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Bacterial colonization of barrier material and periodontal regeneration

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Abstract. The objective of this study was to evaluate the relationship between the presence of bacteria on the tooth-facing surface of ePTFE barriers and the clinical outcome of membrane supported reconstructive periodontal surgery. 20 systemically healthy subjects affected by chronic periodontitis were enrolled. One tooth site per patient, associated with an angular bony defect and a probing attachment loss of >4 mm, was selected to be treated by means of a guided tissue regeneration procedure using an ePTFE barrier membrane. Antibiotics (Augmentin 1 g/day) for 2 weeks were prescribed. In addition to the use of chlorhexidine for post-surgical plaque control, all patients were recalled once a week for professional tooth cleaning. The barrier material was harvested for SEM analysis after 4–6 weeks. Professional tooth cleaning and reinforcement of self-performed oral hygiene measures were given at 1 month intervals after membrane removal. For each treated site, the difference in probing attachment loss between baseline examination and a follow-up examination after 6 months of healing was calculated. The results of the SEM-analysis revealed that bacterial colonization was evident in the collar area of all the retrieved membranes. In the mid part of the membranes 30 out of 60 microscopic fields (50%) demonstrated microbial colonization, and in the most apical part 9 out of 60 fiels (15%). Regression analysis indicated that gain in probing attachment level was positively correlated to initial attachment loss and negatively correlated to microbial colonization of the mid part of the membranes. It was concluded that bacterial colonization in the mid part of the ePTFE membrane reduced the potential gain in probing attachment following GTR-therapy with almost 50%.

Key words: SEM; periodontal regeneration; microbiology

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The objective of a guided tissue regeneration procedure is to promote formation of new connective tissue attachment on a root surface which has been detached due to plaque induced inflammation. To accomplish this objective a barrier material is placed between the root surface and the mucosal flap, giving preference to cells from the periodontal ligament and the alveolar bone to repopulate the wound area adjacent to the root surface (Melcher 1976, Nyman et al. 1980, Boyko et al. 1981, Isidor et al. 1985). Although the validity of the biological principles has been confirmed in a large number of studies in various animal models (Nyman et al. 1982, Gottlow et al. 1990, Aukhil et al. 1983, 1986, Magnusson et al. 1985, Caton et al. 1987), human trials have revealed poor predictability of the results of the surgical procedure (Pontoriero et al. 1988, Schallhorn & McClain 1988, Becker et al. 1988, Caffesse et al. 1990, Lekovic et al. 1990, Anderegg et al. 1991, Metzler et al. 1991, Pini Prato et al. 1992, Yukna 1992, Andersson et al. 1994).

The amount of new attachment achieved by means of guided tissue regeneration procedures, both with respect to absolute value and percentage of the initial depth of the defect, may be influenced by a number of factors (Cortellini et al. 1994) associated with (i) the surgical technique (Becker & Becker 1990, Caffesse & Quinones 1992), (ii) defect characteristics (Gottlow et al. 1986) and (iii) tooth anatomy (Lu 1992). Due to flap recession during the early stages of healing the barrier material may become exposed for colonization of oral microorganisms, which also may adversely affect the treatment outcome. In fact, several studies have shown that the membranes may be heavily colonized by bacteria and that a negative relation-

ship exists between attachment gain and plaque colonization of the barrier material (Selvig et al. 1990, 1992, Mombelli et al. 1993, Demolon et al. 1993, Machtei et al. 1993, Nowzari & Slots 1994). These studies thus indicate that an optimal oral hygiene for prevention of infection of the wound area is essential for periodontal regeneration. However, to what extent bacterial contamination may influence the regenerative potential following GTR-therapy has not been fully elucidated.

The aim of the present study was to evaluate the relationship between bacterial colonization of the inner surface of ePTFE membranes and the clinical outcome of membrane supported reconstructive surgery in patients enrolled in a strict program of plaque control.

