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Opinion

Advances in Bioprinting Technologies for Craniofacial Reconstruction

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Recent developments in craniofacial reconstruction have shown important advances in both the materials and methods used. While autogenous tissue is still considered to be the gold standard for these reconstructions, the harvesting procedure remains tedious and in many cases causes significant donor site morbidity. These limitations have subsequently led to the development of less invasive techniques such as 3D bioprinting that could offer possibilities to manufacture patient-tailored bioactive tissue constructs for craniofacial reconstruction. Here, we discuss the current technological and (pre)clinical advances of 3D bioprinting for use in craniofacial reconstruction and highlight the challenges that need to be addressed in the coming years.

Trends

Conventional craniofacial reconstruction methods still fail to mimic the complex 3D anatomy and biology of native tissues.

Bioprinting can aid in the production of cell-incorporated and patient-tailored bioactive scaffolds that can be used for craniofacial reconstruction.

Currently, there are three different bioprinting technologies (laser-assisted, inkjet, and extrusion-based) used to print craniofacial tissues.

Bioprinting technologies will soon be used to enhance the self-repair capabilities of tissues in the craniofacial area.

Craniofacial Reconstruction Methods

The craniofacial area comprises several different tissue types that include bone, cartilage, muscles, ligaments, and skin, as well as essential supporting structures such as blood vessels and nerves (Box 1 and Figure 1). Traumatic or congenital defects of one or more of these tissues can lead to severe functional impairment and aesthetic problems. Throughout the past decades, the management and treatment of such craniofacial defects has been continuously evolving. A wide range of materials are currently used in reconstructive surgery that range from **autografts** (see Glossary) [1] and **allografts** [2] to metals (e.g., titanium) [3], and polymers (e.g., porous polyethylene) [4]. Despite the good clinical results often attained, these materials have significant disadvantages (Box 2) and often fail to mimic the complex 3D anatomy and biology of native tissues. Such limitations have subsequently led to the development of novel technologies such as **bioprinting**.

Since the introduction of the term bioprinting in 2004 [9], bioprinting technology has been used for the *in vitro* fabrication of various tissue constructs that include bone [10–12], cartilage [13–15], skin [16–18], muscle [19,20], blood vessels [21], and nerves [22]. Translation towards *in vivo* and ultimately clinical application of bioprinted cell-incorporated **bioactive scaffolds** requires three fundamental steps: (i) imaging and design, (ii) choice of adequate biomaterials and cells, and (iii) bioprinting of the tissue construct and implantation into the patient [23].

In this opinion paper, we discuss the current technological developments and possible clinical applications of bioprinting for reconstruction of the craniofacial area.

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Box 1. Craniofacial Anatomy and Physiology

Anatomical Structures of the Face and Skull

The development of the human craniofacial area is critical for the function of the brain, airway, vision, speech, and mastication. In addition, the physiological function of the facial area largely depends on the morphology of the skull, indicating the complex interplay between the two areas [5]. The craniofacial skeleton forms the hard tissue of the face and skull and provides essential structural support and projection for overlying soft tissues, as well as attachments for muscles and guidance and protection of nerves, blood vessels, and sensory organs such as the eyes. In addition to the craniofacial skeleton, several soft tissue compartments exist in the facial area that include fat compartments, fascia, **mimetic and masticatory muscles**, and retaining ligaments (see Figure 1 in main text).

Relevant Cell/Tissue Types

Relevant craniofacial tissue types include bone, cartilage, muscle, and ligaments and their accompanying structures such as blood vessels and nerves. Subsequently, there are a number of different cell types that have to be considered. Bone comprises several cell types that include the bone-forming osteoclasts, the bone-resorbing osteoblasts, and the bone-embedded osteocytes, whereas facial cartilage (i.e., ear and nose) is mainly composed of one cell type, the chondrocyte.

Injuries and Diseases that Affect the Craniofacial Area

Numerous conditions including congenital disease, cancer, and trauma can lead to the physical loss of essential craniofacial tissues. Loss of bone and disruption of bone metabolism, in particular, cause changes in the craniofacial morphology that directly affect physiological function. For example, cancer of the orbital floor may cause problems with eyesight, whereas trauma to the side of the face may lead to damage to the facial nerve and subsequent loss of facial expression due to the denervation of the mimetic muscles. A common congenital disease that affects the craniofacial area is **cleft palate**. Common traumatic injuries to the face include lacerations, blunt force trauma, and burns. Common cancers that affect the craniofacial area include skin cancer, mouth cancer, and esophageal cancer.

