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3D-Printing Technologies for Craniofacial Rehabilitation, Reconstruction, and Regeneration

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Abstract

The treatment of craniofacial defects can present many challenges due to the variety of tissue-specific requirements and the complexity of anatomical structures in that region. 3D-printing technologies provide clinicians, engineers and scientists with the ability to create patient-specific solutions for craniofacial defects. Currently, there are 3 key strategies that utilize these technologies to restore both appearance and function to patients: rehabilitation, reconstruction and regeneration. In rehabilitation, 3D-printing can be used to create prostheses to replace or cover damaged tissues. Reconstruction, through plastic surgery, can also leverage 3D-printing technologies to create custom cutting guides, fixation devices, practice models and implanted medical devices to improve patient outcomes. Regeneration of tissue attempts to replace defects with biological materials. 3D-printing can be used to create either scaffolds or living, cellular constructs to signal tissue-forming cells to regenerate defect regions. By integrating these three approaches, 3D-printing technologies afford the opportunity to develop personalized treatment plans and design-driven manufacturing solutions to improve aesthetic and functional outcomes for patients with craniofacial defects.

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Keywords

Facial prosthetics; craniofacial implants; tissue engineering; regenerative medicine; 3D-printing; scaffolds

1. Introduction

Craniofacial defects arise as a direct result of trauma, oncological resection, or congenital differences. They cause soft tissue or bone deficits, or a combination of both leading to non-healing composite tissue wounds. Defects in the craniofacial region in particular are difficult to treat because of the emphasis on positive aesthetic outcomes and the number of tissue types (bone, cartilage, muscle, and skin) and structures (auricle, orbit, nose, oral cavity) in close proximity. The current options for reconstructive surgery to treat these defects include grafts, local tissue rearrangement which fills defects with adjacent healthy tissue, microsurgical tissue transfer whereby one area of the body is transferred with its blood supply to another area[10,22], and vascularized composite allotransplantation whereby a portion of the body containing skin, muscle and/or bone is transplanted from one patient to another[16]. However, the major challenges with using traditional reconstructive surgery to treat large craniofacial defects are donor-site morbidity and procuring sufficient donor tissue with the same properties, including skin color, quantity and contour of bone, and quantity and quality of subcutaneous tissues, as the surrounding recipient tissue to restore normal anatomic structure and primary organ functions.

The challenge of integrating the various tissues of the face while maintaining or improving aesthetics motivates collaboration between the fields of prosthetic rehabilitation, craniofacial reconstruction, and regenerative medicine. **Prosthetic Rehabilitation** refers to the use of custom-made facial prosthetics to restore normal facial appearance (Figure 1A). **Reconstruction** of the craniofacial region can be performed using a variety of plastic surgery techniques to replace structures and is aided by the precise manufacture of cutting guides, fixation devices, practice models and implanted medical devices (e.g. Figure 1B). **Regeneration** aims to stimulate regrowth of damaged or malformed craniofacial tissues using stem cells and biologically active scaffold materials. (Figure 1C). For a particular defect, these approaches may be employed individually or in conjunction with one another. However, a common thread is the need for patient-specific treatments that fit a particular defect site to achieve both aesthetic cosmesis and functional replacement. As such, 3D-printing techniques that can create highly complex craniofacial geometries with high fidelity are well-suited for addressing particular needs.

Anatomical geometries can be captured using medical imaging such as computed tomography (CT), magnetic resonance imaging (MRI), or light scanning and then 3D-modeled digitally to create useful 3D-printed products. The particular method of 3D-printing affects the print outcome and may be selected based on the particular applications (Table 1). The primary methods for 3D-printing include fused deposition manufacturing (FDM), stereolithography (SLA), selective laser sintering (SLS), inkjet printing, inkjet bioprinting, extrusion bioprinting, and laser assisted bioprinting, which have been reviewed

extensively[50,56]. Briefly, in FDM, molten material is extruded layer-by-layer onto a bed; once the material cools and solidifies, it serves as the foundation for the layer above it. While this method is easily applied to many materials – any material that can be melted and extruded – it requires support structures for printing overhangs. SLA uses a laser to solidify photocurable liquid polymers in a layer-by-layer fashion.[56] In contrast, SLS creates structures by sintering a powder bed layer-by-layer. The powder that is not sintered therefore serves as the support structure. A variation of this method, inkjet writing, also uses a powder bed, but uses a chemical binder instead of a laser to bind the particles together. The similarly named, inkjet bioprinting, uses acoustic, thermal, or electromagnetic forces to eject hydrogel droplets, which could contain cells or biological molecules, onto a platform in an additive fashion, onto a clean print bed or a binding solution.[56] Extrusion bioprinting is similar to inkjet printing, but uses pumps, screws, or pneumatic systems to extrude cell slurries with viscosities too high for inkjet printing. Finally, laser-assisted bioprinting consists of a laser source, a glass “ribbon” covered with a layer of cells in hydrogel solution, and a receiving substrate. The laser vaporizes a small portion of the hydrogel solution, which forms a bubble that can then fall as a droplet onto the platform below.[56]

In this review, we examine how recent developments in 3D-printing enable more effective personalized treatment of complex craniofacial defects. We highlight advances in 3D-printing as applied to prosthetic rehabilitation, surgical reconstruction, and tissue regeneration for non-healing defects in the craniofacial region and identify avenues for further research.

