Trends in Biotechnology

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Opinion

3D Printing in Suspension Baths: Keeping the Promises of Bioprinting Afloat

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Extrusion-based 3D printers have been adopted in pursuit of engineering functional tissues through 3D bioprinting. However, we are still a long way from the promise of fabricating constructs approaching the complexity and function of native tissues. A major challenge is presented by the competing requirements of biomimicry and manufacturability. This opinion article discusses 3D printing in suspension baths as a novel strategy capable of disrupting the current bioprinting landscape. Suspension baths provide a semisolid medium to print into, voiding many of the inherent flaws of printing onto a flat surface in air. We review the state-of-the-art of this approach and extrapolate toward future possibilities that this technology might bring, including the fabrication of vascularized tissue constructs.

Highlights

3D printing in suspension media unlocks the full potential of extrusion-based 3D printers by providing a strategy for fabricating non–self-supporting structures from water-rich, low-viscosity bioinks.

Biomimetic structures representative of native vascular channels have been printed in suspension media, demonstrating that both omnidirectional printing and printing in discrete arbitrary locations are possible with this printing strategy.

Retention of a suspension medium following printing of embedded constructs is achievable through crosslinking. Suspension media are therefore able to double as a 3D cell culture substrate in which printed features such as vessels or cell populations can help with maturing of the engineered tissue.

Current Limitations in the Evolution of 3D Bioprinting

Will we ever be able to engineer functional tissues and organs suitable for *in vivo* transplantation? Will 3D printing have a role in helping to achieve this? These questions have come to the forefront of research in the tissue engineering (TE) field over the past few decades, fueled by the demonstration that conventional 3D-printing technologies can be adapted to control the deposition of high-density cellular populations in 3D space. Of the different technologies, extrusion-based 3D printing has been identified as the most likely technique to realize the TE vision. Specifically, the mild processing conditions, which have a limited impact on cell viability, in conjunction with their flexibility in processing materials with a broad range of properties make this technology an attractive candidate. Although extrusion printers have been used extensively in the **3D-bioprinting** (see Glossary) field, we are a long way from developing whole functional organs. Therefore, it could be hypothesized that a step change is required to harness the full potential of extrusion-based 3D printing in TE.

Looking at the plethora of 3D-bioprinting–related publications, there are currently two predominant, distinct perspectives. The first prioritizes ease of fabrication, leveraging materials that can be extruded into filaments with high shape fidelity to create self-supporting structures. This approach has been extrapolated from 3D printing with stiff plastics for medical devices [1], where extruded filaments will immediately hold their shape. Regarding TE, this perspective is related to printing polymer-rich constructs that either are initially acellular or restrict encapsulated cells in their ability to develop tissue.

The second perspective is tailored toward biomimicry, where replication of cellular and extracellular structures is favored over suitability for fabrication. This second approach has evolved primarily to be the patterning of **bioinks**. Such structures are more permissive to tissue maturation than is the printing of the previously mentioned polymer-rich constructs. However, bioinks are less suited for use as fabrication materials, due to their innate weak mechanical properties, and thus they are generally avoided for printing structures that are greater than several millimeters in size or require a high structural fidelity.

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Over the past few years, a new approach has evolved that has demonstrated the potential to marry the two perspectives described above. 3D printing in **suspension media** provides a platform for the patterning of mechanically weak bioinks into complex, well-defined structures [2,3]. This technology uses an extrusion 3D printer that deposits material not on a flat surface in air, but into a bath that suspends the printed material, preventing settling and collapse (Figure 1). Thus, it offers a paradigm shift in bioprinting by diminishing the need to compromise between material biomimicry and manufacturability.

Suspension Media Marking Next Era of Extrusion-Based 3D Bioprinting

Suspension media hold unique traits that are responsible for their ability to suspend and completely encapsulate printed material. First, suspension media exhibit solid-like characteristics in the absence of an applied stress or at very low stresses such as those induced by gravity [2,4,5]. Application of a stress that overcomes a critical stress, the **yield stress**, will initiate flow and render the media liquid-like. Second, after the disturbance of a suspension medium's microstructure by a passing nozzle and its displacement by any deposited material, the microstructure spontaneously recovers. This **self-healing** capacity permits the medium to transition from a fluidized state back to a solid-like state, thereby encapsulating the deposited material [6]. Ideally, fluidization of the media should be rapid to ensure that no crevasses or air pockets will be trailing from the moving nozzle [7]. As a result, printing of soft materials or low-viscosity fluids, often containing a high water content, is feasible with the use of suspension media. This presents a noticeable increase in the palette of materials that can be printed with extrusion-based printing techniques. Figure 1 provides a schematic representation of the strategy of printing in suspension media, tailored toward fabrication of a coronary arterial tree.

