Three-dimensional bioprinting vascularized bone tissue

Hadis Gharacheh and Murat Guvendiren*

Currently available bone grafts are insufficient to address the demand and lack tissue complexity to mimic the native bone tissue microenvironment. Bone tissue is highly vascular, and vasculature is crucial for bone tissue development and functional integration of the graft to the native defect site. Three-dimensional (3D) bioprinting has emerged as an advanced biomanufacturing technology to develop vascularized bone tissue with architectural, structural, biochemical, and cellular complexity. This article includes currently available 3D bioprinting technologies and corresponding bioinks followed by a detailed review on 3D bioprinting strategies for vasculature. Finally, the current state of the art in 3D bioprinting of vascularized bone tissue is presented.

Introduction

There is a growing need for developing bone graft substitutes, as currently available clinical treatments for bone defects and disease are insufficient to address the demand and show notable limitations.[1–3] A bone autograft—a patient's own bone harvested from another part of the patient at time of the surgery—is known to be the gold standard over decades.[4,5] This is due to their excellent compatibility and non-immunogenic characteristic as well as inherent bioactivity (i.e., osteoconductive and osteoinductive nature), which is essential for bone regeneration and functional integration of the graft to the defect site.[4,5] Despite these advantages, bone autografts are more practical for small defects as they require harvesting bone from other parts of the patient's body, which may not be feasible for large defects. The clinical procedure is associated with additional issues at the tissue-harvesting site (e.g., infection, inflammation, and bleeding). Although bone allograft (i.e., a bone tissue from a donor) is an alternative option, its use is limited due to the possibility of implant rejection (immunogenicity) and transmission of diseases from the tissue donor.[6–8] Conventional bone tissue engineering approaches have been used to develop synthetic bone graft substitutes,[9–12] yet they lack the native bone tissue complexity, including structural, biochemical, mechanical, and cellular.[1,13]

Three-dimensional bioprinting has a significant potential to create a patient-specific living bone tissue that is anatomically and physiologically similar to a patient's native bone tissue.[14–16] Three-dimensional bioprinting enables the use of a patient's own medical image (e.g., magnetic resonance imaging [MRI], computerized tomography [CT] scan, and x-ray) to design a custom digital tissue model and a patient's own cells and tissue to develop a personalized bioprintable ink formulation.[17–19] Thus, This could potentially eliminate clinical issues associated with currently available bone grafts, including tissue compatibility, transplant rejection, and lack of functional tissue integration.[16] The ultimate goal is to create a patient-specific human-scale living tissue using 3D bioprinting. This requires development of fully functional vasculature within the 3D bioprinted tissue.[20] Development and functional integration of vasculature is crucial for 3D tissues as the vascular network is responsible for transferring nutrients and waste products, and failure of the vascular system can lead to cell necrosis and tissue failure.[21–23] It is also known that cell survival decreases drastically when the capillary distances are beyond the range of 60–300 μm. This is not surprising considering that the native bone is a highly vascularized tissue and vasculature is shown to have a significant role in bone

Hadis Gharacheh, Otto H. York Chemical and Materials Engineering, New Jersey Institute of Technology, Newark, USA; hg8@njit.edu
Murat Guvendiren, Otto H. York Chemical and Materials Engineering, New Jersey Institute of Technology, Newark, USA; Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, USA; muratg@njit.edu
*Corresponding author
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maturation, regeneration, and remodeling.[24–26] Thus, it is crucial to understand the current advancements in 3D bioprinting approaches to create vascularized bone tissue, which is the focus of this article. We will provide a brief summary of the commonly used 3D bioprinting technologies and bioink formulations to create bone tissue followed by 3D bioprinting technologies to bioprint vasculature within 3D scaffolds and the use of these technologies for 3D bioprinting of bone tissue.

Three-dimensional bioprinting technologies and bioink formulations

Three-dimensional bioprinting is an additive manufacturing (AM) approach that refers to printing of live cells.[27] It is important not to confuse 3D bioprinting with 3D printing of biomaterials. The latter refers to layer-by-layer printing of biomaterials, including biodegradable polymers, metal and metal alloys, and bioceramics as well as their composites. Three-dimensionally printed biomaterials can be directly implanted or populated with cells *in vitro* prior to implantation. Thus, the 3D printing process does not need to be cell friendly, and a wide range of AM technologies can be utilized based on the biomaterial of interest and form of the biomaterial ink (solution, slurry, powder, etc.).[28–30] However, this is not the case for 3D bioprinting as it strictly refers to bioprinting of cells commonly in the form of cell-only bioinks or cell-laden hydrogel-based bioinks.[17,31–34] Cell-only bioinks include cell suspensions, cell aggregates, and cell spheroids,[35–38] and they rely on cellular self-assembly and self-organization mechanisms due to cell–cell interactions.[39,40] Synthetic, natural, and decellularized extracellular matrix (dECM)-based hydrogels have been used to formulate cell-laden hydrogel bioinks.[41,42] These bioinks can be cross-linked through chemical cross-linking or physical association (including ionic interactions), to form a hydrogel post-printing.[43] A wide variety of biomaterial additives have been incorporated into cell-laden hydrogel bioinks, in particular for bone tissue engineering, to formulate composite bioinks—additives, including bioceramics, metal alloys, and allograft tissue particles.[42,44–50] The selection of the bioink is strictly determined by the bioprinting technology. Currently available technologies include droplet-based, light-based, and extrusion-based bioprinting.[17,18,31]

Inkjet bioprinting requires deposition of cell-laden hydrogels, colloidal suspensions, or cell-only solutions with relatively low viscosities (<10 mPa.s) at high shear rates (10^5–10^6 s^{-1}) in the form of droplets (25–50 μm in diameter).[51] Inkjet bioprinting is one of the first bioprinting technologies to print live cells, yet its application in the bioprinting field is usually limited to two-dimensional (2D) patterning of low viscosity bioinks.[52] There is a continuous effort to address this challenge by enabling inkjet printing of high viscosity inks. For instance, Lewis's group recently reported an acoustophoretic printing method that enables patterning of a broad range of inks, showing yield stress behavior or Newtonian behavior with significantly high viscosities (0.5–25,000 mPa.s), including cell-laden biological matrices.[53]

Light-based bioprinting refers to bioprinting techniques utilizing a light or a laser source,[54–57] in which light is directed (SLA) or projected (DLP) to a viscous photocurable cell-laden hydrogel bioink (<~5000 mPa.s) to cross-link or cure the bioink. This technology includes stereolithography apparatus (SLA) and digital light processing (DLP), and evolved into continuous liquid interface printing (CLIP)[58] and projection stereolithography,[56,59,60] which significantly increase the speed of the process by projecting an entire print layer at once with achievable resolutions below 100 μm. A volumetric bioprinting technology is recently developed to further enhance the print speed enabling centimeter-scale constructs within 30–120 s.[61–63] In this technique, a 3D construct is simultaneously created by irradiating a volume of photocurable ink (within a vat) from multiple angles, which can be achieved either by tomographic reconstruction[61,62] or holographic patterning.[63] Volumetric bioprinting technology has already been applied to bioprint cell-laden hydrogel-based constructs.[64,65] Laser-induced forward transfer (LIFT) utilizes a light source to create a droplet from a viscous cell-laden hydrogel bioink (viscosity value ranging from 1 to 300 mPa.s), which is transferred onto a print substrate.[66–68] LIFT enables bioprinting of viscous cell-laden bioinks with high concentrations of cells up to 1×10^8 cells/mL.[67,68]

