Long-Term Clinical Evaluation and SEM Analysis of the e-PTFE and Titanium Membranes in Guided Tissue Regeneration

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Abstract: Aim: This study aimed to evaluate clinical outcomes of titanium membrane and compare these findings with clinical outcomes of e-PTFE membrane, and to investigate the effect of bacterial contamination on both membranes with SEM during long-term healing.

Results: Sixteen titanium and sixteen e-PTFE membranes were surgically placed adjacent to periodontally involved teeth. Seven titanium and 8 e-PTFE membranes were exposed between 4 and 6 weeks. There were no significant difference between groups for plaque and gingival index. Probing depth and clinical attachment level (CAL) were decreased in both groups when compared with baseline; however, these differences were not statistically significant. The CAL gains between the groups were statistically different in 3rd, 6th, 9th, 12th, and 24th months ($p < 0.05$), and the CAL gain was significantly higher in titanium membrane ($p < 0.05$). There was significant decrease in bleeding on probing from baseline in both groups ($p < 0.05$).

Surfaces of 15 membranes were studied using SEM. The largest amount of bacteria was found on the external cervical surfaces of 15 exposed specimens. The entire surface showed the presence of slough epithelial cells, leukocytes, red blood cells, yeast, and microbial plaque. Thirteen external mid surfaces of the 15 specimens, external apical surfaces of three e-PTFE and 1 titanium membrane, internal collar surfaces of all specimens, internal mid surfaces of 5 e-PTFE and three titanium membranes and internal apical surface of only one e-PTFE membrane were infected. Conclusions: This study demonstrated that titanium membrane is equivalent to e-PTFE membranes for GTR in the treatment of periodontal defects. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 91B: 772–779, 2009

Keywords: guided tissue regeneration; wound healing; titanium; membranes; SEM

INTRODUCTION

The ultimate goal of periodontal therapy is the regeneration of the periodontal support lost due to periodontitis. The World Workshop in Clinical Periodontics in 1989 recognized guided tissue regeneration as a viable technique for achieving new attachments.¹ Caffesse et al. suggest that the objective of guided tissue regeneration (GTR) is to promote cellular growth from the periodontal membrane at the same time that proliferation from the other tissues, especially the epithelium and gingival connective tissue, is blocked.²

The purpose of placing barriers over osseous defects is to exclude gingival epithelium and connective tissue from the entire periodontal space. The space created by these barriers permits cells from the periodontal membrane to populate the root surface. Ideally, these materials should have the following characteristics (described by Scantlebury in 1993): (1) biocompatibility, (2) cell-occlusiveness, (3) space making, (4) tissue integration, and (5) clinical manageability.³ It is important to view biomaterials in terms of their inherent characteristics, as these will, in large part, determine their suitability for any given application. Various types of physical barriers have been suggested for use in GTR and these falls into two categories, resorbable and nonresorbable. The obvious major disadvantage of the latter is the need for a re-entry procedure to remove the material, resulting not only in extra cost, but also in inconvenience to the patient.

Expanded polytetrafluoroethylene (e-PTFE) is the most widely used material for GTR, both in terms of laboratory and clinical trials. A commercial product originally developed for use in general and orthopedic surgery, its
suggested dental use is in the treatment of infrabony and class II and III furcation defects.4,5 e-PTFE membranes have been accepted as the gold standard for human and animal comparative studies.

Use of titanium and titanium alloys for medical and dental applications has increased dramatically in recent years. Because of its high strength and rigidity, its low density and corresponding low weight, its ability to withstand high temperatures and its resistance to corrosion, titanium has been used extensively in aerospace, aeronautic, and marine applications.6 The development of new technologies in many scientific domains also has led to the use of titanium for biomedical devices. Titanium is a highly reactive metal that readily passivates to form a protective oxide layer, which accounts for its high corrosion resistance. This oxide layer provides a highly biocompatible surface.

