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In situ bioprinting: Tailored printing strategies for regenerative medicine

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Abstract

In recent years, three-dimensional (3D) bioprinting has emerged as a revolutionary biological manufacturing technology. Despite significant progress, current bioprinting technologies face critical barriers, such as the need for *in vitro* maturation of printed tissues before implantation and challenges of prefabricated structures not matching the defect shapes. *In situ* bioprinting has been introduced to address these challenges by printing customized structures to the wound shape via direct deposition of biological inks at the tissue interface. This paper reviews strategies to optimize printing performance for enhanced tissue repair and analyzes the advantages, challenges, and future directions of *in situ* bioprinting technologies.

Keywords: Bioprinting; *In situ* bioprinting; Tissue regeneration; Bioinks; Handheld bioprinter

1. Introduction

Over the past decade, bioprinting has gained widespread attention as a powerful tool to precisely control the spatial placement of cells and biomaterials.[1] Bioprinting refers to the bottom-up automated fabrication of scaffolds, containing living cells, drugs, and growth factors, with the aid of additive manufacturing technology in a computer-aided manner.[2-4]

Bioprinting is increasingly demanded due to conditions such as osteosarcoma,[5,6] osteoporosis,[7,8] and skin burns.[9–11] Given the complex hierarchical architectures of human organs and the individual differences among patients, conventional tissue engineering strategies fail to fabricate scaffolds with controlled surface chemistry and complex microstructure.[12,13] Bioprinting can construct artificial tissue grafts with precise cell and regenerative factor placements, overcoming the limitations of donor availability.[14] Bioprinting is also widely used to create tissue models for drug testing[15–18] and disease modeling.[19–22] However, there are still limitations that hinder its development: (i) conventional bioprinting strategies require a computer-aided design (CAD) model, generated by magnetic resonance imaging (MRI) or X-ray computed tomography (CT), before printing and transplantation to the wound site, but the time-consuming nature of MRI or CT scanning makes this approach challenging for time-sensitive clinical cases[23]; (ii) the printed scaffolds may deform or contract after implantation, making it challenging to precisely match the defects[2]; (iii) before surgical implantation, the scaffold requires *in vitro* maturation that lasts several weeks.[24] Hence, it is necessary to overcome these barriers in 3D bioprinting to meet the needs of emergency clinical applications.[25]

Conversely, *in situ*, bioprinting, introduced in 2007 as an emerging strategy for clinical translation of bioprinting, has recently gained traction.[26,27] This technology, also called intraoperative bioprinting, directly prints biomaterials inside tissue defects.[28,29] *In situ* bioprinting bypasses *in*

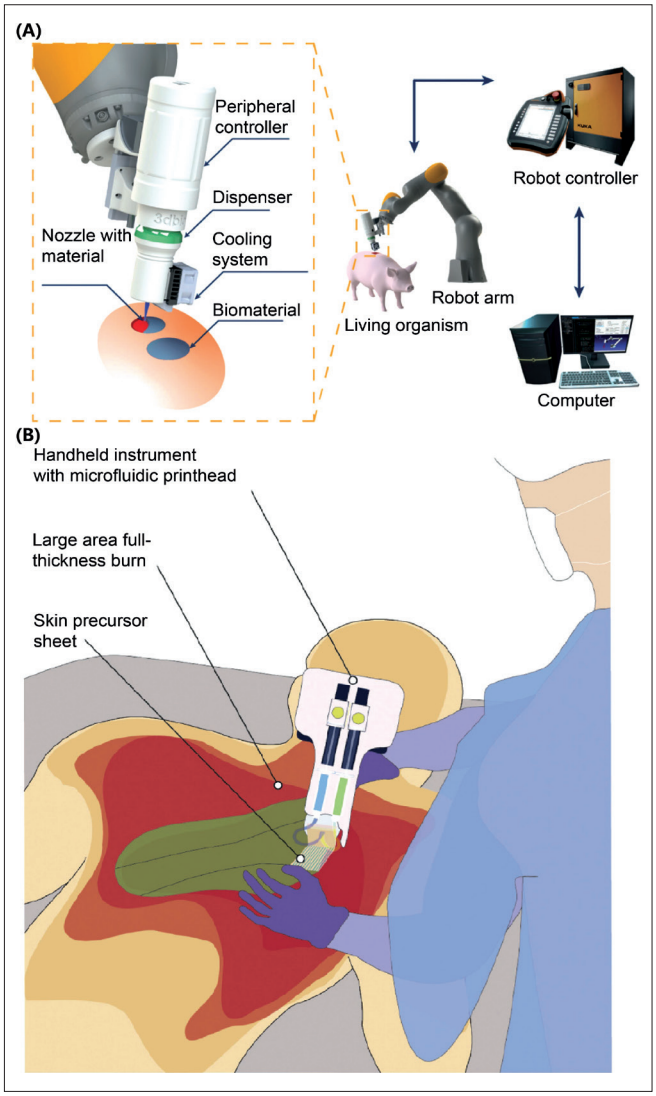

Figure 1. Schematic diagrams of (A) robotic-assisted *in situ* bioprinting system (RASBS) and (B) handheld *in situ* bioprinting system (HISBS). Adapted, with permission, from Levin et al.[34] (A) and Cheng et al.[9] (B).

vitro pre-printing and incubation requirements, reduces contamination risks, and enables real-time adjustments according to the printed structures. Compared with conventional bioprinting, the strategy can precisely match the shape of the wound, crosslink *in situ* for adhesion without *in vitro* culture, facilitate rapid repairment, and minimize fibrosis.[30–32] Furthermore, *in situ* bioprinting can exploit the human body's regenerative potential, providing the physiological environment required for scaffold culture.[33] *In situ* bioprinting systems can be divided into two major categories: robotic-assisted *in situ* bioprinting system (RASBS) (Figure 1A) and handheld *in situ* bioprinting system (HISBS) (Figure 1B). RASBS can be programmed by computer-aided manufacturing (CAM) and usually be used in less mobile environments. This strategy has many advantages, such as high precision,[35,36] multi-material *in situ* printing for significant composite defects,[37] and compatibility with minimally invasive surgery,[38] but is also time-consuming and requires sophisticated equipment. HISBS is an alternative strategy for *in situ* bioprinting that is easy to use without the need for complex equipment and expertise. Although HISBS has a relatively lower printing resolution and limited multi-material processing ability due to the compromise for portability, it has the potential to meet specific requirements of emergency clinical applications. Minimally invasive *in situ* bioprinting combines robotic assistance and human control for non-invasive printing *in vivo*.

In situ 3D bioprinting has been demonstrated to effectively repair tissue defects, addressing the problems of mismatched structures from conventional 3D bioprinting and reducing infection risks, while simplifying the surgical procedure. Despite existing reviews on *in situ* 3D bioprinting technology, recent research has reported new advancements in this technology, highlighting its potential to enhance tissue repair and better promote its clinical application. These studies focus on ensuring the precise fit and mechanical integrity of structures for irregularly shaped wounds, optimizing the printing path, real-time monitoring of the printing process, and accurately positioning and curing bioinks in deep tissues *in vivo*. Herein, this article reviews the utilization of *in situ* bioprinting in real-time monitoring and the optimization of printing performance in terms of automatic printing, handheld printing, human-controlled machine assisted in situ bioprinting and bioinks.

2. Strategies of *in situ* bioprinting

The bioprinting techniques used for *in situ* bioprinting include inkjet bioprinting,[37,39] laser-assisted bioprinting (LAB),[40,41] extrusion bioprinting,[42,43] stereolithography-based (SLA) bioprinting,[44,45] and electrospinning.[46,47] A concise comparison of these techniques is provided in Table 1. Herein, we shall compare the two aforementioned *in situ* bioprinting systems (i.e., RASBS and HISBS) in detail across multiple aspects (Table 2).

2.1. Robotic-assisted *in situ* bioprinting system

2.1.1. System setup

Robotic-assisted *in situ* bioprinting systems (RASBS) are an emerging method for fabricating 3D structures using software codes, reducing human intervention and ensuring higher printing accuracy. The key factors affecting the quality of the printed structure include printing speed, stability, and repeatable positioning accuracy. The printing speed encompasses both the moving speed of the printhead and the extrusion speed of the material, and these parameters require optimization based on the rheological properties of the material.[60] For example, a high moving speed and low extrusion speed will produce discontinuous lines; a low moving speed and high extrusion speed will produce clustered lines. The stability of the printing structure mainly depends on the physical and chemical properties of the material, highlighting the importance of selecting the appropriate bioink.[61] Repeatable positioning accuracy sets high requirements for *in situ* bioprinting systems, necessitating robot-assisted positioning combined with computer vision and sensors to further improve positioning accuracy.[62]

Nonetheless, RASBS offers a range of advantages, such as: (i) superior printing accuracy and dexterity[24] that are crucial for achieving a precise fit with the exact shape of the wound; (ii) rapid production of complex multi-material structures,[63,64] especially in critical situations that necessitate emergency treatment, such as in battlefield or accident scenarios; (iii) reduced human intervention,[65] as RASBS can automate the bioprinting process using computer-aided robotic arms and digital models; (iv) seamless integration with minimally invasive surgical techniques,[29] including endoscopy, facilitating inside body printing; (v) compatibility with process monitoring systems and machine learning techniques,[65–67] contributing to error reduction during the printing process; and (vi) enhanced cell viability by minimizing the exposure of printed cells to external environmental conditions, while the complex topological structures aid in regulating the spatial distribution and growth of cells.

Most reported RASBS are made up of robotic arms,[65,68–71] but some automated *in situ* bioprinting platforms are made up of framework-based systems.[32,72] The *in situ* printing system based on the robotic arm can utilize either multi-axis rigid robot arms[38,73] or flexible robot arms.[74] During the printing process, the structure is printed by computer-

Table 1. Comparison of different bioprinting methods

Parameter	Bioprinting method				
	Inkjet[37,48]	**LAB**[40,41,49]	**Extrusion**[29,50,51]	**SLA**[44,45]	**Electrospinning**[46,47]
Print speed	Fast	Medium	Slow	Fast	Fast
Resolution	High	High	Moderate	High	High
Bioink material	Fibrin/collagen; PEGDA	Collagen; hydroxyapatite	GelMA; Alginate/ gelatin; GelMA/ Laponite/ methylcellulose	GelMA; HCC-PEG; HCC-gelatin	PCL; PLA
Applications	Vessels; skin	Bone	Stomach; skin	Skin; muscle	Skin

Abbreviations: GelMA, gelatin methacryloyl; HCC, 7-hydroxycoumarin-3-carboxylate; LAB, laser-assisted bioprinting; PCL, poly(caprolactone); PEG, poly(ethylene glycol); PEGDA, poly(ethylene glycol diacrylate); PLA, poly(lactic acid); SLA, stereolithography.

Table 2. Comparison between robotic-assisted *in situ* bioprinting systems (RASBS) and handheld *in situ* bioprinting systems (HISBS)

Feature	RASBS	HISBS
Requirements of expertise	Operators require certain professional knowledge and operating experience	Simple and intuitive interface; easy-to-use
Key components	Robotic controller; 3D scanner; CAD/CAM	Rollers, ink cartridges, and print heads the parts are usually integrated into a single unit
Resolution	High	Low
Printed scaffold	Instant production according to the condition of the wound	Instant production according to the condition of the wound
Complexity of scaffold	High	Low
Application	Skin[37,52], bone[1,40], cartilage[1], brain[45], and muscle[44]	Skin[9], skeletal muscle[53,54], cartilage[55,56,57], bone[58], and dental[59]

Abbreviations: CAD, computer-aided design; CAM, computer-aided manufacturing.

aided robot positioning and path planning. The geometry of the defect could be obtained using a high-definition scanner with computer assistance. Slicing software then programs the printing path, which is subsequently executed by the robotic arm comprising a multi-axis movable bioprinting unit.[2]

However, the printing environment of RASBS can be suboptimal, featuring challenges such as wet, irregularly shaped, and potentially moving surfaces (due to patient breathing and twitching), which can cause printed scaffolds to deviate from the wound area and lead to structural weakness.[28] Addressing this issue requires improvements in the fidelity of the printed structure and real-time monitoring of the printing process.[35,50]

2.1.2. Strategies to improve the fidelity of printed structures

A decrease in the fidelity of printed structures results in a mismatch between the structure and defect shape, which can lead to inadequate mechanical support. Achieving accurate *in situ* printing of structures on non-planar surfaces is a challenge. Conventional planar slicing can create a stepped arrangement on inclined surfaces, potentially compromising the mechanical integrity of the structure. Adaptive slicing and multidirectional slicing techniques are employed to reduce this step effect and improve printing precision. Chaudhry et al.[62] presented a print path-planning strategy based on a free-form surface-slicing design. Using this approach, they designed a three-layered skin implant with customizable porosity and mechanical strength. To ensure that the printed structure has a smooth surface, RASBS can also be integrated with sensors and computer vision to improve positioning accuracy, as well as the use of robotic arms with higher degrees of freedom (DOF).[62] Fortunato et al.[71] developed a five-axis *in situ* bioprinting platform to deposit ink via pneumatic injection for simulating skull defect repair. The *in situ* printing system with higher DOF can improve the printing accuracy and enable the deposition of bioink on the curved surface.[68] Ma et al.[66] introduced an extrusion-based six-DOF robotic-assisted 3D bioprinting technology for cartilage regeneration using a fast tool center point calibration method to significantly enhance printing accuracy. This study demonstrated that the robotic system could improve the rate and recovery performance.