Extrusion-based bioprinting, also referred as direct ink writing (DIW) bioprinting, is the most commonly used technique due to its advantages such as ease of use, availability (and cost) of the printers, versatility of bioprintable materials, and ability to bioprint dense cellular structures.[69–71] DIW bioprinting allows extrusion of high viscosity bioinks (30 to 6×10^7 mPa·s) with high cell density (up to 10^8 per mL), including cell-laden curable solutions and cell-laden hydrogels as well as cell suspensions or aggregates, onto a print surface.[69–71] Bioprint resolution (smallest print size) is mainly determined by the needle size (inner diameter of the needle) as well as the bioink formulation (i.e., swelling during extrusion and/or spreading after printing). DIW bioprinting also enables use of multiple print heads during a bioprinting process, usually referred as multimaterial bioprinting, each print head assigned to a different bioink formulation. It is important to mention that bioink and nozzle size determine the resolution of the printing, which is limited to a range of (~100–300 μm). Extruded bioink formulation can be cross-linked during or after the printing process either chemically or physically to ensure the mechanical integrity of the construct.[51] A layer-by-layer bioprinting approach has significant limitations to achieve high structural and architectural complexity in 3D as well as to print soft cell-laden constructs (elastic modulus below 100 kPa) or cells alone. To address these issues, DIW can be performed within a support bath that physically supports the printed structure and eliminates the need for the layer-by-layer printing process enabling omnidirectional bioprinting. This technology is referred as freeform extrusion-based bioprinting

or freeform embedded bioprinting.[72] In this approach, the support bath material (usually a micro-size hydrogel suspension) needs to behave like a solid for physical support, yet behave like a fluid under applied shear stress to allow the nozzle to move freely. It needs to recover immediately after the stress is removed to hold the printed structure in place.

Three-dimensional bioprinting strategies to create vasculature

There are two main bioprinting approaches to create vasculature. The first approach is printing hollow microchannels within cell-laden hydrogels followed by perfusion of endothelial cells within these channels. The latter approach includes 3D bioprinting of

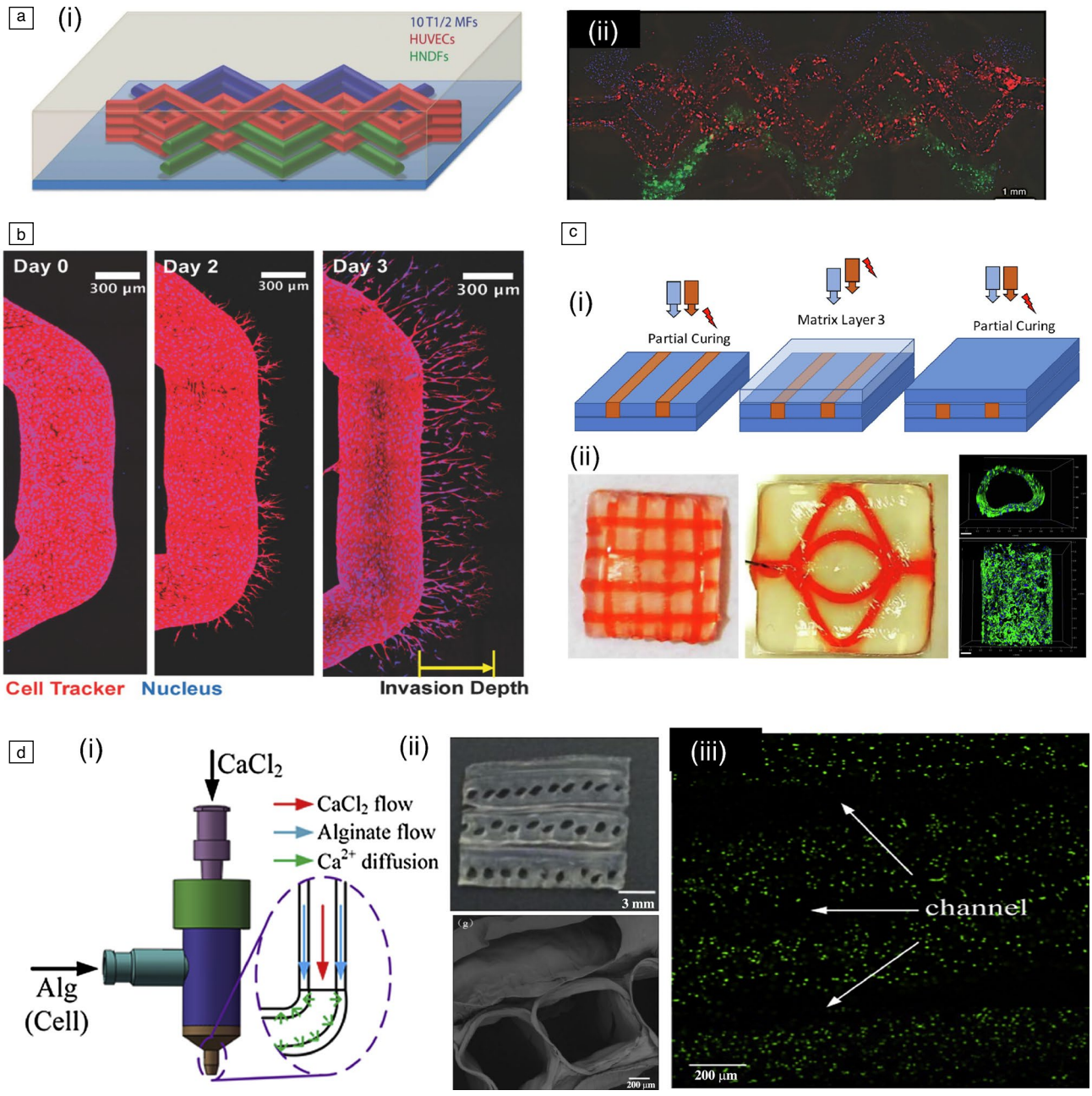

Figure 1. Examples of 3D bioprinting strategies for vasculature. (a) Schematic showing the embedded cellular patterns (i), and corresponding fluorescent image of the cells (ii), including 10T1/2 mouse fibroblasts (MFs) (blue), human neonatal dermal fibroblasts (HNDFs) (green), and human umbilical vein endothelial cells (HUVECs) (red). (b) Free-form embedded printed channels populated with endothelial cells (red) led to sprouting due to cell-mediated degradation of the matrix hydrogel. (c) Schematic of sacrificial ink printing within uncross-linked viscous print layer (i), enabling formation of perfusable channels (red) within cell-laden hydrogels and fluorescent images showing HUVECs (green) forming a monolayer within channels (ii). (d) Schematic of coaxial printing (i), Scanning electron microscopy images of the channels (ii), and fluorescent images of the cells shown in green (iii). Figures are reproduced with permission from Reference 74, © 2014 Wiley-VCH (a), Reference 89, © 2018 Wiley-VCH (b), Reference 80, © 2019 Acta Biomaterialia (c), and Reference 97, © 2015 Elsevier (d).

