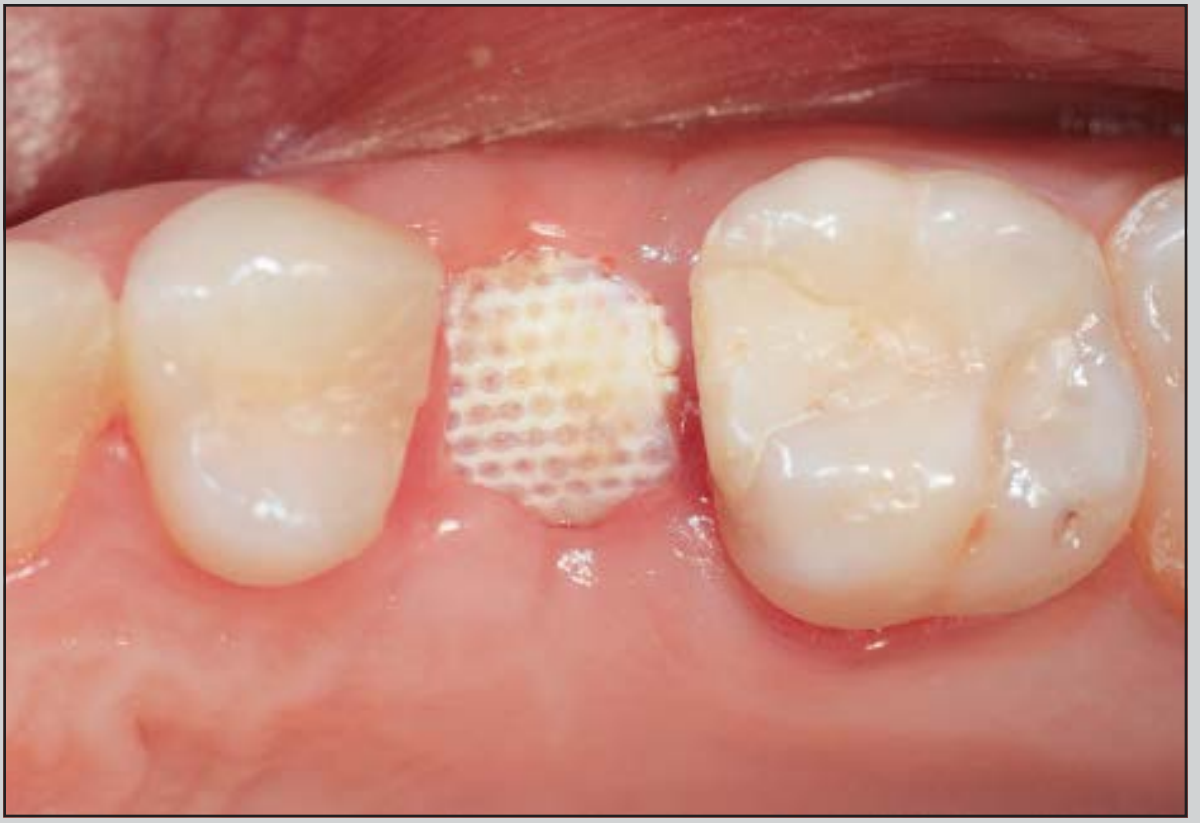
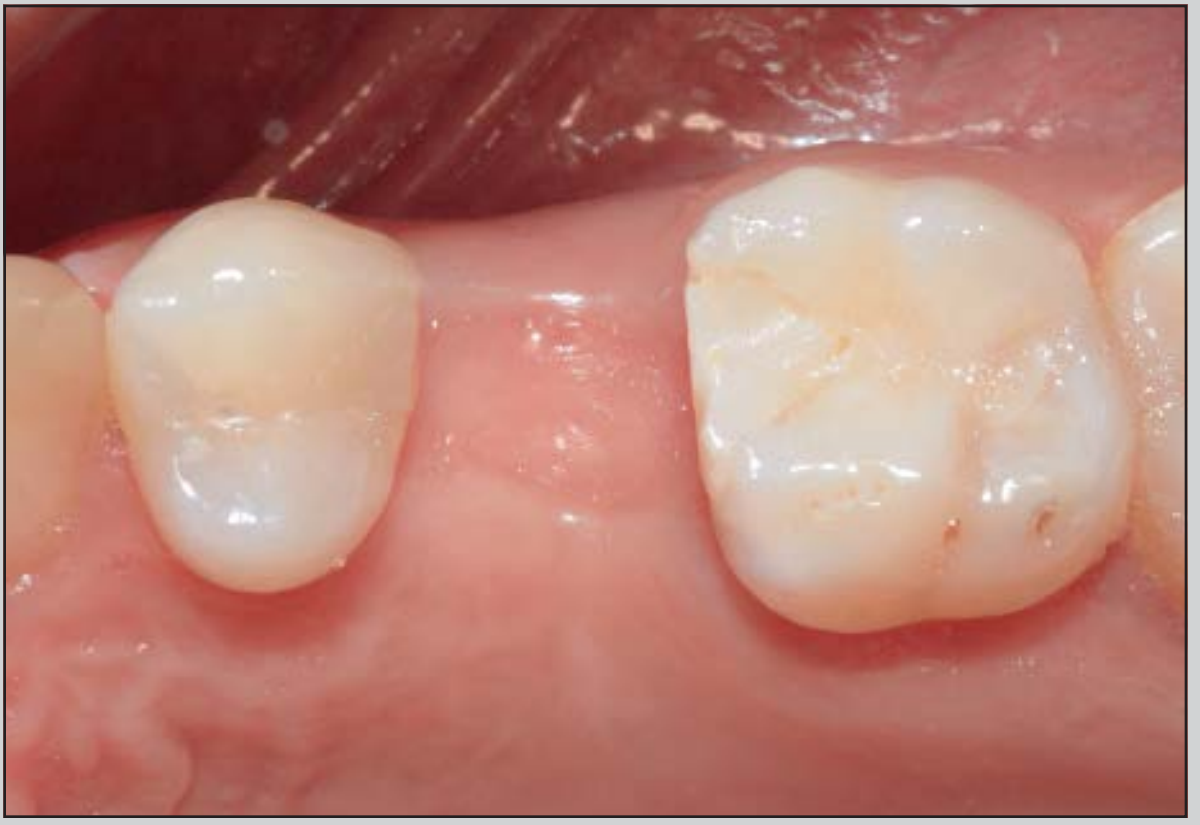
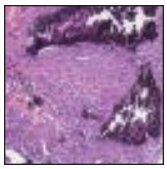