Similarly, Zhao et al.[65] developed a six-DOF bioprinting system (Figure 2A), integrating a 3D scanner and a closed-loop visual system to facilitate rapid healing and high-precision printing. The authors further proposed a seven-axis bioprinting system for *in vivo* underwater bioprinting, specifically designed to operate within the limited space inside the amniotic sac.[38] This seven-axis robot has redundant properties, ensuring high-precision printing with minimal intrusion. The redundant properties of the robot means that the total freedom of each joint is greater than the freedom of the end-effector, and the redundant freedom enables obstacle avoidance and enhances flexibility during the printing process.

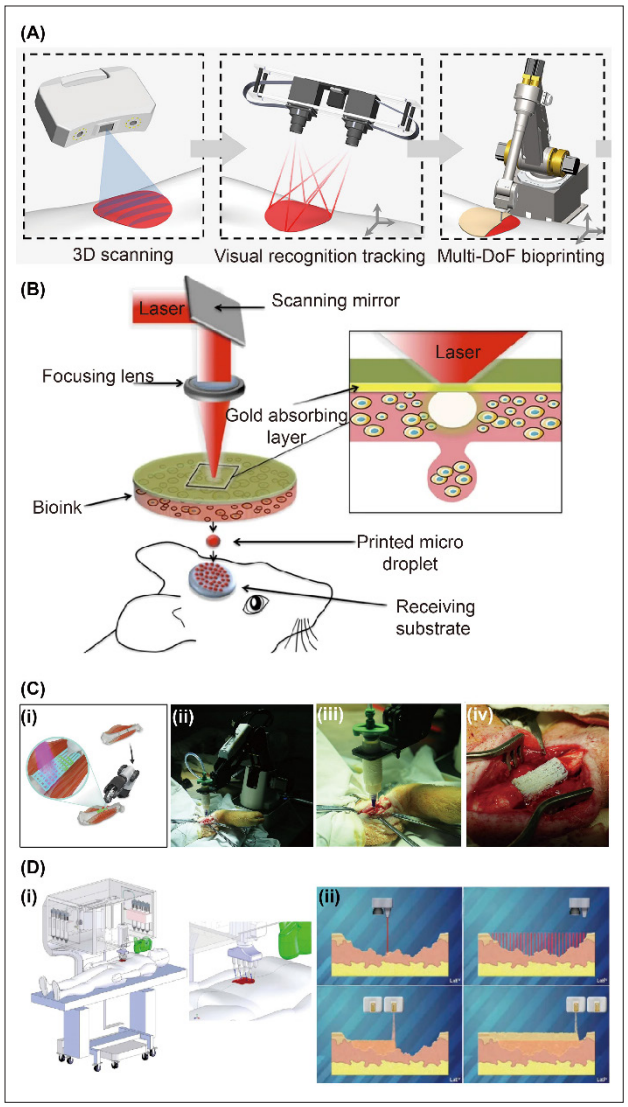

Figure 2. Robotic-assisted *in situ* bioprinting system (RASBS). (A) Schematic of the multi-degree-of-freedom (DoF) bioprinting system. (B) Application of laser-assisted bioprinting (LAB) to directly deposit bioink-encapsulated cells in mice models. (C) The process of *in situ* 3D bioprinting. (C, i) Extruded hybrid hydrogel is photo-polymerized by the ultraviolet (UV) lamp; (C, ii) view of the 3D bioprinting system; (C, iii) the bioprinting process; and (C, iv) the printed scaffold. (D) Treatment of large burn wounds. (D, i) Prior to *in situ* bioprinting, the wound topography was obtained using a handheld 3D scanner; (D, ii) the printhead deposits regenerative materials to specified locations under the guidance of the wound model. Adapted with permission from Zhao et al.[65] (A), Keriquel et al.[40] (B), Li et al.[35] (C), and Albanna et al.[37] (D).

The printing accuracy can also be enhanced using flexible robotic arms, which offer the advantages of not being limited to the DOF of rigid robotic arms and using fewer motors, resulting in smaller robotic arm volumes. Shi et al.[74] developed a flexible soft robotic arm for *in situ* bioprinting, whereby the manipulator can move freely in 3D space along Cartesian and curvilinear coordinates. Moreover, this innovation facilitates the printing of complex structures on curved wounds. For *in situ* printing on a curved surface, the ink is required to be crosslinked immediately. However, optical crosslinking may clog the printhead during printing, which is the primary cause of errors during the printing process.[35] Therefore, achieving instant control of the exposure direction is essential to mitigate this issue. Fortunato et al.[75] developed an *in situ* printing system that is integrated with an automatically activated optical crosslinking system to control the exposure direction according to the print path, ultimately avoiding the risk of needle clogging. Additionally, the potential for printhead blockage also depends on the rheological properties of the bioink. To minimize printhead blockage, selecting a material with shear-thinning characteristics can be advantageous.

2.1.3. Real-time tracking of the printing process

Imaging the tissue defect area is essential to determine the wound structure and construct a model of the implant, prior to printing with the computer-assisted RASBS. The accuracy of the model based on pre-print imaging data directly affects the degree of coincidence between the structure and the target region. During the printing process, mismatches between the printhead ink extrusion and movement speeds can result in deposition errors, thereby reducing the fidelity of the printed structure. Furthermore, correction errors of the print head, instability in the rheological characteristics of the bioink, and control errors stemming from environmental factors can also affect the fidelity of the structure. Hence, it is essential to monitor and implement feedback control mechanisms in the printing process for accuracy and consistency.

The printing procedure would make real-time adjustments according to the printed structures under predefined 3D geometries through CAD and/or CAM to regulate the spatial distribution of all regenerative biomaterials. The calibration process is necessary to reduce

printing errors. In error compensation, a new G-code is generated by comparing the printed structure and the 3D geometry for subsequent modification to the bioprinting process.[28] However, this compensation cannot be adjusted in real time according to the printing condition, especially on wet or deformable surfaces. The lack of process monitoring and immediate feedback adjustment are the main reasons for low structure fidelity. For example, when bioprinting on the surface of the human body, human breathing may cause the movement of the printing base, resulting in an error in the printed structure. Zhu et al.[50] introduced an adaptive 3D bioprinting method that can compensate for the motion of the target surface. The method integrates scanning and *in situ* bioprinting systems, allowing real-time correction of any printing errors based on feedback from the scanning system. Zhao et al.[65] introduced a closed-loop feedback system that enables real-time motion tracking of defects. In this system, the camera identifies the location of the wound and provides feedback to the robotic arm. Kucukdeger et al.[76] proposed a closed-loop control path planning method for micro-extrusion 3D printing based on the real-time perception of local nozzle offset, without pre-characterization of object geometry.

In addition to adaptive *in situ* 3D bioprinting, Yang et al.[77] combined optical coherence tomography (OCT) with *in situ* 3D bioprinting to detect defects layerwise. This approach aims to achieve process monitoring and ensure high structural fidelity. Several common methods used to reconstruct 3D images, such as confocal and multi-photon microscopes, are slow and require additional custom equipment.[78] OCT imaging can be efficiently integrated into *in situ* 3D bioprinting systems to enable real-time and rapid analysis of the printing process. This integrated OCT imaging system can detect print channel blockage, uniformity of printed structures, and defects caused by bubbles.[79] Yang et al.[78] developed a large-field, full-depth imaging system based on OCT. The system features a pre-established feedback control mechanism to perform secondary printing repairs on identified defects. This strategy of *in situ* defect detection and timely repair enhances the fidelity of printed structures improves printing efficiency, and ensures the consistency of the printed structure. Results of finite element analysis revealed that this approach significantly improved the compression modulus of the multi-layer scaffold.

Although OCT imaging features high resolution, it can only scan a depth of 1–2 mm below the surface of biological tissues. In addition to online monitoring of the quality of the printed structure, it is also necessary to track printed cells for bioinks that contain cells, such as using MRI to visualize specific cells deep inside the body. Keriquel et al.[40] demonstrated that LAB can directly deposit collagen/nano-hydroxyapatite loaded with mesenchymal stromal cells inside a murine calvaria defect model (Figure 2B). They also reported that the geometries of the printed cell scaffolds can impact the therapeutic effect in promoting bone regeneration *in vivo*.[80]

2.1.4. Applications

Robotic-assisted *in situ* bioprinting systems (RASBS) are typically utilized in less mobile environments, such as surgical operating rooms, primarily due to their considerable size and limited mobility. Various studies have demonstrated the successful printing of diverse tissues and organs, including skin,[37,50] bone,[40,41,49] and cartilage.[66] Among various *in situ* bioprinting methods, extrusion-based bioprinting stands out as the most widely researched strategy due to its extensive selection of bioinks, low-cost equipment, and versatility.[28,43,81] Li et al.[35] presented an extrusion-based 3D bioprinting system featuring a robotic manipulator to treat the swine's bone defects. The hybrid hydrogel, consisting of sodium alginate, poly(ethylene glycol diacrylate) (PEGDA), and gelatin methacryloyl (GelMA), was extruded directly onto the defect area and photo-polymerized with an ultraviolet (UV) lamp (Figure 2C).

Inkjet bioprinting has also been employed as a strategy for *in situ* bioprinting. Inkjet bioprinting can deposit droplets in predetermined locations,[37,82] facilitating the creation of gradients in cell concentrations.[39,83] Albanna et al.[37] developed an inkjet skin bioprinter for the reconstruction of full-thickness wounds. The bioprinter system comprises two principal components (Figure 2D): (i) a 3D wound scanner and (ii) a printhead. The former can generate a wound map in a single continuous scan that is subsequently compiled with additional wound maps to form a wound model. Likewise, the printhead consists of the *X*-, *Y*-, and *Z*-axis, with the wound area divided into several layers on the *Z*-axis. They printed a fibrin/collagen hydrogel in both murine and porcine total thickness wound models. Their results indicated that combining wound scanners with inkjet bioprinting improves the rate and quality of wound healing. However, as a sequential deposition strategy, inkjet bioprinting requires precise control over the deposition location, which is challenging and time-consuming. Building on inkjet bioprinting, Christensen et al.[39] developed an intersecting jets approach that enables control over the proportion of deposited material at any point in the structure. However, due to inherent spray inconsistencies between reactive hydrogel solutions and suspensions, the printed structures lack shape fidelity. To overcome this hurdle, integrating diverse bioprinting strategies offers a promising approach for achieving *in situ* printing. Moncal et al.[36] proposed a hybrid

extrusion/inkjet-based bioprinting methodology for the reconstruction of intricate craniomaxillofacial defects. In this approach, they employed extrusion bioprinting to directly print an osteogenic hard tissue bioink, while inkjet bioprinting was utilized for the deposition of a soft tissue bioink with lower viscosity. Remarkably, their findings demonstrated approximately 80% skin reconstruction within 10 days and 50% bone regeneration after 6 weeks.

2.1.5. Challenges

While RASBS has demonstrated promise for achieving complex *in situ* bioprinting, there are still some challenges that need to be addressed:

(i) The limited workspace and large setup volume make it difficult to use for internal tissue repair.

