Role of Microvascular Free Flaps Combined with Tissue Engineering

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17.1 Tissue Engineering

The application of tissue engineering (TE) or regenerative medicine methods for reconstruction of maxillofacial continuity defects remains in its infancy. A number of authors have, however, reported successful use of TE for mandible reconstruction in humans and subhuman primates [1]. TE products typically fall within the concept of the tissue engineering triad including constructs, stem cells, and signaling molecules (Fig. 17.1a) [2]. Although significant TE research is ongoing, only a few TE products are available to enhance mandibular continuity defect reconstruction, and none of them provide all aspects of the triad. In this section, we will share some areas where TE products can be beneficial as an adjunct to traditional reconstruction with vascularized grafts. When choosing adjunctive products, some properties should be considered. Constructs should remodel and be completely replaced by viable bone during the regenerative process. Currently the most useful available constructs are allogeneic mineralized cancellous bone particles between 250 and 1000 microns. These American Association of Tissue Bank process-compliant grafts provide a surface area and geometry that is biomimetic and supports cell attachment [2]. Attachment is essential for mesenchymal stem cells to express their phenotype. Initially osteoclasts demineralize these grafts permitting appositional bone formation on the surface followed by definitive remodeling that in most areas will completely replace the graft with vital bone. Stem cells can be delivered in non-concentrated and concentrated forms [3]. Minimally invasive trephine systems are available to procure autogenous cancellous bone plugs that keep stem cells in their native sub-sinusoidal environment (Fig. 17.1b, c). Concentrated stem cells can be procured in the intraoperative environment using bone marrow aspiration coupled with concentration (BMAC, Harvest Technologies Corp., Lakewood, CO) (Fig. 17.1d). Bone inductive signaling molecules are extensively researched, and although their clinical use is regulated by the standard of care, their marketing is regulated by the Food and Drug Administration (FDA). Infuse (rhBMP-2/ACS 1.5 mg/ml, Medtronic, Minneapolis, MN) is the only inductive bone regenerative cytokine available for clinical use (Fig. 17.1e–g). It has maxillofacial indications related to extraction socket defects [4] and

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posterior maxillary alveolar deficiency requiring a sinus lift bone graft [5]. Thus, the application of this technology to mandibular continuity defects is off-label [6, 7]. The package insert further states that Infuse should not be used at the site of an extant or resected tumor when malignancy is present or when a patient is being treated for malignancy. Based upon the literature sited above and our experience, we currently apply Infuse as an adjunct to non-vascularized cancellous particulate bone grafts used to treat continuity defects present from trauma or infection or after the resection of benign tumors [8]. In addition, we use it to treat post-malignant tumor resection in malignancy-free patients having a delayed reconstruction or those in need of reconstruction as a result of osteoradionecrosis [9, 10]. The advantage of combining Infuse with other reconstructive techniques is the rapid formation of an osteogenic granulation tissue that provides a rich connective tissue and vascular environment to support regeneration (Fig. 17.1h).

17.2 Combining Tissue Engineering Principles with Free Tissue Transfer

Numerous technological advances have opened up a wide range of options for maxillofacial reconstruction. Traditional bone graft stood the test of time and is still widely used with success. Bone morphogenetic protein (BMP) whether used alone or in combination with bone graft has greatly contributed to the field of TE. Distraction osteogenesis has been utilized successfully for augmentation of the alveolar ridge and segmental mandibular defects [2]. Prosthetic total joint replacement made possible functional reconstruction of the missing temporomandibular joint (TMJ). The introduction of free vascularized tissue transfer was a game changer and quickly revolutionized oromandibular reconstruction. This treatment modality gained popularity for composite mandibular defects due to their reliable ability to reconstruct simultaneously both missing soft and hard tissues even in a compromised wound.

In addition, the utilization of virtual surgical planning (VSP) greatly added to surgical precision and efficiency [11]. The virtual environment allowed surgeons to accurately and safely plan surgical osseous resection margins, decrease surgical time by giving the reconstructive team the ability to produce a stereolithographic model and pre-bend reconstruction plates, or be able to create a computer-aided design-computer-aided manufacturing (CAD/CAM) plate which is commonly referred to as a custom-made plate. Finally VSP allows regaining of pre-surgical mandibular contour due to the ability of mirroring contralateral, unaffected mandible or utilizing a generic mandibular form [12, 13].

However, none of the existing technological developments alone can perfectly satisfy the complex anatomical and functional needs of the mandible. Each option has specific indications dictated by the disease, defect, patient, and clinician factors. Combining different reconstructive modalities may bring together the advantages of each option.

17.2.1 Hybridized Mandibular Reconstruction (HMR) with FFF and Tissue-Engineered Graft

In HMR, superiorly positioned FFF is combined with inferiorly positioned tissue-engineered graft, i.e., a mixture of BMP, BMAC, and corticocancellous bone graft (Fig. 17.2), in order to precisely recreate the native height of the mandible, to place the dental implants in an ideal restorative relationship to the maxillary arch, to improve the bony union between the fibula-fibula and the fibula-native mandible interface, to recreate the depth of vestibule for manipulation of food and saliva, and to accelerate the bony remodeling of the fibula bone to transform into the shape and quality of the premorbid mandible (Fig. 17.3a–j).

The position of the fibula is dictated by the future prosthetic restoration based on the “reverse engineering” or “prosthetically driven planning” principle [14]. VSP (Medical Modeling Inc., Golden, CO) allows easy translation of this pre-