Titanium dome-shaped barriers have been used in rabbits.7 Successful human clinical trials in persons with advanced resorption of the maxilla in need of endosseous implants were performed in 1994.8 The osseoconductive properties of a full titanium membrane have led to gains in human jaw bone height > 10 mm.8 Animal and clinical data have demonstrated that guided bone neogenesis under a subperiosteally placed titanium barrier could reach large volumes, and the surrounding marginal bone level remained stable up to 5 years after implant placement.8 Removal of nonresorbable barriers requires a second surgical procedure 4 to 6 weeks after the first. However, studies have shown that removal of the barrier does not lead to rapid resorption of the newly formed bone.7–9

One complication of GTR is premature exposure of the nonresorbable membrane. Consequences of the resulting soft tissue dehiscence are compromised bone regeneration, soft tissue ingrowth, bacterial contamination, infection, membrane migration, early membrane degradation, and graft exposure.10

In the light of recent data about guided tissue regeneration with titanium membranes, we hypothesized that titanium guided tissue membranes could provide gains in clinical attachment levels that are superior to e-PTFE membranes. Hence, the aim of this study was to evaluate long-term clinical outcomes of the titanium membrane and compare these findings with clinical outcomes of the e-PTFE membrane as a gold standard. Another purpose was to investigate the effect of the bacterial contamination on titanium and the e-PTFE membrane with a scanning electron microscope (SEM) during long-term healing.

**MATERIALS AND METHODS**

**Study Population**

The study protocol was approved by the Ethics Committee. All subjects were informed about the purpose of the research and all participants provided informed consent. A total of 23 patients (9 females and 14 males) with chronic periodontitis were included in this study. However, only 16 patients (6 females and 10 males; mean age, 42 ± 5.1 years) completed the 2-year evaluation. The other 7 patients were lost to follow-up or to surgery owing to inadequate oral hygiene, exposure of the membranes greater than one fourth of the membrane area in 4–6 weeks, or detection of 1-wall or any circumferential infrabony defect during the surgery. Before enrollment in the study, all patients received oral hygiene instructions and full-mouth supra and subgingival scaling and root planning with ultrasonic and hand instruments. Subgingival scaling was performed under local anesthesia. Patients were recruited according to following criteria: (1) no systemic diseases that could influence the outcome of the therapy, (2) a good level of oral hygiene (plaque index (PI) <1; Löe 1967), (3) compliance with the maintenance program, (4) exhibiting one pair of similar contralateral infrabony defects at the molar region at the same location in the jaw, (5) presence of infrabony defects with a probing depth (PD) of at least 6 mm and an intrabony component of at least 3 mm as detected radiographically, (6) having at least 20 teeth and, (7) free of every kind of dentures. Subjects with a history of hepatitis or HIV infection, diabetes, a need for antibiotic prophylaxis, pregnancy, lactation, or use of chronic anti-inflammatory drugs were excluded. Since cigarette smoking is a severe risk factor for periodontal disease, smokers were excluded. Abnormal occlusal relations were eliminated. Periapical radiographs were taken and bone defects were determined. Evaluation of each subject consisted of personal, medical and dental history, full-mouth periapical radiographs, and dental examinations.

Measurements of probing depth (PD), clinical attachment level (CAL), the gingival margin height and bleeding on probing (BOP) were made at six sites (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, mesiolingual) for each tooth with the same type of periodontal probe (Williams Periodontal Probe, Karl Schumacher Dental Instrument Co. Southampton, PA) using a prefabricated acrylic stent as a reproducible reference point. Gingival index ([GI]; Löe and Silness) and plaque index ([PI]; Löe and Silness) were recorded at four sites (mesial, buccal, distal, and lingual). Recession was measured from the cemento-enamel junction to the gingival margin. BOP was recorded as the percentage of sites with bleeding within 30 s after probing.

Measurements were performed by using acrylic reference stents by the same calibrated investigator and were repeated 3, 6, 9, 12, and 24 months after the first surgery. The defects were randomly assigned before surgery to the 2 membrane types. Defects treated with a titanium membrane were defined as belonging to the test group and e-PTFE applied defects were defined as belonging to the control group.

**Surgical Procedure**

Periodontal surgery was performed first on the defect on the patient’s left side. Treatment on the right side was performed 1–3 weeks later. Each patient received both
treatments, but the sites within each patient were randomly assigned. All surgical procedures were performed under local anesthesia by the same surgeon. Following intracrevicular incisions, full-thickness mucoperiosteal flaps were elevated. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed using hand instruments.

At the test sites, the defects were covered with a titanium membrane (Friatec Titanium Membrane, Mannheim, Germany) so as to cover 2–3 mm of the surrounding alveolar bone. The control defects were covered with e-PTFE membranes (GORE-TEX Regenerative membrane, Arizona) using the same technique. GORE-TEX sutures (GORE-TEX Suture, Arizona) were used to fix and stabilize the e-PTFE membrane. Finally, mucoperiosteal flaps were repositioned coronally and fixed with vertical mattress sutures.