(ii) The process requires complex equipment and is time-consuming, as it involves scanning, 3D geometry construction, printing path optimization, and error compensation.

(iii) Higher criteria for bioinks are required, as not all materials are suitable for *in situ* bioprinting. New materials that are compatible with this technique must be developed, e.g., bioinks for printing on non-planar wounds require a higher viscosity to match the wound shape.

(iv) For successful tissue regeneration, the printed tissue must be able to integrate with the host tissue.

(v) Improving the automation and scalability of *in situ* bioprinting systems can reduce the cost and increase the speed of tissue production. This effort may involve developing new software for designing and printing tissues, as well as creating systems capable of printing multiple tissues simultaneously.

In response to these challenges, artificial intelligence (AI) technology has displayed great application potential in the field of bioprinting. New biomaterials and structural design can be developed through AI and machine learning to be compatible with printing technologies and application environments. Limon et al.[84] established a prediction model of key process parameters of extrusion-based *in situ* 3D bioprinting using machine learning method, and the accuracy of the model to predict the printing wire width was 85%. Qiao et al.[85] used a machine learning model to predict the effect of cryoprotectant formulations on cryoprotected bioinks. Additionally, researchers could predict the number of cells in the printed droplet through machine learning algorithms, achieving real-time evaluation of the number of printed cells during the printing process.[86] AI-mediated real-time monitoring and feedback systems can also improve the degree of automation and printing accuracy of *in situ* bioprinting systems. In the future, advancements are anticipated in remote-controlled robotic surgery, where doctors can program surgical procedures based on patient data and remotely operate robots to perform repairs or treatment.

2.2. Handheld *in situ* bioprinting system

2.2.1. System setup

Handheld *in situ* bioprinting systems (HISBS), also known as hand devices, can be easily manipulated by operators without a professional background. Unlike RASBS, the positioning and movement of the setup during the printing process are typically controlled by operators. HISBS is particularly suitable for minor wounds as it is more flexible, can print structures of any shape, and can adjust the print path in real time. Furthermore, HISBS has a shorter print response time since it does not require prior preparation, such as scanning wound shapes or calibrating path errors.[55,87]

In the actual *in situ* bioprinting process, an initial debridement step is often required, which can result in a mismatch between the prefabricated construct and the defect. To address this challenge, handheld bioprinters have been developed for *in situ* bioprinting applications.

HISBS offers several advantages, including manual control of the printing position and speed, low-cost,[42] portability, lack of computer-aided requirements,[34] ease of sterilization,[66] and suitability for hard-to-reach and non-flat wounds.[24] Handheld printers can directly deposit biomaterials inside the defect to build a tissue scaffold. Handheld bioprinting does not require a high-definition 3D scanner to scan the defect, unlike automated systems. Additionally, handheld devices can be easily operated without requiring specialized knowledge, enabling operators to build constructs using hand movements and adjust the printing strategy in real-time.

2.2.2. Performance optimization strategy of the printed structure

Portable bioprinters, also known as hand bioprinters, have successfully fabricated various types of tissues, including skin,[9] muscle,[53,54] cartilage,[55,56,57] bone,[58] and dental tissue,[59] with most handheld bioprinting utilizing extrusion-based methods. Other bioprinting methods, such as droplet and laser-assisted, are challenged by nozzle clogging and miniaturization. Russell et al.[53] developed a hand bioprinter (Figure 3A) to treat a murine volumetric muscle loss (VML) injury. The hand bioprinter directly printed gelatin-based hydrogels, which were crosslinked *in situ* under UV light. The results indicated that this device could maintain the viability of muscle cells and promote cell proliferation. Using the same printing method, Quint et al.[54] presented a growth factor-eluting bioink to treat

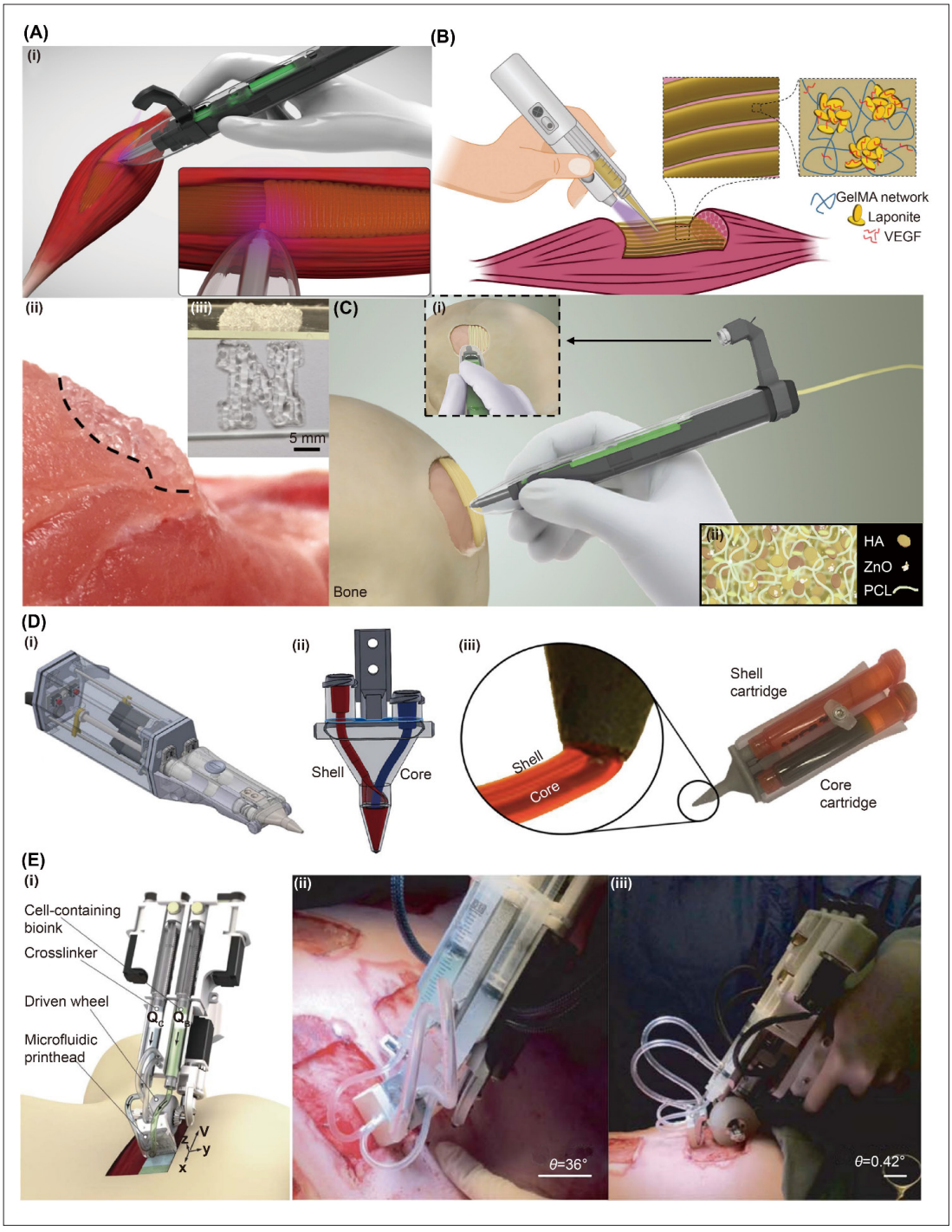

Figure 3. Applications of handheld *in situ* bioprinting systems (HISBS). (A) Utilization of a handheld bioprinter for *in situ* bioprinting of scaffolds. (A, i) Schematic of the *in situ* bioprinting of cell-laden gelatin methacryloyl (GelMA) hydrogels for the treatment of volumetric muscle loss (VML). (A, ii) Photograph of a typical scaffold printed on a non-flat porcine skeletal muscle. (A, iii) Photograph of an N-shaped scaffold (three layers thick). (B) Conceptual diagram of organized scaffold deposition directly into a wound using a handheld printer. (C) Schematic illustration of *in vivo* printing of composite scaffolds. (C, i) Schematic view of an integrated camera on the printing pen. (C, ii) Schematic of the material composition. (D) Core/shell-3D printing via co-axial extrusion. Schematic representation of the (D, i) 3D co-axial handheld printer and the (D, ii) co-axial nozzle. (D, iii) Cartridges for core/shell-loading in the printer. (E) *In situ* formation of precursor skin tissue. (E, i) Image of the handheld device. (E, ii) Isometric view of the handheld instrument during the deposition process. (E, iii) Side view of the handheld device. Scale bars: 5 mm (A, iii); 2.5 cm (E, ii and iii). Abbreviations: HA, hydroxyapatite; PCL, poly(caprolactone); VEGF, vascular endothelial growth factor. Adapted with permission from Russell et al.[53] (A), Quint et al.[54] (B), Mostafavi et al.[58] (C), Duchi et al.[56] (D), and Cheng et al.[9] (E).

VML injuries (Figure 3B). They used laponite nanoclay to control the release of vascular endothelial growth factor. The *in vivo* experimental results suggested that the bioink can promote functional muscle recovery and reduce fibrosis. Mostafavi et al.[58] introduced a hand bioprinter that can deposit melt-spun materials directly within the bone defect site (Figure 3C). The printed scaffolds displayed promising adhesion and biocompatibility in mouse models. To improve the effect of tissue repair, it is generally necessary to add bioactive factors and cells to the bioink. In the case of photo-crosslinked bioinks, the handheld printer deposits the bioink and cells into the tissue defect, and the photoinitiator reduces the activity of the cells. To address this issue, current handheld printers typically use a core-shell structure that is coaxially extruded from the bioink and cell components, isolating the photoinitiator from the cell. Duchi et al.[56] reported a co-axial core-shell handheld device to repair cartilage defects (Figure 3D). Moreover, this strategy maintains high cell viability and has great potential for *in situ* surgical cartilage engineering. Di Bella et al.[55] also introduced a handheld bioprinter featuring a core-shell structure. Their experiment using a full-layer cartilage injury model in sheep demonstrated the feasibility of printing cartilage scaffolds using this device. In addition to reducing the toxicity of the photoinitiator to the cell, the core-shell structure of the handheld printer can also be used to construct multi-layer structures with gradient properties. Besides photo-crosslinked bioinks, there have also been reports utilizing ion-[88] and enzyme-crosslinked[9] bioinks (Figure 3E). Hakimi et al.[88] reported a similar design, but their device incorporates a microfluidic printhead, enabling rapid repair of large skin defects. In addition, the roller is also installed to enhance the stability of the printing process and improve the printing efficiency, and the flow rate of the two ink tanks can be controlled separately. However, this device has its limitations. For instance, it utilizes a pneumatic extrusion, whereby changes in bioink or ambient temperature will affect the rheological properties of the material, necessitating immediate adjustments to the extrusion pressure to maintain a constant flow rate. Therefore, the incorporation of an active temperature control device should be considered. Pagan et al.[87] used a hydraulic-driven injection pump that is separate from the device to maintain constant extrusion flow.

Handheld *in situ* bioprinting systems (HISBS) can be loaded with functional modules, such as a UV light source, positioning device, and ultrasound, to improve the adhesion of printed structures and tissues. Zhou et al.[89] introduced an ultrasound module into HISBS and reported significantly enhanced bio-adhesive performance of the bioink in a diabetic wound model. Their handheld *in situ* bioprinter utilizes a coaxial extrusion strategy, but the method does not guarantee uniform mixing of the bioink. Ultrasound can enhance ink adhesion and facilitate instant mixing of two inks when deposited, thereby ensuring the mechanical strength of the printed structure.

The tissue repair ability of the printed structure can be enhanced by improving its mechanical strength and ensuring that the structure possesses a certain level of porosity. Optimal porosity facilitates efficient transport of nutrients and metabolic waste, thereby promoting improved cell activity. Ying et al.[90] developed an aqueous two-phase emulsion bioink to produce microscale pores via *in situ* photo-crosslinking. Mostafavi et al.[91] prepared a porous bioink by high-speed stirring foaming and reported significantly enhanced viscosity of the bioink for promoting skeletal muscle regeneration in a rat VML model.