Research laboratories that currently use extrusion 3D printers can almost immediately adopt this novel printing strategy with minimal expenditure, where the only additional requirement is the medium itself. Although there are still both challenges and limitations to the strategy of 3D printing in suspension media (Box 1), integration of suspension media with current extrusion 3D printers alleviates several of the limitations associated with this printing platform, tailoring the current extrusion printing strategy toward bioprinting physiologically relevant structures. Promises of this novel printing strategy include preventing collapse of a printed structure; improving continuous extrusion and rapid material deposition; preventing dehydration of printed material and embedded cells; allowing omnidirectional printing (as opposed to layer-by-layer deposition); and permitting printing in arbitrary, discrete locations.

Our view in regard to using suspension media in the 3D-bioprinting field diverges into two distinct pathways, the difference being the fate of the suspension media after printing. The first pathway uses suspension media as a temporary aid during the printing process, requiring the extraction of the printed structure from the media after printing [2,3,5], a notable challenge that is critical to the biological performance of the printed structure (Box 1). The second, lesser-used approach involves retention of the suspension medium after printing.

Suspension Media Enabling Better Biomimicry in 3D Bioprinting

Many current bioinks have been engineered with a focus on ease of processability, where a high viscosity is a preferential characteristic for ensuring accurate, stable deposition during the extrusion process. Although these high-viscosity materials are beneficial for achieving good print fidelity, they often require high extrusion pressures for their deposition, which leads to high shear stresses that hamper cell survival [8,9]. Suspension media provide support to low-viscosity bioinks, helping to ensure cell survival during extrusion, as well as allowing the fabrication of well-defined shapes without requiring a persistent scaffolding material. This is exemplified by a

Glossary

3D bioprinting: controlling the deposition of biologically relevant material, whether living or nonliving, in order to engineer a structure intended to replicate biological functions. Extrusion-based printing is a subclass in which material is deposited through a nozzle that moves relative to the build area.
Bioink: a cell-containing formulation that is processable with 3D-bioprinting techniques.
Crosslinking: formation of permanent or reversible bonds between adjacent polymer chains leading to the formation of a network structure.
Organoids: miniaturized organ-mimicking constructs formed by single self-organizing multipotent stem cells. In 3D cell culture, they recapitulate some of the key characteristics of their representing organ, such as cellular spatial organization.
Self-healing: spontaneous recovery of the microstructure and bulk rheological properties of a fluid after flow.
Suspension media: a yield stress fluid capable of supporting printed material that is extruded within its volume.
Yield stress: a threshold stress below which there is no flow of the fluid.

