Connective Tissue and Bacterial Deposits on Silicone Sheet and ePTFE Barrier Membranes in Guided Periodontal Tissue Regeneration

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ABSTRACT The aim of this study was to compare connective tissue and bacterial deposits on silicone sheets (SS) and expanded polytetrafluoroethylene membranes (ePTFE) used as barrier membranes in guided tissue regeneration (GTR) for periodontal treatment. Eighteen intrabony periodontal lesions from 18 patients were first surgically treated by GTR and either SS or ePTFE were used as barrier membranes. Four to six weeks after the first operation, membranes were retrieved from surgical sites and processed for the scanning electron microscopy. Quantitative study of deposits on the lesion-facing surfaces of membranes was performed. The differences between the number of fields of connective tissue and bacteria on SS and ePTFE were analyzed by the Chi-square test at the level of 0.05 significance. The result showed no significant difference between the number of fields of connective tissue on the surfaces of SS and ePTFE ($p=0.875$). However, the number of fields of bacteria found on SS were significantly less than those found on ePTFE ($p<0.001$). The comparable number of fields of connective tissue deposits on both types of membranes suggests that the degree of healing under both types of membranes was also comparable. Therefore, SS can be used as a barrier membrane in GTR for periodontal treatment.

KEYWORDS: barrier membrane, silicone sheet, polytetrafluoroethylene, connective tissue, bacteria.

INTRODUCTION

The ultimate goals of periodontal treatment are the control of periodontal pathogens and the regeneration of supporting tissues damaged by the periodontal disease. Guided tissue regeneration (GTR) is an efficacious and predictable surgical approach for the treatment of intrabony periodontal defects.[1] This treatment includes the placement of a barrier membrane between the gingival flap and the instrumented root surface. The membrane creates a secluded space for the blood clot and prevents the down-growth of gingival tissue cells. As a result, only cells deriving from the periodontal ligament can migrate and regenerate the periodontal tissue. The commonly used barrier membranes in GTR treatment are expanded polytetrafluoroethylene membranes (ePTFE) and bioabsorbable barrier membranes.[2-7] Since these membranes are costly, hard to adapt to lesions and designed to be used in a single lesion, attempts to find more economical and easily manageable barrier membranes have been performed. Some clinical trials of GTR treatment using rubber dam sheets as barrier membranes have been reported.[8, 9] Our team introduced silicone sheets (SS) to substitute these membranes with clinically satisfactory results.[10, 11]

The connective tissue and bacterial deposits on ePTFE and bioabsorbable barrier membranes used in GTR have been previously reported.[5, 12-21] The connective tissue integration on the outer surface of the membrane prevents membrane exposure and bacterial plaque colonization and thus enhances the clinical outcome following GTR.[19] Products derived from bacterial metabolism may influence and disrupt the blood clot in the early stages of healing[16] and influence the amount of new connective tissue formation. The microbial colonization of barrier membrane has been reported to have a negative influence on the clinical attachment gain.[17-19] Studies of adherent connective tissue and bacterial colonization on SS have not been reported. The aim of this study was to compare connective tissue and

bacterial deposits on the lesion-facing surfaces of SS and ePTFE used as barrier membranes in GTR.

Materials and Methods

Eighteen systemically healthy patients affected by chronic periodontitis were enrolled in this study. The patients were given the initial phase of periodontal treatment comprising oral hygiene instructions, full mouth scaling and root planing. After evaluation of the primary treatment, the remaining intrabony periodontal defects were treated with the GTR using SS (SILIMED-Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda, Rio de Janeiro, Brazil) and ePTFE (Gore-Tex; WL Gore and Associates, Flagstaff, USA) as barrier membranes in nine patients each. The patients were instructed not to brush or floss the operated areas for 4 weeks, and to rinse with 0.2% chlorhexidine solution twice daily for 4 to 6 weeks following surgery. Postoperatively, 200 mg of doxycycline was prescribed, and then 100 mg per day for another 9 days. Due to extensive gingival flap recession during the healing phase of defects treated with SS, the membranes were surgically removed 4 weeks after placement of membranes. For the defects treated with ePTFE, slight gingival flap recession was detected and the healing process was uneventful, therefore the membranes were retrieved 6 weeks after placement of membranes as generally recommended.

