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Biomaterial-based bone regeneration and soft tissue management of the individualized 3D-titanium mesh: An alternative concept to autologous transplantation and flap mobilization

Shahram Ghanaati [a, *], Sarah Al-Maawi [a], Torsten Conrad [b], Jonas Lorenz [a], Ralf Rössler [c], Robert Sader [a]

[a] FORM-Lab, Frankfurt Oral Regenerative Medicine, Department for Oral, Cranio-Maxillofacial and Facial Plastic Surgery, Medical Center of the Goethe University Frankfurt, Germany
[b] Private Office, Heinrichstrasse 10, 55411 Bingen am Rhein, Germany
[c] University for Digital Technologies in Medicine & Dentistry (Luxemburg), Luxembourg

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ABSTRACT

Three-dimensional augmentation in severely atrophic bone and after cancer resection is a challenging clinical indication that is mostly solved using autologous bone transplantation. The development of the digital technique along with the additive manufacturing and three-dimensional (3D) printing opened new avenues for reconstructive oral and maxillofacial surgery. Therefore, patient-specific titanium mesh is a novel means of stabilizing the augmentation region using particulate bone substitute materials (BSMs) combined with autologous bone as a minimally invasive concept. However, dehiscence is a frequently reported complication in this field. Therefore, the aim of the present case series was to introduce a biomaterial-based regenerative concept in terms of exposed open healing to overcome the dehiscence related to 3D-titanium meshes. Additionally, this case series presents a novel protocol using a combination of xenogeneic BSMs with an autologous blood concentrate system (platelet-rich fibrin [PRF]) and collagen matrices without any autologous transplantation. Seven patients with alveolar ridge atrophy with different etiologies (cancer resection, severe atrophy after tooth loss, aplasia, trauma, implant infections) were treated using the open-healing concept. Therefore, after 3D augmentation using the described biomaterials, the flap margins were approximated, and the gap between the flap margins was bridged using a collagen matrix loaded with liquid PRF that was then covered by either a PTFE-based membrane or sterile latex. No periosteum splitting was performed at any time point. After a healing period of 4–8 months, all patients received dental implants as virtually planned. Bone biopsies were performed during dental insertion for histological evaluation. The augmentation area displayed a vital and well-vascularized newly formed bone that incorporated the BSM granules to build a hybrid bone. Additionally, open healing resulted in newly formed soft tissue without any signs of scar formation or fibrosis. The regenerated soft tissue was used to build a new flap during implant insertion and showed good functional and aesthetic results after implant insertion. The open-healing concept of the regeneration of the soft tissue along with bone tissue to regenerate a harmonic implantation bed is a minimally invasive intervention without periosteum splitting or large flap mobilization. However, further controlled clinical studies are needed to evaluate this concept in a larger patient cohort to outline the potential clinical benefit.

1. Introduction

Bone atrophy, trauma and oral cancer resection may cause the loss of bone along with tooth loss (Atwood, 1971; Ghanaati et al., 2013). To achieve good long-term results in dental implantology, adequate bone quality and quantity are needed (Nevins and Langer,

* Corresponding author. Klinik für Mund-, Kiefer- und plastische Gesichtschirurgie Universitätsklinikum Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany. Fax: +69 6301 5644.
E-mail address: Shahram.Ghanaati@kgu.de (S. Ghanaati).

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1993). According to the respective stage of atrophy and defect morphology, several concepts are applied to regenerate bone tissue, including autologous bone transplantation and bone substitute materials (BSMs) in terms of guided bone regeneration (Herford and Nguyen, 2015; Sakkas et al., 2017). Severe atrophic jaw and defects resulting from cancer resection are challenging clinical indications that are usually reconstructed using autologous bone transplants (Stirling Craig et al., 2015). However, transplantation, especially from the extraoral region such as the iliac crest, is a major surgical intervention that results in a second wound at the harvesting site and further complications such as pain, the risk of infection and greater patient morbidity (Young and Chapman, 1989). Horizontal and/or three-dimensional (3D) augmentation is a highly challenging indication. In this scenario, the mechanical stability of the transplant or applied biomaterial is essential for the regeneration process (Angelo et al., 2015). Therefore, the application of particulate BSMs in the form of granules alone reach their limits for this field because of the limited mechanical stability.

When considering BSM granules for complex 3D augmentation, a need for additional mechanical stability became obvious. The alloplastic material titanium was widely implemented for bone stabilization in different indications (Proussaefs and Lozada, 2006). The application of titanium in complex GBR started with the use of flexible titanium mesh (Sumida et al., 2015). In this context, BSM granules are stabilized by a flexible titanium mesh, which can be trimmed and adapted to fit the augmentation area (Proussaefs and Lozada, 2006). Many studies have reported clinical success in different defect morphologies. However, this concept is sensitive to technical performance, and a high dehiscence rate (up to 80%) was observed in many cases (von Arx and Kurt, 1999; Cucchi et al., 2017).

The increased importance of digital techniques and the implementation of 3D computer-aided design/computer-aided manufacturing (CAD/CAM) in dentistry allowed the introduction of a novel stabilization concept that is specifically manufactured to fit into the individual defect morphology (Lorenz et al., 2018b; Sagheb et al., 2017). The patient-specific 3D titanium mesh is manufactured based on virtual planning and design using computed tomography (CT) or cone-beam computed tomography (CBCT) data (Sagheb et al., 2017). In contrast to the aforementioned techniques, flexible titanium mesh, patient-specific titanium meshes, are manufactured of rigid titanium (Lorenz et al., 2018b; Sagheb et al., 2017). This is applicable because this concept predefines a fit accuracy to the planed defect without needing any manual trimming and adaptation by the surgeon. Some case reports and single studies have evaluated the application of the novel patient-specific titanium mesh and reported clinical success in many cases. Nevertheless, a relatively high dehiscence rate (up to 30%) was reported in this context, which is a common clinical challenge in all explained 3D augmentation techniques (Lorenz et al., 2018b; Sagheb et al., 2017). This finding calls for further investigation that is focused not only on the augmentation technique but on the whole surgical concept for this indication.

In addition to the mechanical stability, biological aspects are of great importance in complex 3D augmentation in large defects. Therefore, to date, GBR techniques for 3D augmentation primarily use BSMs in combination with autologous bone at a ratio of 1:1 or 1:2 and never use BSMs alone (Sagheb et al., 2017). The combination of autologous bone with BSMs in complex augmentations should provide an osteogenic capacity to support the regeneration process (Lima et al., 2018).