cell-laden or cell-only vascular bioinks within cell-laden hydrogels. There are several strategies to create microchannels embedded within 3D cell-laden hydrogels. *In a gel-casting strategy*, cell-laden hydrogel is casted and cured within a mold holding a pre-printed sacrificial scaffold, which is removed (dissolved) to create microchannels (150–800 μm) after the hydrogel is cured[73–76] (**Figure 1**a). A wide range of sacrificial inks are available including agarose, gelatin, poly(vinyl alcohol) (PVA), Pluronic, and sugar-based materials to create microchannels.[73–82] It is also possible to directly print the sacrificial inks within 3D cell-laden hydrogels using *freeform extrusion-based bioprinting*, in which case the sacrificial construct can be removed after printing to create microchannels within a cell-laden matrix.[83–89] As previously discussed, the hydrogel matrix (used as a bath) has to allow the needle motion during printing. This requirement significantly limits the available hydrogel formulations for this approach to highly viscous polymer slurries, salt solutions, shear-thinning hydrogels, and microgels.[83–89] For instance, Song et al. 3D-bioprinted microchannels within hydrogels cross-linked with a protease-degradable cross-linker that allowed perfused and attached endothelial cells to spatially degrade the hydrogel matrix to form angiogenic sprouting (Figure 1b).[89] An alternative approach is reported enabling direct printing of a sacrificial ink within an uncross-linked pre-printed photocurable hydrogel matrix layer (Figure 1c).[80] In addition to extrusion-based printing, *light-based bioprinting* can also be used to spatially cure photocurable hydrogels within the vat to create embedded channels within these hydrogels.[59,90] Microchannels (or hollow tubes) can also be fabricated by utilizing *unit stacking* and *coaxial printing* strategies using extrusion-based bioprinting. In unit stacking strategy, microchannels embedded within hydrogels can be fabricated by layer-by-layer bioprinting of a cell-laden hydrogel and a sacrificial ink.[91] In coaxial printing, a coaxial needle is used in extrusion-based printing, which allows printing of two distinct inks simultaneously forming a core–shell filament. The outer layer is usually cross-linked rapidly forming the shell, and the inner layer is used as the support, which can be removed after printing (Figure 1d).[92–97] These hollow channels can be incorporated within a 3D hydrogel matrix by using free-form embedded printing. The free-form embedded bioprinting is also the most commonly used approach to bioprint cell-laden or cell-only vascular bioinks within the cell-laden hydrogel matrix to create vasculature.[37]

Three-dimensional bioprinting vascularized bone tissue

Bone tissue is highly vascularized, and development of bone tissue and vasculature are simultaneous processes. The crosstalk of bone (osteogenic) and vascular cells is known to

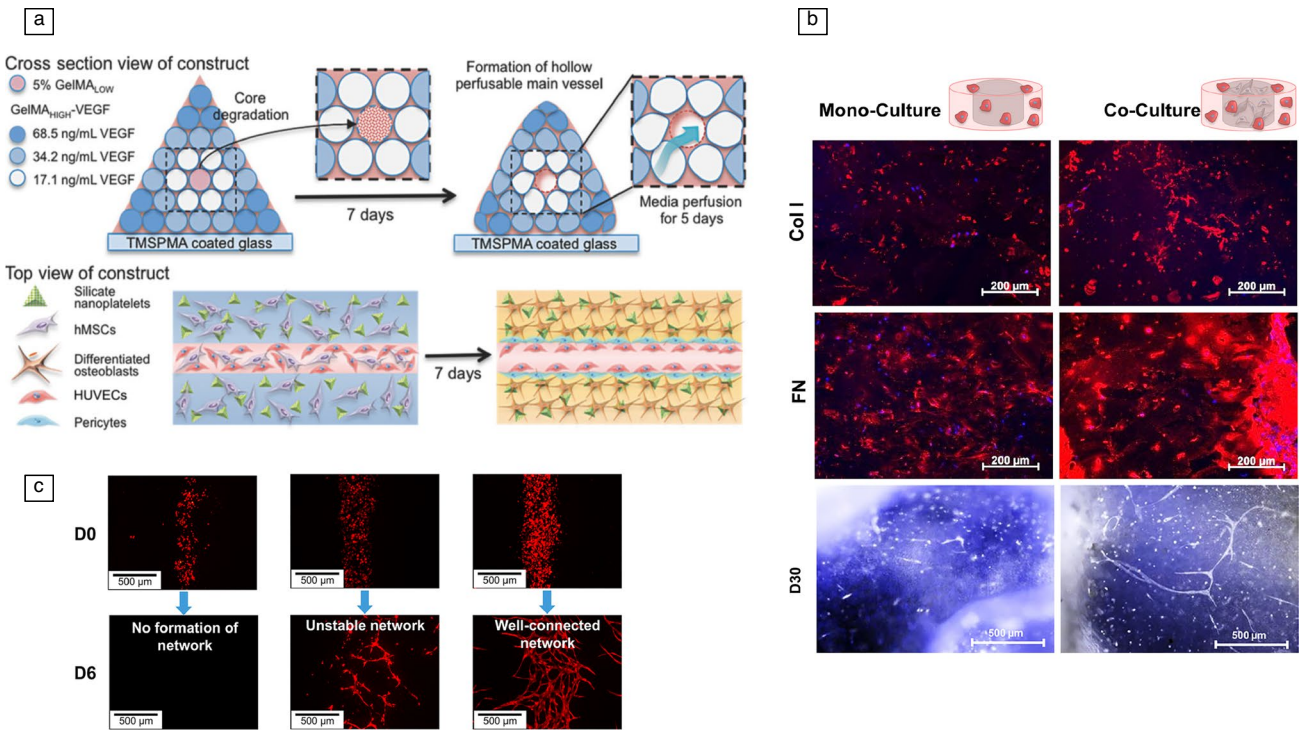

Figure 2. Bioprinting vascularized bone tissue. (a) Schematic summarizing the unit stacking approach to create cell-laden hydrogels with gradient presentation of vascular endothelial growth factor (VEGF) for capillary formation. (b) Schematics of the biphasic construct with mono-culture and co-culture conditions, and corresponding staining images for bone-associated matrix proteins Col I and FN at day 20 (displayed in red, DNA in blue), and for vasculature at day 30 (PECAM-1 in white, DNA in blue). (c) Fluorescent images showing the effect of initial cell density (at day 0) on vascularization/capillary network formation of endothelial cells (red) at day 6. Figures are reproduced with permission from Reference 91, © 2017 Wiley (a), Reference 104, © 2020 Springer Nature (b), and Reference 108, © 2019 Springer Nature (c). hMSCs, human mesenchymal stem cells; HUVECs, human umbilical vein endothelial cells.

