

# Microbiological and FE-SEM Assessment of d-PTFE Membrane Exposed to Oral Environment after Alveolar Socket Preservation Managed with Granular nc-HA

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## ABSTRACT

**Aim:** The aim of this study was to analyze, by the aid of microbiological analysis and the field emission scanning electron microscopical (FE-SEM) analysis, the role of high-density polytetrafluoroethylene (d-PTFE) membranes in avoiding the microbial colonization of a nanocrystalline hydroxyapatite (nc-HA) bone graft and the involvement of this colonization in the healing process.

**Materials and methods:** Six patients underwent extraction of unrecoverable teeth, and a socket preservation technique was carried out with nc-HA synthetic bone graft and then covered with a d-PTFE membrane. After 28 days from surgery, FE-SEM analysis and BioTimer assay technique to assess the microbiological count of streptococci species were carried out. Data were collected and analyzed by the Student's *t* test (confidence interval: 95%).

**Results:** The mean amount of bacteria measured on the upper side of the membrane was  $6.52 \pm 0.50$  CFU, while on the lower side, it was  $6.59 \pm 0.40$  CFU. Significant differences were not found between the two sides of the membrane or between the different sectors ( $p > 0.05$ ). The FE-SEM analysis revealed structured biofilms on both sides of the membrane: species of cocci, bacilli, and fusobacteria were recognizable in occasional settled vegetations.

**Conclusion:** Since the amount of bacteria found was low, the improved impermeability of the d-PTFE membrane permitted the healing process to proceed uneventful and without signs of infection or inflammation.

**Clinical relevance:** The infection of the graft site could lead to a failure of the socket preservation technique which could delay or compromise the rehabilitation following procedures. The use of d-PTFE can improve the bone regeneration thanks to its antimicrobial properties.

**Keywords:** Alveolar socket preservation, Bacterial contamination, Bone graft, Dense polytetrafluoroethylene, Nanocrystalline hydroxyapatite. *The Journal of Contemporary Dental Practice* (2020): 10.5005/jp-journals-10024-2805

## INTRODUCTION

Socket preservation is a guided regenerative technique that aims to minimize the alveolar bone ridge resorption after the extraction of a tooth. For the preservation of the alveolar bone, the major indications are the positioning of dental implants and the prevention of unesthetic soft tissue changes: it is estimated that the post-extractive resorption, in the absence of preservative procedures, could reduce the socket's volume until ~50%.<sup>1</sup>

Actually, many autogenic, allogenic, xenogenic, and synthetic materials are used in the alveolar socket preservation technique like autogenous bone, mineralized or demineralized freeze-dried bone allograft, calcium phosphosilicate putty bone substitutes, and nc-HA. Such materials could be also used alone or in association to reach an improved result, but finally, none of them are considered able to completely stop the alveolar bone loss after tooth extraction.<sup>2,3</sup>

The use of resorbable or non-resorbable membranes is not mandatory for the alveolar preservation, but they could stabilize the bloodclot, allowing the migration of osteoprogenitor cells and preventing the migration of epithelial tissues into the post-extractive socket.<sup>4</sup>

Guided bone regeneration (GBR) adopts membranes to avoid interferences with bone regeneration made by non-osteogenic cells.<sup>5</sup> Resorbable membranes are considered tissue-friendly and do not require surgical removal even if they get exposed to oral

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environment, but the amount of regenerated bone obtained is less in volume when compared with non-resorbable ones. Reasons are due to the impossibility of reaching a stable graft outside the alveolar socket without the help of a titanium reinforcement (present only in the non-resorbable membranes).

Resorbable membranes are usually made of collagen, polyglycolic acid, and polyglactic acid; the non-resorbable ones are made of expanded polytetrafluoroethylene (e-PTFE) or d-PTFE with or without a titanium reinforcement.<sup>6</sup>

While semipermeable e-PTFE membranes consist of dense nodes interconnected by PTFE fibrils and are able to permit a

transmembranous transport of nutrients, dense PTFE membranes consisted of a dense, fibril-free structure, with large spaced indentations which results in an efficient barrier that avoids penetration by fibrous tissue and bacteria.<sup>7</sup> However, the main problem related with this kind of membranes is the exposure to the oral environment, which could allow bacteria to colonize the exposed surface and generate biofilm. To prevent bacterial overgrowth, PTFE membranes are usually dual layered, with an optimized surface developed specifically for the opposed tissue that they would face with.<sup>7,8</sup>

The aim of this study was to analyze, by the aid of microbiological analysis and the FE-SEM analysis, the role of d-PTFE membranes in avoiding the microbial colonization of an nc-HA bone graft and the involvement of this colonization in the healing process.

