Received: 14 January 2022 | Revised: 2 February 2022 | Accepted: 28 February 2022

DOI: 10.1111/aor.14232

Artificial Organs WILEY

REVIEW

Advances in microfabrication technologies in tissue engineering and regenerative medicine

Sara Nadine[1,2] | Ada Chung[3] | Sibel Emir Diltemiz[4] | Brooke Yasuda[1,3] | Charles Lee[1,5,6] | Vahid Hosseini[1] | Solmaz Karamikamkar[1] | Natan Roberto de Barros[1] | Kalpana Mandal[1] | Shailesh Advani[1] | Benjamin Behnam Zamanian[1] | Marvin Mecwan[1] | Yangzhi Zhu[1] | Mohammad Mofidfar[7] | Mohammad Reza Zare[8] | João Mano[2] | Mehmet Remzi Dokmeci[1] | Farshid Alambeigi[9] | Samad Ahadian[1]

[1]Terasaki Institute for Biomedical Innovation (TIBI), Los Angeles, California, USA

[2]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

[3]Department of Psychology, University of California-Los Angeles, Los Angeles, California, USA

[4]Department of Chemistry, Eskisehir Technical University, Eskisehir, Turkey

[5]Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas, USA

[6]Station 1, Lawrence, Massachusetts, USA

[7]Department of Chemistry, Stanford University, Palo Alto, California, USA

[8]Department of Chemical Engineering, Shiraz University, Shiraz, Iran

[9]Walker Department of Mechanical Engineering, University of Texas at Austin, Austin, Texas, USA

Correspondence
Samad Ahadian, Terasaki Institute for Biomedical Innovation (TIBI), Los Angeles, CA 90064, USA.
Email: smd.ahadian@gmail.com

Funding information
Fulbright Commission Portugal and the Portuguese Foundation for Science and Technology, Grant/Award Number: SFRH/BD/130194/2017

Abstract

Background: Tissue engineering provides various strategies to fabricate an appropriate microenvironment to support the repair and regeneration of lost or damaged tissues. In this matter, several technologies have been implemented to construct close-to-native three-dimensional structures at numerous physiological scales, which are essential to confer the functional characteristics of living tissues.

Methods: In this article, we review a variety of microfabrication technologies that are currently utilized for several tissue engineering applications, such as soft lithography, microneedles, templated and self-assembly of microstructures, microfluidics, fiber spinning, and bioprinting.

Results: These technologies have considerably helped us to precisely manipulate cells or cellular constructs for the fabrication of biomimetic tissues and organs. Although currently available tissues still lack some crucial functionalities, including vascular networks, innervation, and lymphatic system, microfabrication strategies are being proposed to overcome these issues. Moreover, the microfabrication techniques that have progressed to the preclinical stage are also discussed.

Conclusions: This article aims to highlight the advantages and drawbacks of each technique and areas of further research for a more comprehensive and evolving understanding of microfabrication techniques in terms of tissue engineering and regenerative medicine applications.

KEYWORDS

3D bioprinting, 4D bioprinting, biomaterials, electrospinning, hierarchical assembly, microfabrication, microfluidics, microneedles, regenerative medicine, soft lithography, tissue engineering

1 | INTRODUCTION

Tissue engineering (TE) and regenerative medicine have employed several natural and synthetic biomaterials as scaffolds to resemble the extracellular matrix (ECM) of the native tissues and organs.[1] In the native tissues, most cells reside within the complex three-dimensional (3D) ECM microenvironment that possesses distinct mechanical and biological properties. However, it is essential to achieve a precise 3D architecture at a micrometer scale to fabricate a functional tissue-engineered construct. The microenvironment of living tissues consists of a heterogeneous cell population and ECM proteins, such as collagen, fibrinogen, and glycosaminoglycans. Moreover, it contains biochemical and biophysical factors, which interact with each other in a well-orchestrated fashion to preserve tissue function.[2] It should be mentioned that most tissues are characterized by repetitive functional units, which either can be highly hierarchically organized or have a less organized structure.[3] Engineered tissue constructs should accurately recapitulate cellular architecture and composition to successfully promote both tissue repair and regeneration. In this regard, an increasing number of research groups have broadly developed microfabrication technologies under different areas of science, engineering, and medicine.[4–6] Approaches at the microscale reduce experimental consumption, leading to lower costs and allowing for high-throughput screening compared to macroscale methods.[7,8] Furthermore, the behavior of cells depends on structural features of biomaterials, such as pore size, shape, and pore distribution, which can range from macroscale to micro-, and nanoscales.[9]

After developing micro-manufacturing techniques for the fabrication of integrated circuits for the semiconductor industry, the methodologies were rapidly employed for different purposes.[10] The last decades have been focused on the translation of electronics, microelectromechanical systems (MEMS), and microfluidic methodologies to the field of TE. In particular, microfabrication devices have been explored for various TE strategies, including muscle, nerve, and bone tissues.[11–14] These devices can offer unparalleled control of multiple aspects of biomaterials' physical properties, including size, structural topography, substrate stiffness, and permeability, which appear mandatory to regulate a range of cellular behaviors.[15–19] The combination of micro- and nanofabrication techniques creates biomaterials with improved and desirable characteristics. These microfabricated structures can also be designed and employed as injectable, minimally invasive carriers for various biomedical applications.[20] Besides replacement and regeneration of tissues, these technologies can be employed to replicate the cancer microenvironment, increasing the tools to fight one of the leading causes of death worldwide.[21]

Based on what was mentioned above, this article aims to highlight some concepts of different microfabrication techniques, such as photolithography, soft lithography, microneedles, self and templated assembly, microfluidics, electrospinning, and 3D/four-dimensional (4D) bioprinting. The advantages and limitations of each technique, as well as the most up-to-date research, are herein discussed. We attempted to cover some of the most exciting research contributions from in vitro tissue engineering research to potential applications in regenerative medicine. In addition, this article provides an in-depth discussion of their applications in biomedical and preclinical practice.

2 | MICROFABRICATION TECHNIQUES

2.1 | Photolithography

Inspired by the lithography principle, photolithography is well-known as a technique utilized to create specific patterns onto a substrate using optical or UV light.[22] Widely employed in the semiconductor industry to fabricate patterns on oxide substrates, photolithographic technology has become a powerful tool in producing micropatterned two-dimensional (2D) and 3D scaffolds for TE strategies.[23–25] Generally, the production of a micropatterned material begins with the deposition of photoresist material on a substrate (usually a silicon wafer). After positioning a photomask containing the desired geometry that defines the pattern, optical or UV light passes through the unmasked areas (Figure 1A). The polymer undergoes structural changes after being irradiated at specific wavelengths. It should be noted that depending on the photoresist type, it can either be positive or negative. In negative resists, the light exposure can induce photoresist crosslinking. Then, the unmasked regions may be eliminated by the developer solution (usually organic solvents). In positive resists, the light exposure induces photoresist dissolution. Afterward, the soluble unwanted areas are washed by the developer solution (usually alkaline solutions).[26,27] Finally, this process results in a solidified patterned material with micrometer-scale features.

Above all, photolithography technique can widely be employed in different fields because of its high versatility regarding the design and size as well as the ability to work at a sub-micron resolution.[22,28] Nevertheless, this technique still encounters some oppositions for its implementation due to its high cost, need for cleanroom facilities, and complex operation.[29] It is well-established that topographical cues of biomaterials play a significant role in cell guidance. Micropatterns created by photolithography with different groove depths and grating pitches were

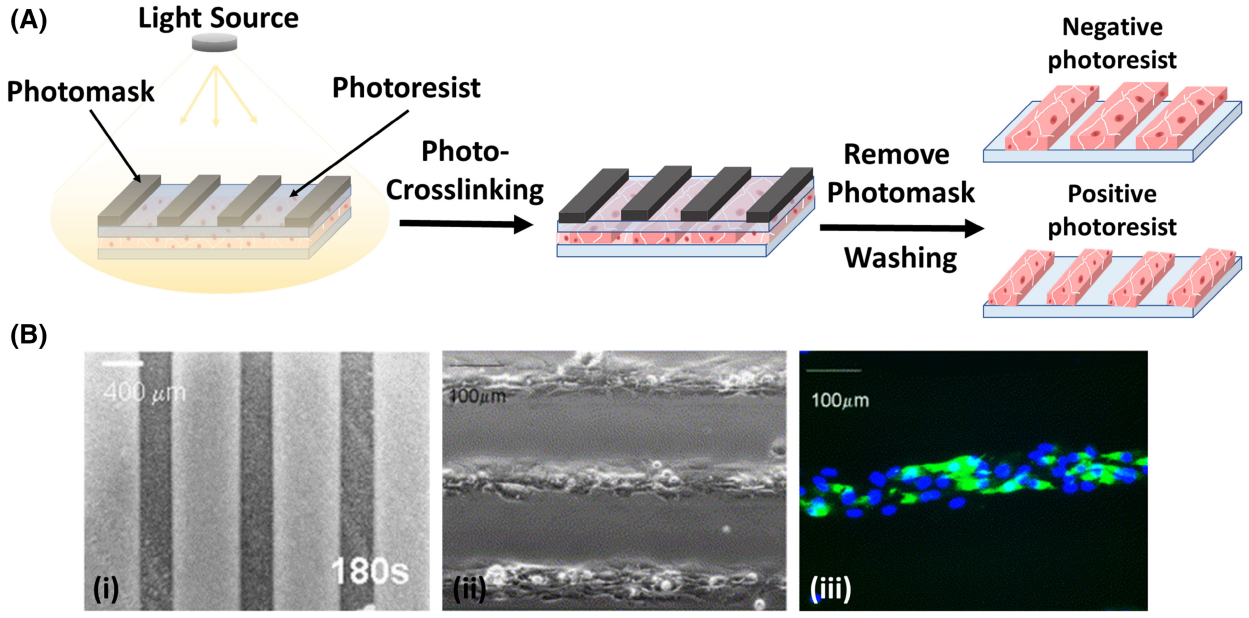

FIGURE 1 (A) A schematic illustration of the fabrication of micropatterned constructs using photolithography. (B) Photolithographic approach to generate cellular micropatterns (i) Crosslinked chitosan pattern after 180 s of UV exposure (315–400 nm), (ii) neonatal rat cardiomyocytes after 8 days of culture, (iii) cardiac troponin I (green) expressed from the patterned cardiomyocytes. Reprinted with permission from Ref. [38] Copyright (2006) Elsevier Ltd.

shown to affect cell alignment.[30] It should be mentioned that the use of topographic cues on biomaterials can have profound implications in stem cell maintenance, differentiation, and wound healing.[31–33]

One of the main advantages of using photolithography for TE strategies is its high compatibility with photocrosslinkable polymers. The extensive employment of synthetic and natural polymers in TE can be attributed to many of its appealing features, such as the resemblance with the ECM of the native tissues, biocompatibility, degradability, and promoting tissue repair.[34,35] For example, crosslinked carboxymethyl cellulose-poly(ethylene glycol) hydrogels encompass several suitable physicochemical properties and cytocompatibility for wound dressing applications. At the same time, glycidyl methacrylate-hyaluronic acid and methacrylic anhydride-hyaluronic acid act as complex ECM-mimicking scaffolds in terms of neuronal tissue applications.[36,37] In this matter, Karp et al. developed a straightforward system to produce patterned culture substrates via photolithographic technique (Figure 1B). Cardiac fibroblasts, cardiomyocytes, and osteoblasts were cultured in the patterned photocrosslinkable chitosan and formed arrays, stable up to 18 days of culture.[38] Additionally, photolithography technology can be employed to fabricate 3D spheroids, which is an essential 3D culture system for many applications in the regenerative medicine field.[39]

Meanwhile, it should be mentioned that some concerns should be considered to engineer photolithographic micropatterned hydrogels. For instance, the light source should efficiently crosslink the precursor solution while keeping cell viability and protein structure intact.[19,40] In the meantime, UV-activated initiators, such as poly(9,9-dioctylfluorene-alt-benzothiadiazole) and poly(9,9-di-n-octylfluorenyl-2,7-diyl), were some of the most widely used light-sensitive compounds for the polymerization of vinyl-based molecules.[41,42] Nonetheless, as these UV-activated initiators can generate dangerous side effects on cells, other alternatives have been proposed, such as camphorquinone, 1-phenyl-1,2-propanedione, Irgacure®819, and VA-086.[43,44] Furthermore, photomask's micropatterns should possess an appropriate resolution to be successfully transferred into the fabricated material.[22] In this way, the development of modern high-resolution printers has allowed photolithography techniques to create more precise patterns at a sub-ten-micrometer resolution to produce structures with higher architectural precision.[45]

Digital Micromirror Device™ (DMD)-based projection printing (DMD-PP) is considered an alternative to the traditional photolithographic approach based on which a DMD chip is utilized in place of the conventional photomask (Figure 2A).[46–48] Note that each DMD chip contains several micromirrors on its surface, which can be tilted ± 10° through the control of computer-aided design (CAD) modeling. In this way, the micromirrors can control the switch mechanism by convention, either by reflecting the light to the sample and inducing a photocrosslinking reaction (on) or by directing the light off collected by a

