Three-dimensional bioprinting for medical applications

Alperen Abaci, Gulden Camci-Unal,* and Murat Guvendiren,* Guest Editors

In recent years, three-dimensional (3D) bioprinting has become an emerging technology to fabricate functional tissues and organs that could replicate native tissue function. Due to its ability to precisely position cellular materials and utilize medical images, 3D bioprinting has enormous potential in biomedical applications, including tissue engineering and regenerative medicine. Three-dimensional bioprinting is a rapidly progressing field that has demonstrated clinically relevant impactful uses. In this article, we provide an overview of important aspects of 3D bioprinting technologies, bioink design, and emerging bioprinting technologies in the field. We also feature five articles that focus on different aspects of 3D bioprinting included in this issue. These articles highlight 3D bioprinted tissue models, 3D bioprinting of organoid and organ-on-a-chip platforms, volumetric bioprinting for large-scale tissues and organs, and applications of 3D bioprinting in bone tissue engineering and otolaryngology.

Introduction

Mimicking the sophisticated biological and architectural features of native tissues is a challenging task. Currently available conventional biofabrication techniques have limitations to address this issue. The unique ability to precisely position bioinks (i.e., cellular materials) into spatially organized living structures renders three-dimensional (3D) bioprinting a practical and widely accessible tool in various medical applications, including tissue engineering and regenerative medicine.[1–3] In addition, 3D bioprinting allows development of personalized scaffolds and implantable grafts by processing patients' medical images obtained by computed tomography (CT) or magnetic resonance imaging (MRI). Biocompatible materials and cells can then be used to fabricate 3D personalized constructs making 3D bioprinting a promising biofabrication approach to address tissue and organ shortage for transplantation as well as *in vitro* disease models.[4,5]

Three-dimensional bioprinting refers to layer-by-layer fabrication of cellular structures using live cell formulations called "bioinks."[6–8] Although it is possible to fabricate polymeric scaffolds by printing biomaterial inks that can be seeded with cells post-printing, this process is not typically considered as "bio" printing because the ink formulations do not incorporate live cells in the printed formulations.[9] Bioinks can contain cellular components in the form of cellular aggregates or spheroids without biomaterial matrices, which is called cell-only bioinks. Bioinks can also include naturally derived or synthetic hydrogels or decellularized extracellular matrix (dECM) to fabricate cell-laden 3D structures.[10] Because live cells are processed during the printing process, the printing technology must be cytocompatible without damaging cells, which limits the available technologies for bioprinting to direct ink writing (DIW), inkjet printing, laser-induced forward transfer (LIFT), stereolithography (SLA), and digital light processing (DLP).[11,12]

DIW is an extrusion-based printing technique where the bioink is loaded into a syringe and extruded through a fine nozzle by pneumatic pressure or mechanical tools.[13] Although it is generally used with high viscosity cell-laden hydrogels ($30–6 \times 10^7$ mPa.s), DIW can also be utilized with cell-only bioinks.[14] The resolution of the printed features depends on parameters such as print speed, extrusion rate, and nozzle size, and the DIW technique generally can provide features as small as ~100 µm.[13,15] As the DIW technology is capable of

Alperen Abaci, Otto H. York Department of Chemical and Materials Engineering, New Jersey Institute of Technology, Newark, USA; aa2848@njit.edu
Gulden Camci-Unal, Department of Chemical Engineering, University of Massachusetts Medical School, Lowell, USA; Department of Surgery, University of Massachusetts Medical School, Worcester, USA; Gulden_CamciUnal@uml.edu
Murat Guvendiren, Otto H. York Department of 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
doi:10.1557/s43577-023-00546-z

working with various types of bioinks simultaneously to fabricate heterogeneous living structures, and also easy to operate and easily accessible, it is the most commonly used bioprinting technology. Besides extrusion-based technologies, 3D structures can also be fabricated using droplet-based bioprinting techniques, namely inkjet and LIFT bioprinting. In inkjet bioprinting, a low viscosity bioink (~10 mPa.s) is deposited in the form of droplets at high shear rates (10^5–10^6 s^{-1}).[14] Droplets as small as 50 µm in diameter can be generated by a piezoelectric or thermal tool.[16] Because low viscosity inks are processed in inkjet bioprinting applications, each individual printed layer needs to be cured rapidly to ensure structural integrity of the printed layer.[17,18] In the LIFT technology, a glass slide is coated with a thin (10- to 100-nm thick) laser-absorbing layer, generally a metal such as gold, titanium, or silver, and the bioink underneath this layer, which is referred as donor substrate. By projecting laser on the laser-absorbing layer, high pressures can be generated due to laser absorption, which allows the bioink underneath to expand rapidly to form droplets and separate from the donor substrate. Ejected droplets are transferred on a receiver substrate, where 3D structures can be fabricated by layer-by-layer deposition of these droplets. With LIFT, features as small as 10 µm can be fabricated.[14,19] Vat photopolymerization-based techniques (SLA and DLP) utilize photocurable cell-laden polymer solutions loaded in a vat that are spatially cross-linked by exposure to a UV light or laser source. Because the resolution of the structures are defined by the size of the projected light, vat photopolymerization-based techniques can provide resolutions as low as 10 µm and highly complex architectures can be fabricated. Despite the possibility of a fast and accurate fabrication process, one of the main challenges of these techniques is incorporating multiple bioinks within the same structure.[20–22]

Despite the strong potential of 3D bioprinting to address tissue and organ shortage and improve personalized medicine, the fundamental challenges of mimicking the native tissues, including the incorporation of biological and topological features similar to the native tissues at a clinically relevant scale limit the translational aspects of 3D bioprinting. To overcome these challenges, bioink design and development of sophisticated 3D bioprinting techniques are crucial toward generating physiologically relevant 3D bioprinted tissue models.