enhance bone tissue formation as well as maintenance and repair of the tissue.[98–100] There has been a significant effort to bioprint bone tissue with built-in vasculature.[16,22,25,101–103] DIW bioprinting was used to bioprint microstructured bone-like tissue constructs containing a perfusable vascular lumen using gelatin methacryloyl (GelMA) (**Figure 2**a).[91] GelMA was conjugated with vascular endothelial growth factor (VEGF) to create a gradient presentation of VEGF for capillary formation, and silicate nanoplatelets were used to promote human mesenchymal stem cell (hMSC) osteogenesis (Figure 2a). Bioprinted construct demonstrated structural stability *in vitro* up to 21 days, and was able to support cell survival and proliferation during tissue maturation.[91] Leucht et al. showed the importance of the crosstalk between osteogenic and vascular cells by fabricating a DIW bioprinted co-culture hydrogel (Figure 2b).[104] Gelatin-based hydrogels are used to formulate allowed bioprinting of co-culture hydrogels including human dermal microvascular endothelial cells (HDMECs) and human adipose-derived stem cells (ASCs) for the vascular compartment, and ASCs and hydroxyapatite for the osteogenic compartment. A self-guided, stabilized assembly of capillary-like networks were reported in the vascular compartment while ASCs differentiated into osteogenic lineage confirmed by expression of bone marker protein collagen type I (Col I), osteopontin (OPN), and fibronectin (FN) (Figure 2b)[104] DIW was used to bioprint two separate osteogenic and vasculogenic cell populations encapsulated in a fibrin bioink to fabricate osteon-like patterns in a biophasic scaffold to enhance neovascularization.[105] *In vitro* studies showed a significant increase in gene expression of angiogenic markers and histological analysis of explanted scaffolds showed a significant increase in the number of blood vessels per area in the 3D printed osteon-like scaffolds. In another study, vascular endothelial cell-laden thermosensitive bioinks were bioprinted on the inner surfaces of interconnected tubular channels of bone mesenchymal stem cell-laden 3D bioprinted scaffolds.[106] The coupling effect between angiogenesis and osteogenesis led to upregulation of osteogenic and angiogenic genes *in vitro*, and vascularized scaffolds promoted new bone formation in a rat calvarial critical-sized defect model.[106] An *in vitro* vascularized bone model was developed by bioprinting a gelatin-nanohydroxyapatite (gel-nHA) hydrogel scaffold seeded with hMSCs.[107] The hMSCs were differentiated *in vitro* for two weeks followed by seeding human umbilical vein endothelial cells (HUVECs) within the macropores to form a capillary-like network during two more weeks of culture. Results confirmed vascular lumen formation and osteogenic commitment of hMSCs in four weeks of culture, and indicated the positive effect of endothelial cells on stem cell osteogenesis.[107] Laser-assisted bioprinting (LAB) was reported to pre-organize endothelial cells into high cell density micropatterns to create a vascular network with defined architecture in collagen hydrogels seeded with mesenchymal stem cells (Figure 2c).[108] Formation of highly interconnected vasculature was achieved and shown to be dependent on the local density of the endothelial cells such that deposition of high density cells (2176 ± 556 cells/mm^2) led to capillary-like structures, whereas low cell density (1447 ± 321 cells/mm^2) patterns formed poorly connected vasculature.[108]

It is common to combine different printing technologies to fabricate vascularized 3D bone scaffolds. Extrusion-based biomaterial printing is used to fabricate support scaffold from polycaprolactone (PCL) mixed with tricalcium phosphate (TCP) particles, which is combined with bioprinting of a cell-laden composite hydrogel (human amniotic fluid–derived stem cells [hAFSC]), gelatin, fibrinogen, glycerol, and HA) and a sacrificial Pluronic hydrogel to fabricate bone scaffolds.[15] Implanted scaffolds in a calvarial bone defect (in Sprague Dawley rats) showed significant bone maturation and vascularization, including formation of large blood vessels inside newly formed bone tissue **Figure 3**a).[15] In another study, extrusion-based filament printing is combined with SLA bioprinting (Figure 3b).[109] First, a honeycomb pore-shaped construct containing several vertical and horizontal vessel-like channels was printed using polylactide (PLA) filaments. Then, cell-laden GelMA hydrogel were bioprinted via SLA to infill the interconnected channels and pores. *In vitro* culture using a bioreactor system along with media perfusion and immobilization of BMP2 (bone morphogenic protein 2) and VEGF within the construct enabled promotion of osteogenesis and angiogenesis simultaneously.[109] Rukavina et al. combined extrusion-based bioprinting with inkjet bioprinting to bioprint ASCs and HUVECs, respectively.[110] *In vivo* evaluation of the bioprinted constructs (in mice) confirmed prevascularized bone formation within the scaffolds. Extrusion-based biomaterial printing is used to fabricate calcium phosphate (CaP) scaffolds (from CaP paste) integrated with coaxial printed GelMA hydrogel microchannels.[111] CaP fibers and microchannels seeded with vascular endothelial cells and mesenchymal stem cells were embedded within GelMA hydrogel. This study confirmed the feasibility of creating vascularized bone scaffolds with enhanced vasculature in the core of the bone scaffolds.[111] Mussel-inspired, hierarchical 3D porous scaffolds were fabricated by sequential dispensing of a PDACS/PCL composite ink (for support), HUVEC laden alginate/gelatin hydrogels, and Wharton's jelly mesenchymal stem cells (WJMSCs).[112] *In vitro* studies confirmed enhanced bone formation and angiogenesis. Finally, *in situ bioprinting* of bone tissue is an emerging technology enabling bioprinting of the tissue directly into or onto the defect site in the operation room.[113–118] This approach utilizes the native microtissue environment as a natural bioreactor for tissue maturation and functional tissue integration into the native site. For instance, *in situ* bioprinted bone tissue demonstrated significant vascularization and bone regeneration in a mouse calvaria bone defect model.[119]