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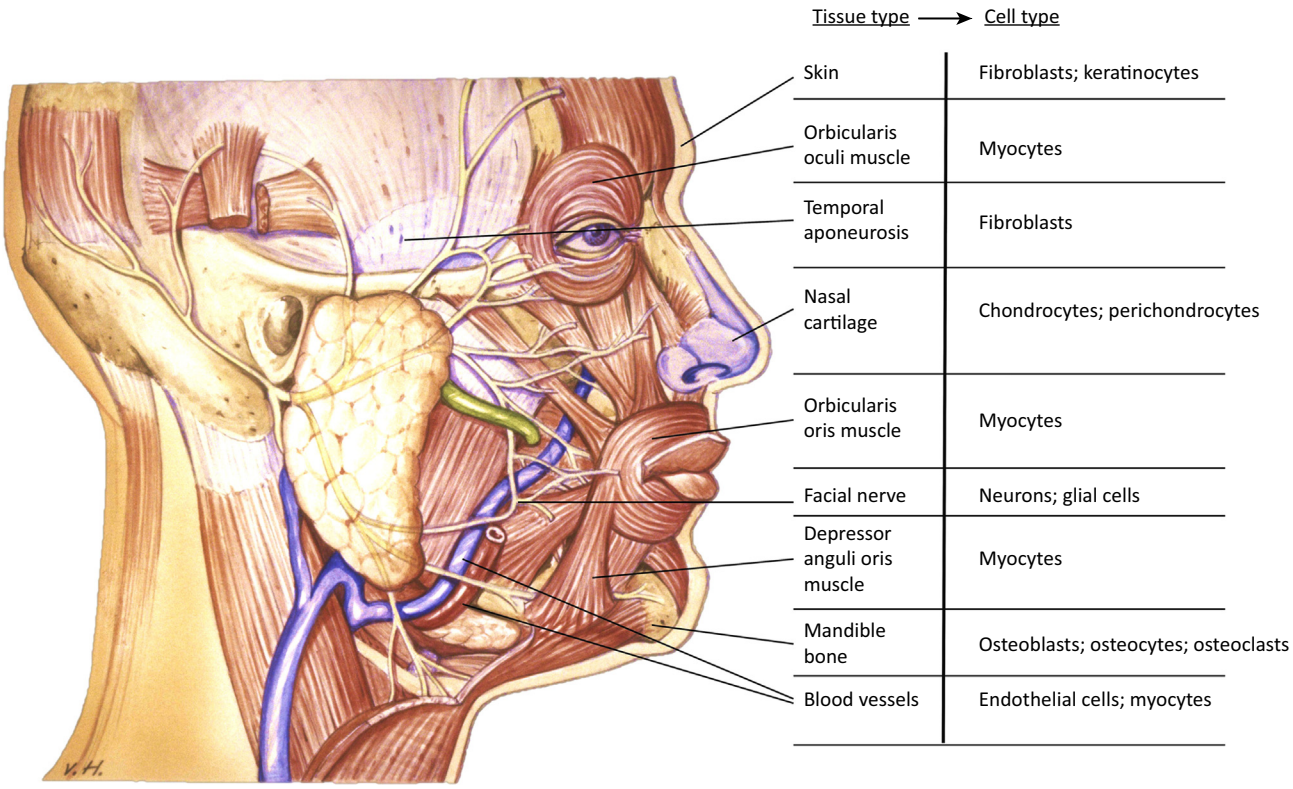

Figure 1. Craniofacial Anatomy. Figure reprinted with permission of the Department of Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA).

Box 2. Advantages and Disadvantages of Current Craniofacial Reconstruction Materials

Autografts and Allografts

Autografts are considered to be the current 'gold standard' material in craniofacial surgery. These grafts can be used for reconstructing bone, cartilage, and skin defects following trauma, tumor resection, or congenital anomalies. A major advantage of autogenous tissue is the lack of immune reaction. However, the harvesting procedure is a tedious process and may cause significant donor site morbidity [1,6].

Allografts are often harvested from the same species and are usually of cadaveric origin, but they may also be harvested from a living donor. The advantages of allogenic grafts are their abundant supply and lack of donor site morbidity. Allografts, however, precipitate immunogenic reactions, and concerns remain regarding the transmission of malignancies and viral diseases [7].

Cement Pastes

The most commonly used cement paste in craniofacial reconstruction is calcium phosphate. Calcium phosphate closely resembles bone and has high biocompatibility. For bone reconstruction, cement pastes are relatively easy to handle and mold. Some minor complications that include the risk of infection and seroma formation have been reported [7].

Metals

At present, titanium-based constructs are the most commonly used metal implants in craniofacial surgery. Titanium is biocompatible and has an excellent strength-to-weight ratio [6]. However, metal implants have low wear resistance, which is problematic in articulating surfaces.

Prefabricated Polymers

The most common polymers used in craniofacial surgery are poly(methyl) methacrylate (PMMA), polyether ether ketone (PEEK), and porous polyethylene (PPE).

PMMA

Polymerization of methyl methacrylate (MMA) yields a durable material that can be contoured and fixed rigidly into a cranioplasty site. Advantages include low cost and a lack of biodegradation. Disadvantages include high bacterial adhesion and thermal tissue injury due to an exothermal mixing reaction [7].

PEEK

PEEK is an organic thermoplastic polymer with high biocompatibility that closely resembles native bone. However, PEEK has no bioactive potential, and therefore there is a significant risk of postoperative infection [8].