Socket Preservation with d-PTFE Membrane: Histologic Analysis of the Newly Formed Matrix at Membrane Removal



Domenica Laurito, DDS, PhD[1]/Riccardo Cugnetto, DDS[2]
Marco Lollobrigida, DDS[1]/Fabrizio Guerra, MD, DDS[3]
Annarita Vestri, PhD[4]/Francesca Gianno, MD[5]
Sandro Bosco, MD[6]/Luca Lamazza, DMD, PhD[2]
Alberto De Biase, MD, DDS[3]

This study aimed to evaluate the efficacy of an exposed high-density polytetrafluoroethylene (d-PTFE) membrane in preventing epithelial migration in postextraction sockets. For this purpose, a histologic description of the newly formed soft tissue underlying the membrane is presented. The periodontal status of the adjacent teeth was also evaluated to assess the gingival response. Ten premolar extraction sockets were treated. After tooth extraction, the sockets were filled with nanocrystalline hydroxyapatite and covered with d-PTFE membranes. Subperiosteal pockets were created to ensure the stability of the membranes. Membranes were left intentionally exposed and were atraumatically removed after 28 days. At that time, a bioptic specimen of the newly formed soft tissue under the membranes was taken. All the histologic samples showed a dense connective tissue without epithelial cells and no signs of foreign body reaction. No significant variation of the periodontal indices was observed on the teeth adjacent to the extraction sites. The study results indicate that exposed d-PTFE membranes can prevent epithelial migration in healing sockets without consequences on the periodontal health.
Int J Periodontics Restorative Dent 2016;36:877–883. doi: 10.11607/prd.2114

[1]PhD Student, Department of Oral and Maxillofacial Sciences, Division of Oral Surgery, Sapienza University of Rome, Rome, Italy.
[2]Private Practice, Rome, Italy.
[3]Associate Professor, Department of Oral and Maxillofacial Sciences, Division of Oral Surgery, Sapienza University of Rome, Rome, Italy.
[4]Full Professor, Department of Public Health and Infectious Diseases, Hygiene Unit, Sapienza University of Rome, Rome, Italy.
[5]Anatomic Pathology Student, Department of Molecular Medicine, Division of Oral Surgery, Sapienza University of Rome, Rome, Italy.
[6]Researcher, Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy.

