A Proposal of Pseudo-periosteum Classification After GBR by Means of Titanium-Reinforced d-PTFE Membranes or Titanium Meshes Plus Cross-Linked Collagen Membranes

Alessandro Cucchi, DDS, MSClin, PhD
Maria Sartori, BSc, PhD
Nicolò Nicoli Aldini, MD
Elisabetta Vignudelli, DDS, MSC, PhD
Giuseppe Corinaldesi, DDS, MD, MSClin

After guided bone regeneration (GBR) with different devices, a layer of connective tissue called pseudo-periosteum can be observed above the newly formed bone. The aim of this study is to evaluate the clinical and histologic features and to suggest a classification of this connective tissue after GBR with nonresorbable membranes or titanium (Ti)-mesh plus resorbable membranes. Forty patients with partial edentulism in the posterior mandible were randomized into two groups: 20 patients were treated by means of Ti-reinforced dense polytetrafluoroethylene (d-PTFE) membrane (group A), while the other 20 patients were treated with Ti-mesh and a cross-linked collagen membrane (group B). After 9 months and during re-opening surgery, bone density and pseudo-periosteum type were recorded. Pseudo-periosteum was classified into Type 1 (no tissue or tissue < 1 mm); Type 2 (regular tissue between 1 and 2 mm); and Type 3 (irregular tissue or tissue > 2 mm). Histologic analyses were performed to identify the features of pseudo-periosteum. Out of 40 patients, 36 (n = 19 in Group A; n = 17 in Group B) with 99 implants were analyzed after GBR and according to the study protocol. The vertical bone gain was 4.2 ± 1.0 mm in Group A and 4.1 ± 1.0 mm in Group B. Group A had a higher bone density and greater amounts of type 1 periosteum than Group B (P = .01 for both). The preliminary results of this study show that both d-PTFE membranes and Ti-mesh plus collagen membranes are two valid options for bone augmentation in the mandible. However, nonresorbable membranes achieve higher bone density and a thinner pseudo-periosteum layer above the newly formed bone. Int J Periodontics Restorative Dent 2019;39:e157–e165. doi: 10.11607/prd.3598

Guided bone regeneration (GBR) is a surgical procedure based on the creation of a protected space where pluripotent and osteogenic cells can migrate to promote new bone formation.1-8 As proposed by Dahlin et al, the GBR therapeutic protocol involves the surgical placement of a space-making barrier device that protects the regeneration site, excluding cells that can impede bone formation (eg, epithelial cells and connective tissue).6 To create and maintain a secluded space, different devices—such as titanium (Ti)-reinforced dense polytetrafluoroethylene (d-PTFE),9 Ti osteosynthesis plates and a resorbable membrane,10 or Ti-mesh—can be used.10-14 Ti-mesh is considered insufficient for maintaining cell exclusion due to the presence of pores, and some authors have proposed the addition of an additional barrier with cell-occlusive properties.15-19 As described by Dahlin et al,7 a layer of connective tissue, called the pseudo-periosteum, can be observed above the newly formed bone. Many other authors reported similar tissue above augmented sites.8,11,12,20–24 Generally, this is a dense connective soft tissue layer with low cellularity and no mineralization.15,25,26 Although it has yet to be formally identified, its histologic features have been reported.11,15,26 The primary aim of the study was...
to observe statistical differences between the two study groups assessing the type of connective tissue layer (pseudo-periosteum) and the density of bone tissue after GBR with Ti-reinforced d-PTFE or Ti-mesh plus cross-linked collagen membranes. The secondary aims were to propose a clinical classification for the pseudo-periosteum and to provide a histologic analysis for each type of this connective tissue.

**Materials and Methods**

Patients referred to the Unit of Oral and Maxillofacial Surgery, Alma Mater Studiorum—University of Bologna (Italy), for restoration of the posterior mandible with a fixed implant-prosthetic rehabilitation were screened for inclusion in this randomized clinical trial (code CMF-01/2013, no. 30/2013/O/Disp, approved by the Ethics Committee of Sant’Orsola-Malpighi Hospital CMF). The study design was previously reported and followed the guidelines of the CONSORT 2010 statement that sought to improve the quality of reporting on randomized controlled trials (http://www.consortstatement.org).