PPE

PPE has become versatile for the reconstruction of the craniofacial area with applications in ear reconstruction, cranial augmentation, nasal, and malar reconstruction. A major advantage of polyethylene implants is that their porous nature allows native tissue ingrowth, which reduces the chance of infection. However, a major disadvantage is the increased infection rate, exposure, and extrusion of the implant in less vascularized areas [7]. Furthermore, unsatisfactory aesthetic appearance has also been reported in up to 10% of patients [4].

Bioprinting Technologies for Craniofacial Application

There are several cell-compatible bioprinting technologies, including laser-assisted bioprinting (LAB), inkjet printing, and microextrusion (Figure 2) that could be used for craniofacial tissue printing in the near future. These promising technologies offer unique opportunities for craniofacial tissue printing and are currently being tested primarily *in vitro*, and in a few cases *in vivo*.

Laser-Assisted Bioprinting

Laser-assisted bioprinting technology (Figure 2A) is based on laser-induced forward transfer (LIFT) and enables the precise printing of individual cells in small constructs [24]. Highly organized craniofacial tissues such as muscle may benefit from LAB technology by generating individual tissue units that can later be assembled into functional tissues using self-assembly or rational design [23]. In addition, tissues that exhibit single cell layers such as the **endothelium** of

Glossary

Allograft: a graft obtained from a donor of the same species.
Aponeuroses: tough layers of dense fibrous tissue that attaches muscles to other muscles or bone.
Autograft: a tissue graft obtained from the same patient.
Basement membrane: a thin fibrous tissue that separates a layer of cells from the underlying tissue (found, for example, in the skin and blood vessels).
Bioactive scaffold: a scaffold or lattice structure with integrated biological function that provides an information-rich support material for tissue engineering.
Bioink: a cartridge filled with a liquid cell suspension used for bioprinting.
Bioprinting: also referred to as 3D bioprinting, encompasses a range of different printing technologies that deposit cells and/or biological materials that are used to manufacture 3D tissue structures.
Bioreactor: an engineered device that mimics a biologically active environment.
Blowout fracture: a traumatic fracture of the floor of the eye socket typically resulting from the impact of a blunt object (e.g., a ball).
Cleft palate: a persistent opening in the roof (palate) of the mouth due to failure of fusion during embryological development.
Crosslinking: a chemical process that induces a change in the physical properties of a polymer (e.g., from soft to hard).
Endothelium: a thin layer of cells (endothelial cells) that line the inner surface of blood and lymphatic vessels.
Epidermis: the outermost layers of cells in the skin.
Hydrogel: a water-based gel suspended with natural or synthetic-based polymers.
Mimetic and masticatory muscles: facial muscles that control facial expression and chewing, respectively.
Scaffold: a 3D structure that supports tissue formation.
Tissue engineering: the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological function.

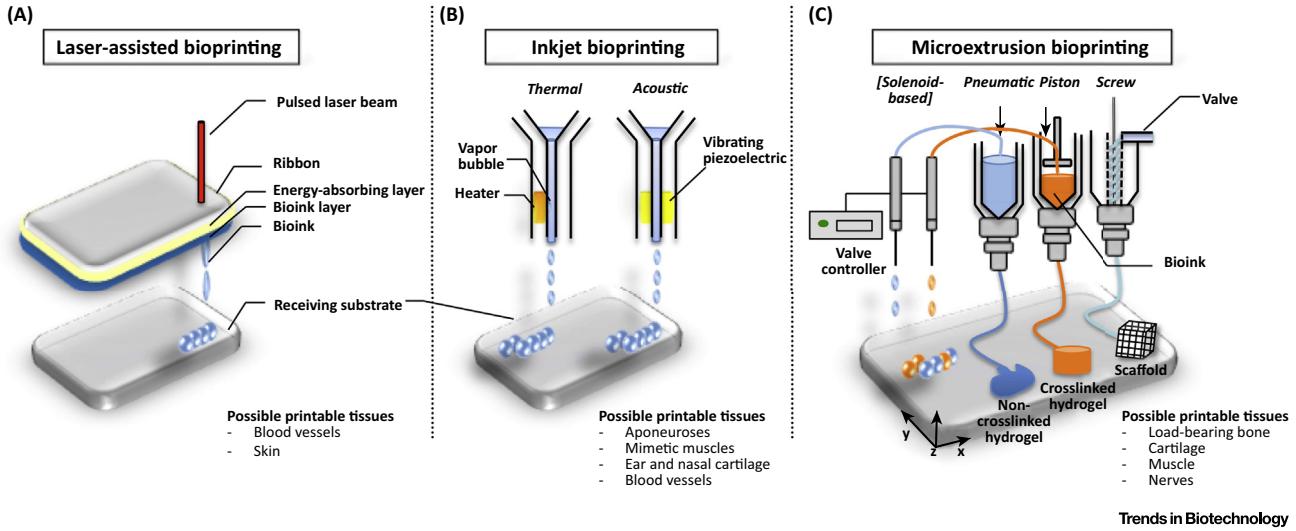

Figure 2. Bioprinting Technologies. (A) Laser-assisted bioprinters use pulsed laser beams focused on an energy-absorbing substrate to generate pressure that propels cell-containing material to deposit onto a receiving substrate. (B) Thermal inkjet bioprinters electrically heat the printhead and produce air pressure pulses to force cell-containing droplets from the nozzle, whereas acoustic inkjet printers use pulses formed by piezoelectric or ultrasound pressure. (C) Microextrusion bioprinters use pneumatic or mechanical (piston or screw) systems to extrude a continuous strand of material and/or cells. Solenoid-based systems rely on pressure and a valve controller to deposit bioink on a receiving substrate. Craniofacial tissues that can possibly be printed in the near future are also specified.

