Topical and Systemic Antimicrobial Therapy in Guided Tissue Regeneration

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Background: Bacterial contamination of membrane material negatively affects healing after guided tissue regeneration (GTR) procedures; conversely, flap connective tissue integration on barrier material improves the clinical outcomes. The objective of this study was to evaluate the effect of topical application of antibiotics on: 1) clinical outcomes of GTR surgical procedures using titanium reinforced expanded polytetrafluoroethylene (ePTFE) periodontal membrane; 2) bacterial colonization of membrane material; and 3) flap connective tissue-membrane integration.

Methods: Fifty-six deep interproximal bony defects were treated with GTR surgical procedures using titanium reinforced ePTFE periodontal membranes. Patients were randomly assigned to 1 of the 2 antimicrobial treatment groups: the test group received weekly topical application of 25% metronidazole gel and the control group received systemic antibiotics (amoxicillin plus clavulanic acid 1g/day for 14 days). Clinical outcomes were assessed at 1 year; the amount of bacterial contamination and connective tissue integration on membrane material was evaluated at time of membrane removal by means of a morphological (SEM) method.

Results: No statistically significant difference was found between test and control groups in terms of clinical attachment (CAL) gain (baseline CAL — 12 months CAL; $P = 0.2$) and probing depth (PD) reduction (baseline PD — 12 months PD; $P = 0.6$). A greater increase in gingival recession (REC) (12 months REC — baseline REC) was found in the test group compared to the control group ($P = 0.003$). The SEM analysis revealed no statistically significant (t test) difference between test and control groups in the number of fields positive to integrated connective tissue ($P = 0.82$), while the number of fields positive to bacteria was statistically higher ($P < 0.001$) in the control group.

Conclusions: Local antibiotic administration is more effective than systemic use in preventing membrane contamination, but it does not improve clinical outcomes due to an interference of the vehicle (gel) with gingival tissues which may reduce the potential benefits derived from better control of the bacterial load. *J Periodontol 1999; 70:239-247.*

KEY WORDS

Metronidazole/therapeutic use; amoxicillin/therapeutic use; clavulanic acids/therapeutic use; bacterial colonization; connective tissue/surgery; outcome assessment; membranes, artificial; membranes, barrier; polytetrafluoroethylene/therapeutic use; surgical flaps.

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A critical point in wound healing, particularly following guided tissue regeneration (GTR) surgery, is the protection of the blood clot. The importance of clot adhesion to the root surface in periodontal repair has been demonstrated in a series of experimental studies.[1] Products derived from bacterial metabolism may influence and disrupt the blood clot in the early stages of healing[2,3] and thus influence the amount of new tissue formation. The negative influence of microbial colonization of the barrier material on the amount of clinical attachment gain,[4-6] together with similar observations reported from microbiological studies,[7-11] indicates the need for effective modalities of plaque control during the healing period.

In order to prevent postoperative wound infection, some investigators have administered systemic antibiotics to patients undergoing GTR therapy during the first and/or second week after membrane placement.[2,9-16] In other studies,[4-6] both local antimicrobial therapy with chlorhexidine rinses and systemic antibiotics have been used to aid in preventing contamination of periodontal wounds.

However, it has been shown that neither systemic antibiotics nor local antimicrobial rinses were effective in preventing bacterial colonization of either bioabsorbable or non-resorbable membranes used for GTR.[2,4-16]

This indicates that either the drugs administered are not directed against the microorganisms responsible for the infection or that the drug does not reach the infected site at a concentration sufficiently high to inhibit the target microorganisms.[17,18]

Metronidazole has been suggested in the treatment of periodontal infection, due to its selective efficacy against obligate anaerobes. Serum and crevicular levels of the drug have been shown to reach minimal inhibitory concentration (MIC) levels for most periodontal pathogens.[19]

More recently, local application of 25% metronidazole gel was proposed[20,21] in treating sites with clinical signs of periodontal disease. In a multi-center randomized clinical study,[20] the efficacy of metronidazole gel was compared to that of subgingival scaling in the treatment of adult periodontitis. At 6 months, the difference between treatments was statistically, but not clinically, significant. The efficacy of metronidazole gel in conjunction with subgingival scaling was demonstrated in another study in which clinical and microbiological parameters were evaluated.[21]

Another study[17] indicated that local application of metronidazole gel had a beneficial effect on clinical healing of periodontal vertical defects treated by GTR, although the measurable microbiological activity of the drug lasted for only 1 week.[18] It was suggested that local application of metronidazole at the target site might have been effective in providing better conditions for the periodontal tissue to regenerate by preventing bacterial colonization of the membrane material at the time of insertion or shortly after.[17,18]

The aim of the present study was to evaluate the effect of metronidazole gel application on: 1) the clinical outcome of GTR surgical procedures using titanium reinforced periodontal membranes; 2) bacterial colonization of membrane material; and 3) flap connective tissue-membrane integration.

