Trends in Biotechnology

CellPress
REVIEWS

Opinion

Intraoperative Bioprinting: Repairing Tissues and Organs in a Surgical Setting

Yang Wu,[1,2,3] Dino J. Ravnic,[4] and Ibrahim T. Ozbolat[2,3,5,6,*]

3D bioprinting directly into injured sites in a surgical setting, intraoperative bioprinting (IOB), is an effective process, in which the defect information can be rapidly acquired and then repaired via bioprinting on a live subject. In patients needing tissue resection, debridement, traumatic reconstruction, or fracture repair, the ability to scan and bioprint immediately following surgical preparation of the defect site has great potential to improve the precision and efficiency of these procedures. In this opinion article, we provide the reader with current major limitations of IOB from engineering and clinical points of view, as well as possibilities of future translation of bioprinting technologies from bench to bedside, and expound our perspectives in the context of IOB of composite and vascularized tissues.

Advances from *in Vitro* Bioprinting to Intraoperative Bioprinting

In the last decade, bioprinting has considerably advanced and a large number of studies have shown attractive outcomes in different applications, such as engineering of various organs for regenerative medicine (i.e., skin [1], cartilage [2], and bone [3]), tissue vascularization [4], drug discovery [5], and disease modeling [6]. However, the majority of bioprinting endeavors have been conducted *in vitro* and, in some cases, further validated *in vivo* [7]. Although bioprinting of solid organs directly into human body remains elusive, some attempts have been practiced in the realm of **intraoperative bioprinting (IOB)** (see Glossary), also known as *in situ* or *in vivo* bioprinting [8–10], which refers to the bioprinting process performed on a live subject in a surgical setting, in which defect imaging, data processing, process planning, and bioprinting are performed consecutively in a single process. IOB of tissue substitutes directly into injury sites is greatly beneficial as it can facilitate the complex tissue heterogeneity in an anatomically accurate manner. Although IOB has not been performed in clinics yet, the field is steadily moving forward owing to the rapid developments in bioprinting technologies with contributions from interdisciplinary teams of researchers from different domains spanning from engineering and materials to medical sciences and surgery.

The realization of IOB will bring about several benefits. First, bioprinted tissue substitutes, especially hydrogel- or cell aggregate-based constructs, usually have weak initial mechanical strengths due to the fluid-rich nature, which makes them difficult to handle with surgical tools and further increases the likelihood of construct disintegration while being transferred to the surgical site and suturing. In contrast, with the assistance of a reverse engineering methodology enabling the real-time design of the graft, IOB is an effective process, where the defect can be repaired with minimum risk of contamination, graft disintegration, and manual interventions such as stacking multiple layers, *in vitro* culturing bioprinted scaffolds, transportation during surgery, or modifying prefabricated scaffolds conforming the defect shape [11]. More importantly, IOB is able to tackle natural defects with irregular topographies, which is common in clinical cases due to trauma and surgical excision. Comparatively, *in vitro* bioprinting usually assumes

Highlights

Recent progress in bioprinting has advanced beyond *in vitro* applications toward intraoperative bioprinting.

Different intraoperative bioprinting modalities possess pros and cons in specific biomedical applications.

The advantages of intraoperative bioprinting bring forth opportunities to clinically translate bioprinting technologies from bench to bedside.

Intraoperative bioprinting of composite tissues has drawn particular interest since the repair of natural defects usually entails the reconstruction of multiple different tissue types.

[1]School of Mechanical Engineering and Automation, Harbin Institute of Technology, Shenzhen 518055, China
[2]Engineering Science and Mechanics Department, The Pennsylvania State University, State College, PA 16801, USA
[3]The Huck Institutes of the Life Sciences, The Pennsylvania State University, State College, PA 16801, USA
[4]Department of Surgery, Penn State Health Milton S. Hershey Medical Center, Hershey, PA 17033, USA
[5]Department of Biomedical Engineering, Penn State University, University Park, PA 16801, USA
[6]Materials Research Institute, Penn State University, University Park, PA 16801, USA

*Correspondence:
ito1@psu.edu (I.T. Ozbolat).

a flat working surface, which is inconsistent with clinical scenarios. Moreover, since intraoperatively repaired defects are surrounded by native tissue, endogenous cells could be directed by proper biochemical and biophysical cues to migrate into the bioprinted constructs and differentiate into target tissue-specific lineages [11,12]. In addition, compared with manual injection of biomaterials into defect sites, IOB enables the precise deposition of cells, genes, or cytokines with localized control and anatomical biomimicry. For tissues that are heterocellular and formed of zonally stratified arrangement of **extracellular matrix (ECM)**, IOB is a powerful technology to precisely reconstitute multiple layers that is quite challenging using manual approaches. Lastly, shape distortion of hydrogels (e.g., high degree of shrinkage of collagen during skin regeneration [13]) can be an issue during *in vitro* maturation of bioprinted constructs. In addition, during *in vitro* culture, cells remodel the matrix and eventually change the shape of the bioprinted constructs, which may pose a significant disruption of predesigned shapes. Therefore, IOB is also advantageous due to the concurrence of *in vivo* integration, where the regenerated tissue may occupy the void space due to shrinkage and regulate the remodeling of matrix.