Bioink design

The bioinks used during the bioprinting process are the building blocks of the tissue models and influence controlling the cellular behavior within the 3D printed constructs. For a successful bioprinting process, the bioink must have the following properties: convenient printability, adequate mechanical properties, desirable degradation rate, biochemical functionality, high cell viability, and biocompatibility.

Printability of the bioink is directly related to the rheological properties of the bioink.[23,24] The bioink must allow replication of desired features of the digital design with high accuracy and print fidelity. For this purpose, generally shear thinning viscous polymer solutions are utilized to print cell-laden hydrogels, as these solutions flow easier under shear during the extrusion process and recover right after printing. These polymers provide the required high viscosity to ensure structural integrity of the printed layer. Besides viscous properties of the bioink, cross-linking strategies are also crucial to fabricating reliable and reproducible structures with high print fidelity.[25,26] Because rapid sol–gel transition is required to ensure structural integrity, cross-linking kinetics of the bioinks should be investigated in detail prior to printing to understand the gelation mechanism and optimize the curing process. If the bioink properties are still not sufficient to self-support the printed shape, they can be printed within a support framework or mold using supportive polymeric inks, such as polycaprolactone (PCL).[27–29] The bioink can also be partially cross-linked to improve the initial viscosity prior to printing,[30] or printed in support suspension baths of shear thinning and self-healing microgels.[31,32]

Adequate mechanical properties are required to obtain self-supporting structures post-printing. Additionally, the stiffness (Young's modulus) of the structures can be influential on cellular behaviors, such as viability, growth, or differentiation.[33–35] To replicate the healthy native tissues, the stiffness of the bioprinted structures should be similar to that of the target tissue for the embedded cells to function properly as in the native environment. A general approach to tune the stiffness of the final structure is the modulation of the cross-linking mechanism or polymer concentration within the bioink formulation.[36]

Post-printing, during the culture, cells within the bioprinted structures start to secrete their own ECM. While the hydrogel-based matrices provide support to the encapsulated cells during the culture period, they should ideally degrade at a physiologically relevant rate to allow cells to form the new tissue. Bioink design strongly influences the degradation process, as the type of polymers or cross-linkers can alter the degradation process.[37] Different degradation approaches can be utilized, such as hydrolytic or enzymatic, and the degree of degradation has a significant effect on cellular behavior as well.[38] Because the cells would have more space to spread within the degradable materials compared to the highly cross-linked nondegradable matrices, the morphology of the encapsulated cells will be affected, possibly influencing the cellular differentiation processes.[39,40]

Another approach to control the cellular behavior is to provide the essential biochemical functionalities to the encapsulated cells through addition of bioactive cues. For example, some widely used polymers in bioprinting approaches do not provide binding sites for the encapsulated cells, which is a required feature for cell survival, growth, and proliferation. The general approach to improve cellular adhesion is to introduce cell-adhesive peptides (e.g., arginine–glycine–aspartic acid [RGD]) to the formulation, which will allow cells to adhere through integrin binding.[41] The addition of RGD sequences typically improves cellular traction and spreading of the cells within the structure.[39] Besides adding peptides, it is possible to incorporate bioactive materials, such as hydroxyapatite, or growth factors to the bioink formulations to direct cellular differentiation.[42,43] Another approach to enable native tissue such as biochemical functionality is to use dECM-based bioinks, as these materials are derived from native tissues by removal of the cellular components while retaining the ECM materials and growth factors within the tissue.[10,44] The composition of these bioinks would otherwise be challenging to replicate, which can enhance the biological function of the encapsulated cells.

Although optimizing the biochemical and mechanical features of the bioink is crucial to control the cell behavior, the survival of the cells during the printing process and culture in the bioprinted structures is also essential. Printing processes and cell culture conditions must be cytocompatible to maintain a high cell viability in the printed structures. In addition, the materials used in the bioink formulations such as the polymers, cross-linkers, or initiators, and the degradation byproducts should be biocompatible to avoid cell damage during the culture period.[45,46]

Although many factors should be considered for an ideal bioink design using cell-laden hydrogels, the alterable properties are limited to cell-only bioinks, as they only contain live cells with no carrier hydrogel. The desired properties of the cell-only bioinks for a successful bioprinting process can be listed as printability, self-assembly post-printing, cell viability, and the type of cells. Printability of the cell-only bioinks is challenging to improve separately, as the formulation contains only cells. Therefore, the cells are generally bioprinted within or on top of the support materials, which degrade or wash away after allowing the self-assembly of the printed cells to hold their shapes and form self-supporting structures.[47–49] The printing technique should be delicate as there is no carrier to protect the cells from the printing process in the cell-only bioinks. The cell-only bioinks in the form of aggregates or spheroids were in various studies with high cell viability.[48,50–53] Additionally, the choice of cells is another important factor in cell-only bioink design, as the cell lines within the formulation must resemble the composition of the targeted native tissue and provide the required bioactivity to the printed structures.