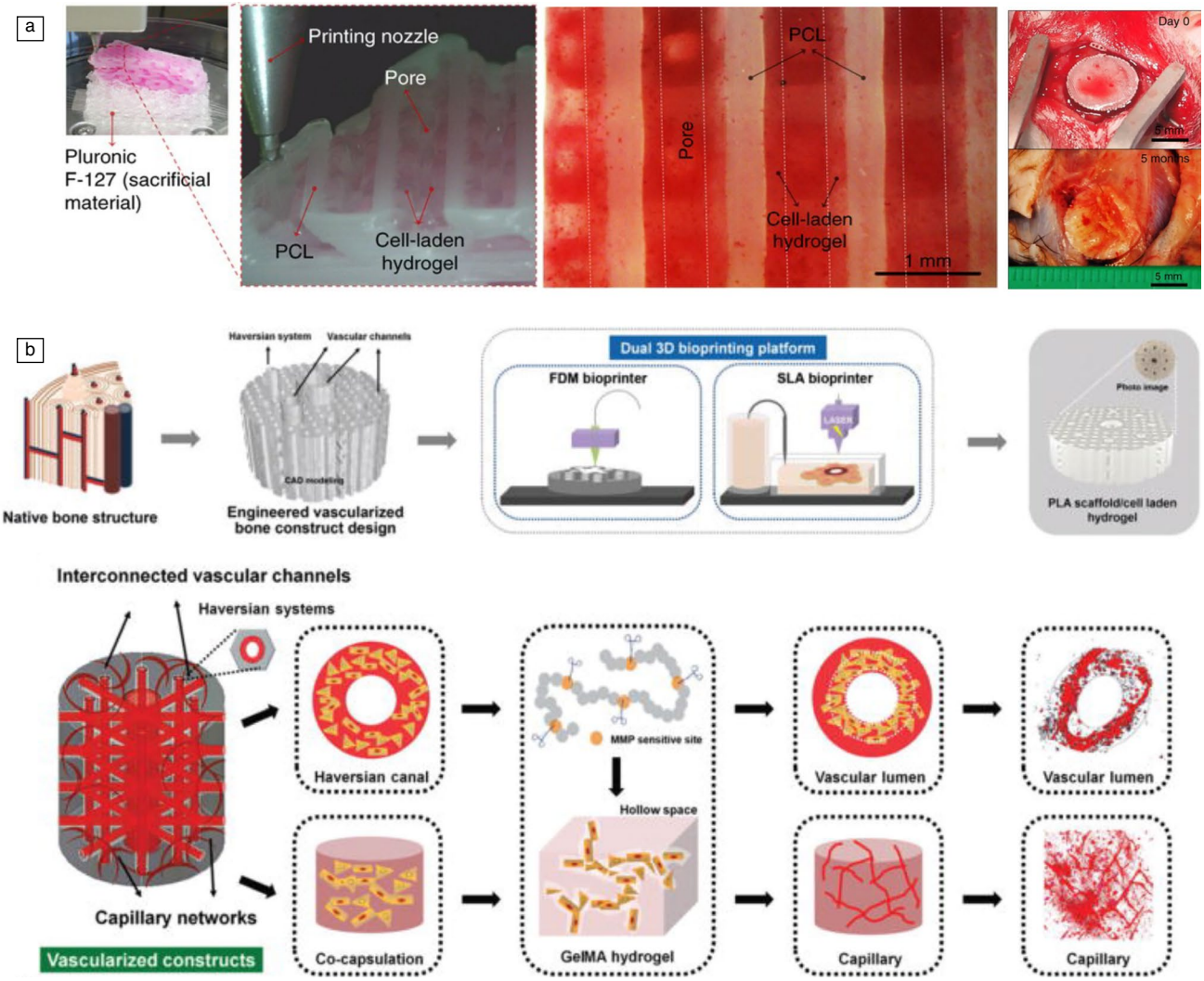

Figure 3. Combining printing technologies for bioprinting vascularized bone tissue. (a) From left to right: Integrated organ printing system to fabricate multimaterial bone scaffolds, picture of the printed material, top image showing the different components of the scaffold, and optical images of the implanted construct at day 0 (top) and after five months (bottom). (b) Schematic showing the design for fabrication of a vascularized bone biphasic construct using filament printing and stereolithography (SLA) 3D bioprinting platform (top) and process flow to create vascularized bone construct using MMP-sensitive GelMA hydrogel to generate a vascular lumen and a capillary network (bottom). Figures are reproduced with permission from Reference 15, © 2016 Springer Nature (a) and Reference 109, © 2016 Wiley-VCH (b). FDM, fused deposition modeling.

Concluding remarks

Three-dimensional bioprinting is at the forefront of research to fabricate bone tissue with built-in vasculature. Several bioprinting strategies have been developed to create hierarchical vasculature in 3D cell-laden hydrogels and these strategies are successfully applied to 3D bioprint bone tissue. Three-dimensionally bioprinted tissues demonstrated the importance of the crosstalk between osteogenic and vascular cells not only for bone tissue formation, but also for tissue maintenance. Current challenges include bioink, bioprinting, and end product related issues. Selection of clinically approved bioprintable hydrogel components and obtaining enough cells especially for cell-only bioinks are major issues for bioink formulation. Bioprinting technologies usually allow formation of microchannels and lack the required resolution to create angiogenesis, which can be achieved by using enzymatically degradable hydrogel systems that allow tubule formation. Recent light-based technologies such as volumetric printing can tackle resolution issues yet face difficulty creating human-scale constructs. Three-dimensionally printed tissues usually require maturation in a reactor, which brings another layer of complexity considering clinical translation. There is a continuous advancement with emerging technologies, including *in situ* bioprinting, which could further move the bioprinted bone tissues to clinic. Overall, fully functional vasculature is the key for enhanced bone formation and functional integration to the defect site.

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Conflict of interest

Authors declare no conflict of interest.