Interestingly, in the last decade, a blood-based autologous system gained increased importance in regenerative medicine (Ghanaati et al., 2014). Platelet-rich fibrin (PRF) is obtained by the centrifugation of patients' own peripheral blood without any additional anticoagulants (Ghanaati et al., 2014). This minimally invasive system provides patients with self-regenerative capacity in the form of a blood concentrate that is composed of platelets, leukocytes and plasma proteins embedded in a fibrin matrix (Ghanaati et al., 2014). The development of the centrifugation protocols using the low-speed centrifugation concept (LSCC) allowed the preparation of highly bioactive PRF matrices by the reduction of the applied centrifugal force (RCF) during centrifugation (Choukroun and Ghanaati, 2018). In this context, PRF matrices that are centrifuged using a low RCF contain a significantly higher number of platelets and leukocytes and release significantly higher concentrations of different growth factors than PRF matrices that are centrifuged using a high RCF (Choukroun and Ghanaati, 2018; Ghanaati et al., 2018a; Kubesch et al., 2018; Wend et al., 2017). Considering the challenges and complications of 3D augmentation outlined here, the aim of the present case series was to introduce a novel surgical technique, including the application of patient-specific titanium mesh. This case series presents for the first time the application of a titanium mesh in combination with xenogeneic BSM and PRF by open healing to overcome dehiscence formation, which is a major complication in relation to 3D augmentation. Additionally, this technique was applied for the treatment of different etiologies in patients needing dental implants.

2. Case series

In the present case series study, PRF is a blood concentrate system gained by the centrifugation of patients' peripheral blood without any additional anticoagulants. According to the respective preparation protocol, it is possible to obtain PRF in both solid and liquid forms (El Bagdadi et al., 2017; Ghanaati et al., 2014; Wend et al., 2017). The application of LSCC by means of a low relative centrifugal force (RCF) allows the preparation of highly bioactive PRF matrices, including a high number of platelets and leukocytes (Choukroun and Ghanaati, 2018). Additionally, PRF matrices that are prepared according to LSCC release high concentrations of different growth factors, including VEGF, EGF, PDGF-BB, PDGF-AB and TGF-β1 (El Bagdadi et al., 2017; Choukroun and Ghanaati, 2018; Ghanaati et al., 2018a; Wend et al., 2017). In the present case series, both liquid and solid PRF matrices were used to biologize bone substitute materials and collagen matrices prior to augmentation. According to the defect size, four to six blood collection tubes of 10 ml each (PROCESS for PRF, NIS, France) were collected from each patient. Subsequently, in small defects, two red tubes (A-PRF,PROCESS for PRF, NIS, France) were used to prepare solid PRF, and two orange tubes (i-PRF, PROCESS for PRF, NIS, France) were used to prepare liquid PRF. For large defects, three tubes were used to prepare each PRF type. Peripheral blood was collected from the medial cubital vein and immediately centrifuged (Duo centrifuge; Process for PRF; radium 110 mm) following the LSCC described by Choukroun and Ghanaati (2018) (Table 1).

After centrifugation, solid PRF matrices were isolated from the resulting red phase, placed on the PRF-box and pressed horizontally to prepare pressed PRF matrices (Fig. 1a and b). Next, the pressed PRF matrices were cut using scissors and combined with the bone substitute granules (Bio-Oss, Geistlich Pharma AG Wolhusen, Switzerland) (Fig. 1c). The liquid PRF was collected using a

Table 1
Centrifugation protocol for platelet-rich fibrin (PRF).

Protocol	Tube	RPM	RCF	Time	Centrifuge radius
Liquid PRF	Orange tube	1200	177×*g*	8 min	110 mm
Solid PRF	Red tube	1200	177×*g*	8 min	110 mm

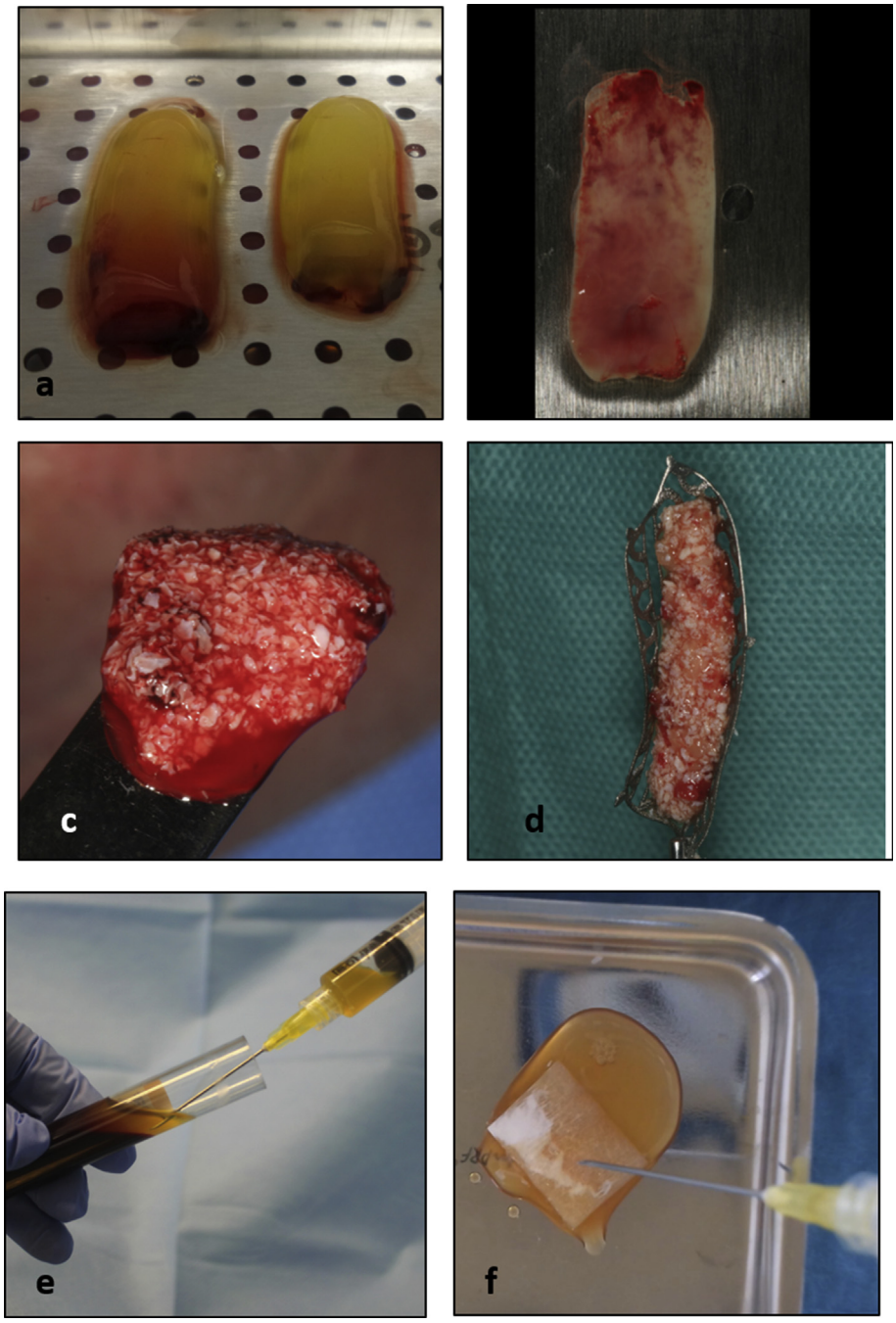


Fig. 1. Preparation of the BSM–PRF mixture. a) Solid PRF matrices directly after isolation from the red phase. b) Pressed solid PRF matrix. c) Xenogeneic BSM granules loaded with solid and liquid PRF, resulting in a sticky bone mixture. d) Titanium mesh initially loaded with the BMS. e) Aspiration of liquid PRF directly after centrifugation to separate from the red phase. f) Collagen matrix loaded with liquid PRF.