From 2013 to 2015, 40 patients with partial edentulism in the posterior regions of the mandible with vertical and horizontal bone resorption requiring three-dimensional bone regeneration to perform implant-supported rehabilitation were selected and enrolled. All patients had a vertical peri-implant bone defect ≥ 2 mm from the alveolar ridge, which required regeneration after implant placement in the three-dimensional ideal position. Patients with general medical conditions that could affect reconstructive bone surgery were excluded from the study.

All patients were enrolled by two examiners (A.C. and G.C.) belonging to the Unit of Oral and Maxillofacial Surgery, Alma Mater Studiorum—University of Bologna, Italy. Every patient received an informational paper and provided written informed consent before any study-related procedures. The study was a double-blinded clinical trial: The patient and statistician were blinded, as was the surgeon before opening and modeling of the membrane or mesh. For obvious reasons, the data collector could not be blinded. Sample size calculation was not performed since no previous studies were published comparing GBR with Ti-reinforced d-PTFE membranes and Ti-mesh plus cross-linked collagen membranes.

The 40 enrolled patients were randomized into 2 study groups: 20 were treated with one-stage GBR using a Ti-reinforced d-PTFE membrane (Group A), and 20 were treated with a one-stage GBR with a Ti-mesh covered by a cross-linked collagen membrane (Group B). All patients were randomly allocated in the study groups using a computer-generated sequence (computerized random numbers). A simple randomization was created using SPSS dedicated software (v. 8 11.5) by an independent blinded statistician. No stratifications, blocks, or other methods were used to implement the random allocation of patients due to the study design.

The assignment of patients to the intervention was done using opaque, sealed, and stapled envelopes that were opened by an external operator during the surgery in order to have a blinded surgical operator (A.C.) before the use of the assigned barrier device.

**Surgical Protocol**

All surgeries were performed as previously described. After implant placement, the randomization envelope was opened and the assigned treatment was revealed to the surgeon. The barrier device was stabilized on the buccal side with two or three mini screws (Pro-fix Membrane Fixation Screws, Osteogenics Biomedical): d-PTFE Ti-reinforced membrane (Cytoplast Ti-250XL, Osteogenics Biomedical) was applied in Group A, whereas a Ti-mesh (Trinon Titaniun) and cross-linked collagen membrane (Osseoguard, Zimmer Biomet) were used in Group B. All sites were grafted with a 50:50 mixture of autogenous bone harvested using a bone-scaper (Saferscraper, META) and bone allograft (EnCore, Osteogenics Biomedical). It was placed and adapted to fulfill the space around the implants under the barrier device. In both groups, a double suture was performed to ensure tension-free primary closure of the surgical wound. Antibiotic, anti-inflammatory, and antiseptic therapies were prescribed. After 9 months, all treated sites were reopened for barrier-device removal and healing screw placement (T1).
Data Collection

All study variables were recorded by a single operator, who was blind to the experimental protocol, using a specific data collection form (CRF) at follow-up visits. All information on the medical histories of patients were recorded in a database that included name (initials), age, sex, American Society of Anesthesiologists class, oral hygiene condition, smoking habits, bruxism habits, periodontitis status, and presence of diabetes or other systemic diseases. All measurements were performed by a single operator using calibrated equipment. Prior to the study, the operator underwent dedicated training.30

Healing Complication Rate

All types of biologic complications, such as device exposure ≤ 3 mm or > 3 mm without purulent exudate, device exposure with purulent exudate, or abscess without membrane exposure, were recorded from T0 (immediately after surgery) to T1 and evaluated in consideration of the newly formed bone during T1. The healing complication rate was calculated as the ratio between the number of sites with one or more complications and the total number of treated sites.

Bone Augmentation Rate

During T0, vertical bone defect (VBD) was assessed considering the vertical distance between the top of the implant shoulder and the first visible bone-implant contact. It was measured at four sites (mesial, distal, buccal, and lingual) around each implant using a UNC-15 periodontal probe (Hu-Friedy) featuring a 1-mm graduated scale, rounding off values to the nearest 0.5 mm. VBD was also measured during T1 to calculate the vertical bone gain (VBG) and the pseudo-periosteum layer above the newly formed bone (Fig 1). The bone augmentation rate was calculated as the ratio between the VBD and VBG, expressed as a percentage.