## MATERIALS AND METHODS

Six patients underwent extraction of unrecoverable teeth (three periodontally compromised teeth, two teeth with deep caries, and one endodontically not-retreatable tooth with periapical lesions affecting both roots), and a socket preservation technique was performed at the Oral Surgery Unit of Policlinico Umberto I of Rome, Italy. The protocol followed the guidelines of the Helsinki's Declaration and received the approval no. 2815/2013 from the Ethical Committee of the Policlinico Umberto I of Rome, Italy. All study participants provided an informed written consent prior to enrollment.

All the patients (three men and three women) included in the study were more than 18 years old ( $46.1 \pm 11.8$ ), declared to be light (less than 10 cigarettes per day) or non-smoker, without systemic pathologies, and with a plaque index <10%. Patients with periodontal disease were excluded. To preserve bone peaks and to obtain a more stable positioning of the membrane, the inclusion criteria for the tooth to be extracted were the presence of two adjacent healthy teeth.

Surgery was performed by expert dental surgeons with experience in socket preservation: all the procedures were done for maxillary and mandibular first molar sectors. Surgical procedure was minimally invasive, without the opening of a flap but only with the elevation of a subperiosteal pocket. After the extraction of the tooth and the intra-alveolar curettage, the post-extractive site was filled with nc-HA synthetic bone graft (NanoBone<sup>®</sup>; Artoss GmbH, Rostock, Germany) and then covered with a d-PTFE membrane (Cytoplast<sup>™</sup> Dense PTFE Membrane TXT-200; Osteogenics Biomedical, TX, USA) positioned over the graft, subperiosteally, underneath the elevated flap, and stabilized with non-resorbable 4-0 sutures (Cytoplast<sup>™</sup> PTFE Sutures, Osteogenics Biomedical) for 14 days (Figs 1A–H). The patients were prescribed to take amoxicillin and clavulanic acid (875 + 125 mg) twice per day. Patients were also prescribed to take painkillers if necessary (paracetamol 1,000 mg) and to apply a 0.3% chlorhexidine gel locally until the membrane removal as suggested by the manufacturer. After 28 days from the surgery, the surgical site was in good conditions, without signs of infection or inflammation and the membrane was removed paying attention to not to contaminate it and immediately sent to the microbiological analysis laboratory.

Removed d-PTFE membranes were put in sterile 1.5-mL tube containing few  $\mu$ L of sterile saline to avoid drying and examined within 1 hour from collection. The d-PTFE membranes were cut lengthwise into two equal halves. One half was subjected to

microbiological analysis to evaluate adherent bacteria, and the other half was examined by FE-SEM (Figs 2A and B).

The d-PTFE membranes were cut into three equal segments, i.e., oral, central, and vestibular (Fig. 2C). To evaluate the microbial population adherent to the upper and lower sides of the segments, a modified semiquantitative technique described by Maki et al. was performed.<sup>9</sup> Briefly, each segment was placed onto a plate with brain heart infusion (BHI) agar for 1 minute. The procedure was repeated once again. The bacteria placed on the surface of BHI plates were collected using a calibrated loop and counted by the Bio Timer assay (BTA) protocol.<sup>10</sup> The bacterial metabolism is measured by the use of the BTA method: it employs the BioTimer-Phenol Red (BT-PR) as medium. The time required for color switch of BT-PR medium (red to yellow) is correlated with the initial bacterial concentration. The required time for color switch is indicative for the amount of bacteria present in the specimen. For the purpose of this article, the correlation line of *Streptococcus oralis* was employed. The correlation line was described by the following equation:  $y = 0.301 \times 9.0615$  ( $r = 0.9999$ ). Therefore, to evaluate the total bacterial load, a total of 0.2 mL of BT-PR reagent was inoculated with the bacteria collected by the calibrated loop. Proper dilutions in BT-PR reagent were done in 96-well plates. The 96-well plates were incubated in Tecan Sunrise<sup>™</sup> Apparatus at 37°C. The time for color switch was recorded and used to estimate the total bacterial load through the correlation line of *Streptococcus oralis*.