blood vessels, the oral mucosa of the mouth, and the **basement membrane** of the **epidermis** of the skin can be printed using LAB technology. In fact, one of the first reported applications of LAB technology was for the bioprinting of the skin [25]. Although skin is a complex tissue that comprises multiple cell layers and appendages such as sweat glands and hair follicles, it has a relatively simple structure that is composed of multiple consecutive cell layers. The major drawbacks of LAB technology are the high costs involved in the printing process and the low flow rates due to the rapid gelation of **hydrogels** after deposition. These drawbacks may explain the lack of advances in this technology (Table 1). Although craniofacial reconstruction may benefit from LAB technology by aiding in skin **tissue engineering**, which may be beneficial for burn victims, it is unlikely that this technology will advance further for the development of load-bearing bone constructs.

Inkjet Bioprinting

Using inkjet bioprinting technology (Figure 2B), cell-containing droplets with a small resolution (20–100 μm) can be produced at exceptionally high speeds (up to ~10 000 droplets/s) [24]. Several craniofacial cell types including bone cells [10], endothelial cells [21], cartilage cells [13], and muscle cells [19] have been printed *in vitro* using this technology. Unfortunately, the droplet viscosity that can be used in inkjet printers is low [41], which limits the thickness and ultimately the mechanical strength that is required for structural support. As a result, inkjet printing technology is less applicable for load-bearing tissues in the craniofacial area such as the mandible and temporomandibular joint (TMJ). Soft, nonload-bearing but highly organized tissues such as **aponeuroses**, particular mimetic muscles (e.g., the orbicularis oculi, orbicularis oris, occipitofrontalis, and depressor anguli oris muscles, which are all commonly affected by ablative surgery and facial trauma), ear and nasal cartilage, and blood vessels may benefit from inkjet printing technologies as these tissues may require a structured deposition of cells at the micrometer resolution that can be accomplished using inkjet printing technology. Inkjet printed tissue constructs cannot be readily implanted as craniofacial constructs due to their lack of mechanical strength, but may benefit from extensive maturation in a **bioreactor** before implantation. Several craniofacial cell types including ear cartilage [42], a TMJ [43], and skeletal muscle [44] have already benefited from *in vitro* bioreactor culturing.

Table 1. Studies on Bioprinting of Human Bone, Cartilage, and Skin Cells[a]

Technique	Tissue	Scaffold Material	Cell Type	Cell Viability	Cell Concentration	Refs
ME	Bone	Alginate; Gelatin; HA	MSCs	84–85%	5×10^6/ml	[26]
ME	Bone	Collagen I; Chitosan; Agarose	MSCs	95–99%	5×10^4–8×10^4/ml	[27]
ME	Bone	PLGA; PEG	MSCs	50–87%	2×10^6/ml	[28]
ME	Bone/Cartilage	Agarose	MSCs	~100%	1×10^7/ml	[29]
ME	Bone/Cartilage	Alginate	Chondrocytes Osteogenic progenitors	70–88%	5×10^6/ml	[30]
ME	Bone/Cartilage	Collagen type I; HA	Nasal chondrocytes Osteoblasts	>90%	2×10^6/ml	[31]
ME	Bone/Cartilage	GelMA	MSCs	~90%	1×10^7/ml	[32]
ME	Cartilage	Nanocellulose; Alginate	Nasal Chondrocytes	73–86%	15×10^6/ml	[33]
ME	Cartilage	PCL; Alginate	Nasal Chondrocytes	85–97%	1×10^6/ml	[34]
ME	Cartilage	PCL; PEG; Alginate	MSCs	95%	1×10^6/ml	[14]
ME	Skin	Bioink (RegenHU)	Fibroblasts Keratinocytes	96.7–99.4%	9×10^6/ml	[18]
ME	Skin	Collagen	Fibroblasts Keratinocytes	85.5–95.0%	1×10^6/ml	[16]
ME	Skin	Fibrin; Collagen	MSCs	–	–	[35]
LAB	Skin	Gelatin	Dermal Fibroblasts	91%	0.6×10^6/ml	[17]
LAB	Skin	MatriDerm®	Fibroblasts Keratinocytes	–	1.5×10^6/ml	[36]
Inkjet	Bone	PEGDMA; Bioglass; HA	MSCs	63.8–86.6%	6×10^6/ml	[37]
Inkjet	Bone/Cartilage	PEG	MSCs	87.9%	6×10^6/ml	[38]
Inkjet	Bone/Cartilage	PEG–GelMA	MSCs	85–90%	6×10^6/ml	[39]
Inkjet	Cartilage	PEGDA	Articular Chondrocytes	–	–	[40]
Inkjet	Cartilage	PEGDMA	Articular Chondrocytes	63.2–89.2%	5×10^6/mL	[13]

[a]Abbreviations: ME, microextrusion; LAB, laser-assisted bioprinting; PEGDMA, poly(ethylene glycol)dimethacrylate; GelMA, gelatin methacryloyl; PCL, polycaprolactone; MSCs: mesenchymal stem cells.