10-ml syringe with an 18-gauge needle. Care was taken to collect the whole liquid PRF phase up to the interface between the liquid PRF and red phase (Fig. 1e). The collected liquid PRF was then combined with the BSM granule–solid PRF mixture that results in a flexible solid sticky bone mixture (Fig. 1c). The mixture was then loaded into the titanium mesh (Fig. 1d). Additionally, liquid PRF was used to biologize the collagen matrix (Fig. 1f).

In total, seven patients in need of dental implants were treated using this technique. Different etiologies, including former cancer patients with severe jaw atrophy and patients with trauma-related tooth loss, were included in this case series (Table 2). All patients gave informed consent and agreed to the use of anonymized data for publication. Different defect sizes were treated using this concept. Titanium meshes used for the augmentation of one to two lost teeth were considered as small meshes, and titanium meshes used for the augmentation of the atrophic area of more than two teeth were considered as large meshes. Additionally, some of the cases were treated in the clinical setting (Department of oral, maxillofacial and plastic facial surgery, Goethe University, Germany) and others in a private practice (Bingen am Rhein) by two of the authors (S.G. and T.C.) to evaluate the suitability of this concept for different working settings, namely, clinic and private practice.

Table 2
Detailed overview of the participating patients, defect localization and treatment indications.|

Patient number	Sex	Age	Anesthesia	Indication	Mesh size	Covering material	Healing period	Inserted implants
1	F	51	Local	Bone atrophy after tooth extraction due to caries Camlog	Small	Latex	7 months	16 (Camlog®Promote® plus)
2	M	18	Local	Tooth loss after trauma Camlog	Small	Latex	6 months	11 (Camlog®Promote® plus)
3	F	46	Local	Bone atrophy after tooth extraction due to caries Camlog	Small	Latex	4 months	11 (Camlog®Promote® plus)
4	F	18	Local	Aplasia of lateral front (Cleft) Camlog	Small	Latex	4 months	22 (Camlog®Promote® plus)
5	F	51	Local	Bone atrophy after tooth extraction due to caries Camlog	Large	Latex	5.5 months	46, 47 (Camlog®Promote® plus)
6	F	62	Local	Periimplantitis/periodontitis Explantation region 44, 46. Extraction tooth 43 Camlog	Large	Latex	5 months	43, 35, 46 (Camlog®Promote® plus)
7	M		General	Cancer resection and irradiation	Large	Titanium reinforced PTFE	8 months	43, 44, 45, 46 (Astra Tech Implant System®, Dentsply Sirona)
Total: 7 patients					4 small 3 large		4–8 months	13 implants

The augmentation was performed either under local or general anesthesia according to the defect size. Special focus was placed on the flap design and soft-tissue management during the augmentation surgery.

After anesthesia, a surgical incision was made in the crestal orientation, and a mucoperiosteal full flap was minimally invasive mobilized to expose the area to be augmented. First, the CAD/CAM-manufactured individualized titanium mesh (Yxoss CBR, Filderstadt, Germany) was tried in situ before loading with the BSM–PRF mixture to verify the accuracy of fit to the defect morphology according to the virtual planning. Subsequently, when loading the titanium mesh with the BSM–PRF mixture, attention was paid to avoid overloading the mesh and to allow precise readaptation to the defect that was virtually planned. In this context, a sufficient and stable placement of the mesh into the defect is essential. The titanium mesh was then stabilized using screws. Next, the titanium mesh was covered with the biologized collagen matrix, and the full flap was then readapted. No periosteum splitting was performed in any of the presented cases. The flap margins were approximated as much as possible without tension or pressure on the flap. The resulting gap between the flap margins was bridged using further biologized collagen matrix (Mucograft, Geistlich Pharma AG Wolhusen, Switzerland) that was not fully covered by the flap in terms of exposed open healing (Fig. 2a–e; Fig. 4a–d). The exposed collagen matrix was covered by a pressed PRF matrix, and the biomaterial–PRF flap was fixed using single sutures. Thereafter, the augmentation area was covered either with a sterile latex piece or PTFE-based membrane (medipac) (Table 2; Fig. 2e; Fig. 4d) that was fixed to the exposed area using a single note suture. After 4–8 months depending on the defect size (Table 2), the titanium mesh was removed, and dental implants were placed in the augmentation area (Fig. 2h; Fig. 4f). In total, 13 implants (Table 2) were placed in the augmentation areas of seven patients. During the implantation surgery, bone biopsies were gained from the augmentation area for histological evaluation. The implants were covered and left to heal without loading for 4–8 weeks before loading with prosthetics. After both surgical procedures, chlorhexidine 0.2% as a mouth rinse and 400 mg of ibuprofen were also prescribed.

The biopsy samples were fixed in buffered 4% formaldehyde for at least 24 h. Next, the samples were processed and dehydrated using alcohol and xylene and then were embedded in paraffin as previously described (Ghanaati et al., 2013; Lorenz et al., 2018a). Histological sections of 3–4 μm were cut along the longitudinal axis using a rotation microtome (Leica, Wetzlar, Germany). The sections were then stained using standard Mayer's hematoxylin and eosin (H&E) and Azan stain as previously described (Al-Maawi et al., 2018; Ghanaati et al., 2010). The analysis was performed using a light microscope (Nikon ECLIPSE 80i microscope; Nikon, Tokyo, Japan) equipped with a digital camera DS-Fi1, together with a Nikon digital sight control unit (Nikon, Tokyo, Japan) to capture representative histological images. Histological analysis was performed by S.G. and S.A.

Wound healing was uneventful in all patients. No signs of infection, necrosis or any adverse reaction were observed at any time point. No material loss or abnormal premature surgical intervention was undertaken during the healing period. The titanium meshes were exposed over the complete healing period. However, soft tissue grew under the titanium mesh and closed the wound along the used collagen and PRF matrices. All patients received the planned number of implants with adequate primary stability without any complications or further augmentation procedure during implant placement. Oral rehabilitation was achieved in all cases including prosthetics. After 1 year, the implant survival rate is 100%.