Material and Methods

20 systemically healthy subjects (age range 37–67 years) affected by chronic periodontitis were enrolled in the study. The participants were selected on a consecutive basis among patients consulting the department of Periodontology, School of Dentistry, Bologna University, during the period January-June 1993. The study design is outlined in Fig. 1. All patients were given an initial phase of treatment comprising oral hygiene instructions and full mouth scaling and root planing. 2 weeks after the completion of the initial therapy a baseline examination was performed and one non-molar tooth site per patient, associated with an angular bony defect and a probing attachment loss (Pre-surgical PAL) of >4 mm, was selected to be treated by means of a guided tissue regeneration procedure using an ePTFE barrier (Gore Periodontal Material™).

Before the start of the study, the examiner was trained and calibrated with respect to the assessments included in the study. The probing attachment loss was measured from the cemento-enamel junction with a calibrated Williams periodontal probe (HuFriedy™) having a 0.5 mm tip diameter and a 1 mm interval scale.

Mucoperiosteal flaps were raised and the exposed root surfaces were carefully scaled and root planed. After placement of the membrane, the flaps were repositioned and sutured to completely cover the barrier material. Antibiotics (Augmentin 1 g per day) for 2 weeks was prescribed and instructions were given to rinse the mouth with a 0.12% solution of chlorhexine twice a day for 2 min. The patients were recalled once a week for professional tooth cleaning until membrane removal, which was carried out after 4–6 weeks.

Immediately before membrane removal, all teeth were polished in order to remove supragingival plaque and reduce the risk for bacterial contamination of the barrier material during the reentry procedure. The amount of barrier material exposed supragingivally was assessed to the nearest 1 mm. The soft tissue covering the membrane was carefully elevated and the barrier material was harvested for SEM analysis (see below). Following flap closure by suturing, the patients continued with the daily rinses with chlorhexine for further 2 weeks. Mechanical tooth cleaning in the surgical treated area was reinstituted after the suture removal 10 days postoperatively. All patients were recalled for professional tooth cleaning and reinforcement of self-performed oral hygiene measures at 1 month intervals during the remaining part of the study. 6 months after the 2nd surgery, probing attachment loss was again assessed for evaluation of the clinical outcome of the regenerative treatment. The difference in probing attachment loss between the baseline examination and the 6 months examination was calculated for each treated site (PAL-change).

The same investigator performed the PAL-measurements at baseline and at 6 months; he was unaware of the microbiological results.

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 mm gold-palladium and mounted on specimen stubs to allow SEM analysis. Observations were made at 15 kV emission voltage and with a specimen tilt angle varying between 15 and 30 degrees.

The tooth facing surface of each membrane was examined at 15× magnification (Fig. 2). 9 randomly selected microscopic fields at 300× magnification were analyzed in each membrane: 3 in the collar area, 3 in the mid portion (corresponding to the coronal half of the occlusive portion of the membrane), and 3 in the apical portion (corresponding to the apical half of the occlusive portion of the membrane). In each microscopic field (0.4×0.3 mm^2) the proportion of membrane surface covered by deposit was evaluated. When 1/3 or more of the examined field was covered by deposits, the magnification was increased up to 6000× in order to determine the nature of the deposit, i.e., bacteria or host cells. Only when bacteria accounted for the majority of the deposit, the microscopic field was considered positive for bacterial colonization (Fig. 3). Conversely, the microscopic field was considered

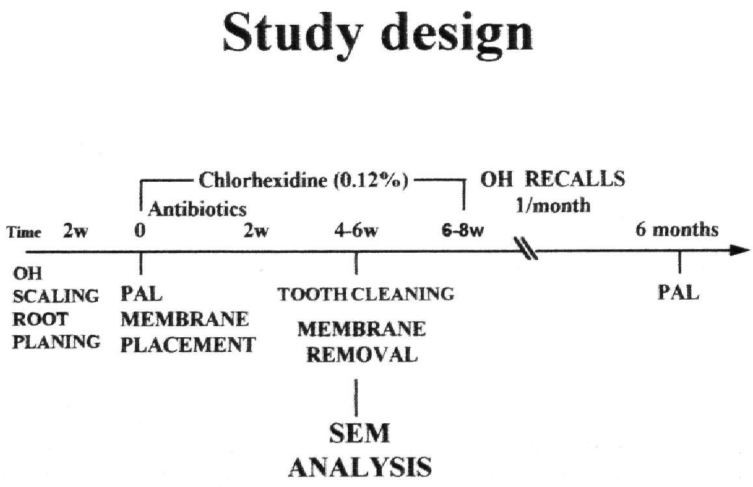

Fig. 1. The study design.