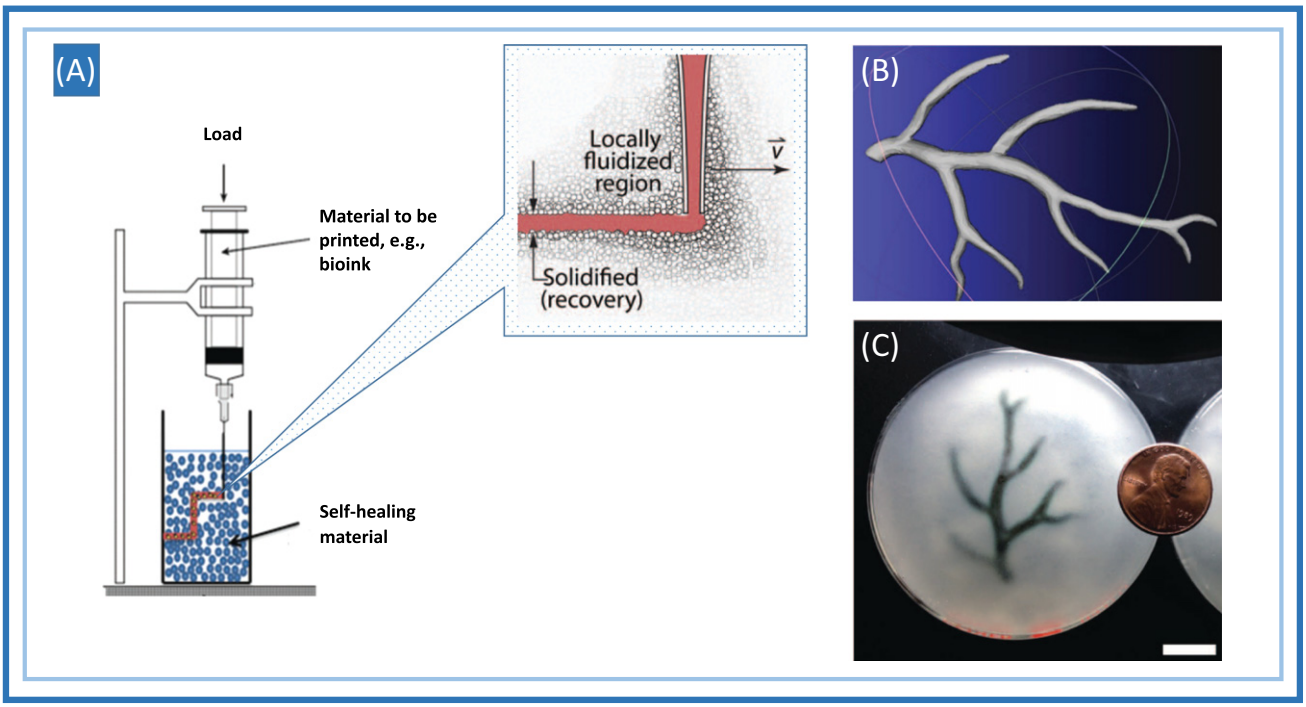

Figure 1. Schematic Representation of the 3D Bioprinting in a Suspension Medium Strategy. (A) Writing of bioink material in a self-healing suspension medium [2,6]. The moving extruder nozzle fluidizes the medium to permit deposition of printed material. The medium then rapidly resolidifies around the printed filament, providing structural support. Yielding of the media occurs at a localized point of injection, with minimal disturbance to the bulk of the medium. Reproduced from the indicated references, licensed under Creative Commons 4.0 (CC 4.0) series. (B) Computer-aided design of intricate, non–self-supporting arterial tree [3]. Adapted from the original, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0). (C) Example of a printed arterial tree that has been printed in a gelatin slurry suspension medium [3]. Reproduced from the original, licensed under CC BY 4.0.

study of printing tissue analogs with decellularized extracellular matrix (ECM)-based bioinks with viscosities of 6.13 and 2.8 Pa·s, which proved too low to be printed on their own and required codeposition with a synthetic polymer supporting framework [10]. Where the printing of these bioinks was not possible using conventional extrusion-based bioprinting, suspension media have allowed the precise printing of collagen [2,11], fibrin [3], and pure cells without a biomaterial [12], all of which are deemed equally as 'unprintable' as the decellularized ECM.

Many organs and tissues exhibit structural features that are not self-supporting outside their native environment. Fabrication of such structures relies on both omnidirectional printing and the deposition of a printed ink in arbitrary discrete locations – freedoms not provided by conventional extrusion technologies. Work carried out by the Angelini group at the University of Florida demonstrated the suitability of their suspension medium, a polyacrylic acid particle-based suspension medium (Carbopol; Lubrizol, Wickliffe, OH) [2], to print structures with unique biological forms in close proximity. Using suspension media harnesses the potential offered by extrusion printers to move in the *x*-, *y*-, and *z*-planes; thus, structures that have a greater resemblance to natural forms can now be printed in 3D.

The transition from 3D printing self-supporting structures to those that are not was pioneered by the Lewis group, who identified the capability of yield stress fluids to facilitate omnidirectional printing of channels [13] in an acellular system that was not self-healing (Figure 2A). Highley and colleagues [5] demonstrated similar capabilities in a self-healing hydrogel system through

Box 1. Challenges and Limitations

Current extrusion 3D printers were originally designed for printing 3D objects using layer-by-layer deposition. Consequently, their accompanying software used to slice a solid 3D object model into a stack of flat layers and define tool paths is developed to create each layer from a series of linear movements in a single plane. Current slicing software presents a challenge to the printing in suspension media strategy because it is not yet fit for the purpose of omnidirectional printing, where continuous printing is required across multiple planes.

A second challenge is that many applications would require an extraction step to remove a printed construct from its embedment in a suspension medium. Current extraction steps include the use of elevated temperatures to melt a medium [3], dilution of the medium [2], changing the pH conditions to cause deswelling of a particulate medium [34], or enzymatic cleavage [35]. Rapid changes to the physical and chemical states of a suspension medium can cause damage to, or in some instances melt, the surface of printed material, lowering the structural integrity of a 3D-printed construct [36]. Injection of salt solutions, previously used to alter the pH of Carbopol suspensions [34], may induce osmotic shock to the cells of printed cell-laden material, whereas dilution of the medium with large volumes of water may also disturb the osmotic balance of cell-laden constructs. It is therefore important to select the most suitable extraction method in order to minimize damage to the printed material.

One inherent limitation of this printing strategy is that the printing temperature of deposited inks is dictated by the temperature of the suspension medium. Inks are therefore not able to be printed at a temperature which differs from that of the medium. This limits the use of hydrogels that rely on temperature changes to promote crosslinking, as well as thermoplastics. The varying temperature required in the printing process for thermoplastics – where deposition of these plastics occurs at higher temperatures than their solidification temperature – cannot be accommodated by current suspension media. The use of thermoplastics to construct mechanically strong components in tissue engineering (TE) limits the applicability of printing in suspension media for fabrication of load-bearing tissues. Other bioprinting applications where printing in a suspension medium has no added value include printing of biological tissues that are flat (2D) or 2.5D, such as skin, where omnidirectional printing and preparing freestanding porous structures are not required.

the printing of fine structures into a modified hyaluronic acid (HA) suspension medium. This medium lends its ability to suspend printed material not from a particulate microcomposition, but from reversible intermolecular bonds; here, HA polymeric chains were modified with either adamantane or β-cyclodextrin, providing guest–host interactions that yield the desired rheology. The development of yield stress fluids that additionally have self-healing properties offers the technological freedom to deposit an ink in areas of the bath that have previously been sheared by the shaft of the extruder nozzle. This freedom is advantageous in bioprinting applications where structures are closely packed together or entangled with one another (Figure 2B–D). One example of a biological form that has been 3D printed in suspension media is a human brain, a structure with a high degree of curvature, intricate folds, and internal void spaces (Figure 2E,F). Hinton and colleagues [3] printed this structure in a gelatin slurry suspension medium. This medium exhibits a particulate microstructure where the generation of a yield stress is the result of closely packed particles. The particulate microcomposition is the most prevalent structure exhibited by suspension media reported in the literature. The most recent studies have departed from printing biology-inspired forms to printing more functional structures (e.g., organ printing), aiming to mimic some of the functional characteristics of the native tissue from which they were inspired (Box 2).