The retrieved membranes were rinsed briefly in normal saline solution to remove the adherent blood and then fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4 for 24 hours. The specimens were rinsed again in 0.1 M PB, pH 7.4, and postfixed in 1% osmium tetroxide in 0.1 M PB, pH 7.4 for 1 hour. They were then dehydrated with graded ethanol, critically point-dried with carbon dioxide, mounted on specimen stubs and sputter-coated with gold particles. Scanning electron microscopic investigation was made at 15 kV emission voltage and a specimen tilt angle of 0 degrees on a Jeol JSM-5410 (Jeol Ltd, Japan).

The lesion-facing surface of each membrane was divided into 3 cervical portions, 3 middle portions and 3 apical portions on the buccal (labial) and lingual (palatal) surfaces (Fig 1A) or on the proximal surfaces (Fig 1B). The central field of each portion was examined at 200X magnification. Each microscopic field covered an area of approximately 0.5 x 0.6 mm^2. When 1/3 or more of each field was covered with deposits, it was considered as a positive field. If less than 1/3 of each field was covered with deposits, it was considered as a negative field. In the positive field, magnification was increased up to 3,500X to determine the prevalent nature of deposits covering the membrane surface as connective tissue (fibroblasts and/or their extracellular matrices), bacteria or others. Only when connective tissue structures dominated, was the field considered positive for connective tissue. When bacteria accounted for the majority of deposits, the field was considered positive for bacteria. The field was considered to be positive for others when the membrane surface was covered with inflammatory cells, epithelial cells, fibrins or unidentified materials.

The total number of fields of connective tissue elements and bacterial deposits and their distributions on each portion of SS and ePTFE were analyzed by the Statistical Package for Social Science version 7.5 for Windows. The Chi-square test was used to test the group difference. Significance of the differences among groups was selected at $p<0.05$.

Results

Scanning electron microscopic investigation was done on 162 microscopic fields from 9 SS and 9 ePTFE barrier membranes. At low magnification, there was variation in the number of fields of deposits among the membranes and different portions on the same membrane. The absence of deposits on some parts of the membrane surface was also evident in some SS. At higher magnification, the deposits were identified as connective tissue, bacteria or others.

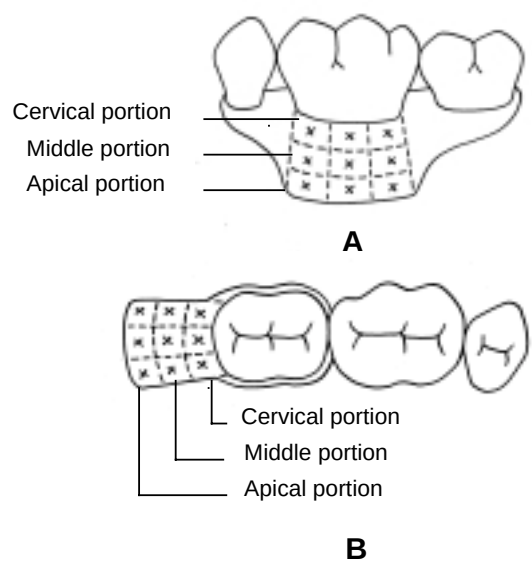

Fig 1. The lesion-facing surface of a barrier membrane was divided into 9 portions. The central field (X) was investigated. A) The buccal lesion B) The distal lesion.

There were fibroblasts and their extracellular matrices forming the connective tissue on SS (Fig 2A) and ePTFE (Fig 2B). Several bacterial forms including cocci, rods and filaments were identified on SS (Fig 3A) and ePTFE (Fig 3B). Spirochete bacteria were observed on ePTFE (Fig 3B). The total number of fields and the distributions of connective tissue and bacterial deposits on SS and ePTFE were shown in Figs 4A and 4B.

Connective tissue was found on 40 out of 81 examined fields of the lesion-facing surfaces of SS membranes, and 41 out of 81 examined fields of those surfaces of ePTFE membranes. Statistical analysis showed that the total number of fields of connective tissue on SS and ePTFE were not significantly different ($p=0.875$). There was no significant difference of the connective tissue distribution among cervical portions (14 fields), middle portions (13 fields) and apical portions (13 fields) of SS ($p=0.952$)(Fig 4A). In contrast, the connective tissue distributions increased significantly from cervical portions (7 fields) to middle portions (15 fields) and apical portions (19 fields) of ePTFE ($p=0.004$).