References

1. M. Alonzo, F. Alvarez Primo, S. Anil Kumar, J.A. Mudloff, E. Dominguez, G. Fregoso, N. Ortiz, W.M. Weiss, B. Joddar, *Curr. Opin. Biomed. Eng.* **17**, 100248 (2021)
2. A. Ho-Shui-Ling, J. Bolander, L.E. Rustom, A.W. Johnson, F.P. Luyten, C. Picart, *Biomaterials* **180**, 143 (2018)
3. A.R. Amini, C.T. Laurencin, S.P. Nukavarapu, *Crit. Rev. Biomed. Eng.* **40**, 363 (2012)
4. A. Brydone, D. Meek, S. Maclaine, *Proc. Inst. Mech. Eng. H J. Eng. Med.*. **224**(12), 1329 (2010)
5. C. Myeroff, M. Archdeacon, *J. Bone Joint Surg. Am.* **93**, 2227 (2011)
6. C. Delloye, O. Cornu, V. Druez, O. Barbier, *J. Bone Joint Surg. Br.* **89**(5), 574 (2007)
7. G. Battafarano, M. Rossi, V. De Martino, F. Marampon, L. Borro, A. Secinaro, A. Del Fattore, *Int. J. Mol. Sci.* **22**, 1128 (2021)
8. J.A. Shibli, B.E. Nagay, L.J. Suárez, C. Urdániga-Hung, M. Bertolini, V.A. Barão, J.G.S. Souza, *Tissue Eng. Part C Methods* **28**, 179 (2022)
9. H. Qu, H. Fu, Z. Han, Y. Sun, *RSC Adv.* **9**, 26252 (2019)
10. H. Jodati, B. Yılmaz, Z. Evis, *Ceram. Int.* **46**, 15725 (2020)
11. K. Jahan, M. Tabrizian, *Biomater. Sci.* **4**, 25 (2016)
12. S. Wu, X. Liu, K.W.K. Yeung, C. Liu, X. Yang, *Mater. Sci. Eng. R Rep.* **80**, 1 (2014)
13. G. Fernandez de Grado, L. Keller, Y. Idoux-Gillet, Q. Wagner, A.-M. Musset, N. Benkirane-Jessel, F. Bornert, D. Offner, *J. Tissue Eng.* **9**, 2041731418776819 (2018)
14. A. Cianciosi, M. Costantini, S. Bergamasco, S. Testa, E. Fornetti, J. Jaroszewicz, J. Baldi, A. Latini, E. Choińska, M. Heljak, C. Zoccali, S. Cannata, W. Święszkowski, A. Diaz Lantada, C. Gargioli, A. Barbetta, *ACS Appl. Bio Mater.* **2**, 5077 (2019)
15. H.-W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, *Nat. Biotechnol.* **34**, 312 (2016)
16. N. Ashammakhi, A. Hasan, O. Kaarela, B. Byambaa, A. Sheikhi, A.K. Gaharwar, A. Khademhosseini, *Adv. Healthc. Mater.* **8**, 1801048 (2019)
17. C. Mandrycky, Z. Wang, K. Kim, D.-H. Kim, *Biotechnol. Adv.* **34**, 422 (2016)
18. S.V. Murphy, A. Atala, *Nat. Biotechnol.* **32**, 773 (2014)
19. S.V. Murphy, P. De Coppi, A. Atala, *Nat. Biomed. Eng.* **4**, 370 (2020)
20. A.K. Miri, A. Khalilpour, B. Cecen, S. Maharjan, S.R. Shin, A. Khademhosseini, *Biomaterials* **198**, 204 (2019)
21. C. Tomasina, T. Bodet, C. Mota, L. Moroni, S. Camarero-Espinosa, *Materials* (Basel) **12**, 2701 (2019)
22. E.P. Chen, Z. Toksoy, B.A. Davis, J.P. Geibel, *Front. Bioeng. Biotechnol.* **9**, 664188 (2021)
23. H.H.G. Song, R.T. Rumma, C.K. Ozaki, E.R. Edelman, C.S. Chen, *Cell Stem Cell* **22**, 340 (2018)
24. F. Simunovic, G. Finkenzeller, *Cells* **10**, 1749 (2021)
25. Y.L. Park, K. Park, J.M. Cha, *Micromachines* (Basel) **12**, 287 (2021)
26. Y. Liu, J.K.Y. Chan, S.-H. Teoh, *J. Tissue Eng. Regen. Med.* **9**, 85 (2015)
27. J. Groll, J.A. Burdick, D.W. Cho, B. Derby, M. Gelinsky, S.C. Heilshorn, T. Jungst, J. Malda, V.A. Mironov, K. Nakayama, A. Ovsianikov, W. Sun, S. Takeuchi, J.J. Yoo, T.B.F. Woodfield, *Biofabrication* **11**, 013001 (2018)
28. C.Y. Liaw, M. Guvendiren, *Biofabrication* **9**, 024102 (2017)
29. C.L. Ventola, *Pharm. Ther.* **39**, 704 (2014)
30. J.-Y. Lee, J. An, C.K. Chua, *Appl. Mater. Today* **7**, 120 (2017)
31. P.S. Gungor-Ozkerim, I. Inci, Y.S. Zhang, A. Khademhosseini, M.R. Dokmeci, *Biomater. Sci.* **6**, 915 (2018)
32. S. Derakhshanfar, R. Mbeleck, K. Xu, X. Zhang, W. Zhong, M. Xing, *Bioact. Mater.* **3**, 144 (2018)
33. Y.S. Zhang, K. Yue, J. Aleman, K. Mollazadeh-Moghaddam, S.M. Bakht, J. Yang, W. Jia, V. Dell'Erba, P. Assawes, S.R. Shin, *Ann. Biomed. Eng.* **45**, 148 (2017)
34. J. Gopinathan, I. Noh, *Biomater. Res.* **22**, 11 (2018)
35. S. Ji, M. Guvendiren, *Front. Bioeng. Biotechnol.* **5**, 23 (2017)
36. A.C. Daly, M.D. Davidson, J.A. Burdick, *Nat. Commun.* **12**, 753 (2021)
37. O. Jeon, Y.B. Lee, H. Jeong, S.J. Lee, D. Wells, E. Alsberg, *Mater. Horiz.* **6**, 1625 (2019)
38. J.A. Brassard, M. Nikolaev, T. Hübscher, M. Hofer, M.P. Lutolf, *Nat. Mater.* **20**, 22 (2021)
39. P.A. Fleming, W.S. Argraves, C. Gentile, A. Neagu, G. Forgacs, C.J. Drake, *Dev. Dyn.* **239**, 398 (2010)
40. R. Burdis, D.J. Kelly, *Acta Biomater.* **126**, 1 (2021)
41. A. Abaci, M. Guvendiren, *Adv. Healthc. Mater.* **9**, 2000734 (2020)
42. J. Yang, Y.S. Zhang, K. Yue, A. Khademhosseini, *Acta Biomater.* **57**, 1 (2017)
43. J.M. Unagolla, A.C. Jayasuriya, *Appl. Mater. Today* **18**, 100479 (2020)
44. H. Gharacheh, M. Guvendiren, *Polymers* (Basel) **14**(18), 3788 (2022)
45. G. Ratheesh, C. Vaquette, Y. Xiao, *Adv. Healthc. Mater.* **9**, 2001323 (2020)