Current Developments in IOB

Although the concept of IOB was first proposed in 2007 [14], very few studies have been reported on this topic, as bioprinting on a live subject is not trivial. Indeed, IOB is more demanding with respect to bioink preparation, bioprinter setup, sterilization, and surgical operation as compared with implanting prefabricated constructs. Thus far, some attempts targeting the repair of cartilage, bone, and skin defects have been reported by different groups. For example, handheld devices have been developed for cartilage repair in a sheep model [15,16] (Figure 1A) and skin repair in murine and porcine models [17]. Although the deposition was performed manually, it has come up with a well-developed strategy to miniaturize the number of external equipment for crosslinking of a cell-laden hydrogel. In terms of bone repair, Keriquel and coworkers [18] have performed IOB in the mouse calvaria defect model using an in-house developed biological laser-based bioprinting (LBB) system (Figure 1B). In their follow-up studies, IOB gave rise to organized microvascular networks and bone regeneration in calvaria defects [19,20]. Currently, the most successful cases on IOB are mostly associated with skin repair due to its ease of access and regenerative potential [21,22]. Most recently, Albanna and colleagues presented a mobile skin bioprinting system with integrated imaging technology for on-site management of murine and porcine full-thickness skin wound models [23] (Figure 1C). Table 1 gives a summary of the past works performed in IOB and their significant outcomes. A few excellent review articles provide more detailed information on the current status of *in situ* bioprinting [8–10]. Beyond the prior reviews, here we focus on the current major limitations of IOB from engineering and clinical points of view, highlighting the potential of future translation of IOB technologies, and also expounding the possibilities of using such a technology in reconstruction of composite and vascularized tissues.

Considerations and Future Outlook for IOB

Limitations of Current Bioprinting Modalities

Bioprinting modalities can be categorized as extrusion-, droplet-, and laser-based, which have been comprehensively reviewed elsewhere [24–26] and are also summarized in Box 1. For IOB, more challenges appear when adapting knowledge gained through *in vitro* systems. Extrusion-based bioprinting (EBB) can be considered an appropriate modality for IOB since manual injection (extrusion) of biomaterials has already been clinically applied for decades [27,28] and current surgical robots, used in some clinical applications such as urology and gynecology [29], can be easily reconfigured to hold EBB tips. However, the print tip might interfere with defect periphery since it is a contact-based technology. In terms of droplet-based bioprinting (DBB), although its resolution is superior than that of EBB, bioink deposition

through a small orifice results in higher risk of nozzle clogging, which may increase the duration of surgery and may bring other complications. Limited by poor structural and mechanical integrity of bioprinted constructs, DBB is more suitable for bioprinting thin tissues such as skin. For LBB, it shares similar advantages (e.g., high resolution) and disadvantages (e.g., low integrity of bioprinted constructs) with respect to DBB. The **miniaturization** of LBB is a major challenge due to its complex setup (such as a laser source and optical components), which constrains its accessibility to internal tissues. Other challenges, such as the immobilization of light sources and the necessity of precise focusing of light, may limit its clinical translation.

and take the place of cells that die or are lost. Popular stem cells in bioprinting include mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), adipose-derived stem cells (ADSCs), etc.
Vacuum assisted closure: a type of therapy to help wounds heal. During the treatment, a device decreases air pressure on the wound, which can help the wound heal more quickly.

(A)

(B)