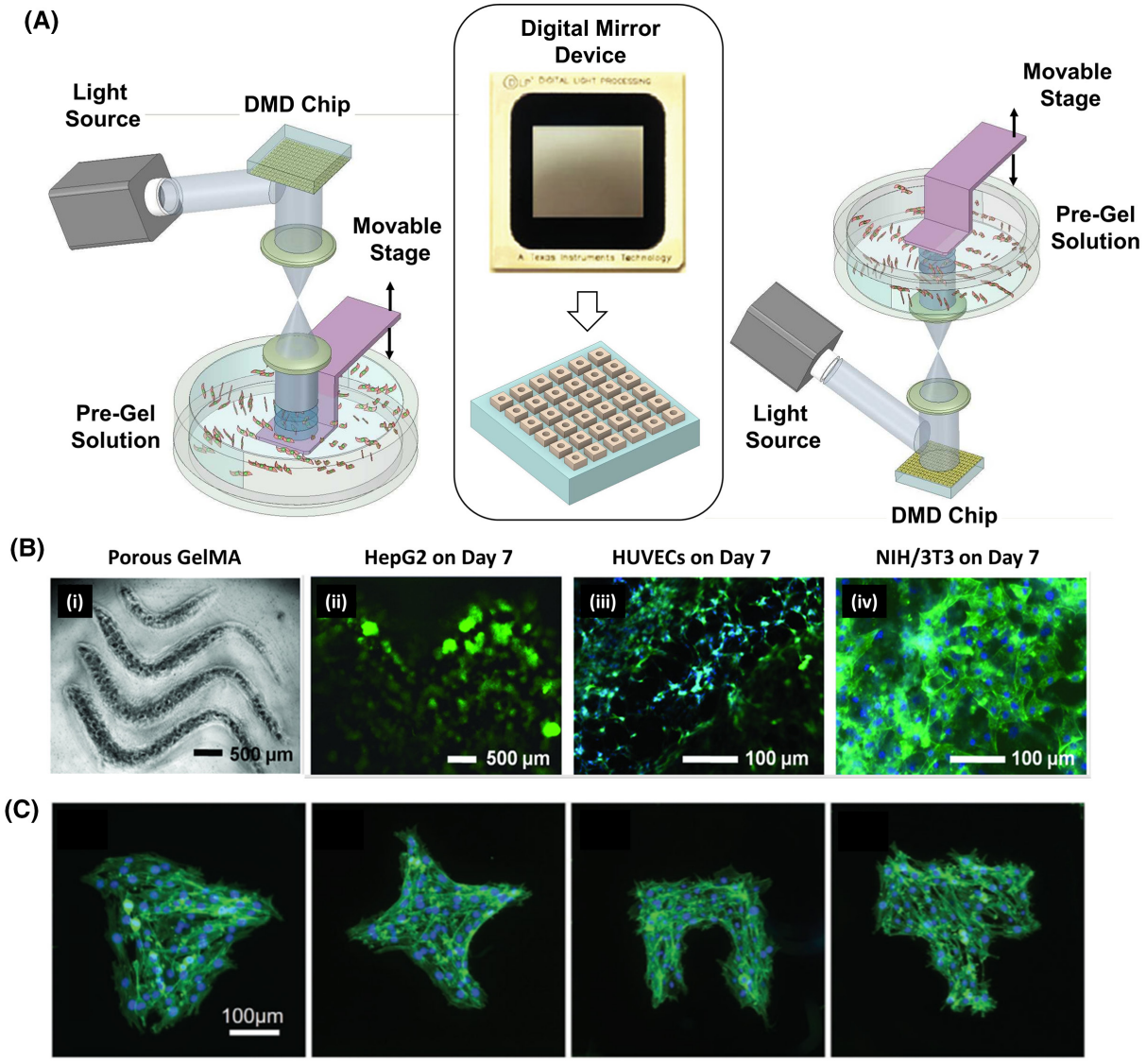

FIGURE 2 (A) A schematic illustration of DMD printing. (B) DMD bioprinting of (i) porous GelMA hydrogel. (ii–iv) bioprinting of HepG2, HUVECs, and NIH/3T3 cells on porous GelMA hydrogel patterns after 7 days of culture. F-Actin filaments were stained in green, and nuclei were counterstained in blue. Reprinted with permission from Ref. [46] Copyright (2018) Wiley. (C) Fluorescent microscopy images showing the encapsulation of NIH/3T3 cells in microgels building blocks fabricated by DMD-based 3D printing technology and microfluidic technology. F-Actin filaments were stained in green, and nuclei were counterstained in blue. Reprinted with permission from Ref. [50] Copyright (2020) Wiley

light absorber (off). Besides, the lateral moveability of DMD chips can expand its fabrication to large-scale 3D constructs.[49] For instance, the encapsulation of human umbilical vein endothelial cells (HUVECs), HepG2, and NIH/3T3 cells on DMD bioprinted porous gelatin methacryloyl (GelMA) structures with a predefined serpentine pattern indicated enhanced cell viability and proliferation (Figure 2B).[46] Yang et al. highlighted the effectiveness of utilizing the DMD technique with microfluidic technology to fabricate modular and customized cell-encapsulated hydrogels with micrometer-scale resolutions.[50] This technique does not require any physical mask as microgels are fabricated in a microfluidic chip using a shadowed light. In this regard, the functional microgels with different shapes (triangle, stars, concave, and convex shape) were easily obtained using an optofluidic maskless lithography system. The encapsulated NIH/3T3 cells were preserved viable and well-distributed inside GelMA blocks after 7 days of culture (Figure 2C).

2.2 | Soft lithography

Soft lithography is a non-photolithographic technique in which micropatterning is performed using elastomeric stamps, molds, or photomasks.[51] It is known as "soft"

because of the extensive use of soft materials. One of the most essential elements of soft lithography is elastomeric molds, typically composed of polydimethylsiloxane (PDMS). Its elasticity, sealing properties, and biocompatibility are significant characteristics that enable PDMS to be an excellent candidate for soft lithography.[52] Other favorable properties of PDMS include low interfacial free energy, malleability, hygroscopic, permeability to gas, optical transparency, and good thermal stability.[53] The following two steps can be implemented to fabricate a PDMS master. First, a pattern on a silicon substrate is obtained using photolithography technology. The silicon wafer is covered with a specific photoresist thickness, followed by photocrosslinking using UV light. The irradiation that goes through the photomask results in fabricating a patterned surface, which acts as the master for PDMS production. The second step aims to generate PDMS stamps with specific patterned surfaces to be applied in soft lithography strategies. For this purpose, followed by the curing process, a mixture of PDMS precursor and its crosslinker is poured into the silicon master, and when it is peeled off after heating, the obtained PDMS stamp presents the desired patterns on its surface.[54]

Compared to other lithographic techniques, soft lithography is more advantageous as it can overcome several limitations inherent to photolithography, such as high cost, time consumption, and resolution.[8] Furthermore, microdevices can be fabricated by utilizing soft lithography without the need for laborious photolithographic steps or clean rooms.[55] Although soft lithography is more capable for industrial applications, this technique can also be practical for micropatterning complex molecules because of flexible PDMS, as it can potentially form tight bonds with substrates.[29] Subsequently, such a feature allows for more reliable replication of patterning. In the field of TE, soft lithographic methods, such as microcontact printing and micromolding, are the most commonly employed strategies because both provide an easy way to control the pattern and the shape of tissues micron- and submicron-scale.[56]

2.2.1 | Microcontact printing (μCP)

μCP can be applied to replicate surface microstructures on planar and non-planar surfaces as well as to fabricate well-defined geometric patterns.[57,58] First proposed in 1993, Lopez et al. proposed μCP as a method to control the spatial distribution and concentration of proteins adsorbed onto patterned self-assembled monolayers.[59] The protein patterning is an essential tool for several biomedical technologies, such as microarrays, lab-on-a-chip, biosensors, and bioMEMS. Afterward, μCP rapidly became a standard method to pattern substrates due to its cost-effectiveness, simple protocol, and high accuracy at a micrometer-scale. Importantly, μCP can generate accurate patterns of proteins, peptides, and deoxyribonucleic acid (DNA) on substrates. Furthermore, different cell types, including cartilage,[60] bone,[61] skin,[62] liver,[63] and cardiac[64] cells, have been successfully manipulated using μCP. Generating such cellular components allows the production of constructs with higher organization and facilitates high precision in controlling the tissue architecture. Therefore, μCP has been extensively exploited to create geometrically organized structures.[65]

The stamp used in μCP can be produced using either photolithography or soft lithography.[57] Then, it is inked with desired bioactive molecules (Figure 3A). Following the stamp coating, the stamp is transferred to a material's surface. μCP is a relatively straightforward technology that ensures an easy ink transfer from the stamp to the material. The features of stamps, such as their mechanical properties, hydrophilicity/hydrophobicity, and polarity, often define their applications in μCP. For instance, elastomeric polymers, such as PDMS, are the most preferred material for μCP stamps due to their flexibility to adapt to any substrate. However, materials as the stamp should not swell or deform during the inking and stamp process.[57]

μCP has been applied for multiple purposes. One example implemented this technique to create microspots without topological features to study the contractile forces generated by cardiomyocytes derived from stem cells.[66] Meanwhile, it has also been widely employed to develop aligned cellular constructs. The development of 3D microtissues based on a thermally expandable hydrogel with microcontact-printed polydopamine patterns was successfully observed after subcutaneous implantation in a mouse model.[62] Compared with a simple monolayer of cells, as depicted in Figure 3B, the cell strips allowed a superior migratory activity and linear merging of translocated cells. Thus, organized microtissues proved their potential in developing structurally defined tissues by natural remodeling in vivo. Furthermore, the μCP scope can be extended to non-planar surfaces. In this regard, Borowiec and colleagues succeeded in fabricating microtopographies onto porous polycarbonate membranes accompanied by the transference of collagen, fibronectin, and laminin proteins into pre-designed geometries in one step, expanding its patterning to the third dimension.[67] The obtained 3D morphologies in the form of channels were cell-friendly and showed promising potential to influence both cell alignment and organization. Besides, different μCP methods can generate different protein-substrate adhesion outcomes. Protein micropatterns from stamp-off and covalent bond μCP methods can impact cells' focal adhesion and proliferation. The stamp-off μCP method

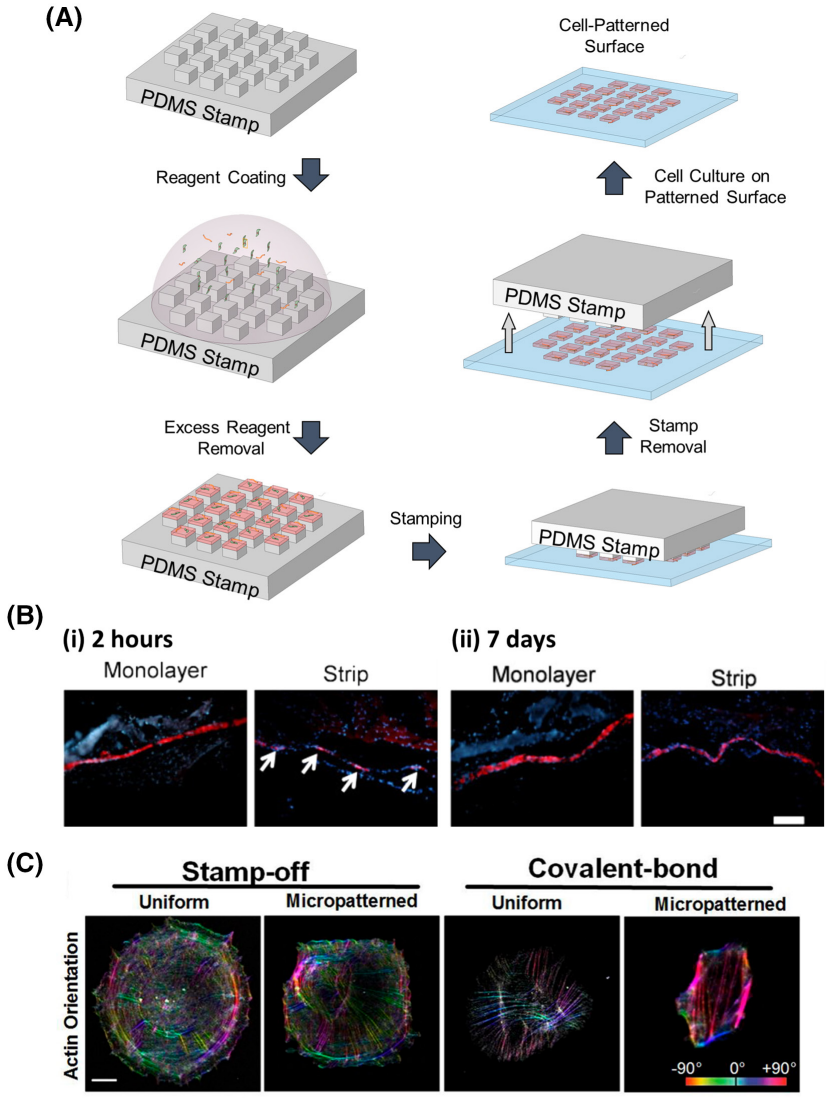

FIGURE 3 (A) Representation of μCP technique for generating patterned substrates for cell culture. (B) Fluorescent histological sections of monolayers and patterned cell microtissues were implanted in a subcutaneous mouse model after (i) 2 h and (ii) 7 days (scale bar: 100 μm). Reprinted with permission from Ref. [62] Copyright (2017) Elsevier Ltd. (C) Spatial maps of Actin filament orientation of cells on stamp-off and covalent bond μCP methods, with uniform and micropatterned fibronectin coatings (scale bar: 10 μm). Reprinted with permission from Ref. [68] Copyright (2018) American Chemical Society

is characterized by direct molecular adsorption while the covalent-bond μCP method, as the name implies, proteins are bonded by covalent bonds. The presence of micropatterns, in conjunction with the use of a covalent bond μCP method, increases the size of focal adhesion and the force of traction. Additionally, it is possible to observe greater viability and actin orientation of cells compared to the stamp-off μCP method (Figure 3C).[68]

Despite the widespread usage of μCP, it still presents numerous limitations affecting the reproducibility and accuracy of the method.[57,69] Patterned areas can be easily changed by the stamping conditions and by the hydrophobic nature of PDMS. Additionally, it is challenging to control the amount of material that is transferred during the printing process. To overcome the hydrophobicity of PDMS, the surface of PDMS stamps can be modified through oxidation using UV radiation, turning hydrophobic into hydrophilic surfaces and allowing water-soluble materials to wet the surface of the elastomer and permeate the bulk.[70] Another method to enhance the polarity of PDMS stamps is by using plasma-assisted polymerization. This process uses a mixture of argon (Ar) and hydrogen (H_2) gases to generate a microwave plasma treatment that activates the surface of the PDMS. Subsequently, it is treated using acrylonitrile grafting. When PDMS stamps are exposed to plasma treatment, surface radicals cause acetonitrile polymerization, creating a cyano-terminated polymer layer, thus increasing surface wettability.[71] For instance, Sadhu et al. produced amino terminated PDMS stamps via plasma polymerization, where the hydrophilic modification was stable for a few months.[72]

To overcome the high-cost limitation, strategies to create lithography-free fabricated stamps were explored simply by using accessible objects, such as injection needles and polystyrene microbeads.[73] Aside from the stamp itself, substrate properties bring some obstacles during the μCP technique.[57] Particularly, hydrogels are challenging target substrates for μCP as they cannot withstand the pressure of stamps. To overcome this challenge, chemical modifications of hydrogels, such as freeze-drying hydrogels or