46. A. Kara, T. Distler, C. Polley, D. Schneidereit, H. Seitz, O. Friedrich, F. Tihminlioglu, A.R. Boccaccini, *Mater. Today Bio* **15**, 100309 (2022)
47. F. Donnaloja, E. Jacchetti, M. Soncini, M.T. Raimondi, *Polymers* (Basel) **12**, 905 (2020)
48. S. Heid, A.R. Boccaccini, *Acta Biomater.* **113**, 1 (2020)
49. X. Xue, Y. Hu, Y. Deng, J. Su, *Adv. Funct. Mater.* **31**, 2009432 (2021)
50. S. Yue, H. He, B. Li, T. Hou, *Nanomaterials* (Basel) **10**, 1511 (2020)
51. S. Agarwal, S. Saha, V.K. Balla, A. Pal, A. Barui, S. Bodhak, *Front. Mech. Eng.* **6**, 589171 (2020)
52. W.C. Wilson Jr., T. Boland, *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **272A**(2), 491 (2003)
53. D. Foresti, K.T. Kroll, R. Amissah, F. Sillani, K.A. Homan, D. Poulikakos, J.A. Lewis, *Sci. Adv.* **4**(8), eaat1659 (2018)
54. W. Sun, B. Starly, A.C. Daly, J.A. Burdick, J. Groll, G. Skeldon, W. Shu, Y. Sakai, M. Shinohara, M. Nishikawa, J. Jang, D.W. Cho, M. Nie, S. Takeuchi, S. Ostrovidov, A. Khademhosseini, R.D. Kamm, V. Mironov, L. Moroni, I.T. Ozbolat, *Biofabrication* **12**, 022002 (2020)
55. L. Elomaa, C.-C. Pan, Y. Shanjani, A. Malkovskiy, J.V. Seppälä, Y. Yang, *J. Mater. Chem. B* **3**, 8348 (2015)
56. R. Gauvin, Y.-C. Chen, J.W. Lee, P. Soman, P. Zorlutuna, J.W. Nichol, H. Bae, S. Chen, A. Khademhosseini, *Biomaterials* **33**, 3824 (2012)
57. B. Guillotin, A. Souquet, S. Catros, M. Duocastella, B. Pippenger, S. Bellance, R. Bareille, M. Rémy, L. Bordenave, J. Amédée, F. Guillemot, *Biomaterials* **31**, 7250 (2010)
58. J.R. Tumbleston, D. Shirvanyants, N. Ermoshkin, R. Janusziewicz, A.R. Johnson, D. Kelly, K. Chen, R. Pinschmidt, J.P. Rolland, A. Ermoshkin, E.T. Samulski, J.M. DeSimone, *Science* **347**(6228), 1349 (2015)
59. R. Raman, B. Bhaduri, M. Mir, A. Shkumatov, M.K. Lee, G. Popescu, H. Kong, R. Bashir, *Adv. Healthc. Mater.* **5**, 610 (2016)
60. C. Sun, N. Fang, D.M. Wu, X. Zhang, *Sens. Actuators A Phys.* **121**(1), 113 (2005)
61. B.E. Kelly, I. Bhattacharya, H. Heidari, M. Shusteff, C.M. Spadaccini, H.K. Taylor, *Science* **363**, 1075 (2019)
62. D. Loterie, P. Delrot, C. Moser, *Nat. Commun.* **11**, 852 (2020)
63. M. Shusteff, A.E.M. Browar, B.E. Kelly, J. Henriksson, T.H. Weisgraber, R.M. Panas, N.X. Fang, C.M. Spadaccini, *Sci. Adv.* **3**, eaao5496 (2017)
64. P.N. Bernal, P. Delrot, D. Loterie, Y. Li, J. Malda, C. Moser, R. Levato, *Adv. Mater.* **31**, 1904209 (2019)
65. J. Gehlen, W. Qiu, G.N. Schädli, R. Müller, X.-H. Qin, *Acta Biomater.* **156**, 49 (2023)
66. R. Xiong, Z. Zhang, W. Chai, D.B. Chrisey, Y. Huang, *Biofabrication* **9**, 024103 (2017)
67. L. Koch, S. Kuhn, H. Sorg, M. Gruene, S. Schlie, R. Gaebel, B. Polchow, K. Reimers, S. Stoelting, N. Ma, P.M. Vogt, G. Steinhoff, B. Chichkov, *Tissue Eng. Part C Methods* **16**, 847 (2010)
68. C. Kryou, I. Zergioti, *Biomed. Mater. Devices*, 1–16 (2022). https://doi.org/10.1007/s44174-022-00040-1
69. S. Ramesh, O.L.A. Harrysson, P.K. Rao, A. Tamayol, D.R. Cormier, Y. Zhang, I.V. Rivero, *Bioprinting* **21**, e00116 (2021)
70. S. Ji, M. Guvendiren, *APL Bioeng.* **5**, 011508 (2021)
71. Y.S. Zhang, G. Haghiashtiani, T. Hübscher, D.J. Kelly, J.M. Lee, M. Lutolf, M.C. McAlpine, W.Y. Yeong, M. Zenobi-Wong, J. Malda, *Nat. Rev. Methods Primers* **1**, 75 (2021)
72. A. McCormack, C.B. Highley, N.R. Leslie, F.P.W. Melchels, *Trends Biotechnol.* **38**, 584 (2020)
73. J.S. Miller, K.R. Stevens, M.T. Yang, B.M. Baker, D.-H.T. Nguyen, D.M. Cohen, E. Toro, A.A. Chen, P.A. Galie, X. Yu, *Nat. Mater.* **11**, 768 (2012)
74. D.B. Kolesky, R.L. Truby, A.S. Gladman, T.A. Busbee, K.A. Homan, J.A. Lewis, *Adv. Mater.* **26**(19), 3124 (2014)
75. D.B. Kolesky, K.A. Homan, M.A. Skylar-Scott, J.A. Lewis, *Proc. Natl. Acad. Sci. U.S.A.* **113**, 3179 (2016)
76. K.A. Homan, D.B. Kolesky, M.A. Skylar-Scott, J. Herrmann, H. Obuobi, A. Moisan, J.A. Lewis, *Sci. Rep.* **6**, 34845 (2016)
77. L.E. Bertassoni, M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A.L. Cristino, G. Barabaschi, D. Demarchi, M.R. Dokmeci, Y. Yang, *Lab Chip* **14**, 2202 (2014)
78. V.K. Lee, D.Y. Kim, H. Ngo, Y. Lee, L. Seo, S.-S. Yoo, P.A. Vincent, G. Dai, *Biomaterials* **35**, 8092 (2014)
79. V.K. Lee, A.M. Lanzi, H. Ngo, S.-S. Yoo, P.A. Vincent, G. Dai, *Cell. Mol. Bioeng.* **7**, 460 (2014)
80. S. Ji, E. Almeida, M. Guvendiren, *Acta Biomater.* **95**, 214 (2019)
81. A.P. Golden, J. Tien, *Lab Chip* **7**, 720 (2007)
82. S.Y. Hann, H. Cui, T. Esworthy, X. Zhou, S.-J. Lee, M.W. Plesniak, L.G. Zhang, *Acta Biomater.* **123**, 263 (2021)