(C)
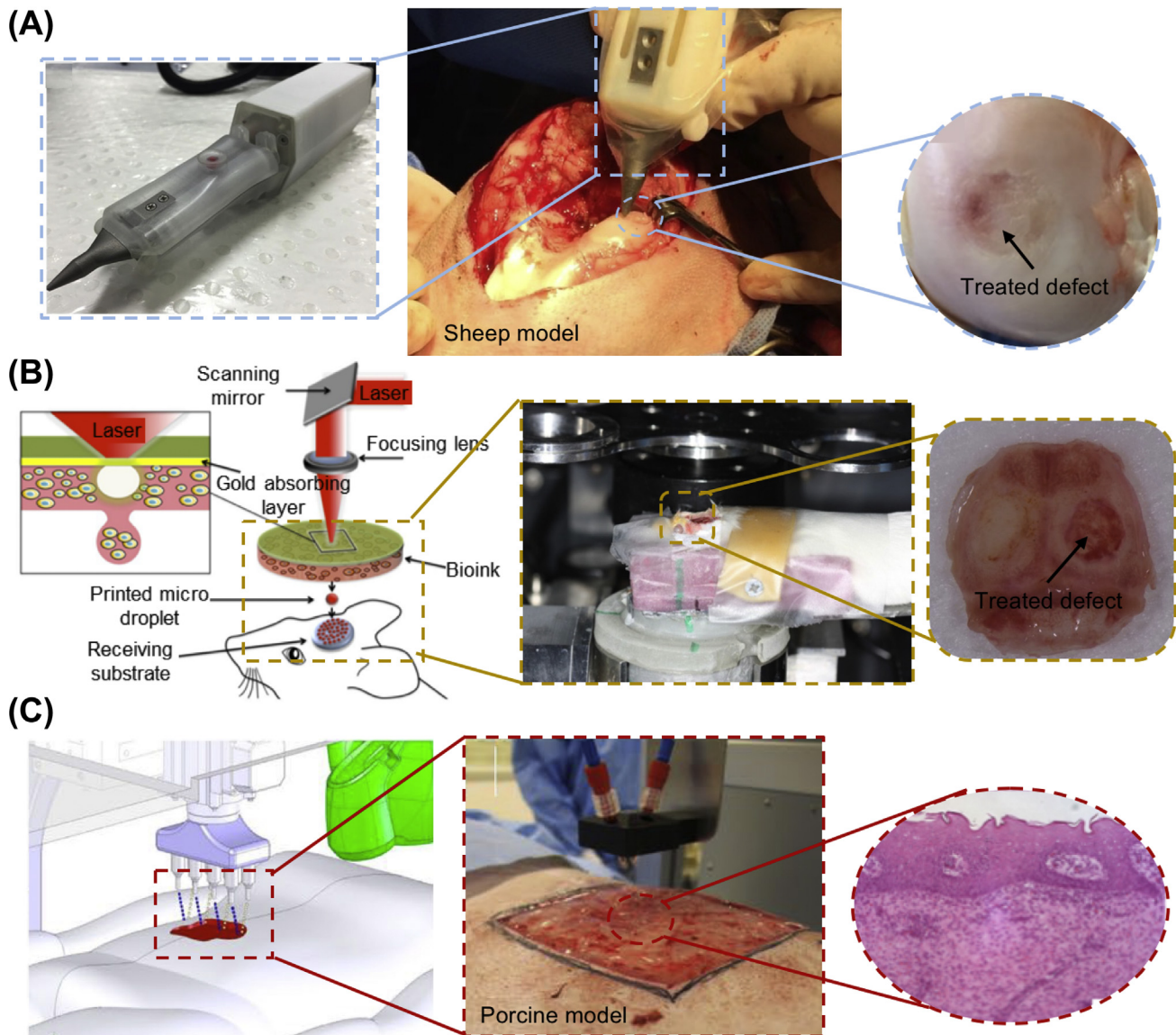
Trends in Biotechnology

Figure 1. Examples for Intraoperative Bioprinting (IOB). (A) IOB using the biopen for treatment of a full-thickness chondral defect in a sheep; adapted, with permission, from [16]. (B) Laser-based IOB for skull repair in a mouse calvarial defect; adapted, with permission, from [18–20]. (C) Droplet-based IOB for skin repair in a porcine model; adapted, with permission, from [23].

Table 1. Summary of Studies Performed in the Context of IOB

Bioprinting modality	Materials	Cell source	Animal type	Defect model	Target tissue	3D scanning	Results	Refs
Extrusion-based bioprinting	Gelatin methacrylamide (GelMA)/hyaluronic acid methacrylate (HAMA)	Mesenchymal stem cells (MSCs)	Sheep	Chondral defect	Cartilage	No	• Better overall macroscopic appearance in the handheld printed group compared with control groups • Handheld printed constructs showed a higher amount of newly regenerated cartilage and the absence of subchondral bone deformation or collapse • Handheld printed constructs showed positive Safranin O and collagen type II staining	[16]
	Alginate/fibrin/collagen/ hyaluronic acid (HA)	Fibroblasts/ keratinocytes	Mice/pig	Skin defect on dorsa	Skin	No	Murine model: • *In situ* deposition of an architected sheet was performed in the form of a fiber array onto a small and compliant wound surface Porcine model: • The porcine model demonstrated successful *in situ* bioprinting to cover full-thickness wounds with a hemostatic barrier where it did not impede normal re-epithelialization or wound contraction • Microscopic analysis of healed wounds on day 20 revealed that both treated and control wounds formed complete granulation tissue and exhibited comparable levels of collagen deposition and cellularity	[17]
Laser-based bioprinting	Nano-hydroxyapatite (n-HA)	–	Mice	Calvaria defect	Bone	No	• Material was present in close contact with dura mater on the test sites after 1 week • After 1 month, newly formed mature and immature bones and n-HA aggregates inside macrophages were observed • Three months after printing, mature bone tissue was observed • From 1 week to 3 months, the amount of n-HA particles observed *in situ* decreased	[18]
	Collagen/n-HA	MSCs	Mice	Calvaria defect	Bone	No	• Both n-HA-collagen and n-HA-collagen + cells, printed in a ring geometry, show only a marginal tissue reconstruction at 2 months postprinting, particularly from the periphery of the defect • The n-HA-collagen + cells, printed in disc geometry, show a substantial new bone formation, well distributed throughout the defect, even in the center of the defect	[19]
	Collagen/vascular endothelial growth factor (VEGF)	Stem cells from the apical papilla (SCAPs)/human umbilical vein endothelial cells (HUVECs)	Mice	Calvaria defect	Bone	No	• Randomly seeded cells were poorly organized within the region of interest in the defect at 2 months • For the 'ring' condition, the geometry of vascularized area was consistent with the initial printed pattern. For the 'disc' and 'crossed circle' patterns, the initial pattern was distorted	[20]

							• Vascularization rate and bone regeneration rate significantly increased in the 'disc' or 'crossed circle' patterns of HUVECs • HUVECs are not the only cells involved in the network formation and host murine cells seem to have a role in this process	
Droplet-based bioprinting	Fibrin/collagen	Amniotic fluid-derived stem cells (AFSCs)/MSCs/HUVECs	Mice	Skin wound on dorsa	Skin	No	• AFS cell- and MSC-driven wound closure and re-epithelialization were significantly greater than wounds treated by fibrin-collagen only • Microvessel density and capillary diameters in AFS cell-treated wounds increased compared with MSC-treated wounds, whereas the skin treated only with gel showed the least microvessels • AFS cells secreted growth factors at higher concentrations than MSCs, resulting in increased wound closure rates and angiogenesis	[21]
	HA/heparin-conjugated hyaluronic acid (HA-HP)	AFSCs	Mice	Skin Wound on dorsa	Skin	No	• Deposition of the HA-HP + AFS cells accelerated closure of wounds faster than HA-only hydrogels and treatment-free controls and induced increased vascularization • The increased levels of elastin, GAGs, and proteoglycans, and decreased relative expression of collagen type I, suggested the regenerated skin using HA-HP + AFS cells is more pliable and elastic compared with control groups	[22]
	Fibrin/collagen	Fibroblasts/keratinocytes	Mice/pig	Skin defect on dorsa	Skin	Yes	Murine model: • Printed skin cells closed the wound by 3 weeks postsurgery compared with 5 weeks for untreated and matrix-only groups • Epithelialization of bioprinted wounds was observed, forming an immature epidermal barrier Porcine model: • Bioprinting of autologous cells resulted in ~3-week acceleration in wound closure compared with other treatments (i.e., untreated, matrix, and allogenic cell-treated) • Bioprinting autologous cells resulted in ~50% reduction in wound contraction compared with other treatments • Bioprinting autologous cells resulted in a 4–5-week acceleration in wound re-epithelialization compared with other treatments • Collagen fibers present in the autologous cell-treated wounds appeared larger and more organized than other groups Comparison with cell spraying: • Bioprinted wounds showed accelerated formation of epidermis and more mature dermis tissue • Staining of collagen fibers were more prominent in the bioprinted wounds at week 4 compared with wounds treated with the sprayed cells	[23]

Box 1. Comparison of Bioprinting Modalities Regarding Intraoperative Bioprinting

Extrusion-based bioprinting: Bioprinting of a bioink solution using an extrusion mechanism, resulting in deposition of cells laden in the bioink in the form of cylindrical filaments of customized 3D structures.