Correspondence to: Prof Alberto De Biase, Department of Oral and Maxillofacial Surgery, Division of Oral Surgery, Via Caserta 6, 00161 Rome, Italy.
Email: alberto.debiase@uniroma1.it

Adequate alveolar ridge volume is a prerequisite for successful implant placement. Postextraction bone resorption can significantly affect implant-related esthetics as well as long-term implant survival.

After tooth extraction, the healing process leads to closure of the socket.[1] However, this process is not purely reparative; it involves an unfavorable loss of hard and soft tissues. The first phase of healing consists of blood clot formation. The clot is then replaced by a granulation tissue that, through migration of mesenchymal cells and synthesis of collagen fibers, leads to the formation of a provisional connective tissue. Osteoid matrix is then deposited and starts to mineralize from the deepest portion of the socket. The bone initially formed is an immature woven bone that will be replaced by a lamellar bone. Throughout this process, bone resorption can occur due to the loss of the periodontal ligament.[2,3] Hence, the overall result of these events is the closure of the socket in association with a dimensional vertical and horizontal loss of the alveolar bone. Lekovic et al[4] reported bone resorption up to 40% of the alveolar height and 60% of the alveolar width for anterior teeth and premolars 6 months after tooth extraction.

Socket preservation (SP) includes several techniques to minimize the dimensional changes in

soft and hard tissues after tooth extraction. Barrier membranes have been used extensively in SP to isolate the extraction sites from the oral environment and to guarantee the blood clot stability.[5–8] To serve these purposes, membranes must be impenetrable to cells and must ensure a space-maintaining effect.[9]

Different techniques have been described in the literature with particular concern for the ridge dimensions and the histologic characteristics of the newly formed bone at the time of implant placement.[10] Conversely, limited histologic data is available with respect to the soft tissue healing in postextraction sockets covered with membranes. The aim of the present pilot study was to investigate the barrier effect of an exposed high-density polytetrafluoroethylene (d-PTFE) membrane in postextraction sockets, histologically evaluating the underlying newly formed soft tissue at 28 days. Periodontal indices on the teeth adjacent to the healing sites were also recorded to investigate the effects of membrane exposure on the periodontal tissue.

Materials and methods

The present study was performed at the Department of Oral and Maxillofacial Science, Division of Oral Surgery, Sapienza University of Rome, Italy. Ethical approval was obtained from the local Ethics Committee for Human Research (protocol number 2815/13.06.2013).

Study design

A total of 10 patients (5 women and 5 men; aged ≥ 18 years; mean age 37.65; age range 24–55), referred for maxillary and mandibular premolar extraction and subsequent single-tooth implant rehabilitation, were enrolled in the present study. The indications for dental extraction included root fracture, advanced periodontal disease, endodontic treatment failure, and nonrestorable hopeless teeth.

Subjects presenting any of the following exclusion criteria were not admitted to the study:

- Systemic diseases/conditions (eg, pregnancy, diabetes, metabolic bone diseases, history of malignancy)
- Long-term steroidal or nonsteroidal anti-inflammatory drug therapy
- Heavy smokers (> 10 cigarettes/day)
- Failure to sign the informed consent
- Unwillingness to return for the follow-up examinations

An informed consent form was signed in accordance with the Helsinki Declaration of 1975, as revised in 2008.

An oral hygiene protocol including scaling, root planing, and oral hygiene instructions was established for each patient 1 week before the surgery (T_0).

A comprehensive periodontal examination of the tooth to be extracted and the adjacent teeth was performed with a periodontal probe (UNC-15, Hu-Friedy) immediately before the surgical procedure (T_1). Plaque Index (PI), Gingival Index (GI), and bleeding on probing (BoP) were recorded.