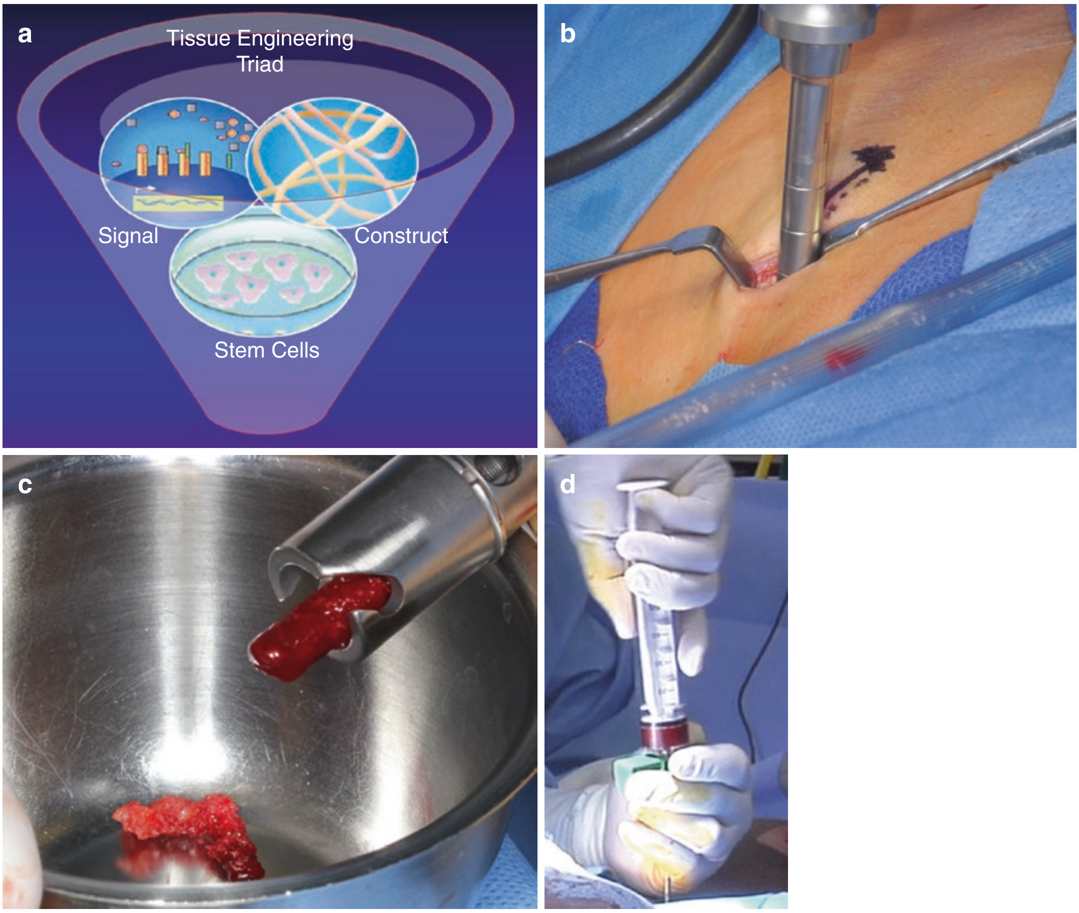
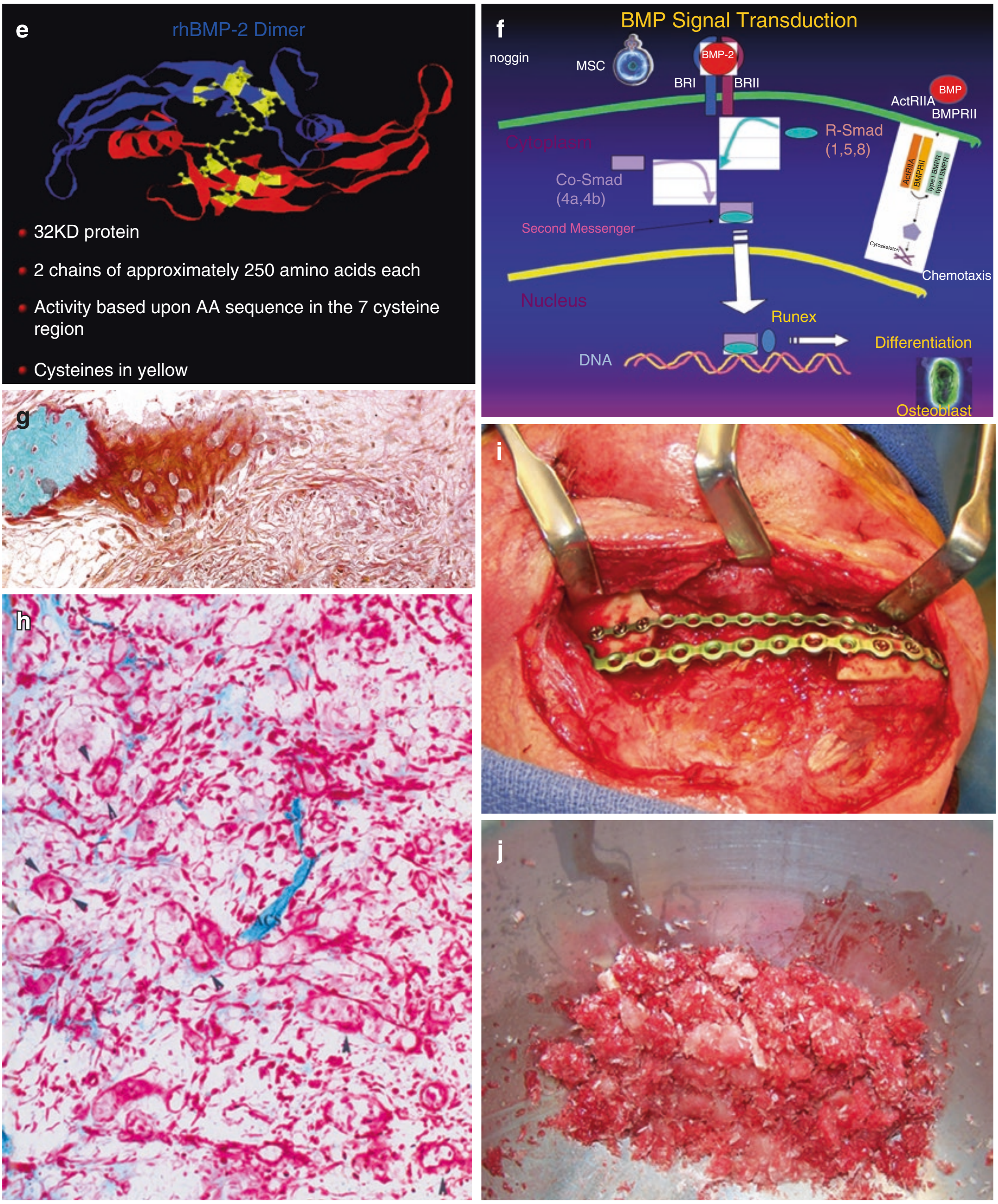
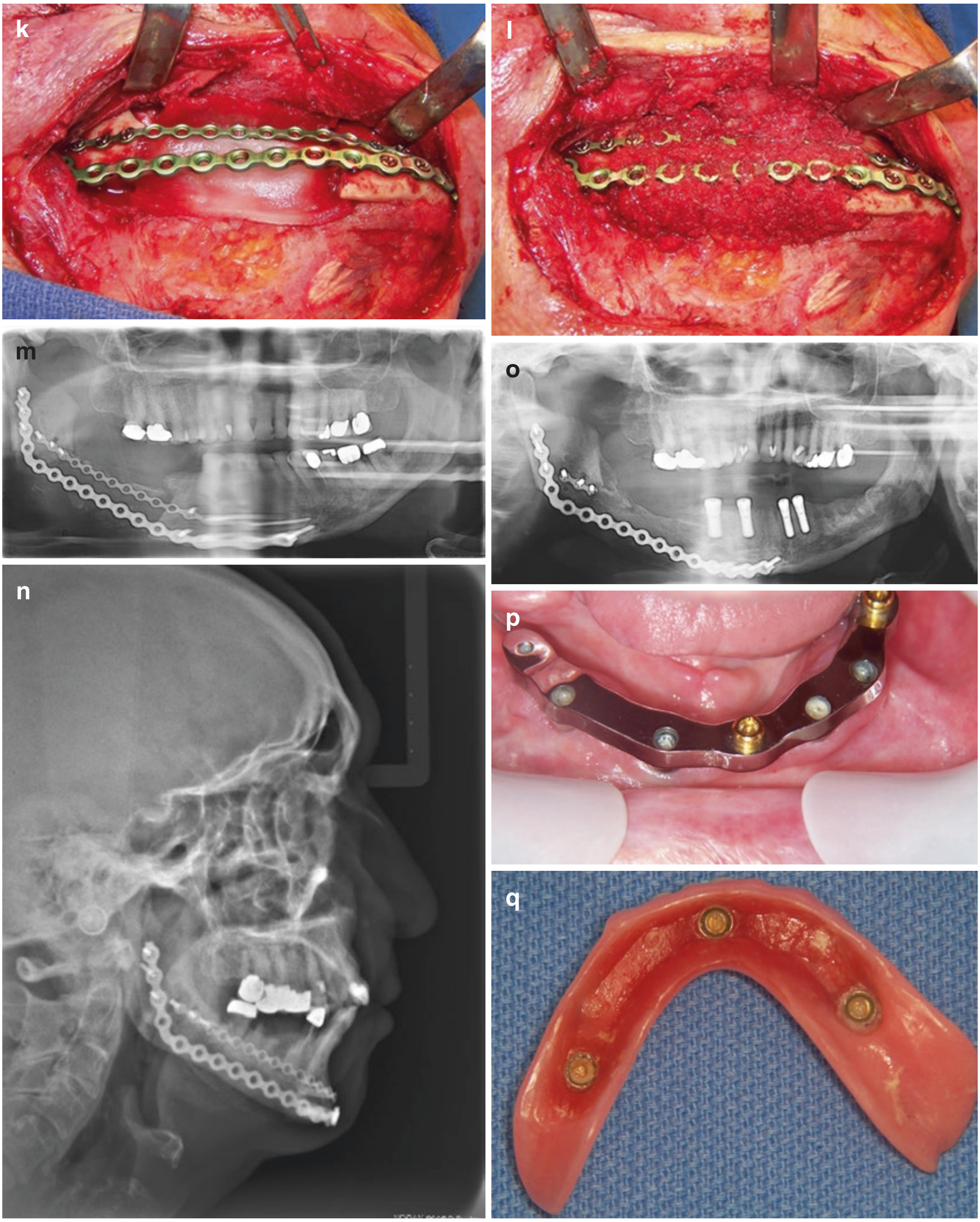


Fig. 17.1 (**a**) The concept of tissue triad with mesenchymal stem cells, scaffolding constructs, and signaling molecules. (**b**, **c**) A trephine system is used to harvest a core of autogenous cancellous bone graft and marrow containing mesenchymal stem cells from the anterior iliac crest. (**d**) Concentrated bone marrow aspirate is collected by the BMAC system from the anterior iliac crest. (**e**) This diagram depicts the molecular structure of rhBMP-2. (**f**) BMP signal transduction leads to osteoblast differentiation of mesenchymal stem cells. (**g**) Microscopic view (40×) of de novo bone formation by BMP 6 months after placement. (**h**) Microscopic view (10×) of the osteogenic granulation created by BMP 10 days after placement in a canine model. (**i**) A 6 cm mandibular continuity defect from osteoradionecrosis resection. Both upper and lower border plates serve as a scaffold for the tissue-engineered graft and define the shape of the mandible. (**j**) Tissue-engineered graft with cancellous bone graft combined with BMP (Infuse, Medtronics). (**k**) The wound bed is first lined with a collagen sponge soaked with BMP. (**l**) Placement of tissue-engineered graft in the defect. (**m**) Postoperative panorex shows adequate alignment of the plates and tissue-engineered graft. (**n**) Postoperative lateral cephalogram shows anatomic recreation of the mandible. (**o**) Dental implants are placed 7 months post-graft placement. Mandibular continuity is reestablished. The superior border plate is removed if necessary for patient comfort. (**p**) An implant bar supra-structure is fabricated. The depth of vestibule on the reconstructed side is similar to the unaffected side. (**q**) The intaglio surface of the denture with locator attachments



