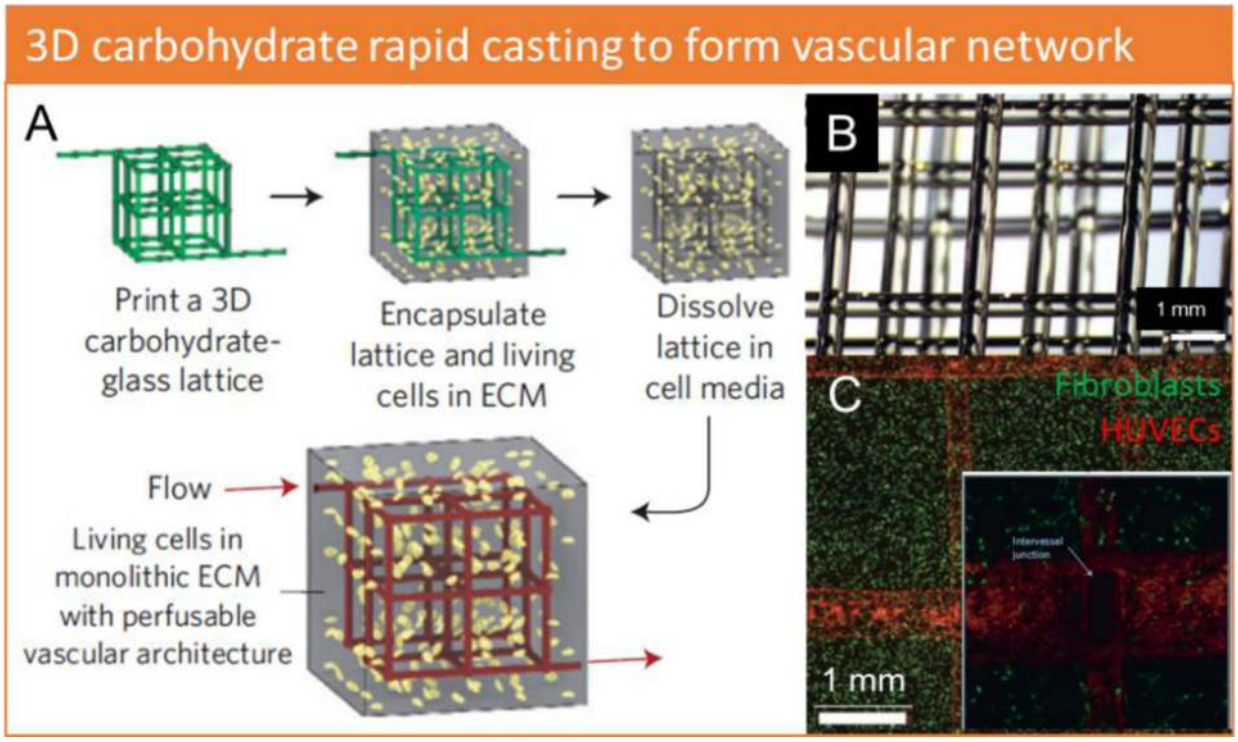

Figure 5.

Alternative 3D printing method for tissue construct vascularization. (A) Direct printed carbohydrate glass lattices (green) are embedded in cell-loaded bioink and dissolve after perfusion, leaving behind perfusable channels (red). (B) Precise, resolute channels can be fabricated with (C) open interchannel junctions (inlay) and endothelialized with HUVECs. Adapted with permission from Miller et al. 2012 [71].