In small titanium meshes, the initial wound healing was uneventful, and the exposed open healing was covered using a latex piece that was removed after 2 weeks. At this time point, the mesh surface was exposed (Fig. 2f). However, a fibrin–collagen layer was observed directly under the titanium mesh, and the BSM was completely covered. No signs of infection or suppuration were observed. The patient did not report any abnormal pain. After 4 weeks, the soft tissue under the titanium mesh was completely regenerated, and the augmented area was completely covered. Although the titanium mesh was still exposed at this time point. After 7 months, the mesh was explanted, and a dental implant was placed. After mesh explantation, thick and healthy soft tissue was found that grew under the titanium mesh (Fig. 2g). The soft tissue showed no signs of scar formation or fibrosis and allowed the preparation of a full flap for implant insertion. During implant insertion, the augmentation area displayed a well-regenerated newly formed alveolar ridge in all dimensions (Fig. 2h). The BSM granules used for augmentation were not macroscopically observable after 7 months (Figs. 2h and 3). A vital well-vascularized bone with adequate quality was found at the

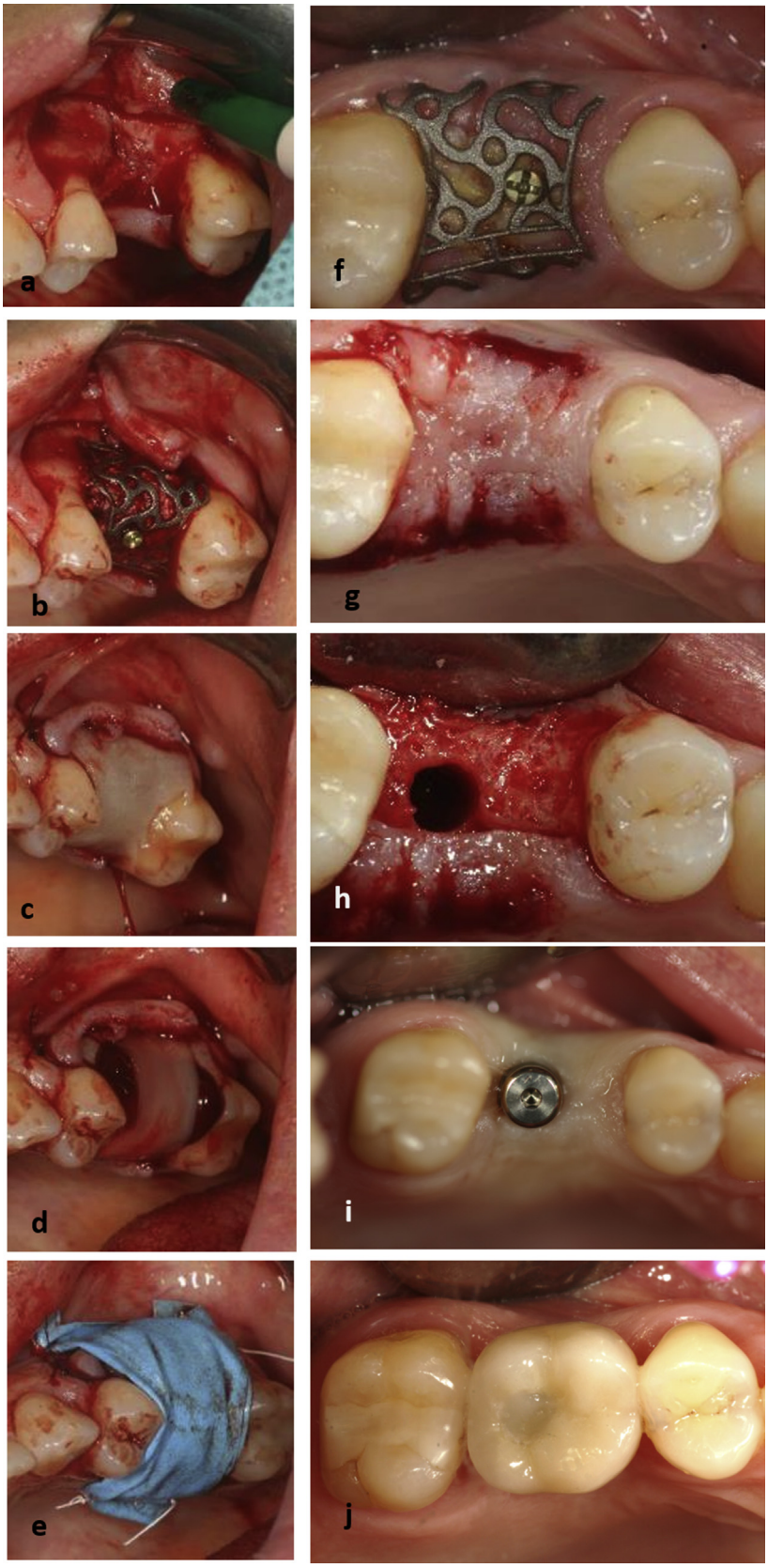


Fig. 2. Representative clinical picture of the augmentation and implantation using a small titanium mesh. a) Preparation of a full thickness flap. b) Placement of the titanium mesh and fixation according to the virtual planning. c) Covering the titanium mesh with PRF-loaded collagen matrix. d) A horizontally pressed solid PRF matrix is placed over the loaded collagen matrix. e) The augmentation area is covered by a sterile latex piece. f) Two weeks after augmentation, newly formed soft tissue is observable under the mesh. g) Seven months after augmentation, the mesh was removed. A healthy and thick soft tissue is observable under the mesh. h) Preparation of the implantation bed in a newly formed bone with good mechanical properties. i) Healing abutment 4 months after implant insertion. j) The prosthetic crown loaded on the inserted implant.

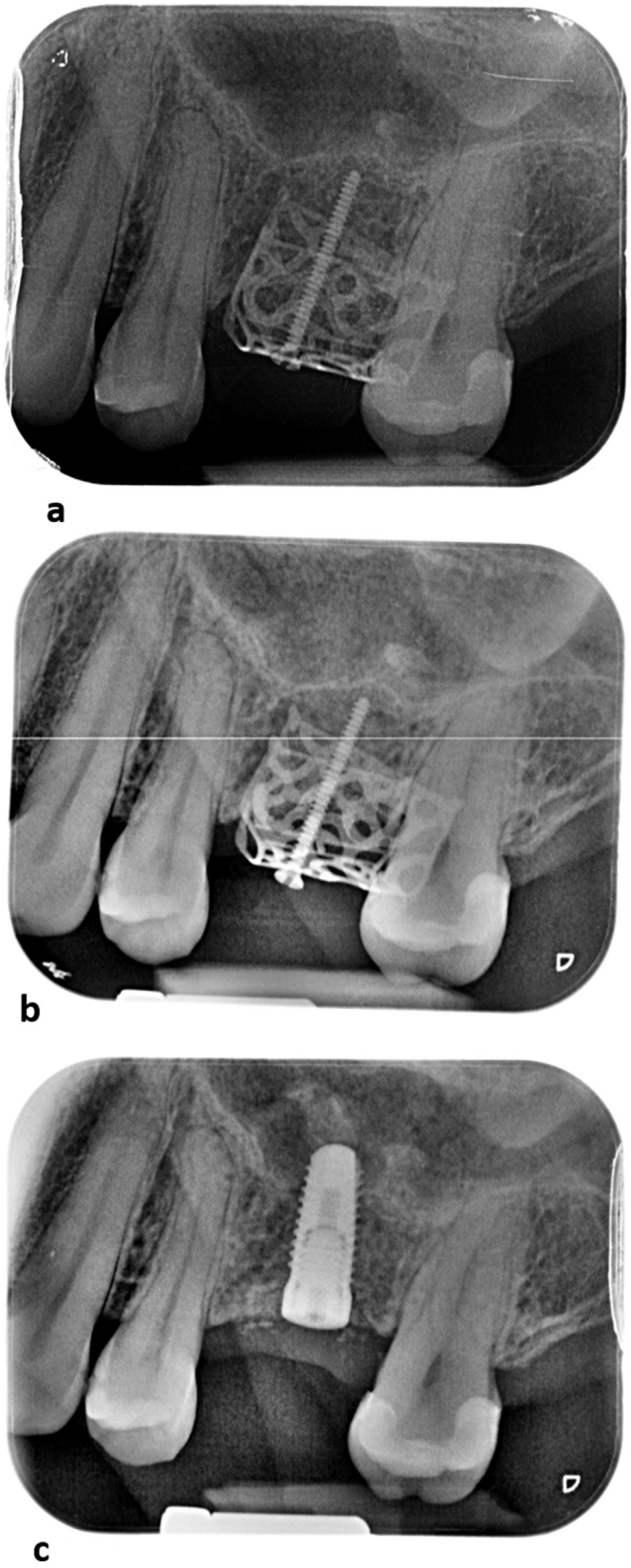


Fig. 3. Radiological picture from the augmentation area. a) Directly after augmentation. b) After 7 months. c) Implant in situ 7 months after augmentation.