## Microscopic Investigation

For FE-SEM observations, the half of the d-PTFE membrane was immersed in 2.0% glutaraldehyde at room temperature without light. After washing with phosphate solution, the d-PTFE membrane was fixed in 1% osmium tetroxide for 2 hours at 4°C in the dark. The specimen was dehydrated in ethanol solutions at crescent concentrations (from 30 to 100%) at 10-minute intervals and dried until the critical point. The FE-SEM analysis of the dehydrated d-PTFE membranes has been performed by the Auriga<sup>®</sup> 405 (Carl Zeiss AG, Oberkochen, Germany), operating at low extracting voltage (2 kV). Before the examination, the samples were coated with 60-nm chromium layer (Q150T; Quorum Technologies Ltd, Laughton, UK) to increase the conductivity and improve the quality of the images.

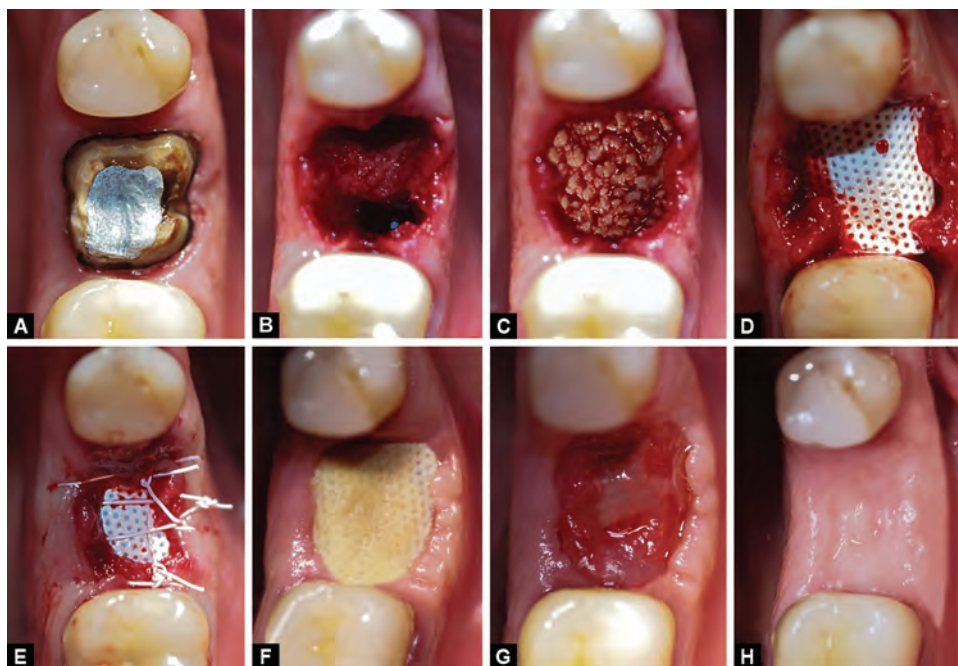
## Statistical Analysis

The correlation line was obtained by a linear regression analysis, and linear correlation coefficients were calculated from the following equation:  $r = (n\sum xy - \sum x\sum y) / \sqrt{[(n\sum x^2 - (\sum x)^2)(n\sum y^2 - (\sum y)^2)]}$ . Results were expressed as mean values  $\pm$  standard deviations (SDs). The statistical analysis was performed using the Student's *t* test, and *p* values  $\leq 0.05$  were considered significant.