2. 3D-printing for Prosthetic Rehabilitation

Recapitulation of patient specific coloring, texture, stiffness, and shape for prostheses is currently a labor-intensive process, which could be streamlined using 3D-printing. Prosthetic rehabilitation may be used in cases where successful surgical reconstruction is not a viable option due to factors such as poor prognosis, co-morbidities, compromised healing due to poor vascularization[79], and patient refusal of further surgical interventions[2]. Further, the economic burden and treatment time for prosthetic rehabilitation is lower than that of surgical reconstruction[72]. Typical sites for craniofacial prosthetic rehabilitation include oral, orbital, nasal, and auricular regions[51,71]. Prosthetic rehabilitation can also serve as an interim strategy during the period of treatment planning for a later surgical reconstruction[69]. Besides providing an aesthetic solution to covering an affected area, prosthetic devices are considered medically necessary due to the functional benefits they offer to warm incoming air, maintain humidity of moisture filled cavities, protect fragile tissue, modulate speech, and provide support for corrective eyeglasses.

Treatment of craniofacial defects with prostheses traditionally involves the creation of a custom made device generally made of polydimethylsiloxane (PDMS) to replace missing tissue and cover underlying tissue[51,71]. The workflow for creating these devices has gone relatively unchanged since the 1970’s. However, the use of advanced 3D imaging techniques (including surface laser scanning and stereo photogrammetry) combined with 3D-printing is changing what was once a traditionally based workflow to include several facets achieved through digitally analogous methods (Figure 2). Only one study to date has reported a

clinically viable workflow for directly 3D-printing these devices[31]. It is still limited, however, as it does not result in a fully colorized prosthesis with physical properties similar to the PDMS devices typically made by traditional methods. An alternate approach has been to 3D-print a negative multiple-piece mold that can be used for casting the final PDMS prosthesis. Advanced digital technologies and additive manufacturing techniques can thus be leveraged in craniofacial cases to increase the quality of outcomes for prosthetic rehabilitation. Future development of methods to directly print fully colorized PDMS prosthetics could significantly improve manufacture time and costs for craniofacial prostheses. A number of companies are developing technologies to directly print PDMS[80–82] and new techniques to precisely color complex and soft constructs (such computational hydrographic printing[77]) offer exciting methods to fully recapitulate the appearance of the prosthetic.

3. 3D-printing for Surgical Reconstruction

3D modeling and manufacturing tools can provide aid in the personalized, surgical reconstruction of complex craniofacial defects by precisely cutting tissues according to preoperative plans, decreasing the total time and cost of surgery, and planning the shape of alloplastic and metal materials. Furthermore, such tools have helped to improve precise shaping and positioning of the newly incorporated tissues and improved the cosmetic and functional outcome of reconstructive operations[45] and are useful for patient education[15]. Tools that are used transiently in the reconstruction process, such as placement or cutting guides, are produced using FDM or SLA out of sterile and bioinert materials such as acrylonitrile butadiene styrene (ABS), poly(methylmethacrylate) (PMMA), or polypropylene[55]. Implanted products additionally require long-term biocompatibility and mechanical strength and are often laser sintered from titanium or bioglass. Both types of products are often accurate to the millimeter scale.

3.1 VSP and Guides

Advances in 3D imaging and manipulation of the resulting datasets have enabled surgeons to plan surgeries using computer models of the patient, virtually moving bones and other tissue to assess different approaches, options, and outcomes (Figure 3). This virtual surgery planning, together with rapid physical modeling of the defects and custom cutting and positioning guides, has vastly improved preoperative planning techniques compared to more traditional approaches, and has significantly aided the surgeon in his or her approach to complex craniofacial reconstruction[20,63]. 3D modeling and virtual planning aids intraoperative precision and efficiency of the surgery to match the preoperative design. Models of the defect site and the transferred bone segments can be manufactured to practice positioning, fixation, and evaluate aesthetic outcomes[66]. Such planning segues easily into precise, custom cutting and placement guides, increasing cosmesis and reducing ischemia and total surgery time. Consider the clinical standard for reconstruction of mandibular bone, the free fibular flap[59]: the fibula and the defect site are first scanned using CT (Figure 3A), then cuts are made in the fibula to adequately position the grafted bone into the defect site (Figure 3C). To aid in the precision of harvesting and repositioning the pieces of fibula, cutting and placement guides are designed and rapidly manufactured, often through FDM

(Figure 3D). Finally, custom surgical guides have been essential in enabling the advent of facial transplants—in addition to the planning and guide fabrication, 3D-printing is essential in preparing an exact fit for the donated face[9].

3.2 Pre-fitting Implants

Rapid prototyped models of the defect, or the predicted defect, are also used to pre-bend generic off-the-shelf implants such as reconstruction plates and titanium meshes to fit the specific anatomy of the patient. Such precise and methodical pre-bending can result in improved functional and aesthetic outcomes[6], decreased subjectivity[29], and reduced surgery and ischemia time[63]. Stereolithographic models of the defect site have also been used to mold PMMA to fashion an alloplastic bone-graft alternative[21]. In addition, 3D-printing models of ideal and patient-specific anatomy produced by mirroring a normal contralateral side has been used to press fit a composite titanium and porous polyethylene implant, and then guide the surgical placement in order to reconstruct the orbital floor after facial trauma[61]. These methods allow for the customization of patient implants without significantly changing the manufacturing process of the device, which is a major regulatory and production hurdle.