- 32KD protein
- 2 chains of approximately 250 amino acids each
- Activity based upon AA sequence in the 7 cysteine region
- Cysteines in yellow

Fig. 17.1 (continued)



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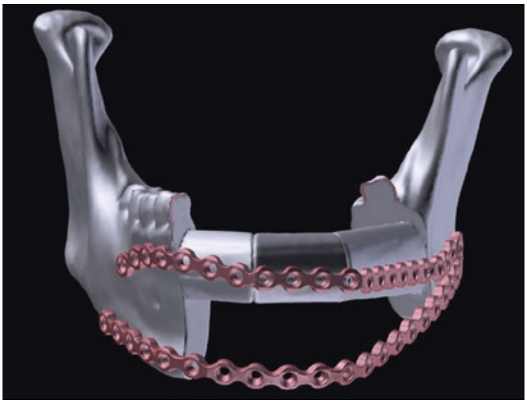


Fig. 17.2 Superiorly positioned fibula provides a sturdy platform for dental implants, while inferiorly placed tissue-engineered graft forms the basal mandibular bone for structural support and lower facial contour. The superior reconstruction plate secures the fibula, while the inferior reconstruction plate provides a scaffold for the tissue-engineered graft

prosthetic plan into the actual surgery (Fig. 17.4). The fibula is placed at an ideal restorative position in relation to the opposing maxillary dentition or a pre-scanned surgical stent in the virtual surgical environment. Even dental implants can be virtually placed if desired. The surgeon is provided with a fibula-cutting guide, a mandibular resection guide, a fibula-positioning guide, and/or a pre-bent plate in order to precisely carry out the plan in the operating room.

One underappreciated downside of FFF is a relatively high incidence of nonunion at the closing osteotomy sites. Due to the straight shape of the fibula bone, the natural contour of the mandible necessitates multiple closing osteotomies of the fibula permitted by its segmental blood supply [2]. Before the VSP technology with cutting guides was available, the osteotomy had to be performed freehandedly and was often less than perfect. Because of the cortical nature of the fibula bone, if bony contact was not adequate, the area often healed with fibrous union. This led to eventual plate fracture and reoperation for the patients. Virtual surgical technology probably has greatly reduced the incidence of nonunion, but many microvascular surgeons agree that achieving perfectly adapted closing osteotomy sites is still challenging even with cutting guides. BMP has shown to improve bony union at the FFF closing osteotomy sites, and HMR with addition of BMAC and corticocancellous bone graft may further ameliorate bony healing [9]. The same rule applies to the FFF reconstruction in mandibular reconstruction in the subsigmoid region. After subsigmoid osteotomy, the proximal segment is usually left with a very narrow cut surface (5–10 mm). When fibula is placed against this surface, either weak bony union or nonunion is expected. When osteotomy surfaces are not perfectly adapted, the use of BMP is likely to improve bony union (Fig. 17.5a–g).

HMR is currently contraindicated in cancer population, due to the possibility of tumor proliferation from BMP, albeit controversial [9, 15], and unpredictable success of immediate bone graft in the face of radiation and oral contamination. In these patients, a prosthetic material such as polyetheretherketone (PEEK) (Fig. 17.6a–d) or a thicker titanium reconstruction plate (Fig. 17.7) may be utilized to fill the inferior border defect below the fibula segments. This technique is less ideal than tissue-engineered graft with de novo bone formation and healing but would satisfy both functional and esthetic requirements of mandibular reconstruction.

17.3 Axially Vascularized Tissue-Engineered Constructs

Despite significant advances in reconstructing maxillofacial ablative defects with the advent of microvascular surgery and the popularization of various osteocutaneous free flaps, some limitations exist in current reconstructive techniques. Currently, the microvascular free flaps most commonly used in reconstructing maxillofacial defects include the fibula, the ilium, and the scapula. These flaps all have unique indications as well as limitations.

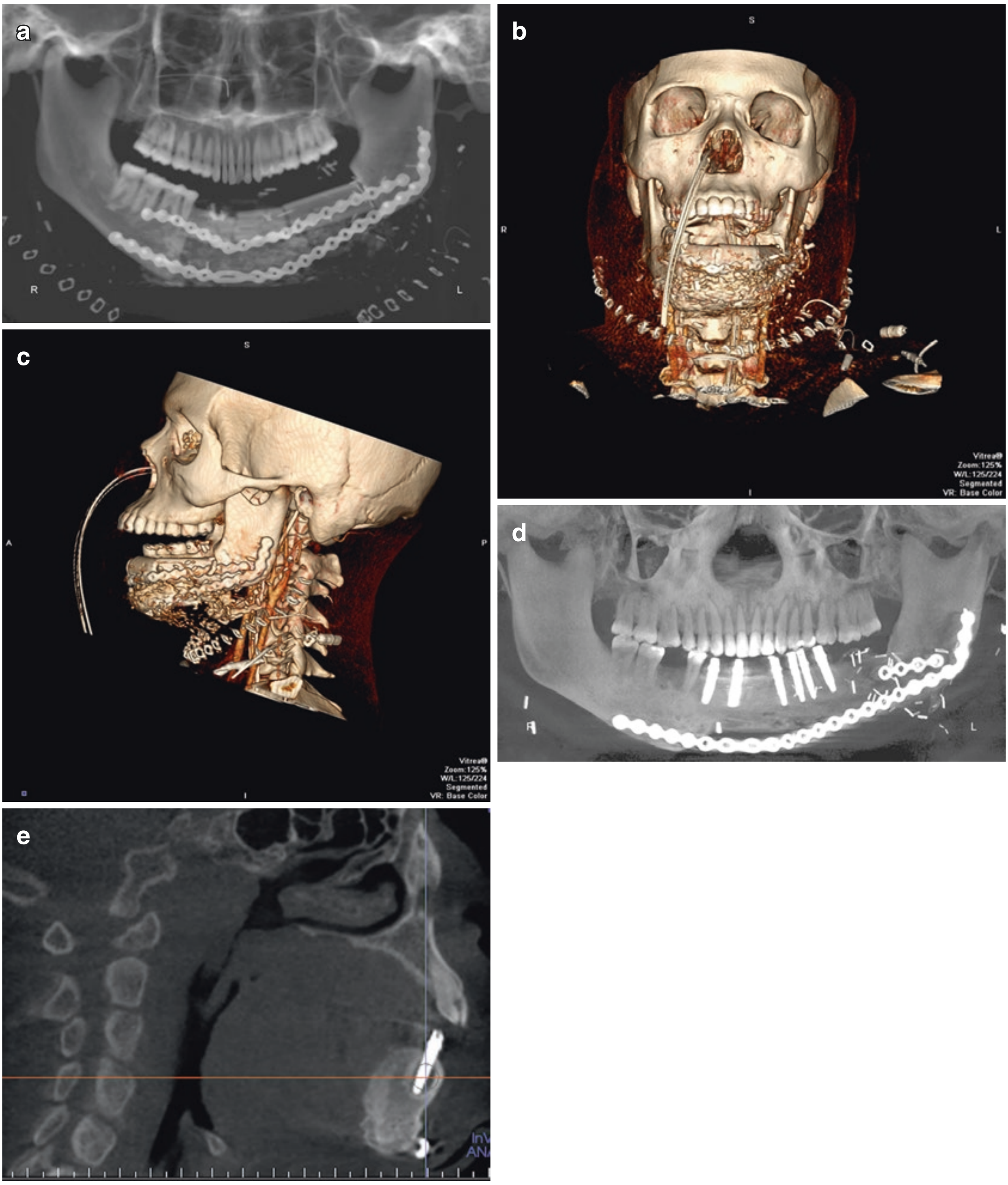


Fig. 17.3 (**a**) This panorex shows HMR after segmental mandibular defect from ameloblastoma. Superiorly positioned fibula segments and inferiorly placed tissue-engineered graft are noted. (**b**, **c**) Three-dimensional images of HMR show excellent anatomic reconstruction of the mandible. (**d**) Complete bony union and remodeling are noted after 6 months. (**e**, **f**) Dental implants are placed at ideal angulations and positions at 9 months, which is possible due to the precise virtual surgical planning. Also, complete remodeling and fusion of the fibula bone with the tissue-engineered graft are noted. The muscular action in the mentolabial region has recreated the B point. (**g**, **h**) Final prosthesis with excellent occlusion is noted in addition to recreation of the premorbid vestibular depth and form. (**i**, **j**) Another HMR case showing recreation of the premorbid vestibular depth and form