The total number of fields of bacterial colonization on SS were significantly less than those on ePTFE (6 fields on SS and 30 fields on ePTFE, $p<0.001$)(Fig 4B). No significant difference was found among their distribution on cervical portions (2 fields), middle portions (1 field) and apical portions (3 fields) of SS ($p=0.583$). Conversely, the distributions of bacterial deposits showed significant differences among cervical portions (17 fields), middle portions (9 fields) and apical portions (4 fields) of ePTFE ($p=0.001$).

The connective tissue and bacterial deposits on cervical portions, middle portions and apical

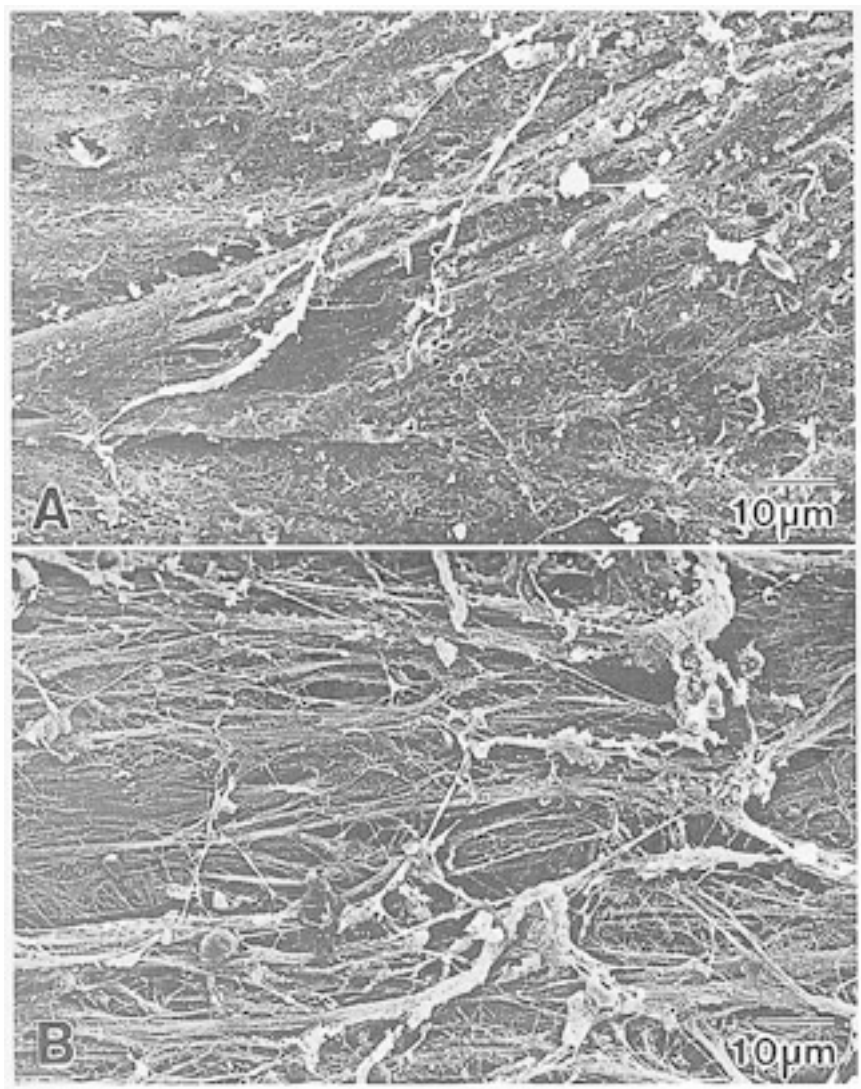

Fig 2. The connective tissue deposits on the surface of SS (A) and ePTFE (B).