MATERIALS AND METHODS

Experimental Design

This was a randomized, controlled clinical trial in which 2 different antimicrobial regimens were associated with the treatment of vertical bony defects by means of GTR surgical procedures using titanium reinforced ePTFE periodontal membranes.§ The test group received weekly repeated topical application of metronidazole gel‖ and the control group received systemic antibiotics¶ (amoxicillin plus clavulanic acid 1g/day for 14 days).

Clinical outcomes were longitudinally followed for 1 year. To avoid randomization imbalances, vertical bony defects were assigned to the 2 treatment groups after controlling for 2 prognostic factors: depth of the intrabony component (INFRA) and clinical attachment level (CAL).[21,22]

Study Population

Patients with systemic disease, who received antibiotics in the 6 months preceding the start of the study, or with a full-mouth plaque score and full-mouth bleeding score greater than 25% after initial therapy were excluded from the study. Following completion of the cause-related therapy consisting of oral hygiene instruction, scaling and root planing, 56 patients affected by chronic adult periodontitis were enrolled in this clinical study (29 female and 27 male; 32 to 65 years of age; mean age 48.2±8.3). All patients gave informed consent to participate in this controlled clinical trial.

One tooth site per patient, associated with an angular bony defect ($\geq$ 4 mm) and an attachment loss $\geq$ 8 mm was selected for GTR treatment using a titanium periodontal membrane.§ Defects did not extend into a furcation.

The tooth population (56 teeth) consisted of 20 incisors, 18 cuspids, 10 bicuspids and 8 molars; 37 teeth were located in the maxillary arch. Baseline full-mouth plaque score was 12.2±2.8; baseline full-mouth bleeding score was 11.1±3.0. Twenty-two patients were smokers (smoking more than 10 cigarettes/day).[23]

Clinical Measurements

Full-mouth plaque score (FMPS) was recorded as the percentage of total surfaces (4 aspects per tooth) which revealed the presence of plaque.[24] Bleeding on probing was assessed dichotomously at a force of 0.3 N with a manual pressure-sensitive probe. Full-mouth bleeding score (FMBS) was recorded as the percentage of total surfaces (4 aspects per tooth) which revealed the presence of bleeding upon probing. The following clinical measurements were taken 1 week before the surgery and at 1-year follow-up: clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ); probing depth (PD), measured from the gingival margin; and marginal gingival recession (REC), measured from the CEJ to the gingival margin.

A single investigator blinded to the treatment performed the clinical measurements at baseline and at 1 year. He was unaware of the morphological results.

Measurements were performed at 6 sites around all teeth; this study, however, reports only local measurements at the deepest interproximal point of the selected defect. All measurements were performed by means of a manual pressure-sensitive probe and were recorded to the nearest millimeter.

The following clinical measurements were taken at the time of the surgery immediately after debridement of the defects:[22] distance from the CEJ to the bottom of the defect (CEJ-BD) and distance from the CEJ to the

§ Gore-Tex regenerative material, W.L. Gore and Associates, Inc., Flagstaff, AZ.
‖ Pernyzol, 25% dental gel, Recordati S.p.A., Milan, Italy.
¶ Augmentin, SmithKline Beecham S.p.A., Milan, Italy.

most coronal extension of the bone crest (CEJ-BC). The intraosseous component of the defects (INFRA) was defined as INFRA = (CEJ-BD) - (CEJ-BC).

Randomization

Before surgery patients were randomly assigned to 1 of the 2 treatment groups using the randomized block approach. Blocking to control for the effect of the prognostic variables, INFRA and CAL, was used to decrease outcome variability.[21,22,25] For randomization purposes INFRA was estimated before surgery on radiographs and by performing transgingival bone sounding.