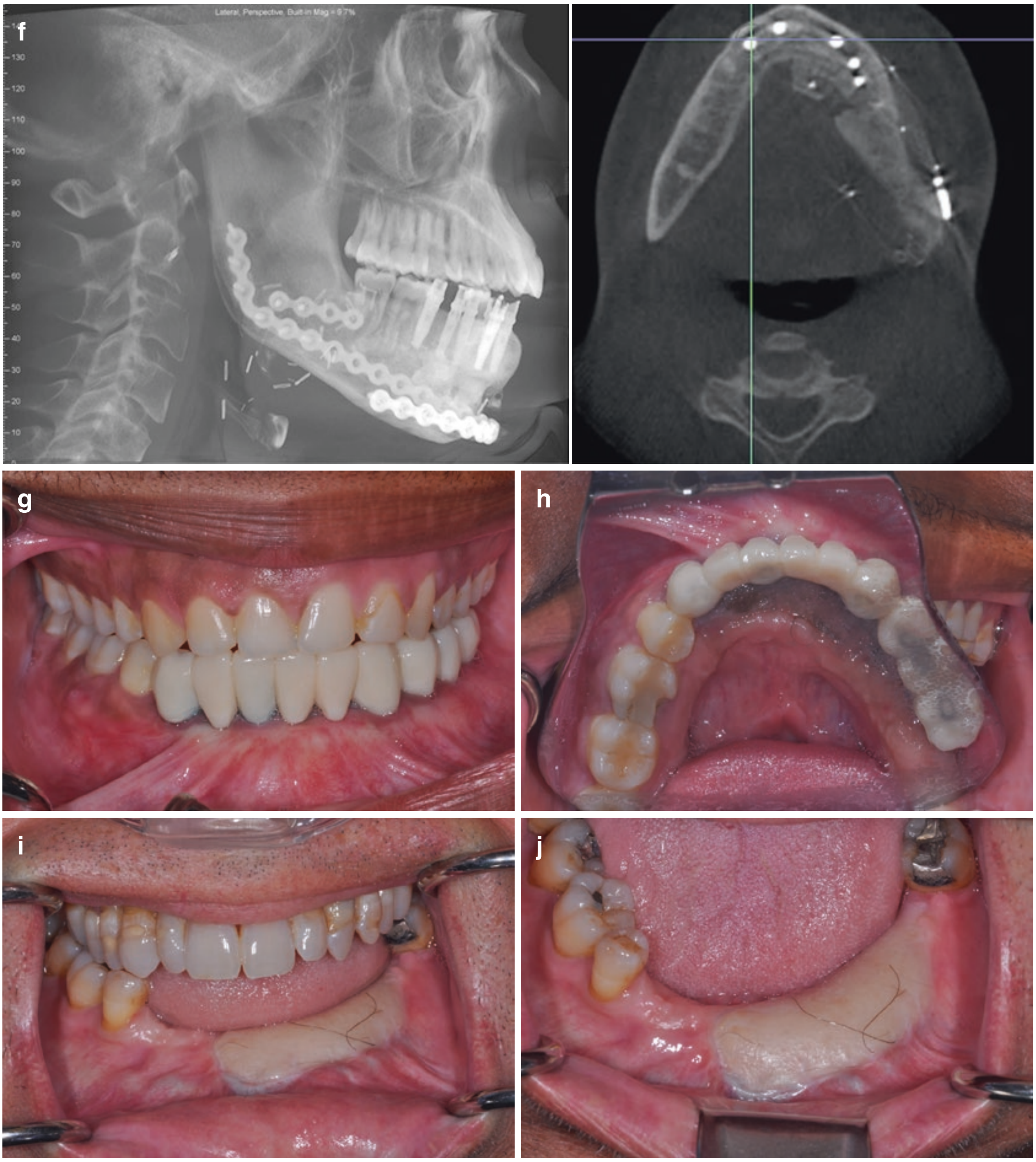


Fig. 17.3 (continued)

A detailed discussion of these flaps is beyond the scope of this text. Briefly, the fibula is able to provide the longest segment of vascularized bone (up to 25 cm) and soft tissue and is thus a popular choice for reconstructing mandibular defects. However, it has significant limitations owing to its limited bone height which makes it difficult to reconstruct mandibular contour while also creating a prosthetically favorable platform for dental reconstruction [2, 11, 16, 17] (Fig. 17.8a–f). Bahr et al. tried to overcome this problem by introducing the double-barrel technique in an attempt to overcome the height mismatch at the interface between the native mandible and fibula free vascularized flap segments. This technique allows improved occlusal relationship with the maxillary dentition but has several limitations as this technique cannot be used for large mandibular segmental defects and leads to significant shortening of the vascular pedicle, which limits your options for microvascular anastomosis [18].

Vascularized iliac crest bone can be used to provide more abundant bony height and width and often has a contour that more accurately recreates the native mandible in comparison to the fibula bone. Nevertheless, it is limited in the length of the bone that can be harvested safely and thus is unsuitable for reconstruction of the long mandibular defects especially with the lack of segmental perforators, which makes closing osteotomies less reliable. Furthermore, it has the potential for significant donor site morbidity such

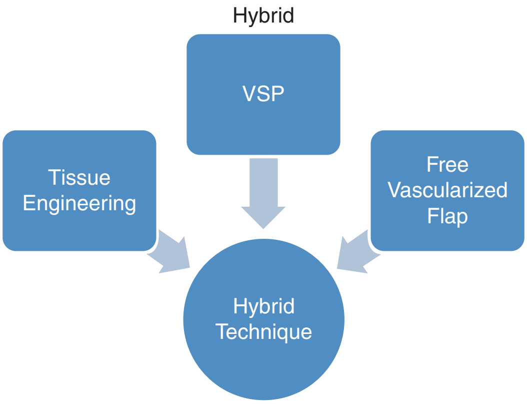


Fig. 17.4 The concept of HMR technique combining FFF, tissue engineering, and virtual surgical planning

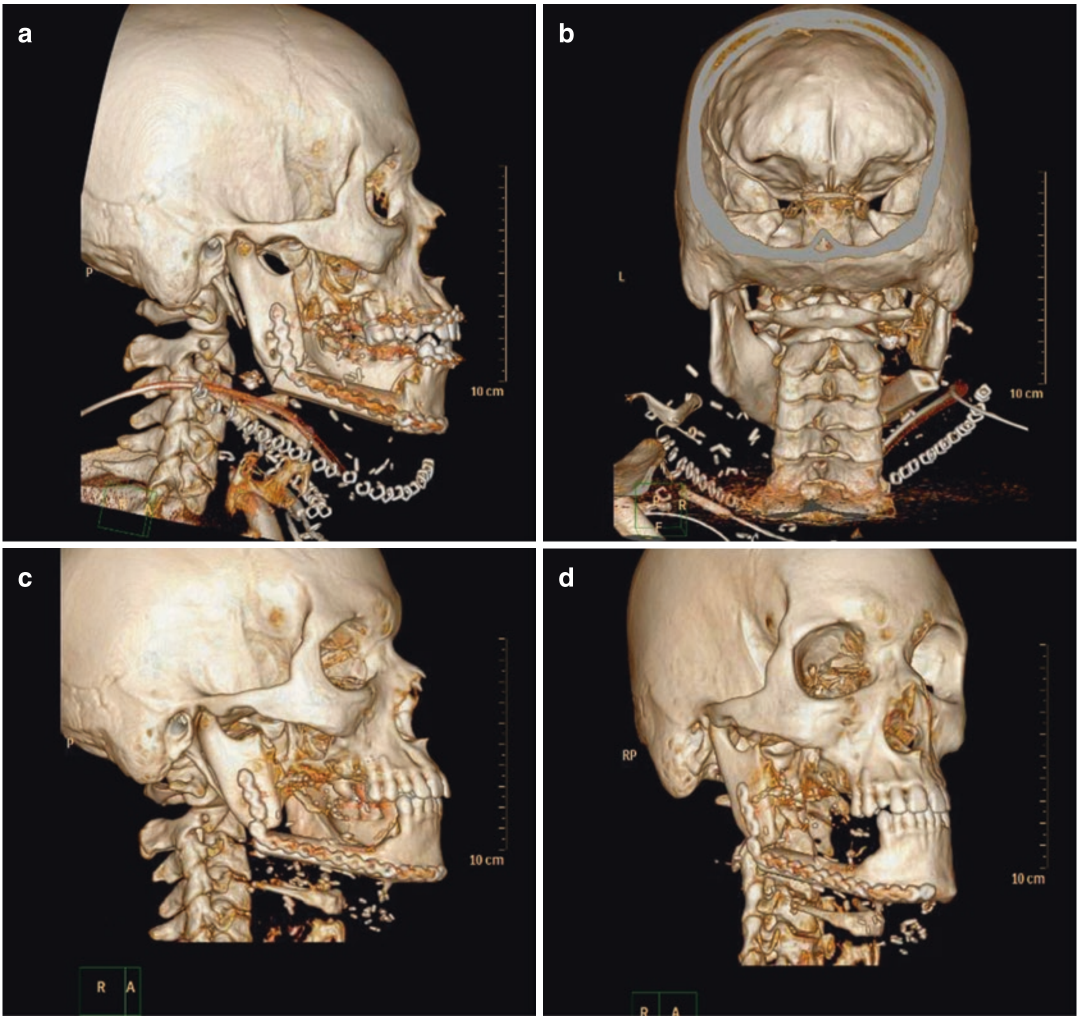


Fig. 17.5 (**a**, **b**) Poor bony contact between the native mandible and the fibula is noted in the subsigmoid region. (**c**, **d**) Nonunion and hardware fracture at 6 months post-op. (**e**–**g**) Reoperation was undertaken with reconstruction plate replacement in addition to the anterior iliac crest bone graft, BMP, and BMAC