augmentation area that allowed implant insertion with satisfactory primary stability. No additional augmentation was needed during implant insertion. After a submucous healing period of 2 months, abutment and prosthetics were placed. The peri-implant soft tissue showed satisfactory red–white aesthetic and functional results (Fig. 2i and j).

During wound healing of the large augmentation using the 3D CAD/CAM titanium mesh, no complication was observed or reported from the patient at any time point. No signs of necrosis, infection or suppuration were revealed during the rehabilitation period. In this case, the soft tissue was regenerated over the titanium mesh. Well-regenerated soft tissue was found without scar formation of fibrosis. Eight months after augmentation, the soft tissue was intact and allowed flap elevation for implant insertion. The regenerated bone showed a vital and well-vascularized bone (Fig. 4e). In this case, the BSM granules were macroscopically observable within the augmentation area (Fig. 4e). However, they were embedded in newly formed bone with adequate mechanical stability. Therefore, 4 implants were successfully inserted in the augmented region and showed adequate primary stability (Fig. 4f; Fig. 5). After implant insertion, the implants were again covered by PTFE-based membrane that was exposed to the oral cavity (Fig. 4g). This technique was applied to prevent tension to the flap during readaptation. After 2 weeks, the PTFE-membrane was removed, and the implants were loaded with prosthetics. The soft tissue displayed a healthy and thick structure that provided satisfactory aesthetic and functional outcomes (Fig. 4h).

In this case series, 13 implants were placed in the augmented regions of 7 patients. During implantation bone biopsies of the augmentation, the area was gained for histological evaluation (Fig. 6a). All biopsy samples displayed newly formed bone around the applied bone substitute granules (Fig. 6b). The granules were embedded in mineralized bone building a hybrid bone (Fig. 6b and c). This specific augmented bone consists of bone substitute granules and a matrix of vital newly formed bone. Osteocytes were found within the relatively wide osteocyte lacunae that are characteristic for newly formed and remodeled bone (Fig. 6d). Moreover, a cell-rich and well-vascularized nonmineralized tissue was found between the bone trabeculae. This specialized tissue exhibits the potential to undergo turnover and may generate newly formed bone at later time points. By contrast, the residual bone in the nonaugmented area displayed atrophic bone with small osteocytes lacunae and a fatty and cell-poor tissue within the intertrabecular area (Fig. 2b).

3. Discussion

The present clinical case series introduced for the first time a surgical concept for biomaterial-based bone regeneration and soft tissue management by means of open healing combined with a 3D CAD/CAM individualized titanium mesh for 3D augmentation in severe atrophic jaw and bone defects for small defects as well as cases after cancer resection within the mandible. Three-dimensional augmentation is still a challenging indication for dental rehabilitation (Lorenz et al., 2018a). This indication is mostly achieved by autologous bone transplantation (Landes et al., 2014). However, the development of the digital technique and its establishment in dentistry and oral/maxillofacial surgery allowed the precise planning of the surgery and exact determination of the implant position (Shenaq and Matros, 2018; Zweifel et al., 2018). Therefore, the backward and virtual planning together with the 3D printing technology enriched the opportunities and capabilities to achieve 3D augmentation without needing autologous bone, because the technique is based totally on biomaterials. This case series study is the first to present a surgical concept in terms of open healing in combination with solid and liquid PRF to achieve 3D augmentation using particulate BSMs without autologous bone.