Surgical Procedure

The intrabony defects were treated according to the principles of guided tissue regeneration with the application of titanium reinforced non-resorbable barrier membrane§ and the modified papilla preservation technique described by Cortellini et al.[23] In brief, full thickness flaps were elevated trying to preserve the marginal and the interdental tissues at the maximum possible extent. Following careful debridement and root planing, titanium reinforced non-resorbable membranes were positioned to completely cover the defects and overlap 2 to 3 mm of the residual bone. Membranes were secured and stabilized to the neighboring teeth with Teflon sutures. Flap elevation was continued as split thickness to permit coronal displacement of the flap and thus complete coverage of the membrane. Sutures were placed in the interproximal areas in order to achieve primary closure of the interdental tissues over the membranes.

Postsurgical Infection Control

In the test group, a slow-releasing dental gel containing metronidazole benzoate (25%)[‖] was applied along the gingival margin with a syringe after completion of the suture of the surgical flap. The gel application was repeated every week for 5 weeks. No systemic antibiotics were prescribed. In the control group, patients received systemic antibiotics[¶] 1g/day for 14 days. All patients (test and control groups) were instructed to rinse with a 0.12% solution of chlorhexidine twice a day up to membrane removal. During this period they were recalled once a week for professional tooth cleaning.

Membrane Removal

Six weeks after the surgery, patients underwent a second surgery to remove the barrier material. Immediately before membrane removal, all teeth were polished to remove supragingival plaque and reduce the risk of bacterial contamination of the membranes during the reentry procedure. The soft tissue covering the membrane was separated from the barrier material with an elevator. Immediately after membrane removal, the distance between the CEJ and the most coronal extension of the newly formed granulation tissue (NFGT) was recorded to the nearest millimeter. The regenerated tissue was covered by coronal positioning of the flaps. The retrieved membranes were processed for scanning electron microscopy (SEM) analysis.

Plaque Control

Patients were recalled once a week for professional tooth cleaning for another month and were instructed to rinse twice daily with 0.12% chlorhexidine for 5 weeks.

Mechanical tooth cleaning in the surgically treated area was reinstituted 4 weeks after the second surgery. Patients were recalled for professional tooth cleaning and reinforcement of self-performed oral hygiene measures at 1-month intervals up to the 1-year reevaluation.

SEM Preparation and Analysis

Following removal, the membranes were rinsed in saline solution containing 3% sodium citrate to remove adherent blood and fixed in 2.5 glutaraldehyde in cacodylate buffer. The specimens were rinsed again in cacodylate buffer, postfixed in 2% osmium tetroxide in phosphate buffer, dehydrated with graded ethanol, critical point dried with CO_2, sputter coated with 20 nm gold-palladium, and mounted at 10 kV emission voltage with a specimen tilt angle varying between 15 and 30 degrees. The flap-facing surface of the membranes was examined by SEM.

After removal each interproximal membrane was divided into half (buccal and palatal/lingual). In each half of the membrane, 3 areas were considered for the SEM analysis: 1 coronal, 1 mid, and 1 apical. Nine randomly selected microscopic fields (at 300x magnification) were analyzed in each area. In each microscopic field, comprising an area of 0.4 x 0.3 mm^2, magnification was increased up to 5,000x in order to determine the prevalent nature of the deposits covering the surface of the membrane: connective tissue structures, bacteria, or other deposits. When connective tissue structures dominated, the field was considered positive for the integrated connective tissue (Fig. 1); that is, connective tissue fibers and cells covering the surface of the membrane. When bacteria (Fig. 2) accounted for the majority of the deposits, the field was considered positive for bacteria. Conversely, microscopic fields showing the membrane surface covered by deposits other than bacteria or connective tissue structures (i.e., fibrin, inflammatory cells, unidentified material) were considered positive for other deposits (Fig. 3). Fifty-four assessments (27 in the buccal half of the membrane and 27 in the palatal half) were made in each membrane. SEM examination and scorings were carried out by an investigator unaware of the clinically recorded data.