Suspension Media Doubling as Extracellular Matrix

Retention of the suspension media after printing provides an opportunity to add a degree of functionality to the suspension medium itself, such as to manipulate the 3D cellular environment [14] or to retain vascular channels embedded in a stabilized suspension medium [15] (Figure 3A). The inclusion of cells throughout a suspension medium conjures up the idea of the medium acting as a platform to position cells in 3D space, as well as providing a bulk matrix fulfilling some of the functions of a native ECM. The noticeable advantage of suspension media being leveraged in this manner is linked to the opportunity to fabricate larger 3D tissue constructs in a shorter time, increasing the throughput of **3D bioprinting**.

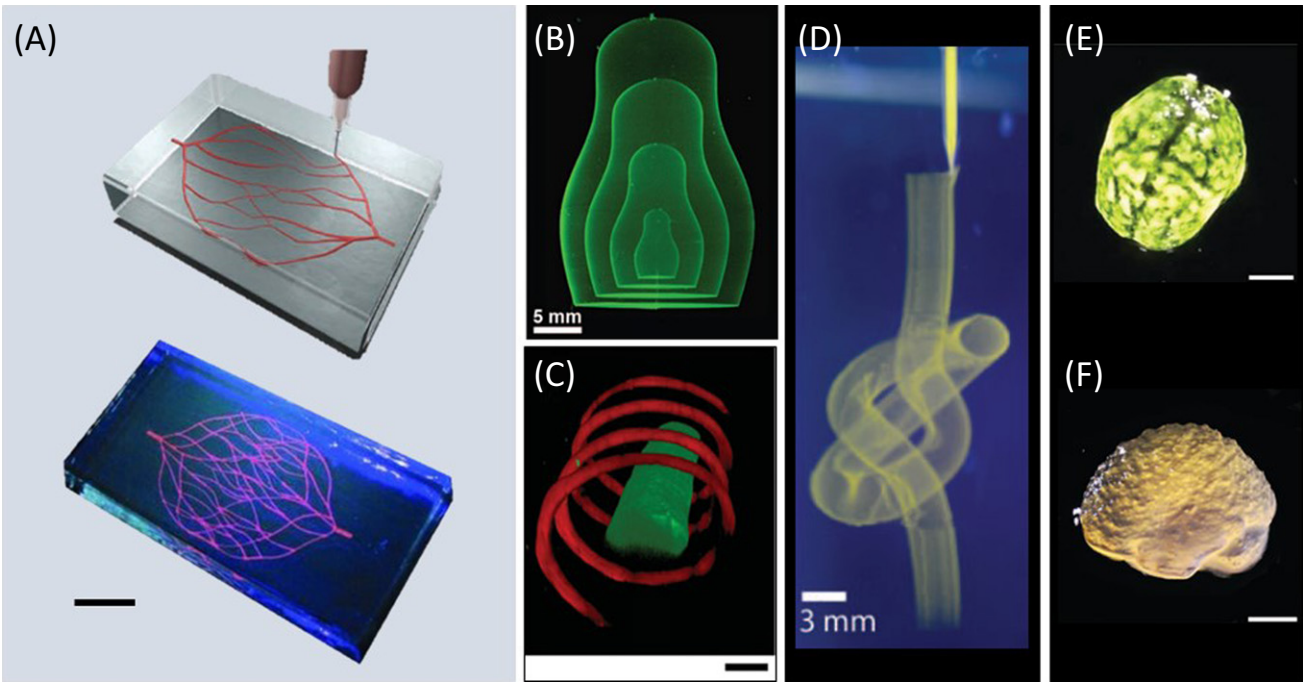

Figure 2. Advancement of Omnidirectional 3D Printing: From Yield Stress Fluids to Suspension Media. (A) Omnidirectional printing in a non–self-healing yield stress fluid. (Top) Schematic for 3D-printed vascular network using a removable ink. (Bottom) A fluorescence image of a 3D microvascular network fabricated via omnidirectional printing of a fugitive ink (dyed red) within a photopolymerizable Pluronic F-127–diacrylate matrix. Scale bar, 10 mm. Reproduced from [13]. (B–F) Omnidirectional printing in suspension media. (B) Miniature Russian dolls printed in a granular suspension medium [2]. Reproduced from the indicated reference, licensed under Creative Commons 4.0 (CC 4.0) series. (C) Printed filament of a fluorescein-labeled ink (in green) surrounded by a continuous spiral structure (in red), printed with a rhodamine-labeled ink [5]. Scale bar, 200 μm. (D) Continuous knot written with fluorescent microspheres in a granular suspension medium. Reproduced from [2]. (E and F) Human brain model 3D-printed in an alginate suspension media. Scale bar, 1 cm. [3]. Reproduced from the original, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0).