Microextrusion Bioprinting

Microextrusion is by far the most commonly used technology for *in vitro* bioprinting (Figure 2C and Table 1) and is based on fused deposition modeling (FDM). Microextrusion technology is typically used to create polymeric **scaffolds** that can be seeded with cells that are expected to create an extracellular matrix (ECM) with the aid of growth factors or mechanical stimulation, traditionally known as the 'top-down' approach [45]. Therefore, microextrusion technology can be very useful for the printing of mechanically strong, patient-tailored polymeric constructs for craniofacial reconstruction that can be directly incorporated in the patient (e.g., to treat bony fragments in **blowout fractures** of the orbital floor). A wide range of different craniofacial cell

types has already been printed *in vitro* using this technology, including important shape-defining tissues such as bone and cartilage (Table 1). Microextrusion technology may be combined with current stem cell technology to generate directly implantable scaffold constructs that may regenerate over time [46]. The results of this may be a one-step surgical procedure [47] by which a construct could be bioprinted and implanted in the patient during craniofacial surgery without the need for extensive bioreactor culturing.

Scaffolds Applicable to Craniofacial Reconstruction

Scaffolds or lattices are 3D structures that form the basis for tissue formation and play pivotal roles in cell–cell interactions and ECM formation [48,49]. Scaffolds for craniofacial reconstruction should exhibit adequate structural and mechanical properties to resist contractive forces to provide a suitable environment for cell and subsequent tissue growth (Figure 3). Owing to the diversity of tissues in the craniofacial area, site-specific scaffolds are required to mimic the surrounding tissue. An ideal craniofacial scaffold comprises a cell-embedded structure with good biocompatibility and adequate structural and mechanical strength that allows for tissue ingrowth. Although several scaffold-free modalities, which may have certain advantages over scaffold-based modalities, exist, scaffold-free approaches are not suitable for certain types of craniofacial tissues such as load-bearing bone.

Scaffold-Based Modalities: Natural versus Synthetic-Based Scaffolds

A variety of common natural-based scaffolds have been used for the *in vitro* bioprinting of craniofacial cells. These scaffolds include collagen type I [27,31], gelatin [12,26,50], fibrin [35], alginate (Table 1), hyaluronic acid [26,31,51], and silk fibers [52] (Table 1). Numerous research