Strengths

- Compatible with a large variety of bioink materials
- High mechanical strength with structural integrity, suitable for reconstruction of hard tissues
- Appropriate modality for intraoperative bioprinting due to its similarity to manual injection, which has been clinically applied
- Enables bioprinting of scaffold-free bioinks such as tissue spheroids
- Commercially available with moderate cost.

Limitations

- Print tip might interfere with defect periphery due to its contact-based mechanism
- Cell damage due to high shear stress
- Low resolution preventing bioprinting of thin layers of tissues, such as the skin, with a stratified arrangement of multiple layers.

Droplet-based bioprinting: Bioprinting of a bioink solution with a droplet deposition mechanism medicated by electrical, thermal, or acoustic energy.

Strengths

- Capable of bioprinting multiple types of cell
- High resolution with high-throughput capability
- Affordable, versatile, and commercially available
- Non-contact bioprinting (the nozzle does not interfere with the defect).

Limitations

- Compatibility with low viscosity bioinks (in the range of 3.5–12 mPa s)
- Poor structural and mechanical integrity of bioprinted constructs
- Small orifice results in higher risk of nozzle clogging, which may increase the duration of surgery and associated risks.

Laser-based bioprinting: Bioprinting of bioink with the laser energy as the major deposition mechanism, allowing high-precision patterning of biologics or fabrication of tissue constructs.

Strengths

- Compatibility with viscosities in the range of 1–300 mPa s
- High resolution with the capability of single cell printing
- Nozzle-free bioprinting resulting in negligible cell damage
- Non-contact bioprinting.

Limitations

- Poor structural and mechanical integrity of bioprinted constructs
- Complicated to operate and difficult to miniaturize for a surgical setting
- Labor intensive and time consuming leading to increased surgery duration
- Higher cost and no commercial availability.

Compatibility of Bioinks to Surgical Settings

Since the deposition is performed directly into a defect in physiological conditions, an ideal bioink should not only meet the general bioink requirements [30] but also possess some features specific to IOB. In particular, such bioinks are expected to be: (i) compatible with and intraoperatively bioprintable by the targeted modality to shorten the surgery duration, (ii) rapidly crosslinkable *in situ* to retain the integrity and resolution of bioprinted constructs in physiological temperature and moist environment, and (iii) commercially available and affordable to minimize the surgery cost. Therefore, most of the popular biomaterials (e.g., polycaprolactone, polylactic acid, etc.), which rely on volatile organic solvent and high melting temperature, become inappropriate. Among hydrogels, although collagen transitions to gel state at 37°C,

slow gelation hinders its use in IOB; however, nano-hydroxyapatite-reinforced collagen has shown promising results in bone regeneration [19]. Fibrinogen is also popular due to its rapid crosslinking when it is interchangeably bioprinted with thrombin into a defect. Photo-crosslinkable bioinks, such as gelatin methacrylamide (GelMA), hyaluronic acid methacrylate (HAMA), and poly(ethylene glycol), have been commonly used in bioprinting [30]. Since exposing UV light directly to a live subject can be dangerous [31], one practical example is to expose UV light towards the side of a transparent nozzle during bioink extrusion [32]. Recently, visible light photo-initiating systems have become popular, which avoided the use of UV light favoring cell viability in photo-crosslinkable bioinks [33–35]. In addition to existing bioinks, more alternatives are expected to be developed to broaden the options for IOB. Newer materials may facilitate retention of the bioprinted constructs at the desired site without the utilization of a support dressing/scaffold, such as a **vacuum assisted closure** device. The manufacture of rapidly integrable bioinks would expand the feasibility of bioprinting into enclosed cavities (e.g., abdomen, thorax), which are not conducive to graft immobilization techniques when compared with more superficial sites, such as the skin.

Automation of IOB Processes

Imaging of defects during IOB should be performed in a minute timescale immediately after a surgical excision since the prolonged exposure of the defect can increase surgical complications [36]. To match this requirement, scanners based on **3D photogrammetry** provide a supreme solution to obtain raw data of the defect topography during IOB. Several models of portable photogrammetric scanners (e.g., Artec Space Spider and CREAFORM HandySCAN) are able to reach a resolution up to ~30 μm, with an extremely short scan time (<5 min), and the portability offers the possibility to get the defect scanned easily. To create a model of tissue constructs, image processing is necessary, including image preprocessing, segmentation, feature extraction, and data mining [37]. During IOB, all these processes should be completed in a few minutes after obtaining the scanned data. Hence, segmentation software is vital for convenient extraction of the region of interest from 3D images [38,39]. Since defects have unique textural features case by case, improper postprocessing of images might generate pointless 3D models, which needs to be carefully evaluated. **Artificial intelligence** and robotics can be incorporated to reduce the process time and variation caused by operators. So far, researchers have strived to optimize **path planning** targeted at IOB [40,41], including printing on a free-moving hand anatomy using motion tracking [42,43]. In the future, machine learning can be used to automatically generate optimal bioprinting strategies [44–47]. The integration of 3D scanner with a fixed relative coordinate to the robotic arm is also required to eliminate the need of prebioprinting calibration to minimize total processing time.