3.3 Materials for Patient Specific Implants

Non-resorbable implants can be designed and manufactured specific to individual patients and can used in lieu of autologous tissue[57]. Many materials including metals, bioglasses, and bioinert plastics can be used in a number of manufacturing processes and maintain biocompatibility over time. For example polyetheretherketone (PEEK) has strong biocompatibility, mechanical strength, and radiographic translucency and can be 3D fabricated into patient specific implants through laser sintering or Computer Numerical Control (CNC) machining[39]. In addition, patient-specific titanium mesh can be manufactured via direct metal laser sintering to hold grafted bone in place and re-create contours and structures of the facial bone[67]. Bioglasses (such as S53P4, 6P53B, and 13–93) have been widely used in craniofacial surgery as a bone graft substitute due to their biocompatibility, strong mechanical strength, and osteoconductivity[1,27]. Bioglass structures can be manufactured by mixing glass particles into a solution, cold-printing in a layer-by-layer fashion, and then dehydrating at high temperatures to sinter the glass particles together and remove the solution[23,33]. Others have reported formulations of bioglass (such as 13–93 which has the composition $53SiO_2$, $6Na_2O$, $12K_2O$, $5MgO$, $20CaO$, $4P_2O_5$; wt.%) which can be laser sintered into anatomic shapes[43]. Hydroxyapatite (the main component of bone) implants, via a resin carrier, can be produced through SLA and have been used to reconstruct large (>20 cm^2) defects with resolutions less than 0.4mm[8]. Finally, in 2012 a titanium mandible was laser sintered and implanted into an 83-year-old patient. The patient was able to speak and swallow the same day, and exhibited excellent restoration of facial aesthetics[28]. While titanium is the industry standard in orthopedic implants, the cost of materials, unknown long term efficacy, and manufacturing remain limiting. There is particular concern of implant exposure and infection over time as there is often only a thin layer of soft tissue covering the implant.

As the intersection of 3D imaging, manipulation, design, and manufacturing develops further, these tools for surgeons will broaden from individual case studies to common practice. The past decade of developing these tools apace with the maturation of 3D technology will likely revolutionize surgical standards, just as 3D-printing has revolutionized transradial prostheses[17,83]. Increased efficiency and accuracy provided by these tools will be driving factors of their widespread adoption while regulatory, biocompatibility, and reimbursement challenges remain[4]. Innovation stimulated and facilitated by these 3D technologies will also continue, leading to techniques as impressive as the recent total face and jaw transplants[9,16].

4. 3D Printing for Craniofacial Bone Regeneration

The goal of a 3D-printed construct for regeneration is to fill the defect with biological tissue. To accomplish this, an appropriately shaped construct can be produced that is populated uniformly with tissue-forming cells that are signaled to regenerate tissue. This can be accomplished in two ways: printing of acellular scaffolds that can be populated with cells prior to implantation or the printing of living, cellular constructs, termed '*bioprinting*'.

4.1 Acellular printing

Several key parameters should be considered and optimized for scaffold development: (1) macro-geometry (Figure 1C), (2) micro-architecture, (3) bioactivity, and (4) mechanical properties (Figure 4). The strengths and weaknesses of these currently investigated printing approaches to achieving the four considerations outlined above are discussed below.

Incorporating micro-architecture, which encompasses pore geometry and pore size, is critical for uniform cell distribution and cell migration into the scaffold; interconnected pores can improve integration of regenerated tissue with native tissue.[46] For bone tissue engineering *in vivo*, higher porosity has been correlated with increased bone ingrowth into scaffolds.[36] Designing pore architecture results in higher pore connectivity and uniform cell distribution compared to random architecture resulting from salt-leaching methods, despite similar porosity, pore size, and surface area[52]. Pore size and interconnectivity also improves nutrient diffusion into and waste diffusion out of scaffolds.[60] Scaffold vascularization, a critical component of tissue survival, has been shown to increase with increasing pore size; pore sizes between 160–270 μm resulted in extensive vessel formation in both mathematical and experimental models[3,13]. Osteoblast proliferation and migration through collagen-glycosaminoglycan scaffolds also depends on pore size, with larger pores around 300 μm resulting in higher cell numbers throughout the scaffold[54]. In the context of 3D-printing, some methods are better suited to creating defined pores. For instance, FDM relies on rapid cooling of an extruded molten material, resulting in well-defined scaffold struts and well-defined pores[70]. In contrast, chemical binding-based approaches rely on dispensing a liquid binder onto a powder substrate and result in pore sizes less than 100 μm due to binder flow[38].

The scaffold should also provide biological signals to resident cells to form tissue. For bone, the most widely used strategy is incorporation of mineral phases in scaffolds for osteoinductivity[5]; similar strategies have been investigated with 3D-printed scaffolds. For

example, a phosphoric acid binder was used to bind calcium phosphate together, creating a mineralized structure that can house cells[38]. Another method used polycaprolactone (PCL) with incorporated tricalcium phosphate particles in FDM[65]. In addition, incorporation of bioactive molecules, such as bone morphogenetic proteins (BMPs), have been investigated; however, given most 3D-printing methods rely on high temperatures, up to 1300 °C for sintering methods[68], use of growth factors in 3D-printing remains a challenge. Chemical binding methods have the distinct advantage of printing at room temperature, creating potential for application of the method to growth factor incorporation, though careful choice of binder is required to prevent pH-related damage. A second approach is to load growth factors onto a scaffold post-printing, which circumvents these issues but adds another step to scaffold manufacturing.