Developing 3D printing techniques

Although bioink design is one of the crucial steps of bioprinting as it directly impacts the cellular behavior, developing advanced bioprinting techniques are also important to replicate the inherent tissue architecture. The main challenges in the fabrication of large-scale structures include the mechanically soft nature of bioinks, which cause loss in structural integrity, and incorporation of a vascular network to provide sufficient nutrient support to the encapsulated cells within the printed structure. In addition, due to the layer-by-layer fabrication approach, 3D bioprinting is limited for incorporation of complex features, such as hollow channels and overhanging structures.[58,59] However, multiple types of bioinks are required to be positioned in the printed structures to incorporate the needed biological heterogeneity while replicating architectural features of the respective tissue model. Although some biofabrication methods such as gel casting or unit stacking can overcome these issues to an extent, they have not been able to provide the required structural complexity.[60] In recent years, there have been emerging bioprinting approaches, such as embedded printing, volumetric printing, organ-on-a-chip platforms, four-dimensional (4D) printing, and *in situ* printing, to improve the fabricated tissue models.

Embedded printing

To overcome the challenges due to the limitations of conventional biofabrication techniques, embedded printing approaches within support baths were developed by Angelini[32] and Feinberg[31] groups around the same time. In these approaches, soft hydrogels are printed within a suspension bath that contain microgels which show Herschel–Bulkley or Bingham plastic behavior (**Figure** 1a). These support baths act as a liquid under shear where the needle moves during the extrusion process and act as solid when the needle departs and shear is removed. Because the microgels utilized in embedded printing applications show shear-thinning and self-healing properties, the needle can freely move within the support bath and the deposited features are held within the printed location without spreading as microgels recover rapidly to hold the printed material without losing their structural integrity. Due to this unique feature, embedded printing can be applied to various types of soft materials that are challenging to fabricate architecturally complex self-supporting structures.[61,62] With the potential of fabricating overhanging structures, embedded printing can also be used to fabricate vascularized tissues by depositing sacrificial materials, which can be removed after the printing process, within a matrix to generate hollow channels.[40,63] These channels can then be seeded with endothelial cells to form vascular structures that can enhance nutrient support.

Although cell-laden hydrogels have been convenient bioink choices as they provide cells a 3D matrix that resembles the ECM and applied in embedded printing applications, the use of hydrogel-based inks limits cellular interactions, and can lead to inhomogeneous distribution of cells and low cell density within the bioprinted structure.[47] To overcome these issues, cell-only bioinks started to receive growing interest to fabricate densely packed, scaffold-free cellular structures. Due to their soft nature, it is challenging to utilize cell-only bioinks in air with layer-by-layer printing approaches to create self-supporting structures. Thus, cell-only bioinks are generally printed within suspension baths using embedded printing approaches that act as a support during the bioprinting printing process. For example, using cell-only bioinks, Jeon et al. showed that highly complex structures could be