Professional supragingival plaque control was performed weekly until membrane removal. Chlorhexidine digluconate 0.12% was used twice daily to facilitate proper plaque control until 2 weeks after membrane removal.

Exposure of the membrane was observed by visual examination at weekly recall visits. The extent of membrane exposure was recorded, and the subjects were divided into nonexposure and exposure groups. The exposed membranes were removed at the end of the 4 weeks while the nonexposed membranes were removed with a second surgical procedure 6 weeks after the GTR procedure.

**SEM Preparation and Analysis**

Exposed and nonexposed membranes were chosen from both groups. Samples were cut into three parts as collar, middle, and deep. A scanning electron microscope (SEM; JSM-6400 Scanning Electron Microscope. JEOL, Tokyo) was used to evaluate inner (against alveolar bone) and outer (against the gingiva) parts of the samples for tissue integration, cell components, surface features, and bacterial contamination.

Removed membranes were rinsed gently with normal saline. The retrieved membranes were further rinsed with sodium citrate to remove adhered blood and then fixed in glutaraldehyde 2.5%. Samples were then washed in sodium phosphate buffer 3 times in 1 hour. The specimens were serially dehydrated with 70, 75, 80, 90, 95, and 100% alcohol, for 5 min each. Postfixation was accomplished using osmium tetroxide 2% and then washing in distilled water. After dehydration was complete, specimens were placed in a chamber under CO$_2$ pressure and critical point drying was performed. Samples were sputter coated with gold and examined under a SEM at 20 kV emission voltages.

Under SEM, the specimens were examined in collar, mid-part and apical directions.

Membrane surfaces facing the bone (inner surface) and the mucoperiosteal flap (outer surface) were examined. According to the criteria described by Listgarten and Hellen, 4 types of bacterial morphology were identified under SEM: cocci, rod, filaments, and spirochete.$^{11}$ If specimens were infected with 1 of these bacterial types on SEM analysis they were considered positive (+). If there was no bacterial contamination they were considered negative (−).

**Data Analyses**

For each continuous variable, normality was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests and by histograms. Since the data were not normally distributed, the Mann-Whitney $U$ test was performed to compare the data between the test and control groups. Differences between the clinical parameters achieved at baseline and 3, 6, 9, 12, and 24 months after the procedure were evaluated using the Friedman test. The Wilcoxon signed rank test was performed to compare the inner group data between baseline and 24th month. Statistical analyses were performed using commercially available software program (Statistical Package for the Social Sciences, version 11.0, SPSS, Chicago, IL).

**RESULTS**

**Clinical Results**

Wound healing was uneventful in both groups. Seven titanium membranes and 8 e-PTFE membranes were exposed during 4–6 weeks.

There were no statistically significant differences between groups for PI ($p > 0.05$) and GI ($p > 0.05$) for all time points during the 24 months. PD, CAL, GR, and CAL gain values for each membrane is seen in Table I and there was no statistically significant difference between groups for PD, CAL, and GR in any time point ($p > 0.05$). Only CAL gains were statistically significant different at 6th, 9th, 12th, and 24th months between the groups (Figure 1).

In both groups, PD, CAL, and CAL gain values decreased over time from baseline measurements; these results were statistically significant ($p < 0.05$). However, there was no significant difference between time points for GR in each group ($p > 0.05$). There was a statistically significant decrease in BOP from baseline in both groups. This difference was not significant between the groups for each time point.

Table II shows contamination of the exposed and nonexposed membranes according to surfaces. The attachment gains were greater in the exposed titanium group than in the exposed e-PTFE group, but the difference did not reach significance. Also, recession was less in the test group than it was in the control group. However, there was no significant difference between the exposed and nonexposed membranes for CAL gain and GR ($p > 0.05$).

**Ultrastructural Analysis**

Surfaces of 30 membranes were studied using a SEM. External surfaces were divided into three surfaces exposed
to the oral cavity (collar, middle, apical) and three internal surfaces (collar, middle, and apical).