Finally, in the replacement of craniofacial bone, the scaffold must provide structural support for both resident cells and for transduction of mechanical forces through the craniofacial skeleton. Target scaffold stiffness depends on anatomical location, with the elastic modulus of human trabecular bone within the mandibular condyle ranging between 120–450 MPa or within the mandible from midline to ramus ranging from 112–910 MPa.[32] Many current 3D-printed scaffolds have achieved stiffness within the 10–100 MPa range[32,37,38,78]. Testing mechanical properties of polymeric scaffolds under physiological conditions is crucial as groups have shown changes in compressive moduli at different temperatures and in aqueous media.[37] It should be noted that increased porosity leads to lower mechanical properties – a study using sintered PCL reported that the stiffness of printed porous scaffolds was around 15 MPa, compared to 300 MPa for a solid PCL piece[18]. As such, the importance of porosity for cellular ingrowth and proliferation must be balanced against the importance of structural scaffold properties for mechanical support and force transduction.

The importance of these four criteria is clearly demonstrated in the clinical regeneration of soft and osseous tissue holding the left mandibular cuspid in place[62]. Using the patient's CT scan the exact macroscopic geometry of the scaffold was determined. The scaffold was printed using SLS of PCL containing 4% hydroxyapatite for osteoinductivity. In addition, the scaffold was designed to release platelet-derived growth factor BB (PDGF-BB), a factor known to support vascularization and mineralization[30,34], in a burst manner from pre-formed channels. Due to the high printing temperatures associated with sintering, the scaffold was first printed without growth factor and immersed in PDGF-BB solution for 15 minutes after printing. The use of PCL as the main biomaterial was justified from a mechanics standpoint: the stiffness of PCL scaffolds manufactured using SLS has been reported to be ~15 to 300 MPa, depending on porosity[18], values that fall within the reported range for human trabecular bone.

The scaffold porosity or micro-architecture was not reported, though the lack of interconnected pores was noted as a limitation of the approach. The implantation of the scaffold was successful – the image-based geometry fit the defect well – and the printed channels for growth factor release successfully dispensed PDGF-BB in a burst manner.[62] As a shortcoming, the patient presented with scaffold exposure and wound failure past 13 months post-implantation. Upon removal of the scaffold, histological analysis indicated a preponderance of connective tissue formation and little bone regeneration, suggesting the

lack of internal micro-architecture prevented the infiltration of regenerative and vascular cells and therefore precluded regeneration. Combined with the slow-degrading properties of PCL, the authors concluded that the scaffold's low porosity served to block tissue regeneration. As such, while the macro-geometry and mechanical properties were appropriate (over the 13-month period, the scaffold did not fail mechanically despite being in a region of load), the lack of micro-architecture inhibited the bioactive and regenerative properties of the scaffold.

This example of the clinical application of 3D-printing scaffolds for craniofacial regeneration highlights strengths and necessary improvements. The combination of image-based extraction of craniofacial geometry and the ability to 3D-print shapes with high fidelity resulted in a scaffold tailored to the specific defect. The ability to incorporate bioactive factors into the printed scaffold was also demonstrated. Finally, the choice of PCL as a printable biomaterial illustrated the ability to print mechanically appropriate scaffolds for load-bearing craniofacial regions. In addition to the group featured in this case study, other groups have commercialized FDA-approved PCL scaffolds fabricated by FDM.[84]

A relatively underexplored area of 3D-printed scaffolds involves printing biological and mechanical gradients. For example, printing scaffolds with hydroxyapatite gradients could improve bone formation with exterior areas having more mineral to encourage growth of compact bone and interior areas having more diffuse mineral to mimic trabecular bone. While printing with growth factors has been a challenge due to printing conditions for many techniques surpassing biological pH and temperatures at which these molecules are stable, several groups have printed bioactive ceramics or extracellular matrix (ECM)[35,47]. The incorporation of ECM enhanced scaffold bioactivity, but high ECM concentrations decreased scaffold mechanics. Printing extracellular matrix proteins in 3D spatial gradients has been achieved by using maskbased SLA to stimulate assembly of genetically engineered photoactive proteins, though this was used as a surface modification for tissue culture rather than an implantable 3D construct[73]. Another group used inkjet printing to create gradients of laminin and used their materials to study cell alignment[11].

Printing mechanical gradients by varying pore structure and size could also assist with building tissue that mimics native function, particularly in the bone example. One group has recently demonstrated that gradient pore sizes created by FDM can slightly improve both chondrogenesis[49] and osteogenesis[48], although they did not investigate different geometries. By designing scaffold pore sizes and geometries based on biological mechanical requirements, these improvements may be further enhanced.

4.2 Bioprinting

Bioprinting differs from the traditional tissue engineering approach of seeding cells onto preformed scaffolds by depositing cell and scaffold simultaneously, forming a predesigned structure[24]. Bioprinting is the computer-aided deposition of living cells into 3D patterns. It is currently performed with micron-scaled precision[50]. As cell viability must be maintained during the printing process, the methods used for bioprinting differ from those used for traditional 3D-printing. Important parameters of 3D-bioprinting scaffolds include (1) cell positioning, (2) bioink selection, and (3) mechanical strength. In many cases, the type of

bioink used and the required resolution dictates the optimal printing technique for a particular application.