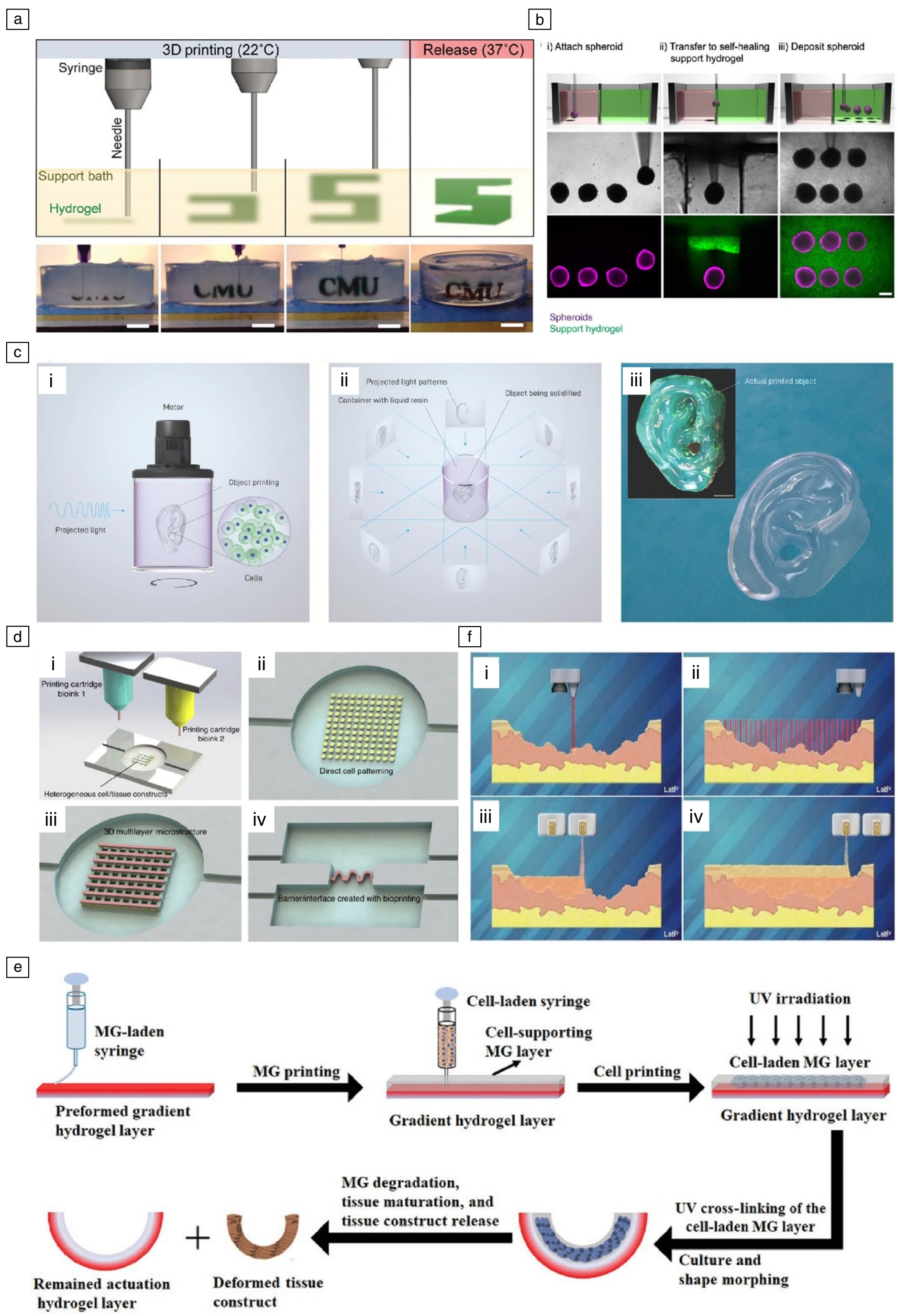

Figure 1. (a) Freeform bioprinting approach to print embedded soft hydrogel (alginate) and collect the printed features by releasing the gelatin support bath. Scale bars = 1 cm. Adapted with permission from Reference 31. © 2015 The Authors, licensed under a Creative Commons Attribution (CC BY 4.0) license. (b) Embedded bioprinting using mesenchymal stem cell-only bioinks (spheroids) to fabricate cellular structures with high cell density within hyaluronic acid-based support bath. Scale bars: 250 µm. Adapted with permission from Reference 49. © 2021 The Author(s), licensed under a CC BY 4.0 license. (c) Volumetric bioprinting approach where (i) the bioink is loaded into a rotating platform to (ii) to project light patterns. (iii) Bioprinted ear model in 22.7 s. Scale bar: 500 µm. Adapted with permission from Reference 54. © 2019 Wiley. (d) Organ-on-a-chip approach where (i) multiple bioinks are deposited to create heterogeneous structures, (ii) reproduce direct cell patterning or (iii) 3D microstructures, and (iv) used to model tissue barrier/interface. Adapted with permission from Reference 55. © 2019 Elsevier. (e) Four-dimensional bioprinting approach with cell-only bioinks to generate preprogrammed cellular shapes. MG, microgel. Adapted with permission from Reference 56. © 2022 Wiley. (f) *In situ* bioprinting approach where the defect site can be scanned for precise bioprinting and directly filled with bioinks. Adapted with permission from Reference 57. © 2019 The Author(s).

fabricated within oxidized methacrylated alginate microgel support using an hMSCs-only bioink. The 3D printed hMSCs were then differentiated into cartilage and bone tissues.[48] Brassard et al. developed an approach called bioprinting-assisted tissue emergence (BATE) where macroscale intestinal tubes within Matrigel-collagen supports could be formed through self-organization of bioprinted cell aggregates.[53] Besides using cell aggregates as cell-only bioinks, spheroids were also utilized (Figure 1b) to fabricate densely populated osteogenic and chondrogenic tissues,[50–52] as well as cardiac tissue models[49] using embedded printing techniques. Although the use of cell-only bioinks along with suspension baths improve complexity of the tissues by incorporating high cell density to the fabricated structures, the current approaches use the suspension bath technique generally as a mechanical support only and the printed structures lack the relevant bioactive cues and heterogeneity to guide cellular behavior, such as differentiation into a specific lineage or cellular morphology.

Current embedded printing approaches limit the printable formulations due to the lack of tunable material properties of the support baths. To improve the embedded printing methods, Ji et al. developed a novel printing approach that could be used to create complex channels within cell-laden hydrogels.[63] In this approach, the support bath was replaced with a support matrix that is printed layer-by-layer with commercially available photocurable methacrylated alginate (MeAlg) and methacrylated hyaluronic acid (MeHA) hydrogels while partially cross-linking each layer by blue light (405-nm wavelength) exposure to create a self-supporting layer. At the desired height of the printed structure, a sacrificial ink (Pluronic F-127) was deposited within the matrix layer prior to partial cross-linking. After this layer was stabilized by partial cross-linking again, more layers could be deposited, and the final structure was fully cross-linked. After the 3D printing process is complete, the sacrificial material could be washed away to obtain embedded hollow channels within cell-laden hydrogels that could be seeded with human umbilical vein endothelial cells (HUVEC) to form a confluent endothelial layer on the channel walls. This approach facilitates the incorporation of user-defined and tunable channels, while improving the number of usable support materials in embedded printing techniques, as the developed approach does not require use of shear thinning and self-healing microgels.

Volumetric printing

Despite the improvements in the printable features with embedded printing techniques, it is still challenging to fabricate living tissues with inherent tissue architecture at clinically relevant sizes in centimeter-scale rapidly. Recently, Kelly et al. developed a method called computed axial lithography, where a set of 2D images are projected onto a photocurable resin from multiple angles (Figure 1c), which eliminates layer-by-layer deposition and allows fabrication of centimeter-scale structures within minutes.[64] This approach was utilized by Bernal et al. to fabricate cellular meniscus shaped constructs using chondroprogenitor cells along with a methacrylated gelatin (GelMA)-based ink. Fabricated structures could be cultured up to 28 days *in vitro* and showed high cell viability and neotissue formation.[54] Later, in another study, Bernal et al. used a similar approach to bioprint hepatic organoids within a GelMA-based ink with complex perfusable architectures that showed liver-specific functionality, such as improved urea and albumin secretion.[65] These advanced bioprinting techniques demonstrate significant promise for volumetric bioprinting to generate large-scale tissue models in minutes by using patient-specific medical images that can be used for regenerative medicine applications as well as disease models and drug screening.