Data Analysis

A series of Student *t* tests were used to control the effectiveness of the random assignment of the subjects to the test and control groups as long as potentially relevant variables were involved. Initial CAL, PD, REC,

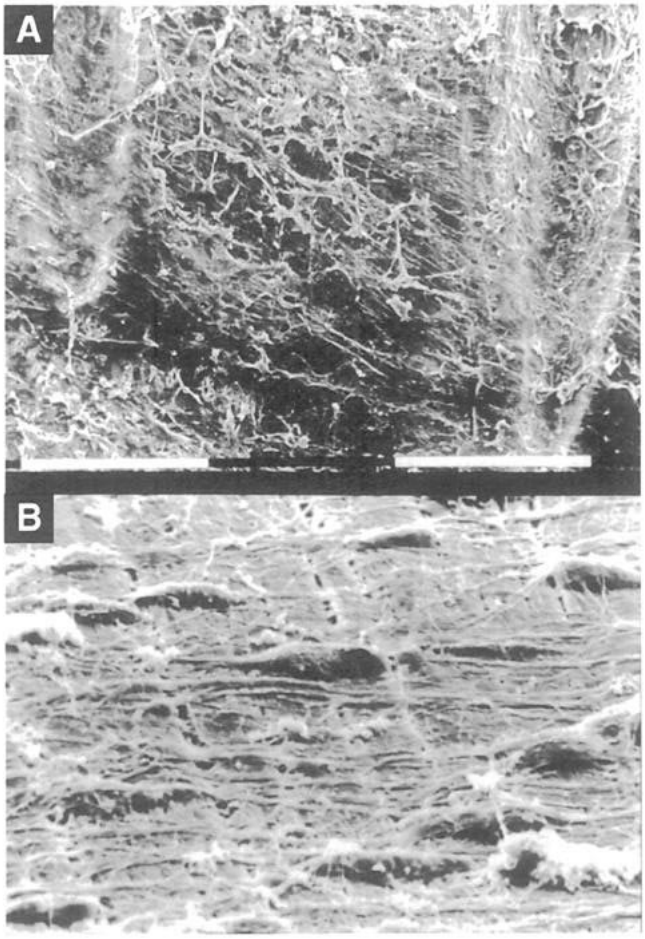

Figure 1.
Test membrane. A. Microscopic field positive to integrated connective tissue; connective tissue structures (cells and fibers) can be seen on the flap-facing surface of the mid portion of the membrane (300x original magnification). B. A higher (2,500x) magnification of connective tissue cells colonizing the surface of the membrane.

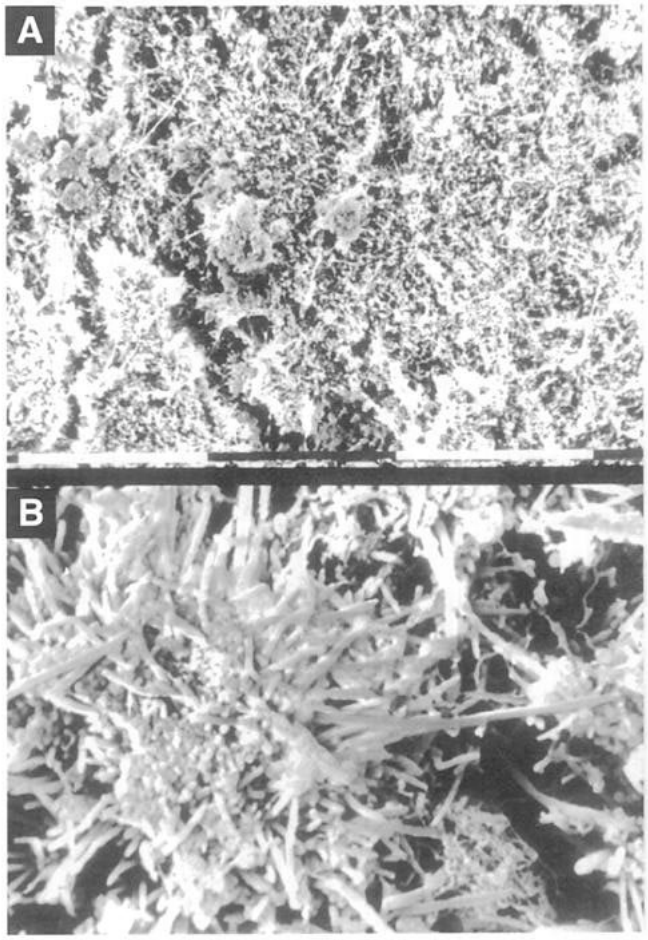

Figure 2.
Control membrane. A. Microscopic field positive to bacteria. The surface of the membrane (collar area) is completely covered by a thick layer of plaque (300x original magnification). B. Different bacterial morphotypes can be distinguished; long rods and filaments predominate (4,200x magnification).

CEJ-BD, and INFRA were tested for significance of the differences between the means of test and control groups. The significance of the difference in the proportion of smokers between the groups was tested using the approximation to the normal distribution.