2.2.3. Challenges

While HISBS is appropriate for regenerating superficial trauma and minor damage, handheld devices are limited in treating more severe damage and accessing internal trauma. This limitation can potentially increase the risk of infection.[24] Furthermore, several challenges still need to be addressed, such as low resolution, poor repeatability, high dependence on operator skills, and difficulty in rapidly covering large areas of tissue defects. Notably, most handheld printers only have simple extrusion and coating functions. For repairing tissue defects, the accurate construction of scaffolds is essential for wound healing and functional recovery. To address these challenges, several strategies can be employed. For example, reducing the speed of the printhead movement and using thinner print needles can improve resolution. Introducing programmed design and stepper motors enables controlled movement of the printhead, reducing reliance on human influence. Additionally, multi-channel printhead enables rapid coverage of large wounds. Taken together, HISBS has significant potential for further development in addressing these challenges.[55,91]

2.3. Minimally invasive *in situ* bioprinting

Minimally invasive *in situ* bioprinting utilizes human-controlled robotic systems for *in vivo* tissue repair. Automated *in situ* bioprinting systems can be combined with minimally invasive surgery to enhance printing accuracy and flexibility. Minimally invasive *in situ* bioprinting is crucial as it can mitigate the risk of infection associated with traditional surgical procedures.[60] Minimally invasive bioprinting can be achieved by integrating non-invasive surgical tools with automated strategies, such as extrusion bioprinting[29,60,51] and SLA.[44,45] To clinically apply minimally invasive *in situ* bioprinting, two issues need to

be addressed: (i) delivery and curing of scaffolds in deep tissue and (ii) real-time monitoring of the printing process.

2.3.1. Delivery and curing of scaffolds in deep tissue

Minimally invasive *in situ* bioprinting combines automated printing systems with human control for *in vivo* printing. This approach demands high flexibility and miniaturization of the printing system. However, a key challenge that needs to be addressed is accurately transporting the material to the designated area in the body. Zhao et al.[29] incorporated a micro-bioprinting platform into an endoscope and demonstrated the feasibility of *in situ* 3D printing at a specified location. The printing platform consists of a fixed base with three actuators and a laminated mobile platform that accurately deposits the bioink to the damaged area of the stomach wall via extrusion (Figure 4A). The study

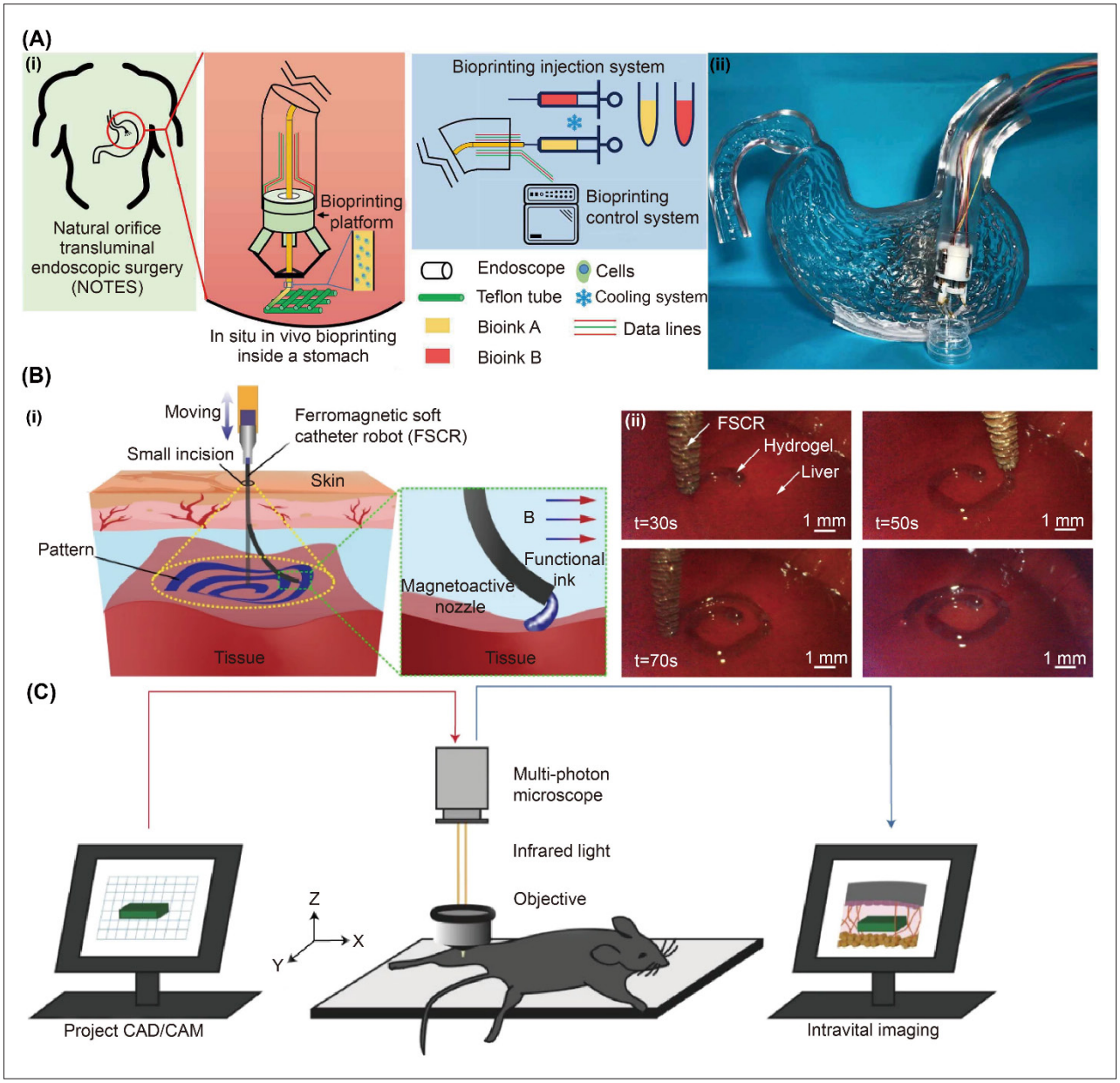

Figure 4. Minimally invasive *in situ* bioprinting combined with automated systems. (A) Schematic illustration of *in situ* bioprinting inside a stomach. (A, i) Schematic of the bioprinting and injection system. (A, ii) A bioprinting platform installed in a curved pipe mimicked an endoscope to perform bioprinting inside a stomach model. (B) Minimally invasive surgery controlled by a ferromagnetic soft catheter robot. (B, i) Schematic illustration of *in situ* bioprinting with functional bioinks under a magnetic field through a small incision. (B, ii) Photographs illustrating the minimally invasive bioprinting process on the liver surface at different times. (C) Implementation of bio-orthogonal two-photon photo-polymerization of polymers. Abbreviations: CAD/CAM, computer-aided design/computer-aided manufacturing. Adapted with permission from Zhao et al.[29] (A), Zhou et al.[60] (B), Urciuolo A et al.[45] (C).

used a stomach model to demonstrate the feasibility of the printing system and indicated further optimization of the printing platform is necessary to achieve better *in vivo* bioprinting. Several optimization strategies have been proposed, such as reducing the platform size to match the size of the endoscope before integrating into the endoscope and *in situ* real-time monitoring system. In addition, the alginate/gelatin bioink can only form stable structures at low temperatures; the use of Ca^{2+} as a crosslinking agent can affect cell activity; and other gel systems should be explored for repairing gastric wall damage. Shi et al.[92] added magnetic complexes to gelatin/sodium alginate hydrogels, which have the opposite charge to gastric juices. This approach increased the curing in the acidic environment of gastric juices without requiring external conditions. By dispersing magnetic complexes in the hydrogel, an external magnetic field can be applied to precisely locate and control the position of the hydrogel, thereby achieving the sealing of gastric perforations. The practicability of the printing method was validated in a pig model with an artificially perforated stomach, while the biosafety of the ink was confirmed in a rat model. Zhou et al.[60] developed a ferromagnetic soft catheter robotic system for minimally invasive *in vivo* printing with a magnetic drive and assessed its performance in a pig tissue model and a live rat liver (Figure 4B). Nonetheless, the minimally invasive surgical method is still in its early stages. It is necessary to miniaturize the device to fit narrow spaces in the body and develop closed-loop systems for real-time imaging combined with machine vision and structured light to enhance the accuracy of printed structures. Yang et al.[93] integrated micro-CT into a ferromagnetic soft catheter robot for printing path scanning and planning, as well as printing irregular complex structures. Electroactive bioinks were printed in a living rat model of partial hepatectomy, and the results demonstrated that the printed scaffolds significantly promoted tissue regeneration. However, the equipment used in this ferromagnetic soft catheter robot is complex and expensive. Based on simple mechanical engineering design principles, Shi et al.[74] developed a flexible robotic arm for *in vivo* bioprinting. However, the disadvantage of this flexible robot arm is its requirement for complex control and software tracking to effectively plan the printing path.

In vivo scaffold printing typically involves light-based non-invasive polymerization, but this approach is mainly limited to superficial tissues. Chen et al.[44] developed a minimally invasive *in situ* printing system based on digital near-infrared light polymerization, demonstrating the ability to construct auricle structures *in vivo*. Similar to UV and blue light, near-infrared light can also induce photo-polymerization. The system uses near-infrared light for digital light-processing *in situ* 3D bioprinting, leveraging its high penetration to induce photo-crosslinking and *in situ* polymerization of the bioink. In another study, Urciuolo et al.[45] implemented bio-orthogonal two-photon photo-polymerization of polymers (Figure 4C). They demonstrated that photosensitive biopolymers, consisting of cell-laden branched polyethylene glycol (PEG) and gelatin, can generate newly formed myofiber bundles in mice, compatible with a functional vascular network. Notably, minimally invasive *in situ* bioprinting employing SLA is constrained by the requirement of photo-crosslinkable bioinks and the effects of printing depth on the printing process. Another strategy of *in situ* bioprinting involves external ultrasound-mediated sound-sensitive bioink polymerization to achieve high-resolution, non-invasive *in situ* printing deep within the body.[94] The ultrasound-induced polymerization process does not damage the body, and ultrasound can also control the microstructure and pore size of the scaffold. Moreover, the system can also be used to achieve continuous drug release by regulating the induction time.

2.3.2. Real-time monitoring of the printing process

In minimally invasive surgery, the patient's anesthetized deep breathing can cause incision displacement, potentially leading to misalignment with the robot's remote motion center. This misalignment can increase tissue stress and the risk of postoperative hernia. Therefore, it is necessary to monitor and adjust the small incision on the patient's body in real time. In an earlier study, Zhao et al.[38] developed a seven-axis robot-assisted bioprinting system that can actively control the alignment of the remote motion center with the incision. On this basis, an adaptive closed-loop minimally invasive *in vivo* 3D printing strategy based on precise incision positioning is proposed, incorporating accurate positioning and attitude estimation through binary color ring array labeling.[95] This strategy enables notch sensing and robot printing to constitute a closed-loop control system, facilitating adaptive calibration.

2.4. Vascularization of bioprinted structures

Adequate vascularization is essential for promoting tissue defect repair, as the microvasculature provides nutrients and oxygen to tissues and promotes metabolism.[96,97] For *in situ* 3D-bioprinted structures, there are currently two ways to induce vascular tissue formation: (i) growth factor-induced vascularization and (ii) microporous structure-guided vascularization. Some studies have demonstrated that copper-epigallocatechin gallate (Cu-EGCG) promotes the secretion of growth factors from vascular endothelial cells. Hu et al.[98] prepared an extracellular matrix (ECM)-based 3D-bioprinted scaffold loaded with Cu-EGCG to promote diabetic wound healing. The ECM scaffold has

good mechanical properties and pore size distribution, but it is not sufficient to control the position and geometry of the microvascular network. To improve vascularization, it is necessary to form a predetermined microchannel in the printed structure. Mostafavi et al.[91] used high-speed stirring to control the pore size and distribution of bioinks. Coaxial printing, used to fabricate hollow structures and predetermined microchannels, has the disadvantage of low resolution. There are studies using sacrificed-template to print structures with submicron-sized capillaries. This method leverages the different solubility or temperature sensitivity of two bioinks, such as GelMA/poly(ethylene oxide)[99] or GelMA/gelatin[100] bioinks. After printing the bioinks side by side, the sacrificial bioink is removed, retaining the desired structure. However, this method is insufficient to develop complex structures. Enrico et al.[97] proposed a method of cavitation molding using femtosecond infrared laser pulses to generate cavitation bubbles in the bioink to form microchannels, subsequently filling them with endothelial cell suspension to form continuous cell layers after cell culture.