**External Collar Surfaces.** The largest amount of the bacteria was found in the 15 exposed specimens. An overview of the entire surface showed the presence of slough epithelial cells, leukocytes, red blood cells, yeast, and microbial plaque. Figure 2 shows contamination external collar surface of the exposed e-PTFE membrane according to surfaces described earlier.

**External Mid Surfaces.** Thirteen of the 15 specimens were infected. The overview was similar to that described for the cervical surfaces. Figure 3 represents the area in which the largest amount of bacteria was found in the 15 membrane specimens.

**External Apical Surfaces.** Three e-PTFE and titanium membranes were infected. There was a predominance of coccobacilli and rods.

**Internal Collar Surfaces.** The overview was similar to the outer surfaces except that there was less bacterial mass found here than on the outer surfaces. All specimens were infected.

**Internal Mid Surfaces.** Five e-PTFE and 3 titanium membranes were infected. The morphotypes seen on these surfaces were similar to those seen on the outer surfaces; these were mainly cocci, coccobacilli, and rods (Figure 4).

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**TABLE I. Mean, Standard Deviation and Median Values of Each Membrane for Probing Depth (PD), Clinical Attachment Level (CAL), Gingival Recession (GR) and Clinical Attachment Gain (CAL Gain) in 3rd, 6th, 9th, 12th, and 24th months**

<table>
<thead>
<tr>
<th>Time (month)</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3rd</td>
<td>6th</td>
</tr>
<tr>
<td>PD</td>
<td>e-PTFE</td>
<td>8.3 ± 2.2</td>
<td>3.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Titanium</td>
<td>8.9 ± 2.3</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>p-value*</td>
<td></td>
<td>0.423</td>
<td>0.239</td>
</tr>
<tr>
<td>CAL</td>
<td>e-PTFE</td>
<td>9.4 ± 2.1</td>
<td>4.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Titanium</td>
<td>11.0 ± 2.0</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>p-value*</td>
<td></td>
<td>0.028</td>
<td>0.026</td>
</tr>
<tr>
<td>GR</td>
<td>e-PTFE</td>
<td>1.1 ± 0.9</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Titanium</td>
<td>2.0 ± 1.2</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>p-value*</td>
<td></td>
<td>0.034</td>
<td>0.846</td>
</tr>
<tr>
<td>CAL Gain</td>
<td>e-PTFE</td>
<td>4.8 ± 1.3</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Titanium</td>
<td>4.3 ± 1.0</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>p-value*</td>
<td></td>
<td>0.115</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Friedman test between time measurements
* Wilcoxon test between baseline and 24th month measurements in the groups.
* Mann–Whitney U test between groups.

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Figure 1. Distribution of mean and standard deviation for CAL gains between groups and time (O; represents outliers, *; represents extreme values).
Internal Apical Surfaces. One e-PTFE membrane was infected. There were also fibroblastlike cells in both groups. Large, branching cells and fibrous structures were interpreted as connective tissue elements adhering to the membranes (Figure 5).

Figure 6 shows perforations of the nonexposed titanium membrane at ×200 magnification.

**DISCUSSION**

The biological principle of GTR relies on different regenerative capacities of different periodontal tissues. Placing membrane barriers prevents undesirable tissues such as gingival connective tissue cells and epithelial cells from migrating into wound and allows the specific cells (ie, periodontal ligament cells) to produce connective tissue attachments that are guided into contact with the root surface.12–16 Previous data suggest that the specific physical characteristics of different types of GTR membranes may influence the clinical handling properties that could influence the degree of regeneration of the periodontal attachment apparatus. The GORE-TEX soft-tissue Patch (GORE-TEX™ soft tissue, W. L. Gore and Ass., Flagstaff, Arizona) is an expanded polytetrafluoroethylene membrane used in general surgery for hernia repair, vascular surgery, and cardiac surgery. It has several characteristics that make it well suited for periodontal surgery.

Titanium foils are highly stable and malleable. Microperforations prevent soft tissue in-growth into defects and permit diffusion of interstitial fluid.17 Titanium membranes are clinically used with bone substitution material under-neath, which may lessen the passive malleability and possible micromovements by the pressure of soft tissue of the mucoperiosteal flap. Strietzel et al. evaluated the healing pattern of bone defects covered by different membrane types and suggested that by applying a dense barrier material, the defects could be occupied by new woven bone that started from the defect walls.18

This is the first clinical and SEM study to evaluate the long-term clinical outcomes of titanium membranes compared with e-PTFE membranes. This study also investigated the effects of exposure on healing outcome in different membranes types.

