GUIDED TISSUE REGENERATION COMBINED WITH A CREATION OF AN EXTRA SUPRACRESTAL BONE SPACE IN SURGICALLY PRODUCED DEHISCENCE - TYPE DEFECT. AN EXPERIMENTAL STUDY IN THE DOG.

PROCEDIMENTO DE REGENERAÇÃO TECIDUAL GUIADA COMBINADO COM A CRIAÇÃO DE ESPAÇO SUPRACRESTAL ACESSÓRIO EM DEFEITOS CIRÚRGICOS TIPO DEISCÊNCIA CRIADO CIRURGICAMENTE. ESTUDO EXPERIMENTAL EM CÃES.

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The purpose of the present investigation was to evaluate the use of mechanic barrier far from the radicular surface to achieve a supracrestal space in the guided tissue regeneration-procedure over teeth with dehiscence-type defects in 3 mongrel dogs. Two 4th premolars were used in each dog. Following the elevation of a mucoperiosteal flap, the buccal and the proximal bone was removed to a level 6mm apical to the cemento-enamel junction and the exposed root surface was scaled and planned. Prior to suturing, in each dog, a mechanic barrier (Millipore filter) was placed over the denuded root surface. Between the barrier and the root, a space was allowed by means of a strip of steel matrix band bent and adjusted to the root surface. The other premolar was treated in the same way, but the matrix and the barrier weren't applied. The histological analysis after 3 months revealed a limited new connective tissue attachment formation in both groups, a consistently bone loss and recession in the test group, and a similar lenght of dento-gingival epithelium for both groups. There was statistically significant difference in the histological parameters, except for the lenght of the dento-gingival epithelium. The results of the present study suggest that the mode of mechanic barrier application used to achieve a supracrestal space is critical to its proper performance in the guided tissue regeneration-procedure.

UNITERMS

Guided tissue regeneration; Periodontal therapy.

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INTRODUCTION

The search for the regeneration of the periodontal tissue lost as consequence of the periodontal disease gained in recent years a strong impulse with the development of a treatment modality called guided tissue regeneration (GTR)[6,13,14]. This treatment prevents the dento-gingival epithelium and gingival connective tissue from reaching the curetted root surface during healing. At the same time, a space is created into which the periodontal ligament cells can migrate coronally on the root surface and form a new attachment. Several reports in animals and humans indicated that such treatment results in a reasonably predictable new connective tissue attachment formation[1,14]. However, despite of the good results achieved by the above cited studies regarding to the new connective tissue attachment formation, the same doesn't occur in respect to the bone regrowth. The majority of the studies didn't show any relationship between the amount of new connective tissue attachment and bone regrowth [1,3,8,11].

Recently, NOJIMA et al. (1990) [12] investigated whether periodontal ligament cells can differentiate into osteoblasts or cementoblasts in freshly isolated periodontal tissues and in cultured cells derived from periodontal ligament. This study demonstrated that periodontal ligament cells have phenotypes typical of osteoblasts, indicating that they can differentiate into osteoblasts. These findings support the hypothesis that the coronal new connective tissue attachment achieved after the GTR-procedure can be accomplished with the same amount by bone regrowth. But, it does't occur.

Many reports have indicated that the amount of bone regrowth is related to the defect type, in such way that more the walls have a defect, more the bone regrowth occurs[2,7,8,14]. One of the reasons which tries to explain this fact is that the source of bone cells increases with the number of walls of the defect. Another reason for that may be the amount of space achieved between the mechanic barrier of the bone wall and the root surface. This space seems to create a better environment to the bone regeneration. Actually, one of the major morphological determinants of the bone structure is its finite thickness below which bone will not survive and will be resorbed. So, it suggests that in non-vertical defects there aren't enough supracrestal space for a proper bone regeneration.

The purpose of the present study was to evaluate the effect of a supracrestal space achieved by a mechanic barrier maintained far from the root surface in the GTR-procedure on the periodontal tissue regeneration over teeth with dehiscence-type defect.

MATERIAL AND METHODS

Two maxillary or mandibular fourth premolar (each tooth providing 2 roots) in 3 female mongrel dogs were used in the study. During the operative procedures the animals were sedated with intramuscular injection of Ketalar* (10mg/kg body weight). Following an intramuscular incision, a mucoperiosteal flap was raised over the experimental and adjacent teeth. Using a bone chisel, the buccal and proximal alveolar bone between the mesio-buccal and disto-buccal line angles of each individual experimental root was removed to a level 6mm apical to the cemento-enamel junction. The exposed root surface was carefully scaled and planed to remove all cementum (Figure 1A).