Organ-on-a-chip platforms

Organ-on-a-chip platforms contain 3D cellular microfluidic structures that allow cellular micron-scale tissue models to act as model organs and closely mimic the mechanical and physiological responses of the native tissues and organs. It is possible to fabricate multicellular structures and recapitulate the tissue–tissue interface using 3D bioprinting, which gives an opportunity to perform complex *in vitro* studies for functional tissues and organs (Figure 1d).[55,66,67] These platforms are also a great tool to simulate external conditions, such as shear stress, and mechanical or electrical stimulus. These aspects render organ-on-a-chip models promising candidates for high-throughput disease modeling and drug screening experiments. Three-dimensional bioprinting, especially extrusion-based techniques, is a convenient approach to fabricate organ-on-a-chip models, as it can print multiple materials to form distinct tissues in a single chip. Although it is possible to fabricate organ on a chip for a single target tissue, the main strength of the organ-on-a-chip models is to incorporate multiple tissues or organs on a single chip to generate integrated functional living structures, where response of multiple organs can be examined during drug screening or disease progression of a target organ. For example, Skardal et al. developed an organ-on-a-chip system, including liver, heart, and lung in a single microfluidic chip.[68] It was shown that when lung specific toxicity was incorporated through addition of bleomycin, cardiac tissue was affected by the released inflammatory cytokines by the lung organoids within the integrated system.

Four-dimensional printing

In the native environment, properties of tissues and organs are dynamic and change during new tissue formation or disease progression processes. To incorporate the dynamic nature, properties of the bioprinted structures, such as shape or cellular behavior, should evolve in time, which can be included as the fourth dimension into the printing process. Four-dimensional printing refers to the fabrication of preprogrammed 3D structures that show changes in shape or function in response to external stimuli, such as temperature, humidity, pH change, or enzymatic response.[69,70] Recently, Ding et al. showed that 4D printing can be used to print cell-only bioinks within a shape morphing bilayer hydrogel disc consisting of cell-supporting oxidized and methacrylated alginate (OMA) and a printed gradient hydrogel layer, including OMA and

GelMA (Figure 1e).[56] In the bilayer hydrogel system, the hMSC-only structures could be collected after 21 days of culture with preprogrammed shape and they were differentiated into chondrogenic cells. This study shows the promising potential of 4D printing in fabricating scaffold-free systems with preprogrammed structural changes. Although the shape and structural change is mostly investigated with 4D bioprinting, stimuli responsive bioinks that contain bioactive cues can potentially be incorporated to regulate and instruct the cellular behavior within the printed structures dynamically during the culture.

In situ printing

One of the end goals of 3D bioprinting is to perform surgical procedures on the defect site by bioprinting directly on the damaged tissue and organ. Although *ex situ* bioprinting provides significant advantages for fundamental studies, the incorporation of *in situ* conditions provide better integration of the bioprinted implants on the defect site due to direct connection with the natural microenvironment of the tissue and an enhanced maturation process in the body, which acts as a natural bioreactor for the bioprinted implants, compared to *in vitro* culture condition or bioreactors.[71,72] During *in situ* printing, either an automated robotic arm or handheld device is used. In the robotic arm approach, the defect size is imaged using various scanning approaches, such as CT, MRI, or structured-light scanning, which is later used to directly print on with an automated system (Figure 1f). In the handheld system, the bioink is deposited at a controlled rate but the location of the deposition is controlled by a surgeon. *In situ* printing was successfully applied to various types of tissues, such as bone,[73,74] cartilage,[75–77] muscle,[78–80] skin,[57,81,82] brain[83] and dental pulp.[84]

Concluding remarks

With the immense potential of 3D bioprinting to fabricate functional tissue mimetics, the design of bioinks and development of novel printing technologies became an important research direction in the emerging 3D bioprinting field. In this issue, we present five articles focusing on 3D bioprinting for medical applications. Walters-Shumka et al.[85] provided recent advances in personalized 3D bioprinted tissue models for cardiac, cancer, skin, and neural tissue applications highlighting the use of 3D bioprinting and patient-derived cells as a promising new avenue for disease modeling, drug discovery, and regenerative medicine. Skardal et al.[86] focused on 3D bioprinting of *in vitro* tumor organoid and organ-on-a-chip models. They provided bioprinting and biomaterial technologies to fabricate organoids and organ-on-a-chip platforms, and described the uses of these model systems for different types of tumors, immune-oncology studies, and personalized medicine. Jang et al.[87] discussed volumetric bioprinting strategies to create large-scale tissues and organs, and provided a critical evaluation of advantages and limitations of this technology. Gharacheh and Guvendiren[88] discussed current bioprinting technologies and ink formulations to create vasculature within 3D living constructs and current efforts to utilize these technologies for fabrication of vascularized bone tissues. Finally, Gottardi[89] and his colleagues provided a comprehensive review on the use of 3D and 4D bioprinting to address specific challenges in otolaryngology, mainly focusing on ear, nose, and throat.

Acknowledgments

This work is partially funded by the National Science Foundation CAREER Award (No.2044479, MG).

Funding

Division of Materials Research (2044479).

Data availability

Data sharing not applicable to this article as no data sets were generated or analyzed during the current study.

Conflict of interest

Authors declare no conflict of interest.