The differences between the mean values of the outcome variables in test and control groups were also tested with Student *t* test. CAL gain was the main variable of interest and was used for the real hypothesis testing of this trial, the alternate hypothesis being a greater CAL gain in the test group. Other variables were investigated with an explorative purpose: PD reduction (baseline PD — 12 months PD); REC increase (baseline REC — 12 months REC); CEJ-NFGT, tissue gain (CEJ—BD — NFGT); regenerated CAL (CEJ-BD — 12 months CAL); tissue loss (tissue gain — regenerated CAL); and number of fields positive to bacteria, integrated connective tissue or other deposits.

Other explorative analyses included ordinary least-square multiple linear regressions which were carried out in order to provide explanatory hypotheses for the observed facts.

RESULTS

Mean age in the test group and control group was 49.2±7.3 and 47.9±8.1, respectively. There were 14 female patients in the test group and 15 in the control group. There were 10 cigarette smokers in the test group and 12 in the control group. All patients remained until completion of the study.

Oral hygiene and defect characteristics are shown in Table 1. No statistically significant difference was observed between the 2 groups in any of the considered clinical parameters, indicating that the randomization process had been effective.

Tissue Formation Under the Membrane

Healing in all control cases was uneventful. In 5 of the test patients, soft tissue swelling with pocket formation between the membrane and the covering gingival tissue was observed. Membrane exposure occurred in 17 out

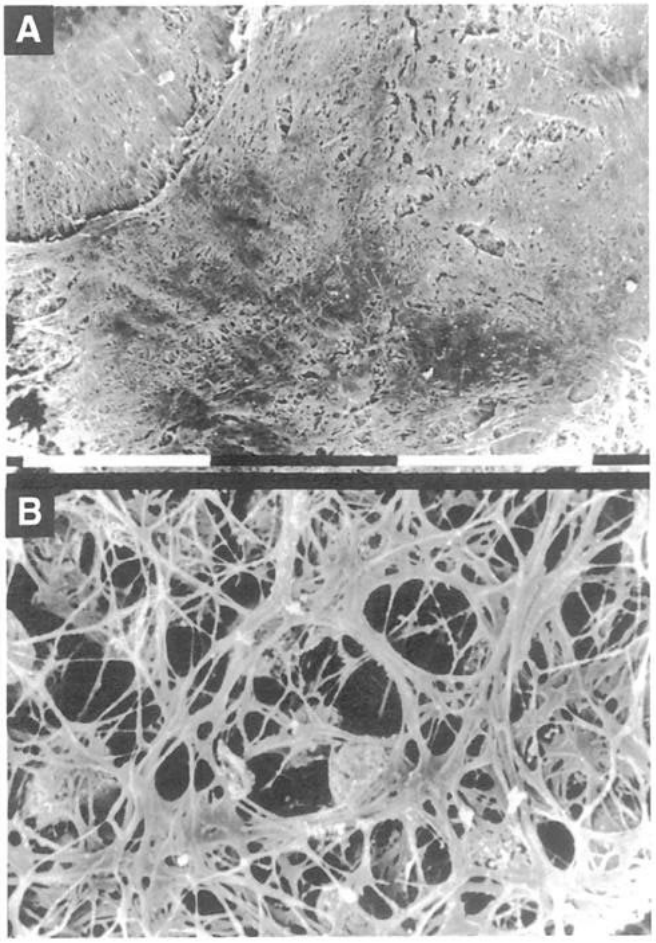

Figure 3.
Test membrane. A. Microscopic field positive to other deposits. The surface of the membrane (collar area) is covered by deposits (300x magnification). B. The higher (2,500x) magnification shows that these deposits consist of structures other than bacteria or connective tissue. Some inflammatory cells can be recognized in the fibrin net covering the surface of the membrane. No bacteria can be observed.

of 26 cases (65%) in the test group and 13 out of 30 cases (43%) in the control group.

At membrane removal, the distance between the CEJ and the most coronal extension of regenerated tissue was 3.5±1.1 in the test group and 3.8±1.4 in the control group. The difference was not statistically significant ($P = 0.32$, t test).

Clinical Outcomes at 1 Year (Table 2)

At 1 year FMPS were 10.2±2.4 in the test group and 9.2±3.1 in the control group; FMBS were 8.1±2.7 in the test group and 7.4±3.0 in the control group. A statistically significant difference was observed between baseline and 1 year FMPS and FMBS in both groups ($P = 0.0008$ and $P < 0.0001$ for the test group and $P < 0.0001$ and $P < 0.0001$ for the control group, respectively), indicating that the recall program was effective in further improving oral hygiene.