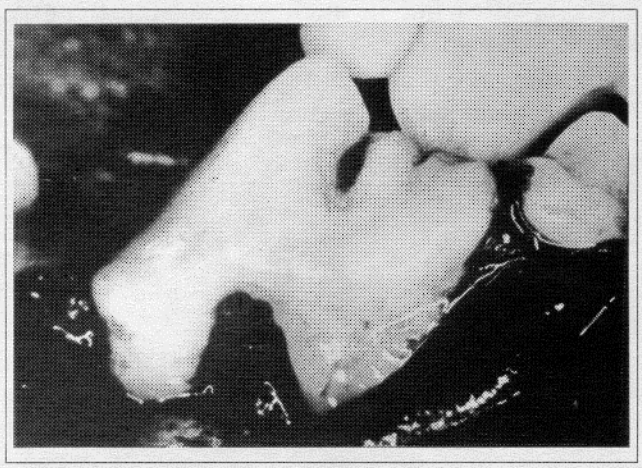

FIGURE 1A- Root surface after bone defect surgical creation and scaling and root planning.

A notch, serving as a landmark for measurements to be made in histological sections, was prepared in the buccal root surface at the level of the surgically reduced bone crest. In one of the 2 teeth in each dog, a mechanic barrier, Millipore filter (Millipore S.A. Molsheim, França), was placed over the denuded root surface to a level 3 to 4mm apical to the alveolar crest and 1 to 2mm coronal to the cemento-enamel junction. Between the barrier and the root surface, a space was allowed by a bent strip of steel matrix band (S.S. White, Rio de Janeiro, RJ). The matrix band was positioned onto the interradicular bone, extending from the

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cemento-enamel junction to a level 2mm apical to the bone crest and was fixed by means of a metal wire sling suture (Figure 1B).

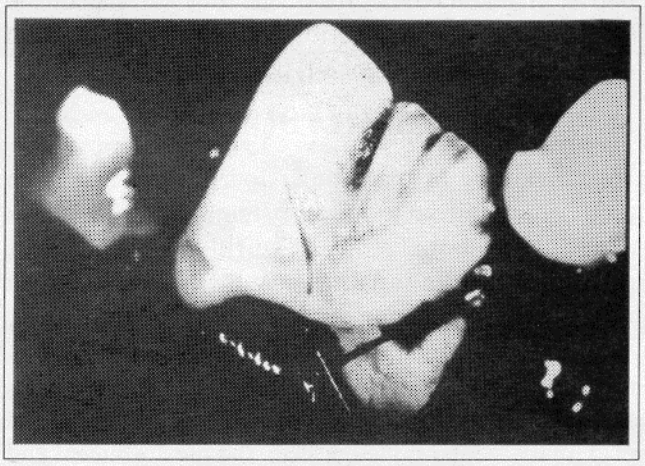

FIGURE 1B - Matrix bend adapted to the root surface.

The concave side of the matrix band faced the interradicular bone. Thus, the Millipore filter was adjusted over the matrix band as a tent over the root surface, allowing in such way a significantly supracrestal space. The flaps were sutured by resorbable sutures and positioned 1mm apical to the Millipore filter margin (Figure 1C).

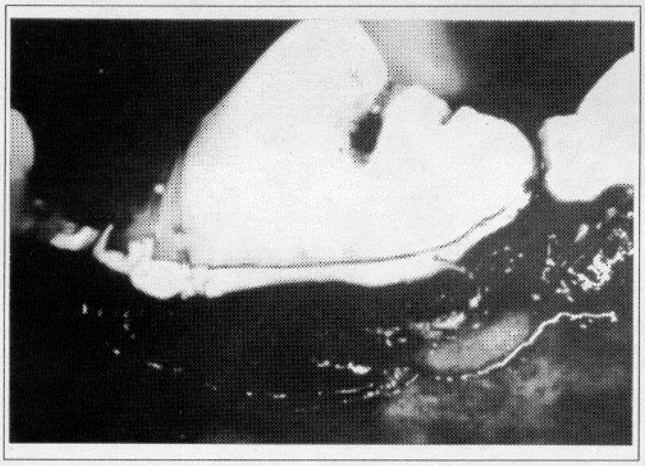

FIGURA 1C - Millipore filter in position and the sutured flap.

The other premolar (control teeth) was treated in the same way, except that the matrix and filter weren't applied. After 3 months of healing, the animals were sacrified with an overdose of Nembuntal** and the jaws were removed. The specimens containing test and control teeth with their periodontal tissue were dissected free and placed in 10% buffered formalin. The specimens were decalcified in electrolytic solution of tricloroacetic acid 0,3M, dehydrated and embedded in parafin. Buccal-lingual serial sections of each root were prepared with the microtome set at 8 micrometers.The sections were stained with hematoxylin and eosin. From each tooth, 5 sections (80 apart) representing the mid portion of the roots were used for histological assessments.