Bioprinting offers a key advantage over the traditional approach of seeding cells into 3D-printed scaffolds: digitally designing layer-by-layer deposition of cells to precisely regulate 3D cell distribution. This is advantageous when designing vascularized soft tissue, as adequate nutrient and oxygen supplies are necessary during tissue regeneration.[42] For example, Kolesky et al. developed a bioprinter that could print up to four cell types simultaneously and created complex 3D patterns of fluorescently labeled human dermal fibroblasts and human umbilical vein endothelial cells[44]. However, there are also several challenges associated with cellular printing.

Another disadvantage of bioprinting compared to acellular printing is that the mechanical strength of bioinks is typically lower than thermoplastic polymers. Originally, the majority of bioinks were natural hydrogel polymers, particularly alginate and fibrin, which when printed have compressive moduli of approximately 5 kPa[12]. Human bone and cartilage typically have moduli of about 10–20 GPa and 700 kPa, respectively[25]. In order to print tissues having similar load-bearing capacities to native bone and cartilage, PEG-based hydrogels have been printed with compressive moduli between 300–350 kPa[26]. Another method used to improve mechanical strength is integrating acellular and cellular bioprinting. Merceron et al. used a combination of FDM and extrusion bioprinting to print two thermoplastic polymers along with C2C12 and NIH/3T3 cells to create a 3D-printed muscle-tendon unit[53] and Kang et al. integrated FDM and extrusion bioprinting to print vascularized bone, muscle, and cartilage[40]. Printing hybrid scaffolds with cellular and acellular components may be one way to improve mechanical strength of bioprinted scaffolds. These limitations are some of the reasons that bioprinting has not yet been used to regenerate craniofacial tissues in human patients.

Of the tissues necessary for craniofacial reconstruction, skin bioprinting is the nearest towards clinical translation, with studies conducted *in vivo* using mice and pigs. One study of note compared bioprinted scaffolds to a commercially available engineered skin graft (Apligraf)[75]. A current limitation of engineered skin grafts such as Apligraf is that they lack microvasculature to maintain cell viability over time and instead rely upon diffusion to transport oxygen and nutrients to cells. Bioprinting can overcome this limitation by precisely patterning microvascular structures for skin grafts. Bioprinted scaffolds were trilayered with the top layer composed of collagen and printed keratinocytes, the middle layer composed of fibrin and endothelial cells, and the bottom composed of collagen and fibroblasts. Apligraf is a bilayered material cast with two collagen layers: one containing dermal fibroblasts and the other containing keratinocytes[74]. The group found that wound contraction, which if excessive can be a marker for joint contraction, malfunction, and poor aesthetic outcomes decreased in the bioprinted scaffolds compared to Apligraf and no treatment, which were statistically similar. Additionally, the mice with printed grafts healed between 14–16 days, whereas those with no grafts or with Apligraf healed within 21 and over 28 days, respectively. Histologically, the printed groups showed microvessel formation by implanted human endothelial cells in the printed scaffolds. Macroscopic images of skin regeneration in Apligraf and bioprinted groups can be seen in Figure 5A–F. Patterning endothelial cells to

form lumenized microvessels to improve graft viability could allow for scale up in terms of graft thickness and area by reducing oxygen and nutrient diffusion limitations. Binder *et al* have developed a promising *in situ* bioprintier for skin, but initial preclinical tests in pigs demonstrated unsatisfactory healing outcomes in wound closure rates, which the authors suggested was due to an insufficient cell density (2.0×10^5 cells/cm^2).[7] While the bioprinted materials have improved skin wound healing in terms of decreasing wound contraction and healing time *in vivo* is a promising advance for skin bioprinting, but such methods are still inferior or comparable to cell spraying techniques[7].

Bioprinting of bone has also moved forward, with some preliminary bioprinting studies conducted *in vivo*. Of particular import is a pilot study conducted by Keriquel *et al.* that investigated the use of laser assisted bioprinting to manufacture hydroxyapatite scaffolds directly into a calvarial defects in mice, as seen in Figure 5G, H[41]. When bone formation was measured by X-ray micro-tomography at the group observed considerable variation in bone formation between individual mice and did not provide quantitative data for bone ingrowth. Though these results are preliminary, they do show that bioprinting *in vivo* is possible and may have potential for clinical use with the proper bioink and cell source.

The precise patterning of biological molecules and cells through bioprinting may be useful in creating tissues with complex spatial orientations. Though the field is young, promising results have been achieved for skin and bone engineering *in vivo.* Studies have investigated cartilage[14], muscle[53], and adipose[58] tissue engineering using bioprinting, though these have not yet advanced to *in vivo* studies. The expensive specialized equipment necessary to use bioprinting technologies and the added regulatory burden of incorporating cells into a biomaterial, acellular printing may be the preferred regenerative method for treating craniofacial defects. Bioprinting could be further improved by widening the selection of available bioinks, decreasing print time, increasing print resolution, and moving more studies towards *in vivo* models.

5. Conclusion

Craniofacial deformities, when they arise, are particularly debilitating as they impact emotional, psychosocial, and functional well-being of the affected individual. They are difficult to treat due to the geometrical requirements and multiplicity of tissue types that are impacted. However, recent advances in 3D-printing technologies hold tremendous promise for advancing treatment options available to patients. The requirements of 3D-printed products differ depending on the size and severity of the defects, which together with patient-specific factors determine whether the primary treatment modality is prosthetic rehabilitation, surgical reconstruction, or regeneration. For rehabilitation, the use of 3D-printing technologies to either directly create PDMS prosthetics or print molds has the potential to significantly streamline the associated workflows for this process. The prostheses are flexible, non-degradable, and need to incorporate patient-specific skin tones. They differ considerably from 3D-printed guides or alloplastic implants used in reconstructive surgeries. Perhaps the most transformative applications, of 3D-printing lie in the realm of tissue regeneration. This area remains relatively nascent to date and significant research efforts are being dedicated to its continue rapid advancements that include the

development of biodegradable scaffolds as well as bioinks used for printing live cells. The successful implementation of these technologies clinically will expand the treatment options available to patients.