References

1. A. Shafiee, A. Atala, *Trends Mol. Med.* **22**, 254 (2016)
2. S. Vijayavenkataraman, W.-C. Yan, W.F. Lu, C.-H. Wang, J.Y.H. Fuh, *Adv. Drug Deliv. Rev.* **132**, 296 (2018)
3. Y. Huang, X.-F. Zhang, G. Gao, T. Yonezawa, X. Cui, *Biotechnol. J.* **12**, 1600734 (2017)
4. S.V. Murphy, A. Atala, *Nat. Biotechnol.* **32**, 773 (2014)
5. I.T. Ozbolat, Y. Yu, *IEEE Trans. Biomed. Eng.* **60**, 691 (2013)
6. S. Ji, M. Guvendiren, *Front. Bioeng. Biotechnol.* **5**(23) (2017). https://doi.org/10.3389/fbioe.2017.00023
7. C. Mandrycky, Z. Wang, K. Kim, D.-H. Kim, *Biotechnol. Adv.* **34**, 422 (2016)
8. M. Hospodiuk, M. Dey, D. Sosnoski, I.T. Ozbolat, *Biotechnol. Adv.* **35**, 217 (2017)
9. J. Groll, J.A. Burdick, D.W. Cho, B. Derby, M. Gelinsky, S.C. Heilshorn, T. Jüngst, J. Malda, V.A. Mironov, K. Nakayama, A. Ovsianikov, W. Sun, S. Takeuchi, J.J. Yoo, T.B.F. Woodfield, *Biofabrication* **11**, 013001 (2019)
10. A. Abaci, M. Guvendiren, *Adv. Healthc. Mater.* **9**, 2000734 (2020)
11. 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)
12. I.T. Ozbolat, K.K. Moncal, H. Gudapati, *Addit. Manuf.* **13**, 179 (2017)
13. I.T. Ozbolat, M. Hospodiuk, *Biomaterials* **76**, 321 (2016)
14. K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu, A. Ovsianikov, *Biofabrication* **8**, 032002 (2016)
15. M.A.S.R. Saadi, A. Maguire, N.T. Pottackal, M.S.H. Thakur, M.M. Ikram, A.J. Hart, P.M. Ajayan, M.M. Rahman, *Adv. Mater.* **34**, 2108855 (2022)
16. R.E. Saunders, B. Derby, *Int. Mater. Rev.* **59**, 430 (2014)
17. K. Zub, S. Hoeppener, U.S. Schubert, *Adv. Mater.* **34**, 2105015 (2022)
18. H. Gudapati, M. Dey, I. Ozbolat, *Biomaterials* **102**, 20 (2016)
19. C. Kryou, I. Zergioti, *Biomed. Mater. Dev.* (2022). https://doi.org/10.1007/s44174-022-00040-1
20. Y. He, F. Yang, H. Zhao, Q. Gao, B. Xia, J. Fu, *Sci. Rep.* **6**, 29977 (2016)
21. S. Derakhshanfar, R. Mbeleck, K. Xu, X. Zhang, W. Zhong, M. Xing, *Bioact. Mater.* **3**, 144 (2018)
22. J. Zhang, Q. Hu, S. Wang, J. Tao, M. Gou, *Int. J. Bioprint.* **6**(1), 242 (2020)
23. N. Paxton, W. Smolan, T. Böck, F. Melchels, J. Groll, T. Jungst, *Biofabrication* **9**, 044107 (2017)
24. A. Schwab, R. Levato, M. D'Este, S. Piluso, D. Eglin, J. Malda, *Chem. Rev.* **120**, 11028 (2020)
25. K.S. Lim, J.H. Galarraga, X. Cui, G.C.J. Lindberg, J.A. Burdick, T.B.F. Woodfield, *Chem. Rev.* **120**, 10662 (2020)
26. A. GhavamiNejad, N. Ashammakhi, X.Y. Wu, A. Khademhosseini, *Small* **16**, 2002931 (2020)
27. H.-W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, *Nat. Biotechnol.* **34**, 312 (2016)
28. T.K. Merceron, M. Burt, Y.-J. Seol, H.-W. Kang, S.J. Lee, J.J. Yoo, A. Atala, *Biofabrication* **7**, 035003 (2015)
29. F. Pati, J. Jang, D.-H. Ha, S. Won Kim, J.-W. Rhie, J.-H. Shim, D.-H. Kim, D.-W. Cho, *Nat. Commun.* **5**, 3935 (2014)