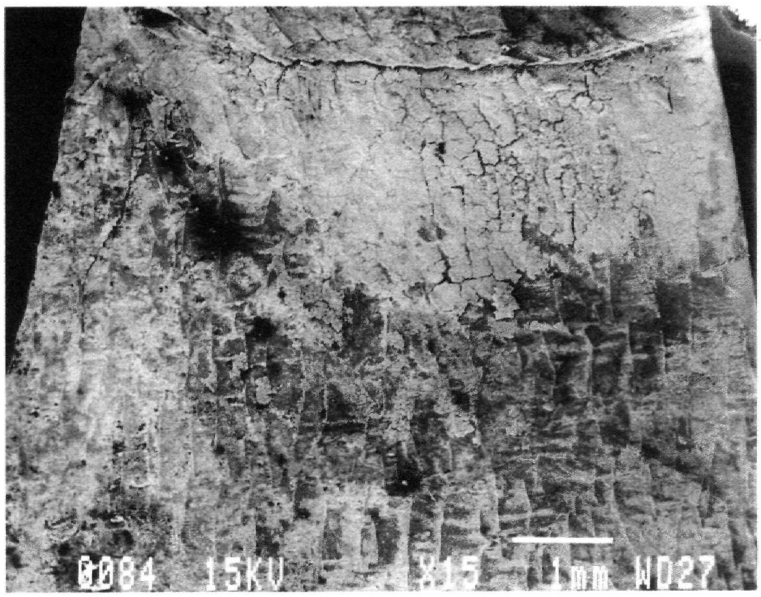

Fig. 2. A 15× magnification of a clinically unexposed membrane. Note the decreasing amount of organic deposit covering the barrier surface in coronal-apical direction.

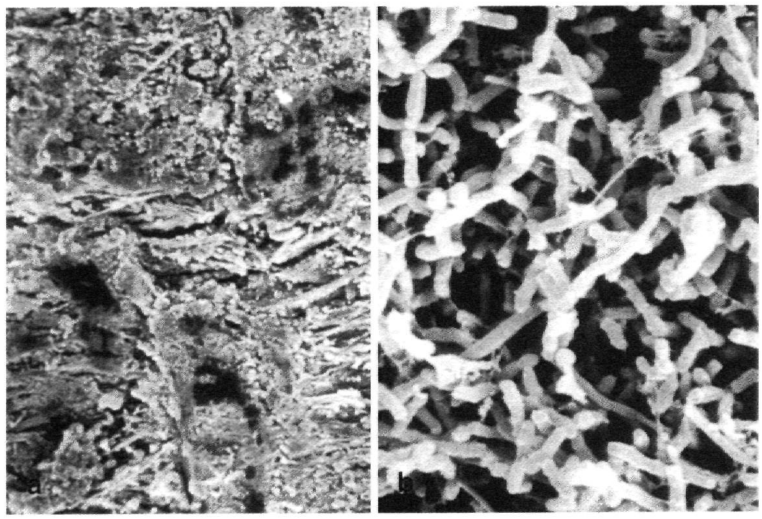

Fig. 3. Microscopic field *positive* for bacterial colonization. (a) At 300× magnification the surface of the membrane is fully covered by organic deposit. (b) this deposit mainly consists of bacteria (6000× magnification).

negative when other structures (host cells or unidentified material) were predominant. Microscopic fields (at 300× magn.) showing a clean membrane surface or only small areas (<1/3 of the field) covered by deposit were considered negative (Fig. 4). SEM examination and scorings were carried out by one investigator who was unaware of the clinically recorded data.

Data analysis

Basic descriptive statistics were calculated for the clinical variables (pre-surgical PAL and PAL-change). Frequency distribution of microscopic fields dominated by microorganisms was determined for exposed and non-exposed membranes, respectively.