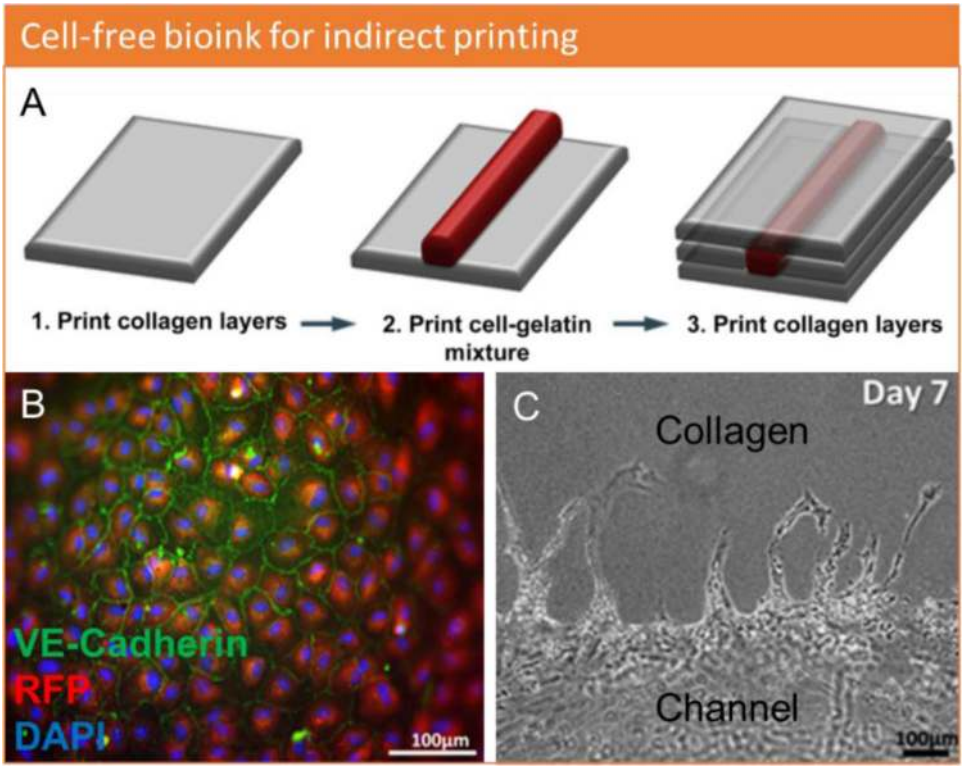

Figure 6.

Use of cell-free bioink to promote cellular integration. (A) Indirect printing was used with cell-free bioink (i.e., collagen) around a sacrificial gelatin channel. (B) The collagen channel provided a biocompatible surface for endothelialization and (C) supported angiogenic sprouting. Adapted with permission from Lee et al. 2014 [53].

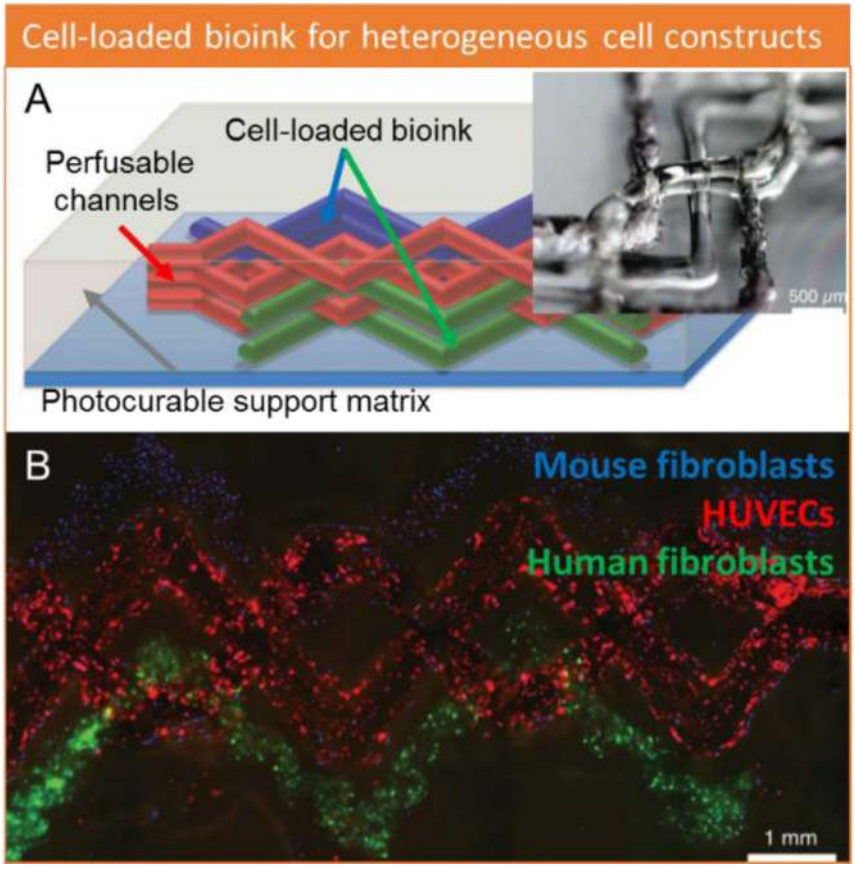

Figure 7.

Cell loaded bioink for use in vascularized tissue constructs. (A) Combinatorial approach combines perfusable channels with direct printing of vascular support cells (i.e., fibroblasts) supported by a photocurable matrix (i.e., gelatin methacrylate). (B) Fibroblasts are bioprinted to support HUVEC channels and provides proof-of-concept for tissue-specific cell type organization. Adapted with permission from Kolesky et al. [46].