Due to shape distortions caused by the sol–gel transition of hydrogels and movement of the live subject during surgery (such as breathing), automation of IOB with tight control on high resolution is also challenging. This issue might be addressed by real-time monitoring technologies to observe construct deformation and provide feedback signal for subsequent deposition [43]. Commercially available bioprinters with three-axis coordinates are usually not sufficient for IOB for irregular-shaped defects, which necessitates the use of bioprinters with higher degrees of freedom (DOF), where robotic arms (such as surgical robots) can be the ideal solution. Although BioAssemblyBot with a six-axis robotic arm from Advanced Solutions Inc. (Figure 2A, Key Figure) is currently the only commercially available bioprinter with higher DOF capability, there are many companies specialized in robotic surgeries (e.g., Intuitive Surgical, MAKO Surgical Corp, etc.) with the potential of integrating bioprinting capabilities into surgical robots in the future.
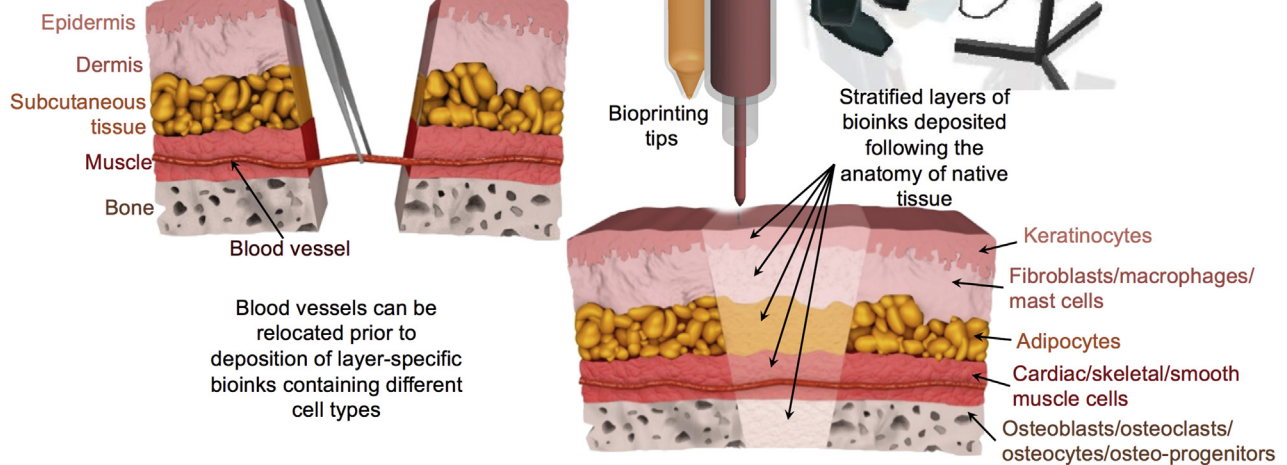
Key Figure

Intraoperative Bioprinting (IOB) in a Surgical Setting and its Application for Repair of Composite Tissues

(A)

(B)
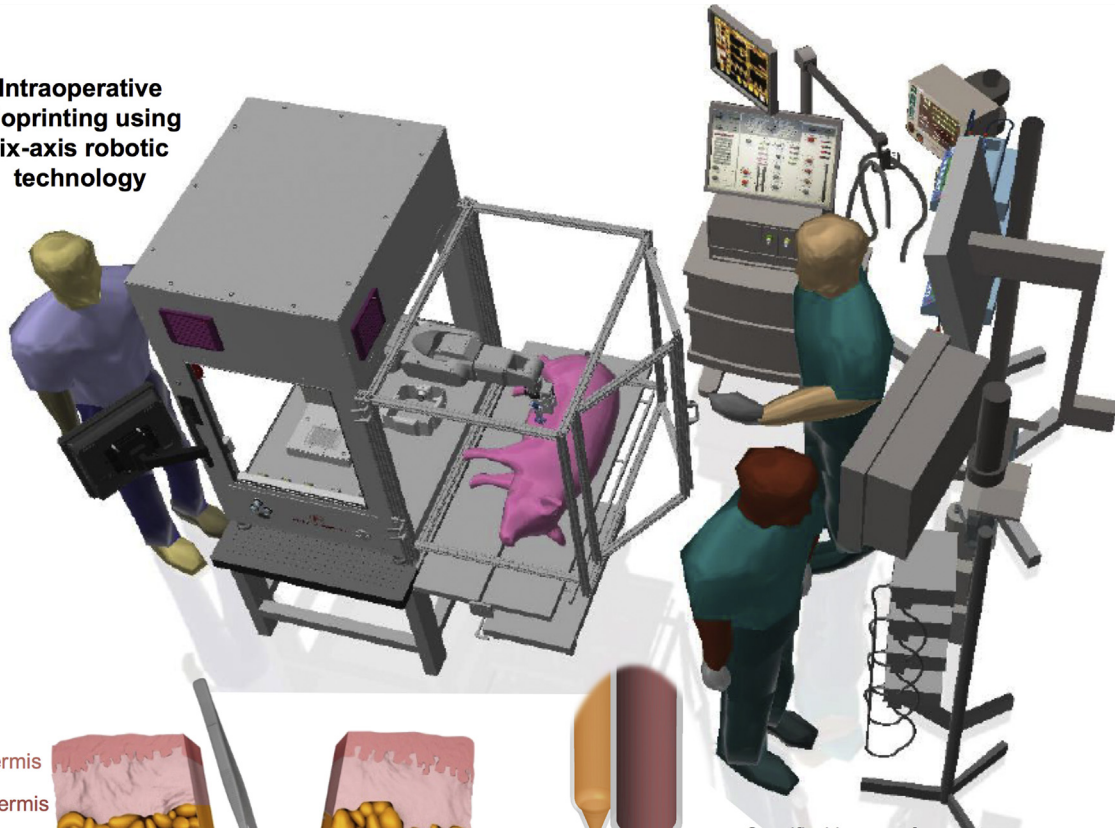
Figure 2. Conceptual schematic diagram of (A) IOB in a surgical setting, and (B) the regeneration of composite tissues in a stratified manner. In the case where blood vessels are retained in host tissue; they can be relocated (left) prior to the deposition of layer-specified bioinks containing different cell types (right).