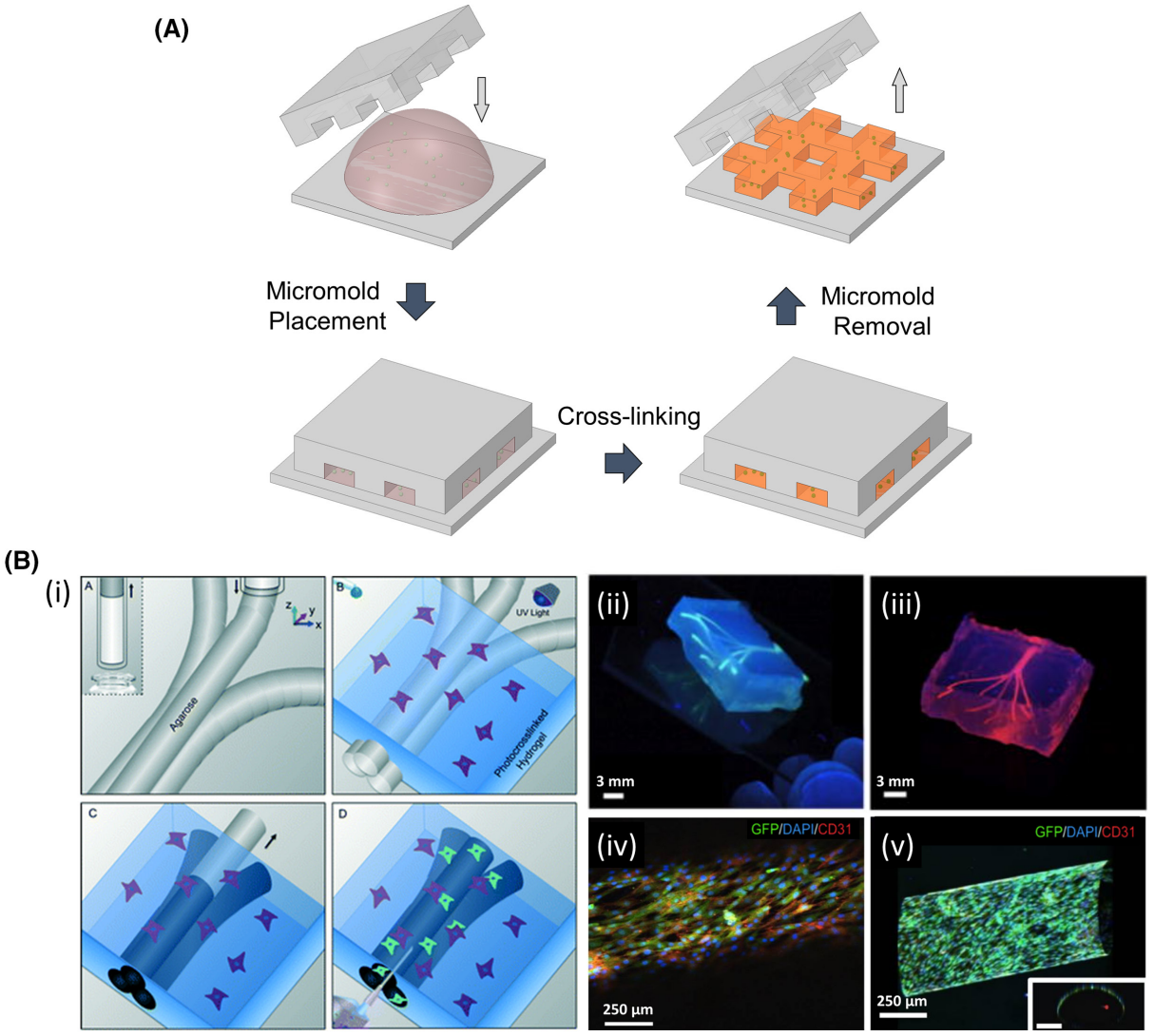

FIGURE 4 (A) A schematic representation of micromolding method for producing 3D microtissues in vitro. (B) (i) bioprinted agarose template fibers were used as templates to create a microchannel network in 3D hydrogels. (ii) 3D agarose templates (stained in green) embedded in the photocrosslinkable GelMA hydrogel. (iii) 3D branching microchannels fabricated inside of the hydrogel perfused with a fluorescent pink solution. (iv) confocal image and (v) longitudinal view section of lined endothelial cells inside the fabricated microchannels, stained for GFP/DAPI/CD31 markers. Reprinted with permission from Ref. [80] Copyright (2014) Royal Society of Chemistry

delivering pre-printed materials on poly(vinyl alcohol) (PVA), have been extensively considered in the literature to improve both µCP and hydrogel compatibility.[62] Moreover, bioprinting of cell-adhesive molecules, such as integrins that facilitate cell–cell and cell-ECM adhesion onto biomaterial substrates, should also be considered.[74]

2.2.2 | Micromolding

Micromolding is a methodology to manufacture hydrogels with the desired size and shape efficiently and reproducibly. Different patterns can be easily obtained by utilizing distinct varieties of molds.[75] Molds utilized to create microtissues in vitro are commonly made of polymers, such as PDMS and poly(methyl methacrylate) (PMMA).[76] However, other types of materials can also be used as molds. Notable examples include gelatin and sugar.[77,78] Generally, this technique relies on placing a polymer solution between a flat and a patterned substrate, followed by UV light or temperature-assisted crosslinking (Figure 4A). A successful micromolding technique is only achieved if an appropriate mold is produced. Hence, before fabricating molds, some important factors should be considered, such as the reactivity and biocompatibility of the material. In the broadest sense, micromolding is an easy-to-use approach that offers the advantage of decreasing the processing time. Photolithography and laser cutting are the

most common techniques used to fabricate micromolds. For instance, micropatterned photoresist molds produced on silicon wafers may be employed either as the micromolds or masters for fabricating PDMS molds. The main advantage of using a template relies on easy and fast replication of identical molds.[75,79]

Micromolding technique can be used to fabricate microvascular networks with several architectural features within hydrogels.[80] As vascularization is still one of TE strategies' main challenges, agarose fibers can be printed with a specific geometric form. They can then be used as a mold to create 3D perfusable tubes inside cell-laden hydrogels (Figure 4B). Briefly, after embedding the agarose microtubes in GelMA, the hydrogel was polymerized by UV light, and the agarose mold was detached. The perfusion of fluorescent microbeads demonstrated the successful fabrication of microtubes, mimicking the natural vascularization network of the native tissues. Additionally, the lumen of microtubes could be cultured using a monolayer of HUVECs, indicating a great expression of CD31 and confluent lining of cells, while the channels were completely perfusable. In another approach, vascular networks from mulberry leaves were used as a replica mold in order to create microchannels in various synthetic matrices.[81] Such a strategy allowed endothelial cells to grow and spread within the patterned network. Moreover, the proposed micromolding technique can engineer perfusable pathways for culture medium in hydrogels to address the long-term cell viability issues associated with most encapsulation systems. The micromolding method has been employed in other applications, notably the patterning of hydrogels and different types of substrates to improve cell orientation and develop functional microtissues and the fabrication of building blocks for organ-on-chip applications.[82–84]

2.3 | Microneedles

Microneedles have been widely employed as transdermal drug and vaccine delivery systems.[85] Microneedling intends to overcome the outermost skin layer that is impermeable to hydrophilic and large molecules. The expansion of high precision fabrication techniques in the 1990s gave rise to the diversity of microneedles to facilitate fluid transport. Before the arrival of microneedle technology, collecting body fluids and delivering drugs were associated with physical discomfort, as the instrument of choice was hollow hypodermic needles. The stratum corneum and epidermis are barriers to the permeation of topically applied drugs, limiting drug delivery through the skin.[86] Unlike conventional hypodermic needles, advancements in microneedle technology can overcome these limitations. They can avoid any interactions with nerve fibers and blood vessels when penetrating the skin, granting a more pain-free experience.[87] Furthermore, microneedling approaches can be applied not only to the skin but also to different organs or tissues. One excellent example is the use of microneedles for vascular drug administration, simply by penetrating the external surface of blood vessels.[88,89] For instance, the penetration of rhodamine B can be easily visualized by using rabbit abdominal aortas (Figure 5A). Generally, microneedles are between 25 and 2000 μm in height but can vary significantly in terms of functionality due to their different designs, manufacturing methods, and materials.[90]

Microneedles can be classified depending on their structure as either solid, hollow, dissolving, or coated microneedles.[91] Table 1 shows some advantages and disadvantages of different types of microneedles. Briefly, solid microneedles have been used as skin pretreatment to form microchannels on the skin surface, enhancing the permeation of drugs by allowing a direct diffusion into the dermal layer.[92,93] Hollow microneedles are a miniaturized version of hypodermic needles. In this type, liquid formulations can be injected through bores in the center of microneedles.[94–96] Although high doses can be delivered into the dermal layer, the fabrication of hollow microneedles is complicated as their structure is highly fragile.[91] Dissolving type microneedles are fabricated with biodegradable materials. When dissolving microneedles are used, the loaded drug is released through microneedle degradation.[97,98] Coated microneedles are solid-based microneedles with a coating of drug solutions that are released after microneedle insertion into the tissue.[99,100]

Most microneedles are fabricated using lithographic methodologies. To develop sharp microneedles, researchers have applied two different techniques, namely wet and dry etching. Wet etching is mainly used to manufacture solid microneedles in which unwanted material is retrieved after being immersed into a liquid chemical etchant. Conversely, in dry etching, which can be used for hollow and solid microneedles, inert or reactive gases are used at low pressures involving reactive ion etching.[91]

Various biodegradable and biocompatible materials, such as silicon,[94] dextrin,[101] glass,[102] ceramic,[103] maltose,[104] and galactose,[105] have been used to manufacture microneedles. The value of biodegradable biomaterials over non-degradable ones relies on their ability to decompose easily through the actions of living organisms. Biocompatibility is another crucial feature to avoid an exacerbated immune response and potential materials rejection. Therefore, polymeric materials are also commonly used for microneedle fabrication due to their biocompatibility and reusability. For instance, a detachable hybrid microneedle depot was proposed to deliver mesenchymal

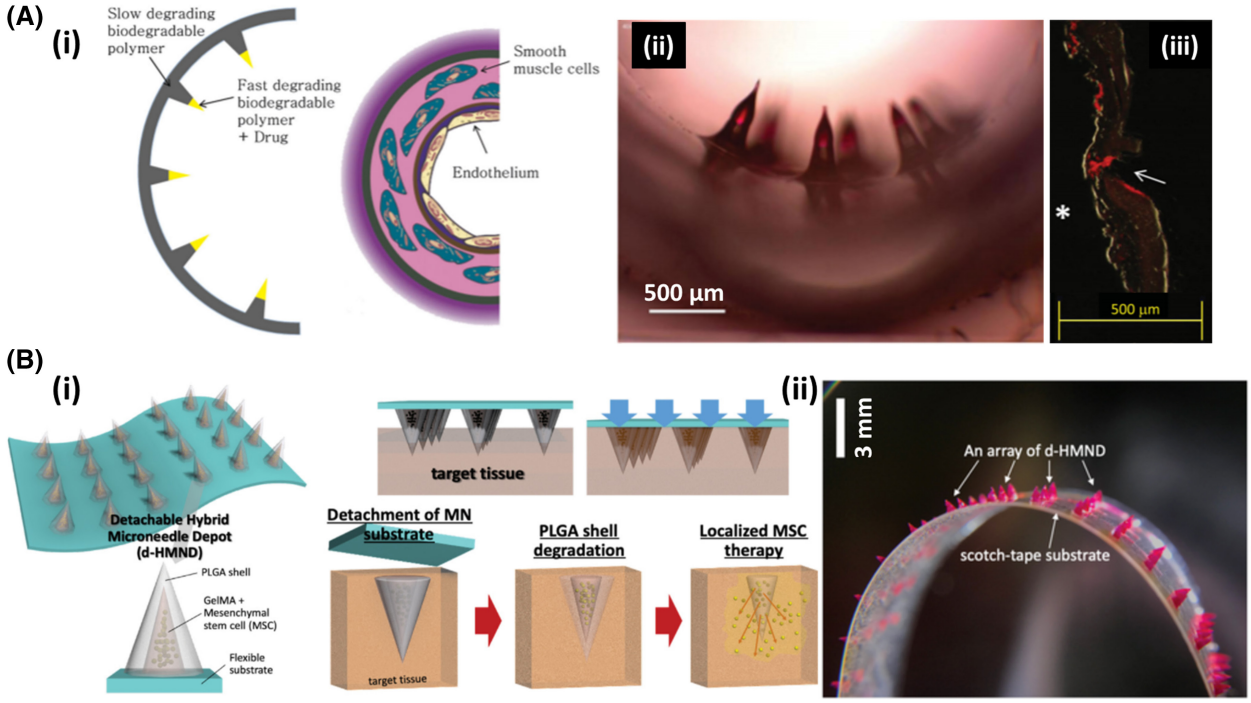

FIGURE 5 (A) (I) a schematic representation of vascular microneedles. (ii) biodegradable microneedle cuff coated with rhodamine B. (iii) penetration of rhodamine B using microneedles in a rabbit abdominal aorta. Reprinted with permission from Ref. [89] Copyright (2012) Wiley. (B) (i) a schematic illustration of detachable hybrid microneedle depot (d-HMND) and respective working mechanism for the delivery of MSCs into the injured tissue. (ii) image of the hybrid microneedles encapsulating MSC-laden GelMA surrounded by a pink-dyed PLGA shell. reprinted with permission from Ref. [106] Copyright (2020) Wiley

stem cells (MSCs) aiming for tissue regeneration.[106] These hybrid microneedles are surrounded by a poly(lactic-co-glycolic) acid (PLGA) shell while encapsulating MSCs embedded in GelMA hydrogel (Figure 5B). While the synthetic polymer facilitates the microneedle penetration and protects the encapsulated cells, the gelatin-based core preserves the viability of cells, providing an appropriate ECM microenvironment for cell migration and proliferation. After the degradation of the PLGA shell, the immunomodulatory MSCs start to migrate to the target tissue and release regenerative factors. Note that this strategy shows enormous potential for the treatment of skin wounds.

2.4 | Hierarchical assembly of tissue microstructures

Developing tissue-engineered structures by a classical "top-down" approach, where a scaffold is first conceptualized and subsequently seeded by cells, presents various limitations.[107] In contrast, "bottom-up" strategies, where smaller components are assembled to create larger constructs, address the hurdles of top-down approaches.[108] Bottom-up approaches can benefit TE applications as living tissues are often characterized by repetitive functional units. Despite the extensive use of bottom-up strategies, the challenge remains yet in fabricating highly complex structures to mimic the native tissues and organs.[109] As a result, self-assembly and templated assembly have been proposed for the microfabrication of 3D biomimetic engineered tissues. Additionally, it can be considered as an alternative for lithographic techniques as it allows fabricating features and structures for potential use in scaffolds for tissue engineering applications. The combination of micromolding and photolithographic techniques is also reported to generate structures for templated assembly.

2.4.1 | Self-assembly

Self-assembly operates on the principle that small, unrelated components are utilized to construct large and complex structures. It is a promising approach to create biomimetic 3D tissue constructs due to its high scalability. Essentially, self-assembly can occur through different mechanisms, including surface tension minimization, geometric and chemical recognition, and biological interactions.[110] Several bottom-up approaches to control the self-assembly of cells and cell-laden microgels have been proposed to generate complex tissues with specific microarchitectures. For instance, a successful self-assembly strategy was proposed using thermodynamic properties

TABLE 1 Advantages, disadvantages, and drug delivery methods of different microneedle strategies

Microneedle types	Advantages	Disadvantages	Drug delivery methods	References
Solid	Different types of materials can be used	Fracture of microneedles and limited surface area for drug absorption	Drug diffusion through the created microchannels	92,93
Hollow	High drug load	Fragility	Pressure-driven flow through needles	94–96
Dissolving	Facile manufacturing	Only biodegradable materials can be used	Dissolving needles after insertion to the target tissue	97,98
Coated	Enhanced peptide/protein stability	Temperature limitations, drug loss during the manufacturing process	Drug release from coating	99,100

of multiphase liquid–liquid systems to decrease the surface free energy of cell-laden hydrogels.[109] Briefly, these higher-order structures were generated because of the high surface tension of aqueous suspensions when cell-laden poly(ethylene glycol) (PEG) microgels were suspended in a hydrophobic liquid (Figure 6A). Aside from the biocompatibility, this approach allows the direct assembly of microgels, which was confirmed using a "lock-and-key" design.