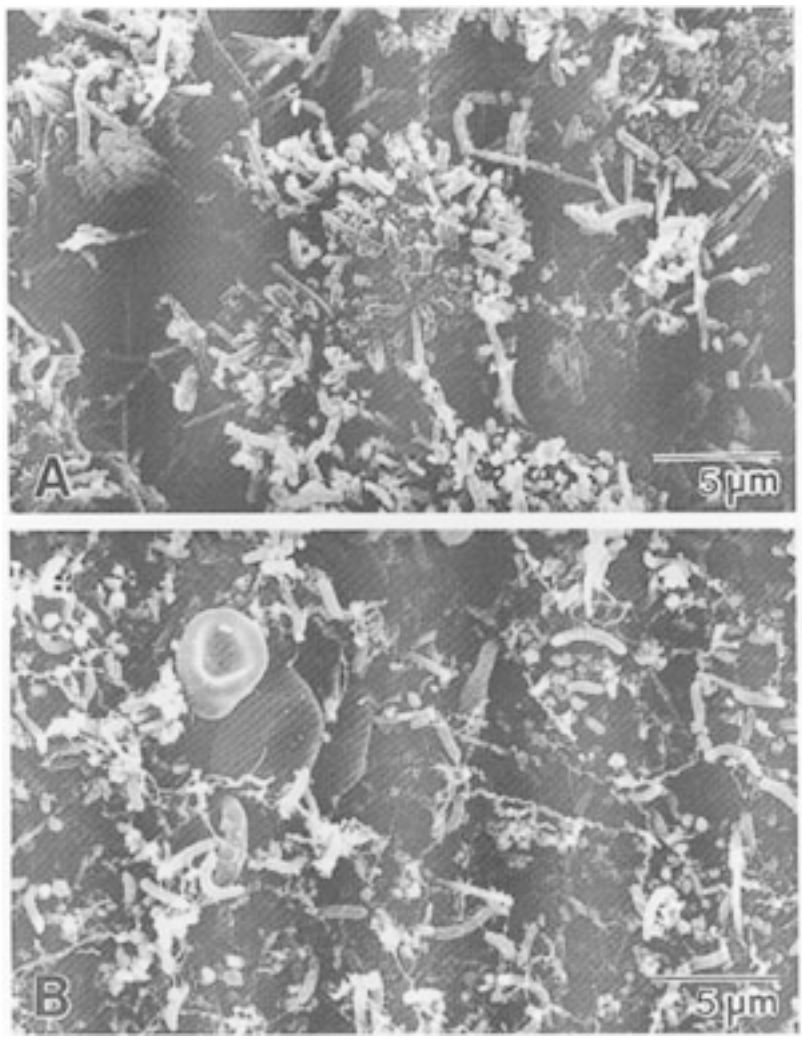

Fig 3. The bacterial colonization on the surface of SS (A) and ePTFE (B).

portions were compared between SS and ePTFE (Figs 4A and 4B, respectively). There was no significant difference in the connective tissue deposits between both types of membranes on cervical, middle and apical portions ($p=0.51$, $p=0.586$ and $p=0.097$, respectively). The bacterial distributions on cervical and middle portions of SS were significantly less than those on ePTFE ($p<0.001$, $p=0.005$), but not on apical portions of both membranes ($p=0.685$).

The total number of fields of other deposits were significantly more on SS than on ePTFE (35 fields on SS and 10 fields on ePTFE, $p<0.001$). There was no significant difference in the number of fields of inflammatory cells (11 fields on SS and 6 fields on ePTFE, $p=0.200$) and epithelial cells (3 fields on SS and 3 fields on ePTFE, $p=1.000$) between both types of membranes.

Discussion

The material used as a barrier membrane must be biocompatible or inert.[7] SS are synthetic materials which are commonly used in reconstructive and orthopedic surgery,[22-24] and have been shown to have good biocompatibility to human gingival fibroblasts.[10] Therefore, we introduced SS as barrier membranes in GTR for periodontal treatment. The clinical trial indicated a favourable clinical outcome.[11]

Many reports have suggested that connective tissue and bacterial deposits on retrieved membranes may help indicate the clinical outcome of GTR treatment.[5, 12-21] In the present study, connective tissue and bacterial deposits on SS were examined using ePTFE as the gold standard, because of its well-known characteristics and clinical effectiveness.[2,3] Our study showed that the structure of connective