The following linear distances were measured in each 5 histological sections: (a) the apical border of the notch in the root surface to the apical termination of the dento-gingival epithelium (i.e. the amount of new attachment formation); (b) the apical border of the notch to the alveolar bone crest (i.e. the amount of bone regrowth); (c) the apical portion of the dento-gingival epithelium to the gingival margin (i.e. the length of the dento-gingival epithelium) and the amount of gingival recession which was calculated by subtracting 6mm from the sum of b + c.

The measurements were given in milimeters(mm), with plus or minus indicating, respectively, a coronal or apical position in relation to the apical border of the notch. The results were statistically analysed using the Student t-test for paired observations.

RESULTS

CLINICAL OBSERVATION

Recession of the gengival flap margins occurred on the buccal aspects of both groups, during the first week of healing; being particularly pronounced in the test groups. At this time, some test specimens exposed almost completely the Millipore filter. At the end of the second week, all specimens had exfoliated the Millipore filter and the strip of matrix band. In the following week of healing, no other clinical changes occurred in the experimental group. The control group healed uneventfully, although the recession of gingival margin was observed during the initial period of healing.

HISTOMETRIC OBSERVATIONS

Due to failure in either histological preparation, sample collection or in surgical procedure, some histological measurements couldn't be performed in control groups. Thus, this left a total of 6 roots in the test group and 6 in the control, but in the later some measurements had to be excluded. The results are presented in Table1.

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TABLE I - Histological Parameters Measured in mm.

	N	MEAN	RANGE	t
A Test	30	-1,58	(-2,10-0,94)*	16,78
Control	20	0,21	(-0,31-0,87)	
B Test	30	0,33	(-0,40-1,92)*	6,7
Control	25	1,37	(0,24-2,06)	
C Test	30	1,31	(0,52-2,62)	1,26
Control	30	1,51	(0,45-2,69)	
D Test	30	4,36	(2,21-5,81)*	4,88
Control	25	3,40	(2,01-4,22)	

* $p < 0,0001$
(A) amount of bone regrowth; (B) amount of new connective tissue attachment formation; (C) length of dento-gingival epithelium; (D) amount of gingival recession.

In this table N represents the total number of histological sections analysed. New connective tissue attachment had occured in 66% of the test groups. The average gain was 0.33mm, ranging from -0.40 to 0.92mm. The coronal portion of the attachment formation stopped at the apical portion at the dento-gingival epithelium in all test roots (Figure 2).

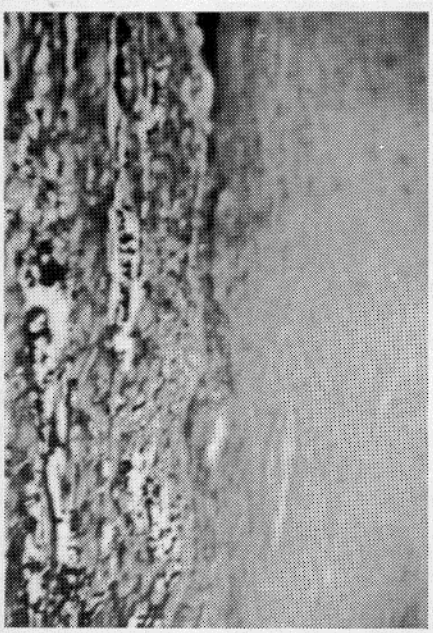

FIGURE 2 - Experimental root surface treated with Millipore filter and steel strip of matrix band. A thick layer of cementum is present on the root surface immediately apically to the dento-gingival epithelium (DE). (H & E 200X).

Small and sparse areas of root resorption were observed. The dento-gingival lenght was 1.31mm in average, ranging from 0.52 to 2.63mm. The gingival margin was loçated in average at a level 4.35mm apical to the cemento-enamel junction. Bone loss had occured in all test roots and was in average -1.58mm (Figure 3).