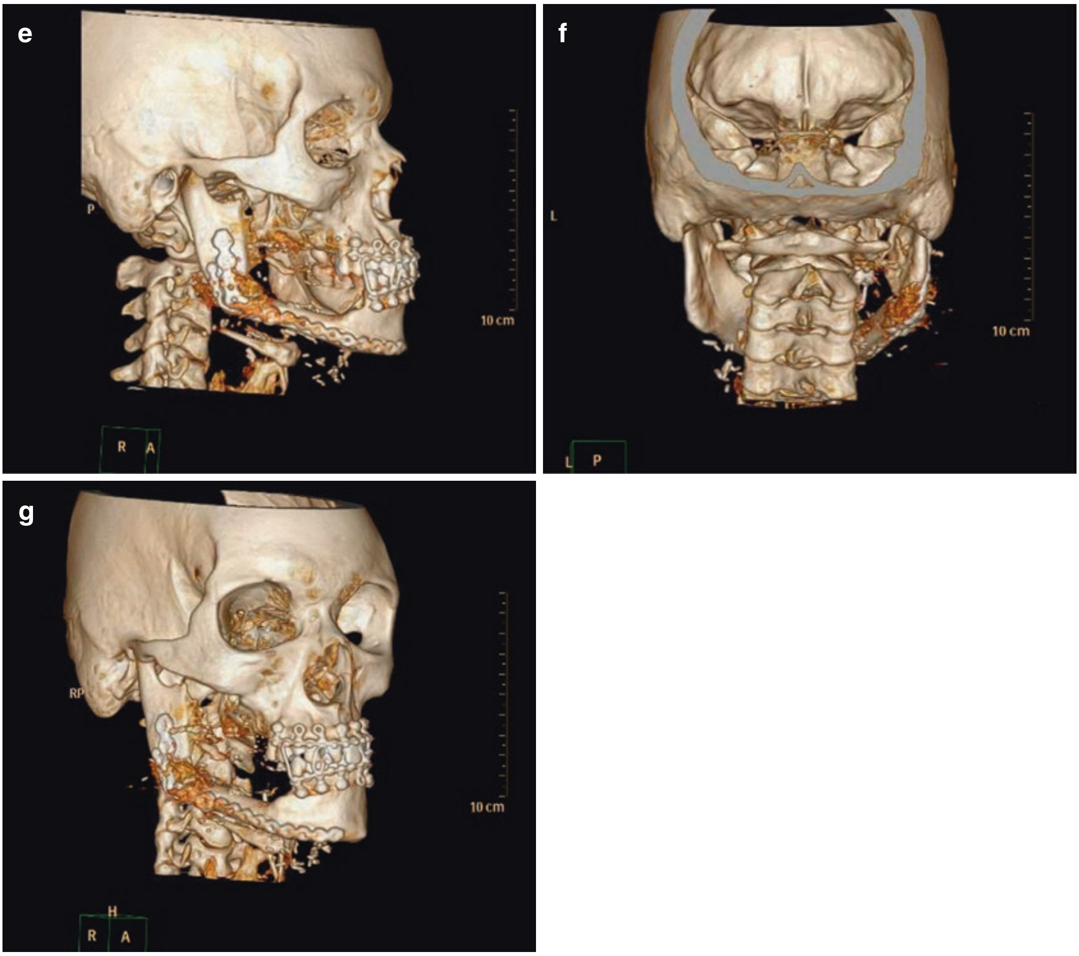


Fig. 17.5 (continued)

as the development of incisional or inguinal hernias and gait disturbance and is limited by a shorter pedicle length [14, 19, 20] (Fig. 17.9a–f).

The osseocutaneous scapula flap has a limited bone stock and is often too thin to accommodate dental implants. The scapular tip is an excellent choice for reconstruction of palatal defects as its shape closely approximates that of the palate (Fig. 17.10) [19, 20]. In summary all the current reconstructive options suffer from limited anatomic compatibility with the defects they intend to reconstruct. Furthermore, the harvest of composite flaps will always result in varying degrees of donor site morbidity. Thus, it is evident that novel approaches need to be developed.

Regenerative medicine and tissue engineering attempt to reconstruct diseased tissue or ablative defects with viable healthy tissue, similar in function and appearance to the native tissue being reconstructed. Consequently, there is a need for a method to create a three-dimensional medium that provides structural support and allows for the migration and differentiation of cells and appropriate release of growth factors [21–23]. In tissue engineering, such a structure is known as a scaffold. Early techniques for creating scaffolds involved solvent casting, particulate leaching, gas foaming, phase separation, melt molding, and emulsion freeze-drying [21–23]. These techniques had limitations as they were unable to produce precise geometry, appropriate pore sizes, and mechanical strength [21–23]. The advent of multiple 3D printing techniques including direct 3D printing, fused deposition modeling, stereolithography, and selective laser sintering has allowed researchers to produce structures with

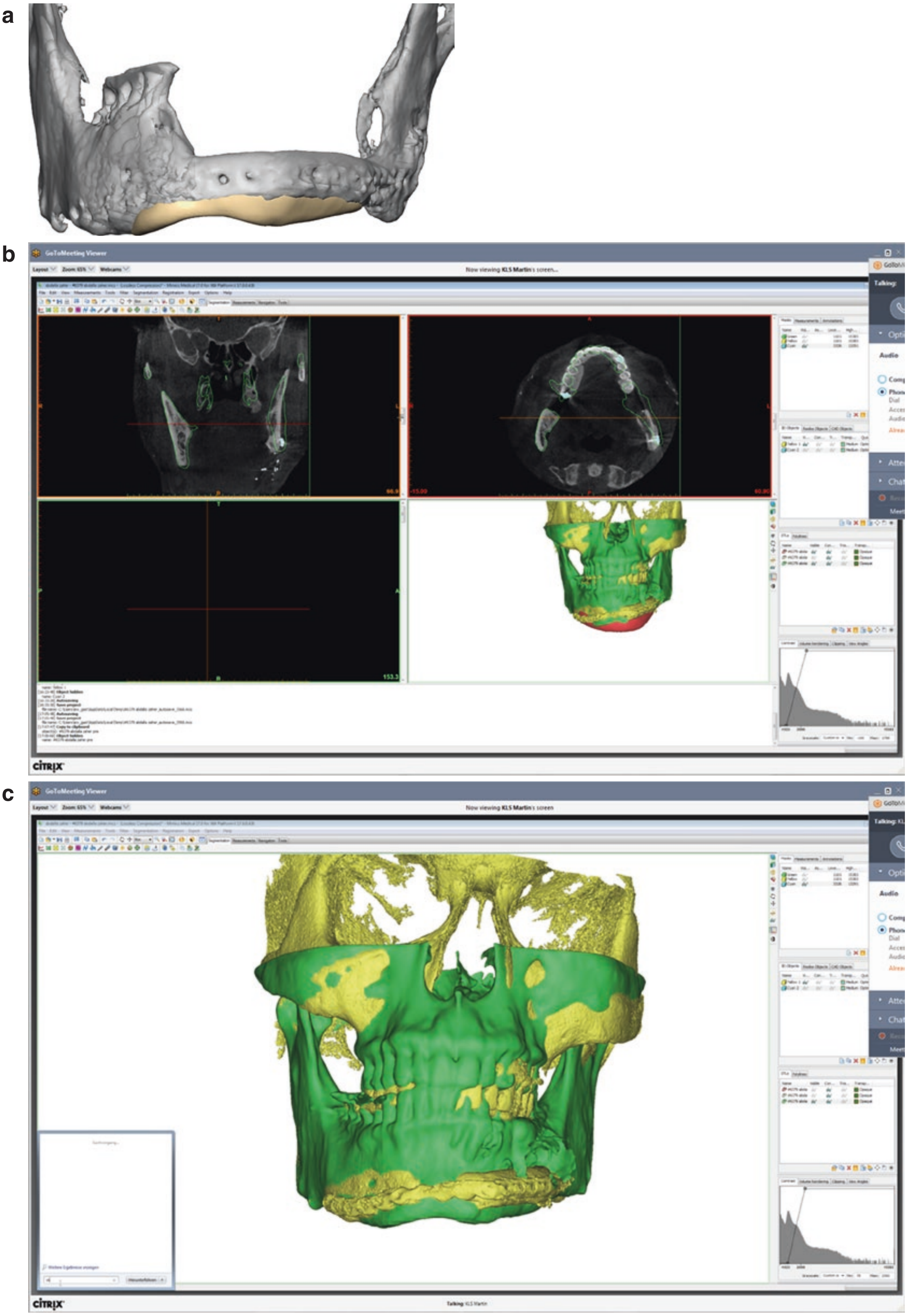
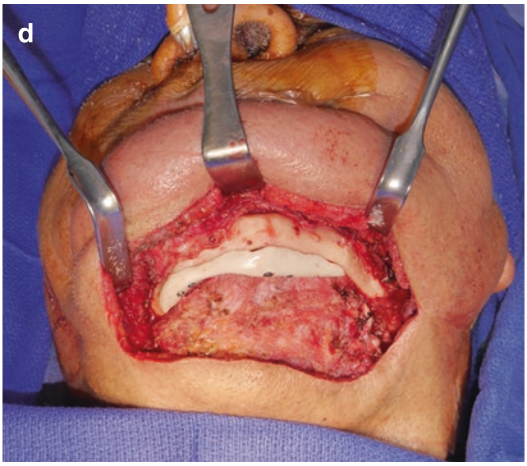