Pseudo-periosteum Type

The pseudo-periosteum was considered the connective tissue between the regenerated bone and barrier device. The pseudo-periosteum was clinically assessed during T1 and divided into three types to facilitate classification using the Murphy system, which evaluates utility, adequacy, disjointedness, and simplicity31: Type 1: no pseudo-periosteum or a layer of soft tissue thinner than 1 mm; Type 2: a regular soft tissue layer between 1 and 2 mm; and Type 3: an irregular layer of soft tissue and/or a layer thicker than 2 mm (Fig 2). In some cases, a biopsy sample was taken and a histologic evaluation was performed to characterize the features of the pseudo-periosteum layer.

Clinical Bone Density

The clinical bone density was evaluated based on the resistance of the newly formed bone to probe penetration. It was assessed during T1 by a previously calibrated examiner at the top of the crest in the vertical direction and at the buccal side in the horizontal direction using a calibrated probing force of 30 g. Clinical bone density was grouped into three classes: high density, no probe penetration; medium density, partial probe penetration into newly
formed bone; and low density, total probe penetration.  

**Histologic Analysis**

Pseudo-periosteum biopsy samples were fixed in 10% buffered formalin, dehydrated in a graded series of alcohols, and then processed for paraffin embedding. Five-micrometer-thick sections were obtained using a Microm HM340E rotary microtome (Microm International). Sections were stained with h&ē, and images were acquired using a digital scanner system at different magnifications to evaluate the histologic characteristics of the pseudo-periosteum after GBR with d-PTFE membranes (Group A) or Ti-mesh plus collagen membranes (Group B).

**Data Management and Statistical Analyses**

The authors employed a data collection form and data management system using Excel (v. 14.0.0, 2011, Microsoft Windows). Data were entered by a single operator (E.V.) who was blind to the experimental protocol. Before entry, data were evaluated for accuracy and completeness; logical consistency was verified, and the range of quantitative data was computed. Data were analyzed using SPSS software (v. 8 11.5). The results obtained in the two study groups (A and B) were subjected to statistical description and analysis using specific tests to observe any significant differences between the two groups. All analyses were accomplished comparing the two study groups; no additional clusters or subgroups were identified. An external blinded statistician performed all data analyses. Power analyses revealed that patient numbers were sufficient to detect a 35% difference in the complication rate and a 1-mm difference in vertical bone gain between the two groups, with a standard deviation \( s = 1 \), a significance level \( \alpha = .05 \), and a power of 80%, as previously reported. Both the intent-to-treat population and per-protocol population were analyzed. The implant was considered the statistical unit of analysis. The statistical differences in clinical bone density and pseudo-periosteum quality were investigated using Fisher exact test. Statistical significance was set at \( \alpha = .05 \).

**Results**

In total, 40 patients (13 males, 27 females) with a mean age of 52 years were treated according to the previously described protocol, and 108 implants were placed. As a result of complications or patient drop-outs, data from 36 patients (19 in Group A and 17 in Group B) with 99 implants were analyzed for the study variables recorded at T1; one patient dropped out due to a car accident immediately after GBR; one refused to continue the study for logistic and economic reasons; and three patients had a major complication, stopping the trial and needing barrier removal before T1. In Group A, the VBD was 3.8 ± 0.7 mm (range: 2.7 to 4.9 mm) and the VBG was 4.2 ± 1.0 mm (range: 2.7 to 5.8 mm); in Group B, the VBD was 4.0 ± 0.8 mm (range: 2.9 to 5.4 mm) and the VBG was 4.1 ± 1.0 mm (range: 2.6 to 6.3 mm). In most cases, bone density was similar, but in some cases, there was a significant difference. The classification of pseudo-periosteum is shown in Figure 2.
overgrowth was observed after barrier-device removal, and an osteotomy was required to expose the submerged implants; consequently, the bone augmentation rates were 110.5% and 102.5% in Groups A and B, respectively (Figs 3 and 4). In Group A, 11.1% of implants (n = 6) showed a low regenerated-bone density, 16.7% (n = 9) showed a medium bone density, and 72.2% (n = 39) showed a high bone density. Moreover, 40.7% of those implants (n = 22) showed a type 1 pseudo-periosteum, 37.0% (n = 20) showed type 2, and 22.2% (n = 12) showed a type 3. In Group B, 6.7% of implants (n = 3) showed a low regenerated-bone density, 42.2% (n = 19) showed a medium bone density, and 51.1% (n = 23) showed a high bone density. Finally, 15.6% of those sites (n = 7) showed a type 1 pseudo-periosteum, 40.0% (n = 18) showed type 2, and 44.4% (n = 20) type 3 (Figs 5 and 6). The difference between the two groups was statistically significant based on periosteum quality and bone density ($P = .01$ for both). Figures 5 and 6 show the types of pseudo-periosteum in, and the bone densities of, the two study groups.