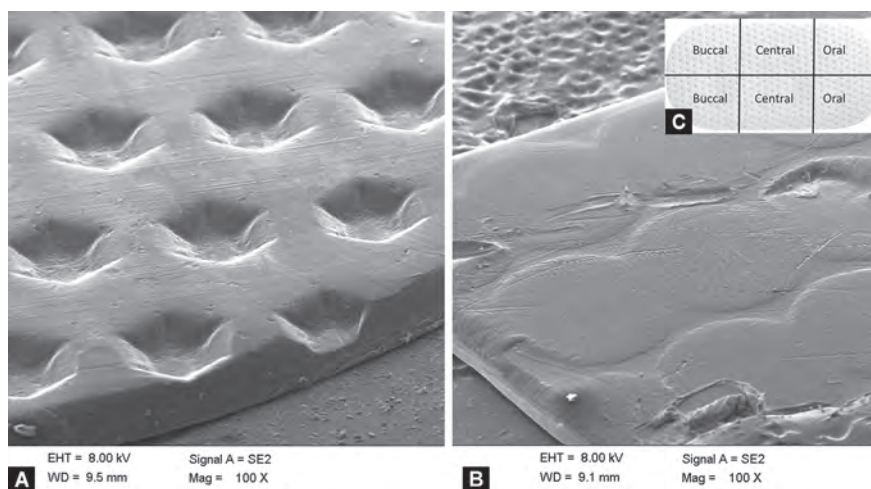
## RESULTS

All the socket preservation procedures were successful, and no patients experienced discomfort or needed to remove the membrane before the expected day. Since different amounts of plaque were visible on membranes' surface, no inflammatory conditions like redness, swelling, pus, or pain were diagnosed at the removal.

The total bacterial counts were performed on both the upper and the lower sides of the extracted d-PTFE membranes. Results showed that both sides of the d-PTFE membranes were colonized. The bacterial populations of the upper sides ranged from  $3.6 \times$



**Figs 1A to H:** Surgical procedures: (A) A mandibular first molar; (B) The socket after its extraction; (C) nc-HA in the post-extractive socket; (D) d-PTFE membrane covering the graft; (E) PTFE suture; (F) Aspect of the membrane; (G) The underlying gingival tissues after 28 days; (H) Gingival aspect after 7 months



**Figs 2A to C:** Aspect of the sterile membrane at FE-SEM: (A) Upper; (B) Lower layers; (C) Subdivision of the d-PTFE membrane for the microbiological and microscopical analyses

**Table 1:** Total bacterial counts of the upper and lower sides of the extracted membranes

Patient (sample no.)	Total counts log CFU (upper side)	Total counts log CFU (lower side)
V. A. (1)	6.86	7.16
P. A. (2)	7.30	6.96
C. O. (3)	6.36	6.05
F. U. (4)	6.00	6.47
P. A. (5)	6.55	6.45
M. P. (6)	6.05	6.42
Mean values	6.52	6.59
SD	±0.50	±0.40
p		0.3947

$10^6$  to  $6.9 \times 10^7$  CFU and those of the lower parts from  $3.5 \times 10^6$  to  $4.5 \times 10^7$  CFU. However, no significant difference between the populations of upper and lower sides was found (Table 1).

Thereafter, we evaluated if differences existed between the biofilm populations adherent to the oral, central, and vestibular parts of the d-PTFE membranes.

The data (Table 2) showed that the bacterial populations adherent on the different parts of the d-PTFE membranes ranged from  $4.5 \times 10^5$  to  $4.1 \times 10^7$  CFU on the upper side and from  $6.4 \times 10^5$  to  $2.9 \times 10^7$  CFU on the lower side. However, the central parts of the both sides were colonized at higher extent and less in the oral and vestibular ones. Significant differences were noticed between the central and both the oral and vestibular parts of the lower side of the membranes.