Fig. 17.6 (**a**) A PEEK implant is designed for the inferior border defect due to the patient's refusal of tissue-engineered graft. (**b**, **c**) VSP images. (**d**) Intraoperative picture of the implant, augmenting the inferior border of the fibular reconstruction



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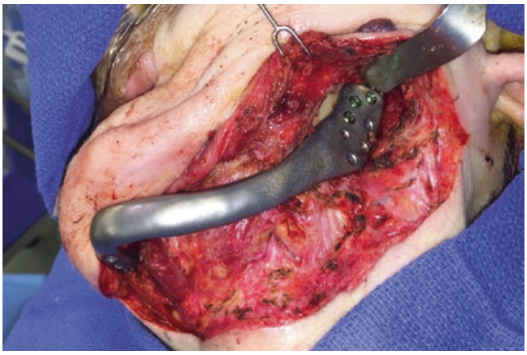

Fig. 17.7 A custom-designed plate with a thicker profile was used in this medically compromised patient in order to prevent hardware fracture in the long term

complex morphologies and precisely controlled pore sizes, on a nanometer scale, that are able to more accurately reproduce the native extracellular matrix, thus creating an ideal environment for tissue regeneration [21–24].

There are a variety of materials used for scaffold creation. Perhaps the most promising in terms of applications for maxillofacial reconstruction is the use of biocompatible ceramics. These are promising because of their osteoconductive potential and mechanical strength, making them an excellent substrate for regeneration of the maxillofacial skeleton. Hydroxyapatite (HA) in particular is the predominant mineral compound in both teeth and the skeleton. Animal studies suggest that scaffolds with pore sizes similar to that of newly formed trabecular bone, in the range of 250–400 μm, are effective at conducting osteoblasts and regenerating bone in critically sized defects [25]. Other researchers have demonstrated that similar scaffolds fabricated out of tricalcium phosphate (TCP) with a pore size in the 300 μm range are able to allow nutrient diffusion to the cells contained within [21, 25].

The advent of 3D printing has allowed researchers to create structures that are precisely designed to promote osseoconduction (Fig. 17.11), release inductive factors, and precisely conform to the complex anatomy of the maxillofacial region. Early research in such grafts relied on the graft obtaining neovascularization through peripheral ingrowth of blood vessels [26]. This understandably limits the theoretical application of such grafts to smaller defects and defects with a healthy soft tissue envelope and precludes use in irradiated tissues [26].

Attempts to overcome limited neovascularization of these structures have involved the addition of growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-B) [27–31]. However these techniques have drawbacks, as they rely on recruiting local cells for neo-angiogenesis which may be limited depending on the amount of progenitor cells available in the particular subject [27]. Furthermore, more research is necessary to precisely determine the proper concentrations as well as timing and duration of cytokine release necessary to produce optimum outcomes [27]. The implantation of endothelial progenitor cells (EPCs) into these constructs has shown promise. However, ensuring proper blood supply to tissue-engineered constructs remains a challenge [27].

One novel area of research involves the fabrication of axially vascularized tissue-engineered grafts that can then be harvested and transplanted to the defect site using traditional microsurgical techniques. These techniques aim to implant an osse-

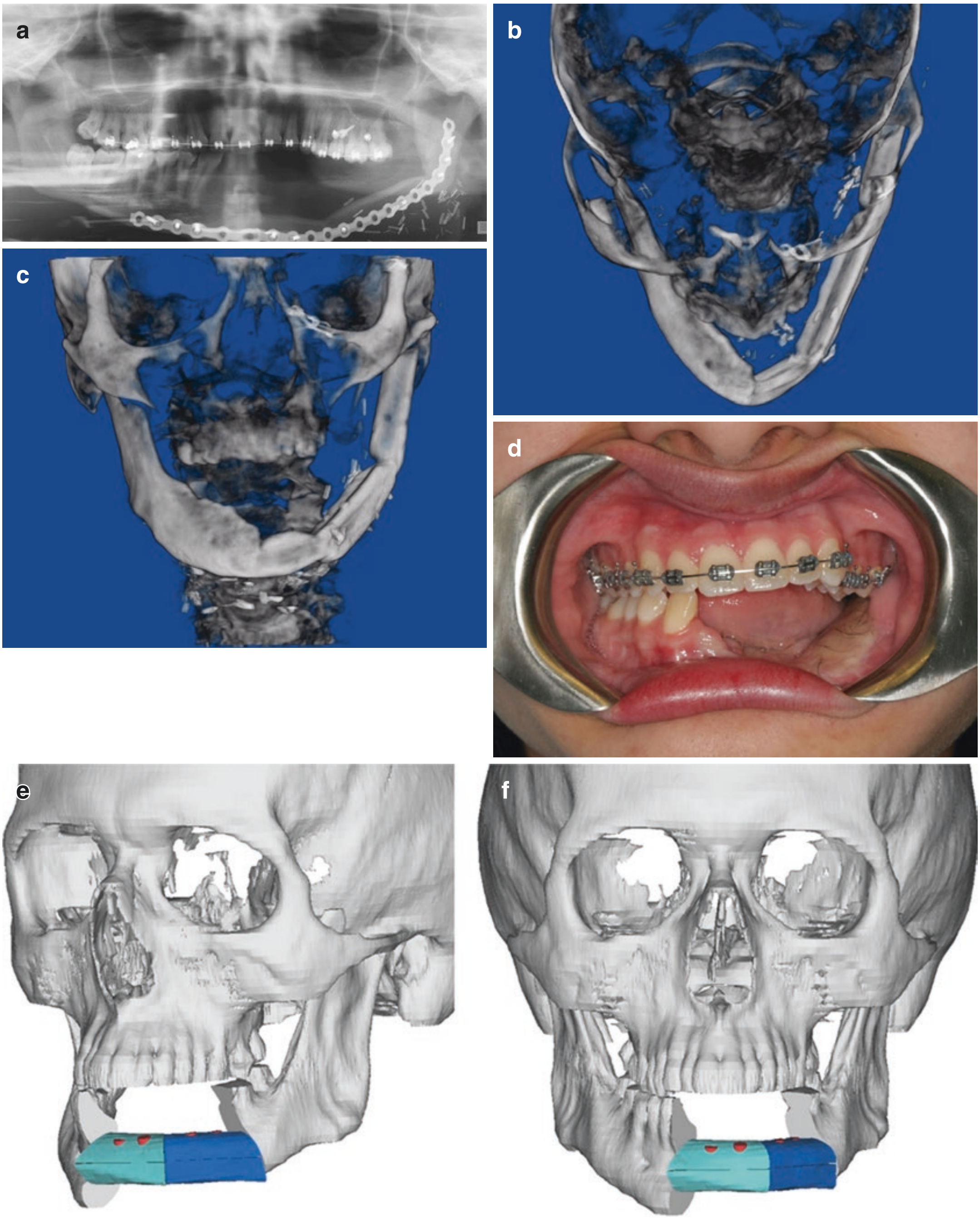

Fig. 17.8 (**a**) The fibula placed at the inferior border. A significant height mismatch with the native mandible leads to a high crown-to-implant fixture ratio if dental implants are placed. (**b**) The fibula is placed too anteriorly in relation to the native maxillary arch. This relation subjects the future prosthesis to a significant cantilever force. (**c**) The fibula is placed too laterally in relation to the native maxillary arch. (**d**) The lack of vestibule results from the inferior positioning of the fibula. (**e**) Superior positioning of the fibula may compromise the lower facial contour