In 2D cell culture, cells are more restricted in their morphological development and motility than in a 3D environment. There is a growing awareness in the scientific community of the resulting stark differences in terms of cellular differentiation and tissue organization, where 3D cell culture is more reflective of the complex native cellular environment. The feasibility of a suspension medium providing a platform for 3D cell culture was highlighted by the work on liquid-like solids (LLSs) [14], where granular microgels (made of granules 6–7 μm in diameter) packed in close proximity in cell culture media (>99% volume) were used as a 3D cell culture substrate. Undisturbed, these LLSs are capable of providing support and stability to cell assemblies while the interstitial space between the microparticles allows for the unrestricted diffusion of nutrients and other elements throughout the volume, thus providing metabolic support to cells [14]. This paper also excellently characterized cell migration and division in LLS media. The authors observed that the mechanical properties of an LLS suspension medium, such as the low yield stress, enable cellular division to occur with negligible physical resistance from the media, detailing the moving apart of daughter cells at an approximate rate of 15 μm/h [14]. Similarly to some of the pioneering studies on granular hydrogels [16–18], they found cellular migration to occur via the pore space between microparticles. This differs from the movement of cells in a **crosslinked** network of polymer chains, where cells typically create tunnels by enzymatic degradation through the interstitial nanoscale mesh between the chains [14]. Such work details the possibility of a suspension medium for 3D bioprinting doubling as 3D cell culture substrate, such as one in which mechanical properties can be tuned for 3D studies of collective cell mechanics [19]. In