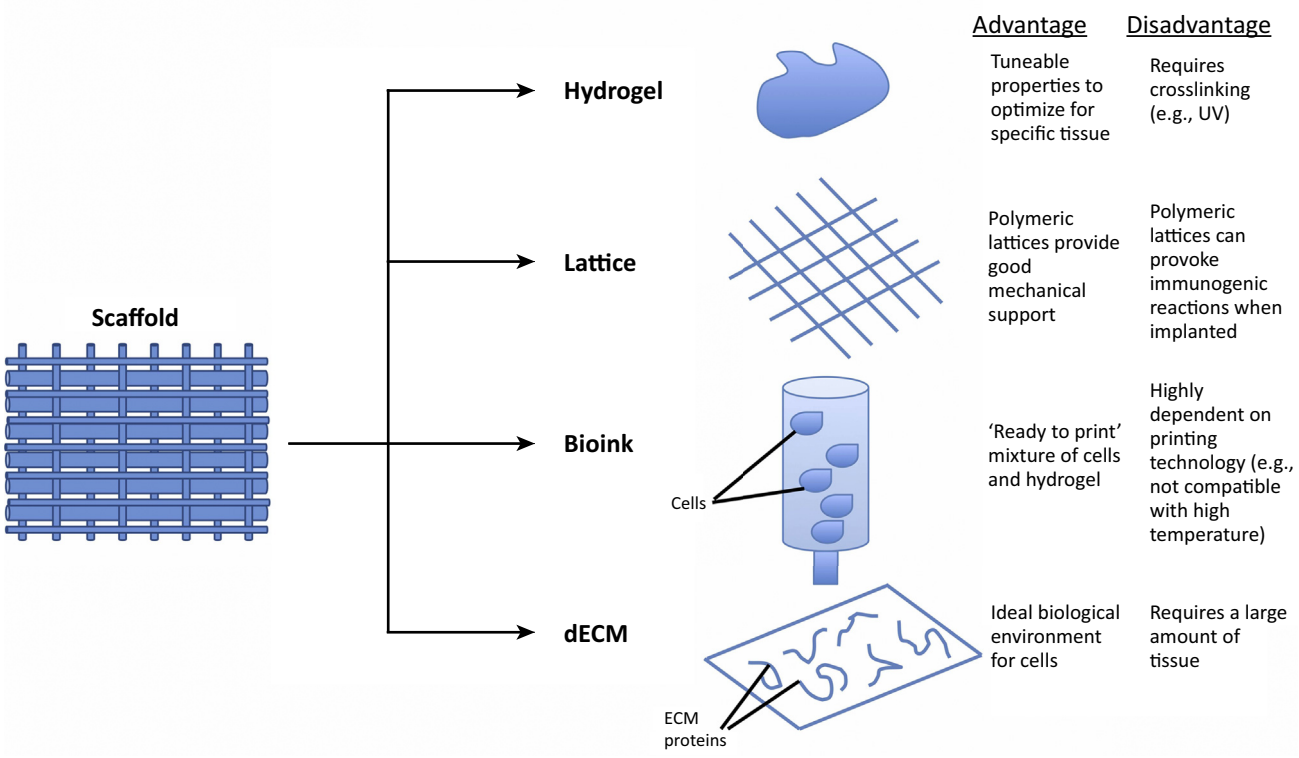

Figure 3. Advantages and Disadvantages of Different Scaffold Types Used for Craniofacial Bioprinting. The scaffold is a supporting structure for cells. Hydrogels, lattices, and dECM are all considered scaffolds. The biomaterial that makes up a bioink is also considered a scaffold. The different bioprinted scaffolds each have advantages and disadvantages when it comes to application for craniofacial reconstruction. Abbreviation: dECM, decellularized extracellular matrix.

groups have developed chemical and mechanical tools to customize the viscosity and mechanical strength of such scaffolds. For example, modification of a collagen scaffold with calcium phosphate can increase cell adhesion and mineralization [53]. In addition, simultaneous **crosslinking** of methacrylated gelatin (GelMA) and polyethylene glycol (PEG) can increase the properties of bioprinted bone and cartilage tissue [39].

Natural-based scaffolds provide an adequate environment for cell growth and matrix deposition but require crosslinking to preserve gel integrity [27,54,55]. This (i) decreases the production speed of bioprinted constructs and (ii) may affect cell viability because UV light, chemicals, and/or heat are often required to crosslink such constructs.

Despite the advantages of natural-based scaffolds, bioprinted low-viscosity gels are not sufficient for direct implantation and reconstruction of large load-bearing craniofacial tissues (e.g., mandible). However, injectable [56] or topically applied **bioink** [57] that can be bioprinted directly into the patient (*in situ* bioprinting) may become a reality for the reconstruction of facial burns and the filling of small traumatic cartilaginous or bony defects.

As opposed to natural-based scaffolds, synthetic-based scaffolds such as polylactic acid (PLA) [58], poly-ε-caprolactone (PCL) [14], PEG [14], and polyether urethane (PU) [59] (Table 1) have the ability to withstand large forces. Therefore, synthetic scaffolds are ideal candidates for the reconstruction of complex anatomical tissues such as bone (e.g., maxilla) and nasal cartilage tissue in the craniofacial area that require mechanical strength. Unfortunately, long-term studies on the biocompatibility and toxicity of such materials are still lacking. In addition, synthetic polymers require higher extrusion temperatures that range from 60°C to 200°C. Owing to the high extrusion temperatures, the synchronous bioprinting of cells and polymers is not feasible.

Combining synthetic-based polymers with natural-based bioinks in separate printing cartridges can, however, combine the best of both worlds and seems to be a feasible method to bioprint anatomically accurate and mechanically stable ear and mandibular constructs that can be implanted *in vivo* [20].

Another approach to scaffold-based bioprinting is the use of decellularized extracellular matrices (dECMs) that are composed of proteins and polymers that provide structural support to the tissue and allow interaction with cell receptors. In recent years, dECM has been used as bioink for the printing of multiple craniofacial cell types including bone, cartilage, and fat cells [60,61]. However, the excessive loss of important ECM proteins during printing [61] and the large amounts of initial ECM required to create a relevant tissue construct makes clinical translation of this approach difficult and likely irrelevant.

Scaffold-Free Modalities

Scaffold-free tissue engineering relies on the ability of cells to fuse and develop the entire ECM without the use of supporting scaffolds. Multiple cell types and combinations of cells that include nerve tissue [22] and blood vessels [62] have been bioprinted *in vitro* using this technology. Although the same bioprinting technologies can be applied to both scaffold-based and scaffold-free approaches, the latter requires a confined temporary space where the cells are printed (i.e., a mold). Despite the many advantages of scaffold-free bioprinting such as direct cellular interaction, biomimicry, and quick tissue formation [63], this modality is better reserved for organ printing rather than craniofacial tissue printing for two reasons: (i) the scaffold-free approach requires large numbers of cells that have to be harvested from the patient. This increases morbidity and requires extensive culturing and eliminates the possibility of a one-step surgical procedure. (ii) The mechanical strength of cell aggregates is low, which

makes direct implantation of the bioprinted construct, especially in load-bearing locations, impossible.