DNA base pairing has also proven to be a suitable tool in self-assembly techniques.[111] Unlimited programmability properties of DNA allow for the construction of various macrostructures. In combination with microfabrication technology, Qi and colleagues showed the potential of controlled self-assembly of hydrogel modules using sequence-specific DNA to create complex structures.[112] The self-assembly was guided by the hybridization of the complementary DNA sequences (Figure 6B). This strategy is highly programmable and manageable, with the ability to fabricate structures with specific microarchitectures. Moreover, DNA-directed assembly of cells has generated more complex ECM structures with a better spatial resolution.[113] Unequivocally, the DNA-directed assembly approach holds great promise for TE applications. One research group implemented image-guided intra-scaffold cell assembly and DNA-directed assembly to fabricate biomimetic spinal cord tissue.[114] Results demonstrated that DNA assembly rather than a layer-by-layer technique allowed better cell viability. A more recent application of DNA self-assembly was described in the development of chemotherapy. Employing DNA self-assembly strategies to create a drug-conjugated DNA hydrogel reduces the tumor relapse rate.[115] Another programmed assembly consists of the presence of acrylate groups on the surface of microgels. In the presence of thiolated polypeptide crosslinkers, the microgels can be grouped into porous 3D macroconstructs aiming for different regenerative purposes.[116]

Moreover, amino acids and carbohydrates are capable of self-assembly. As such, carbohydrates possess highly specific oligosaccharides and glycopeptides, thus, allowing for complex arrangements.[117] This type of assembly mechanism relies on non-covalent interactions, such as hydrogen bonding and electrostatic interactions. Interestingly, peptides can be used to change specific functional groups of the building blocks. This type of self-assembly relies on the derivatives of arginine–alanine–aspartic amino acid sequences, which have a crucial role in regulating interactions between cells and ECM.[118] The use of biological interactions is another strategy for assembling tissue constructs. As exemplified, the self-assembly of MSC-laden microniches within gelatin structures was employed to produce macroscale tissue constructs.[119] Here, the increase in

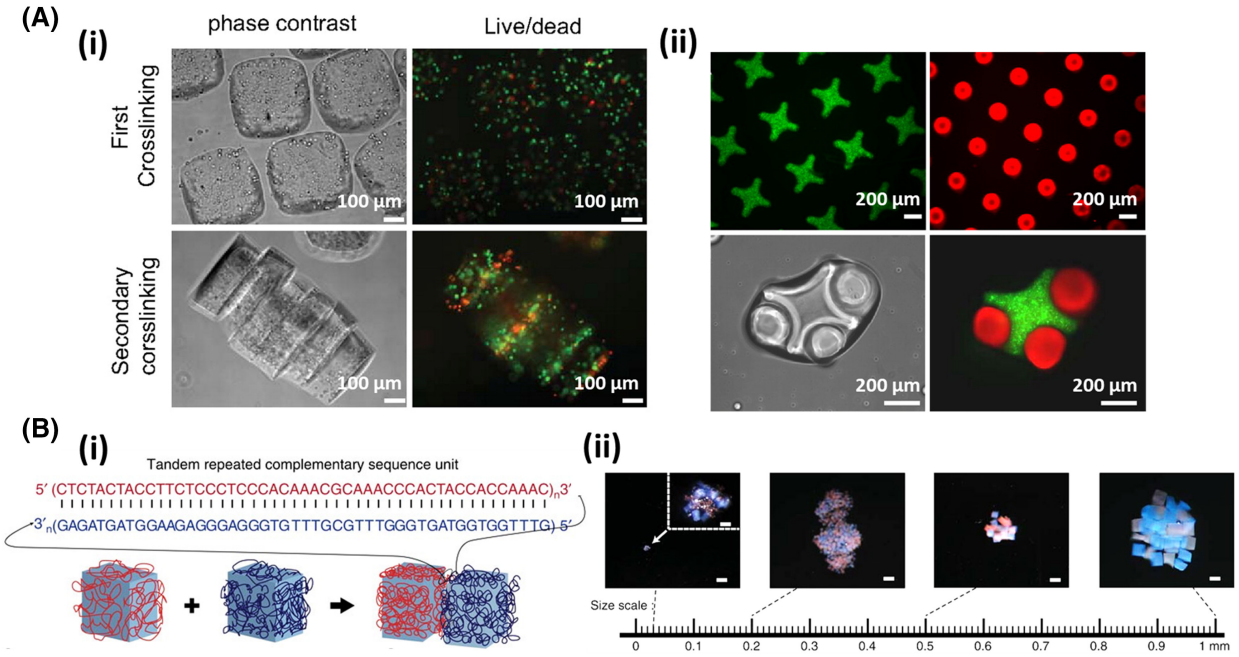

FIGURE 6 Self-assembly of micrometric scale tissues to generate macrostructures for TE purposes. (A) Assembly of cell-laden microgels. (i) Phase-contrast and corresponding live-dead images of cell-laden PEG microgels before and after assembly. (ii) "lock-and-key" design to generate a direct assembly of cross- and rod-shaped hydrogels (stained with FITC-dextran and Nile red, respectively). Reprinted with permission from Ref. [109] Copyright (2008) National Academy of Sciences. (B) DNA-based self-assembly. (i) Illustration of microgels conjugated with giant-DNA. (ii) Giant-DNA-directed cubes assembly. Reprinted with permission from Ref. [112] Copyright (2013) nature publishing group

cell–cell or cell-ECM interactions allowed for the self-assembly of the microniches. This self-assembly method is a potential solution to persisting problems in TE relating to cytotoxicity caused by traditional crosslinking methods and the problem of the loss of MSC's functional phenotype.

Besides the construction of larger tissues, the self-assembly of peptides can modulate the topography of specific surfaces and scaffolds by changing the molecular structure in which the self-assembly occurs. For instance, the self-assembly of peptide amphiphiles has been studied in order to generate aligned nanoscale fibers suitable for cell culture.[120] Briefly, Stupp's group developed plaques with filamentous textures that spontaneously break into large arrays of aligned nanofibers through a thermal reaction. When mixed with cells and extruded into a salt solution, it generates monodomain gels of aligned filaments, where cells direct their orientation in a 3D environment. Such approach was already established for different tissue engineering strategies, including for neural, bone, and cartilage therapeutic applications.[121–123]

2.4.2 | Templated assembly

Templated assembly is a technique that follows the idea of using a basic template and lock framework to generate macrostructures on a large scale.[124] More specifically, this technique allows researchers to utilize a pre-existing guide to build additional structures. The high matching precision of the method permits the creation of complex hierarchical structures. The encapsulation of mouse embryoid bodies within templated constructs using a combination of micromolding and photolithography techniques was proposed to understand how different biomaterials can interact with cells. For that, embryoid bodies were encapsulated within the interface between GelMA and PEG substrates. Interestingly, results showed that the sprouting of the embryoid bodies was more favorable toward the GelMA microgels.[125] One of the most common strategies to create porous structures relies on the use of sacrificial bead templates, which introduce well-ordered and monodisperse pores into materials.[126] In the past, the attempts to assemble complex tissue structures were less specific. Having a high spatial microenvironmental control over biological systems is essential, especially for processes that involve the migratory formation of vasculature, where gradient patterns should guide the cell movement.[127] Thus, a templated assembly can be beneficial for vascularization and endothelial cell stabilization.

Templated assembly has also been envisioned for making vaccine and viral serology reagents through viral recreation.[128] For example, McCoy and colleagues used a

template assembly to create a virus-like particle. A protein macromolecular framework was made on an electrostatic template using amine-terminated dendrimers. Then, it was locked in with a cementing protein capable of binding to the P22 virus-like particle. Upon the removal of the dendrimer, the remained framework was entirely made of protein.[129] Templated assembly has also been explored on liquid surfaces. More specifically, liquid surfaces have been manipulated to form reconfigurable templates. Chen et al. explored microscale assembly using liquid-based surface controlling via chamber shape changes, waveform, and symmetric modes, and harmonic order of standing waves.[130] This liquid-based bottom-up technique can be used for TE by involving polystyrene beads as microcarriers for cell assembly. This liquid-based method is unique as it utilizes the frequency and acceleration of a liquid surface rather than a more traditional surface. Independent on the symmetry of chambers and the presence or absence of the pillars, the assemblies were easily achieved. Furthermore, low- and high-ordered structures were generated depending on different vibrational parameters, based on symmetric modes and harmonic orders. The structure of the assembly could be modified by resetting the vibrational parameters.

2.5 | Microfluidics

The use of microfluidics approaches has been increasing in the last several years within the TE field. Microfluidics is a powerful technology characterized by fluid flow control through microchannels with dimensions of tens to hundreds of microns.[131] Additionally, it is possible to encapsulate multiple cells or even create single-cell constructs for multiple TE purposes. Regarding the design of microfluidic devices, the standard material platform is made of PDMS.[132] To develop microfluidic devices, a PDMS elastomer and a glass surface are often used. The surface is treated with oxygen plasma to increase its hydrophilicity.

The three most common geometries to produce droplets using microfluidics are T-junction, co-flow, and flow-focusing (Figure 7A).[133] In a T-junction geometry, a perpendicular channel injects a dispersed phase into a channel that carries a continuous phase.[134] In contrast, in a co-flow geometry, a droplet is obtained by injecting the dispersed phase in an inner channel within a larger capillary in which flows the continuous phase. Ultimately, in a flow-focusing device, two counterflowing channels containing a continuous phase are used to squeeze a dispersed phase through the main channel.[135] To control the size of developed droplets, the flow rate of the continuous phase should be changed. For example, a higher flow rate generates more force, resulting in smaller droplets.

Microfluidics technology is essential due to its broad application in different fields, such as chemistry, biological analytics, and MEMS.[136] The use of microfluidics has been noted for studying concentration gradients, mimicking the natural vascular network of living tissues, and recreating in vitro models for drug research.[137] Microfluidic devices can be used to solve many challenges encountered in TE strategies. Some of these issues include gathering sufficient cells and ensuring cell viability and physiological functionality.[56,138,139] These issues become more challenging to control as tissue constructs become larger or the diffusion of essential biomolecules is limited. Therefore, a microfluidic device named microfluidic perfusion bioreactor can alleviate these mentioned challenges by providing cell culture media to the cells in an exact and directed manner through the manipulation of fluid flow in microchannels.[140,141] Some advantages of the bioreactor include high spatiotemporal control of cell culture media delivery, the ability to use various cell culture media types, and the high-throughput study of different cell phenotypes due to the availability of multiple cell culture chambers. Finally, a microfluidic perfusion bioreactor has excellent application to TE due to its ability to effectively mimic the natural cellular environments by connecting stacked microfluidic devices through membranes or channels.[142]

Polymeric particles are valuable as injectable carriers of biological molecules, such as cells, proteins, and DNA.[143] The production of particles using solvent emulsion techniques faces multiple difficulties due to widespread polydispersity, heterogeneous droplet sizes, and damage to encapsulated species, threatening its use for biomedical applications. To confront such problems, microfluidics has been used to generate monodispersed droplets containing a specific amount of biological materials, which can be utilized as templates for assembling microparticles with distinct physical and chemical properties.[144] After acquiring the monodisperse droplets, large constructs can be obtained using them through several methods, including polymerization, temperature gelation, ionic crosslinking, solvent evaporation, and colloid assembly, among others.[143] For instance, a gelation method was proposed to produce monodisperse self-assembling peptide microbeads using a microfluidic device. The technology relied on incorporating powdered salts in a continuous (oil) phase using an axisymmetric flow-focusing device. Results revealed the successful production of the microbeads, being monodispersed in size and shape (see Figure 7B for more detail). Additionally, the viability of encapsulated cells remained uncompromised, demonstrating their capabilities for TE strategies.[145] Microfluidics was also used to generate porous microbeads to be employed as injectable cell-laden scaffolds. Interestingly, a pulsed electric field (EF) combined with a microfluidic oil-in-water (O/W) emulsion generator is a potential solution to overcome the

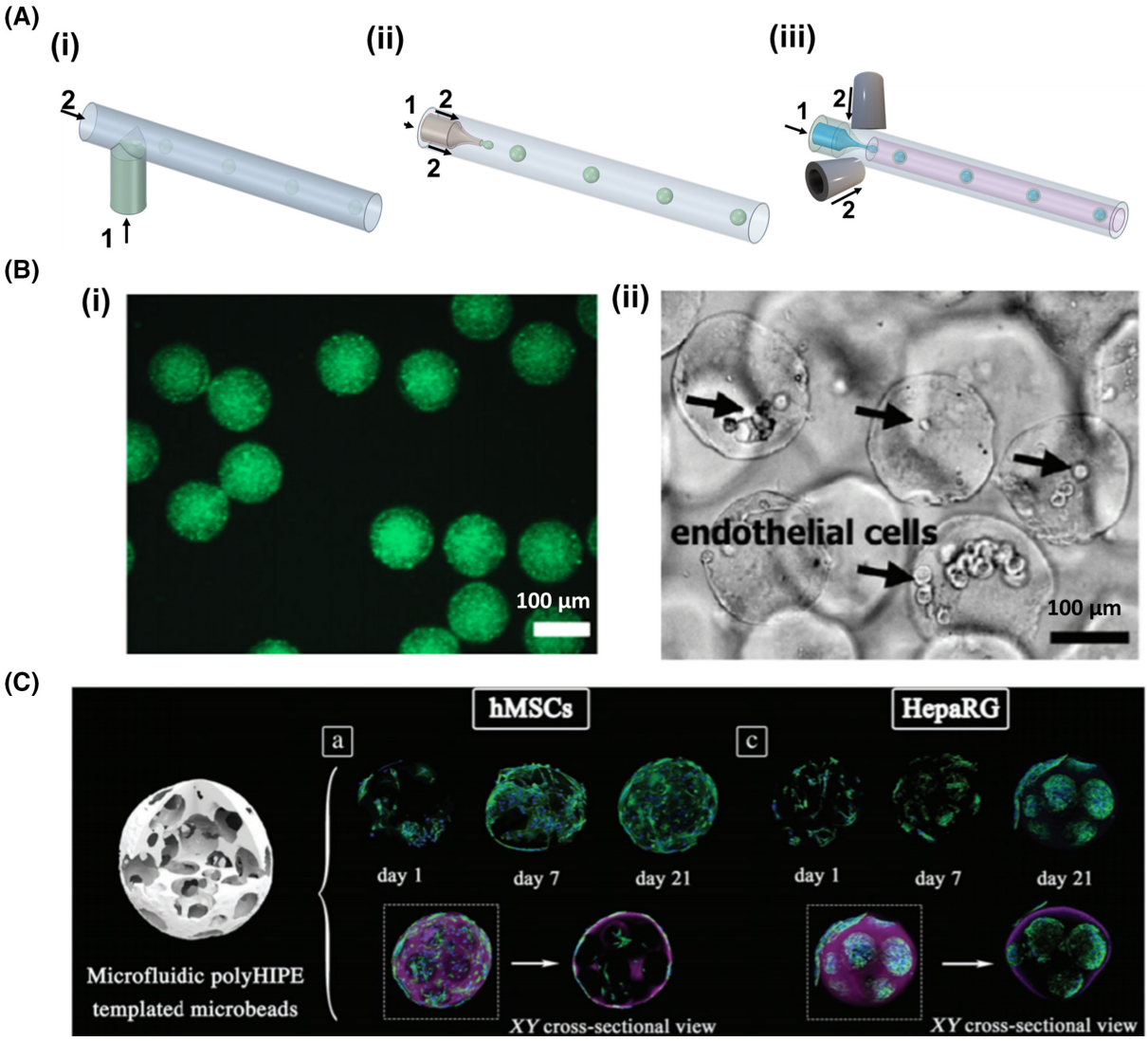

FIGURE 7 (A) Microfluidic device with (i) T-junction, (ii) co-flow, and (iii) flow-focusing geometries to fabricate microdroplets (1 = dispersed phase; 2 = continuous phase). (B) (i) fluorescent staining of self-assembling peptide microbeads produced by microfluidics. (ii) phase contract microscopy of microbeads encapsulating endothelial cells. Reprinted with permission from Ref. [145] Copyright (2010) American Chemical Society. (C) Fluorescence images of MSCs and HepaRG cells cultured on porous microbeads templated produced by microfluidics. F-Actin is represented in green and nuclei in blue. Reprinted with permission from Ref. [146] Copyright (2018) Wiley

production of poorly interconnected or many tiny pores. After testing with MSCs, the porous beads revealed high cell viability and non-cytotoxic characteristics and allowed for superior cell infiltration and growth compared to traditional microbeads (Figure 7C). Similarly, when cultured with HepaRG cells, the porous microbeads showed non-cytotoxic properties, thus highlighting its potential for therapeutic applications as a cell carrier.[146]

