Michaela Maintz[+]*, Yukiko Tomooka[+], Manuela Eugster, Nicolas Gerig, Neha Sharma, Florian M. Thieringer[§], and Georg Rauter[§]

In situ minimally invasive 3D printing for bone and cartilage regeneration - a scoping review

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Abstract: Advancements in personalized medicine, three-dimensional (3D) printing, miniaturization, and robot-assisted surgery are driving innovation in tissue engineering. A novel approach, known as *in situ* printing, focuses on the direct deposition of materials at the surgical site. Using the *in situ* printing approach, bone and/or cartilage defects can be addressed with high precision. Furthermore, highly customized 3D printed tissue constructs or implants can be deposited directly inside the body. Currently, most applications of *in situ* printing are limited to areas near the skin or open surgeries. Even though a minimally invasive approach would bring clinical benefits, only a few research groups have focused on this field. In this scoping review, we provide an overview of the current state of *in situ* minimally invasive 3D printing technology for bone and cartilage regeneration and discuss its advantages and current challenges.

Keywords: Robot-assisted surgery, Bioprinting, Biofabrication, Tissue engineering, Three-dimensional printing, Keyhole surgery

1 Introduction

The advances of three-dimensional (3D) printing in healthcare using 3D modeling and computer-assisted design enable implants to be tailor-made for the patient's anatomy [1, 2]. The developments in bioprinting are fuelling the interest in directly printing tissues inside the patient's body. We define the term minimally invasive 3D printing (MI3DP) as the deposition of biomaterials on the intended anatomical location in the living body through small surgical incisions. A conceptual illustration of this vision is depicted in Figure 1. MI3DP could unlock new opportunities in tissue regeneration to locally heal the body or support the body to heal itself.

Through MI3DP, materials that fit into irregular crevices can be brought to areas of the body that are difficult to reach, minimizing damage to the surrounding healthy tissue. With this technology, biomaterials, cells, growth factors, and/or pharmaceuticals can be precisely deposited *in situ* and do not need to be transported from the 3D printer into the operating room. An *in vitro* laboratory fabricated tissue scaffold could be affected by structural deterioration or contamination during storage, and transfer to the operating room. Additionally, the fabrication process can take several hours, prolonging the duration until surgery can take place. Furthermore, implantation surgery often requires large incisions, posing particular disadvantages for deep-seated defect locations or large implant dimensions. A minimally invasive procedure can reduce healing time, and reduce the risk of infections and scarring. The *in situ* integration of an implant into native tissue can be improved with adhesives [3, 4], complex geometrical intersections, or the possibility to suture native tissue to the printed construct [5]. Based on patient-specific anatomical data, the volume to be *in situ* 3D printed can be preoperatively planned and the material can be directly deposited into a printing site. Moreover, combining 3D printing with intraoperative imaging techniques allows the adaptation of the 3D printing process during surgery.

***Corresponding author: Michaela Maintz[+],** Swiss Medical Additive Manufacturing Research Group (Swiss MAM), Department of Biomedical Engineering, University of Basel, Hegenheimermattweg 167C, Allschwil, Switzerland and University of Applied Sciences and Arts Northwestern Switzerland FHNW, School of Life Sciences HLS, Institute of Medical Engineering and Medical Informatics IM^2, Hofackerstrasse 30, Muttenz, Switzerland, e-mail: michaela.maintz@unibas.ch
Yukiko Tomooka[+], Manuela Eugster, Nicolas Gerig, Georg Rauter[§], BIROMED-Lab, Department of Biomedical Engineering, University of Basel, Hegenheimermattweg 167C, Allschwil, Switzerland, e-mail: yukiko.tomooka@unibas.ch, nicolas.gerig@unibas.ch, manuela.eugster@unibas.ch, georg.rauter@unibas.ch
Neha Sharma, Florian M. Thieringer[§], Clinic of Oral and Cranio-Maxillofacial Surgery, University Hospital Basel, Petersgraben 4, Basel, Switzerland, and Swiss Medical Additive Manufacturing Research Group (Swiss MAM), Department of Biomedical Engineering, University of Basel, Hegenheimermattweg 167C, Allschwil, Switzerland, e-mail: neha.sharma@usb.ch, florian.thieringer@usb.ch

[+] Michaela Maintz and Yukiko Tomooka contributed equally to this work as shared first authors.

[§] Georg Rauter and Florian M. Thieringer contributed equally to this work as shared last authors.