**Histologic Findings**

No histology of pseudo-periosteum type 1 was provided, as it was not possible to obtain tissue biopsy samples at T1 when the thickness of the layer was less than 1 mm. The histology of pseudo-periosteum type 2 is shown in Fig 7. A regular layer of connective tissue characterized pseudo-periosteum type 2 (Fig 7a), in which blood vessels and capillaries were detected (Fig 7b). A small fragment of bone tissue was also visible and surrounded by connective tissue composed of fibers with a multidirectional orientation (Figs 7b and 7c). Regarding pseudo-periosteum type 3 (Figs 8 and 9), biopsy samples consisted of irregular bulks of connective tissue (poorly or not at all vascularized), and small fragments of bone graft used to fill the surgical sites were present and appeared intensely stained (Figs 8b, 8c, and 9b). In one sample, a mild adipose infiltrate was also detected in the inner part of the biopsy sample surrounded by connective tissue (Fig 9c). No inflammatory reaction or infiltrate was detected in any type of pseudo-periosteum.

**Discussion**

GBR before implant placement is considered a successful and predictable technique for vertical and horizontal ridge augmentation. One of the fundamental biologic principles of GBR is cell exclusion. Even when a cell-occlusive space-making device is used to perform GBR, a layer of connective tissue, called the pseudo-periosteum, is commonly observed above the newly formed bone at the re-opening surgery. This was first described by Dahlin et al. and suggested an interesting hypothesis about its nature. Histologic animal studies have shown that the pseudo-periosteum under Ti-mesh is a dense, connective soft tissue with several fiber groups.
Fig 5 Distribution of pseudo-periosteum class in the two groups.

Fig 6 Distribution of clinical bone density in the two groups.

Fig 7 Histologic images of pseudo-periosteum type 2. (a) General overview of the retrieved tissue (×0.8 magnification; 3-mm scale bar). (b, c) Higher magnifications (×8 magnification; 3-μm scale bar) of different portions of the tissue; blood vessels, bone fragments, and connective tissue organization are highlighted. *: blood vessels and capillaries; TC: connective tissue; black arrows: bone fragment. Images were stained with h&e and acquired with a digital scanner.

Fig 8 Histologic images of pseudo-periosteum type 3. (a) General overview of the bulk tissue (×1.4 magnification; 2-mm scale bar). (b, c) Higher magnifications (b: ×4 magnification and 500-μm scale bar; c: ×10 magnification and 200-μm scale bar) of connective tissue organization, showing a presence of the bone graft used to fill the surgical site. TC: connective tissue; black arrows: bone graft. Images were stained with h&e and acquired with a digital scanner.