Vascularization in Intraoperatively Bioprinted Tissues

Vascularization is crucial for maturation of bioprinted constructs, especially in segmental defects. Although bioprinted constructs with an embedded microvasculature have been described [4], it is still not feasible to directly anastomose them to the recipient vasculature. Therefore, engineered

grafts still rely on **inosculation** from the recipient bed for perfusion. In order to drive microvascularization within intraoperatively bioprinted tissues, there are multiple ways, such as bioprinting of endothelial progenitor cells, hypoxia-inducible factor (HIF), or vascular endothelial growth factor (VEGF). Since it usually takes more than 10 days for angiogenesis to take place [48], temporary strategies can be followed to extend the oxygen supply prior to inosculative angiogenesis. For example, oxygen-filled microparticles or **oxygen-generating biomaterials (OGBs)** can be bioprinted within the bioink, which are expected to feed the cells until capillaries can actively transfer the blood [49,50]. Oxygenation rate of OGBs (e.g., peroxides) is the key parameter for viable outcome of organs, which can be controlled by factors such as pH, temperature, and solubility of peroxides [51]. Involvement of porous internal structures is an advantage of bioprinting and another option to facilitate infiltration of blood from host tissue, which offers the possibility for IOB of porous constructs. Introduction of macropores in IOB can be realized by bioprinting filaments in crosshatched patterns similar to what has been achieved in conventional *in vitro* bioprinting [52].

However, the ultimate goal is to create a bioprinted construct that includes an embedded microvasculature with a continuous anastomosable artery and vein. With the development of super-microsurgical techniques, it is now feasible to perform a direct anastomosis on vessels with an internal diameter <150 µm [53]. This advancement may provide a mechanism for the rapid establishment of graft blood flow without requisite exposure to main vascular tree. This is significant as the vascular pedicle of the graft can be kept quite short, simplifying the bioprinting process. To facilitate graft anastomosis and vascularization, the implanting surgeon can also utilize a variety of autologous conduits, including the creation of **arteriovenous loops**.

IOB of Composite Tissues and Their Translational Potential

Currently, seamless reconstruction of tissues with multiple components such as **craniomaxillofacial (CMF) defects** (skin, bone, and muscle), osteochondral defects (cartilage and bone), and musculoskeletal defects (bone, muscle, tendon, and skin) possesses several limitations. Different tissue types exhibit local variations in terms of physiological, anatomical, and histological aspects and precise layer-by-layer stacking of multiple tissue compartments is not trivial. Such compartmentalization necessitates the precision and effective use of **stem cells** and differentiation factors, since differentiating stem cells into multiple lineages is crucial in order to recapitulate the native tissue anatomy. Taking a major musculoskeletal defect as an example, it comprises layers of bone, blood vessel, muscle, subcutaneous fat, and skin tissues from inside out, which contains more than 10 cell types (Figure 2B) and has traditionally required an autologous composite fibula free-flap for correction.

IOB of composite tissue should allow rapid acquirement of the defect information with minimum manual interventions, enabling personalized reconstructions in an anatomically accurate and cosmetically appealing manner immediately after characterization of the defect. Although bioprinted constructs are usually designed based on the compartmentalization of native tissues, the maturation of bioprinted tissues may alter its anatomy and phenotype, leading to differences from the native tissue. Since composite tissues are usually thick, vascularization is particularly vital to enhance the proper tissue regeneration, such as bone formation in CMF defects, as necrotic scar tissue would take over if vascularization is not sufficient [54]. Currently, free-flap surgery is the standard of care for the repair of composite segmental defects. A free-flap is an autologous tissue transplant where expendable donor tissue along with its feeding vascular pedicle (artery and vein) is transferred to a remote recipient site [55]. This approach

has revolutionized the treatment of large traumatic and oncologic defects. However, free-flap surgery can be restricted by limited donor site supply and donor site morbidity. Additionally, although the goal is to replace like tissue with like tissue, this is often impossible. For example, a fibula free-flap can be used for intra-oral bone and soft tissue replacement in mandible reconstruction but the amount of bone stock is reduced, sensation is lost, and skin is not equivalent to native mucosa. Therefore, it may be better to combine the principles of reconstructive microsurgery and IOB. The surgeon could configure the recipient vasculature to perfuse an adjacent bioprinted construct either via direct anastomosis or angiogenic induction. This would eliminate the concerns of donor supply and morbidity while providing an exact match of the desired replacement tissue. In the future, it is anticipated that bioprinted or tissue engineered grafts will be available instead of harvesting autologous flaps.

Another concern about IOB of composite tissues is the integration of bioprinted tissues to native tissue, especially for avascular tissues, such as cartilage due to its low metabolism and anti-adhesive ECM [56]. Vertical integration of cartilage to underlying bone occurs due to the innate repair capability of bone [56]. In order to enhance the healing capability, intraoperatively bioprinted composite tissues with a histologically similar osteochondral interface will be a promising solution [2], which can be integrated into full-thickness bone-cartilage defects. However, lateral integration of the bioprinted cartilage to the adjacent cartilage is a major roadblock to achieve permanent cartilage replacement [56]. Strategies to enhance lateral integration may include bioink functionalization to enable direct bonding to the adjacent cartilage [57].