2.6 | Fiber spinning

The fabrication of fibers has attracted much interest due to their capabilities in developing high-ordered nanomaterials and textiles.[147–149] The presence of interconnected pores in fiber meshes allows controlling cell distribution while avoiding the formation of necrotic cores. The fibers' diameter can vary between micro- and nanoscale. Moreover, fibers can be used as a drug delivery approach or guides for cell alignment, a critical feature to induce functionality for some tissues.[150] Different fiber fabrication techniques have been widely utilized for TE applications, such as electrospinning, wetspinning, microfluidic spinning, and meltspinning.[151]

Electrospinning has gained interest due to its potential application in various fields, particularly in TE, because such a technique can be applied to construct nanofibers that mimic the native ECM. Electrospinning is a process

to fabricate ultrathin fibers by forcing a polymeric solution through an electric field, creating a stretched and thinned charged jet, ultimately resulting in solid micro- and nanofibers (Figure 8A).[152] Electrospun fibers are characteristically defined by their large surface areas, high porosity, small pore size, and low density.[153,154] These characteristics are desirable because they can be utilized for many biomedical applications, especially in wound dressings, drug delivery systems, cell differentiation and alignment, and antibacterial films.[155,156] Thickness and fiber orientation may be controlled by manipulating various parameters. Pre-processing parameters, such as solvent type and concentration, or the processing parameters, such as flow rate, the distance between spinnerets and collector, and voltage, can be modified to control such properties.[157,158] As these areas are further explored, the classical electrospinning process has been improved to produce more advanced techniques, such as blend, coaxial, and emulsion electrospinning, thus expanding the portfolio of future applications.[159] Beyond the valuable properties of these fibers, electrospinning is also comparatively cost-efficient, capable of using a multitude of solvents, easily scalable in regards to production, and easy to use.[160,161] Furthermore, this technique can be applied to biological moieties that can be easily denatured, such as proteins, growth factors, drugs, and even genes.[162] Despite these advantages, there are still challenges related to the implementation of electrospinning technology. It is imperative to understand the transformation process of a liquid solution into a solid fiber to precisely tune the geometry, mass production, and characteristics of the fibers. Moreover, obtaining fibers with a diameter in the nanometer scale using melt electrospinning is also challenging.[157]

Cells have also been incorporated into the electrospinning process to construct 3D cell-laden scaffolds. For instance, cells were added into four different polymeric solutions, namely pullulan, gelatin, collagen, and poly(ethylene oxide) PEO, to generate cell-laden scaffolds through electrospinning. It was found that the cells incorporated into the pullulan and gelatin were more protected from high voltages used in the process. In addition, cell viability following encapsulation was higher using these types of materials.[163] A large number of other synthetic polymers (such as PEG and PVA) or natural polymers (namely hyaluronic acid, silk, and alginate) have also been used to fabricate electrospun fibrous scaffolds.[164,165] For example, the fabrication of composite nanofiber structures using gelatin-polyaniline (PANI) nanofiber doped with camphorsulfonic acid (CSA) was envisioned to culture myoblasts.[166] Gelatin has weak electrical conductivity;

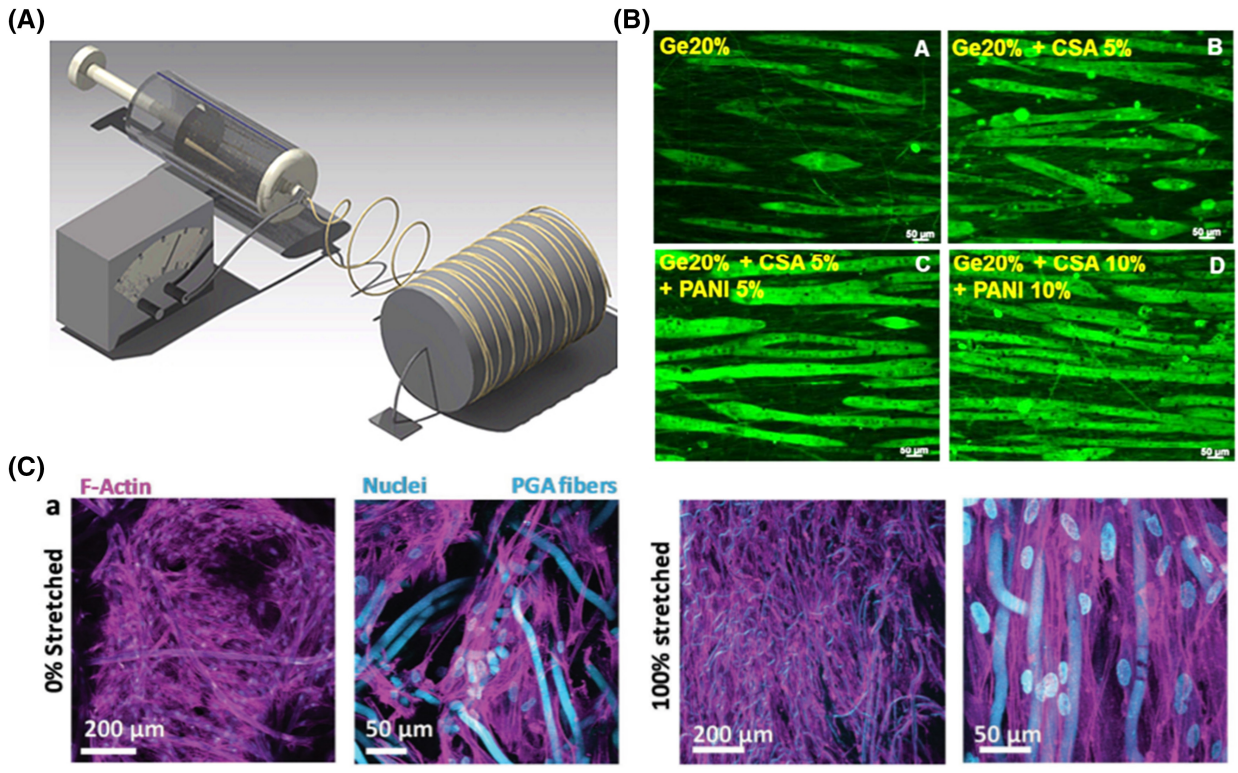

FIGURE 8 (A) A schematic illustration of a conventional electrospinning set-up. Reprinted with permission from Ref. [152] Copyright (2016) Wiley. (B) Myotube formation on aligned electrospun in different composition conditions. Reprinted with permission from Ref. [166] Copyright (2017) American Chemical Society. (C) Confocal images of fibroblasts cultured over non-stretched and stretched PGA scaffolds. Reprinted with permission from Ref. [176] Copyright (2018) Wiley

however, electrical conductivity is crucial when skeletal muscle cells are cultured. To overcome this problem and obtain electrically conductive nanofibrous scaffold structures, PANI nanofibers were used. The composite gelatin-CSA-PANI nanofiber could improve myoblast differentiation and myotube formation compared to gelatin or gelatin-CSA nanofibers (Figure 8B).

The wetspinning is a non-solvent–induced phase inversion technique that uses a coagulation bath to extrude a pre-polymeric solution. After the extrusion of a thin polymeric filament, the polymer solidifies, giving rise to a long fiber, with a diameter usually in the order of tens to hundreds of micrometers.[167] Wetspinning has been proposed to produce fibers for TE applications utilizing different natural and synthetic polymers, including chitosan,[168] poly(ε-caprolactone) (PCL),[169] PLGA,[170] and starch-based materials.[171] The microfluidic spinning or co-axial flow system is also a spinning technique similar to wetspinning. However, instead of using a coagulation bath, a crosslinking agent is provided by a coaxial flow. Both microfluidic spinning and wetspinning can overcome issues related to high voltage necessary for electrospinning technique.[172] An all-aqueous microfluidic spinning method was recently proposed for producing biomimetic perfusable microtubes. Different sizes and shapes of perfusable microtubes were obtained simply by manipulating the fluid dynamics at the microscale.[173] However, meltspinning is a technique that melts polymers and extrudes them through a micron-sized spinneret with a specific geometry to fabricate thin fibers continuously.[150] Although this technique works only with synthetic polymers, such as poly(lactic acid) (PLA)[174] and polyethylene terephthalate (PET),[175] the obtained fiber strands present relatively high mechanical properties. Fabricated meshes using meltspinning have been used as scaffolds for TE applications. For instance, commercially available polyglycolic acid (PGA) meshes fabricated by meltspinning have been used for tissue regeneration and cell alignment.[176–178] Notably, a simple strategy to align fibers was proposed by Hosseini and colleagues by coating a PGA mesh with poly(4-hydroxybutirate) (P4HB) acid, heating above the glass-transition temperature of both polymers, and then stretching to produce aligned scaffolds. The increase in cell alignment was observed after fibroblasts cultured on the stretched fibers (Figure 8C).[176]

2.7 | Bioprinting

The rapid progress in biotechnology has allowed automated systems to dominate the traditional manual manufacturing processes.[179] Automating processes have significantly changed the course of microfabrication by increasing productivity and quality while reducing costs.[180] The use of 3D bioprinting techniques has contributed positively to the rising demands to fabricate biomimetic and functional tissues and organs.[141] While such techniques hold great promise, barriers such as clinical trial approval still stand in the way of full commercialization.[181,182] To date, different non-living printing scaffolds have been applied in patients for bone and cartilage reconstruction or even for oral pill manufacturing. However, the clinical translation of 3D-printed living tissues is still challenging due to the maintenance of mechanical properties of the fabricated tissues and difficulties in achieving physiological vasculature and heterogeneity.[183] However, 3D bioprinting has numerous advantages for the production of customized patient-specific cellular constructs.[184] The production of 3D biological structures is usually made from biomaterials, cells, and biomolecules termed bioinks. Bioinks can be made of natural, synthetic, and hybrid materials.[185] In general, the following steps are required to engineer a tissue structure using 3D bioprinting: (1) generating a computer-aided design (CAD) for the 3D model of choice, (2) choosing and applying bioinks to the apparatus, (3) following the fabrication steps from the CAD model, and (4) assembling the 3D structure.[186,187] In the next sections, different techniques used for bioprinting living tissues will be addressed.

2.7.1 | Stereolithography bioprinting

The intersection between material fabrication and stereolithography came about in 1986 when Charles W. Hull invented an apparatus necessary for creating 3D objects via stereolithography.[188] Nowadays, stereolithography bioprinting is a technique similar to stereolithography microfabrication that utilizes a light source, usually UV light, to polymerize biomaterials during the printing process. A stereolithography-based bioprinter is composed of a reservoir containing photocurable resin, a laser source, and a computer that tracks the resin liquid surface under the action of a deflection mirror (Figure 9A).[189] Through single-photon absorption onto the photocrosslinkable material, a 2D structure with specific patterns is developed. Then, 3D designs are fabricated using a layer-by-layer build-up. Stereolithography 3D bioprinting has multiple advantages, such as keeping high cell viability and resolution, as well as its rapidness.[190]

Multi-material stereolithography bioprinting opens opportunities for creating highly complex 3D structures, such as tissues with different phenotypes of cells. However, the current technology still encounters various challenges, as using various materials is inefficient and time-consuming. Regarding this, a promising strategy is