30. A.L. Rutz, K.E. Hyland, A.E. Jakus, W.R. Burghardt, R.N. Shah, *Adv. Mater.* **27**, 1607 (2015)
31. 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)
32. T. Bhattacharjee, S.M. Zehnder, K.G. Rowe, S. Jain, R.M. Nixon, W.G. Sawyer, T.E. Angelini, *Sci. Adv.* **1**, e1500655 (2015)
33. A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, *Cell* **126**, 677 (2006)
34. N. Huebsch, E. Lippens, K. Lee, M. Mehta, S.T. Koshy, M.C. Darnell, R.M. Desai, C.M. Madl, M. Xu, X. Zhao, O. Chaudhuri, C. Verbeke, W.S. Kim, K. Alim, A. Mammoto, D.E. Ingber, G.N. Duda, D.J. Mooney, *Nat. Mater.* **14**, 1269 (2015)
35. N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, J. Rivera-Feliciano, D.J. Mooney, *Nat. Mater.* **9**, 518 (2010)
36. M. Guvendiren, J.A. Burdick, *Nat. Commun.* **3**, 792 (2012)
37. S. Ji, A. Abaci, T. Morrison, W.M. Gramlich, M. Guvendiren, *Bioprinting* **18**, e00083 (2020)
38. P.M. Kharkar, K.L. Kiick, A.M. Kloxin, *Chem. Soc. Rev.* **42**, 7335 (2013)
39. S. Khetan, M. Guvendiren, W.R. Legant, D.M. Cohen, C.S. Chen, J.A. Burdick, *Nat. Mater.* **12**, 458 (2013)
40. K.H. Song, C.B. Highley, A. Rouff, J.A. Burdick, *Adv. Funct. Mater.* **28**, 1801331 (2018)
41. M. Guvendiren, J.A. Burdick, *Curr. Opin. Biotechnol.* **24**, 841 (2013)
42. A. Wenz, K. Borchers, G.E.M. Tovar, P.J. Kluger, *Biofabrication* **9**, 044103 (2017)
43. S.T. Bendtsen, S.P. Quinnell, M. Wei, *J. Biomed. Mater. Res. A* **105**, 1457 (2017)
44. B.S. Kim, S. Das, J. Jang, D.-W. Cho, *Chem. Rev.* **120**, 10608 (2020)
45. S. Boularaoui, G. Al Hussein, K.A. Khan, N. Christoforou, C. Stefanini, *Bioprinting* **20**, e00093 (2020)
46. E. Lepowsky, M. Muradoglu, S. Tasoglu, *Bioprinting* **11**, e00034 (2018)
47. C. Norotte, F.S. Marga, L.E. Niklason, G. Forgacs, *Biomaterials* **30**, 5910 (2009)
48. O. Jeon, Y.B. Lee, H. Jeong, S.J. Lee, D. Wells, E. Alsberg, *Mater. Horiz.* **6**, 1625 (2019)
49. A.C. Daly, M.D. Davidson, J.A. Burdick, *Nat. Commun.* **12**, 753 (2021)
50. B. Ayan, N. Celik, Z. Zhang, K. Zhou, M.H. Kim, D. Banerjee, Y. Wu, F. Costanzo, I.T. Ozbolat, *Commun. Phys.* **3**, 183 (2020)
51. B. Ayan, D.N. Heo, Z. Zhang, M. Dey, A. Povilianskas, C. Drapaca, I.T. Ozbolat, *Sci. Adv.* **6**, 5111 (2020)
52. B. Ayan, Y. Wu, V. Karuppagounder, F. Kamal, I.T. Ozbolat, *Sci. Rep.* **10**, 13148 (2020)
53. J.A. Brassard, M. Nikolaev, T. Hübscher, M. Hofer, M.P. Lutolf, *Nat. Mater.* **20**, 22 (2021)
54. P.N. Bernal, P. Delrot, D. Loterie, Y. Li, J. Malda, C. Moser, R. Levato, *Adv. Mater.* **31**, 1904209 (2019)
55. F. Yu, D. Choudhury, *Drug Discov. Today* **24**, 1248 (2019)
56. A. Ding, S.J. Lee, R. Tang, K.L. Gasvoda, F. He, E. Alsberg, *Small* **18**(36), 2202196 (2022)
57. M. Albanna, K.W. Binder, S.V. Murphy, J. Kim, S.A. Qasem, W. Zhao, J. Tan, I.B. El-Amin, D.D. Dice, J. Marco, J. Green, T. Xu, A. Skardal, J.H. Holmes, J.D. Jackson, A. Atala, J.J. Yoo, *Sci. Rep.* **9**, 1856 (2019)
58. P. Zorlutuna, N. Annabi, G. Camci-Unal, M. Nikkhah, J.M. Cha, J.W. Nichol, A. Manbachi, H. Bae, S. Chen, A. Khademhosseini, *Adv. Mater.* **24**, 1782 (2012)
59. H.C. Ko, B.K. Milthorpe, C.D. McFarland, *Eur. Cell Mater.* **14**, 1 (2007)
60. S. Ji, M. Guvendiren, *APL Bioeng.* **5**, 011508 (2021)
61. L.-Y. Zhou, J. Fu, Y. He, *Adv. Funct. Mater.* **30**, 2000187 (2020)
62. J. Zhao, N. He, *J. Mater. Chem. B* **8**, 10474 (2020)
63. S. Ji, E. Almeida, M. Guvendiren, *Acta Biomater.* **95**, 214 (2019)
64. B.E. Kelly, I. Bhattacharya, H. Heidari, M. Shusteff, C.M. Spadaccini, H.K. Taylor, *Science* **363**, 1075 (2019)
65. P.N. Bernal, M. Bouwmeester, J. Madrid-Wolff, M. Falandt, S. Florczak, N.G. Rodriguez, Y. Li, G. Größbacher, R.-A. Samsom, M. van Wolferen, L.J.W. van der Laan, P. Delrot, D. Loterie, J. Malda, C. Moser, B. Spee, R. Levato, *Adv. Mater.* **34**, 2110054 (2022)
66. S.N. Bhatia, D.E. Ingber, *Nat. Biotechnol.* **32**, 760 (2014)
67. Q. Wu, J. Liu, X. Wang, L. Feng, J. Wu, X. Zhu, W. Wen, X. Gong, *Biomed. Eng. Online* **19**, 9 (2020)
68. A. Skardal, S.V. Murphy, M. Devarasetty, I. Mead, H.-W. Kang, Y.-J. Seol, Y. Shrike Zhang, S.-R. Shin, L. Zhao, J. Aleman, A.R. Hall, T.D. Shupe, A. Kleensang, M.R. Dokmeci, S. Jin Lee, J.D. Jackson, J.J. Yoo, T. Hartung, A. Khademhosseini, S. Soker, C.E. Bishop, A. Atala, *Sci. Rep.* **7**, 8837 (2017)
69. S. Amukarimi, M. Mozafari, *Bioprinting* **23**, e00161 (2021)
70. Q. Yang, B. Gao, F. Xu, *Biotechnol. J.* **15**, 1900086 (2020)
71. M. Samandari, A. Mostafavi, J. Quint, A. Memić, A. Tamayol, *Trends Biotechnol.* **40**, 1229 (2022)
72. S. Singh, D. Choudhury, F. Yu, V. Mironov, M.W. Naing, *Acta Biomater.* **101**, 14 (2020)
73. L. Li, J. Shi, K. Ma, J. Jin, P. Wang, H. Liang, Y. Cao, X. Wang, Q. Jiang, *J. Adv. Res.* **30**, 75 (2021)
74. K.K. Moncal, R.S. Tigli Aydın, K.P. Godzik, T.M. Acri, D.N. Heo, E. Rizk, H. Wee, G.S. Lewis, A.K. Salem, I.T. Ozbolat, *Biomaterials* **281**, 121333 (2022)
75. C.D. O'Connell, C. Di Bella, F. Thompson, C. Augustine, S. Beirne, R. Cornock, C.J. Richards, J. Chung, S. Gambhir, Z. Yue, J. Bourke, B. Zhang, A. Taylor, A. Quigley, R. Kapsa, P. Choong, G.G. Wallace, *Biofabrication* **8**, 015019 (2016)
76. S. Duchi, C. Onofrillo, C.D. O'Connell, R. Blanchard, C. Augustine, A.F. Quigley, R.M.I. Kapsa, P. Pivonka, G. Wallace, C. Di Bella, P.F.M. Choong, *Sci. Rep.* **7**, 5837 (2017)
77. C. Di Bella, S. Duchi, C.D. O'Connell, R. Blanchard, C. Augustine, Z. Yue, F. Thompson, C. Richards, S. Beirne, C. Onofrillo, S.H. Bauquier, S.D. Ryan, P. Pivonka, G.G. Wallace, P.F. Choong, *J. Tissue Eng. Regen. Med.* **12**, 611 (2018)
78. J.P. Quint, A. Mostafavi, Y. Endo, A. Panayi, C.S. Russell, A. Nourmahnad, C. Wiseman, L. Abbasi, M. Samandari, A. Sheikhi, K. Nuutila, I. Sinha, A. Tamayol, *Adv. Healthc. Mater.* **10**, 2002152 (2021)
79. 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)
80. A. Mostafavi, M. Samandari, M. Karvar, M. Ghovvati, Y. Endo, I. Sinha, N. Annabi, A. Tamayol, *Appl. Phys. Rev.* **8**, 041415 (2021)
81. R.Y. Cheng, G. Eylert, J.-M. Gariepy, S. He, H. Ahmad, Y. Gao, S. Priore, N. Hakimi, M.G. Jeschke, A. Günther, *Biofabrication* **12**, 025002 (2020)
82. G. Ying, J. Manríquez, D. Wu, J. Zhang, N. Jiang, S. Maharjan, D.H. Hernández Medina, Y.S. Zhang, *Mater. Today Bio.* **8**, 100074 (2020)
83. A. Urciuolo, I. Poli, L. Brandolino, P. Raffa, V. Scattolini, C. Laterza, G.G. Giobbe, E. Zambaiti, G. Selmin, M. Magnussen, L. Brigo, P. De Coppi, S. Salmaso, M. Giomo, N. Elvassore, *Nat. Biomed. Eng.* **4**, 901 (2020)
84. D.F. Duarte Campos, S. Zhang, F. Kreimendahl, M. Köpf, H. Fischer, M. Vogt, A. Blaeser, C. Apel, M. Esteves-Oliveira, *Connect. Tissue Res.* **61**, 205 (2020)
85. J.P. Walters-Shumka, S. Sorrentino, H.B. Nygaard, S.M. Willerth, *MRS Bull.* **48**(6) (2023). https://doi.org/10.1557/s43577-023-00551-2
86. S. Anderson, E. Stagner, A. Skardal, *MRS Bull.* **48**(6) (2023)
87. D. Kim, D. Kang, D. Kim, J. Jang, *MRS Bull.* **48**(6) (2023). https://doi.org/10.1557/s43577-023-00541-4
88. H. Gharacheh, M. Guvendiren, *MRS Bull.* **48**(6) (2023). https://doi.org/10.1557/s43577-023-00547-y
89. I. Chiesa, C. De Maria, D. Vozzi, R. Gottardi, *MRS Bull.* **48**(6) (2023). https://doi.org/10.1557/s43577-023-00544-1