3. Bioinks for *in situ* bioprinting

3.1. Performance requirements

Bioinks, containing active biomaterials and cells, serve as scaffolds to accelerate wound or defect recovery, playing an essential role in driving biological interactions. Bioinks should meet specific essential characteristics to address the challenge of complex tissue regeneration effectively. Traditional biomaterials have been biocompatible but often lack the ability to effectively promote interactions between cells, materials, and tissues.[101] Similarly, bioinks used for *in situ* bioprinting should essentially possess remarkable rheological properties to enhance the resolution of the printing structure and maintain a specific mechanical strength. Other critical factors include rapid gelation, mechanical properties, shape fidelity, biocompatibility, and biofunctionality.[43,63] In some personalized medicine applications, bioinks should contain autologous bioactive factors from the patients. Hydrogels have been widely used as matrices for *in situ* bioprinting due to their excellent biocompatibility, ability to encapsulate cells, high permeability, large water content, and similarity to native ECM.[102–106] The *in situ* formation of hydrogels has significant advantages over traditional pre-formed hydrogels, such as being minimally invasive, excellent adaptation to wound margins, accurate filling of defects, and simple cell encapsulation.[101,103] Current research primarily focuses on meeting specific characteristics, such as electroconductivity,[103,107] physiological stimulus-responsive ability,[43] and shear-thinning ability.[81,105] Shear-thinning hydrogels are ideal for maintaining cell viability after injection.[81] Some materials, such as platelet-rich plasma (PRP), have been investigated for hydrogel integration. Zhao et al.[63] incorporated PRP into sodium alginate/gelatin bioink for repairing skin defects by releasing various growth factors and active ingredients. However, PRP degrades rapidly in the wound environment and cannot sustainably release growth factors. Lai et al.[108] prepared a dressing with three layers of core-shell fiber through coaxial 3D printing and fixed PRP in the core layer of the fiber to achieve continuous release of growth factors. By optimizing bioink characteristics, *in situ* bioprinting can be further developed and expanded for various applications in tissue engineering and regenerative medicine. The bioinks currently used for *in situ* bioprinting and their specific applications in tissue engineering are summarized in Table 3. Based on the bioink sources, the materials reported for *in situ* bioprinting can be divided into either natural or synthetic polymers.

3.2. Challenges

Current bioink research faces challenges such as viscosity,[121] rheological properties,[73,81] and the difficulty of producing intricate pores. High-viscosity bioinks can significantly improve the mechanical strength of printed structures, leading to a higher extrusion pressure and lower cell viability. Although a larger diameter nozzle can be used, the printing accuracy will be reduced. Thermo-sensitive bioinks are required to crosslink at body temperature for *in situ* bioprinting. Crosslinked bioinks should have low mechanical strength to protect cell activity, but simultaneously require high mechanical strength to maintain shape and match the defect. Rheological properties require optimization according to the properties of the material itself, the loaded cells, the bioprinting approach, bioprinting conditions (e.g., temperature, pH, and crosslinking mode), and other factors.[43,63,122] Some inks, such as gelatin, have problems creating complex pores due to their high water content and thermal sensitivity.[42]

3.3. Optimization of cellular compatibility and mechanical strength

The performance of bioinks varies according to the *in situ* bioprinting technology applied. For example, inkjet-based *in situ* bioprinting utilizes bioinks with low viscosity or shear-thinning characteristics to ensure the smooth formation of droplets, thereby limiting material selection.[37,39] Hydrogels with high water content are widely used in inkjet-based *in situ* bioprinting. Additionally, photo-crosslinked bioinks, such as GelMA, have reportedly been used in inkjet bioprinting but are prone to nozzle clogging. A multiple-nozzle system can be designed to separate the photoinitiator from the ink, or a coating can be applied to the nozzle surface to reduce clogging and adhesion.

Table 3. Bioink materials utilized for *in situ* bioprinting

Source	Materials	Category	Gelation mechanism	Bioprinting approach	Advantages	Disadvantages	Applications
Natural	Collagen	Natural proteins	pH and temperature-induced	Extrusion, LAB, and inkjet	Highly bioactive	Uncrosslinked solution lacks stability	Bone[41,105], dermis[109], skin[37,34], and cartilage[4] defect repair
	Gelatin		Enzyme-induced	Extrusion	Facilitate cellular attachment and growth	Poor mechanical properties	Muscle tissue engineering[110]
	GelMA		Photo-induced	Extrusion and SLA[111]	Fast gelation; biocompatible	Poor mechanical properties at low concentrations	Muscle[112], cartilage[56], bone[35,113], and skin[69,90] tissue engineering
	Fibrin		Photo-induced	Extrusion	Biocompatible; nanofibrous structural properties	High viscosity hindering extrusion	Cartilage[114] defect repair
	PRP		Enzyme-induced	Extrusion	Rich in growth factors and active ingredients	Rapid degradation	Skin repair[63,108]
	Silk fibroin		Enzyme-, ultrasound-, and photo-induced	Extrusion	Excellent mechanical and biological properties	Poor cell attachment	Tendon[115] repair
	HA/HAMA	Natural polysaccharides	Chemical-induced	Extrusion	Biocompatible	Slow gelation	Cartilage[66,55,116] and bone[1] regeneration"
	Alginate		Chemical-induced	Extrusion and LAB[117]	Biocompatible; fast gelation	Poor cell attachment; low mechanical strength	Bone[118] and skin[88] defect repair
Synthetic	PCL	Synthetic polymer-based hydrogel	Temperature-induced	Extrusion	Biocompatible; low melting temperature	Not bioactive	Bone repair[58]
	PLA		Temperature-induced	Electrospinning	Excellent mechanical properties	Poor cell attachment	Skin repair[46]
	PEGDA		Photo-induced	Extrusion and SLA[111]	Biocompatible; hydrophilic	Poor cell attachment	Cartilage[119] and bone[120] tissue engineering

Abbreviations: GelMA, gelatin methacryloyl; HA, hyaluronic acid; HAMA, hyaluronic acid methacrylate; LAB, laser-assisted bioprinting; PCL, poly(caprolactone); PEGDA, poly(ethylene glycol diacrylate); PLA, poly(lactic acid); PRP, platelet-rich plasma; SLA, stereolithography.

Bioinks used in extrusion-based *in situ* bioprinting require specific printability, i.e., to possess viscosity that supports the maintenance of extrusion line shape. Photo-crosslinked bioinks, such as GelMA, often employ pre-crosslinking strategies to achieve suitable viscosity. Conversely, the viscosity of bioinks should be reduced when printing to improve the biocompatibility of bioinks, which compromises the mechanical strength required to maintain the support structure. At present, microgels have garnered attention due to their good rheological properties and biocompatibility. Xie et al.[73] developed a microgel-based bioink, consisting of (i) a GelMA microgel to load cells and (ii) a GelMA precursor solution with a photoinitiator to ensure fluidity and provide mechanical strength. The pore morphology of bioinks is essential for many biological processes, such as cell migration, infiltration, printability,[123] and tissue vascularization.[77,102] Porous scaffolds can also be printed by customizing the stiffness and composition of microgels. Jalandhra et al.[113] developed a porous microgel scaffold to control the direction of stem cell differentiation by adjusting the microgel stiffness and gap-filling hydrogel volume. Other researchers have used lyogels, or freeze-dried hydrogels, to introduce microchannel structures. Lyogels offer an advantage over conventional hydrogels in that they can be stored in a dry state and used readily.[102] The pore morphology of lyogels is

determined by the size and shape of the ice crystals during the freeze-drying process. Notably, the pores of the printed structure affect tissue repair because scaffolds with good porosity are conducive to oxygen transport and promote cell adhesion and proliferation. Several studies have reported oxygen supply strategies for adding inorganic peroxides to scaffolds, but these oxygen supply systems are limited in their ability to provide sufficient oxygen. Wang et al.[124] developed a self-supplying oxygen system that prints photosynthetic microalgae *in situ* at the wound site, thereby providing continuous oxygen for wound healing. The system could also promote cell proliferation, migration, and differentiation under hypoxic conditions and accelerate wound healing in chronic diabetic wounds.

3.4. Intelligent materials for 4D bioprinting

Different soft tissue injuries require specific complex structures for repair. For *in situ* bioprinting, the surgical site is often exposed to unavoidable damage, and cells are lost during the implantation process.[125] Therefore, 4D printing technology, which allows structures to be implanted into the damaged site in a compact form, has garnered significant attention. 4D printing technology combines smart materials (i.e., stimuli-responsive materials) with 3D printing technology to compress 3D structures into 1D or 2D structures *in vitro* and implant them in the body to restore programmable shapes under specific stimuli (temperature, humidity, magnetic field, pH, etc.).[126] Shi et al.[92] developed a magnetic hydrogel for treating stomach injuries that control bioink delivery through a gastroscope nozzle. The magnetic bioink accumulates at the damaged site under the influence of an external magnetic field, facilitating sutureless tissue sealing. Compared to external stimuli, such as magnetic fields and high temperatures, endogenous stimuli in response to body temperature or body fluids are more convenient and biofriendly. Hydrogels expand due to water absorption, making them the preferred material for 4D printing. Using water-induced programmable deformation, Joshi et al.[126] prepared hydrogels using alginate and methylcellulose at specific ratios for different expansion rates. The hydrogels were then used to construct 4D-printed catheters for repairing peripheral nerves. Liu et al.[127] developed an amphiphilic dynamic thermosetting polyurethane that transitions from 2D to 1D structures in a body temperature environment and programmatically transforms into 3D structures upon exposure to water after implantation *in vivo*. Furthermore, the material has water-hardening properties, suggesting good mechanical properties. The structure is printed using melt deposition modeling, employing a layer-by-layer printing strategy that can lead to weak interlayer bonding in the printed structure. Thermally reversible dynamic covalent bonds were subsequently introduced to enhance the adhesion between the component layers. Luo et al.[128] introduced cinnamic acid groups to a polylactic acid/PEG-copolyester blend to induce photo-crosslinking, enhancing interlayer bonding, and thereby improving the printing accuracy and stability of the structure.

Overall, 4D-printed dynamic scaffolds are still in the early stages of development, with a key challenge being the design of materials that are both programmable and biocompatible. Future advancements are expected to integrate AI or machine learning techniques to develop new materials, design functional structures, and optimize printing parameters.

4. Future perspectives

While notable advancements have been made in *in situ* bioprinting, several challenges remain in promoting vascularization within printed structures, automating RASBS procedures, developing highly modular designs for HISBS, and optimizing the bioink system. Printed structures for tissue repair should promote vascularization, and *in situ* 3D bioprinting technology can combine multiple materials and cells to print complex structures, creating microchannels that promote vascularization. Microfluidic technology can also be integrated, such as using a microfluidic chip needle to mix multiple bioinks and cells and print a scaffold with a specific concentration gradient.

For RASBS, incorporating AI could enhance path planning to achieve more detailed and automated *in situ* bioprinting. For *in situ* bioprinting on curved and inclined planes, flexible robotic arms may represent the future direction of development. These arms offer higher degrees of freedom compared to rigid robotic arms, effectively mitigating the step effect caused by printing on curved structures. In addition, machine learning algorithms can optimize non-planar automatic segmentation, reconstruct defects in damaged parts, and obtain print paths. *In situ* bioprinting platforms can also be integrated with machine vision and depth cameras to improve recognition accuracy. Traditional bioprinting technology can print *in vitro* and perform print quality checks, capabilities that are currently limited with *in situ* bioprinting strategies. Therefore, achieving *in situ* quality inspection and control of printed structures is also a future development trend. There have been studies using MRI to track printed cells and assess the healing process.[80] For evaluating the quality of printed scaffolds, OCT can be used for rapid real-time imaging and process feedback control according to the monitoring data.[79]

Considering the portability of handheld bioprinters, such devices should be designed to be highly modular

and easy to disassemble, clean, and disinfect to meet the operational requirements of surgical procedures. Handheld *in situ* bioprinters are typically used in emergency trauma scenarios (e.g., car accidents, battlefields), where users are generally non-professionals. A smartphone can be combined with a handheld *in situ* bioprinter, and the smartphone's high-definition camera and computing power can be used to scan the damaged area and plan the print path.[129] Deep learning can also be combined with cloud computing to monitor and calibrate printhead movements in real time, improving print accuracy. In the future, the handheld *in situ* bioprinter may become an essential tool for astronauts during space emergencies, such as the extraction and storage of biological products containing blood or stem cells before astronauts embark on missions.[130]

At present, a few studies are focusing on real-time monitoring of *in situ* printing processes, utilizing large imaging devices and complex equipment. In the future, it is necessary to miniaturize imaging detection systems to integrate them with minimally invasive printing platforms to enhance the fidelity of printed structures. The use of external magnetic fields to control the precise positioning of magnetic bioinks in the body is a promising technology, and this strategy does not require complex minimally invasive printing robotic arms. Technical validation and optimization for more complex geometric defect printing is also required in the future. To further reduce the volume of the minimally invasive printing platform, the injection device can also be placed outside the body and connected to the pipe through the dispensing nozzle to achieve *in situ* printing. However, the effect of the material temperature at the dispensing nozzle and the ambient temperature on the printing performance of the material will be a challenge.[131] For *in vivo* bioprinting, the selection of suitable bioink depends on the specific tissue repair area. For example, in the acidic environment of the stomach, polyelectrolytes with opposite charges can be added to the bioink to achieve instant curing, without the need for external conditions, such as near-infrared light or ultrasound, to mediate polymerization.