Table 1.

Baseline Oral Hygiene and Defect Characteristics (means ± SD)

	Test (26)	Control (30)	*P* (*t* test)
FMPS (%)	12.4 ± 3.0	13.6 ± 2.8	0.32*
FMBS (%)	10.8 ± 2.7	11.6 ± 2.8	0.28*
CAL (mm)	10.4 ± 1.4	10.3 ± 2.0	0.97*
PD (mm)	9.1 ± 1.4	8.9 ± 1.8	0.74*
REC (mm)	1.3 ± 1.0	1.4 ± 1.0	0.64*
CEJ-BD (mm)	12.1 ± 1.3	11.8 ± 1.8	0.47*
CEJ-BC (mm)	5.5 ± 1.2	5.0 ± 1.3	0.17*
INFRA (mm)	6.6 ± 1.1	6.8 ± 1.8	0.71*

* Not significant

The mean gain of clinical attachment (CAL gain) was 4.8±1.2 in the test group and 5.3±1.7 in the control group. No statistically significant difference ($P = 0.2$) was found between test and control mean AG using a 1-tailed Student t test. Therefore, the null hypothesis (no difference between test and control as far as AG is concerned) could not be rejected. The 95% confidence intervals of the difference in attachment gain are 0.899591433 and 0.085023951, both in the direction of a greater attachment gain in the control group.

The mean PD reduction (baseline PD - 1 year PD) was 6.7±1.2 in the test group and 6.5±1.6 in the control group. No statistically significant 2-tail (t test) difference was found between groups ($P = 0.6$).

The mean increase in gingival recession (1 year REC - baseline REC) was 1.8±0.9 in the test group and 1.2±0.7 in the control group. A statistically significant greater increase of gingival recession ($P = 0.003$) was found in the test group compared to the control group.

Table 3 shows that the mean tissue gain (CEJ-BD - NFGT) was 8.6±1.3 in the test group and 8.0±1.6 in the

Table 2.

Clinical Changes at 1 Year (means ± SD)

	Test (26)	Control (30)	*P* (*t* test)
FMPS (%)	10.2 ± 2.4	9.2 ± 3.1	0.47*
FMBS (%)	8.1 ± 2.7	7.4 ± 3.0	0.42*
CAL gain (mm)	4.8 ± 1.2	5.3 ± 1.7	0.22*
PD reduction (mm)	6.7 ± 1.2	6.5 ± 1.6	0.62*
REC Increase (mm)	1.8 ± 0.9	1.2 ± 0.7	0.004

* Not significant

Table 3.

Newly Formed Tissue Gain, Regenerated CAL, and Tissue Loss (in mm) (means ± SD)

	Test (26)	Control (30)	P (*t* test)
Tissue gain (CEJ-BD - NFGT)	8.6 ± 1.3	8.0 ± 1.6	0.1*
Regenerated CAL (CEJ-BD - CAL)	6.5 ± 1.2	6.7 ± 1.6	0.6*
Tissue loss (tissue gain - regenerated CAL)	2.1 ± 0.9	1.2 ± 0.6	< 0.001

* Not significant

control group. The difference was not statistically significant (2-tail *t* test) (*P* = 0.1). The mean regenerated CAL (CEJ-BD - 12 months CAL) was 6.5±1.2 in the test group and 6.7±1.6 in the control group. The difference was not statistically significant (*P* = 0.6). The mean tissue loss (tissue gain - regenerated CAL) was statistically (*P* <0.001) greater in the test compared to the control group.

Morphological (SEM) Results

The mean number of fields positive to integrated connective tissue was 3.0±1.3 and 3.4±1.6 in the test and control membranes, respectively, while the fields positive to other deposits were 4.8±1.2 in the test membranes and 2.5±0.8 in the control membranes. The mean number of bacteria-positive fields was 0.9±1.2 in the test membranes and 3.1±1.3 in the control membranes.

No statistically significant (*t* test) difference was found between test and control group in the number of fields positive to integrated connective tissue (*P* = 0.82), while the number of fields positive to bacteria was statistically higher (*P* <0.001) in the control group. A statistically significant (*P* <0.001) higher number of "other deposits" fields were demonstrated in the test group (Fig. 4). Interestingly, the connective tissue integration variable did not show a statistically significant difference, while the bacteria variable was significantly higher in the control group and other deposits in the test group.