A reassessment was planned for 6 weeks after the SP procedure (T_5). All measurements were taken by one dental hygienist who was previously calibrated after an initial training on casts. A clinical follow-up of the complete healing was finally carried out at 6 months (T_6).

Surgical procedure and materials

After a mouthrinse with 0.20% chlorhexidine for 1 minute prior to surgery, local anesthesia (2% mepivacain with 1:100.000 epinephrine) was administered. All cases were treated by the same operator (D.L.) (Fig 1). Atraumatic extractions were performed with a dedicated piezoelectric device to prevent fracture or fenestration of the facial bone walls.

After debridement and irrigation with sterile saline solution, the sockets were filled with nanocrystalline hydroxyapatite (ncHA) (NanoBone, Artoss).

Nonresorbable d-PTFE membranes (Cytoplast TXT-200 Singles, Osteogenics) were trimmed to fit and cover the sockets. Two subperiosteal pockets were created buccal and lingual to each extraction site to insert the edges of the membrane. For this purpose, the gingival margins were undermined without a releasing incision. A space of 1.0 mm between the membrane and the adjacent teeth was maintained to

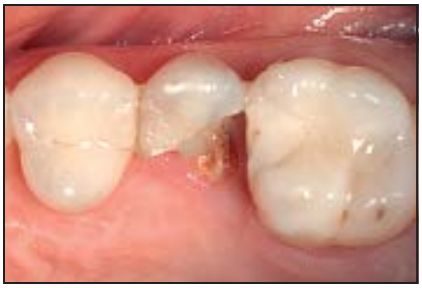


Fig 1 *A maxillary second premolar to be extracted.*

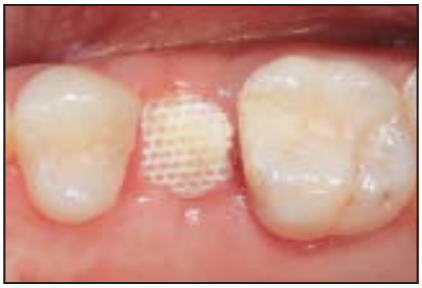


Fig 2 *Clinical view before membrane removal (28 days). Plaque accumulation was detected on the exposed surface of the membrane.*

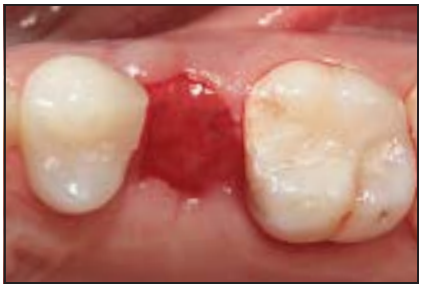


Fig 3 *Clinical view immediately after membrane removal.*

enhance the reattachment of the papillae. Membrane stabilization was obtained with two interrupted sutures at the interdental papillae and a horizontal mattress suture across the socket (Cytoplast PTFE, Osteogenics). The membrane was left intentionally exposed until removal.

Postoperative therapy consisted of antibiotics (amoxicillin 875 mg + clavulanic acid 125 mg, twice a day for 7 days), anti-inflammatories (nimesulide 100 mg, twice a day for 3 days), and 0.20% chlorhexidine mouthrinses (twice a day for 14 days). Patients were observed at 7 days (T_2), and sutures were removed at 14 days (T_3).

Membrane removal

Membranes were atraumatically removed at 28 days (T_4) without anesthesia (Figs 2 and 3). After the removal, a biopsy of the underlying tissue was performed in the middle of the socket with a #11 blade (Bard Parker). The specimens were minimally dimensioned (2 × 2 mm in width and 2 mm in depth). The center of the socket was determined with a periodontal probe as the cross point between the buccolingual and mesiodistal dimensions from the midpoint of the alveolar crests. No postoperative therapy was given after this procedure.

Histologic analysis

Specimens were fixed in 10% neutral buffered formalin, dehydrated through alcohol baths of increasing concentration (70% to 100% ethylic alcohol and xylene) and included in paraffin. Sections of 4-µm thickness were prepared and stained with hematoxylin-eosin.