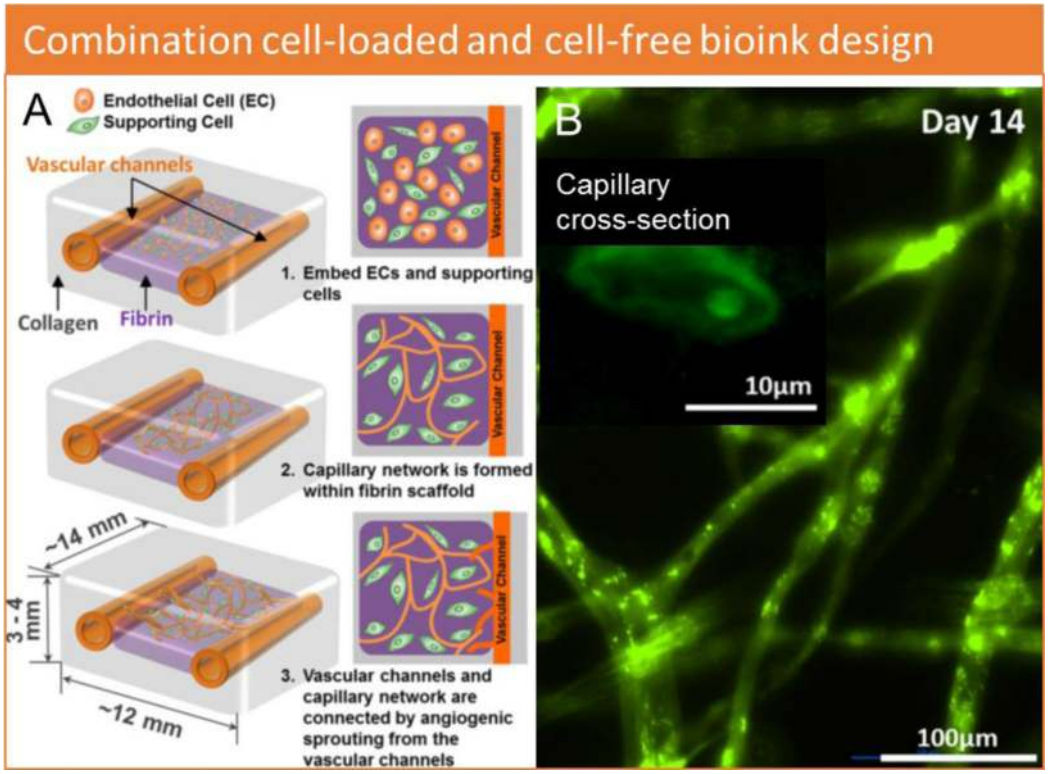

Figure 8.

Multi-scaled approach for vascular fabrication. (A) Indirect printing of channels using cell-free bioink is advanced by using cell-loaded bioink to deliver vascular cell types. (B) HUVECs assemble into capillary network and connect with perfused channels. Adapted with permission from Lee et al. 2014 [54].

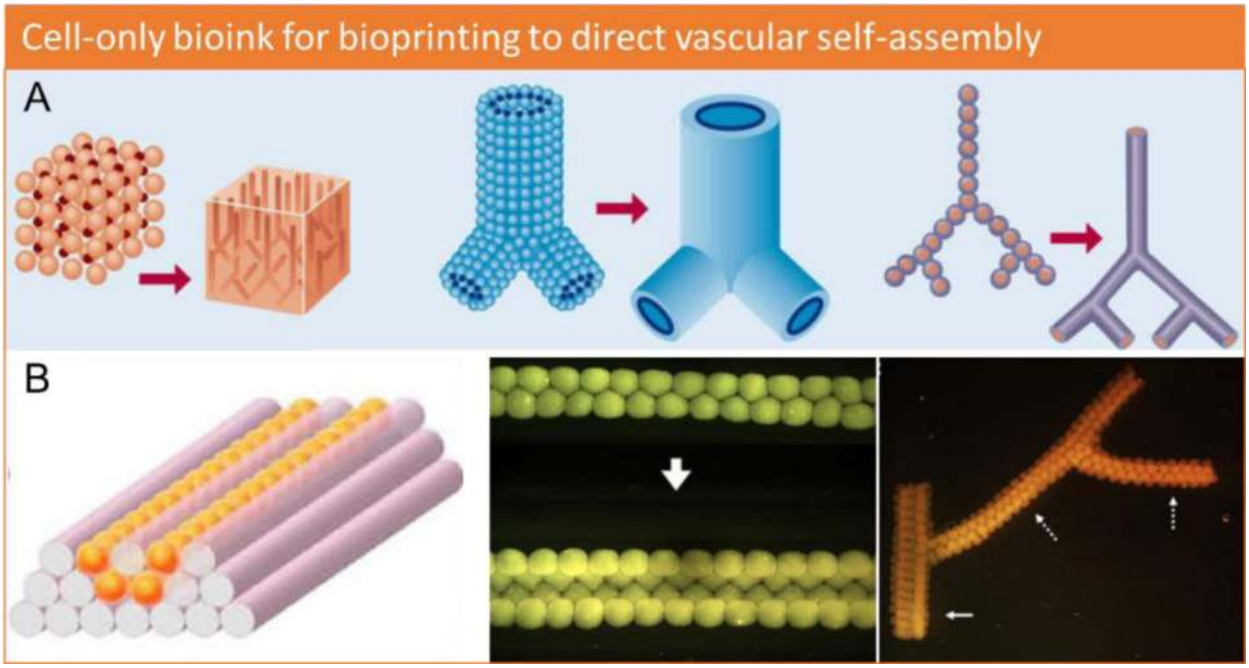

Figure 9.