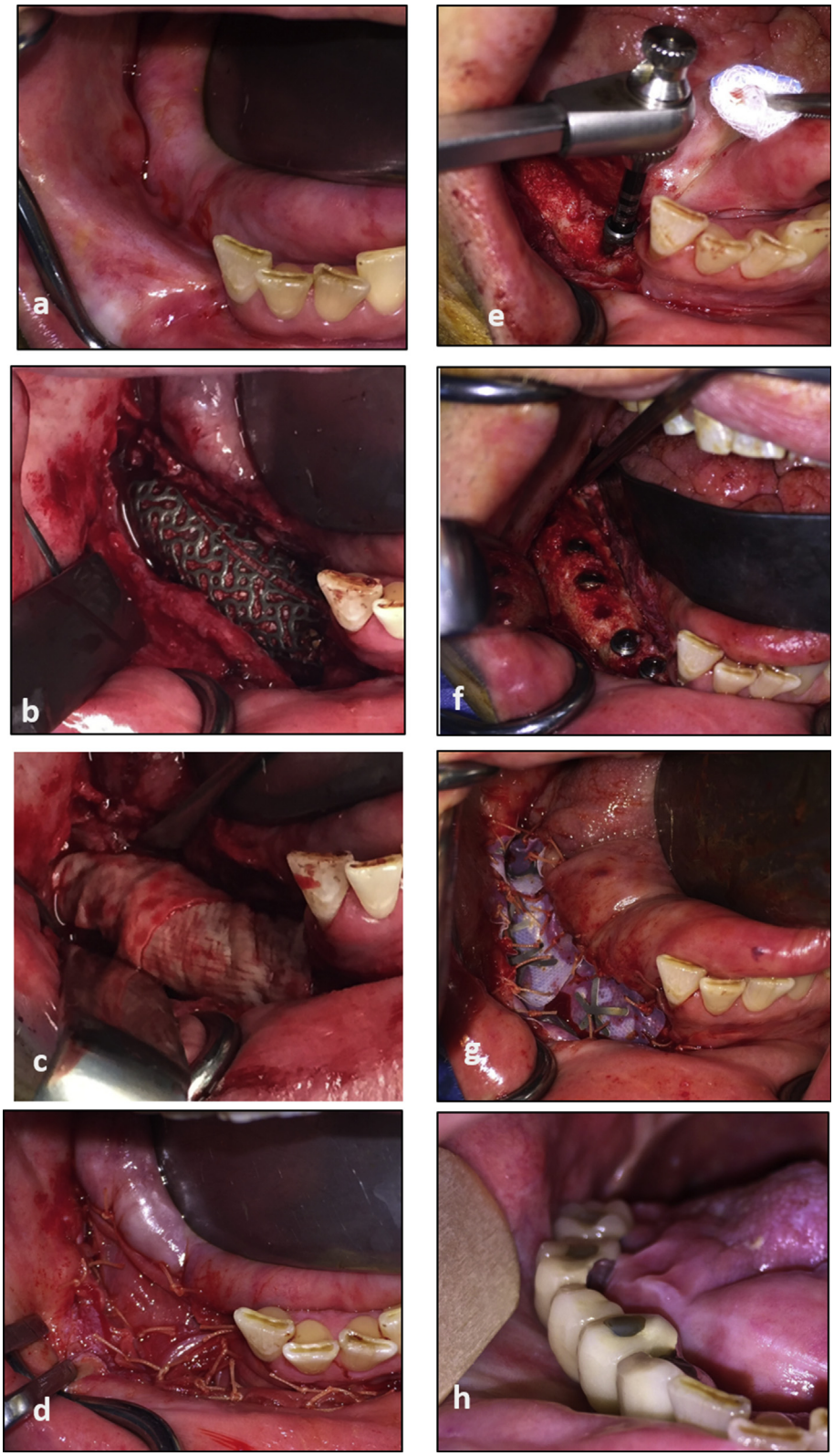


Fig. 4. Representative clinical picture of the augmentation and implantation using a large titanium mesh in a former cancer patient. a) Clinical situation 18 months after cancer resection. b) Placement and fixation of the loaded titanium mesh. c) The titanium mesh is covered by PRF-loaded collagen matrices. d) The gap between the flap margins is bridged by PRF-loaded collagen matrices showing the open-healing concept. e) Implant placement 8 months after augmentation. f) Four implants in situ 8 months after augmentation showing a hybrid bone with good mechanical properties. g) The open-healing concept showing PRFE-based titanium-reinforced membrane after implant insertion. h) Prosthetic single crowns are loaded on the inserted implants.

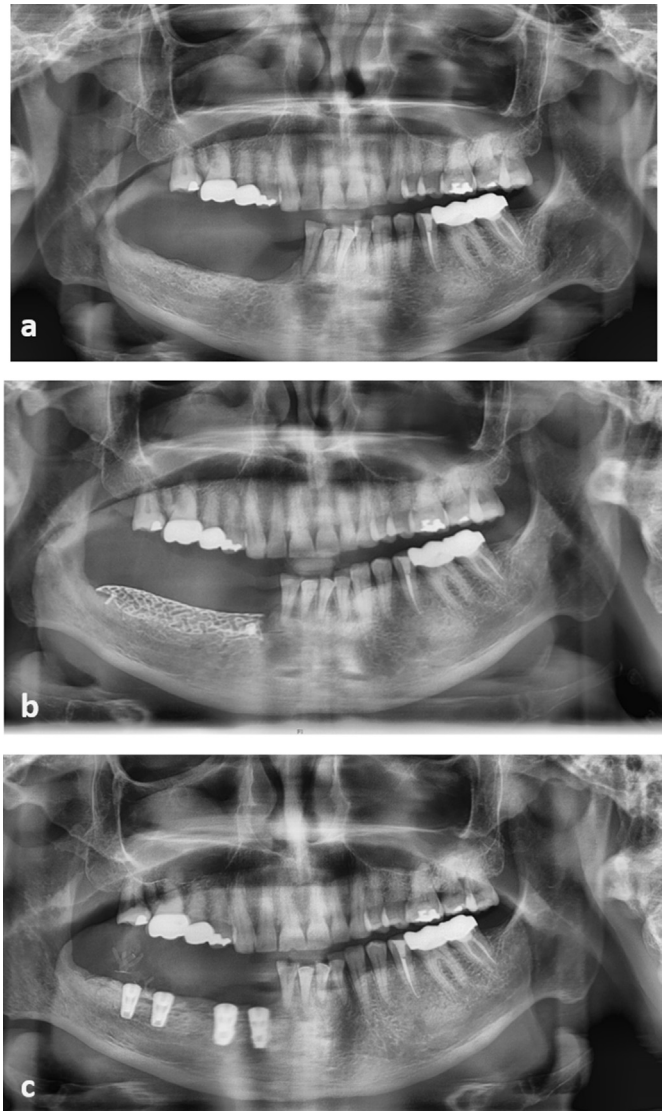


Fig. 5. Radiological picture from the augmentation area. a) Directly after augmentation. B) After 7 months. c) Implant in situ 7 months after augmentation.

The preoperative virtual planning and patient-specific tailored titanium mesh in combination with solid and liquid PRF without autologous transplantation or complex flap raising led to a significantly shorter operation time, reduced pain and swelling, and fewer complications than autologous bone transplantation (Lorenz et al., 2018c; Weibull et al., 2009). Additionally, using digital technology and virtual planning to design a rigid titanium mesh with a high accuracy of fit is highly beneficial for clinical application. In this case, no further trimming or adaptation is required during the surgery. By contrast, the use of flexible titanium meshes or prefabricated bone blocks needs manual trimming and adaptation to fit the defect morphology, which is a technically sensitive procedure and a widely discussed topic in the literature to find the most suitable trimming technique and to achieve high accuracy (Venet et al., 2017). Additionally, a previous clinical study evaluated the clinical benefit of individually customized titanium meshes compared to prefabricated titanium meshes. The results showed that the use of individually customized titanium meshes significantly reduced the operation time and led to less pain, swelling and postoperative complications (Sumida et al., 2015). Although manually trimmed autologous and allogeneic bone blocks showed successful clinical outcomes, a relatively high rate of material loss is still observed due to clinical complications such as infection and dehiscence (Khojasteh et al., 2012; Wallowy and Dorow, 2012; Zhou et al., 2018). By contrast, the use of titanium mesh in combination with particulate BSMs and autologous bone shows a high survival rate despite soft tissue dehiscence in an early or late stage (Sagheb et al., 2017; Sumida et al., 2015; Zhou et al., 2018). The present surgical protocol suggested the benefits of particulate BSM granules. The granules allow exact filling of the titanium mesh without gaps and are easy to shape within the mesh. Additionally, BSM granules build intergranular areas that allow osteogenic cells from the peri-augmentation area to penetrate into the BSMs and support neoosteogenesis and regeneration (Barbeck et al., 2014). This process accelerates the integration of the BSM into the augmentation region and allows the vitalization of the BSMs by means of vascularization, making particular BSMs less sensitive to exposure and dehiscence than bone blocks (Barbeck et al., 2014; Lorenz et al., 2018; Sagheb et al., 2017). This is evidenced by the histological results of the present case series that showed the integration of the BSMs granules into a mineralized matrix of newly formed bone, illustrating a hybrid bone composed of the BSMs and patients own bone. The integration of the BSM granules provide mechanical stability to the newly formed bone and stimulate the bone to preserve the augmented dimension without undergoing premature resorption (Fig. 7).