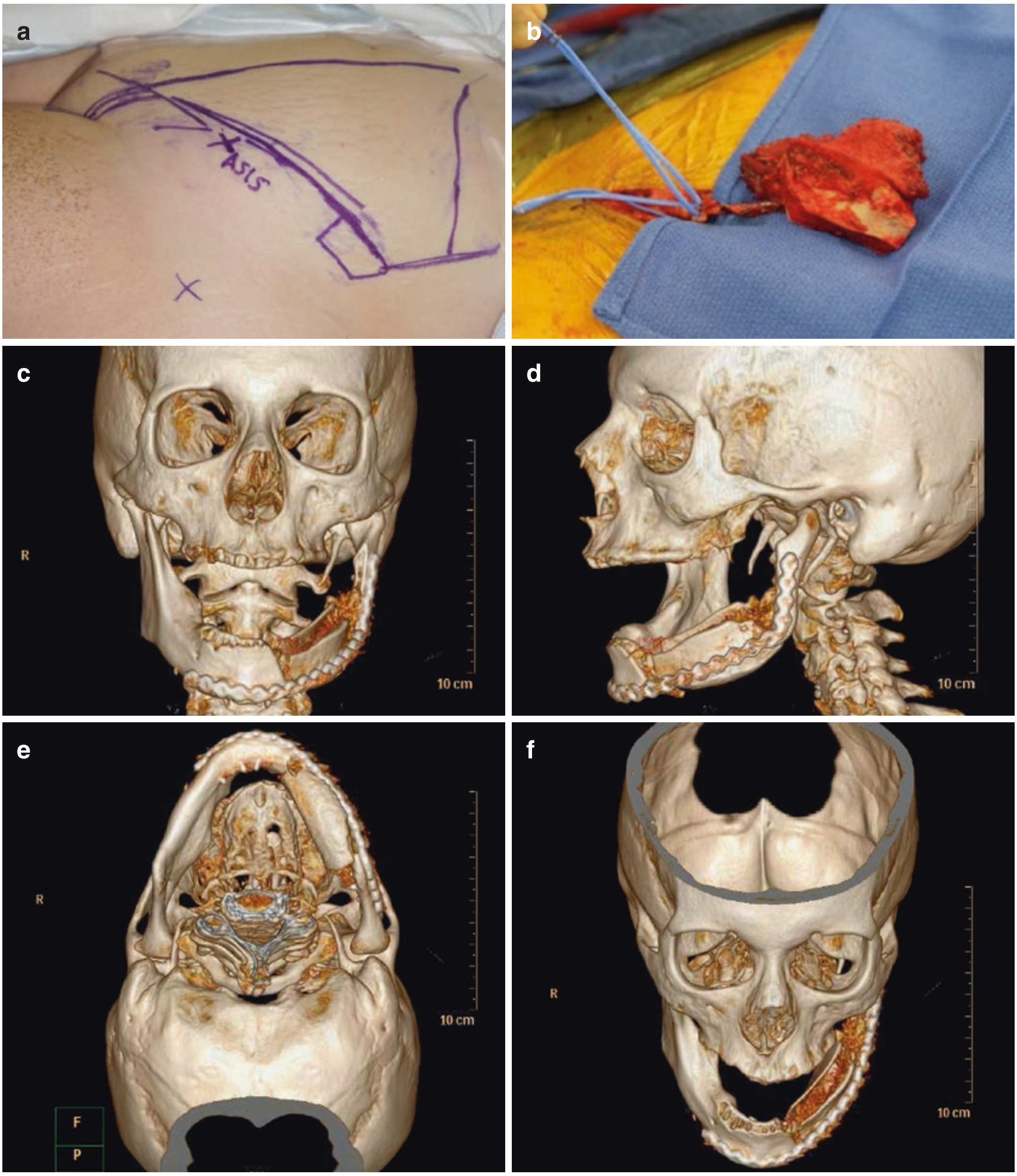

Fig. 17.9 (**a**) Skin markings for the deep circumflex iliac artery flap (DCIAF). (**b**) The iliac crest bone and the internal oblique muscle are harvested in one unit based on the DCIA. Excellent bone stock is noted. The muscle mucosalizes promptly once placed in the oral environment. (**c**, **d**) These three-dimensional images show an excellent height match between the native mandible and DCIAF. (**e**, **f**) The natural curvature of the iliac crest allowed the bone to be placed without closing osteotomies in this case. The width of the bone is more than adequate for dental implantation at the crest level of the neo-mandible

ous scaffold within an axially vascularized territory; thus when transplanted for reconstruction, the construct can be nourished through its axial blood supply in the same manner as a vascularized free flap. Further, these vascularized constructs have the added advantage of perfectly conforming to the defect and avoiding donor site morbidity in a way that a traditional free flap is unable to [26, 32].

There are two main techniques for creating an axially vascularized tissue-engineered construct,

Fig. 17.10 3D CT of a palatal defect reconstructed with a scapular tip flap demonstrating good anatomic conformation but limited bone stock for implant placement

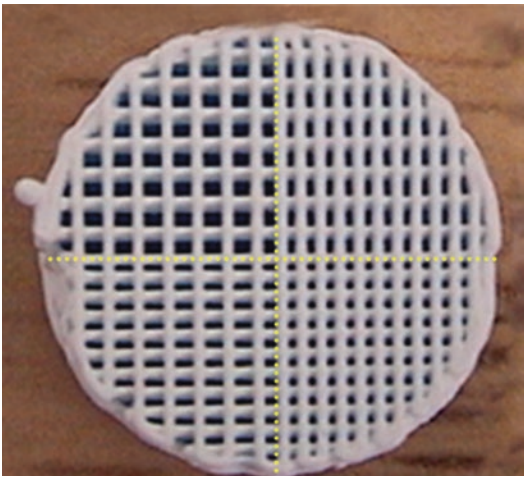

Fig. 17.11 An experimental 3D-printed HA scaffold with controlled porosity

prelamination and prefabrication. Prelamination was first described in 1994 by Pribaz and Fine which most accurately refers to the implantation of a customized structure into the vascular territory of a flap, allowing for extrinsic vascularization of the construct with subsequent transfer to the planned reconstructive site [26, 32, 33]. As an example of this concept, observe Fig. 17.12. In this case a costochondral graft was implanted into the vascular territory of the radial forearm and subsequently used to reconstruct a complete auricular avulsion. There are several reports in the literature where prelamination techniques were used to reconstruct composite mandibular defects. Warnke et al. implanted xenograft blocks with BMP and BMAC into the latissimus dorsi muscle of a patient and then successfully used this construct to reconstruct a composite mandibular defect [26, 32, 34].

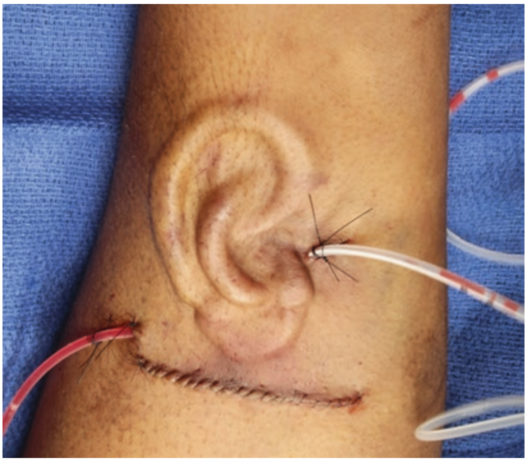

Fig. 17.12 An example of a prelaminated flap designed by implanting a costochondral framework into the vascular territory of a radial forearm free flap

Prefabrication of a flap refers to the implantation of a vascular pedicle into a graft construct such as a custom-designed scaffold that aims to reconstruct a composite osseous defect. Recently, several papers have suggested that the creation of arteriovenous loop (AVL) and implantation of the fistula into the scaffold construct provide for improved vascular supply to the graft. This concept has been supported through several promising animal studies [26, 32, 35–38]. Furthermore, recent studies have demonstrated that such AVL-vascularized constructs are able to maintain viability after exposure to ionizing radiation, suggesting that these techniques may 1 day be used to reconstruct oncologic defects in patients who are likely to undergo adjuvant radiation [35].

17.4 Practical Applications

The theoretical workflow for creating an axillary vascularized construct depends on the clinical situation. If one were to create a prelaminated flap, this necessitates a secondary surgical procedure where the construct is implanted into a flap's vascular territory and time must be allotted for integration and neovascularization to take place [37, 38]. A second operation needs to take place to transplant the construct into the surgical defect. Thus, a major advantage of free tissue transfer, the ability to perform immediate reconstruction, is negated. One suggested workflow for prefabricated flaps using an AVL mitigates this issue. In an experiment conducted in a sheep animal model, Eweida was able to demonstrate the viability of creating an AVL utilizing the facial vessels at the time of mandibular ablation, to successfully revascularize an immediately implanted osseous scaffold [36]. Such a technique may show promise in humans. It remains to be seen how such a technique would address the reconstruction of a composite defect involving soft tissue loss as well as bone.