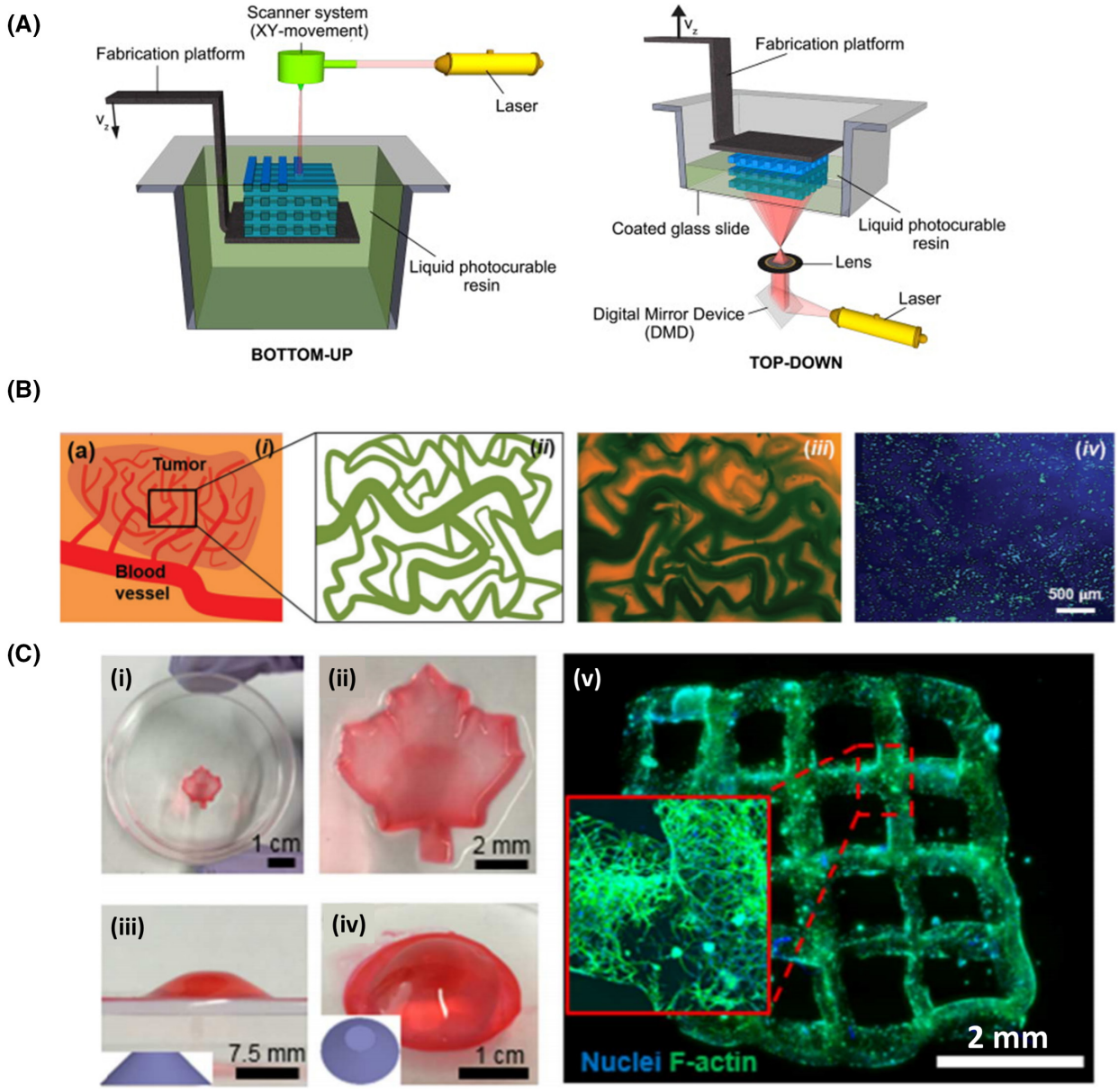

FIGURE 9 (A) Schematic representation of bottom-up and top-down stereolithographic bioprinting setups for the fabrication of tissue-engineered structures. Reprinted with permission from Ref. [189] Copyright (2012) Elsevier. (B) (i,ii) representation of a tumor angiogenic model. (iii) vasculature design bioprinted in the hydrogel. (iv) encapsulation of MCF7 cells (blue) inside of the microvasculature and seeded with HUVECs (green) in the channels. Reprinted with permission from Ref. [192] Copyright (2018) Wiley. (C) 3D bioprinting of (i,ii) a maple leaf pattern and (iii,iv) truncated cone structure. (v) Fluorescence image of NIH-3T3 cell-laden bioprinted structure after 5 days of culture. Reprinted with permission from Ref. [193] Copyright (2018) American Chemical Society

using a dynamic fluidic system, which controls numerous liquid photopolymers. Here, complex 3D structures are rapidly produced through an effective material exchange method.[191] To speed up the fabrication of multimaterial constructs, another group used a DMD-based bioprinter combined with a microfluidic device. The microfluidic platform was made with a PDMS chamber between PMMA sheets, where multiple inlets allowed the injection of different bioinks.[192] The presence of a PDMS elastomeric membrane in the microfluidic chip along with the programmed insertion of bioinks allowed the formation of 3D constructs. Then, the bioprinting of structures resembling biological tissues was achieved using two to four different bioinks with a smooth transition among them. A bioprinted pattern imitating the tumor angiogenesis was obtained by encapsulating breast cancer cells (MCF7) in GelMA and then seeded with HUVECs in vascular channels (Figure 9B).[192] Such strategy has potential

for better understanding the cancer progression and angiogenesis. Like many stereolithography bioprinters, photopolymerizable hydrogels that require photoinitiators with a peak absorbance in the UV range are commonly employed, which poses problems due to the UV radiation's ability to damage cellular DNA as well as the maintenance of cell viability. To solve this problem, an eosin Y (EY) to induce the photoinitiation in the visible light range and cell-adhesive GelMA hydrogels were used for stereolithography bioprinting.[193] In the latter study, a maple leaf and cone patterns were used (Figure 9C) to demonstrate the capability of the system to print various EY-GelMA patterns. During this implementation, researchers observed good cell proliferation and the formation of a 3D intercellular network within the EY-GelMA hydrogels, indicating the excellent potential of the system for the biofabrication of tissues.

2.7.2 | Inkjet bioprinting

Originating from image and text printing, inkjet bioprinting is a non-contact technique that has been gradually adapted for the fabrication of 3D tissue-engineered constructs. During inkjet bioprinting, biomaterial droplets are layered onto a substrate in a predesigned manner to create tissue structures.[194] Analogous to a traditional printer, an ink cartridge storing a hydrogel pre-polymer solution is used to form a pattern of the desired 3D structure.[195] Inkjet 3D bioprinters are categorized as either continuous jet or drop-on-demand printers. In continuous jet printers, ink droplets are continuously streamed by a pressure pump. Conversely, drop-on-demand printers are more efficient as they produce an exact amount of ink for the printing process.[196] Drop-on-demand printers are further classified by how they extrude bioink droplets. Most conventional approaches include thermal, piezoelectric, or electrostatic methodologies. Inkjet printers that utilize thermal techniques rely on air bubbles that generate enough pressure to eject ink droplets from a nozzle. In contrast, piezoelectric inkjet printers rely on piezoelectric ceramic pressure, limiting the type of biomaterials that can be used.[197] To overcome that limitation and increase the compatibility of inks, Hoch and colleagues modified gelation through methacrylation and acetylation groups. As less methacrylated gelatin generates highly viscous hydrogels, responsible for nozzle clogging, researchers added acetyl functionalities to highly viscous methacrylated gelatin to decrease the viscosity and prevent gel formation. Besides creating a proper printable hydrogel for piezoelectric inkjet bioprinters, the insertion of photochemically reactive and inert groups did not change the biocompatibility of cells.[195] Electrostatic inkjet bioprinting operates on a similar principle. Rather than experiencing a pressure wave, the absence of a voltage pulse triggers the shape restoration of pressure plate and releases the droplet.[198]

Inkjet bioprinting has notable advantages, such as a plethora of cell and material types that can be printed, cost-effectivity and efficiency, and its relatively high yield of cell viability, ranging from 80% to 90%.[199,200] However, there are still challenges in using inkjet printers to print mammalian cells. For instance, piezoelectric inkjet printers have a working frequency ranging from 15 to 25 kHz, a sonification frequency that may cause damage to the cellular membrane.[201] Besides thermal and mechanical stress to which cells are exposed, clogging is frequently found in this printing technique.[48] Another limitation to consider about inkjet bioprinting is related to the viscosity requirement of bioinks. Generally, an ideal bioink should be viscous enough to pass through the nozzle without compromising the overall structural integrity. A related problem to bioink viscosity lies in the cell density. Typically, a high cell density is ideal in bioprinting because it yields better viability of tissues and some tissues require high cell density to be functional. However, this means that the frequency of clogging will be higher.[202]

Inkjet bioprinting has been used to fabricate constructs with superior mechanical properties to form bone and cartilage tissues. Instead of using multiple steps for scaffold synthesis and cell encapsulation, Gao and colleagues produced a biocompatible PEG scaffold that includes acrylated Arg-Gly-Asp (RGD), acrylated matrix metalloproteinase (MMP), and MSCs. By developing a photopolymerizable PEG hydrogel bioink in one step, the clogging problem decreased while the bone and cartilage differentiation were significantly enhanced.[203] Later, the same group added GelMA to this one-step printing technology. Likewise, the inkjet bioprinting of MSCs in a GelMA and PEG photocrosslinked hydrogel was indicated to promote an excellent osteogenic and chondrogenic differentiation capacity.[204] In a different strategy, the embedding of MSCs in a less stiff agarose-collagen hydrogel (18.1 ± 3.5 kPa) was envisioned to promote the modulation of cell morphology and MSC differentiation into an osteogenic phenotype. After the inkjet bioprinting, the results revealed improved osteogenic differentiation and increased cell spreading and branching compared with stiffer agarose-collagen hydrogels, indicating that this bioink could be a potential approach for successful bone tissue formation.[205]

2.7.3 | Laser-assisted bioprinting

Laser-assisted bioprinting utilizes a laser as the energy source to stream biomaterials and cells. Laser-assisted bioprinting devices are comprised of (1) a pulsating laser

source; (2) a target or ribbon layer containing material that can absorb the laser energy (i.e., gold, silver, or titanium) along with a layer with the biological material; and (3) collector support.[206] The laser pulse induces the metal film's vaporization that results in droplet formation. Then, the material is subsequently deposited onto the receiving substrate. The resolution of laser-assisted bioprinting is at the picolitre level, and the organization of fabricated 3D structures can be controlled up to a single-cell level. Additionally, the proposed technology can be performed directly in situ (Figure 10A).[207] Increased bone formation was found by printing MSC-laden particles of nano hydroxyapatite-collagen into a mouse calvaria defect. Having the bioink in a disk geometry was significantly better to obtain mineralized bone tissue than in a ring geometry (Figure 10B).

As laser-assisted bioprinting does not contain nozzles, no clogging problem occurs during the bioprinting process.[208] Aside from being nozzle-free, laser-assisted bioprinting is also a non-contact printing technique. Such features allow for better cell survival rates by minimizing the shear stress that cells sense compared to other bioprinting strategies.[209] However, some limitations are associated with laser-assisted bioprinting, including its time consumption and high cost.[210]

Laser-assisted bioprinting has effectively developed 2D and 3D patterns for several applications, including for human adipose tissue-derived stem cells (ASCs) differentiation, bone repair, and creating complex skin substitutes.[211–213] In one example, Michael et al. successfully generated skin substitutes via laser-assisted bioprinting. After 11 days of transplantation in mice, the developed 3D-like skin was connected entirely with surrounding tissues.[213] Laser-assisted bioprinting has also been used to construct layered bioprinted tissues that imitate the natural human corneal epithelium and stroma structure.[214] The bioprinting of embryonic stem cell-derived limbal epithelial stem cells (hESC-LESC) and ASCs in the bioink

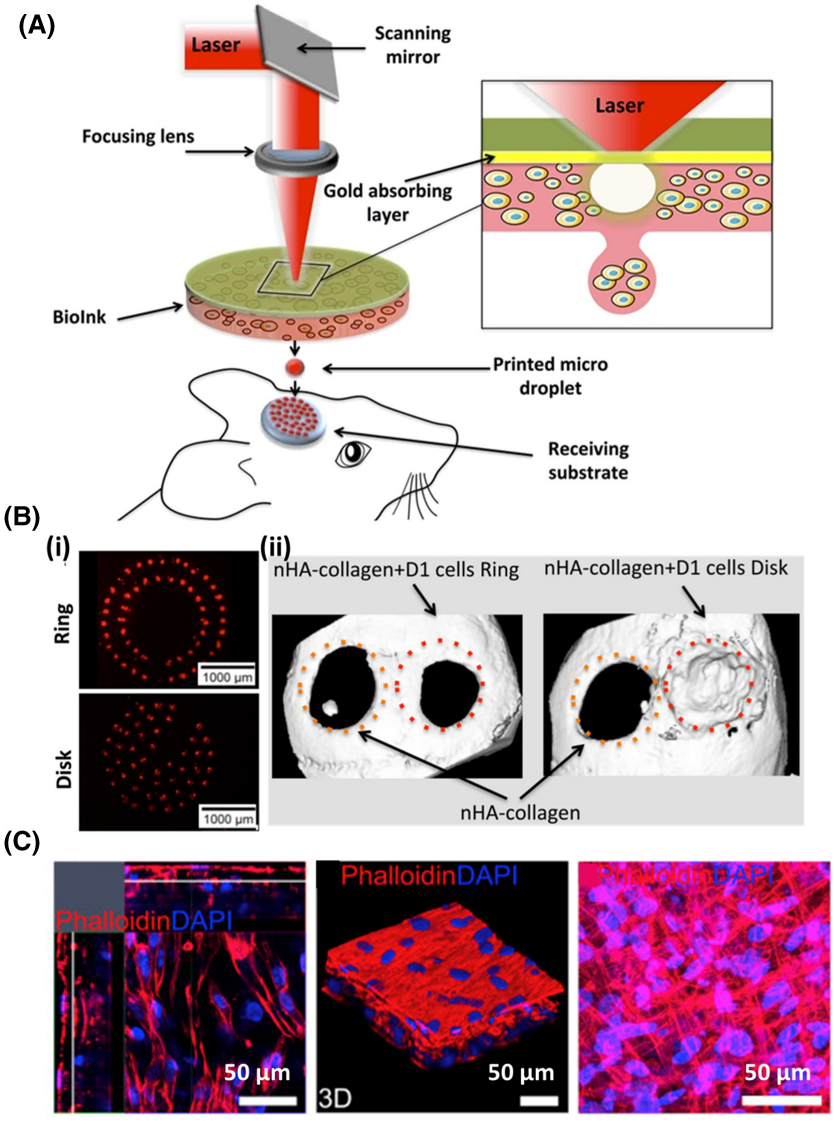

FIGURE 10 (A) A schematic diagram of laser-assisted bioprinting. Reprinted with permission from Ref. [207] Copyright (2017) springer nature. (B) (i) fluorescence images of a ring- and disk-shaped bioprinting of cells containing TdTomato protein gene (red). (ii) X-ray microtomography after 2 months of in situ bioprinting of ring- and disk-shaped nano hydroxyapatite-collagen with or without cells. Reprinted with permission from Ref. [207] Copyright (2017) springer nature. (C) Fluorescent images of laser-assisted bioprinting of ASCs that resemble the native corneal stroma. (i–iii) staining of Actin filaments (red) and nuclei (blue) of different perspectives of the 3D bioprinted stroma after 7 days of culture. Reprinted with permission from Ref. [214] Copyright (2018) Elsevier Ltd.

composed of recombinant laminin and collagen I showed an excellent survival rate. Additionally, the results showed that the printed corneal stromal presented the cellular organization into lamellae. Overall, the 3D-printed structures' cell organization was similar to the native human corneal stroma (Figure 10C). Laser-assisted bioprinting has also been recognized as a powerful tool for constructing more similar tumor environments through 3D pancreatic ductal adenocarcinoma (PDAC) models.[215] In the past, there have been concerns related to reproducibility and ability to create homogenous spheroids. However, laser-assisted bioprinting has bridged this issue by being a highly accurate cellular positioning tool that can reduce variability in 3D model construction. The successful production of tumor spheroids could be observed with the expression of epithelial growth factor receptor (EGFR), a marker associated with the progression of pancreatic ductal adenocarcinomas.