So far, animal models for IOB are almost non-load-bearing, such as skin and calvarial defects (Table 1). IOB for repairing load-bearing defect models, such as segmental bone defects in long bones and joint defects, will gain more attention in the future. Mechanical stiffness of intraoperatively bioprinted constructs depends on their inherent properties determined by the bioink, which can be resolved by developing new materials such as tough hydrogels [58] or integrating mechanically strong thermoplastics. In this regard, bioprinting of soft bioinks can be coupled with thermoplastics [e.g., polycaprolactone (PCL) or poly(lactic-co-glycolic acid) (PLGA)], where one can envision a technology that can facilitate rapid cooling of deposited thermoplastics into a defect in a safe manner (such as laser-based cooling systems [59]). In addition, postoperative care and rehabilitation are still necessary (as in conventional surgeries) until intraoperatively bioprinted tissue restores sufficient mechanical strength.

Concluding Remarks

IOB has already shown promise for regeneration of tissues, including cartilage, skin, and bone, in animals. However, regeneration of composite tissues, which are composed of hard and soft tissues, and the interface layers in between, have hardly been explored, requiring the development of new bioinks with rapid and stable crosslinking and the integration of advanced bioprinting modalities.

In addition, seeking a practical way to facilitate and enhance vascularization is vital for long-term functionality of intraoperatively bioprinted tissues. It is especially appealing to combine IOB with vascular anastomosis to repair composite segmental defects, which is currently treated by free-flap surgeries. In the long term, automation of IOB not only relies on the integration of sequential processes, but also requires a large amount of clinical cases to optimize the bioprinting strategies (see Outstanding Questions). Beyond scientific considerations, clinical studies will be impeded by ethical and regulatory issues and sharing of patient-specific information for database

Outstanding Questions

What are some efficient methods to facilitate vascularization and microcapillarization in an intraoperatively bioprinted construct?

How can bioprinting modalities be integrated and miniaturized for intraoperative bioprinting of composite tissues?

How can image processing, robotics and artificial intelligence contribute to the automation of intraoperative bioprinting technologies?

How can current surgical methods be combined with intraoperative bioprinting to promote its clinical translation, especially in the context of repairing of composite tissues?

Beyond the scientific barriers, how can ethical, regulatory, and intellectual property issues be addressed for successful clinical translation of intraoperative bioprinting technology?

development will be related to the protection of private information and intellectual properties. Although significant efforts will be required to address all these issues, we do not doubt that IOB will be a game changer in regenerative surgical care.