Bioprinting Cell types for Craniofacial Tissue Formation

As previously mentioned, the craniofacial area comprises several different tissues. To date, the majority of the research published has focused on the bioprinting of bone, cartilage, and skin. The greatest advancements have also been made in bioprinting of these cell types. Importantly, cancer and trauma in the craniofacial area often specifically damage these tissues, resulting in significant aesthetic problems.

Mesenchymal Stem Cells Used for Bioprinting Bone Tissue

To date, mesenchymal stem cells (e.g., bone marrow derived and adipose tissue derived) are the most commonly used cell types for the bioprinting of bone tissue (Table 1). Such cells are often isolated and subsequently expanded to acquire enough bone-forming cells. These cells are then combined with natural-based scaffolds (Table 1) and can be printed as bioink. However, because natural-based scaffolds lack mechanical strength, materials such as Bioglass [37,50], PEG [28], PLA [58], and combinations thereof, are used to enhance the mechanical stability of these constructs (often called 'composite scaffolds'). For example, Wüst *et al.* reported using hydroxyapatite (HA) and gelatin/alginate hydrogels to increase the stiffness and printability of the gel without affecting cell viability [26].

Recently, there has been considerate interest in incorporating growth factors such as bone morphogenic protein-2 (BMP-2) in bioprinted bone constructs [10,64]. BMP-2 is FDA (US Food and Drug Administration) approved and has been used clinically to reconstruct craniofacial hard tissue defects such as the frontal sinus, cranium, mandible, and nasal septum [65]. The ability to print bone-forming cells (i.e., stem cells) seems promising with regard to stability and cell viability, which generally lies between 60% and 100% (Table 1).

Cartilage Cells

Several attempts have been made to bioprint human craniofacial cartilage using a variety of cells including human nasal chondrocytes [33,34] and adipose-derived stromal cells differentiated into chondrocytes [14]. These chondrocytes, however, require a scaffold for stabilization. To date, several novel scaffolds have been developed to fabricate cartilage-based bioinks. For example, nanocellulose [33], Pluronic F-127 [15], and glycosaminoglycan-based hydrogels [66] have shown promising results with regard to increasing the mechanical stability of the bioprinted cartilage constructs required for ear and nasal reconstruction.

There is a noticeable trend towards using higher cell numbers for bioprinting [15,33]. One major drawback of using high cell numbers is that it requires extensive *ex vivo* expansion that can cause a deterioration in the characteristics and potency of the cell. As an alternative, the body can be used as a bioreactor for *in vivo* growth of cell-seeded scaffolds and subsequently transferred to the craniofacial reconstruction area [67].

Skin Cells

Human fibroblasts and keratinocytes are the most commonly used cell types for skin bioprinting [18,36]. Several bioinks composed of cells combined with collagen or fibrin have been used. In addition, bovine collagen–elastin (MatriDerm®) has also been used for the bioprinting of skin cells [36]. MatriDerm® may have advantages over hydrogels as it has already been clinically approved for use with facial burn patients [68]. However, it is an animal-derived product that may contain growth factor contamination as well as batch-to-batch variation. Although skin bioprinting is still at a very early stage in its development, it could be beneficial for the treatment of facial burn wounds.

Clinical Application of Bioprinted Constructs in the Craniofacial Area

Clinical applications of bioprinting are at a very early stage and still require further development (Figure 4, Key Figure). Nevertheless, a number of promising preclinical and animal studies have already been performed. For example, Michael *et al.* [36] engineered cellularized skin substitutes using LAB technology and transplanted these into full-thickness skin wounds in mice resulting in migration of fibroblasts, blood vessel formation, and collagen production. Furthermore, recent studies have reported on the applicability of bioprinting for the deposition of tissue *in situ*. Keriquel *et al.* [69] used *in situ* LAB technology to deposit nano-HA in a mouse calvarium model. Skardal *et al.* [35] used the laser printing of amniotic fluid stem cells to regenerate skin defects. Inkjet bioprinting technology has also been applied to the *in situ* repair of skin defects in rats

Key Figure

Schematic of Steps Involved in Clinical Translation of Bioprinted Craniofacial Constructs