An ordinary least square linear multiple regression model was used to test the hypothesis that the attachment gain is influenced by the presurgical degree of attachment loss and the extent of microbial colonization of the mid-portion of the membrane. The presurgical PAL was included as the first predictor, as its influence on attachment gain was already known. Only the mid portion of the membrane was considered in the regression analysis because this is the area of the membrane which directly faces the detached root surface and the regenerating tissue as well. The hypothesis was rejected at $p>0.05$. The regression of probing attachment gain on presurgical PAL and bacterial contamination was also analyzed.

Results

At the time of membrane removal, 16 out of the 20 membranes were partly exposed supragingivally. The maximal exposure recorded was 2 mm.

The data generated from the clinical assessments and from the SEM analysis are presented in Table 1. The average pre-surgical loss of probing attachment for the 20 defects treated was 7.3 mm (S.D. 1.7). Defect sites that showed partly exposed barrier material at time of removal of the membrane (4–6 weeks) had somewhat greater initial loss of attachment than those in the unexposed group. The mean gain in probing attachment assessed 6 months after membrane removal was 3.4 mm (1.1), and no difference was found beetween sites with exposed and non-exposed barrier material during the initial healing phase.

The microscopic analysis revealed that all 48 fields examined in the collar area of the 16 exposed membranes were positive for bacterial colonization (Table 1). Also 8 (66%) out of the 12 examined microscopic fields in the 4 clinically unexposed membranes were positive. In the mid-portion of the membranes, 29 (57%) of the fields in the exposed membranes were positive, while only one field (17%) in the unexposed membranes was considered positive with respect to bacterial colonization. The corresponding figure for the apical part of the membranes was 9 (19%) for the exposed membranes,

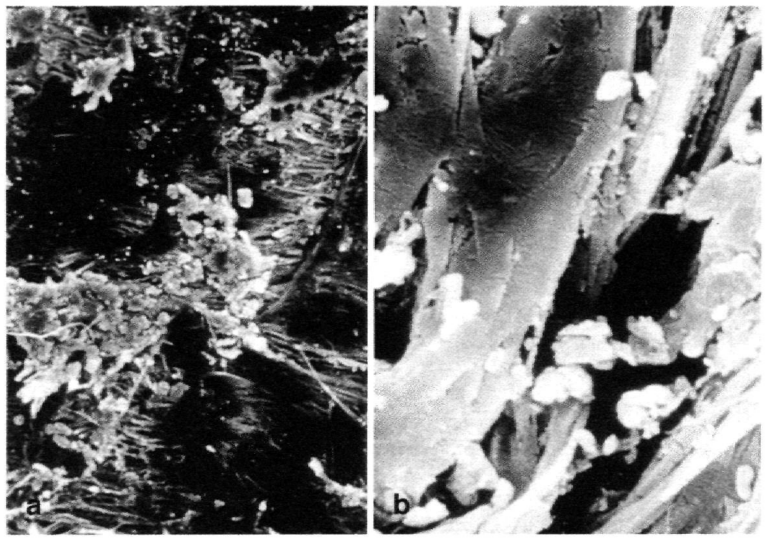

Fig. 4. Microscopic field *negative* for bacterial colonization. (a) At 300× magnification only small areas of the surface (<1/3 of the fields) are covered by organic deposit. (b) At 6000× magnification no bacteria can be observed.

whereas all fields examined in the non-exposed membranes were negative.

Cocci and short rods were the predominant morphotypes of microorganisms found in the collar area of the membranes (Fig. 5). More apically, on the unexposed occlusive portion of the membranes, filaments, short and long curved rods, and a varying number of spirochetes were also present (Fig. 6). Although there was a variation in amount of microorganisms between membranes, the different pattern of colonization in the collar, mid and apical parts of the membranes was in common.

A regression analysis was performed in order to evaluate the effect of the pre-surgical depth of the angular defect and the proportion of bacteria-positive fields in the mid portion of the barrier material on the gain of probing attachment (Table 2). Most of the variance, (more than 70%) of the dependent variable (PAL-gain) could be explained when including the two factors as explanatory variables. The calculated estimates and *p*-values for the independent variables indicated that (i) the depth of the angular defect was positively associated with the amount of PAL-gain, while (ii) microbial colonization was negatively associated. Furthermore, the estimates showed that the influence of microbial colonization on PAL-gain was 4× as strong as the pre-surgical depth of the defect, and in presence of microbial colonization the amount of PAL-gain was reduced by almost 50%.