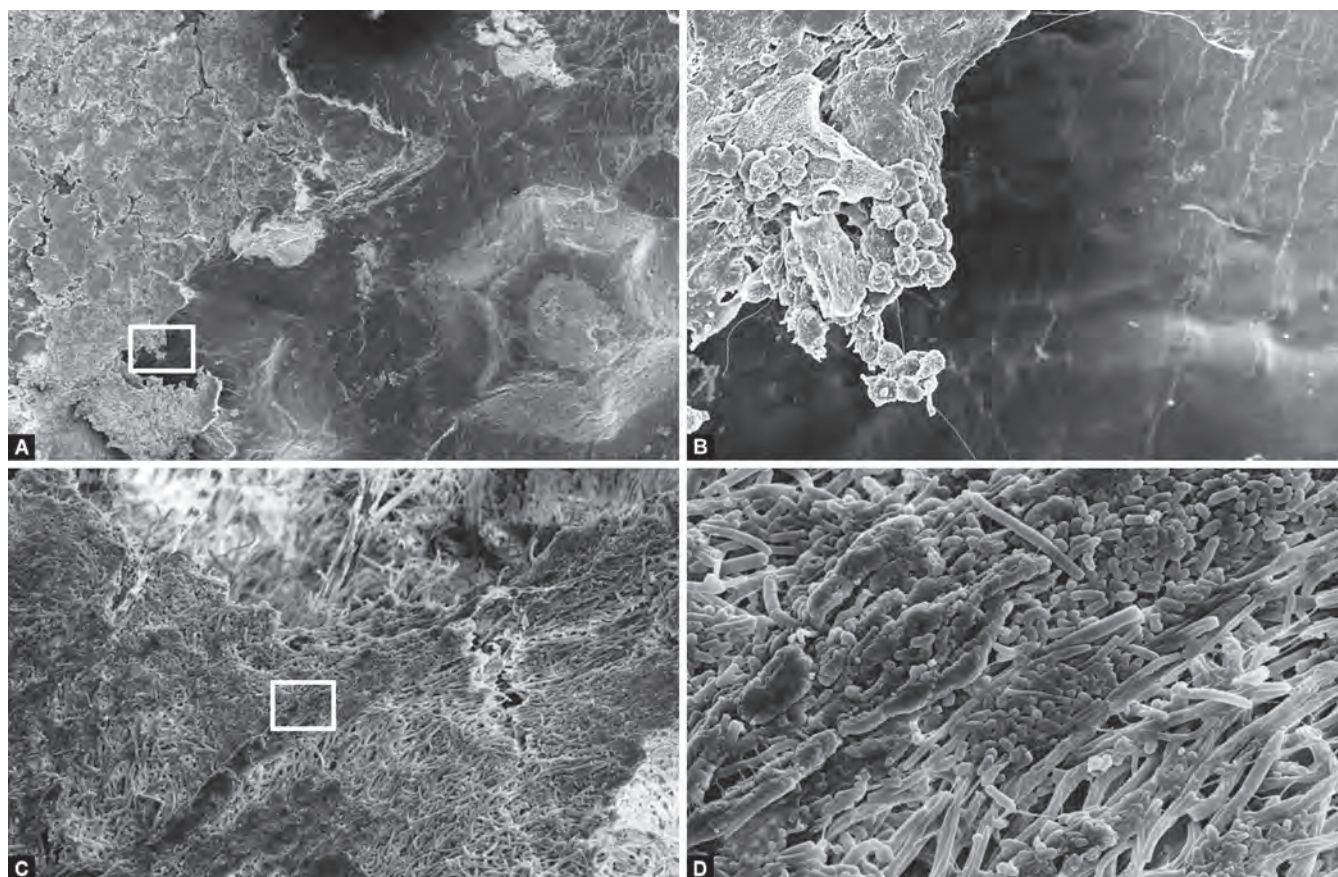
In Figure 2, the FE-SEM images of sterile d-PTFE membrane are shown. Microscopic observations of removed membranes showed that both upper and lower sides of the d-PTFE membranes were colonized by adherent biofilm. Moreover, the FE-SEM images confirmed that the biofilm colonization was at higher extent in the central part and less in the vestibular and oral ones as depicted in the representative figures (Figs 3 and 4).