Moreover, the present case series study introduced the combination of solid and liquid PRF matrices that are centrifuged according to LSCC (Choukroun and Ghanaati, 2018; Wend et al., 2017). The addition of PRF to the used biomaterials provides technical and biological benefits. The combination with fibrin gives the BSM granules a sticky consistency that is technically favorable during the surgical procedure and allows precise shaping of the BSMs inside the titanium mesh. Moreover, the bioactivity of PRF as a source of autologous regenerative capacity accelerates and supports new bone formation (Ghanaati et al., 2018b). In this context, PRF releases high concentrations of different growth factors (e.g., EGF, PDGF, TGF, EGF) that are essential for wound healing and regeneration. EGF was shown to support epithelialization (Arda-Pirincci and Bolkent, 2014; Bedford et al., 2015), which is highly needed in this context, especially when considering the concept of open healing. Enhancing the epithelialization will lead to rapid soft-tissue regeneration to close the wound in the early stage of wound healing and will allow the augmentation area to regenerate without delay. This effect of PRF was also observed in the treatment of medication-related osteonecrosis of the jaw, where epithelization is essential for wound healing to prevent the progression of necrosis (Soydan and Uckan, 2014). PDGF is involved in the early regeneration and osteogenesis process (DiGiovanni and Petricek, 2010). An in vitro study showed that osteoblasts that were treated with PRF showed a significantly higher proliferation and differentiation capacity than the untreated control (Wang et al., 2017). Additionally, the combination of PRF with endothelial cells showed a significantly increased vascularization rate compared with that of endothelial cells without PRF in vitro (Dohle et al., 2017). Another in vivo study demonstrated the angiogenic effect of PRF matrices prepared with low RCF compared to those prepared with high RCF and outlined the role of PRF in enhancing the vascularization and recruitment of cells from the peri-implantation area (Kubesch et al., 2018). In this context, PRF, as an autologous concentrate of different inflammatory cells and a source of growth factors, was shown to support the process of regeneration on different levels. The biologization of biomaterials using PRF with its potential in angiogenesis and epithelialization may have accelerated the regeneration process in this case series study.

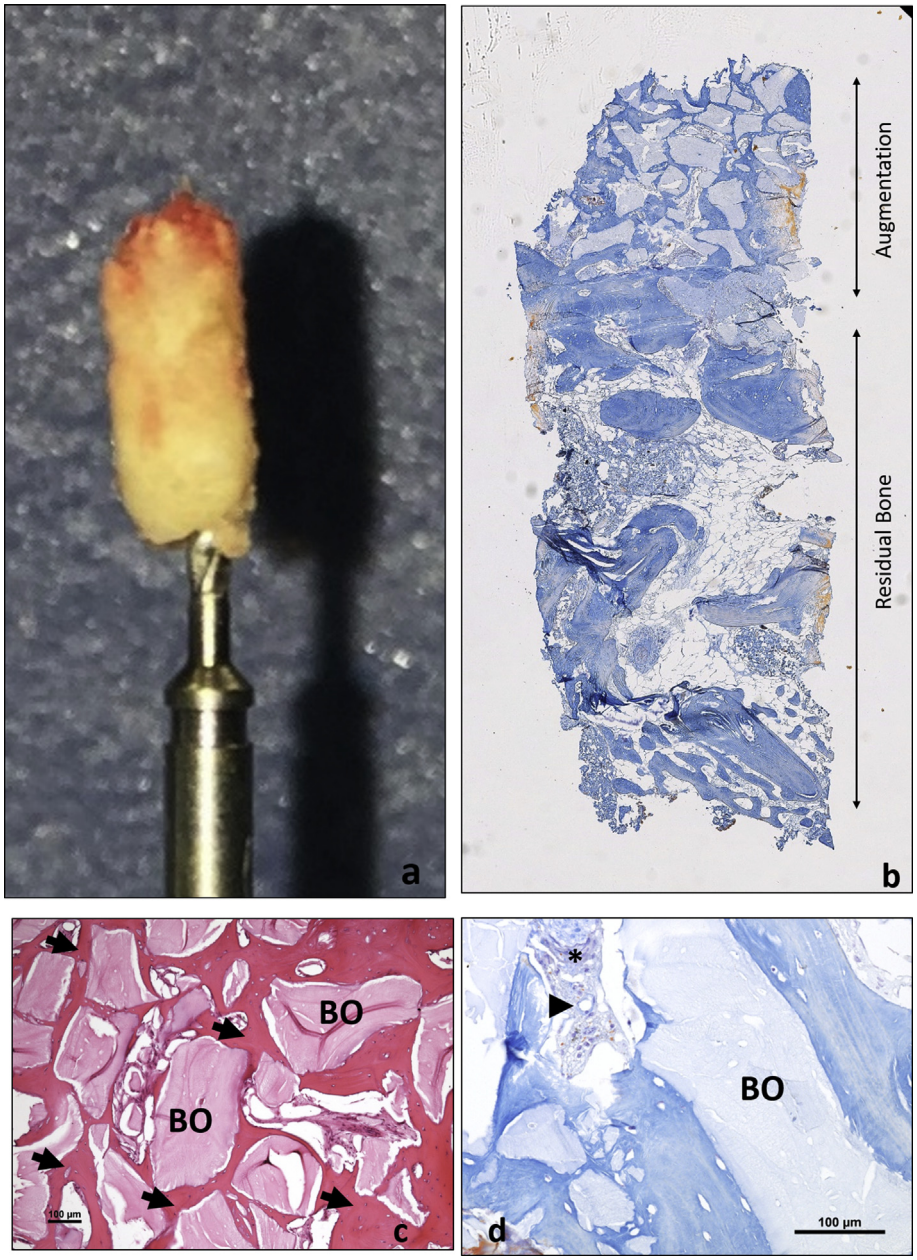
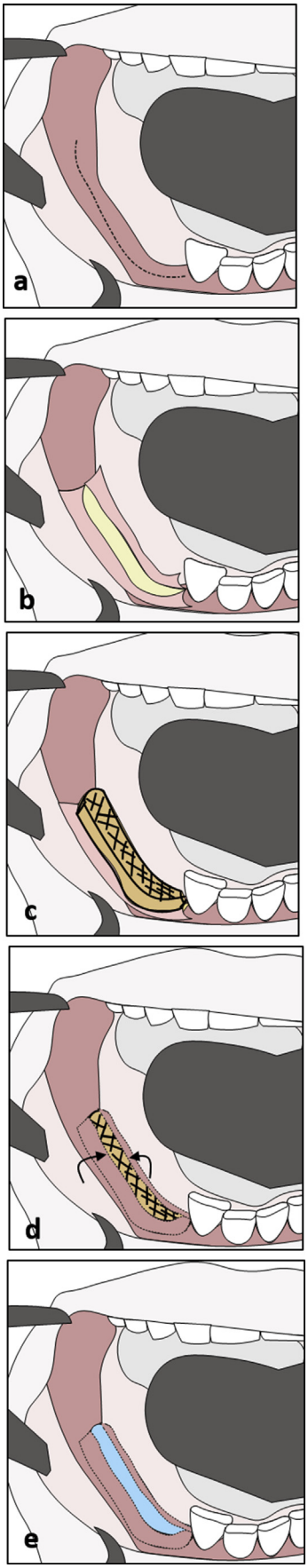


Fig. 6. Representative histological picture showing the augmented newly formed hybrid bone. a) Biopsy of the augmented region. b) Total scan showing the whole histological biopsy, including the non-augmented residual bone and augmented region; Azan staining; ×40 magnification. c) A closer section of the augmented region showing the hybrid bone, including the BSM granules (BO) and newly formed mineralized bone matrix (arrows). H and E staining; ×100 magnification. d) High magnification histological picture shows the integration of the BSM granules (BO) into the augmentation region and the specialized connective tissue (asterisks) including a high number of cells and vessels (arrow head). Azan staining; ×200 magnification.