Cell-only bioink for vascularized tissue fabrication. (A) Bioprinting allows for precise organization of cell aggregates to facilitate a variety of vessel formation strategies. (B) Bioprinting cell spheroids around agarose support structures demonstrate vessel formation through cellular self-assembly. Adapted with permission from Mironov et al. 2009 [73] and Norotte et al. 2009 [82].

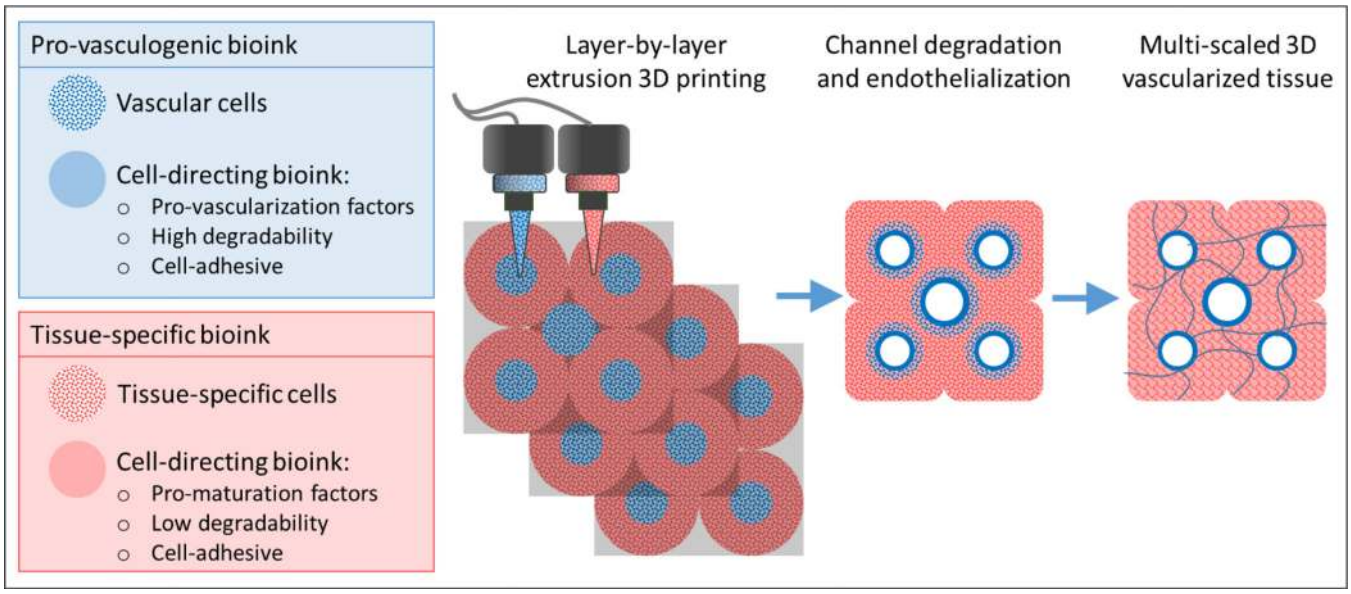

Figure 10.

Proposed 3D bioprinting strategy for vascularized tissue fabrication. Combining 3D extrusion printing with cell-directing materials would provide for a multi-scaled approach for tissue assembly. Layer-by-layer, cell-specific positioning guides large-scale design, and cell-directing materials support vascularization post-printing.

Table 1

Properties of bioprinting approaches for vascularized tissue fabrication

Bioprinter Method for vascularized tissue fabrication	Indirect or Direct	Approximate Resolution: printing/vessel diameter	Bioink compatibility (1-Cell-free, 2-Cell-loaded, 3-Cell-only; high or low viscosity)	3D bioprinting capability	References
Extrusion	Indirect and Direct	200 μm/200 μm	1, 2, 3; high and low viscosity	+++	4,5,24,40,44,46,53,54,104,105,109
Inkjet	Direct	30 μm/200 μm	1, 3; low viscosity	+	12,13,14,77,124,126
Other					
Laser	Direct	50 μm/“cell-width” (10 μm)	3; low viscosity	+	27,28,29,122
Rapid casting of carbohydrate glass	Indirect	NA/150 μm	1, 2 for post-printing embedding	+++	71