In situ printing has developed rapidly in recent years and has been investigated for various applications, such as soft tissue [3, 4, 6–8], bone [9–12], and cartilage [13–17] regeneration. Handheld printers have been developed, which, compared to robot-assisted systems, are more intuitive, flexible in their application, and portable. Handheld printers have shown potential in the treatment of skin [8] and muscle injuries [4], however, their use is limited when i) the defects are located within confined areas in deep-seated anatomical locations, ii) more than one biomaterial is required, or iii) when the material deposition requires high precision. Robot-assisted *in situ* printing allows the exact fabrication of structures that fit into a defect. The geometry of the 3D printed structure must be predefined using the anatomical data recorded by intraoperative scanners and/or medical imaging techniques such as computed tomography or magnetic resonance imaging. Through a multi-axis system, the bioink, or the biomaterial can be deposited into the defect. Currently, few papers focus on the minimally invasive approach for *in situ* 3D printing.

Our goal is to develop a minimally invasive *in situ* bioprinting system [18] based on a miniature parallel robot [19, 20]. We performed a scoping review of the relevant literature in the Web of Science database with the combinations of keywords involving but not limited to "minimally invasive", "3D printing", "bioprinting", "*in vivo*", "*in situ*", "intraoperative", "bone" and "cartilage". In this scoping review, we provide a brief overview of the current state of the technology and discuss the current challenges of MI3DP in bone and cartilage regeneration using robot-assisted 3D printers.

2 System requirements

MI3DP is expected to allow faster recovery of patients and better integration of the printed structures into surrounding tissues, however, its application in clinical settings remains a challenge. The main challenges of MI3DP are attributed to the complexity of the working environment inside the patient's body. For instance, non-planar printing surfaces, confined workspaces, gravity, risk of damage to surrounding tissue, and limited options of post-processing methods must be faced. To overcome these challenges, there are certain requirements for MI3DP systems (Figure 2, System Requirements).

For instance, miniaturization of printing and monitoring systems is required for MI3DP to access the printing sites through a small incision. In addition, the use of a multi-axial positioning system could be advantageous to minimally invasively print complex 3D structures on irregular surfaces. Robot-assisted multi-axial *in situ* printers with a wide range of motion have been developed to enhance surgical dexterity for open surgical procedures [9, 13].

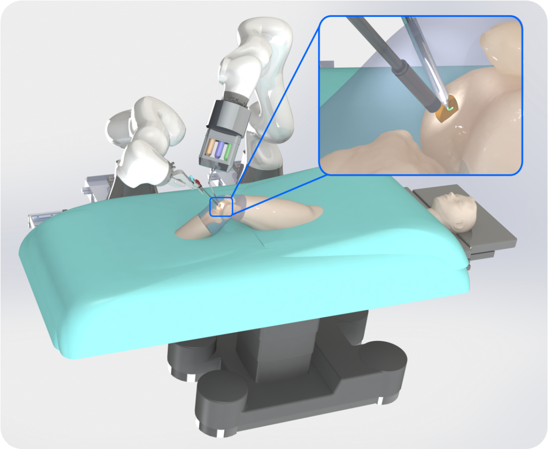

Fig. 1: Conceptual vision of robot-assisted *in situ* minimally invasive 3D printing for femoral cartilage replacement as an exemplary application.

In robot-assisted MI3DP, preoperative and intraoperative defect imaging and planning are essential. The printing process requires continuous monitoring to avoid unexpected outcomes (e.g., crack, delamination of layers, or layer shift) due to possible printing errors (e.g., nozzle clogging or misalignment). The risk of damage to surrounding healthy tissues in the body (e.g., collision, penetration, or overheating) should also be minimized through intraoperative observation. Camera-based monitoring systems were integrated into some *in situ* printing systems, however, their usage is limited to areas near the skin or open surgical procedures [10, 11].

For MI3DP, printing materials and curing methods need to be selected considering the *in situ* printing process. Along with fulfilling the structural requirements for implants, it is important to keep the surrounding healthy tissue intact. Materials must be printable and curable at human body temperature, and curing methods should not be harmful to surrounding native tissue.