In addition, the present study focused on a novel concept of soft tissue management by means of exposed open healing under the PTFE-based membrane or sterile latex piece. This concept was developed to manage the frequently observed dehiscence during the application of titanium meshes (Sagheb et al., 2017). One important aspect that may lead to dehiscence formation is the overloading of the titanium mesh with more BSMs than initially planned. Therefore, in the present case series, attention was paid to the precise placement of the titanium mesh according to the virtually planed protocol. Therefore, it may be beneficial to try the titanium mesh in situ and to verify the accuracy of fit before loading the BSMs. In addition, the material thickness of the rigid titanium mesh has to be considered when loading the mesh. In this context, the authors find it beneficial not to overfill the mesh with BSM to allow precise placement of the mesh and preadaptation according to the planned augmentation volume without overcompensation. However, 3D augmentation predefines a large enhancement of the bone volume in all dimensions. In this case, the question arises concerning whether this enhancement also requires enhancement of the amount of available soft tissue along with the bone tissue. Consequently, bone and soft tissue have to harmonize to reach good clinical outcomes. To date, the only compensation used during the surgical procedure for the soft tissue to overcome the limited soft tissue amount and to provide tension-free flap closure is periosteum splitting. This technique enhances the soft tissue flexibility but not the amount of the available soft tissue. Further



modifications of the flap design were introduced to overcome complications associated with dehiscence when performing 3D augmentation, such as a deposition of the incision to the vestibular area, so that the mesh does not become exposed once the flap margins undergo dehiscence (Seiler et al., 2018). Other authors have suggested high mobilization of the flap to allow tension-free flap closure. However, even when respecting these technical requirements and the achievement of initially tension-free flap closure, early dehiscence may still result, in many cases. These observations outline that the initial apparent tension-free closure of the flap is not necessarily sufficient to prevent dehiscence formation. Intraorally, wound and flaps are subject to many mechanical manipulations as an outcome of different forces resulting from the daily mastication and phonation actions, which may cause dehiscence, although the flap was closed apparently tension free. Therefore, in the present case series, the authors present an alternative concept using collagen-based matrix and PRF to enhance the available amount of soft tissue and to bridge the gap between the flap margins without a high rate of flap mobilization or periosteum splitting. The exposed collagen-based matrix was initially covered using a piece of sterile latex or PTFE-based membranes. In both cases, rapid wound closure was achieved without any infection of material loss. Additionally, this technique was successfully performed in patients under local and general anesthesia, in private practice and hospital settings alike. The collagen-based matrix used herein was evaluated in a translational research series. In an animal study, the cellular reaction induced by this collagen matrix was evaluated in vivo using a subcutaneous implantation model. The matrix induced only physiological mononuclear cells, including leukocytes, macrophages, lymphocytes and fibroblasts. No signs of foreign body reaction were observed over the study period of 60 days. Additionally, the matrix showed a stable structure over 60 days and did not undergo premature degradation (Ghanaati et al., 2011). The same matrix was used for recession coverage in patients in need of recession coverage but were otherwise healthy and demonstrated successful clinical outcomes (Ghanaati et al., 2011). A further clinical study used this collagen matrix to expand the peri-implant soft tissue in former cancer patients after oral cancer resection. The application of this matrix displayed satisfactory functional and aesthetic outcomes (Lorenz et al., 2017). This study also included histological evaluation of soft tissue biopsies that demonstrated a similar physiological reaction, including those of macrophages, monocytes and fibroblasts, as previously shown in vivo (Lorenz et al., 2017). In another application field, this collagen matrix was used to regenerate extraoral skin defects after cancer resection in different facial localizations. The matrix supported skin regeneration and showed an uneventful healing pattern without material loss, resulting in good aesthetic and functional results (Ghanaati et al., 2016). Using this collagen matrix in the extraoral skin regeneration was not covered by any type of flap, making the concept similar to the presented open-healing concept to support soft tissue regeneration and to bridge the gap between the flap margins without the need for periosteum splitting or tension in flap closure. Similarly, the results of the present case series showed that it is possible to regenerate the soft tissue using collagen-based biomaterial in terms of open healing, thereby supporting bone regeneration in the underlaying augmentation region without material loss or infection at any time point. In this context,

Fig. 7. Illustrative artwork demonstrates the surgical procedure of the open healing concept. a) Incision line in the crestal orientation. b) Minimally invasive flap preparation to expose the area of interest without large mobilization. c) Fixation of the loaded titanium mesh. d) Approximation of the flap margins display the gap between the margins. No periosteum splitting is performed. e) Application of a PRF-loaded collagen matrix to bridge the gap between the flap margins and left to heal exposed.

no high mobilization of the flap is needed. This makes this procedure rather minimally invasive and safe to the adjacent anatomical structures, such as nerves. Additionally, the soft tissue preserves its initial anatomic position without any translocations. Interestingly, the observations of the present case series showed that the soft tissue regeneration was achieved under the titanium mesh and not over the mesh, so that the mesh was exposed for almost the whole healing period. These observations outline that it is important to respect the physiologic anatomic aspects without overcompensation. Therefore, the soft tissue seemed to function physiologically in direct proximity to the bone. Additionally, avoiding periosteum splitting preserves the anatomy and function of the flap and supports bone regeneration when the intact flap is readapted to the augmentation and periosteum faces the BSMs. Furthermore, preserving the anatomy of the flap prevents flattening of the vestibulum that mostly occurs when large flaps are translocated and indicates a further surgical intervention in terms of vestibulopathy, which can be avoided when using the open-healing concept in combination with biomaterials. The high rate of dehiscence in relation to 3D augmentation using bone blocks or titanium meshes shows that conventional flap designs and periosteum splitting are effective up to a specific augmentation volume but may have reached their limits in large 3D augmentation volumes. In the latter case, dehiscence seems to be pre-preprogrammed. Therefore, it is important to search for alternatives to overcome this complication proactively and not only focus on finding solutions to treat the already occurred dehiscence. In this indication, open healing may be a good concept to achieve satisfactory clinical results without invasive flap preparation and autologous transplantation. Additionally, the use of collagen-based matrix loaded with PRF allowed the regeneration of a vital thick soft tissue without any signs of scars of fibrosis. This newly formed soft tissue can be used after implantation to form a healthy peri-implantation tissue that is essential for the long-term survival of implants. However, further controlled clinical studies are needed to evaluate this concept in a larger patient cohort and to examine the long-term clinical outcomes.

4. Conclusion

The open healing concept presented herein suggests the use of a bioactive blood concentrate (platelet-rich fibrin [PRF]) and a collagen matrix to provide soft-tissue regeneration along with the bone augmentation without high flap mobilization and periosteum splitting. This technique seems to be sufficient in complex 3D augmentation to regenerate bone and soft tissue with satisfactory aesthetic and functional outcomes after implant insertion.

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