Statistical analysis

Periodontal indices were statistically analyzed. Continuous variables are presented as median and interquartile range because the distribution is not normal. Wilcoxon test was used to evaluate differences within the groups. The probability values are two-sided, and $P < .05$ was considered statistically significant. All analyses were carried out with Stata 12 software.

Results

Each of the 10 patients in the study contributed one extraction site. Of the subjects, 5 were smokers (< 10 cigarettes per day). All the procedures were successfully carried out with no postoperative complication.

Clinical evaluation

None of the patients reported any unusual pain and no clinical signs of infection (swelling and suppuration) were observed until membrane removal. Mild inflammation of the gingival margins was noted at T_4, and plaque accumulation was constantly detected on the exposed surface of the membranes. Partial epithelialization occurred at T_5 without signs of inflammation

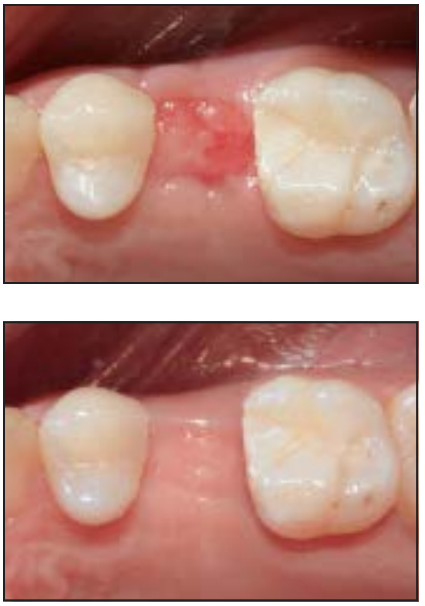


Fig 4 (top) *Follow-up at 42 days.*

Fig 6 (bottom) *Follow-up at 6 months. Keratinized tissue had completely covered the socket.*

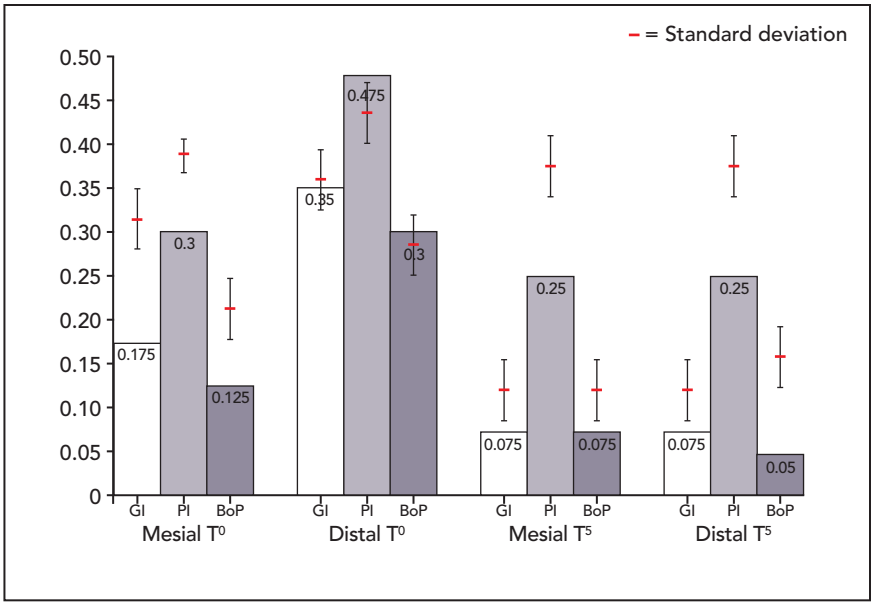
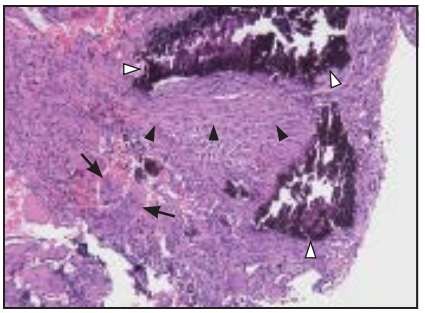