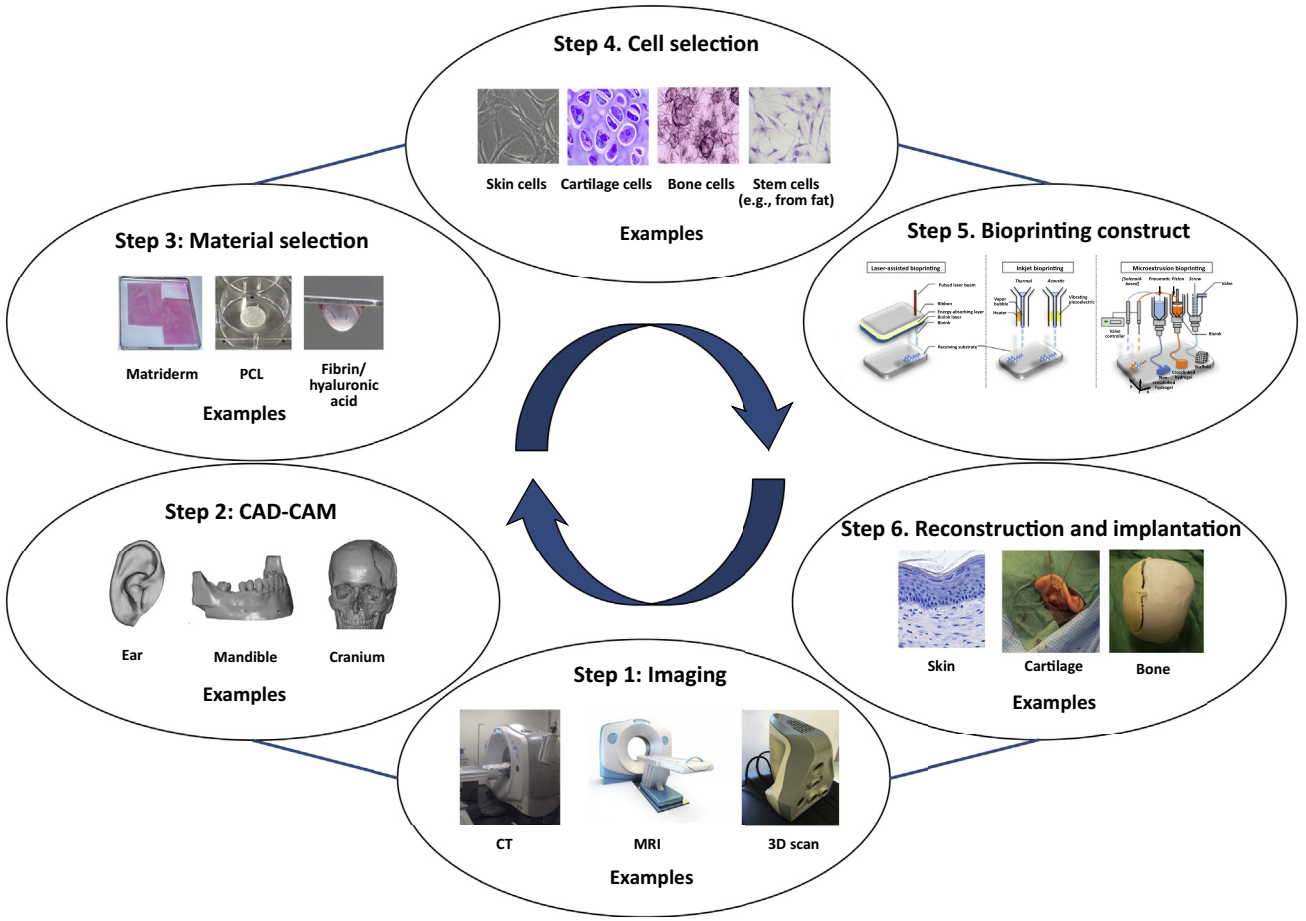

Figure 4. First, an image is acquired of the tissue using medical imaging. Next, computer-aided design (CAD) and computer-aided manufacturing (CAM) are used to prepare the acquired medical image for bioprinting. Then, the material is chosen based on the tissue type (e.g., load-bearing bone requires mechanically stronger materials than skin). Next, the appropriate cells are harvested from the patient and ideally used directly for bioprinting or alternatively first cultured *in vitro* to obtain an adequate number of cells. Finally, the construct is printed using the appropriate bioprinting technology and implanted in the patient. Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; PCL, poly-ε-caprolactone.

resulting in acceptable cell survival and fast skin recovery (K. Binder, PhD thesis, Wake Forest University, 2011).

A recent clinical study reported on the treatment of 13 patients with adipose-derived mesenchymal stem cell-seeded resorbable scaffolds at four different anatomical sites within the craniofacial skeleton [65]. In some cases, individualized 3D printed constructs were used to bridge large defects in the lower jaw. This treatment method appears to be a logical first step towards the treatment of craniofacial defects using stem cell-incorporated bioprinted scaffolds.

Concluding Remarks and Future Perspectives

In this opinion paper, we have presented the state-of-the-art bioprinting technologies required to fabricate bioactive scaffolds for craniofacial reconstruction. The different bioprinting technologies show promise, but because each tissue currently requires a specified technology, the printing of multicellular tissue constructs is difficult. In addition, the mechanical stability of current hydrogels and bioinks are not optimized for craniofacial reconstruction (see Outstanding Questions). With the need for long-term (pre)clinical studies, intelligent polymers, and ultimately good manufacturing production (GMP) of bioprinted constructs, there is still a long road ahead. A promising future approach for the treatment of external craniofacial tissues could be a handheld bioprinting device that will enable the delivery of cells into tissues such as skin or cartilage. For now, focusing on the optimization of bioprinting technologies to enhance the self-repair capabilities of tissues in the craniofacial area seems a logical first step in clinical bioprinting.

Outstanding Questions

Can we apply and combine currently available biomaterials and incorporate cells from the patient to create clinically applicable bioactive scaffold constructs for craniofacial reconstruction?

How far should we aim to create tissue complexity *in vitro*? Or should we aim at simple constructs that regenerate within a limited time after implantation?

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