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Abbreviations

3D	Three Dimensional
ABS	Acrylonitrile Butadiene Styrene
BMPs	Bone Morphogenetic proteins
CNC	Computer Numerical Control
CT	Computed Tomography
ECM	Extracellular Matrix
FDM	Fused Deposition Modeling
MRI	Magnetic Resonance Imaging
PCL	Polycaprolactone
PDMS	Polydimethylsiloxane
PDGF-BB	Platelet-Derived Growth Factor BB
PEEK	Polyetheretherketone
PMMA	Polymethyl Methacrylate
PLA	Polylactide
SLA	Stereolithography
SLS	Selective Laser Sintering
VSP	Virtual Surgery Planning

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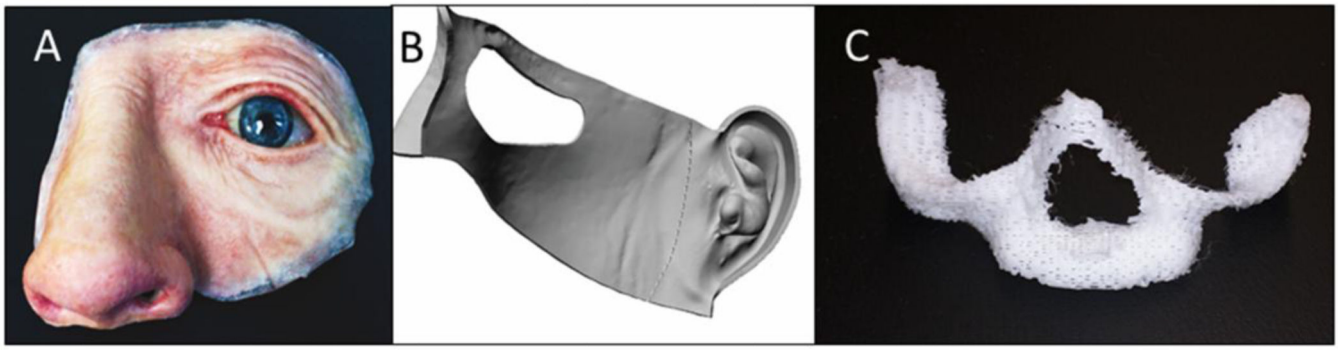

Figure 1.

Examples of Rehabilitation, Reconstruction, and Regeneration. (**A**) Custom PDMS midfacial and ocular prosthesis. (**B**) Cutting and placement guides for auricular autogenous reconstruction. (**C**) 3D-printed maxilla, porous PCL scaffold.

Figure 2.

(**A**) Orbital mold 3D model obtained through a fully digital workflow. (**B, C**) Resulting 3D-printed 3-piece mold that can be used for casting PDMS prosthesis. **(D)** The final PDMS prosthesis can be colored and provide satisfactory cosmesis. Photos used with author's permission. (Perry, R. The Development of an Orbital Prosthesis Workflow Using Advanced Digital Technologies, A thesis submitted to Johns Hopkins University in conformity with the requirements for the degree of Master of Arts, Baltimore, Maryland October, 2015)