Regression analysis of CAL gain on groups (dummy variable), bacteria and baseline PD revealed a significant negative correlation with the treatment (less CAL gain in the test group) and with bacteria and a positive correlation with baseline PD (Table 4).

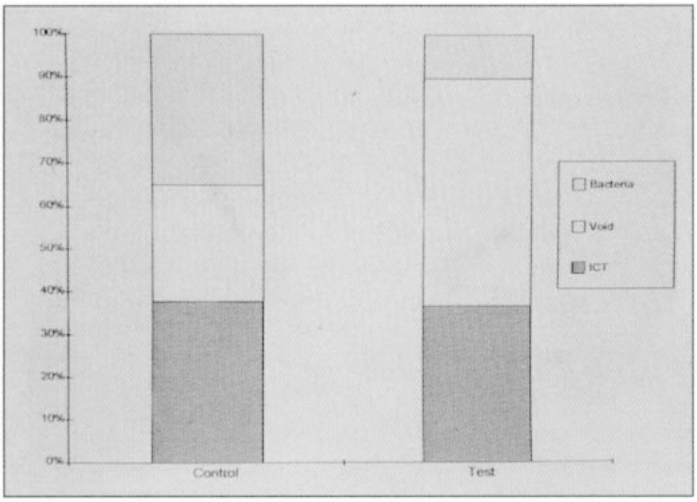

Figure 4.
Relative (%) distribution of the microscopic fields positive to integrated connective tissue (ICT), bacteria, and other deposits in test and control membranes. The number of fields positive to bacteria was statistically (P <0.001) greater in the control membranes, while the number of "other deposits" fields was greater (P <0.001) in the test membranes. No statistically significant difference (P = 0.82) was demonstrated in the number of fields positive to connective tissue structures between test and control membranes.

DISCUSSION

The aim of the study was to compare the clinical outcomes of GTR procedures using titanium reinforced ePTFE membranes in patients undergoing different antimicrobial treatments: systemic and topically applied antibiotics.

The rationale for antimicrobial therapy in GTR therapy has not yet been established. Conversely, bacterial colonization of membrane material has been demonstrated to be the major limiting factor for tissue regeneration.[4-11]

Different antibiotics and administration protocols have been suggested[2,9-16] to prevent bacterial colonization of membranes. In addition, 2 to 3 daily (0.12 to 0.2%) chlorhexidine mouthrinses have been frequently recommended in patients undergoing GTR.[4-6] Both treatments have been demonstrated to be ineffective in preventing bacterial colonization of both bioabsorbable and non-resorbable materials.[2,4-16] Conversely, some authors reported better clinical outcomes after GTR procedures in patients using systemic antibiotics compared to patients not receiving antibiotics.[11,13] In particular, the administration of antibiotics prior to surgery[11-12] has been shown to improve clinical outcomes following GTR. This may be due to the eradication of the pathogens present in the surgical site.[12]

On the other hand, the systemic route of antibiotic therapy may induce some, even severe, problems due to development of bacteria resistance or allergic reactions. This fact, together with the need to reach a higher concentration of the drug at the target site, has suggested the use of local antibiotics.[17]

Sander et al.[17] reported on the use of a slow-releasing dental gel containing metronidazole benzoate (25%) in patients undergoing GTR therapy. In this study, the

Table 4.

Results of the Ordinary Least-Square Multiple Linear Regression CAL Gain on Test/Control Groups, Preoperative PD, and Bacterial Colonization of the Membrane (Number of Positive Fields)

R^2 = 0.72	*F* -value: 45.5		*P* <0.001
Degrees of Freedom: 55			
Adjusted R^2 = 0.71			
	Estimate	S.E.	*P* Value
Intercept	1.626	0.823	0.05
Test/Control	-1.603	0.301	<0.001
Bacterial colonization of the membrane (sum of positive fields)	-0.465	0.096	<0.001
Presurgical PD	0.574	0.072	<0.001

Test = 1; control = 0.

gel was applied filling the angular bony defect at the time of the surgery, on the outer surface of the membrane before suturing the flap and along the gingival margin at the end of the surgery. Gel application was not repeated during the healing period. The 6-month results demonstrated that the gain of probing attachment, reported as a percentage of the initial defect, was greater in patients treated with metronidazole gel compared to control (no antibiotics) patients. No differences were reported between the 2 groups in terms of pocket reduction, gain in bone height, or recession of gingival margin.