## DISCUSSION

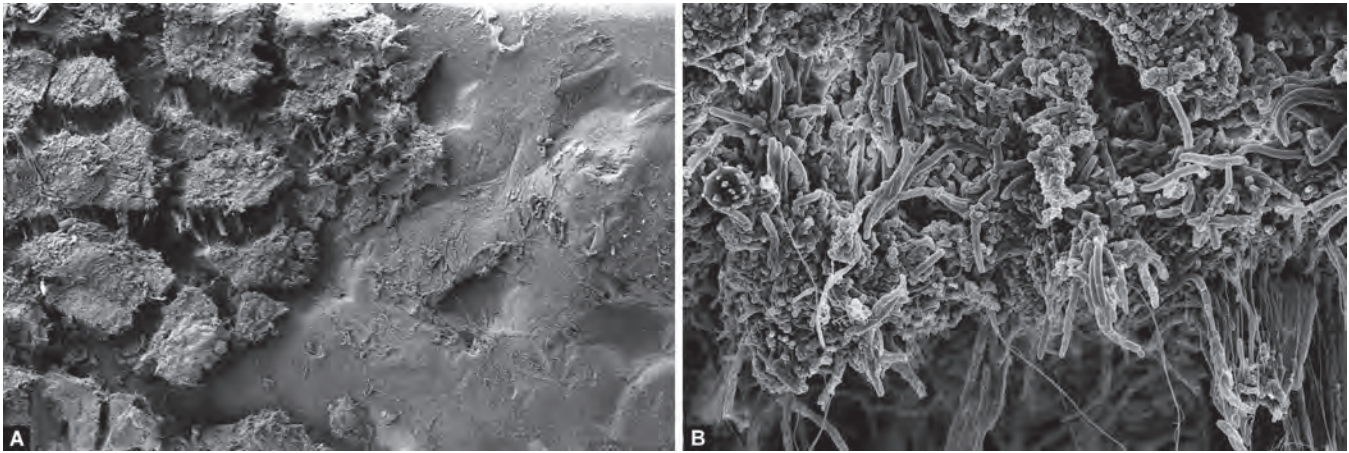
The use of membranes associated with GBR techniques is commonly considered a valid option to obtain hard and soft tissue preservation after tooth extraction.<sup>11</sup> Since d-PTFE is characterized by a peculiar low porosity, microbial contamination is considered a rare opportunity when compared with the less dense e-PTFE

**Table 2:** Bacterial counts of oral (1), central (2), and vestibular (3) parts of the upper (UP) and lower (LW) sides of the extracted membranes

Patient (sample no.)	Log CFU					Log CFU					p values UP vs LW
	UP 1	UP 2	UP 3	Mean values	SD	LW 1	LW 2	LW 3	Mean values	SD	
V. A. (1)	6.56	7.15	6.86	6.86	±0.30	7.15	7.18	7.15	7.16	±0.02	0.135
P. A. (2)	7.15	7.15	7.61	7.30	±0.27	6.26	7.15	7.46	6.96	±0.62	0.187
C. O. (3)	6.26	6.56	6.26	6.36	±0.17	5.95	6.26	5.95	6.05	±0.18	0.009
F. U. (4)	6.40	5.65	5.95	6.00	±0.38	6.26	6.56	6.59	6.47	±0.18	0.098
P. A. (5)	6.51	6.70	6.45	6.55	±0.13	6.26	7.15	5.95	6.45	±0.62	0.465
M. P. (6)	6.26	5.95	5.95	6.05	±0.18	5.81	6.56	6.88	6.42	±0.55	0.093
Mean values	6.52	6.53	6.51			6.28	6.81	6.66			
SD	±0.33	±0.62	±0.64			±0.47	±0.40	±0.62			
p values											
UP 1 vs UP 2 and UP 3		0.399	0.399	LW 2 vs LW 1 and LW 3		0.005		0.005			
UP 2 vs UP 3			0.399	LW 1 vs LW 3				0.05			
UP (1, 2, 3) vs LW (1, 2, 3)						0.121	0.090	0.235			



**Figs 3A to D:** Field emission scanning electron microscopical image (A) of the central (left)/vestibular (right) margin of the upper side of the d-PTFE membrane (×242). A higher magnification (×2.00k) of the delimited area present in the center of the previous figure (B). Central part of the lower side of the d-PTFE membrane at 2.00k (C) with the higher magnification (×10.00k) represented in the white square (D). Patient C. O., sample 3



**Figs 4A and B:** Field emission scanning electron microscopical image of the central (left)/oral (right) margin of the lower side of the d-PTFE membrane (A); Higher magnification ( $\times 5.00k$ ) of the central part (B). Patient V. A., sample 1

membranes. This study aimed to investigate the role of d-PTFE membranes to avoid microbial contamination that could affect the healing of a post-extractive dental socket filled with grafting material.

Despite the exposure of a membrane is considered a risk factor for contamination and failure for bone formation,<sup>8</sup> d-PTFE membranes are commonly used even in the presence of soft tissue dehiscences that allow oral fluids to contaminate the membrane surface: d-PTFE is able to avoid liquids, particulates, or bacteria to migrate through it.<sup>12</sup>

d-PTFE membranes are also easy to remove, while its nonporous nature did not permit cellular overgrowth, but due to this aspect, a firm stabilization is mandatory. Furthermore, it should be considered that the periosteal intake of nutrient will be avoided by the dense nature of the matrix.<sup>13</sup>

For what concerns this study, all interventions reported no complications, and the patients referred only moderate pain or swelling in the postoperative phase.