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Alperen Abaci is a doctoral candidate in the Department of Chemical and Materials Engineering at the New Jersey Institute of Technology. He received his BS degree from Boğaziçei University, Turkey, in 2018. His research focuses on development of novel bioinks and three-dimensional bioprinting approaches for regeneration of osteochondral interface. Abaci can be reached by email at aa2848@njit.edu.

Gulden Camci-Unal is an associate professor in the Department of Chemical Engineering at the University of Massachusetts Lowell. Her research interests include design and implementation of engineered biomaterials for regeneration of tissues, unconventional materials and medical devices for personalized medicine, and low-cost point of care diagnostics. She received the Robert and Gail Ward Endowed Professorship, University of Massachusetts Lowell Teaching Excellence Award, Iowa State University Teaching Excellence Award, Runner-Up for the Massachusetts Medical Device Development Center (M2D2) $200 K Challenge, and finalist for the BioFlux Innovation Award. Camci-Unal is an associate editor for *Bioactive Materials*, and an editorial board member of *Scientific Reports*, *PLoS One*, *Bioengineering*, *Gels*, *Regenerative Engineering and Translational Medicine*, and the *International Journal of Bioprinting*. Camci-Unal can be reached by email at Gulden_CamciUnal@uml.edu.

Murat Guvendiren is an associate professor in the 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. His awards include 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.