Acknowledgments

References

1. Vijayavenkataraman, S. et al. (2016) 3D bioprinting of skin: a state-of-the-art review on modelling, materials, and processes. Biofabrication 8, 032001
2. Wu, Y. et al. (2018) Three-dimensional bioprinting of articular cartilage: a systematic review. Cartilage. Published online October 29, 2018. https://doi.org/10.1177/1947603518809410
3. Ashammakhi, N. et al. (2019) Advancing frontiers in bone bioprinting. Adv. Healthc. Mater. 8, 1801048
4. Datta, P. et al. (2017) Bioprinting for vascular and vascularized tissue biofabrication. Acta Biomater. 51, 1–20
5. Peng, W. et al. (2017) 3D bioprinting for drug discovery and development in pharmaceutics. Acta Biomater. 57, 26–46
6. Vijayavenkataraman, S. et al. (2018) 3D bioprinting of tissues and organs for regenerative medicine. Adv. Drug Deliv. Rev. 132, 296–332
7. Hong, N. et al. (2018) 3D bioprinting and its in vivo applications. J Biomed Mater Res B Appl Biomater 106, 444–459
8. Ashammakhi, N. et al. (2019) In situ three-dimensional printing for reparative and regenerative therapy. Biomed. Microdevices 21, 42
9. Singh, S. et al. (2019) In situ bioprinting–bioprinting from benchside to bedside? Acta Biomater. 101, 14–24
10. Wang, M. et al. (2015) The trend towards in vivo bioprinting. Int. J. Bioprinting 1, 15–26
11. Ozbolat, I.T. (2015) Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol. 33, 395–400
12. Qu, F. et al. (2019) Cell migration: implications for repair and regeneration in joint disease. Nat. Rev. Rheumatol. 15, 167–179
13. Yan, W.-C. et al. (2018) 3D bioprinting of skin tissue: from pre-processing to final product evaluation. Adv. Drug Deliv. Rev. 132, 270–295
14. Campbell, P.G. and Weiss, L.E. (2007) Tissue engineering with the aid of inkjet printers. Expert. Opin. Biol. Ther. 7, 1123–1127
15. O'Connell, C.D. et al. (2016) Development of the Biopen: a handheld device for surgical printing of adipose stem cells at a chondral wound site. Biofabrication 8, 015019
16. Di Bella, C. et al. (2018) In situ handheld three-dimensional bioprinting for cartilage regeneration. J. Tissue Eng. Regen. Med. 12, 611–621
17. Hakimi, N. et al. (2018) Handheld skin printer: in situ formation of planar biomaterials and tissues. Lab Chip 18, 1440–1451
18. Keriquel, V. et al. (2010) In vivo bioprinting for computer-and robotic-assisted medical intervention: preliminary study in mice. Biofabrication 2, 014101
19. Keriquel, V. et al. (2017) In situ printing of mesenchymal stromal cells, by laser-assisted bioprinting, for in vivo bone regeneration applications. Sci. Rep. 7, 1778
20. Kérourédan, O. et al. (2019) In situ prevascularization designed by laser-assisted bioprinting: effect on bone regeneration. Biofabrication 11, 045002
21. Skardal, A. et al. (2012) Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl. Med. 1, 792–802
22. Skardal, A. et al. (2017) A tunable hydrogel system for long-term release of cell-secreted cytokines and bioprinted in situ wound cell delivery. J. Biomed. Mater. Res. B Appl. Biomater. 105, 1986–2000
23. Albanna, M. et al. (2019) In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds. Sci. Rep. 9, 1856
24. Ozbolat, I.T. and Hospodiuk, M. (2016) Current advances and future perspectives in extrusion-based bioprinting. Biomaterials 76, 321–343
25. Gudapati, H. et al. (2016) A comprehensive review on droplet-based bioprinting: past, present and future. Biomaterials 102, 20–42
26. Mandrycky, C. et al. (2016) 3D bioprinting for engineering complex tissues. Biotechnol. Adv. 34, 422–434
27. Clark, J.M. and Cook, T.A. (2002) Immediate reconstruction of extruded alloplastic nasal implants with irradiated homograft costal cartilage. Laryngoscope 112, 968–974
28. Besalti, O. et al. (2005) The role of extruded disk material in thoracolumbar intervertebral disk disease: a retrospective study in 40 dogs. The Canadian Veterinary Journal 46, 814
29. Hussain, A. et al. (2014) The use of robotics in surgery: a review. Int. J. Clin. Pract. 68, 1376–1382
30. Jungst, T. et al. (2015) Strategies and molecular design criteria for 3D printable hydrogels. Chem. Rev. 116, 1496–1539
31. Duchi, S. et al. (2017) Handheld co-axial bioprinting: application to in situ surgical cartilage repair. Sci. Rep. 7, 5837
32. Ouyang, L. et al. (2017) A generalizable strategy for the 3D bioprinting of hydrogels from nonviscous photo-crosslinkable inks. Adv. Mater. 29, 1604983
33. Wang, Z. et al. (2015) A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication 7, 045009
34. Lim, K.S. et al. (2016) New visible-light photoinitiating system for improved print fidelity in gelatin-based bioinks. ACS Biomaterials Science & Engineering 2, 1752–1762
35. Wang, Z. et al. (2018) Visible light photoinitiation of cell-adhesive gelatin methacryloyl hydrogels for stereolithography 3D bioprinting. ACS Appl. Mater. Interfaces 10, 26859–26869
36. Cheng, H. et al. (2017) Prolonged operative duration increases risk of surgical site infections: a systematic review. Surg. Infect. 18, 722–735
37. Sun, W. et al. (2004) Computer-aided tissue engineering: application to biomimetic modelling and design of tissue scaffolds. Biotechnol. Appl. Biochem. 39, 49–58
38. Ozbolat, I. and Gudapati, H. (2016) A review on design for bioprinting. Bioprinting 1–14
39. Xu, S. et al. (2013) In Imaging in Cellular and Tissue Engineering (Yu, H. and Rahim, N.A.A., eds), pp. 223, CRC Press
40. Ding, H. and Chang, R.C. (2018) Simulating image-guided in situ bioprinting of a skin graft onto a phantom burn wound bed. Addit. Manuf. 22, 708–719
41. Li, X. et al. (2017) Development of a robotic arm based hydrogel additive manufacturing system for in-situ printing. Appl. Sci. 7, 73
42. O'Neill, J.J. et al. (2017) 3D bioprinting directly onto moving human anatomy. In 2017 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS), pp. 934–940, IEEE
43. Zhu, Z. et al. (2018) 3D printed functional and biological materials on moving freeform surfaces. Adv. Mater. 30, 1707495
44. Shi, J. et al. (2019) Multi-objective optimization design through machine learning for drop-on-demand bioprinting. Engineering 5, 586–593

45. Gu, G.X. *et al.* (2018) Bioinspired hierarchical composite design using machine learning: simulation, additive manufacturing, and experiment. *Mater. Horiz.* 5, 939–945

46. Menon, A. *et al.* (2019) Optimization of silicone 3D printing with hierarchical machine learning. *3D Print. Addit. Manuf.* 6, 181–189

47. Dernowsek, J. *et al.* (2017) BioCAE: a new strategy of complex biological systems for biofabrication of tissues and organs. *J Tissue Sci Eng* 8

48. Staton, C.A. *et al.* (2004) Current methods for assaying angiogenesis in vitro and in vivo. *Int. J. Exp. Pathol.* 85, 233–248

49. Kheir, J.N. *et al.* (2012) Oxygen gas–filled microparticles provide intravenous oxygen delivery. *Sci. Transl. Med.* 4, 140ra88

50. Pedraza, E. *et al.* (2012) Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials. *Proc. Natl. Acad. Sci. U. S. A.* 109, 4245–4250

51. Ward, C.L. *et al.* (2013) Oxygen generating biomaterials preserve skeletal muscle homeostasis under hypoxic and ischemic conditions. *PLoS One* 8, e72485

52. Lee, J.M. and Yeong, W.Y. (2016) Design and printing strategies in 3D bioprinting of cell-hydrogels: a review. *Adv. Healthc. Mater.* 5, 2856–2865

53. Walløe, L. (2016) Arterio-venous anastomoses in the human skin and their role in temperature control. *Temperature* 3, 92–103

54. Hankenson, K.D. *et al.* (2011) Angiogenesis in bone regeneration. *Injury* 42, 556–561

55. Shandalov, Y. *et al.* (2014) An engineered muscle flap for reconstruction of large soft tissue defects. *Proc. Natl. Acad. Sci. U. S. A.* 111, 6010–6015

56. Huey, D.J. *et al.* (2012) Unlike bone, cartilage regeneration remains elusive. *Science* 338, 917–921

57. Wang, D.-A. *et al.* (2007) Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration. *Nat. Mater.* 6, 385

58. Hong, S. *et al.* (2015) 3D printing of highly stretchable and tough hydrogels into complex, cellularized structures. *Adv. Mater.* 27, 4035–4040

59. Nemova, G. and Kashyap, R. (2010) Laser cooling of solids. *Rep. Prog. Phys.* 73, 086501