Hydrogels, including collagen, gelatin, and alginate, have been widely used for *in situ* bioprinting. Most of these materials have excellent biocompatibility and low toxicity, but a single biomaterial cannot meet the requirements of tissue repair. Therefore, developing a multi-material *in situ* bioprinting system could expand its applications significantly. For photocured hydrogels, near-infrared light is required to induce bioink polymerization for minimally invasive bioprinting *in vivo*. Hence, it may be crucial to optimize the type and concentration of photoinitiator, light wavelength, and irradiation time. For minimally invasive printing *in vivo*, ultrasound can be used to mediate *in situ* curing of sound-sensitive inks. In this regard, other sound-sensitive materials with good biocompatibility may be developed to enhance tissue regeneration on printed scaffolds. *In situ*-bioprinted tissue scaffolds require uniform pore structure and mechanical strength, both of which share an inverse correlation. Therefore, alternative pore-forming methods need to be developed, such as optimizing microgels to serve as porous scaffolds.[113]

5. Conclusion

In this review, we introduced 3D *in situ* bioprinting to fabricate complex structures for tissue regeneration. Conventional 3D bioprinting strategies require a long incubation period for pre-printed structures in a large working space, potentially leading to a mismatch in the shape of the wound. *In situ* bioprinting can compensate for these deficiencies by using the recipient body as a bioreactor where living biomaterials and cells of scaffolds can be further cultured. The *in situ* bioprinting approach can be divided into three types: RASBS, HISBS, and minimally invasive *in situ* bioprinting. RASBS has higher printing accuracy with less human intervention and can adjust printing models and paths according to the actual printing conditions. Furthermore, combined with minimally invasive tools, RASBS can achieve *in situ* deposition of bioinks without open wounds. Driven by human hand movement, handheld bioprinters are easier to operate but are limited in their application to internal trauma and complex structures. Bioinks normally contain living biomaterials and cells as a matrix to rearrange regenerative factors. Bioinks should have optimal rheological properties for *in situ* bioprinting, ensuring sufficient mechanical strength and printing resolution. Overall, *in situ* bioprinting holds great promise as an emerging technology for tissue repair. This technology is expected to make significant progress in the coming years with technological advances in AI, medical robotics, and biomaterials.

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Conflict of interest

The authors declare no conflicts of interest.

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References

1. Li L, Yu F, Shi J, et al. In situ repair of bone and cartilage defects using 3D scanning and 3D printing. *Sci Rep.* 2017;7(1):9416. doi: 10.1038/s41598-017-10060-3
2. Singh S, Choudhury D, Yu F, Mironov V, Naing MW. In situ bioprinting—bioprinting from benchside to bedside? *Acta Biomater.* 2020;101:14-25. doi: 10.1016/j.actbio.2019.08.045
3. Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. *Trends Biotechnol.* 2015;33(7): 395-400. doi: 10.1016/j.tibtech.2015.04.005
4. Gatenholm B, Lindahl C, Brittberg M, Simonsson S. Collagen 2A type B induction after 3D bioprinting chondrocytes in situ into osteoarthritic chondral tibial lesion. *Cartilage.* 2021;13(2_SUPPL):1755S-1769S. doi: 10.1177/1947603520903788
5. Yang BW, Yin JH, Chen Y, et al. 2D-black-phosphorus-reinforced 3D-printed scaffolds: a stepwise countermeasure for osteosarcoma. *Adv Mater.* 2018;30(10):1705611. doi: 10.1002/adma.201705611
6. Chow T, Wutami I, Lucarelli E, et al. Creating in vitro three-dimensional tumor models: a guide for the biofabrication of a primary osteosarcoma model. *Tissue Eng Part B Rev.* 2021;27(5):514-529. doi: 10.1089/ten.teb.2020.0254
7. Li ZH, Zhao Y, Wang ZH, et al. Engineering multifunctional hydrogel-integrated 3D printed bioactive prosthetic interfaces for osteoporotic osseointegration. *Adv Healthc Mater.* 2022;11(11):e2102535. doi: 10.1002/adhm.202102535
8. Yvanoff C, Willaert RG. Development of bone cell microarrays in microfluidic chips for studying osteocyte-osteoblast communication under fluid flow mechanical loading. *Biofabrication.* 2022;14(2):025014. doi: 10.1088/1758-5090/ac516e
9. Cheng RY, Eylert G, Gariepy J-M, et al. Handheld instrument for wound-conformal delivery of skin precursor sheets improves healing in full-thickness burns. *Biofabrication.* 2020;12(2):025002. doi: 10.1088/1758-5090/ab6413
10. Jiao T, Lian Q, Lian W, et al. Properties of collagen/sodium alginate hydrogels for bioprinting of skin models. *J Bionic Eng.* 2023;20(1):105-118. doi: 10.1007/s42235-022-00251-8
11. Liu J, Zhou Z, Zhang M, et al. Simple and robust 3D bioprinting of full-thickness human skin tissue. *Bioengineered.* 2022;13(4):10087-10097. doi: 10.1080/21655979.2022.2063651
12. Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. *Biotechnol Adv.* 2016;34(4):422-434. doi: 10.1016/j.biotechadv.2015.12.011
13. Sithole MN, Kumar P, du Toit LC, et al. A 3D bioprinted in situ conjugated-co-fabricated scaffold for potential bone tissue engineering applications. *J Biomed Mater Res Part A.* 2018;106(5):1311-1321. doi: 10.1002/jbm.a.36333
14. Kilian D, Sembdner P, Bretschneider H, et al. 3D printing of patient-specific implants for osteochondral defects: workflow for an MRI-guided zonal design. *Bio-Des Manuf.* 2021;4(4):818-832. doi: 10.1007/s42242-021-00153-4
15. Kurzyk A, Szumera-Cieckiewicz A, Miloszewska J, Chechlinska M. 3D modeling of normal skin and cutaneous squamous cell carcinoma. A comparative study in 2D cultures, spheroids, and 3D bioprinted systems. *Biofabrication.* 2024;16(2):025021. doi: 10.1088/1758-5090/ad2b06.
16. Fu Z, Hai N, Zhong Y, Sun W. Printing GelMA bioinks: a strategy for building in vitro model to study nanoparticle-based minocycline release and cellular protection under oxidative stress. *Biofabrication.* 2024;16(2):025040. doi: 10.1088/1758-5090/ad30c3
17. Shukla P, Bera AK, Yeleswarapu S, Pati F. High throughput bioprinting using decellularized adipose tissue-based hydrogels for 3D breast cancer modeling. *Macromol Biosci.* 2024;24:2400035. doi: 10.1002/mabi.202400035
18. Wu D, Pang S, Berg J, et al. Bioprinting of perfusable vascularized organ models for drug development via

sacrificial-free direct ink writing. *Adv Funct Mater*. 2024:2314171.
doi: 10.1002/adfm.202314171

19. Gvaramia D, Fisch P, Flegeau K, et al. Evaluation of bioprinted autologous cartilage grafts in an immunocompetent rabbit model. *Adv Ther*. 2024;7(6):2300441.
doi: 10.1002/adtp.202300441

20. Bhar B, Das E, Manikumar K, Mandal BB. 3D bioprinted human skin model recapitulating native-like tissue maturation and immunocompetence as an advanced platform for skin sensitization assessment. *Adv Healthc Mater*. 2024;13(15):e2303312.
doi: 10.1002/adhm.202303312

21. Di Buduo CA, Lunghi M, Kuzmenko V, et al. Bioprinting soft 3D models of hematopoiesis using natural silk fibroin-based bioink efficiently supports platelet differentiation. *Adv Sci*. 2024;11(18):2308276.
doi: 10.1002/advs.202308276

22. Edri S, Frisch AN, Safina D, et al. 3D bioprinting of multicellular stem cell-derived constructs to model pancreatic cell differentiation. *Adv Funct Mater*. 2024;34:2315488.
doi: 10.1002/adfm.202315488

23. Li H, Cheng F, Orgill DP, Yao J, Zhang YS. Handheld bioprinting strategies for in situ wound dressing. In: Jang J, ed. *Essays in Biochemistry. Vol. 65*. London: Portland Press; 2021:533-543.
doi: 10.1042/EBC20200098

24. Thai MT, Phan PT, Tran HA, et al. Advanced soft robotic system for in situ 3D bioprinting and endoscopic surgery. *Adv Sci*. 2023;10(12):2205656.
doi: 10.1002/advs.202205656

25. Agostinacchio F, Mu X, Dire S, Motta A, Kaplan DL. In situ 3D printing: opportunities with silk inks. *Trends Biotechnol*. 2021;39(7):719-730.
doi: 10.1016/j.tibtech.2020.11.003

26. Akilbekova D, Mektepbayeva D. Chapter 5-patient specific in situ 3D printing. In: Kalaskar DM, ed. *3D Printing in Medicine*. Cambridge: Woodhead Publishing; 2017:91-113.
doi: 10.1016/b978-0-08-100717-4.00004-1

27. Campbell PG, Weiss LE. Tissue engineering with the aid of inkjet printers. *Expert Opin Biol Ther*. 2007;7(8):1123-1127.
doi: 10.1517/14712598.7.8.1123

28. Samandari M, Mostafavi A, Quint J, Memic A, Tamayol A. In situ bioprinting: intraoperative implementation of regenerative medicine. *Trends Biotechnol*. 2022;40(10): 1229-1247.
doi: 10.1016/j.tibtech.2022.03.009

29. Zhao W, Xu T. Preliminary engineering for in situ in vivo bioprinting: a novel micro bioprinting platform for in situ in vivo bioprinting at a gastric wound site. *Biofabrication*. 2020;12(4):045020.
doi: 10.1088/1758-5090/aba4ff

30. Nuutila K, Samandari M, Endo Y, et al. In vivo printing of growth factor-eluting adhesive scaffolds improves wound healing. *Bioact Mater*. 2022;8:296-308.
doi: 10.1016/j.bioactmat.2021.06.030

31. Wu Y, Ravnic DJ, Ozbolat IT. Intraoperative bioprinting: repairing tissues and organs in a surgical setting. *Trends Biotechnol*. 2020;38(6):594-605.
doi: 10.1016/j.tibtech.2020.01.004

32. Chen H, Zhang Y, Zhou D, et al. Mechanical engineering of hair follicle regeneration by in situ bioprinting. *Biomater Adv*. 2022;142:213127.
doi: 10.1016/j.bioadv.2022.213127

33. Gaharwar AK, Singh I, Khademhosseini A. Engineered biomaterials for in situ tissue regeneration. *Nat Rev Mater*. 2020;5(9):686-705.
doi: 10.1038/s41578-020-0209-x

34. Levin AA, Karalkin PA, V. Koudan E, et al. Commercial articulated collaborative in situ 3D bioprinter for skin wound healing. *Int J Bioprint*. 2023;9(2):380-393.
doi: 10.18063/ijb.v9i2.675

35. Li L, Shi J, Ma K, et al. Robotic in situ 3D bio-printing technology for repairing large segmental bone defects. *J Adv Res*. 2021;30:75-84.
doi: 10.1016/j.jare.2020.11.011