Fig 9 Histologic images of pseudo-periosteum type 3. (a) General overview of the bulk tissue (×2.6 magnification; 800-μm scale bar). (b, c) Higher magnifications (b: ×8 magnification and 300-μm scale bar; c: ×10 magnification and 200-μm scale bar) of connective tissue organization, showing the presence of bone graft and adipose tissue infiltration in the inner part of the biopsy sample. *: adipose tissue; TC: connective tissue; black arrows: membrane fragments. Images were stained with h&e and acquired with a digital scanner.
Circumferential and dense fibers interposing through the pores were observed around the Ti-mesh. Underneath the circumferential fibers, the straight fibers were parallel to the Ti-mesh surface, and slightly loose and oblique fibers were observed below. Few blood vessels and no mineralized structure were observed. A similar layer of connective tissue has been observed under different types of nonresorbable membrane barriers.\textsuperscript{8,23} In particular, in human and animal studies, Simion et al\textsuperscript{8,23} showed that most coronal portions of implants placed with a one-stage GBR using nonresorbable Ti-reinforced membranes were immersed in a dense fibrous tissue over the crestal level of the regenerated bone. Histologic analysis at 9 months showed that this tissue consisted of densely packed collagen fibers with few cells and scarce blood vessels. No inflammatory reactions or epithelial tissue were present.\textsuperscript{23,32} In the present study, histologic analyses were performed on biopsy samples of types 2 and 3 pseudo-periosteum obtained from patients at T1. Type 1 pseudo-periosteum was not used because it was not an adequate thickness. In agreement with previous reports, the pseudo-periosteum was composed of connective tissue with a multidirectional fiber organization, a variable degree ofcellularity, little or no vascularization, and an absence of inflammatory reactions. Moreover, a pseudo-periosteum was detected in both study groups, although with a significant difference. In Group A, treated with d-PTFE, the majority of cases showed a type 1 pseudo-periosteum, representing a tissue that is absent or thinner than 1 mm. By contrast, in Group B, the majority of cases showed a pseudo-periosteum thicker than 3 mm. In addition, the bone under the d-PTFE membrane was significantly more dense than that under the Ti-mesh and collagen membrane. These differences may be associated with host-related factors, such as the major regenerative site potential. Due to the small sample size, statistical analysis did not reveal any significant correlations. As reported previously, different degradation timing and cell-occlusive effects may affect the amount of connective tissue under the membranes.\textsuperscript{33} Further studies should investigate the role of the membrane placed over the mesh, comparing mesh with membranes and mesh alone. More histologic analyses are needed to confirm these preliminary results that suggest a reduction of pseudo-periosteum and an improvement of bone density with long-lasting membranes. Although native collagen membranes have excellent cell affinity and biocompatibility, they have obvious drawbacks for GBR applications, including the loss of space-maintaining ability, inferior mechanical strength, and rapid biodegradation.\textsuperscript{34} To reinforce the mechanical and biodegradable stability and achieve acceptable biocompatibility for use as GBR membranes, various chemical, physical, and biologic cross-linking methods have been introduced to cross-link collagen. Among the chemical cross-linkers, glutaraldehyde, ethyl-dimethylaminopropyl-carbodiimide, and diphenyl-phosphorylation azide are the most commonly used.\textsuperscript{34} The choice to use a cross-linked collagen membrane can be questionable because it has a low degradation and long-lasting effects, but it requires a more complicated degradation and, consequently, more severe tissue inflammation. Moreover, the micromovements between the bone and any implanted material prevent bone formation, resulting in the development of fibrous tissue.\textsuperscript{14} On the other hand, some authors proposed the addition of another barrier membrane with cell-occlusive properties over the Ti-mesh, which is similar to the present study.\textsuperscript{15–17,21} The results of the present study showed pseudo-periosteum in both groups, but it was significantly lower under d-PTFE than under the Ti-mesh plus collagen membrane. Based on these observations, the authors concluded that the use of a completely occlusive Ti barrier could improve the regenerated bone quality and reduce the pseudo-periosteum tissue layer. In addition, the d-PTFE membranes could limit the stress and micromovements on the interface between the devices and tissue, which may be implicated in the genesis of this soft tissue layer during the fibrointegration of the implants.\textsuperscript{16,18} In general, shrinkage of the blood clot under the membrane during the initial stage of healing or an insufficient healing period could influence formation of the connective tissue layer. Although the pseudo-periosteum has histologically described,\textsuperscript{11,13,22} the clinical role of this tissue remains unclear.\textsuperscript{15} In the present study, three types of tissue (varied according to thickness)
were identified. The presence of the pseudo-periosteum under a barrier device corresponds to a lack of bone regeneration with respect to the planned reconstructive bone volume. At this time, it remains unclear whether the soft tissue under the barrier devices undergoing mineralization after an extended time is due to the low cellularity and lack of mineralized structure.\(^{18}\) Lim et al\(^{15}\) suggested that pseudo-periosteum formation facilitates secondary-intention healing in cases of barrier-device exposure, and some authors believe this tissue should be maintained to protect newly formed bone and avoid bone exposure in cases of secondary healing after membrane removal.\(^{18,24}\) The histologic images showed blood vessels and fibroblast cells that can protect the newly formed bone. As this tissue could protect the bone and prevent graft infection and resorption, the authors suggested leaving the tissue in place after barrier device removal, irrespective of the tissue type. The limits of this study are small sample size, calibration of examiner, and absence of histomorphometric analysis and can be avoided in following studies to improve the validity of these findings.

Conclusions

The results of this RCT showed e-PTFE membranes or Ti-mesh plus collagen membranes form three different types of pseudo-periosteum above the newly formed bone. However, d-PTFE membranes showed a significantly better bone density and thinner pseudo-periosteum compared to Ti-mesh and collagen membranes.

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