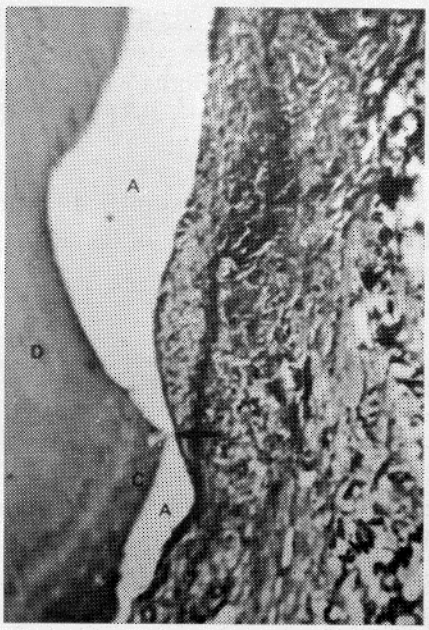

FIGURE 3 - Experimental root surface treated with Millipore filter and steel strip of matrix band. There is not bone beneath the apical portion of the notch (arrow), indicating bone resorption. (A) artefact; (C) cementum; (D) dentin, (H & E 312,5X).S

There was no correlation between the amount of gain or loss of new connective tissue attachment and the amount of loss of bone support. New connective tissue attachment had occured in all control roots 1,37mm in average, ranging from 0.24 to 2.06mm (Figure 4).

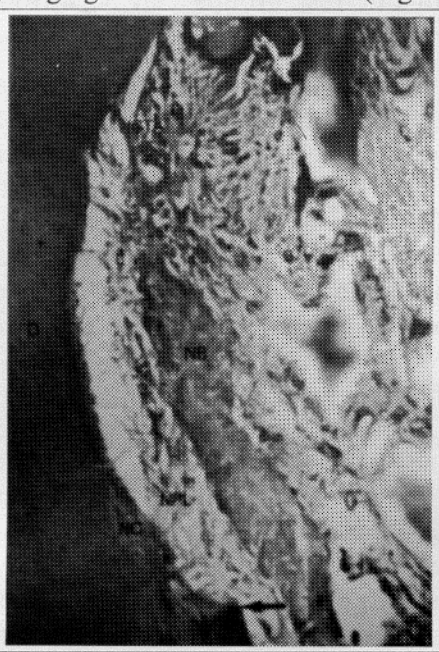

FIGURE 4 - Control root surface. Limited periodontal regeneration at the apical portion of the root surface (NTCA). New connective tissue attachment; (NB) new bone; (NPL) new periodontal ligament; (NC) new cementum; (D) dentin (H & E 312,5X).

The length of dento-gingival epithelium was in average 1.51mm (0.45 to 2.69mm). Some small and scattered resorption areas were observed on the radicular notch. The gingival marginal recession was in average 3.40mm (2.01 to 4.22mm). Both loss and gain of bone height with in average 0.21mm (-0.31 to 0.37mm) were identified.

There was a statistically significant difference between the two groups except for the dento-gingival epithelium measurements.

DISCUSSION AND CONCLUSIONS

The present study failed to demonstrate any benefitial effect of a supracrestal space achieved by using a mechanic barrier maintained far from the root surface in the GTR-procedure over dehiscence-type defects. The amount of new connective tissue attachment formation was limited to the apical portion of the root surface in the test group, some times occuring a loss of attachment. The results are inconsistent with other studies that had reported favorable results with GTR-procedure in animals and humans[1,3,9,11]. These studies have demonstrated an amount of new connective attachment formation ranging from 1.3 to 4.2mm, which are significantly superior to the present results. In the other hand, the control group showed similar results to other studies, indicating that poor results in our test groups can be atributted to experimental procedures.

In all test root surfaces a bone loss occured (X=-1.58). Differently from some studies that observed an average bone regrowth of 1.0mm [1,3,8,11]. However, other investigations also revealed bone loss, like those of CAFFESSE et al. (1988)[3] that frequently observed it in their study (-0.87mm). These authors suggested that bone loss was due to trauma resulting from the surgical procedure, since bone loss was also observed in the control root. Although this explanation can not be excluded, probably there are other factors that might have influenced our results. With resorbable membrane in GTR-procedure, WARRER et al. (1992)[19] observed a minimal bone regrowth and frequently a bone loss in the test groups, suggesting that it may be a consequence of the inability of the membrane used for the GTR-procedure. In the present study Millipore filter and a strip of steel matrix band were used. There are no indication in the literature that the Millipore filter may be incontestably inferior to other non-resorbable membranes[5,6]. The possible influence of the matrix band in healing is difficult to determine and not so evident as were not connective tissue attachment or bone height loss in the interradicular bone where it was positioned.

WARRER; KARRING (1992)[18] also studied periodontal regeneration after the creation of a supracrestal space, but a bone graft was applied to maintain the supracrestal space between the membrane and the root surface. Their results were similar to the present study, the majority of root surfaces revealing bone loss. WARRER; KARRING (1992)[18], suggested that poor results were due to flap displacement and re-opening of the wound, allowing bacterial contamination and food impaction. This suggestion may explain in some extent the results of our investigation.