Discussion

The results of the study demonstrated that bacterial colonization on the tooth facing surface of ePTFE membranes used in periodontal regeneration is common and critical for the outcome of

Table 1. Positive (+) and negative (−) microscopic areas with regard to bacterial colonization in various parts of the *tooth-facing surface* of the retrieved barrier material for each of the 4 non-exposed and 16 exposed membranes and amount of gain of probing attachment (mm)

Membrane exposure	Bacterial colonization			Pre-surgical probing attachment loss	Gain of probing attachment
	Collar	Middle	Apical		
unexposed	−++	−−−	−−−	5	2
unexposed	−+−	+−−	−−−	5	3
unexposed	+++	−−−	−−−	6	4
unexposed	+−+	−−−	−−−	8	5
% positive fields	66%	17%	0%		
mean (SD)				6.0 (1.4)	3.5 (1.2)
exposed	+++	+−+	−−−	7	3
exposed	+++	−−+	−−+	7	4
exposed	+++	+++	+−+	8	2.5
exposed	+++	+++	−−−	6	2
exposed	+++	+++	++−	10	3
exposed	+++	+−+	−−−	5	1.5
exposed	+++	+−−	−−−	6	3
exposed	+++	++−	+−−	9	4
exposed	+++	++−	−+−	9	5
exposed	+++	+−+	−−−	10	4
exposed	+++	+++	−−−	9	4
exposed	+++	+−−	−−−	7	4
exposed	+++	+−+	−−−	6	2
exposed	+++	−+−	+−−	10	5
exposed	+++	+−−	−−−	6	3
exposed	+++	+−−	−+−	8	4
% positive fields	00%	57%	19%		
mean (SD)				7.7 (1.7)	3.4 (1.0)

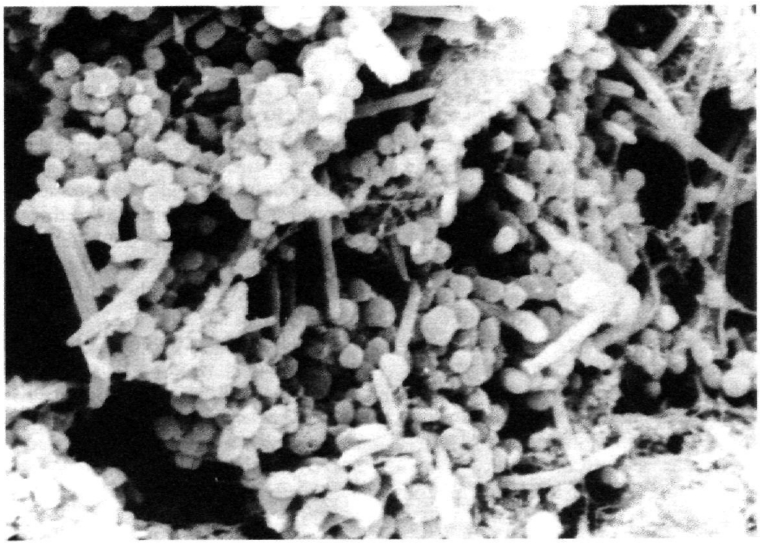

Fig. 5. Cocci represent the prevalent bacterial morphotype in the collar part of clinically exposed membranes (6000× magnification).