3 3D Printing modalities & materials

Current 3D printing modalities can be mainly categorized into laser-based, extrusion-based, and inkjet-based modalities (Figure 3), and the comparison of these modalities was repeatedly summarized in literature [21, 22]. The extrusion-based printing modality has been the most commonly selected modality for *in situ* bone or cartilage printing [9, 10, 12–16]. The fact that extrusion-based printing allows for a relatively high

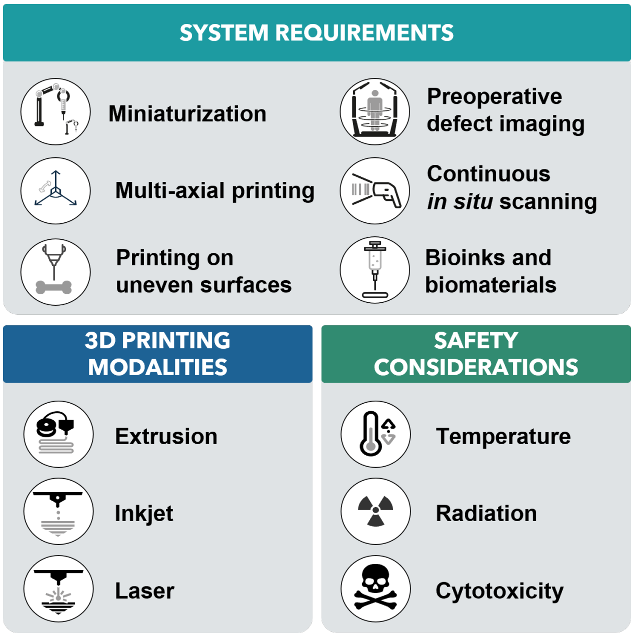

Fig. 2: Overview of major system requirements, 3D printing modalities and safety requirements.

mechanical and structural material integrity and short printing time [21] could be the reason for the preference for this modality.

In situ printing involves either handheld or robot-assisted devices. Several studies demonstrated *in situ* 3D printing using handheld or robot-assisted approaches for bone or cartilage repair with animals, however, these studies remained open surgical procedures [9, 10, 12–14]. Only a few MI3DP systems have been proposed so far. For example, MI3DP has been researched for cartilage [15, 16, 18] and abdominal organs [3, 6, 7] using the extrusion-based printing modality. Lenatowicz et al. showed a proof of concept of a MI3DP approach for cartilage repair [15]. They proposed an arthroscopic handheld 3D printing tool used with a secondary tool holding a camera and ultraviolet (UV) source for monitoring and curing materials. Lipskas et al. developed a robot-assisted system for cartilage repair allowing MI3DP through a small incision [16]. They demonstrated 3D printing *in vitro* with an open surgical environment and showed an average defect imprint geometrical error of $0.06 \pm 0.14\,\mathrm{mm}$ which is comparable to other studies in the field of *in situ* printing for cartilage or bone regeneration [17]. Tomooka et al. developed an experimental 3D printing platform consisting of an extrusion system with a tube connecting the extrusion syringe to the dispensing nozzle (tube-based material transfer) [18]. This tube-based material transfer system conceptually allows MI3DP by placing space-consuming components (e.g., extrusion syringe) outside of the patient body and inserting a small diameter device with a printing nozzle at the tip to reach the printing site minimally invasively. Tomooka et al. demonstrated the feasibility of 3D printing structures using the tube-based material transfer system in an open experimental setup and discussed the challenges of tube-based material transfer.

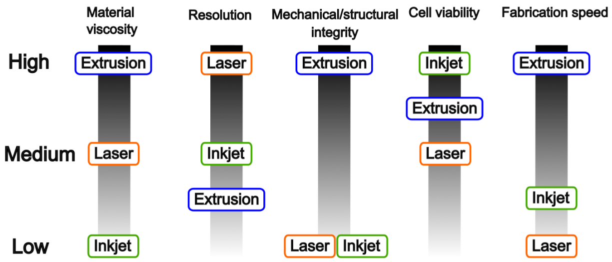

Fig. 3: Relative comparison of printing modalities based on the results presented in the review article by Dababneh et al. [21]: extrusion-based, laser-based, and inkjet-based printing.

To our best knowledge, research evaluating the performance of a MI3DP system for bone or cartilage regeneration in a minimally invasive setting has not yet been published. However, several groups developed MI3DP systems for abdominal organs and evaluated their performance in minimally invasive settings [3, 6, 7]. Zhou et al. proposed a ferromagnetic soft catheter robot capable of controlling material deposition directions using a magnetic actuator [3]. They printed various patterns with their system *in vitro* and successfully demonstrated MI3DP in a liver of a living rat. They noted that the achievable printing speed, resolution, and complexity of printed patterns are currently limited with their system, and further optimization and miniaturization are required. Zhao et al. designed an endoscopic MI3DP system for gastric wall injury treatments using gelatin-alginate hydrogels [6]. In their system, a miniaturized delta robot-assisted 3D printing platform was fixed at the tip of the endoscope and printing material was delivered through a polytetrafluoroethylene (PTFE) tube from a syringe. They evaluated the performance of the device with a stomach phantom and demonstrated a proof of concept of their system. Although the size of the presented system was slightly larger than existing endoscopes ($16-20\,\mathrm{mm}$ in diameter), they mentioned the possibility of downsizing to $12\,\mathrm{mm}$ by miniaturizing the parallel mechanism in the future. Thai et al. developed a flexible MI3DP system capable of printing, water injection, and dissection for gastric injury treatment [7]. They successfully printed various patterns on different surfaces including a fresh porcine kidney and artificial colon using various materials such as food-grade chocolate, liquid silicone elastomer, gel composite, and commercial bioink.