In the present study, the metronidazole gel was applied only along the gingival margin at the end of the surgery and its application repeated every week up to the time of membrane removal. The results of the study indicated that the same amount of attachment gain can be achieved in GTR patients using systemic or local antibiotics. Furthermore, the amount of attachment gain obtained in the present study is similar to that reported in another study[23] on guided tissue regeneration with titanium reinforced ePTFE membranes in which patients received tetracycline HCL for 1 week. Conversely, the results of the present study differ from those of Sander et al.[17] Differences in the surgical procedure might have affected the interpretation of the data. In fact, it cannot be ruled out that the mere space-keeping effect of the gel applied into the angular bony defect could influence the results of surgery. Furthermore, the true biocompatibility of metronidazole in the periodontal wound has not been demonstrated; rather, a possible interference of this drug with connective tissue cells' metabolism cannot be excluded. The importance of connective tissue-membrane integration on the clinical outcomes of guided tissue regeneration has been recently demonstrated.[6] It is conceivable that the presence of the gel (and/or the physical shift during time) above the membrane, due to its physical characteristics, could jeopardize the integration between the flap connective tissue and the outer surface of the membrane and thus affect the regeneration process.

Nevertheless, our data suggest that the local application of antibiotics is preferred to systemic administration, since similar clinical results can be obtained with a much lower dosage than that needed for systemic treatment.

The results of the present study also demonstrated that both the increase in gingival recession (from baseline to 1-year follow-up) and the amount of regenerated tissue lost during the maturation phase after membrane removal were statistically greater in patients receiving local metronidazole gel application.

The fact that less "tissue gain" became "regenerated CAL" in the test group compared to the control group could be ascribed to poorer coverage of the newly formed tissue under the membrane in patients treated with metronidazole. This is in accordance with another study[22] which indicated that the lack of coverage of the regenerated tissue under the membrane is a major factor negatively influencing tissue maturation.

The greater tissue loss, together with the greater increase in gingival recession in the test group, is indicative of gingival tissue alterations taking place during the healing process.

It could be speculated that the metronidazole itself or its vehicle, the gel, or its subsequent physical modifications may have interfered with soft tissue healing processes.

Support of this hypothesis comes from the following data: 5 test patients showed soft tissue swelling and pocket formation between the membrane and the covering gingival tissue; and the percentage of membrane exposure was higher (65% versus 43%) in the test compared to the control group.

The morphological (SEM) results of the study demonstrated that the number of bacteria-positive fields was greater in the control membranes, while the number of fields positive to other deposits was higher in the test membranes. No difference was demonstrated in the number of fields positive to integrated connective tissue between test and control groups.

Almost all the fields positive to connective tissue structures were located in the mid and apical portions of the membranes (far from the gingival margin) in both test and control groups, while the marginal portions of the membranes were heavily colonized by bacteria in the control membranes and by other deposits in the test membranes. Thus it can be assumed that when applied once a week along the gingival margin, the metronidazole gel is effective in preventing bacterial colonization of membrane material during the healing process, but it does not increase the integration of flap connective tissue with the outer surface of the membrane. This may be due to an inhibitory effect of the drug or to the physical characteristics of its vehicle, which render the bacteria-free sites unavailable for connective tissue structure integration.

Regression analysis identified the application of metronidazole gel as a predictor of poorer attachment gain once adjusted for bacterial contamination and initial probing depth. This result can be interpreted as follows: baseline probing depth is a usual predictor (or may be a limit) of attachment gain;[4,22] bacterial contamination is a well-known negative predictor;[4,5] metronidazole itself reduces the bacterial contamination, thereby enhancing attachment gain, but this effect is masked by a negative effect of the gel. This negative effect may also explain the fact that the greater amount of bacteria-free fields in the test group is associated with an increase in the other deposits fields and not in the integrated connective tissue fields.

It is conceivable that the local route of antibiotic administration could be more effective than the systemic route in preventing periodontal wound infection, but it does not improve the clinical outcomes of surgery due to an interference of the vehicle (the gel) with gingival tissues which may delete the potential benefits deriving from the better control of the bacterial load.

Further research is needed to study the effect of the gel without the active drug and possibly the effect of metronidazole in a different preparation.

The goal of preventing, or at least reducing, bacterial colonization of the membrane material by a local (topically applied) antimicrobial agent which does not interfere with the healing process should be further explored.

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