Each membrane type was applied on one of a pair of similar contralateral infrabony defects in the same patient. Thus, we eliminated individual differences that might have affected the healing process. While there was no difference in PI, GI, and BOP measurements between the test and control subjects, there was a significant difference in baseline CAL between the test and control groups with the test group having a greater starting CAL. Pocket depths decreased significantly in both groups. Numerous studies have evaluated e-PTFE membranes. Investigators usually compared e-PTFE membranes with periodontal flap operations. These studies demonstrated the clinical success of

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**TABLE II. Contamination of the Exposed and Nonexposed Membranes According to Surfaces**

<table>
<thead>
<tr>
<th></th>
<th>e-PTFE Membrane</th>
<th>Titanium Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internal Surfaces</td>
<td>External Surfaces</td>
</tr>
<tr>
<td></td>
<td>C  M  A</td>
<td>C  M  A</td>
</tr>
<tr>
<td>Exposed</td>
<td>8  +5  +2  +1</td>
<td>8  +7  +5</td>
</tr>
<tr>
<td>Nonexposed</td>
<td>8  -    -    -</td>
<td>-    -    -</td>
</tr>
</tbody>
</table>

A, apical; C, collar; M, mid; +, infected; -, noninfected.
the guided tissue regeneration technique with e-PTFE membrane versus flap surgery.\textsuperscript{19,20} The e-PTFE membrane was thought to be better than the other nonresorbable and resorbable membranes.\textsuperscript{21–25}

There was a significant difference between groups for CAL at the baseline and third months and this difference was tended towards greater values in the test group. Differences between groups for attachment gains were statistically significant in every time points except third month. At 24 months, attachment gain was statistically greater in those in whom titanium membrane was applied compared with controls, but CAL at 24th month was not significant between groups. It is not known whether the deeper baseline CAL for the test group could be related to the subsequent greater increase in attachment gain. Additional studies are needed to clarify the affect of starting CAL on amount of attachment gain.

There was no difference for gingival recession values at 24 months. In a study by Cortellini et al., titanium-reinforced e-PTFE membranes, enamel matrix protein, bioabsorbable membranes with a graft, and bioabsorbable membranes were compared in terms of gingival recessions, clinical attachment gains, and elimination of deep pockets.\textsuperscript{26} While the clinical results were satisfying, there were no statistically significant differences between groups.

In another study performed by Cortellini et al., clinical attachment gain was higher in titanium than it was in reinforced e-PTFE membranes in agreement with the current study.\textsuperscript{27} Lins et al. evaluated titanium-reinforced membranes in gingival recession treatment and found that GTR resulted in significantly greater alveolar crest levels.\textsuperscript{28} However, the amount of root coverage obtained with a coronally positioned flap was greater than that observed with GTR.

Few studies have evaluated the efficiency of titanium membranes in periodontal regeneration. To evaluate the efficacy of GTR around exposed implant threads, 16 implants were placed into fresh extraction sockets in beagle dogs.\textsuperscript{29} e-PTFE membranes and titanium membranes were used to cover the defects around the implants. The titanium membranes used in this study had different surface characteristics as they did not have structured perforations on their surfaces, hence insufficient blood supply and nutrition resulted in mean gains in bone height of 2.1 mm for e-PTFE sites and 0.8 mm for titanium membranes. However, the titanium membranes used in the present study have perforations. We analyzed the structural perforations of the titanium membrane using a SEM. We did not observe any necrotic tissue that would have been due to an insufficient blood supply to the flap that covered the membranes during the 4- to 6-week study. These results regarding successful tissue integration and sufficient vascular supply from use of the perforated titanium membrane are encouraging.