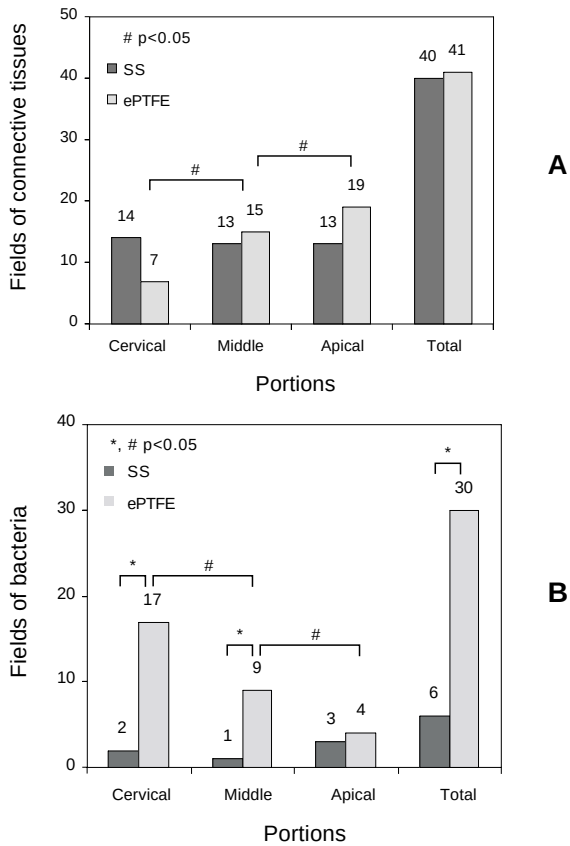

Fig 4. Comparison of the number of fields and the distributions of the connective tissues (A) and the bacterial colonizations (B) on SS and ePTFE.
*p<0.05 comparison between SS and ePTFE (Chi-square test).
#p<0.05 comparison between portions of ePTFE (Chi-square test).

tissue formed on SS did not differ from that on ePTFE. Furthermore, the number of fields covering with the connective tissue formation on SS was comparable to those found on ePTFE (p=0.875). These data indicated that SS did not interfere in the healing process. In addition, it provided the appropriate environment for the connective tissue formation. This evidence confirmed the favourable outcomes of the *in vitro* and the clinical studies of our previous investigations on SS.[10, 11]

In this study, there was no penetration of connective tissue into SS due to its nonporous nature, which is different from what we have found and others have previously reported in ePTFE.[25] From our clinical observation, the amount of gingival flap recession of SS was more than that of ePTFE. This result implied that the tissue integration on the outer surface of SS might be less than that of ePTFE. The membrane exposure from extensive gingival flap recession is an undesired effect of the GTR technique using SS, because it may increase the chance for bacteria invasion into the newly regenerated tissues. However, exposure of the membrane during the early healing phase does not severely affected the healing response. The appearance of the new tissue at the time of membrane removal has been shown to be the single most important consideration for healing pattern determination.[26] The new connective tissue under both SS and ePTFE had a pink rubber-like surface. In this study, there was no significant difference on the number of fields of connective tissue deposits between on SS and ePTFE. The data indicated that the healing process can occur underneath SS in a similar manner to that under ePTFE.

The present study showed that the distributions of connective tissue and bacteria on each portion of SS were not significantly different because of the close adaptation to the root surface and the nonporous surface of SS. Conversely, the connective tissue deposits on ePTFE increased from cervical portions to apical portions significantly (p=0.004) and the bacterial deposits on ePTFE showed the reverse distributions (p=0.001). It has been suggested that the difference in distribution of the deposits might be affected by the texture and structural surface characteristics of the different membrane materials.[16, 17, 21] The highest accumulation of bacteria was found on cervical portions and lowest on apical portions of ePTFE in this study, which was consistent with previous studies.[12, 17, 21] Selvig et al[12] reported that the pattern of bacterial distribution may be caused by the configuration of cervical collar of ePTFE and the shrinkage and recession of readapted gingiva, which resulted in marginal exposure with plaque accumulation. De Sanctis et al[18] studied bacterial colonization on bioabsorbable Polyglactin 910 membranes and reported that the corresponding cervical portion was always completely colonized in exposed membranes.

In conclusion, the structure and amount of connective tissue deposits on SS and ePTFE barrier membranes were comparable. This result suggests that the healing process at the surgical site occurred similarly underneath SS and ePTFE. Although SS showed less tissue integration on the outer surface, they were economical, have good biocompatible with human tissue, and could provide the suitable environment to form the underlying regenerated periodontal tissue. Therefore, the data confirm the previous *in vitro* and clinical studies that SS can be used as barrier membranes in GTR.

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