36. Moncal KK, Gudapati H, Godzik KP, et al. Intra-operative bioprinting of hard, soft, and hard/soft composite tissues for craniomaxillofacial reconstruction. *Adv Funct Mater*. 2021;31(29):2010858.
doi: 10.1002/adfm.202010858

37. Albanna M, Binder KW, Murphy SV, et al. In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds. *Sci Rep*. 2019;9(1):1856
doi: 10.1038/s41598-018-38366-w

38. Zhao W, Hu C, Xu T, et al. Subaqueous bioprinting: a novel strategy for fetal membrane repair with 7-axis robot-assisted minimally invasive surgery. *Adv Funct Mater*. 2022;32(51):2207496.
doi: 10.1002/adfm.202207496

39. Christensen K, Compaan A, Chai W, Xia G, Huang Y. In situ printing-then-mixing for biological structure fabrication using intersecting jets. *ACS Biomater Sci Eng*. 2017;3(12):3687-3694.
doi: 10.1021/acsbiomaterials.7b00752

40. Keriquel V, Oliveira H, Remy M, et al. In situ printing of mesenchymal stromal cells, by laser-assisted bioprinting, for in vivo bone regeneration applications. *Sci Rep*. 2017;7(1):1778.
doi: 10.1038/s41598-017-01914-x

41. Kerouredan O, Hakobyan D, Remy M, et al. In situ prevascularization designed by laser-assisted bioprinting: effect on bone regeneration. *Biofabrication*. 2019;11(4):045002.
doi: 10.1088/1758-5090/ab2620

42. Ding H, Chang RC. Simulating image-guided in situ bioprinting of a skin graft onto a phantom burn wound bed. *Addit Manuf*. 2018;22:708-719. doi: 10.1016/j.addma.2018.06.022

43. Liu Y, Luo X, Wu W, et al. Dual cure (thermal/photo) composite hydrogel derived from chitosan/collagen for in situ 3D bioprinting. *Int J Biol Macromol*. 2021;182:689-700. doi: 10.1016/j.ijbiomac.2021.04.058

44. Chen Y, Zhang J, Liu X, et al. Noninvasive in vivo 3D bioprinting. *Sci Adv*. 2020;6(23):eaba7406. doi: 10.1126/sciadv.aba7406

45. Urciuolo A, Poli I, Brandolino L, et al. Intravital three-dimensional bioprinting. *Nat Biomed Eng*. 2020;4(9):901-915. doi: 10.1038/s41551-020-0568-z

46. Chen H, Zhang H, Shen Y, et al. Instant in-situ tissue repair by biodegradable PLA/gelatin nanofibrous membrane using a 3D printed handheld electrospinning device. *Front Bioeng Biotechnol*. 2021;9:684105. doi: 10.3389/fbioe.2021.684105

47. Tianyuan Y, Yi Z, Zhian J, Yuanyuan L. A novel handheld device: application to in situ bioprinting compound dressing for the treatment of wound. Paper presented at: *Journal of Physics: Conference Series*; 2021. doi: 10.1088/1742-6596/1965/1/012059

48. Li X, Lian Q, Li DC, Xin H, Jia SH. Development of a robotic arm based hydrogel additive manufacturing system for in-situ printing. *Appl Sci*. 2017;7(1):73. doi: 10.3390/app7010073

49. Keriquel V, Guillemot F, Arnault I, et al. In vivo bioprinting for computer-and robotic-assisted medical intervention: preliminary study in mice. *Biofabrication*. 2010;2(1):014101. doi: 10.1088/1758-5082/2/1/014101

50. Zhu Z, Guo S-Z, Hirdler T, et al. 3D printed functional and biological materials on moving freeform surfaces. *Adv Mater*. 2018;30(23):e1707495. doi: 10.1002/adma.201707495

51. Adib AA, Sheikhi A, Shahhosseini M, et al. Direct-write 3D printing and characterization of a GelMA-based biomaterial for intracorporeal tissue engineering. *Biofabrication*. 2020;12(4):045006. doi: 10.1088/1758-5090/ab97a1

52. Skardal A, Mack D, Kapetanovic E, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl Med*. 2012;1(11):792-802. doi: 10.5966/sctm.2012-0088

53. Russell CS, Mostafavi A, Quint JP, et al. In situ printing of adhesive hydrogel scaffolds for the treatment of skeletal muscle injuries. *ACS Appl Bio Mater*. 2020;3(3):1568-1579. doi: 10.1021/acsabm.9b01176

54. Quint JP, Mostafavi A, Endo Y, et al. In vivo printing of nanoenabled scaffolds for the treatment of skeletal muscle injuries. *Adv Healthc Mater*. 2021;10(10):e2002152. doi: 10.1002/adhm.202002152

55. Di Bella C, Duchi S, O'Connell CD, et al. In situ handheld three-dimensional bioprinting for cartilage regeneration. *J Tissue Eng Regen Med*. 2018;12(3):611-621. doi: 10.1002/term.2476

56. Duchi S, Onofrillo C, O'Connell CD, et al. Handheld co-axial bioprinting: application to in situ surgical cartilage repair. *Sci Rep*. 2017;7(1):5837. doi: 10.1038/s41598-017-05699-x

57. Onofrillo C, Duchi S, O'Connell CD, et al. Biofabrication of human articular cartilage: a path towards the development of a clinical treatment. *Biofabrication*. 2018;10(4):045006. doi: 10.1088/1758-5090/aad8d9

58. Mostafavi A, Abdullah T, Russell CS, et al. In situ printing of scaffolds for reconstruction of bone defects. *Acta Biomater*. 2021;127:313-326. doi: 10.1016/j.actbio.2021.03.009

59. Campos DFD, Zhang S, Kreimendahl F, et al. Hand-held bioprinting for de novo vascular formation applicable to dental pulp regeneration. *Connect Tissue Res*. 2020;61(2):205-215. doi: 10.1080/03008207.2019.1640217

60. Zhou C, Yang Y, Wang J, et al. Ferromagnetic soft catheter robots for minimally invasive bioprinting. *Nat Commun*. 2021;12(1):5072. doi: 10.1038/s41467-021-25386-w

61. Abdelrahim AA, Hong S, Song JM. Integrative in situ photodynamic therapy-induced cell death measurement of 3D-bioprinted MCF-7 tumor spheroids. *Anal Chem*. 2022;94(40):13936-13943. doi: 10.1021/acs.analchem.2c03022

62. Chaudhry MS, Czekanski A. Surface slicing and toolpath planning for in-situ bioprinting of skin implants. *Biofabrication*. 2024;16(2):025030. doi: 10.1088/1758-5090/ad30c4

63. Zhao M, Wang J, Zhang J, et al. Functionalizing multi-component bioink with platelet-rich plasma for customized in-situ bilayer bioprinting for wound healing. *Mater Today Bio*. 2022;16:100334. doi: 10.1016/j.mtbio.2022.100334

64. Liu X, Wang X, Zhang L, et al. 3D liver tissue model with branched vascular networks by multimaterial bioprinting. *Adv Healthcare Mater*. 2021;10(23):e2101405. doi: 10.1002/adhm.202101405

65. Zhao W, Chen H, Zhang Y, et al. Adaptive multi-degree-of-freedom in situ bioprinting robot for hair-follicle-inclusive skin repair: A preliminary study conducted in mice. *Bioeng Transl Med*. 2022;7(3):e10303. doi: 10.1002/btm2.10303

66. Ma K, Zhao T, Yang L, et al. Application of robotic-assisted in situ 3D printing in cartilage regeneration with HAMA hydrogel: an in vivo study. *J Adv Res*. 2020;23:123-132. doi: 10.1016/j.jare.2020.01.010

67. Al-Kharusi G, Dunne NJ, Little S, Levingstone TJ. The role of machine learning and design of experiments in the advancement of biomaterial and tissue engineering research. *Bioengineering (Basel)*. 2022;9(10):561. doi: 10.3390/bioengineering9100561

68. Albouy M, Desanlis A, Brosset S, et al. A preliminary study for an intraoperative 3D bioprinting treatment of severe burn injuries. *Plast Reconstr Surg Glob Open*. 2022;10(1):e4056. doi: 10.1097/GOX.0000000000004056

69. Chen H, Ma X, Gao T, et al. Robot-assisted in situ bioprinting of gelatin methacrylate hydrogels with stem cells induces hair follicle-inclusive skin regeneration. *Biomed Pharmacother*. 2023;158:114140. doi: 10.1016/j.biopha.2022.114140

70. Simeunovic A, Wolf K, Tierling K, Hoelzle DJ. A surgical robot for intracorporeal additive manufacturing of tissue engineering constructs. *IEEE Rob Autom Lett*. 2022;7(3):7495-7502. doi: 10.1109/LRA.2022.3183752

71. Fortunato GM, Sigismondi S, Nicoletta M, et al. Analysis of the robotic-based in situ bioprinting workflow for the regeneration of damaged tissues through a case study. *Bioengineering (Basel)*. 2023;10(5):560. doi: 10.3390/bioengineering10050560

72. Colosi C, Costantini M, Barbetta A, Dentini M. Microfluidic bioprinting of heterogeneous 3D tissue constructs. *Methods Mol Biol*. 2017;1612:369-380. doi: 10.1007/978-1-4939-7021-6_26

73. Xie M, Shi Y, Zhang C, et al. In situ 3D bioprinting with bioconcrete bioink. *Nat Commun*. 2022;13(1):3597. doi: 10.1038/s41467-022-30997-y

74. Shi E, Lou L, Warburton L, Rubinsky B. Three-dimensional printing in combined cartesian and curvilinear coordinates. *J Med Device*. 2022;16(4):044502. doi: 10.1115/1.4055064

75. Fortunato GM, Batoni E, Pasqua I, et al. Automatic photo-cross-linking system for robotic-based in situ bioprinting. *ACS Biomater Sci Eng*. 2023;9(12):6926-6934. doi: 10.1021/acsbiomaterials.3c00898

76. Kucukdeger E, Johnson BN. Closed-loop controlled conformal 3D printing on moving objects via tool-localized object position sensing. *J Manuf Processes*. 2023;89:39-49. doi: 10.1016/j.jmapro.2023.01.020.

77. Yang S, Wang L, Chen Q, Xu M. In situ process monitoring and automated multi-parameter evaluation using optical coherence tomography during extrusion-based bioprinting. *Addit Manuf*. 2021;47:102251. doi: 10.1016/j.addma.2021.102251

78. Yang S, Chen Q, Wang L, Xu M. In situ defect detection and feedback control with three-dimensional extrusion-based bioprinter-associated optical coherence tomography. *Int J Bioprint*. 2023;9(1):47-62. doi: 10.18063/ijb.v9i1.624

79. Tashman JW, Shiwarski DJ, Coffin B, et al. In situ volumetric imaging and analysis of FRESH 3D bioprinted constructs using optical coherence tomography. *Biofabrication*. 2023;15(1):014102. doi: 10.1088/1758-5090/ac975e

80. Kerouredan O, Ribot E, Fricain J-C, Devillard R, Miraux S. Magnetic resonance imaging for tracking cellular patterns obtained by laser-assisted bioprinting. *Sci Rep*. 2018;8(1):15777. doi: 10.1038/s41598-018-34226-9

81. Zhou Y, Liao S, Chu Y, et al. An injectable bioink with rapid prototyping in the air and in-situ mild polymerization for 3D bioprinting. *Biofabrication*. 2021;13(4):045026. doi: 10.1088/1758-5090/ac23e4

82. Kotlarz M, Ferreira AM, Gentile P, Russell SJ, Dalgarno K. Droplet-based bioprinting enables the fabrication of cell-hydrogel-microfibre composite tissue precursors. *Bio-Des Manuf*. 2022;5(3):512-528. doi: 10.1007/s42242-022-00192-5

83. Zhao D-k, Xu H-q, Yin J, Yang H-y. Inkjet 3D bioprinting for tissue engineering and pharmaceutics. *J Zhejiang Univ Sci A*. 2022;23(12):955-973. doi: 10.1631/2023.A2200569

84. Limon SM, Quigley C, Sarah R, Habib A. Advancing scaffold porosity through a machine learning framework in extrusion based 3D bioprinting. *Front Mater*. 2024;10:1337485. doi: 10.3389/fmats.2023.1337485

85. Qiao Q, Zhang X, Yan Z, et al. The use of machine learning to predict the effects of cryoprotective agents on the GelMA-based bioinks used in extrusion cryobioprinting. *Bio-Des Manuf*. 2023;6(4):464-477. doi: 10.1007/s42242-023-00244-4

86. Huang X, Ng WL, Yeong WY. Predicting the number of printed cells during inkjet-based bioprinting process based on droplet velocity profile using machine learning approaches. *J Intell Manuf*. 2023;35(5). doi: 10.1007/s10845-023-02167-4

87. Pagan E, Stefanek E, Seyfoori A, et al. A handheld bioprinter for multi-material printing of complex constructs. *Biofabrication*. 2023;15(3):035012. doi: 10.1088/1758-5090/acc42c.