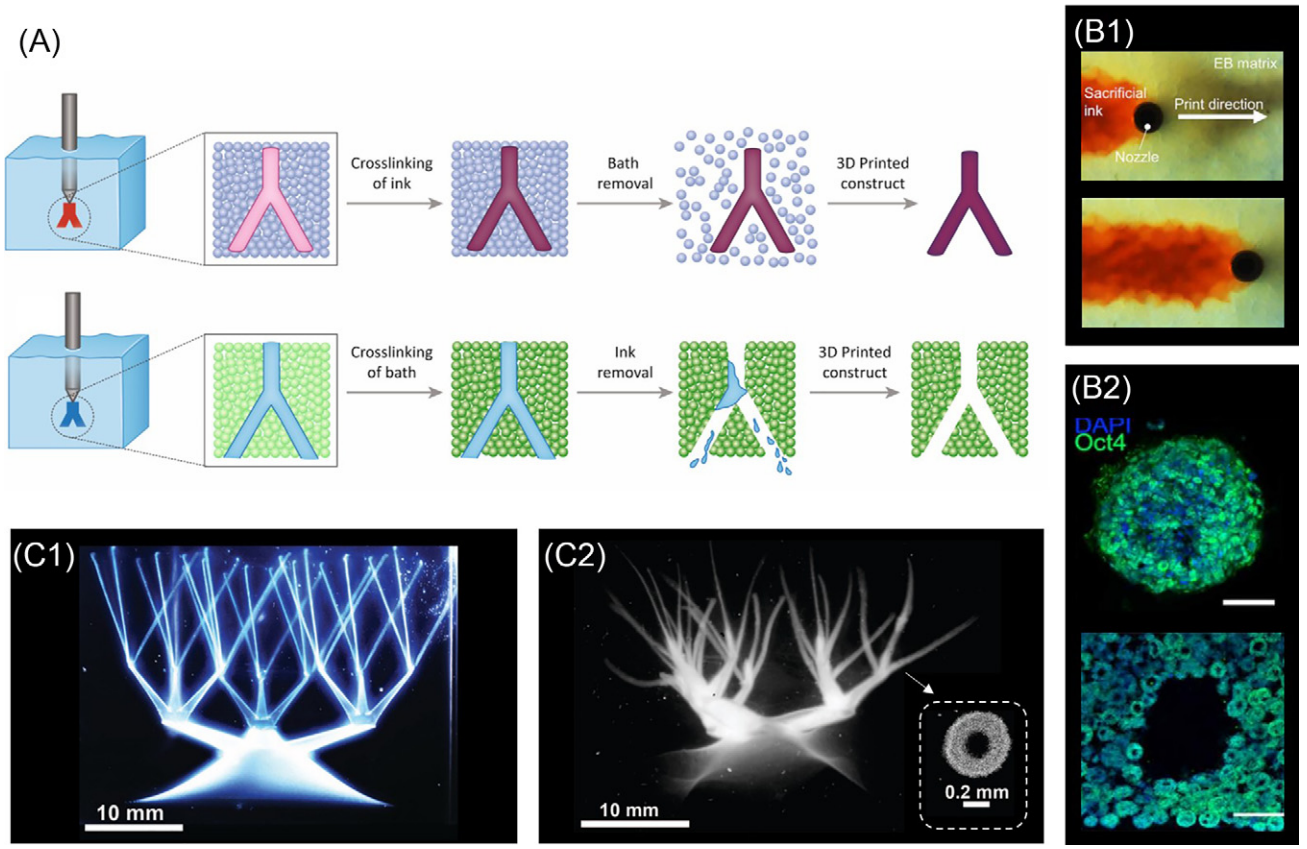

Figure 3. Suspension Media Used as a Strategy to Aid Better Biomimicry in the 3D-Bioprinting Field. (A) Overview of the 3D-printing pathways that can be followed when printing in a suspension medium. (Top) Pathway is defined by removal of the medium after printing. Suspension medium provides mechanical stability to printed ink while crosslinking of the ink takes place (e.g., by exposing the embedded ink to ultraviolet light). The medium is subsequently removed in order to extract the printed construct. (Bottom) Pathway is defined by retention of the medium after printing. Following deposition of a sacrificial ink, the medium is crosslinked to form a single construct. In this crosslinked state, the medium has lost its ability to flow. The sacrificial ink can then be extracted, such as with a syringe needle, leaving behind hollow channels embedded within the construct. (B1) Writing of sacrificial ink within an embryoid body suspension medium [27]. Reproduced from the original, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0). (B2) Row 1: Singular organ building block. Scale bar, 50 μm. Row 2: Cross-section of channel printed in an embryoid body suspension medium following removal of the sacrificial printed ink. Scale bar, 500 μm [27]. Reproduced from the original, licensed under CC BY 4.0. (C1 and C2) Example of a highly branched tubular network printed in a Carbopol suspension medium where (C2) is the structure freed from the media [2]. Reproduced from the original, licensed under CC BY-NC 4.0.

the case of an LLS medium, the microparticles are not interconnected, and thus the medium may fluidize over extended culture periods. Engineering a medium that can provide mechanical stability over long culture periods is critical to the 3D bioprinting and maturation of large tissue constructs. Such was demonstrated by Jeon and colleagues, who added photoreactive groups (**crosslinking** moieties) to alginate microparticles [12]. They 3D bioprinted human mesenchymal stem cells (hMSCs) into a suspension of these particles, followed by crosslinking through low-level ultraviolet irradiation. In the TE field, photoactivated crosslinking has been used widely because it allows the rapid formation of a network structure under mild conditions [20,21]. The advantage of this method is that the crosslinking process can easily be controlled in space and time because photo-initiated polymerization occurs only where an area is irradiated with a light source. Eliminating the ability of the suspension medium to flow prevents shear yielding of the medium, thus providing a mechanically robust matrix for extended cell culture. Interestingly, the Alsberg group have also used these crosslinkable microparticles, laden

Box 2. Suspension Media Enabling Free-Form Printing of a Human Heart

The most recent advances relating to 3D bioprinting of organs have seen the use of suspension media as a technological printing aid, specifically within the cardiac TE field [11,35]. Recapitulation of the functional characteristics inherent to the heart, such as contractility, rely on the use of bioink formulations rich in parenchymal cells. Maintaining the phenotype of these cells is critical to their performance, and thus bioink formulations rich in parenchymal cells exhibit a low stiffness to avert any transfer of stress from the carrier material (e.g., a gel or cell culture media) to the cells. Dvir and colleagues [35], as a proof of concept, demonstrated printing of induced pluripotent stem cell–derived cardiomyocytes and endothelial cells to print a cellularized human heart (Figure I), proving that use of a suspension medium was the enabling technology to fabricate complex anatomical structures from patient-derived, mechanically weak bioinks. The bath was composed of alginate microparticles surrounded by a solution of xanthan gum dissolved in cell culture media. Although this heart was miniaturized (with a height of 20 mm and diameter of 14 mm), the resolution achieved from printing in this suspension medium permitted perfusion of both the major blood vessels and the heart ventricles. This achievement in combination with the structure being printed from a patient-derived bioink has led to many news outlets deeming this "the world's first 3D-printed human heart."

Gelatin particulate–based media have been used to aid printing of functional components of the heart, such as fabrication collagen-printed trileaflet heart valves [11]. This work, carried out by Feinberg and colleagues, adapts their original FRESH (freeform reversible embedding of suspended hydrogels) technique [3] to establish an advanced version, FRESH v2.0, centered on control of the microparticle homogeneity of their gelatin suspension medium (Figure I). Compared with v1.0, in which a gelatin block was blended to produce particles with a mean diameter of 65 μm, v2.0 uses a coacervation approach to produce particles of a smaller diameter (~25 μm) and lower polydispersity [11]. The FRESH technique demonstrates the feasibility of printing anatomical structures of the heart from mechanically weak materials such as extracellular proteins.