While most clinicians agree that primary soft tissue closure which is maintained throughout the course of regeneration is ideal, opinions vary greatly concerning how best to accomplish this goal.\textsuperscript{30} Opinions differ as to the necessity for the guided tissue regeneration technique with e-PTFE membrane versus flap surgery.\textsuperscript{19,20} The e-PTFE membrane was thought to be better than the other nonresorbable and resorbable membranes.\textsuperscript{21–25}

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of achieving primary soft tissue closure in guided tissue regeneration procedures. In the present study, 8 out of 16 titanium membranes and 7 out of 16 e-PTFE membranes were exposed but there was no difference for clinical attachment gain and gingival recession between exposed and nonexposed membranes. However, the attachment gains were greater and recession was less in the exposed titanium group than in the exposed e-PTFE group. In a study by Becker et al., out of 49 implants, three were missed before loading. These implants showed early membrane exposure. Sites where the barrier remained unexposed showed greater amount of bone fill in the peri-implant region when compared with sites where membranes became prematurely exposed in Becker et al. study. In another study, Celletti et al. used e-PTFE membranes and titanium membranes to cover the defects around implants. A control group did not receive any membranes. Results were evaluated histologically and the greatest gain in bone levels was seen for 2 sites that received e-PTFE membranes and remained covered for the entire evaluation interval. It was concluded that when primary coverage is maintained, the use of e-PTFE membranes can significantly enhance bone regeneration around implants. In the present study, primary closure of soft tissues was achieved at the end of the surgery. In the event that the membrane was exposed in the first 4–6 weeks of healing, chlorhexidine digluconate 0.12% was administered twice daily to facilitate proper plaque control until 2 weeks after membrane removal. Professional supragingival plaque control was performed weekly until membrane removal. Membranes were removed before 6 weeks postoperatively only if they became problematic with regard to continuing pain or exudate. A partial thickness flap was made adjacent to the augmented site and the barrier was removed, then the flap margins were resutured. Attention should be paid not to disturb the granulation tissue beneath the membrane.

Similar attachment gain between nonexposed and exposed membranes in the present study may be due to patients’ compliance to the surgery. The patients followed the visits carefully, and complied with the instructions regarding oral hygiene procedures, and medications.

Whenever a regenerative barrier is applied, primary closure of soft tissues is considered a prerequisite to the success of the guided bone regeneration process. We agree with this statement, however the results of the present study showed that under the proper circumstances it is possible to obtain attachment gain even after the membrane is exposed.

Bacterial colonization of the exposed membrane, followed by the spread of infection to the underlying treated tissues may cause complete failure or only partial regeneration. We also evaluated the clinical healing of the exposed membranes. The attachment gains were also greater in the exposed titanium group than in the exposed e-PTFE group. Also, recession was less in the test group than it was in the control group. However, these results were not statistically significant. Greater attachment gain and lesser recession could be explained by the improved tissue integration, which prevents bacterial penetration. In our study, the cervical part of the internal and the external surfaces of the membranes were infected in all specimens. Bacterial contamination was lower at the mid parts of the membranes than it was at the cervical parts as was expected. Good tissue integration of the titanium membrane might be the reason for fewer infected titanium membranes at the apical parts.

A SEM was used by Zucchelli et al. to evaluate early bacterial colonization on 3 different membranes. They suggested that quantitative differences in early plaque accumulation on various membranes seemed to be related to the textural and structural characteristics of the surface. The same group also published a study in 1996 that evaluated the relationship between the presence of bacteria on the inner surface of e-PTFE barriers and the clinical outcome of membranes supported by reconstructive periodontal surgery. The results of the SEM analysis revealed that bacterial colonization was evident in the collar area of all the retrieved membranes. In the middle part of the membranes, 30 of 60 microscopic fields demonstrated microbial colonization, and in the most apical part 9 of 60 fields demonstrated microbial colonization. The gain in probing attachment level was positively correlated with initial attachment loss and negatively correlated with microbial colonization of the middle part of the membranes. It was concluded that bacterial colonization in the middle part of the e-PTFE membrane reduced the potential gain in probing attachment following GTR therapy by nearly 50%.

This study has some limitations regarding the microbiologic aspect of the SEM analysis. The presence of gram-negative bacterial colonization in deep periodontal tissue via the exposed membrane surface in the oral cavity is one of the crucial factors that affects periodontal wound healing. The size of our study population also was too small for a more particular statistical analysis, but it is difficult to follow a study population for 24 months.

In conclusion, the use of nonresorbable or a resorbable membranes can enhance tissue regeneration and provide long-term predictable results. In addition, the results of this study demonstrate that titanium membrane is equivalent to e-PTFE membranes for GTR and would be one of the potential alternative membranes for the treatment of periodontal defects. Although the more rigid structure of titanium complicates the clinic application of the membrane, good space maintenance and tissue integration are the principal characteristics for the success of the GTR.

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