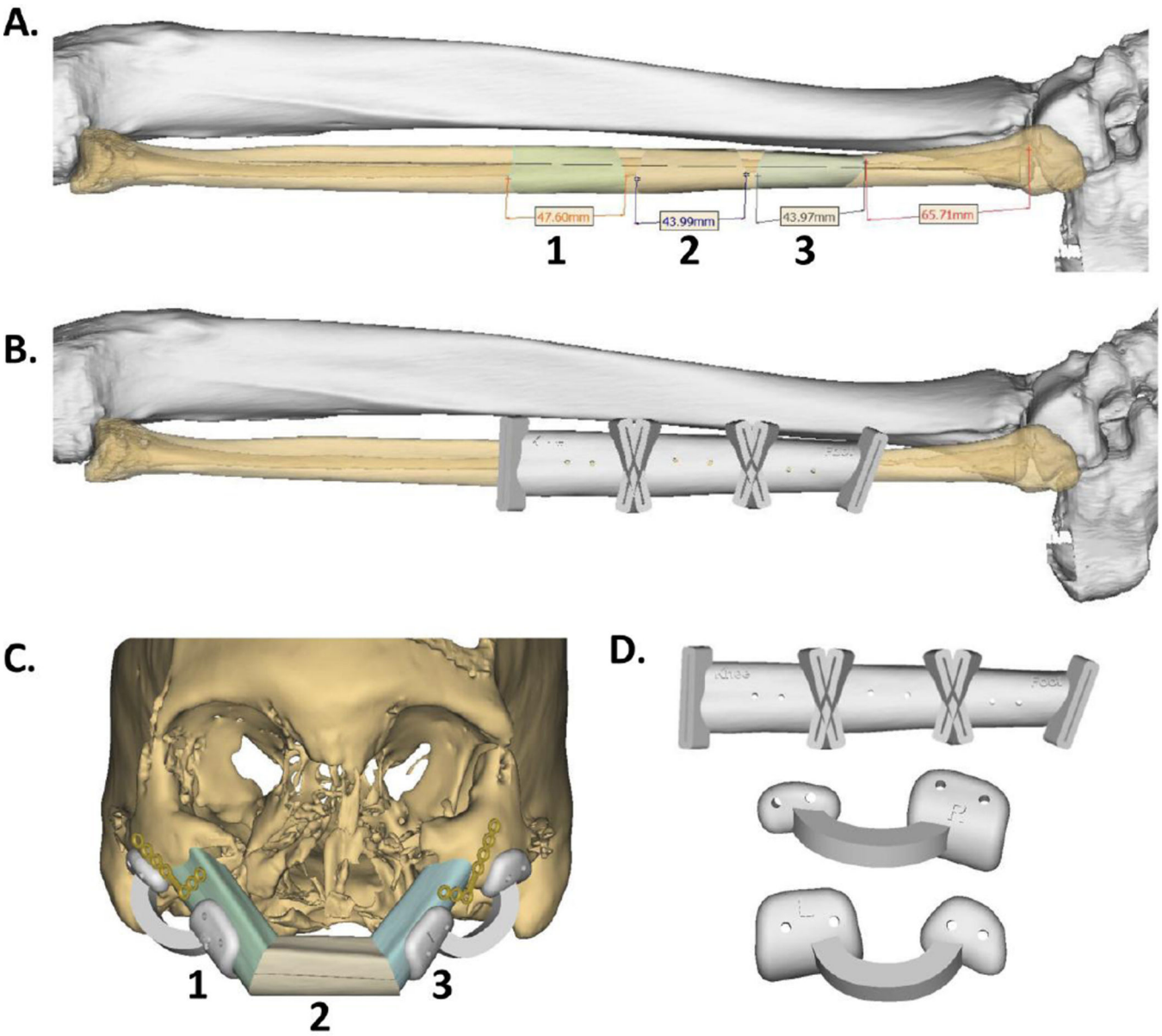

Figure 3.

Synthes Pro Plan Virtual Surgery Plan and 3D-printing Cutting and Placement Guides. (**A**) Pre-operative CT Scan of the right fibula. Graft pieces are labeled beginning 6.6 cm from the distal end of the fibula. (**B**) Planned cutting guide superimposed over the fibula. (**C**) Planned fibular flaps in the context of the remaining zygoma, using the positioning guides and exact graft pieces. (**D**) 3D-printed parts delivered to the surgeon include an anatomic guide, the fibula cutting guide, and positioning guides.

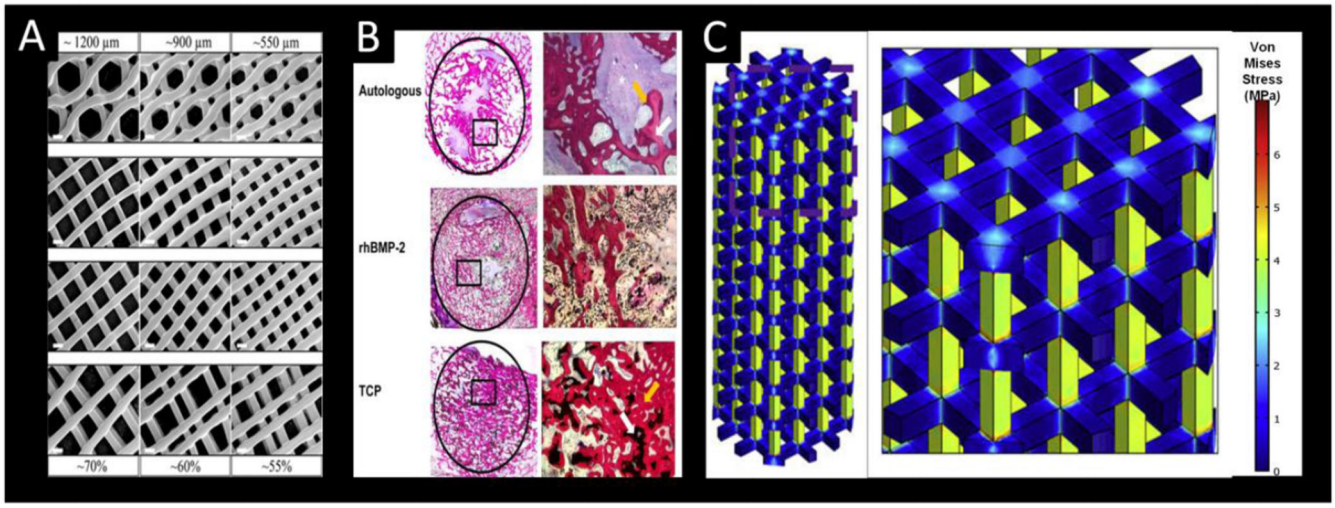

Figure 4.

3D-printed scaffolds. (**A**) The scaffold should have appropriate micro-architecture, encompassing pore size and porosity. Using direct ink writing of a ceramic powder in a viscoelastic solution, different well-defined pore geometries were manufactured and visualized under scanning electron microscopy. Scale bars represent 500 µm. Adapted from[64]. (**B**) Cells residing within the scaffold should be signaled appropriately to regenerate tissue. Sintered tricalcium phosphate scaffolds were implanted in critically sized iliac defects in sheep. Bone formation by resident cells, denoted by the red stain, is evident when compared against other osteoinductive materials (bone morphogenetic protein and autologous bone graft). Adapted from[76]. (**C**) The mechanical properties of the scaffold must be appropriate for the tissue being regenerated. Selective laser sintering of polycaprolactone was used to fabricate a porous cylinder, which was tested mechanically to result in a stiffness of 15 MPa, within the range of trabecular bone. Adapted from[19].