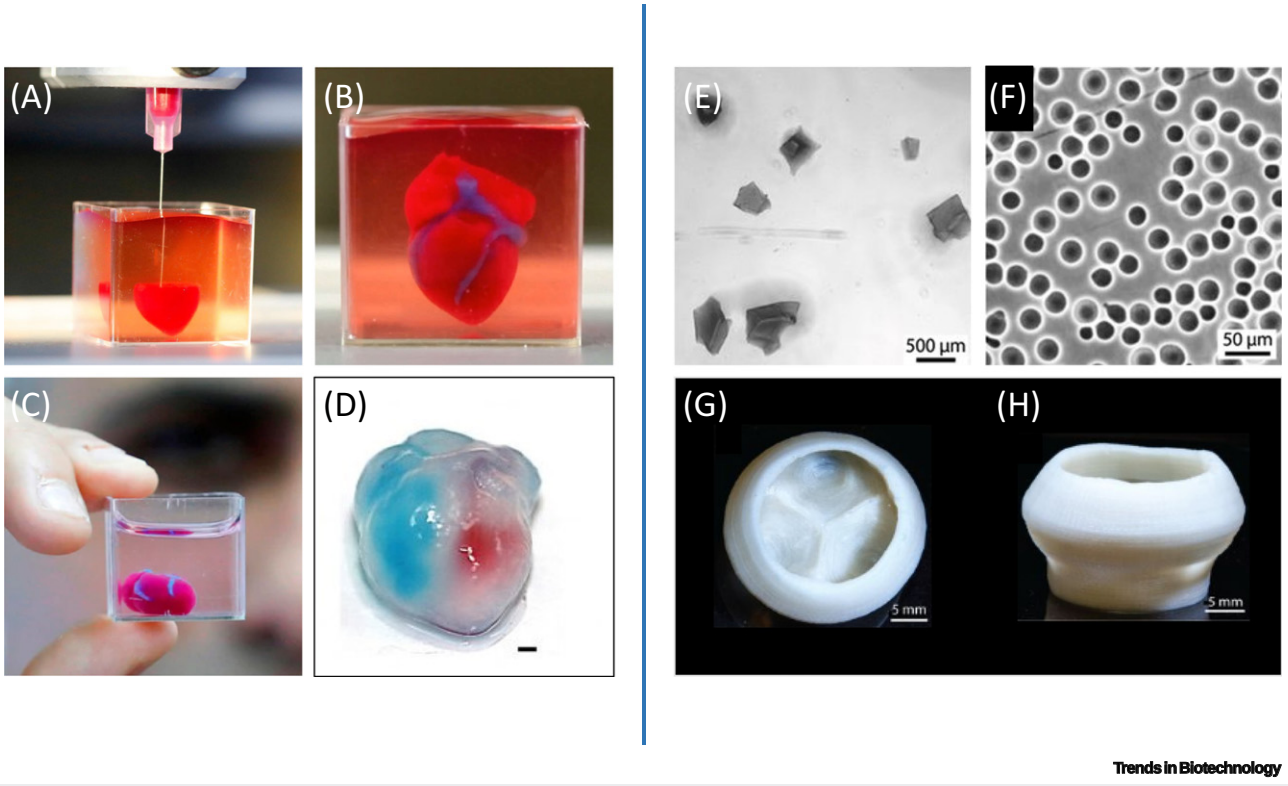

Figure I. Suspension Media Used as Technological Aid for 3D Bioprinting of a Human Heart. (A–C) Example of a 3D bioprinted, miniaturized, cell-laden human heart [35]. Figure is reproduced from the indicated references, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0). (D) 3D-printed heart extracted from the medium, then perfused with red and blue dyes to demonstrate hollow chambers within the construct [35]. Scale bar, 1 mm. Figure is reproduced from the indicated references, licensed under CC BY 4.0. (E and F) Gelatin microparticles that comprise FRESH v1.0 and v2.0 suspension media, respectively. From [11]. Reprinted, with permission, from the American Association for the Advancement of Science (AAAS). (G and H) Collagen-printed heart valve, printed within a FRESH v2.0 medium. From [11]. Reprinted, with permission, from AAAS.

with hMSCs, as a bioink for printing into a suspension medium [22]. In this instance, a gelatin slurry was used as suspension medium for printing, followed by photo–crosslinking of the cell-laden particles to form a singular continuous structure.

Need for Speed

Many tissues are hierarchical in nature, meaning their reproduction requires different resolutions for differently sized features. With current extrusion-based printing methods, the relationship between print time and resolution is a fundamental limitation where an increase in resolution results in an exponentially longer print time [23,24]. If a large tissue construct is to be formed wholly by extrusion at relatively high resolution, building a relevant volume of tissue will require a long time in which the deterioration of cells and biological matter is a likely occurrence along the way. Similarly to the printing of detailed small features combined with casting of a bulk tissue [25,26], using a suspension medium that doubles as a bulk matrix seems a promising pathway to engineering hierarchical tissues in a rapid way, because the bulk of the tissue does not need to be extruded line-by-line. The ability of suspension media to suspend on both the macro- and microscales permits the inclusion of cells within the bulk volume of the bath before printing. Such an advantage allows a suspension medium to act as a cell-populated foundation in which additional features can be printed. Skylar-Scott and colleagues [27] took this to the extreme by leaving out the biomaterial altogether and developing a medium consisting solely of embryoid bodies from induced pluripotent stem cells (Figure 3B1 and B2). These embryoid bodies were cultured to form granular-shaped organ building blocks (OBBs) that were compacted via centrifugation, which resulted in rheological properties suited for use as a suspension medium. Through engineering a suspension medium composed of OBBs that fuse into tissues after printing, this work elucidates the possibility to achieve a construct with high cellular densities comparable to those *in vivo*.