83. T.E. Angelini, B.S. Sumerlin, C.S. O'Bryan, W. Gregory Sawyer, T. Bhattacharjee, Organic microgel system for 3d printing of silicone structures (Patent No. US20180057682A1)(2021)
84. C.B. Highley, C.B. Rodell, J.A. Burdick, *Adv. Mater.* **27**, 5075 (2015)
85. T.J. Hinton, Q. Jallerat, R.N. Palchesko, J.H. Park, M.S. Grodzicki, H.-J. Shue, M.H. Ramadan, A.R. Hudson, A.W. Feinberg, *Sci. Adv.* **1**, e1500758 (2015)
86. C.S. O'Bryan, T. Bhattacharjee, S. Hart, C.P. Kabb, K.D. Schulze, I. Chilakala, B.S. Sumerlin, W.G. Sawyer, T.E. Angelini, *Sci. Adv.* **3**, e1602800 (2017)
87. L. Ouyang, C.B. Highley, C.B. Rodell, W. Sun, J.A. Burdick, *ACS Biomater. Sci. Eng.* **2**, 1743 (2016)
88. W. Wu, A. DeConinck, A. Lewis Jennifer, *Adv. Mater.* **23**, H178 (2011)
89. K.H. Song, C.B. Highley, A. Rouff, J.A. Burdick, *Adv. Funct. Mater.* **28**, 1801331 (2018)
90. K. Arcaute, B.K. Mann, R.B. Wicker, *Ann. Biomed. Eng.* **34**, 1429 (2006)
91. B. Byambaa, N. Annabi, K. Yue, G. Trujillo-de Santiago, M.M. Alvarez, W. Jia, M. Kazemzadeh-Narbat, S.R. Shin, A. Tamayol, A. Khademhosseini, *Adv. Healthc. Mater.* **6**, 1700015 (2017)
92. Y. Zhang, Y. Yu, A. Akkouch, A. Dababneh, F. Dolati, I.T. Ozbolat, *Biomater. Sci.* **3**, 134 (2015)
93. Y. Zhang, Y. Yu, H. Chen, I.T. Ozbolat, *Biofabrication* **5**, 025004 (2013)
94. Y. Zhang, Y. Yu, I.T. Ozbolat, *J. Nanotechnol. Eng. Med.* **4**, 020902 (2013)
95. Y. Gao, X. Jin, *Mar. Drugs* **17**(10), 557 (2019)
96. L. Shao, Q. Gao, C. Xie, J. Fu, M. Xiang, Y. He, *Biofabrication* **12**, 035014 (2020)
97. Q. Gao, Y. He, J.-Z. Fu, A. Liu, L. Ma, *Biomaterials* **61**, 203 (2015)
98. N. Koike, D. Fukumura, O. Gralla, P. Au, J.S. Schechner, R.K. Jain, *Nature* **428**, 138 (2004)
99. L.H. Nguyen, N. Annabi, M. Nikkhah, H. Bae, L. Binan, S. Park, Y. Kang, Y. Yang, A. Khademhosseini, *Tissue Eng. Part B Rev.* **18**, 363 (2012)
100. D.N. Heo, M. Hospodiuk, I.T. Ozbolat, *Acta Biomater.* **95**, 348 (2019)
101. B. Bisht, A. Hope, A. Mukherjee, M.K. Paul, *Ann. Biomed. Eng.* **49**, 1128 (2021)
102. F. Shahabipour, N. Ashammakhi, R.K. Oskuee, S. Bonakdar, T. Hoffman, M.A. Shokrgozar, A. Khademhosseini, *Transl. Res.* **216**, 57 (2020)
103. A. Joshi, S. Choudhury, S.B. Gugulothu, S.S. Visweswariah, K. Chatterjee, *Biomacromolules* **23**, 2730 (2022)
104. A. Leucht, A.-C. Volz, J. Rogal, K. Borchers, P.J. Kluger, *Sci. Rep.* **10**, 5330 (2020)
105. C. Piard, H. Baker, T. Kamalitdinov, J. Fisher, *Biofabrication* **11**, 025013 (2019)
106. M. Shen, L. Wang, Y. Gao, L. Feng, C. Xu, S. Li, X. Wang, Y. Wu, Y. Guo, G. Pei, *Mater. Today Bio* **16**, 100382 (2022)
107. I. Chiesa, C. De Maria, A. Lapomarda, G.M. Fortunato, F. Montemurro, R. Di Gesù, R.S. Tuan, G. Vozzi, R. Gottardi, *Biofabrication* **12**, 025013 (2020)
108. O. Kérourédan, J.-M. Bourget, M. Rémy, S. Crauste-Manciet, J. Kalisky, S. Catros, N.B. Thébaud, R. Devillard, *J. Mater. Sci. Mater. Med.* **30**, 28 (2019)
109. H. Cui, W. Zhu, M. Nowicki, X. Zhou, A. Khademhosseini, L.G. Zhang, *Adv. Healthc. Mater.* **5**(17), 2174 (2016)
110. P. Rukavina, F. Koch, M. Wehrle, K. Tröndle, G. Björn Stark, P. Koltay, S. Zimmermann, R. Zengerle, F. Lampert, S. Strassburg, G. Finkenzeller, F. Simunovic, *Biotechnol. Bioeng.* **117**, 3902 (2020)
111. C. Twohig, M. Helsinga, A. Mansoorifar, A. Athirasala, A. Tahayeri, C.M. França, S.A. Pajares, R. Abdelmoniem, S. Scherrer, S. Durual, J. Ferracane, L.E. Bertassoni, *Mater. Sci. Eng. C Mater. Biol. Appl.* **123**, 111976 (2021)
112. Y.-W. Chen, Y.-F. Shen, C.-C. Ho, J. Yu, Y.-H.A. Wu, K. Wang, C.-T. Shih, M.-Y. Shie, *Mater. Sci. Eng. C Mater. Biol. Appl.* **91**, 679 (2018)
113. S. Singh, D. Choudhury, F. Yu, V. Mironov, M.W. Naing, *Acta Biomater.* **101**, 14 (2020)
114. A. MacAdam, E. Chaudry, C.D. McTiernan, D. Cortes, E.J. Suuronen, E.I. Alarcon, *Front. Bioeng. Biotechnol.* **10**, 940896 (2022)
115. L. Li, F. Yu, J. Shi, S. Shen, H. Teng, J. Yang, X. Wang, Q. Jiang, *Sci. Rep.* **7**, 9416 (2017)
116. V. Keriquel, H. Oliveira, M. Rémy, S. Ziane, S. Delmond, B. Rousseau, S. Rey, S. Catros, J. Amédée, F. Guillemot, J.-C. Fricain, *Sci. Rep.* **7**(1), 1778 (2017)
117. V. Keriquel, F. Guillemot, I. Arnault, B. Guillotin, S. Miraux, J. Amédée, J.-C. Fricain, S. Catros, *Biofabrication* **2**, 014101 (2010)
118. C.S. Russell, A. Mostafavi, J.P. Quint, A.C. Panayi, K. Baldino, T.J. Williams, J.G. Daubendiek, V. Hugo Sánchez, Z. Bonick, M. Trujillo-Miranda, S.R. Shin, O. Pourquie, S. Salehi, I. Sinha, A. Tamayol, *ACS Appl. Bio Mater.* **3**, 1568 (2020)
119. O. Kérourédan, D. Hakobyan, M. Rémy, S. Ziane, N. Dusserre, J.-C. Fricain, S. Delmond, N.B. Thébaud, R. Devillard, *Biofabrication* **11**, 045002 (2019) □

Hadis Gharacheh is a doctoral candidate in the Department of Chemical Engineering at the New Jersey Institute of Technology (NJIT). Her research focuses on designing advanced composite bioinks and bioprinting bone tissue scaffolds for bone regeneration applications. Her research also involves printing biomaterial inks for in vitro bone tissue disease models. She received her master's degree in chemical engineering from Rowan University. Gharacheh can be reached by email at hg8@njit.edu.

Murat Guvendiren is an associate professor in the Department of Chemical and Materials Engineering Department at the New Jersey Institute of Technology (NJIT). His research focuses on the development of instructive biomaterials and three-dimensional bioprintig. He is the recipient of several awards, including the NJIT Excellence in Research (2022), Newark College of Engineering Rising Star in Research (2022), National Science Foundation CAREER (2021), NJIT Excellence in Innovative Teaching (2021), Innovation in Engineering Education Award (2020), and MTF Biologics Junior Investigator (2019). He has been an elected senior member of the National Academy of Inventors since 2022. Guvendiren can be reached by email at muratg@njit.edu.