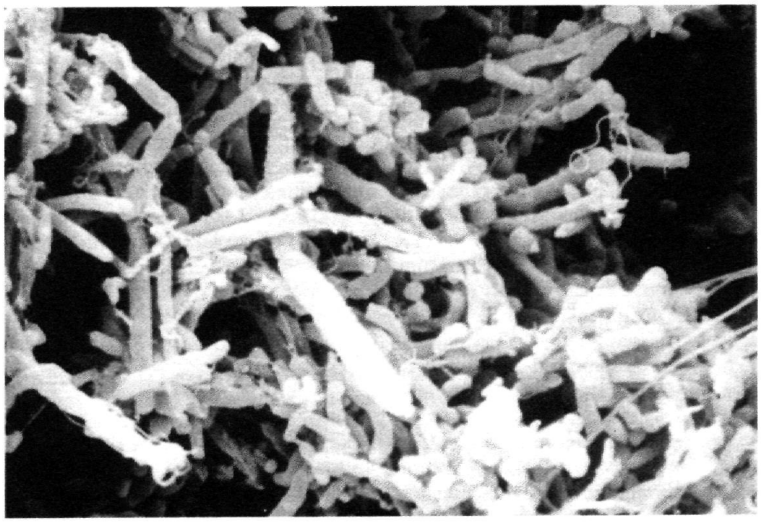

Fig. 6. Long and short rods, filaments and spirochetes in a microscopic field of the middle part of a clinically exposed membrane (6000× magnification.

the regenerative procedure. Despite the fact that the patients following surgical treatment were kept on a plaque control regimen which, in addition to weekly recalls for professional tooth cleaning, included 14 days of antibiotic therapy and chlorhexidine rinsings 2× a day for the entire 4–6 weeks period the membrane was kept in place, a majority of the membranes demonstrated presence of microorganisms at time of removal of the barrier material. This was the case not only in the collar part of the membranes, which in 16 out of the 20 treated sites had been partly exposed supragingivally during the healing period, but also in the subgingivally positioned part of the material, i.e., in the area of tissue regeneration. Furthermore, even in those cases where the barrier material had remained unexposed during the entire healing period, microorganisms were found in the collar portion of the membrane. Whether this finding is due to a contamination of the barrier material during the surgical retrieval of the membranes or in fact represents a true microbial colonization during the healing period can be disputed. A control experiment was therefore carried out in which 5 un-exposed ePTFE membranes, retrieved from sites of ridge augmentation, were analyzed. On none of these membranes did microscopic examination reveal the presence of microorganisms. Furthermore, the fact that microorganisms were observed not only on the surface of the membranes retrieved from the periodontal defects but also in the interstice area, supports the hypothesis of bacterial colonization rather than contamination during the retrieval of the membranes. Hence, it seems most likely that the membranes, which remained covered with soft tissue during the entire healing period, have been exposed for microbiological colonization through the "gingival pocket", or from residual microbial foci in the treated periodontal lesion as proposed by Nowzari & Slots (1994). However, since microbial colonization was more pronounced in the collar than in the deeper parts of the membranes the former source for infection seems to be the most likely one in the our material. The major pathway may have been between the membrane and the tooth surface, although the possibility exists, as demonstrated in vitro by Simion et al. (1994), that microorganisms also can have penetrated through the membrane.

A critical point in wound healing, and particularly following regenerative surgery, is the protection of the blood clot. The importance of clot adhesion to the root surface in periodontal repair has recently been demonstrated in a series of experimental studies by Wikesjö (1991). Products derived from bacterial metabolism may influence and disrupt the blood clot in early stages of healing (Slots & Genco 1984, Persson et al. 1990), thus influencing the amount of new tissue formation. Mombelli et al. (1993) examined the microbiological profile at ePTFE membranes in 10 patients 6 weeks after insertion and found a composition similar to that of an untreated periodontal pocket with high prevalence of gram-negative anaerobes. Similar observations were made by Nowzari & Slots (1994). Wang et al. (1994) studied the microbial adherance to ePTFE membranes and reported a high adherance of *P. gingivalis*, *Prevot-*

Table 2. Results of the ordinary least square multiple linear regression analysis with gain of probing attachment as dependent variable (20 teeth); the first predictor included in the equation was pre-surgical probing attachment loss

R^2=0.82	*F*-value: 40.2		p<0.001
degrees of freedom: 17			
adjusted R^2=0.74			
dependent mean value: 3.4 mm			
	Estimate	S.E.	*p*-value
intercept	0.458	0.452	NS
bact. colonization of the middle portion (sum of positive fields)	−0.647	0.107	<0.001
pre-surgical prob. attachment loss	0.540	0.065	<0.001

ella melaninogenica, *Selenomonas sputigena* and *Actinomyces viscosus*.