88. Hakimi N, Cheng R, Leng L, et al. Handheld skin printer: in situ formation of planar biomaterials and tissues. *Lab Chip*. 2018;18(10):1440-1451. doi: 10.1039/c7lc01236e.

89. Zhou FY, Xin LJ, Wang SY, et al. Portable handheld "SkinPen" loaded with biomaterial ink for in situ wound healing. *ACS Appl Mater Interfaces*. 2023;15(23):27568-27585. doi: 10.1021/acsami.3c02825

90. Ying G, Manriquez J, Wu D, et al. An open-source handheld extruder loaded with pore-forming bioink for in situ wound dressing. *Mater Today Bio*. 2020;8:100074. doi: 10.1016/j.mtbio.2020.100074

91. Mostafavi A, Samandari M, Karvar M, et al. Colloidal multiscale porous adhesive (bio)inks facilitate scaffold integration. *Appl Phys Rev*. 2021;8(4):041415. doi: 10.1063/5.0062823

92. Shi Y, Tang S, Yuan X, et al. In situ 4D printing of polyelectrolyte/magnetic composites for sutureless gastric perforation sealing. *Adv Mater*. 2023;36:2307601. doi: 10.1002/adma.202307601

93. Yang Y, Yu Z, Lu X, et al. Minimally invasive bioprinting for in situ liver regeneration. *Bioact Mater*. 2023;26:465-477. doi: 10.1016/j.bioactmat.2023.03.011

94. Debbi L, Machour M, Dahis D, et al. Ultrasound mediated polymerization for cell delivery, drug delivery, and 3D printing. *Small Methods*. 2024:2301197. doi: 10.1002/smtd.202301197

95. Zhao W, Hu C, Lin S, et al. A closed-loop minimally invasive 3D printing strategy with robust trocar identification and adaptive alignment. *Addit Manuf*. 2023;73:103701. doi: 10.1016/j.addma.2023.103701

96. Goker M, Derici US, Gokyer S, et al. Spatial growth factor delivery for 3D bioprinting of vascularized bone with adipose-derived stem/stromal cells as a single cell source. *ACS Biomater Sci Eng*. 2024;10(3):1607-1619. doi: 10.1021/acsbiomaterials.3c01222

97. Enrico A, Voulgaris D, Ostmans R, et al. 3D microvascularized tissue models by laser-based cavitation molding of collagen. *Adv Mater*. 2022;34(11):e2109823. doi: 10.1002/adma.202109823

98. Hu Y, Xiong Y, Zhu Y, et al. Copper-epigallocatechin gallate enhances therapeutic effects of 3D-printed dermal scaffolds in mitigating diabetic wound scarring. *ACS Appl Mater Interfaces*. 2023;15(32):38230-38246. doi: 10.1021/acsami.3c04733

99. Lu S, Wang X, Li W, Zu Y, Xiao J. Injectable 3D-printed porous scaffolds for adipose stem cell delivery and endometrial regeneration. *Adv Funct Mater*. 2023;33(34): 2303368. doi: 10.1002/adfm.202303368

100. Ouyang L, Armstrong JPK, Chen Q, Lin Y, Stevens MM. Void-free 3D bioprinting for in situ endothelialization and microfluidic perfusion. *Adv Funct Mater*. 2020;30(1):1908349. doi: 10.1002/adfm.201908349

101. Muhammad M, Willems C, Rodriguez-Fernandez J, Gallego-Ferrer G, Groth T. Synthesis and characterization of oxidized polysaccharides for in situ forming hydrogels. *Biomolecules*. 2020;10(8):1185. doi: 10.3390/biom10081185

102. Baptista M, Joukhdar H, Alcala-Orozco CR, et al. Silk fibroin photo-lyogels containing microchannels as a biomaterial platform for in situ tissue engineering. *Biomater Sci*. 2020;8(24):7093-7105. doi: 10.1039/d0bm01010c

103. Chen X, Ranjan VD, Liu S, et al. In situ formation of 3D conductive and cell-laden graphene hydrogel for electrically regulating cellular behavior. *Macromol Biosci*. 2021;21(4):e2000374. doi: 10.1002/mabi.202000374

104. Li C, Faulkner-Jones A, Dun AR, et al. Rapid formation of a supramolecular polypeptide-DNA hydrogel for in situ three-dimensional multilayer bioprinting. *Angew Chem Int Ed*. 2015;54(13):3957-3961. doi: 10.1002/anie.201411383

105. Moeinzadeh S, Park Y, Lin S, Yang YP. In-situ stable injectable collagen-based hydrogels for cell and growth factor delivery. *Materialia (Oxf)*. 2021;15:100954. doi: 10.1016/j.mtla.2020.100954

106. Alruwaili M, Lopez JA, McCarthy K, Reynaud EG, Rodriguez BJ. Liquid-phase 3D bioprinting of gelatin alginate hydrogels: influence of printing parameters on hydrogel line width and layer height. *Bio-Des Manuf*. 2019;2(3):172-180. doi: 10.1007/s42242-019-00043-w

107. Handral HK, Natu VP, Cao T, et al. Emerging trends and prospects of electroconductive bioinks for cell-laden and functional 3D bioprinting. *Bio-Des Manuf*. 2022;5(2): 396-411. doi: 10.1007/s42242-021-00169-w

108. Lai S, Wu T, Shi C, et al. Triple-layered core-shell fiber dressings with enduring platelet conservation and sustained growth factor release abilities for chronic wound healing. *Regener Biomater*. 2024;11:rbae034. doi: 10.1093/rb/rbae034

109. Kang MS, Kwon M, Lee SY, et al. In situ crosslinkable collagen-based hydrogels for 3D printing of dermis-mimetic constructs. *ECS J Solid State Sci Technol*. 2022;11(4): 045014. doi: 10.1149/2162-8777/ac6897

110. Kang MS, Kang JI, Thi PL, et al. Three-dimensional printable gelatin hydrogels incorporating graphene oxide to enable spontaneous myogenic differentiation. *ACS Macro Lett*. 2021;10(4):426-432. doi: 10.1021/acsmacrolett.0c00845

111. Miri AK, Nieto D, Iglesias L, et al. Microfluidics-enabled multimaterial maskless stereolithographic bioprinting. *Adv Mater*. 2018;30(27):e1800242. doi: 10.1002/adma.201800242

112. Hwangbo H, Lee H, Jin E-J, et al. Bio-printing of aligned GelMa-based cell-laden structure for muscle tissue regeneration. *Bioact Mater*. 2021;8:57-70. doi: 10.1016/j.bioactmat.2021.06.031

113. Jalandhra GK, Molley TG, Hung T-t, Roohani I, Kilian KA. In situ formation of osteochondral interfaces through "bone-ink" printing in tailored microgel suspensions. *Acta Biomater*. 2023;156:75-87. doi: 10.1016/j.actbio.2022.08.052

114. de Melo BAG, Jodat YA, Mehrotra S, et al. 3D printed cartilage-like tissue constructs with spatially controlled mechanical properties. *Adv Funct Mater*. 2019;29(51):1906330. doi: 10.1002/adfm.201906330

115. Li C, Wang J, Yang W, et al. 3D-printed hydrogel particles containing PRP laden with TDSCs promote tendon repair in a rat model of tendinopathy. *J Nanobiotechnol*. 2023;21(1):177. doi: 10.1186/s12951-023-01892-5

116. Li J, Huang Y, Song J, et al. Cartilage regeneration using arthroscopic flushing fluid-derived mesenchymal stem cells encapsulated in a one-step rapid cross-linked hydrogel. *Acta Biomater*. 2018;79:202-215. doi: 10.1016/j.actbio.2018.08.029

117. Blaeser A, Million N, Campos DFD, et al. Laser-based in situ embedding of metal nanoparticles into bioextruded alginate hydrogel tubes enhances human endothelial cell adhesion. *Nano Res*. 2016;9(11):3407-3427. doi: 10.1007/s12274-016-1218-3

118. Khoshnood N, Shahrezaee MH, Shahrezaee M, Zamanian A. Three-dimensional bioprinting of tragacanth/hydroxyapaptite modified alginate bioinks for bone tissue engineering with tunable printability and bioactivity. *J Appl Polym Sci*. 2022;139(36):e52833. doi: 10.1002/app.52833

119. Bandyopadhyay A, Mandal BB, Bhardwaj N. 3D bioprinting of photo-crosslinkable silk methacrylate (SilMA)-polyethylene glycol diacrylate (PEGDA) bioink for cartilage tissue engineering. *J Biomed Mater Res Part A*. 2022;110(4):884-898. doi: 10.1002/jbm.a.37336

120. Zhang M, Qian T, Deng Z, Hang F. 3D printed double-network alginate hydrogels containing polyphosphate for bioenergetics and bone regeneration. *Int J Biol Macromol*. 2021;188:639-648. doi: 10.1016/j.ijbiomac.2021.08.066

121. Koo Y, Kim G. New strategy for enhancing in situ cell viability of cell-printing process via piezoelectric transducer-assisted three-dimensional printing. *Biofabrication*. 2016;8(2):025010. doi: 10.1088/1758-5090/8/2/025010

122. Krishnadoss V, Kanjilal B, Masoumi A, et al. Programmable bio-ionic liquid functionalized hydrogels for in situ 3D bioprinting of electronics at the tissue interface. *Mater Today Adv*. 2023;17:100352. doi: 10.1016/j.mtadv.2023.100352

123. Yin J, Zhao D, Liu J. Trends on physical understanding of bioink printability. *Bio-Des Manuf*. 2019;2(1):50-54. doi: 10.1007/s42242-019-00033-y

124. Wang X, Yang C, Yu Y, Zhao Y. In situ 3D bioprinting living photosynthetic scaffolds for autotrophic wound healing. *Research (Wash D C)*. 2022;2022:9794745. doi: 10.34133/2022/9794745

125. Hann SY, Cui H, Esworthy T, Zhang LG. 4D thermo-responsive smart hiPSC-CM cardiac construct for myocardial cell therapy. *Int J Nanomed*. 2023;18:1809-1821. doi: 10.2147/IJN.S402855

126. Joshi A, Choudhury S, Baghel VS, et al. 4D printed programmable shape-morphing hydrogels as intraoperative self-folding nerve conduits for sutureless neurorrhaphy. *Adv Healthcare Mater*. 2023;12(24):e2300701. doi: 10.1002/adhm.202300701

127. Liu B, Li H, Meng F, et al. 4D printed hydrogel scaffold with swelling-stiffening properties and programmable deformation for minimally invasive implantation. *Nat Commun*. 2024;15(1):1587. doi: 10.1038/s41467-024-45938-0

128. Luo K, Wang L, Wang M-X, et al. 4D printing of biocompatible scaffolds via in situ photo-crosslinking from shape memory copolyesters. *ACS Appl Mater Interfaces*. 2023;15(37):44373-44383. doi: 10.1021/acsami.3c10747

129. Li W, Wang M, Mille LS, et al. A smartphone-enabled portable digital light processing 3D printer. *Adv Mater*. 2021;33(35):2102153. doi: 10.1002/adma.202102153

130. Warth N, Berg M, Schumacher L, et al. Bioprint FirstAid: a handheld bioprinter for first aid utilization on space exploration missions. *Acta Astronaut*. 2024;215:194-204. doi: 10.1016/j.actaastro.2023.11.033

131. Tomooka Y, Spothelfer D, Puiggali-Jou A, et al. Minimally invasive in situ bioprinting using tube-based material transfer. *at - Automatisierungstechnik*. 2023;71(7):562-571. doi: 10.1515/auto-2023-0060