Fig 5 *Periodontal indices variation. GI = Gingival Index; PI = Plaque Index; BoP = bleeding on probing.*



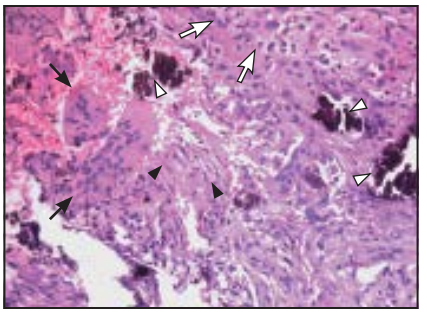


Fig 7 (left) *Histologic specimen showing fibroblasts (solid arrowheads) surrounded by connective tissue matrix, with some ncHA granules (open arrowheads) and multinucleated giant cells (arrows). Hematoxylin-eosin staining; original magnification ×10.*

Fig 8 (right) *At a higher magnification, acute inflammatory cell infiltration of polymorphonuclear granulocytes (open arrows) can be noted. Hematoxylin-eosin staining; original magnification ×20.*

(Fig 4). Although all the periodontal indices were reduced at T_5 on both the mesial (GI from 0.18 to 0.08; PI from 0.30 to 0.25) and the distal tooth (GI from 0.35 to 0.08; PI from 0.48 to 0.25), only the BoP reduction on the distal tooth (from 0.30 to 0.05) was statistically significant (P = .046) (Fig 5). A complete epithelialization was observed at T_6 in all cases (Fig 6).

Histologic evaluation

Ten tissue samples were harvested at the time of membrane removal. At the microscopic analysis, no epithelial cells were detected. All the specimens exhibited a dense connective tissue with a large number of fibroblast and inflammatory cells (both lymphoplasma cells and neutrophil granulocytes), forming phlogistic areas with granulation aspect. No signs of foreign-body reaction were present. Giant multinucleated osteoclastic-like cells were also detectable in association with various ncHA granules. A network of small blood vessels within the connective tissue was observed. No bacterial contamination was observed in any case (Figs 7 and 8).

Discussion

In the present study, the effect of a d-PTFE membrane associated with ncHA was evaluated via clinical examination and histologic analysis of the newly formed soft tissue underlying the membrane.

At membrane removal, the original position of the gingival margins was unvaried and an underlying soft tissue was clinically observable. In all cases, the d-PTFE membranes contained the graft material and prevented epithelial migration into the cavity. No infective complications occurred in the postextraction sites during the follow-up. The histologic analysis of the soft tissue samples showed the presence of a dense connective tissue matrix with mainly fibroblasts and no epithelial cells. The GI and PI recorded on the adjacent teeth showed no significant differences at the 6-week follow-up, while a BoP reduction on the distal tooth was noted.

The rationale for adopting SP techniques is the maintenance of a sufficient alveolar bone volume for implant placement. Different protocols, including the use of membranes, graft materials, or a combination of both, have been described in the literature. However, there is currently no agreement about the procedure or the timing. Although some authors have proposed the use of graft materials alone to maintain the alveolar volume,[11,12] the association with barrier membranes has been claimed as giving better results.[13]

One of the main advantages of resorbable membranes (eg, polylactic acid, collagen membranes) is that a second surgical procedure is not necessary for membrane removal. On the other hand, they require a primary tension-free closure, which can create more problems during the primary surgery.[14] Nonresorbable expanded PTFE (e-PTFE) membranes have been reported to be most effective in providing bone regeneration, but a second surgery is needed for their retrieval.[15] Similar to resorbable membranes, e-PTFE membranes need a complete coverage but present a higher incidence of premature spontaneous exposure.