A Strategy for Vascularization

The material properties of a suspension medium facilitate omnidirectional extrusion of a printed ink in 3D space. Subsequently, this provides a platform to address vascularization of large tissue constructs, one of the most prominent challenges in the TE field [28,29]. Vascularization is particularly significant when implanting a thick tissue analog (larger than several millimeters in size) into a host tissue [30]. Generally speaking, it has been observed that spontaneous vessel ingrowth into an implanted artificial construct is too slow to prevent formation of a hypoxic core within the implant, resulting in cell death before a complete vascular bed is established [31]. Previous discussions on vascularization of tissue analogs [31,32] stressed the importance of prevascularizing a construct in order to ensure high cell survival rates *in vivo*. Typically, vascular networks are dense, often exhibiting structural features such as entanglements and complex branching. Replication of such features by 3D printing in a suspension medium relies on the structural integrity of printed channels in close proximity to be unaffected as subsequent channels are printed. Through balancing print speed and extrusion pressure with the rheological properties of the suspension medium, fluidization of the medium can be localized within an extremely small distance from the extruder nozzle, thus minimizing disturbance to both the medium and previously printed channels.

Attempts at fabricating vascular channels in biomaterials using extrusion-based 3D-printing approaches have primarily produced channels with a square lattice or 'woodpile' arrangement [26,30]. These approaches have used the printing of sacrificial materials that are surrounded by a casted biomaterial matrix. The sacrificial material is then dissolved/liquefied to leave behind hollow channels that can be seeded with endothelial cells. However, such constructs lack the complex, hierarchical morphologies of a vascular network. The use of yield stress fluids as a platform to facilitate omnidirectional printing, established by the Lewis group [13], was pivotal to achieving channels with high degrees of biomimicry. The existence of a yield stress exhibited by suspension media has resulted in recognition of these media as a tool for the printing of biomimetic channels. In one such example, Bhattacharjee and colleagues used the capability of their granular suspension medium [2] both to exhibit a yield stress and to self-heal to fabricate a hierarchical

arrangement of a structure with 40 connected vessels (Figure 3C1 and C2). This structure had three levels of division, which ended in very narrow channels of 100 μm in diameter. However, the printed vasculature was subsequently freed from the suspension medium, and thus the vascularization was not providing perfusion of a matrix in this example.

Concluding Remarks and Future Perspectives

In recent years, the advance of TE using extrusion-based 3D-bioprinting technologies has continued to grapple with well-appreciated limitations inherent to printing soft, water-rich biomaterials. 3D printing in suspension media promises to alleviate some of these limitations by providing a platform to allow the controlled deposition of cell-laden inks with a high water content. Early adopters of this novel printing strategy have begun to demonstrate the value of a suspension medium for engineering complex biological structures [2,3,6], exploiting advantages such as the structural support provided by the medium's yield stress and the medium's ability to spontaneously self-heal. In the short term, this printing strategy will disrupt the current perspective on what is achievable with extrusion-based 3D-printing technologies.

Looking forward, the application of printing in a suspension medium to 3D cell culture seems both exciting and highly feasible, as demonstrated by the Alsberg and Angelini research groups (see Outstanding Questions). We foresee the exploitation of suspension media to double as a bulk matrix providing an enabling technology to engineer large functional tissues. Additionally, we believe there is the potential to embed **organoids** and print surrounding vascular channels within the suspension medium. Work by the Lutolf research group in Switzerland showed that separate stages in organoid formation require different mechanical environments [33]. The opportunity to alter the mechanical properties of the suspension medium over time, such as by either the addition of crosslinking chemistries or dilution of a microparticle medium, may be permissive to mimicking the dynamic character of the organoid microenvironment.

As we have begun to outline, the use of suspension media has the potential to shift the current 3D-bioprinting paradigm. Analogous to the upsurge of research in the biofabrication field with the introduction of the various 3D-printing platforms, we believe suspension media can provide the required technological platform that will support the next level of 3D-bioprinting research.

Outstanding Questions

How does the optimal rheology of the suspension media relate to what is being printed? Will different biomaterials or cells in their culture media be printable into a single suspension medium?

Will the interconnected micropore space between particles in the media facilitate perfusion, cell migration, and tissue maturation upon culture?

Will it be possible to integrate subtractive manufacturing technologies with the strategy of 3D printing in suspension media? For example, can we exploit lithography to control precise photolysis of the media to generate microscale vascular channels?

Will this printing strategy help progress the research on engineering tissues from organoids?

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