2.7.4 | Microextrusion bioprinting

Microextrusion bioprinting has been viewed as an attractive approach in biofabrication. Extrusion 3D printing involves the deposition of cell-laden bioinks by applying a force to dispense the biomaterial from a syringe or nozzle.[216] The applied force can be either pneumatic or mechanical to extrude a continuous stream of the bioink. Then, the extrusion can be broken down into different types, including direct ink writing, coaxial bioprinting, printing in a bath, and free-form reversible embedding. One significant benefit of utilizing microextrusion bioprinting is its ability to use high viscosity bioinks, which reduces the shear stress experienced during printing.[217] Microextrusion bioprinting is easy to use, can facilitate high cell density printing, and can utilize materials with different polymerization methods. Furthermore, microextrusion has an advantage over other bioprinting methods due to its superior speed, which allows for the creation of large, complex constructs such as cardiovascular tissue constructs.[218,219] Despite these aforementioned advantages, microextrusion bioprinting has some limitations. For example, the type of bioinks is limited because some characteristics, such as biocompatibility, shear-thinning, and printability, should be met for maximum performance.[218] Crosslinking duration and maintaining the structure's structural integrity after printing are additional characteristics considered when choosing a bioink.[220] To overcome some of these limitations, a cost-effective bath consisting of a pseudo-plastic matrix of xanthan-gum was proposed to fabricate a freeform suspended 3D bioprinted construct. Such a strategy allowed the fabrication of complex 3D structures within the three orthogonal axes while cell viability was uncompromised.[221]

Recently, the utilization of induced pluripotent stem cells and SWIFT (Sacrificial Writing Into Functional Tissue) biomanufacturing methods has allowed multiple organ building blocks.[222] In the latter study, the researchers manufactured a perfusable embryoid bodies-based tissue in a perfusion chamber that facilitates the diffusion of medium rich in nutrients and oxygen through the embedded microchannels and around the periphery of the tissue. Interestingly, endothelial cells adhered to the luminal surface after seeding the cells in the SWIFT constructs. Additionally, they bioprinted a perfusable cardiac tissue that could beat synchronously for a week-long period. Microextrusion bioprinting has also been applied to construct heterogeneous, biomimetic liver lobules. More specifically, these 3D tissue constructs were obtained by a combination of alginate, cellulose nanocrystal, and GelMA, incorporating NIH/3 T3 and HepG2 cell lines. The biocompatibility of these microextrusion bioprinted structures was confirmed, and no change in cell morphology was found due to the printing. Notably, the results demonstrated that these compatible bioinks could potentially create complex 3D structures composed of different cell types and extracellular matrices, which holds tremendous power for clinical translation and the field of TE overall.[219]

2.7.5 | 4D bioprinting

The insertion of conformational variations in bioprinted constructs in a programmed manner using stimuli-responsive biomaterials makes 4D bioprinting superior to 3D printing. A schematic representation of 3D and 4D printing techniques is exhibited in Figure 11A. Integrating stimuli-responsive materials into the foundational principles of 3D printing will give rise to the fabrication of bioprinted tissues capable of changing their morphology based on the changes that the tissue is experiencing.[223]

4D bioprintable bioinks should have the ability to be responsive to changes in temperature, pH, humidity, electricity, magnetic field, or acoustics.[223] Poly(*N*-isopropylacrylamide) (PNIPAm)-based polymers are classic examples of temperature-responsive materials.[224] To create biomaterials responsive to pH, materials containing carboxyl, pyridine, sulfonic, phosphate, and tertiary amines chemical groups are often sought after because they can accept protons following a pH fluctuation. Humidity-responsive materials have the capability of relaxing and contracting depending on humidity.[225] Some materials are responsive to an electric field, including polyelectrolyte hydrogels and carbon-based nanomaterials. Other inks for 4D bioprinting include light-responsive materials that depend on UV, infrared, or near-infrared light.[226] Such a strategy can be particularly useful in

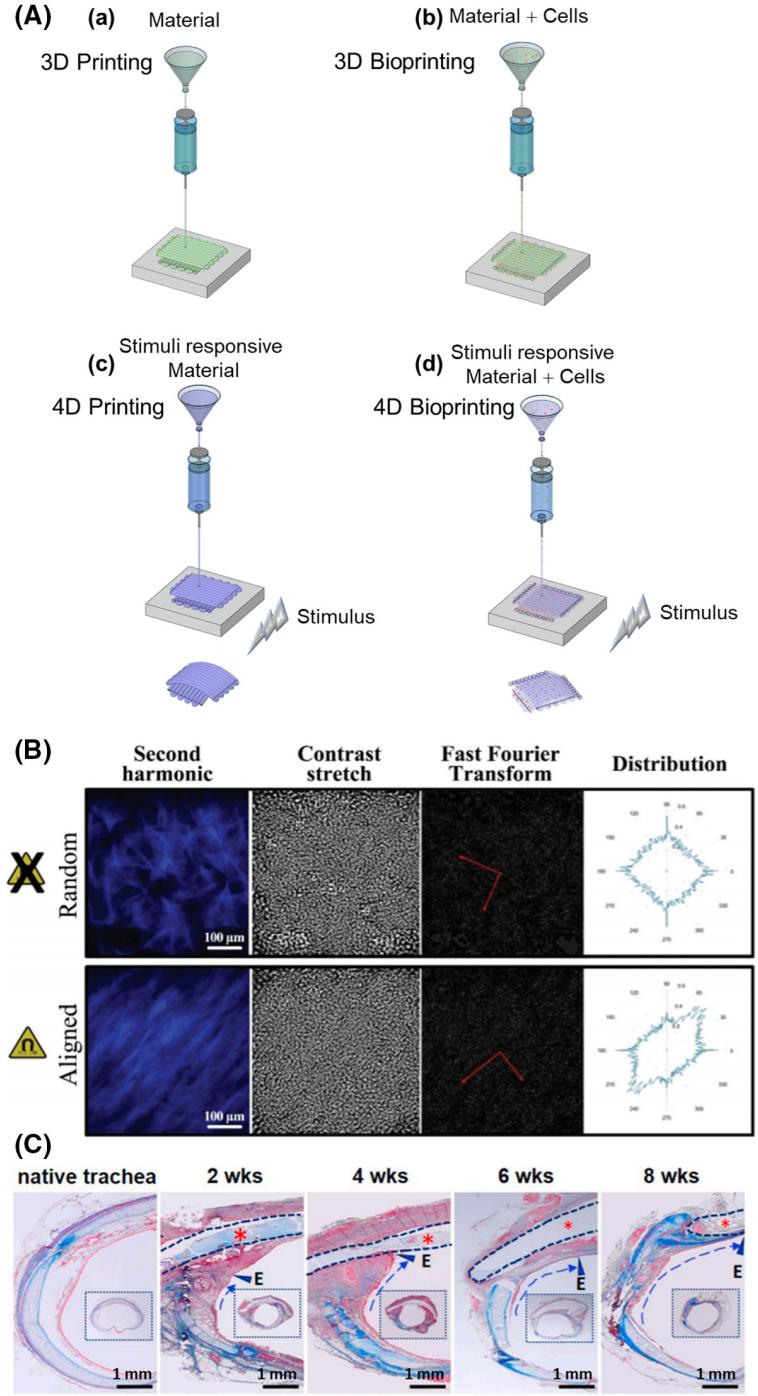

FIGURE 11 (A) Schematic representation of different printing technologies (3D printing, 3D bioprinting, 4D printing, and 4D bioprinting) using conventional materials, cells, and stimuli-responsive materials. (B) Alignment of collagen fibers in printable bioinks in the presence or absence of a magnetic field. Reprinted with permission from Ref. [227] Copyright (2018) Wiley. (C) Masson's trichrome staining of implanted 4D bioprinted trachea after 2, 4, 6, and 8 weeks of implantation. Reprinted with permission from Ref. [229] Copyright (2020) Elsevier

controlling the degradation rate of hydrogels and, hence, allowing the creation of dynamic tissues.

More recently, 4D bioprinting has been explored for multiple applications in TE and regenerative medicine. For instance, a 4D microstructure re-organization system using a drop-on-demand 3D bioprinter and magnetic fiber alignment method was proposed for articular cartilage bioprinting.[227] To achieve this, magnetic nanoparticles were embedded in hydrogel blends of agarose and collagen type I. In the presence of a magnetic field, the collagen fibers of printed bioinks could be re-aligned simultaneously during bioprinting (Figure 11B). The parallel alignment of the collagen fibers occurred due to the unidirectional traveling motion of the magnetic nanoparticles across the printable hydrogel. This method allowed cells to sense a better biomimetic microenvironment, expressing markedly more collagen II than solely randomly oriented fiber constructs. Another challenge provided by conventional 3D structures is related to the efficacy of populating microstructure scaffolds with cells. A 4D self-folding cell encapsulating mechanism to transform 2D into 3D microstructures was proposed to fulfill this limitation.[228] Such a process was achieved using

an alginate-based layer-by-layer sacrificial coating, enabling self-folding to occur precisely after cells adhere, grow, and form a monolayer on the 3D scaffolds. This method holds an excellent application to engineering microscopic tissues because it allows for specific cell organization. While there are numerous examples of 4D bioprinting, many of these systems lack biocompatibility and characteristics suitable for TE applications. By using a digital light processing (DLP) 3D bioprinting system with photocurable silk fibroin (Sil-MA) hydrogel, constructs with excellent performance in terms of biocompatibility were obtained. Conformational changes were induced by changing the interior and exterior properties of the hydrogel.[229] Ultimately, the applicability of the process was tested by creating a multi-cellular trachea structure followed by its implantation into rabbits. After 8 weeks, the implants were well integrated within the host trachea, and both epithelium and cartilage were formed at the implanted sites (Figure 11C).

The actin cytoskeleton of cells allows them to generate protrusive and contractile forces within the ECM.[230] Taking advantage of this biological-driven stimulation, printed structures can be shaped simply using cell traction forces. For instance, a 3D cell origami construct was developed based on the traction forces principle in which plates were lifted and folded according to a prescribed pattern. Several cell-laden microcontructs with different geometries were obtained without disturbing the cell viability.[231] Such stimuli-responsive biomaterials can also be envisioned as a potential strategy for 4D bioprinting.

3 | APPLICATIONS OF MICROFABRICATION TECHNIQUES

As microfabrication techniques enable control over the surface morphology of biomaterials, they can be beneficial for fabricating tissue-engineered microtissues.[232,233] In particular, the use of different microfabrication techniques alone or in combination with other approaches demonstrates their potential for preclinical and clinical applications of engineered tissues and organs. Besides tissue repair and replacement, microfabrication technologies have been gathering attention in the scientific community as an alternative strategy for meat and leather-like materials production.[234] In the following sections, some preclinical studies and organ-on-a-chip approaches using microfabrication techniques will be addressed.

3.1 | Preclinical applications

Microfabrication technologies are widely expanding in the medical field. Multiple technologies have been tested, including personalized implants and prostheses, scaffolds for tissue regeneration, anatomical models, and personalized drug delivery systems.[5,19] With the rise in donor shortages for organ transplantation, the demand for artificial implants and organs has intensified.[186] There are important considerations that should be made when choosing an appropriate material for application in clinical practice. Aside from physical and mechanical properties and biocompatibility, the strategies should fulfill the commercial requirements and clinical needs. For that, any proposed strategy should be user-friendly, scalable, and cost-effective.[235] Contemplating all the previous information, various microfabrication technologies have been proposed to fabricate constructs targeting clinical applications.

3.1.1 | Bioprinting

The use of various 3D-printed structures has been approved for clinical practice.[236] However, most proposed constructs function as structural support without regenerative and integration capacity. Thus, the bioprinting of living cells shows enormous potential to mimic the large and complex organs characterized by the presence of vasculature, innervation, and lymphatics. In general, there are four main applications of 3D (bio)printing in the medical field.[237] The first one is the use of organ models to aid in the preoperative planning and analyzing surgical treatments. In this context, it raises an essential point on collaborative communication between physicians and engineers. Researchers can create plastic medical models to learn the preoperative process. For example, Zhao et al. constructed an in vitro cervical tumor model using 3D bioprinting technology with HeLa cells.[238] The obtained structure can improve the knowledge about cervical cancer by better understanding how cells proliferate, differentiate, and spread to other parts of the body. The second level of 3D printing in medical applications is about developing and implementing permanent nonbioactive implants.[239] This type of construct is mainly used in dentistry and orthopedics. The overarching goal is to avoid an exacerbated immune response, mainly to prevent the rejection of implanted materials. The third level of application of bioprinting technology is the fabrication of biodegradable scaffolds that can present local bioactivity.[240] For instance, a sustained release of bone morphogenic protein-2 (BMP-2) in bioprinted hydrogel-based constructs showed enhanced osteogenic differentiation in vitro and in vivo.[241] The last application for bioprinting and the most anticipated is to construct fully functional tissues and organs.[237] Cardiac diseases remain the leading cause of death worldwide, which urgently prompts

advancements in cardiac TE. In one study, HUVECs and induced pluripotent cell-derived cardiomyocytes (iPSC-CMs) were embedded in a heterogeneous, multi-cellular 3D cardiac bioprinted construct composed of alginate and PEG monoacrylate-fibrinogen.[242] A significant issue regarding heart TE centers around the extensive biological and mechanical specificity required to mimic cardiac function. Nevertheless, the bioprinting of this cardiac tissue showed improved cardiomyocyte organization and differentiation. When the constructs were grafted in vivo, results showed promising vascular network development and a precise cellular organization by controlling the orientation of the myocardiocytes. 3D bioprinting has also contributed to the development of vascularized microtissues.[141] Current techniques employed to incorporate vascularization and angiogenesis in scaffolds include microfabrication of cellular networks, functionalization of biomaterials, employment of proangiogenic growth factors, and promoting vascularization with different cell types. For instance, Gold and colleagues were able to bioprint 3D cylindrical blood vessels that can recapitulate thromboinflammatory responses observed in vivo.[243] To achieve this, HUVECs and human umbilical artery smooth muscle cells (VSMCs) were printed in a colloidal bioink, comprised of GelMA, poly(ethylene glycol) diacrylate (PEGDA), and nanosilicates. Such a strategy allows the generation of different free-standing vessels with various lumen sizes and geometries, mimicking the native human vascularization. It is also important to note that bioprinting can be utilized directly in wound sites for skin repair.[244] Although autographs are the gold standard for severe skin defects, this approach is associated with patient discomfort, morbidity, and lack of availability. In contrast, allographs are challenging due to the possibility of immune rejection.[245] Dermal substitutes have been widely proposed as an alternative option to skin autographs. A cutting-edge technology is the combination of imaging and bioprinting systems.[246] Essentially, the proposed technology utilizes an imaging system to screen the wound topography while bioprints in situ to improve skin regeneration. After the delivery of dermal fibroblasts and epidermal keratinocytes in a fibrin/collagen hydrogel, results showed good cell cytocompatibility, accelerated wound closure, and re-epithelization.[246] Another extension of this microfabrication technology is personalized cartilage reconstruction. However, the fabrication of cartilage engineered tissues that resemble the native cartilage is still challenging. The in situ printing of a photocrosslinkable PEG dimethacrylate and human articular chondrocytes clarified some properties similar to the native human articular cartilage. The precise delivery of cells and biomaterial scaffolds through a layer-by-layer assembly resulted in uncompromised viability, integration with the surrounding cartilage tissue, and enhanced proteoglycan deposition.[247] Overall, the 3D bioprinting has been shown promising and relevant results for clinical applications.