Membranes were removed after 28 days, following common considerations aiming to avoid an excessive time of membrane exposure and plaque-related adverse reactions or infections.<sup>14,15</sup>

The amount of bacteria colonizing the surfaces of a d-PTFE membrane was investigated by the use of a common technique adopted for the measurement of bacterial contamination of endovenous catheters.<sup>16</sup>

Results from the microbiological analysis revealed that there were no differences between the whole specimens, and this could be due to the proper nature of the d-PTFE which does not allow the cellular adhesion.<sup>17</sup> However, the results evidenced that the lower side was the most colonized, in particular at the central segment of the membrane. This could be due to a more favorable environment that allows the bacterial replication and growth. Furthermore, the areas underneath the membrane are impossible to reach with the daily cleaning procedures engaged by the patient.

At 28 days from the surgery, the removal of the membrane revealed a completely closed wound with healing granulation tissues underneath. Since it is expected to find redness, swelling, and pain when infection is set, the absence of signs of inflammation suggested that the low amount of bacteria colonizing the layers was unable to initiate infection or interfere with the healing.

Soft amounts of visible plaque were found on the external surface, which was directly exposed to the oral cavity.

Microbiological and microscopic analyses revealed that an amount of bacteria was present in the internal layer, underneath the peri-alveolar gingival tissues. This finding suggested that bacteria could migrate underneath the gingiva and colonize the internal layer of the membrane, by finding a path between the external layer and the gingival tissues that cannot adhere to the smooth d-PTFE surface. Another possibility is that the colonization takes place during the surgical procedures: however, in both circumstances, this did not affect the healing process, probably due to the small number of bacteria present in the site.

nc-HA is a nonorganic graft used through years for the osteoinductive and osteoconductive characteristics given by its similarity to the bone mineral matrix. Besides the long time to resorb and the possible interference with the mucoperiosteal flap adhesion in the initial healing phases,<sup>18,19</sup> a synthetic nc-HA is still considered an effective material for jaws bone graft.<sup>20</sup> The use of nc-HA in this study seemed to not affect the healing process; however, the presence of the membrane avoided a direct inspection of the underlying wounds, during the postoperative days.

Previous histologic and histomorphometric studies by Laurito et al.<sup>21,22</sup> on nc-HA graft covered by d-PTFE membranes found a dense connective tissue with a large amount of fibroblast, lymphoplasma cells, and neutrophil granulocytes, without the presence of epithelial cells or signs of foreign body reaction or bacteria.<sup>21</sup> Osteoclastic-like cells were present in association with the nc-HA granules, and a network of blood vessels was forming within the connective tissue. Newly formed bone, with osteoid tissue embedding particles of nc-HA, was clearly identifiable.<sup>22</sup>

Histomorphometric studies also revealed that the quantity of vital bone is greater or fully comparable with other techniques and when the graft is covered with a d-PTFE membrane, the adverse effects caused by its exposure could be reduced.<sup>23,24</sup>

Despite the limited number of cases, results from this study are in accordance with previous researches by Bartee,<sup>14</sup> Krauser,<sup>25</sup> and a revision of the literature by Carbonell et al.,<sup>26</sup> which revealed the effectiveness of d-PTFE membranes to reach a proper preservation of the alveolar bone, even when exposed to oral environment. Even if the presence of the membrane avoided a direct visual inspection of the wounds in the initial phase, the use of nc-HA in this study seemed to not affect the healing process in any cases.

The CFU measured in this study are in line with the measurements of bacteria performed in the oral cavity of healthy subjects by van Houte and Green.<sup>27</sup>

In conclusions, since a mild colonization from oral bacteria should not affect negatively the healing process of the alveolar socket preservation made with an nc-HA bone graft, the d-PTFE membrane surface resulted should be able to prevent the surgical site infection due to the refractoriness of its surfaces that will avoid bacterial overgrowth.

## CLINICAL RELEVANCE

The infection of the graft site could lead to a failure of the socket preservation technique which could delay or compromise the rehabilitation following procedures. The use of d-PTFE can improve the bone regeneration thanks to its antimicrobial properties.

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