References

1. Spagnoli DB, Marx RE. Dental implants and the use of rhBMP-2. Oral Maxillofac Surg Clin North Am. 2011;23(2):61, vii. https://doi.org/10.1016/j.coms.2011.02.003.
2. Hidalgo DA, Pusic AL. Free-flap mandibular reconstruction: a 10-year follow-up study. Plast Reconstr Surg. 2002;110(2):1.
3. Marx RE, Harrell DB. Translational research: the CD34+ cell is crucial for large-volume bone regeneration from the milieu of bone marrow progenitor cells in craniomandibular reconstruction. Int J Oral Maxillofac Implants. 2014;29(2):201. https://doi.org/10.11607/jomi.te56.
4. Fiorellini JP, Howell TH, Cochran D, et al. Randomized study evaluating recombinant human bone morphogenetic Protein-2 for extraction socket augmentation. J Periodontol. 2005;76(4):605–13. https://doi.org/10.1902/jop.2005.76.4.605.
5. Triplett RG, Nevins M, Marx RE, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. J Oral Maxillofac Surg. 2009;67(9):1947–60. https://doi.org/10.1016/j.joms.2009.04.085.
6. Carter TG, Brar PS, Tolas A, Beirne OR. Off-label use of recombinant human bone morphogenetic protein-2 (rhBMP-2) for reconstruction of mandibular bone defects in humans. J Oral Maxillofac Surg. 2008;66(7):1417–25. https://doi.org/10.1016/j.joms.2008.01.058.
7. Herford AS, Boyne PJ. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). J Oral Maxillofac Surg. 2008;66(4):616–24. https://doi.org/10.1016/j.joms.2007.11.021.
8. Seto I, Asahina I, Oda M, Enomoto S. Reconstruction of the primate mandible with a combination graft of recombinant human bone morphogenetic protein-2 and bone marrow. J Oral Maxillofac Surg. 2001;59(1):3.
9. Sweeny L, Lancaster WP, Dean NR, et al. Use of recombinant bone morphogenetic protein 2 in free flap reconstruction for osteonecrosis of the mandible. J Oral Maxillofac Surg. 2012;70(8):1991–6. https://doi.org/10.1016/j.joms.2011.08.037.
10. Howard BK, Brown KR, Leach JL, Chang CH, Rosenthal DI. Osteoinduction using bone morphogenic protein in irradiated tissue. Arch Otolaryngol Head Neck Surg. 1998;124(9):985–8.
11. Roser SM, Ramachandra S, Blair H, et al. The accuracy of virtual surgical planning in free fibula mandibular reconstruction: comparison of planned and final results. J Oral Maxillofac Surg. 2010;68(11):2824–32. https://doi.org/10.1016/j.joms.2010.06.177.
12. Tarsitano A, Mazzoni S, Cipriani R, Scotti R, Marchetti C, Ciocca L. The CAD-CAM technique for mandibular reconstruction: an 18 patients oncological case-series. J Craniomaxillofac Surg. 2014;42(7):1460–4. https://doi.org/10.1016/j.jcms.2014.04.011.
13. Bao T, He J, Yu C, et al. Utilization of a pre-bent plate-positioning surgical guide system in precise mandibular reconstruction with a free fibula flap. Oral Oncol. 2017;75:133–9.
14. Carini F, Francesconi M, Saggese V, Monai D, Porcaro G. Implant-supported rehabilitation of a patient with mandibular ameloblastoma. Ann Stomatol (Roma). 2012;3(Suppl 2):21–5.
15. Kokorina NA, Zakharkin SO, Krebsbach PH, Nussenbaum B. Treatment effects of rhBMP-2 on invasiveness of oral carcinoma cell lines. Laryngoscope. 2011;121(9):1876–80. https://doi.org/10.1002/lary.21914.
16. Stosic S. Mandibular reconstruction--state of the art and perspectives. Vojnosanit Pregl. 2008;65(5):397–403.
17. Terry Sua RF. Microvascular reconstruction of the mandible: an argumentfor the fibula osteocutaneous free flap. Rev Esp Cir Oral Maxilofac. 2014;2(36):1–8.
18. Bahr W, Stoll P, Wachter R. Use of the "double barrel" free vascularized fibula in mandibular reconstruction. J Oral Maxillofac Surg. 1998;56(1):38–44.
19. Frodel JL, Funk GF, Capper DT, et al. Osseointegrated implants: a comparative study of bone thickness in four vascularized bone flaps. Plast Reconstr Surg. 1993;92(3):8.
20. Urken ML, Buchbinder D, Costantino PD, et al. Oromandibular reconstruction using microvascular composite flaps: report of 210 cases. Arch Otolaryngol Head Neck Surg. 1998;124(1):46–55.
21. Do AV, Khorsand B, Geary SM, Salem AK. 3D printing of scaffolds for tissue regeneration applications.

Adv Healthc Mater. 2015;4(12):1742–62. https://doi.org/10.1002/adhm.201500168.

22. Sachlos E, Czernuszka JT. Making tissue engineering scaffolds work. Review: the application of solid free-form fabrication technology to the production of tissue engineering scaffolds. Eur Cell Mater. 2003;5:40.
23. Wust S, Muller R, Hofmann S. Controlled positioning of cells in biomaterials-approaches towards 3D tissue printing. J Funct Biomater. 2011;2(3):119–54. https://doi.org/10.3390/jfb2030119.
24. Peltola SM, Melchels FP, Grijpma DW, Kellomaki M. A review of rapid prototyping techniques for tissue engineering purposes. Ann Med. 2008;40(4):268–80. https://doi.org/10.1080/07853890701881788.
25. Simon JL, Michna S, Lewis JA, et al. In vivo bone response to 3D periodic hydroxyapatite scaffolds assembled by direct ink writing. J Biomed Mater Res A. 2007;83(3):747–58. https://doi.org/10.1002/jbm.a.31329.
26. Eweida AM, Horch RE, Marei MK, et al. Axially vascularised mandibular constructs: is it time for a clinical trial? J Craniomaxillofac Surg. 2015;43(7):1028–32. https://doi.org/10.1016/j.jcms.2014.10.018.
27. Zan T, Li Q, Dong J, et al. Transplanted endothelial progenitor cells increase neo-vascularisation of rat pre-fabricated flaps. J Plast Reconstr Aesthet Surg. 2010;63(3):474–81. https://doi.org/10.1016/j.bjps.2008.11.076.
28. Li QF, Reis ED, Zhang WX, Silver L, Fallon JT, Weinberg H. Accelerated flap prefabrication with vascular endothelial growth factor. J Reconstr Microsurg. 2000;16(1):45–9.
29. Gurunluoglu R, Meirer R, Shafighi M, Huemer GM, Yilmaz B, Piza-Katzer H. Gene therapy with adenovirus-mediated VEGF enhances skin flap prefabrication. Microsurgery. 2005;25(5):433–41. https://doi.org/10.1002/micr.20142.
30. Haws MJ, Erdman D, Bayati S, Brown RE, Russell RC. Basic fibroblast growth factor induced angiogenesis and prefabricated flap survival. J Reconstr Microsurg. 2001;17(1):4.
31. Iwasawa M. Accelerated maturation in prefabricated flaps by transforming growth factor-beta: an experimental study in the rabbit. Ann Plast Surg. 1993;31(1):72–5.
32. Eweida AM, Nabawi AS, Elhammady HA, et al. Axially vascularized bone substitutes: a systematic review of literature and presentation of a novel model. Arch Orthop Trauma Surg. 2012;132(9):1353–62. https://doi.org/10.1007/s00402-012-1550-3.
33. Pribaz JJ, Fine NA. Prelamination: defining the prefabricated flap--a case report and review. Microsurgery. 1994;15(9):618–23.
34. Warnke PH, Wiltfang J, Springer I, et al. Man as living bioreactor: fate of an exogenously prepared customized tissue-engineered mandible. Biomaterials. 2006;27(17):3163–7.
35. Eweida A, Frisch O, Giordano FA, et al. Axially vascularized tissue-engineered bone constructs retain their in vivo angiogenic and osteogenic capacity after high-dose irradiation. J Tissue Eng Regen Med. 2016;12(2):e657–68. https://doi.org/10.1002/term.2336.
36. Kokemuller H, Jehn P, Spalthoff S, et al. En bloc prefabrication of vascularized bioartificial bone grafts in sheep and complete workflow for custom-made transplants. Int J Oral Maxillofac Surg. 2014;43(2):163–72. https://doi.org/10.1016/j.ijom.2013.10.013.
37. Eweida AM, Nabawi AS, Abouarab M, et al. Enhancing mandibular bone regeneration and perfusion via axial vascularization of scaffolds. Clin Oral Investig. 2014;18(6):1671–8. https://doi.org/10.1007/s00784-013-1143-8.
38. Leibig N, Wietbrock JO, Bigdeli AK, et al. Flow-induced axial vascularization: the arteriovenous loop in angiogenesis and tissue engineering. Plast Reconstr Surg. 2016;138(4):825–35. https://doi.org/10.1097/PRS.0000000000002554.