One major problem occurring with flap dehiscence and membrane exposure is infection of the healing site. However, different outcomes have been described with exposed nonresorbable and resorbable membranes. Exposed e-PTFE membranes, due to their high surface roughness and microporosity, are susceptible to bacterial penetration and need to be removed.[16] On the contrary, exposed collagen membranes usually do not lead to infection, although premature degradation causes a loss of the barrier function and a reduction in bone regeneration.[17] From this perspective, one of the most relevant topics in recent years is the use of intentionally exposed occlusive membranes to protect healing extraction sites. Bartee and Carr[18] were the first authors to extensively describe the use of d-PTFE membranes in GBR and SP techniques. Due to the 0.2-μm nanopores, dense membranes have been claimed to resist bacterial penetration with a low risk of infection even when exposed to the oral environment.[19] This assumption is based on the disparity between the average size of bacteria (about 1–2 μm) and the pore diameter. Examining d-PTFE membranes with a scanning electron microscope after 21 days exposure, Krauser[20] observed no bacterial contamination on the internal surface. In the present study, no bacterial cells were observed in the specimens although plaque accumulation was constantly detected on the external surface of the membranes. Furthermore, the external contamination did not negatively affect the periodontal indices on the adjacent teeth. The observed reduction may be related to the postoperative therapy with chlorhexidine. These findings support the idea of a minimal or absent inflammation induced by the exposed membranes on the surrounding tissue.

In the esthetic area, SP techniques with d-PTFE membranes were demonstrated to preserve the keratinized gingiva (KG). On the contrary, different studies have reported a reduction in KG with e-PTFE and resorbable membranes.[21,22] Maintenance of the original position of the gingival margins may contribute to preserve the existing keratinized tissue. In addition, the secondary epithelialization that follows membrane removal can further increase the keratinized tissue.[23] In a series of 420 cases treated with exposed d-PTFE membranes after tooth extraction, Barboza et al[24] reported the formation of a normal KG and a preserved mucogingival junction position for all patients at the time of implant placement. The

results of the present study are in accordance with these findings, since no infection occurred in any case and a complete epithelialization could be clinically observed at 6 months.

Another advantage of exposed d-PTFE membranes is that they do not require a second surgical phase to be removed.[25] The removal is also facilitated by the smooth internal surface. In the present study, membrane removal was scheduled at 28 days. According to Barber et al[23] longer membrane persistence may lead to an apical migration of the flap or a delay in bone formation. For this reason, the authors suggested a time range for membrane removal between 4 and 6 weeks after surgery.

To the authors' knowledge, no histologic analyses of specimens retrieved at the removal of exposed d-PTFE membranes have been performed to date. In a recent study on GBR with d-PTFE membranes, Ronda et al[26] observed a thin fibrous tissue layer (< 1 mm) between the membrane and the regenerated bone after 6 to 7 months. However, this tissue has not been further characterized. The soft tissue features observed in the present study are comparable to those of the spontaneous wound healing process of extraction sockets at 4 weeks.[3] At this phase of healing, the initial granulation tissue is progressively replaced by a dense fibrous tissue that constitutes a provisional matrix. In this regard, d-PTFE membranes seem not to have impaired the healing process of the newly formed tissue. In the authors' opinion, the importance of this tissue layer should be highlighted since it represents the only separation between the graft material and the oral environment until the complete epithelialization.

Conclusions

The present study focused exclusively on soft tissue. The histologic analysis of 10 tissue specimens revealed no epithelial ingrowth or bacterial contamination after 28 days of membrane exposure in postextraction sockets filled with ncHA. Although the specimens may be not representative of the whole of the soft tissue under the membranes, the histologic findings indicate that the use of d-PTFE can exclude epithelial and bacterial cells from the healing sites.

Acknowledgments

The authors reported no conflicts of interest related to this study.