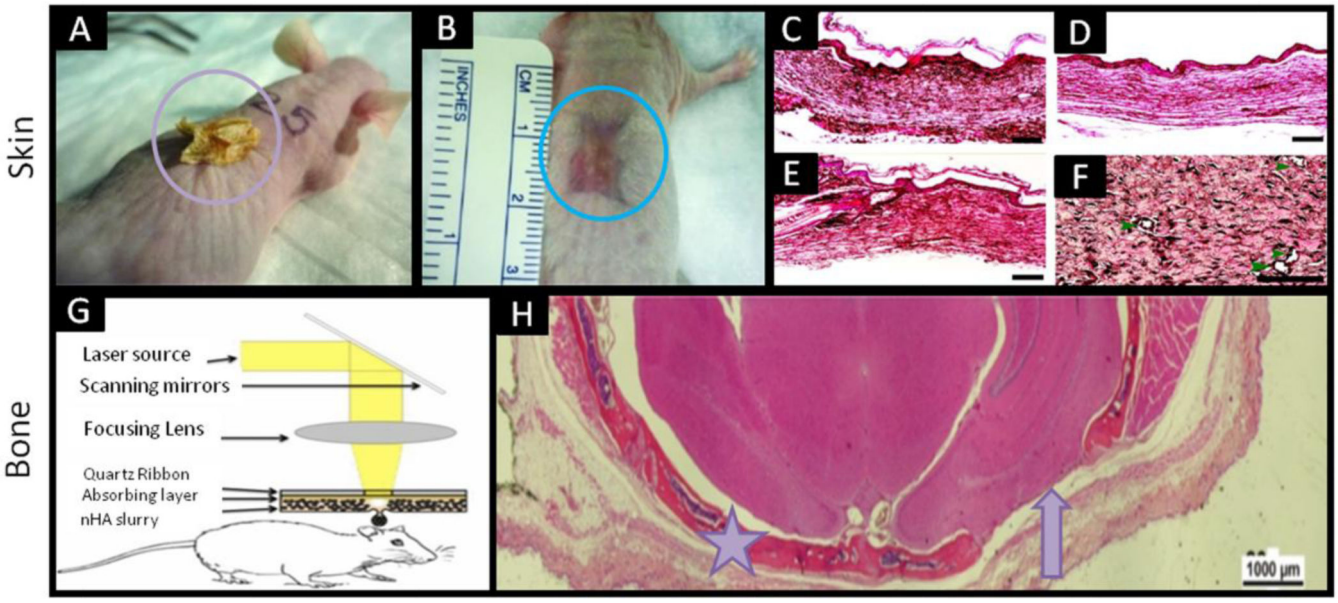

Figure 5.

Bioprinting for engineering skin and bone tissues. Full thickness dermal wounds after 4 weeks of healing with (**A**) Apligraf applied, denoted by the yellow circle and (**B**) 3D-bioprinted skin applied denoted by the blue circle. Severe wound contraction and scaffold drying took place in the Apligraf scaffold compared to the bioprinted scaffold with microvessels. **C–E.** H&E stains of (**C**) Apligraf, (**D**) no treatment, and (**E**) 3D-bioprinted skin scaffold. (**F**) A higher magnification image of 2 weeks of healing following application of 3D bioprinted skin. Adapted from[75]. (**G**) Schematic of laser-assisted bioprinting directly into mouse calvarial defect. nHA slurry refers to a nano-hydroxyapatite suspended in a glycerol solution for printing. (**H**) H&E stain 3 months after calvarial defects were made. Bone healing observed in the area where the 3D bioprinted scaffold was applied (denoted by the star) and no bone healing in the no scaffold control (denoted by the arrow). G and H adapted from[41].

Table 1

Summary of 3D-printing technologies used for treating craniofacial deformities

Treatment Type	3D Printing Applications	Printing Methods	Materials	Qualities of
Prosthetic Rehabilitation (Improve patient aesthetics)	☐ Molds for casting duplicates ☐ Printed prostheses ☐ Surgical guides ☐ Auricle, orbit, and nose rehabilitation	☐ Inkjet ☐ FDM	PDMS ABS PMMA	The material should non-degradable, anatomic shape or create a rigid to case PDMS
Reconstruction (Tissue grafting)	☐ Surgical positioning and cutting guides ☐ Custom metal implants ☐ Bone reconstruction	☐ FDM, ☐ Stereolithography ☐ Laser sintering ☐ Direct-ink writing	Titanium PEEK Polypropylene Bioglasses PLA ABS PMMA Hydroxyapatite	The material should degradable, and that the surgeon graft into place.
Tissue Regeneration (Recapitulate native tissue structure and function)	☐ Scaffold generation ☐ Cellular constructs ☐ Bone, cartilage, skin, muscle ☐ Composite craniofacial tissues	**Acellular:** ☐ SLS ☐ FDM	PCL Calcium Phosphate	The material should and porous. Bioactivity, properties, and should mimic healthy
		Bioprinting: ☐ Inkjet ☐ Extrusion ☐ Laser-assisted ☐ Stereolithography	Fibrinogen Gelatin Alginate	The material should Bioactivity and of the native tissue mimicked.