Polymers are the most commonly applied materials in 3D printing due to biocompatibility and biodegradability.

Polymers in bioprinting are generally classified into either synthetic polymers, e.g., pluronic and polyethylene glycol, or natural polymers, e.g., collagen, gelatine, and hyaluronic acid [22]. Natural polymer-based materials containing gelatine, or hyaluronic acid are often used for *in situ* 3D printing for cartilage and bone regeneration [9, 12–14, 17]. Materials are generally cured by light, thermal or chemical transitions in 3D printing. The photo-initiated curing method is one of the most commonly used methods in *in situ* 3D printing for bone or cartilage regeneration [9, 13, 14, 17].

3.1 Safety requirements

A system that operates inside a human body is considered a medical device, therefore, the respective legal safety requirements must be met. According to the Safety of Surgical Robots and IEC 80601-2-77 standard, risks to patients and medical staff must be reduced to an acceptable level [23]. For instance, electric shocks must be prevented by robotically assisted surgical equipment. In the case of endoscopic surgery, the main surgeon must be able to watch the endoscopic view while other staff monitors any potentially dangerous situation outside the patient's body. According to the guidelines, operability, and intervention by the surgeon should be ensured at any time. For instance, the operator should be able to constantly monitor the device's movements and stop the procedure quickly. Sterility and biocompatibility of the material and system must be ensured. Toxic and contaminated material must not be brought into the body.

Furthermore, regulatory requirements for implantable medical devices should be followed. Unnecessary radiation of the patient must be avoided. Cross-linking with high energy UV light could cause cell damage, therefore, longer wavelengths such as visible light polymerization could be a viable option [24]. When using thermoplastic polymers, the tissue should ideally not be exposed to heat above 40 °C for long periods, to prevent cell damage.

4 Open challenges & discussion

Currently, in our literature review, research involving *in situ* printing for bone and cartilage regeneration has been exclusively performed in animal studies in open surgical procedures. No reports on the systems in clinical use were found. 3D printing in small cavities seems to remain a challenging task. To successfully implement the MI3DP technology, certain challenges must be overcome. For instance, the biological response of the printed construct should be studied in depth for optimal tissue regeneration. For an improved healing process and tissue regeneration, further research is necessary to enhance the complexity of the 3D printed constructs to mimic the complex structural and chemical composition of native tissue. As for many new emerging technologies, long-term effects will be challenging to assess. Therefore, patients should be informed of the risks of this procedure and the effects of the potential removal of the 3D printed structure.

From a technological standpoint, miniaturization is a significant challenge to overcome. For instance, the printing modality should be selected considering the potential for miniaturization in addition to the characteristics summarized in Figure 3. Extrusion-based printing was the only modality utilized in the MI3DP systems found at the time of our literature research. This selection of extrusion-based modality for MI3DP systems is likely because it allows the placement of space-consuming components outside of the patient's body. Specifically, the material reservoir and deposition control unit (e.g., extrusion syringe) can be distally placed from the dispensing nozzle. The MI3DP systems developed for abdominal organs [3, 6, 7] could be also adapted to cartilage or bone regeneration through further miniaturization and modification for these applications.

In addition, intraoperative scanning, monitoring, and image processing techniques need to be improved to track and anticipate internal tissue movements such as muscular contractions, breathing, peristalsis, and blood flow. Solutions must be found to address obstructions of the printing process through surrounding tissue or body fluids. The best course of action must be decided in the event that the 3D printing process is interrupted, which entails restarting the process precisely where it left off in order to obtain the desired geometry.

To ensure the feasibility of this technology and its application in the operating room, the benefit of MI3DP must be maximized to compensate for the higher cost of the equipment, the potentially longer duration of the intervention, and the effort to train the staff to develop 3D models and operate the device during surgery.

Despite the challenges, *in situ* minimally invasive 3D printing shows promise for a large range of medical applications. With further research and development, this technology will boost a more personalized approach to medical treatment, reducing the risk of complications and recovery time.

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