In the present study, the barrier and the matrix band were exfoliated prematurely at the second week of healing. This also may have contributed to the poor results, but in the study of AUKHIL et al. (1983)[1], although it had occured premature exfoliation of the membranes, it did not occur bone loss and it was even observed a consistently new attachment formation and some bone regrowth. Another reason for bone loss in the present study may be the mode of application of the mechanic barrier that could have impaired the proper vascularization of the bone margin.

The lenght of the dento-gingival epithelium in both groups was relatively short and there was no statistically significant difference between the groups. This lenght was similar to the other studies with GTR-procedure. However, it isn't possible to affirm that the mechanic barrier in the present study was effective in preventing the apical migration of the dento-gingival epithelium due to the significant gingival margin recession in both groups.

The gingival margin recession contributed directly to the poor results of the present study. The recession prevented significantly the amount of root surface available to the periodontal regeneration. This was greater in the present study than in the previous ones [2,8,10]. Although some studies suggested that the gingival recession after GTR-procedure isn't frequent or is even inexistent[8,15], other studies demonstrate that it occurs[2,10,17] and may be related to the defect morphology and to the amount of keratinized gingival tissue[8,17]. In the study of GOTTLOW et. al. (1990)[8], similar defects to the present study were produced and treated with GTR-procedure, but without gingival recession. The authors suggested that these defects

surgically produced and limited to the buccal aspect of roots, while the height of approximal bone was maintained, provide a proper support for flap adaptation preventing the gingival recession. Thus, the defect morphology doesn't seem to have any influence in the results of the present study. The main reason for the gingival recession in the test root in our study seems to be the mode of application of mechanic barrier, which could have interfered significantly in the blood supply to the gingival tissue and, probably, to the marginal bone. This interference may be due to the lack of contact between the buccal flap and the lingual tissue in interproximal area and to the supracrestal space that may have impaired the proper flap revascularization. Also, it allowed bacteria and food to penetrate the wound, disturbing the healing.

The result of the present study should be analysed with caution. The supracrestal space may not demonstrate any benefitial effects for other reasons than itself. The poor results may be due to the type of membrane used, mode of barrier application and blood supply problems. The mode of barrier application in our study suggests to be inadequate to the GTR-procedure. Thus, it is necessary to modify the mode of mechanic barrier application to achieve a supracrestal space to one that can be more appropriate to GTR-procedure.

RESUMO

O objetivo do presente trabalho foi avaliar a utilização de barreiras mecânicas mantidas afastadas da superfície radicular durante o procedimento de regeneração tecidual guiada e seu efeito na regeneração dos tecidos periodontais de suporte em defeitos ósseos em forma de deiscência de 3 cães. Neste trabalho foram utilizados dois 4^{o} pré-molares em cada cão. Após o rebatimento de um retalho muco-periósteo, o osso vestibular dos 4^{o} pré-molares estudados foram removidos até um nível 6mm apical à junção cemento-esmalte e a superfície radicular exposta foi raspada e alisada cuidadosamente para remoção de ligamento periodontal e cemento radicular. Antes da sutura, em um dos dentes de cada cão, uma barreira mecânica (filtro Millipore) foi adaptada sobre o defeito ósseo estendendo-se de um nível 3 a 4mm apical à crista óssea remanescente até 1 a 2mm além da junção cemento-esmalte. Foi garantido um espaço entre a barreira mecânica e a raiz através de uma tira de matriz de amálgama curva e ajustada sobre a superfície radicular. O outro pré-molar serviu como controle, recebendo o mesmo tratamento, porém sem a utilização da barreira e da tira de matriz. Nas duas primeiras semanas de cicatrização houve grande recessão de retalho, exposição e esfoliação da barreira mecânica e da tira de matriz no grupo experimental. Após três meses de cicatrização, as arcadas dentárias foram removidas e cortes histológicos das raízes estudadas foram realizados. A análise histológica mostrou uma formação limitada de nova inserção conjuntiva nos 2 grupos, porém esta foi maior no grupo de controle. As raízes experimentais apresentaram perda óssea em todas as espécimes e o crescimento ósseo no grupo controle foi reduzido. Não houve diferença estatisticamente significante quanto ao comprimento epitélio dento-gengival entre os dois grupos. Ocorreu recessão gengival nos 2 grupos, porém essa foi significantemente maior no grupo teste. Os resultados deste trabalho permitiram concluir que o procedimento de regeneração tecidual guiada é sensível ao modo de aplicação da barreira mecânica para obtenção de espaço acima da crista óssea.

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Regeneração tecidual guiada; Terapia periodontal

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