The negative influence of microbial colonization of the barrier material on the amount of gain of probing attachment found in the present study, together with similar observations reported from other recent studies (Selvig et al. 1990, 1992, Mombelli et al. 1993, Demolon et al. 1993, Machtei et al. 1993, 1994, Nowzari & Slots 1994), indicates the need of effective modalities of plaque control during the healing period. Cortellini et al. (1994) also demonstrated in a clinical study the importance of supragingival plaque control for the healing outcome of regenerative periodontal therapy. In the present study, the patients used a 0.12% solution of chlorhexine twice daily for supragingival plaque control and were recalled for polishing of the teeth on a weekly basis during the entire healing period. In addition, antibiotics (Augmentin 1 g/day) were prescribed for 14 days postoperatively. Dispite this stringent plaque control regimen, the majority of the membranes showed bacterial colonization at time of removal of the barrier material. This may indicate that the antibiotic treatment given was inappropriate in terms of the time period of drug delivery and/or the effectiveness against putative pathogens. Recent studies have shown an association between the presence of *A. actinomycetemcomitans*, *B. forsythus* and *P. micros* and impaired clinical results of GTR treatment (Machtei et al. 1993, 1994, Nowzari & Slots 1994), and the antibiotic regimen used in our study may not have been sufficient to eliminate and/or prevent the recolonization of these pathogens. Neither doxycycline (100 mg/day) given during a 3-week period following membrane insertion was found to be sufficient to prevent colonization (Nowzari & Slots 1994). Maybe an antibiotic therapy with a broader spectrum of activity, e.g., a combination of amoxicillin and metronidazole (Van Winkelhoff et al. 1989), can offer a better protection against microbial colonization of the barrier material. Furthermore, antibiotic therapy over periods longer than 2–3 weeks may be indicated for achievement of optimal healing conditions. On the other hand, it could be important to have optimal concentrations of the drug in the wound area and in the blood clot during and immediately after the operation, e.g., by giving the antibiotic 1 h before the surgery as generally recommended for prophylactic use. Since data on appropriate administration of antibiotic therapy in conjunction with GTR treatment is lacking in the literature, studies should be carried out to evaluate if the treatment outcome with respect to regeneration of the periodontal tissues can be improved by the use of different administrations of antibiotic therapy.

An improved antimicrobial effect might be obtained by local application of a proper antibiotic providing a sufficiently high concentration at the membrane site. A recent study by Sander et al. (1994) indicated that local application of metronidazole gel has a beneficial effect on clinical healing of periodontal vertical defects treated by guided tissue regeneration although the measurable microbiological activity of the drug lasts for only 1 week (Frandsen et al. 1994). It has been suggested that local application of metronidazole at the target site might be effective in providing better conditions for the periodontal tissue to regenerate preventing bacterial colonization of the membrane material at the time of insertion or shortly after. Further studies are needed to test the efficacy and the biocompatibility of metronidazole gel in periodontal wounds.

Although care was taken at time of surgery to properly cover the barrier material with the soft tissue flap, the collar area of 16 out of the 20 membranes became partially exposed supragingivally during the healing period. It is obvious that not only the function of the collar as a preventer of epithelial downgrowth through the ingrowth of connective tissue thereby is lost, but also that the collar becomes a retention site for plaque bacteria. The fact that all 4 unexposed membranes also showed presence of microorganisms in the collar area, and that a strong negative correlation was demonstrated between the presence of bacteria on the tooth facing surface apical to the collar area and gain of probing attachment, may raise questions as to the beneficial effect of the collar structure on the outcome of regenerative periodontal treatment. However, since no microbiological data on the barrier material without the collar structure are available, the present study offers no answer on this question.

In conclusion, the present study demonstrated that in the absence of bacterial plaque on the membrane, the gain in probing attachment achievable with the GTR procedure was on the average one half of the initial defect depth plus 0.45 mm. However, in the presence of bacteria on the submerged portion of the barrier material the potential gain of probing attachment is reduced with almost 50%.