3.1.2 | Soft lithography

Soft lithography techniques have also been employed to construct microtissues for future medical applications. For instance, an osteoconductive double network hydrogel with micropatterns of hydroxyapatite was fabricated by soft lithography.[51] While the patterned hydrogel showed cell adhesion and migration in vitro, a selective and robust bonding to a rabbit bone was observed in vivo. Micromolding has also been proposed as a strategy to support myocardial mass loss, which is a strong indicator for possible heart failure.[248] Due to the limited ability for myocardium regeneration, and the absence of appropriate scaffolds to help cardiac tissue repair, there is a demand for ideal biomaterials for cardiac tissue engineering. The generation of a novel photocrosslinkable methacrylated tropoelastin (MeTro) gel that can maintain in vitro contractile properties, electrical stimulation, and micropatterns has been a promising technology in the cardiovascular field. In fact, these human-based MeTro hydrogels present integrin-mediated adhesion sites, allowing attachment and proliferation of cardiomyocytes. Moreover, the nanogrooves in the hydrogel's surface generated by the micromolding technique improved cell elongation, resembling the native cardiac tissue, while synchronous beating was obtained following electrical field stimulation.[248] Zheng and colleagues developed a microfluidic vascular network model that improved the understanding of the interaction of blood cells with tumors and stem cells.[249] The approach combined soft lithography to construct the microfluidic structure in a collagen I gel, and HUVECs seeded in the microchannels. By creating these microvascular networks, it was possible to observe that angiogenesis could be promoted by forming a functional endothelium layer. Soft lithography has been envisioned for several other tissue-engineered constructs, including oral mucosa, tendon, muscle, among others.[250–252]

3.1.3 | Microfluidics and fiber spinning

Microfluidics and fiber spinning techniques have demonstrated potential results for clinical applications. In a high-throughput manner, both strategies allow the fabrication of monodispersed cell-laden microgels and microfibers with tunable sizes. Mao and colleagues utilized microfluidics for the generation of single-cell laden microgels to be injected

intravenously, targeting a variety of tissue engineering applications.[253] The encapsulation of allogenic MSCs in C57/BL6 mice improved cell maintenance after intravenous injection, while a desirable diffusion of cytokines was observed through the gel. The same microgels can also be delivered through an intraperitoneal route approach. In a similar strategy, the single-cell encapsulation of MSCs in alginate microgels showed promising results for the treatment of bone defects in a minimally invasive procedure.[254] The microgels increased bone osteogenesis and mineralization in a rat tibial marrow ablation model. A different successful approach was proposed for cartilage tissue engineering.[255] Monodisperse MSCs-laden microcapsules were generated via a droplet-based microfluidic approach by mixing MSCs with thiolated gelatin and vinyl sulfonated hyaluronic acid. After hypodermic injection into nude mice, microgels self-assembled into cartilage-like tissues, with reduced vascularization and hypertrophy, characteristic of chondral repair. Meniscus tears are common injuries, but the self-regeneration of cartilage tissue is difficult due to poor vascularization. To enhance meniscus regeneration, decellularized meniscus ECM/polycaprolactone (DMECM/PCL) fibers were combined to fabricate DMECM scaffolds.[256] The aim was to provide extra mechanical support to produce a multi-layer scaffold for meniscus regeneration by electrospinning. The results revealed that meniscus cells could proliferate within the implanted scaffolds generating meniscus-like structures, and showed similar histology scores to the native rabbit menisci. Furthermore, the implanted scaffold was implicated in decreasing joint space narrowing and providing cartilage protection. Electrospun fibers were also proposed for wound healing applications.[257] Core-shell nanofibers of polyurethane/starch (PU/St) and PU/St (hyaluronic acid) were generated with a coaxial electrospinning methodology. When mice wounds were covered with the fabricated nanofibers, the scaffolds revealed a higher healing rate potential than the control. PU/St (hyaluronic acid) condition presented the best results. Alternatively, cellulose/CNCs nanocomposite nanofibers combined with BMP-2 were studied for bone tissue applications.[258] Aligned nanofibers were obtained utilizing a rotating collector. These biocompatible aligned nanofiber scaffolds instructed the endogenous progenitor cells guidance and improved cortical bone formation after being implanted in a rabbit cranial defect model. In fact, the scaffolds loaded with BMP-2 allowed the generation of newly aligned collagen fibers, showing full integration with the host's bone tissue.

3.1.4 | Microneedles

The use of microfabrication for drug delivery has created multiple advantages on various levels for preclinical and clinical applications. For instance, doxorubicin (DOX) is a commonly used antitumor drug, but its use for treating cancer has adverse effects on patients and can cause toxicity due to non-targeted delivery. Thus, the use of dissolvable PVA microneedles was proposed to improve the transdermal delivery of DOX.[259] This polymer has been shown to enhance microporation of the stratum corneum and epidermis compared to other dissolving microneedle types. The results showed that the DOX was dissolved entirely in the PVA matrix, conducive to uniform drug distribution. Furthermore, after qualifying the PVA microneedle poration, its effectiveness was confirmed with a 100% penetration result. Through the latter study, the PVA microneedles were found to reach the superficial dermis layer by passing through the stratum corneum and epidermis layers, but not yet reaching pain receptors or blood capillaries, allowing for the maximized drug delivery and minimized invasiveness. Another application for microneedles is the delivery of exogenous nucleic acids, which presents great potential for diverse pathologies. Qu and colleagues fabricated a GelMA microneedles patch for the controlled transdermal delivery of plasmid DNA.[260] The intracellular delivery of DNA was accomplished by poly(β-amino ester) (PBAE) nanoparticles. Utilizing a C57Bl/6 mouse model, it was possible to test the efficacy of penetration of microneedles in the epidermal layer of the skin. Moreover, the delivery of DNA was confirmed by immunofluorescence staining, with a cell transfection rate of cells around 31%. The proposal of successful microneedle patches has been indicated for several other applications, including delivery of cells and anesthetic agents,[261,262] cardiac and cartilage tissue regeneration,[263–265] cancer treatment,[266] among others.

3.2 | Organs-on-a-chip

There is an extensive process to validate a certain drug, ranging from in vitro research to animal testing. As in vitro studies do not recapitulate the native environment of tissues, and in vivo experiments are ethically complex, time-consuming, and challenging to mimic diseases, models of human organs and diseases are urgently required. Thus, organ-on-a-chip platforms are being fabricated to simulate the mechanics and the physiological responses of different tissues and organs.[19,267] Besides drugs and cosmetics development, these platforms can be utilized as microsensors to monitor a particular tissue or organ or as models to study diseases. Such platforms can be applied in preclinical phases while giving better control than traditional in vitro assays.[268] Moreover, the platforms are acceptable engineered tissues as they replicate the microarchitecture of functional units of a given tissue while incorporating mechanical and biochemical stimuli. In fact,

they can reproduce special features and functions of organs, including lungs,[269] gut,[270] blood vessels,[271] liver,[272] brain,[273] and heart.[274] In this way, organ-on-a-chip may provide us a better understanding of drug delivery, physiological functions, and transport mechanisms.[267,275–278]

3.2.1 | Microfluidics

Microfabrication technologies are essential for developing organ-on-a-chip platforms. Briefly, an organ-on-a-chip is a microfluidic cell culture device employed as functional units of human tissues or organs in vitro. These devices can reproduce one type of tissue function by culturing one cell phenotype in a single perfused microfluidic chamber. If the purpose is to mimic interfaces between two tissues, distinct cell phenotypes should be cultured in separated microchannels connected by a porous membrane.[279] Additionally, other important features can be incorporated in organ-on-a-chip devices, including fluid shear force, concentration gradient, mechanical stress, and cell patterning, to better reproduce the physiological interactions of native organs. In theory, any type of cell, from human and mammalian to plant and insect cells, is suitable for organ-on-a-chip fabrication.[267]

Microfluidic devices were first created with inorganic substrates; however, other substrates, such as elastomers, thermoplastics, and paper, are becoming more commonly used.[280] These approaches need to consider function, degree of integration, and application.[268] When evaluating the materials for organs-on-a-chip, it is essential to assess their main criteria, including electrochemical detection, production costs, and reusability.[281] For example, silicon and glass have proper electrochemical detection and are reusable, but they are expensive. In contrast, elastomers have low electrochemical detection capability and are not reusable, but they have slightly lower production costs. Thermoplastics and paper generally have moderate electrochemical detection and low production costs, but they differ in reusability properties.[281] As mentioned above, photolithography and soft lithography are classic examples of microfabrication techniques used to construct microfluidic platforms.[282] The generation of patterning in PDMS pieces is advantageous because it allows precise control in directing fluids and cells to appropriate positions within a chip.[283,284] Generally, organs-on-a-chip are composed of several components, including chambers for the guided spatial confinement of cells, fluid shear stress, and microsensors, which describe the microenvironment conditions of cultured cells. The monitorization of pH, O_2, CO_2, biomolecules, metabolites, as well as electrical and mechanical stimulation, which creates a controlled microfluidic environment, are other features found and applied in such platforms.[285]

3.2.2 | Bioprinting

Although lithography techniques are essential, 3D bioprinting has also been useful due to its layer-by-layer construction, enabling greater flexibility in the design.[284] The 3D bioprinting benefits from freeform construction and microfluidic manipulation, allowing for the concrete production of complex structures. For example, 3D bioprinting has been shown to create a heterogeneous system that could mimic both biochemical and biophysical properties of glioblastoma.[286] This cancer-on-a-chip device matched the expected clinical results following radiation and showed sensitivity against drug combinations, highlighting its potential for patient-specific modeling. Furthermore, the proposed cancer-on-a-chip model could facilitate point-of-care testing because of how rapidly it can be produced via 3D printing. This technology can hold incredible power in clinical settings.[286] To better simulate in vivo models, 3D bioprinting has also been used with organ-on-a-chip devices. For example, a vessel-on-a-chip device was combined with bioprinting to produce a system that could mimic the in vivo vessel wall structure and blood flow through vessels. The results highlight the importance of the proposed technology due to its potential to create highly realistic vascularized tissues that mimic in vivo microenvironments for biological system modeling.[287] Overall, organ-on-a-chip platforms are being widely studied, and significant scientific advances have been made. The idealistic main purpose is to develop a chip containing multiple organs, to achieve a "human-on-a-chip."

4 | CONCLUSION AND FUTURE PERSPECTIVE

Microfabrication techniques have several advantages compared with other macro-engineered strategies, such as reduced costs, high levels of control, and outstanding efficiency. Although microfabrication was born out of technologies, such as 3D printing, microfluidics, and photolithography, which were primarily used in electronics, it transcended the biological and chemical sciences.

Different microfabrication processes offer unique advantages that can be exploited in biology and chemistry-related fields. The use of microfabrication allows for the creation of biomaterials and microtissues with greater complexity, more robust biological compatibility while mimicking the structure of living tissues. Minimizing the number of biomaterials is a smart strategy for tissue regeneration. The "minimalistic-engineering" approaches can guide the performance and recruitment of a patient's

cells to the injury site to balance the regenerative niche toward the healing process.[288] Microfabrication has made remarkable advances in the medical field. With the aid of different microfabrication techniques, improvements in the bioprinting of organs and tissues continue to expand. Additionally, better drug delivery systems have evolved and improved due to microfabrication research. All the advances in microfabrication technologies can in the future support the building of effective tissues and regeneration of damaged tissues at a clinically relevant scale.

Although there have been many achievements in this field, the lack of fluid flow and vascularization inside the constructs is still an issue. For this purpose, researchers have been incorporating materials within bioprinted construct as a sacrificial template and then removing them by dissolution.[78] The created void spaces can act as channels to improve the diffusion of essential molecules for cell survival and enhance vascularization.[208] Another important aspect is related to cell viability. For example, in a study using extrusion-based bioprinting to engineer liver tissues, increasing gelatin concentration and the needle diameter allowed cells to avoid exposure to toxic glutaraldehyde, enhancing the survival rate by 5%.[289] In fact, higher cell survival rates can be obtained by improved cell–cell contact, cell-matrix interactions, and appropriate cell densities. However, to bioprint 3D living tissue efficiently, it is required to bear in mind the necessity to reproduce the 3D architecture of the native tissue, containing the appropriate phenotypes of cells embedded in a bioink with suitable biomechanical properties, while the function of the tissue that should be similar to the natural tissue is uncompromised following implantation.[183] There are many microfabrication techniques and materials that need to be improved. The fabrication of organ-on-a-chip platforms is still relatively expensive and laborious. Thus, the type of cells and materials chosen should support the scale-up of the system and the extrapolation to the clinical practice. Although PDMS is widely employed for chip fabrication, flexibility, gas permeability, and small-drug absorption capacity are features that bring some disadvantages. Moreover, the culture medium of these chips needs to be carefully explored to find the most suitable formulation for different organs.

For further research, the advancement of TE constructs will continue rising. Although many medical microfabrication applications are still in the preclinical stages, microfabrication has come a long way. As more technological advancements and research discoveries are made, microfabrication will continue evolving and encompassing more and more aspects of medicine and biological systems. Though promising, future steps entail working toward high throughput microfabricated structures for drugs and diagnoses. To meet the demands of personalized medicine, these technologies need to become even more precise and reproducible.

ACKNOWLEDGMENTS

SN acknowledges the financial support given by the Fulbright Commission Portugal and the Portuguese Foundation for Science and Technology (FCT) with the doctoral grant SFRH/BD/130194/2017. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. SED thank the financial support from the Scientific and Technological Research Council of Turkey (TÜBİTAK-1059B191800324).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

AUTHOR CONTRIBUTIONS

Sara Nadine and Ada Chung conceptualized the manuscript, performed the literature research, and wrote the review article. Sibel Emir Diltemiz, Brooke Yasuda, Charles Lee, Vahid Hosseini, Solmaz Karamikamkar, Natan Roberto de Barros, Kalpana Mandal, Shailesh Advani, Benjamin Behnam Zamanian, Marvin Mecwan, Yangzhi Zhu, Mohammad Mofidfar, Mohammad Reza Zare, João Mano, and Mehmet Remzi Dokmeci assisted in writing and revising the manuscript. Samad Ahadian conceptualized the manuscript and assisted in the revision of the manuscript. All authors have approved the final version of the manuscript.

ORCID

Samad Ahadian https://orcid.org/0000-0002-6784-5716

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How to cite this article: Nadine S, Chung A, Diltemiz SE, Yasuda B, Lee C, Hosseini V, Advances in microfabrication technologies in tissue engineering and regenerative medicine. Artif Organs. 2022;46:E211–E243. doi:10.1111/aor.14232