References

1. Amler MH. The sequence of tissue regeneration in human extraction wounds. Oral Surg Oral Med Oral Pathol 1969;27:309–318.
2. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 2005;32:212–218.
3. Trombelli L, Farina R, Marzola A, Bozzi L, Liljenberg B, Lindhe J. Modeling and remodeling of human extraction sockets. J Clin Periodontol 2008;35:630–639.
4. Lekovic V, Camargo PM, Klokkevold PR, et al. Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. J Periodontol 1998;69:1044–1049.
5. Mardas N, Chadha V, Donos N. Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: A randomized, controlled clinical trial. Clin Oral Implants Res 2010;21:688–698.
6. Barone A, Aldini NN, Fini M, Giardino R, Calvo Guirado JL, Covani U. Xenograft versus extraction alone for ridge preservation after tooth extraction. A clinical and histomorphometric study. J Periodontol 2008;79:1370–1377.
7. Fotek PD, Neiva RF, Wang HL. Comparison of dermal matrix and polytetrafluoroethylene membrane for socket bone augmentation: A clinical and histologic study. J Periodontol 2009;80:776–785.
8. Becker W, Lynch SE, Lekholm U, et al. A comparison of ePTFE membranes alone or in combination with platelet-derived growth factors and insulin-like growth factor-I or demineralized freeze-dried bone in promoting bone formation around immediate extraction socket implants. J Periodontol 1992;63:929–940.
9. De Biase A, Catalano G, Vozza I, Lamazza L. Rigenerazione ossea guidata in 40 sedi implantari ritardate mediante l'utilizzo di Biostite e membrana Paroguide. Implantologia 2007;2:33–39.
10. Vignoletti F, Matesanz P, Rodrigo D, Figuero E, Martin C, Sanz M. Surgical protocols for ridge preservation after tooth extraction. A systematic review. Clin Oral Implants Res 2012;23 (suppl):s22–s38.
11. Nemcovsky CE, Serfaty V. Alveolar ridge preservation following extraction of maxillary anterior teeth. Report on 23 consecutive cases. J Periodontol 1996;67:390–395.
12. Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: Histomorphometric evaluations at 9 months. J Periodontol 2000;71:1015–1023.
13. Morjaria KR, Wilson R, Palmer RM. Bone healing after tooth extraction with or without an intervention: A systematic review of randomized controlled trials. Clin Implant Dent Relat Res 2014;16:1–20.
14. Wang HL, Carroll MJ. Guided bone regeneration using bone grafts and collagen membranes. Quintessence Int 2001;32:504–515.
15. Simion M, Scarano A, Gionso L, Piattelli A. Guided bone regeneration using resorbable and nonresorbable membranes: A comparative histologic study in humans. Int J Oral Maxillofac Implants 1996;11:735–742.

16. Moses O, Pitaru S, Artzi Z, Nemcovsky CE. Healing of dehiscence-type defects in implants placed together with different barrier membranes: A comparative clinical study. Clin Oral Implants Res 2005;16:210–219.
17. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP. Histological assessment of augmented jaw bone utilizing a new collagen barrier membrane compared to a standard barrier membrane to protect a granular bone substitute material. Clin Oral Implants Res 2002; 13:587–594.
18. Bartee BK, Carr JA. Evaluation of a high-density polytetrafluoroethylene (n-PTFE) membrane as a barrier material to facilitate guided bone regeneration in the rat mandible. J Oral Implantol 1995; 21:88–95.
19. Carbonell JM, Martín IS, Santos A, Pujol A, Sanz-Moliner JD, Nart J. High-density polytetrafluoroethylene membranes in guided bone and tissue regeneration procedures: A literature review. Int J Oral Maxillofac Surg 2014;43:75–84.
20. Krauser JT. High-density PTFE membranes: Uses with root-form implants. Dent Implantol Update 1996;7:65–69.
21. Bartee BK. Extraction site reconstruction for alveolar ridge preservation. Part 2: Membrane-assisted surgical technique. J Oral Implantol 2001;27:194–197.
22. Bartee BK. Evaluation of a new polytetrafluoroethylene guided tissue regeneration membrane in healing extraction sites. Comp Cont Ed Dent 1998;19: 1256–1264.
23. Barber HD, Lignelli J, Smith BM, Bartee BK. Using a dense PTFE without primary closure to achieve bone and tissue regeneration. J Oral Maxillofac Surg 2007;65: 748–752.
24. Barboza EP, Stutz B, Ferreira VF, Carvalho W. Guided bone regeneration using nonexpanded polytetrafluoroethylene membranes in preparation for dental implant placements--A report of 420 cases. Implant Dent 2010;19:2–7.
25. Bartee BK. The use of high density polytetrafluoroethylene membrane to treat osseous defects: Clinical reports. Implant Dent 1995;4:21–26.
26. Ronda M, Rebaudi A, Torelli L, Stacchi C. Expanded vs. dense polytetrafluoroethylene membranes in vertical ridge augmentation around dental implants: A prospective randomized controlled clinical trial. Clin Oral Implants Res 2014;25: 859–866.