Zusammenfassung

Bakterielle Besiedelung von Membranmaterial und parodontale Regeneration

Das Ziel dieser Studie war die Bewertung des Zusammenhanges zwischen der Anwesenheit von Bakterien auf der dem Zahn zugewandten Seite von ePTFE-Membranen und dem klinischen Ergebnis der membranunterstützten rekonstruktiven Parodontalchirurgie. Zwanzig Personen mit gesundem Allgemeinzustand, die von chronischer Parodontitis befallen waren, wurden in die Studie aufgenommen. Eine Tasche pro Patient, die einen angulären Knochendefekt und einen Attachmentverlust von >4 mm aufwies, wurde ausgewählt, um mittels gesteuerter Geweberegeneration unter Verwendung von ePTFE-Membranen behandelt zu werden. Für zwei Wochen wurden Antibiotika (Augmentan 1 g pro Tag) verschrieben. Zusätzlich zur Verwendung von Chlorhexidin zur postoperativen Plaquekontrolle bekamen die Patien-

ten einmal pro Woche eine professionelle Zahnreinigung. Nach 4–6 Wochen wurde die Membran zur REM-Untersuchung entnommen. Professionelle Zahnreinigung und Remotivation der Mundhygienemaßnahmen wurde nach der Membranentfernung in einmonatigen Intervallen durchgeführt. Für jedes behandelte Parodontium wurde die Differenz des klinischen Attachmentverlustes zwischen der Ausgangsuntersuchung und einer Nachuntersuchung 6 Monate nach der Heilung berechnet. Die Ergebnisse der REM-Untersuchung zeigten, daß die bakterielle Besiedelung in der Kragenregion von allen entnommenen Membranen offensichtlich war. Im Mittelteil zeigten 30 von 60 mikroskopischen Blickfeldern (50%) mikrobielle Kolonisierung und am apikalen Teil waren dies 9 von 60 (15%) Blickfeldern. Die Regressionsanalyse zeigte, daß der klinische Attachmentgewinn positiv mit dem initialen Attachmentverlust und negativ mit der mikrobiellen Kolonisierung des Mittelteils der Membranen korrelierte. Es wurde die Schlußfolgerung gezogen, daß die bakterielle Kolonisierung des Mitteltels der ePTFE-Membran den potentiellen klinischen Attachmentgewinn nach GTR-Therapie um fast 50% reduziert.

Résumé

La colonisation bactérienne du matériau des membranes barrières et la régénération parodontale

Le but de la présente étude était d'évaluer la relation existant entre la présence de bactéries sur la face des barrières d'ePTFE tournée vers la dent et les résultats cliniques de l'intervention chirurgicale de reconstruction supportée par une membrane. L'étude a porté sur 20 sujets en bonne santé générale, atteints de parodontite chronique. On a sélectionné chez chacun des patients 1 site associé à une lésion osseuse angulaire et à une perte d'attache au sondage >4 mm, site destiné à être traité par une méthode de régénération tissulaire guidée (RTG) en utilisant une membrane barrière d'e-PTFE. Un antibiotique (Augmentin 1 g par jour) a été prescrit. En plus de l'usage de chlorhexidine pour le contrôle post-chirurgical de la plaque, tous les patients ont été convoqués une fois par semaine pour un nettoyage dentaire professionnel. Le matériau barrière a été recueilli après 4–6 semaines pour analyse au MEB. Après la dépose de la membrane, des nettoyages dentaires professionnels et des séances de renforcement des mesures d'hygiène bucco-dentaire pratiquées par les sujets eux-mêmes ont eu lieu 1 fois par mois. Pour chacun des sites traités, on a calculé la différence entre les pertes d'attache mesurées par sondage à l'examen initial (baseline) et lors de l'examen de rappel après 6 mois de cicatrisation. Les résultats de l'analyse au MEB ont mis en évidence une colonisation bactérienne manifeste dans la région du collet de toutes les membranes recueillies. Dans la partie moyenne des membranes, 30 des 60 champs microscopiques (50%) présentaient une colonisation microbienne, et, dans la partie la plus apicale, 9 des 60 champs (15%). Une analyse de régression indiquait que le gain du niveau d'attache au sondage était en corrélation positive avec la perte d'attache initiale et en corrélation négative avec la colonisation microbienne de la partie moyenne des membranes. En conclusion, la colonisation bactérienne dans la partie moyenne de la membrane d'e-PTFE réduisait d'environ 50% le gain potentiel d'attache au sondage résultant du traitement par RTG.

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