Differences in collagen distribution of healthy and regenerated periodontium. Histomorphometric study in dogs

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Summary. Previous studies have shown that there is a relationship between periodontal disease and the distribution of collagen fibers. This study evaluated the distribution of collagen types I and III in regenerated bone and periodontal ligament, comparing them to the tissues near the regenerated area and to the healthy periodontium. In the third (P3) and fourth (P4) mandibular premolars of 5 healthy mongrel dogs, bilaterally, buccal class 2 furcation lesions were surgically created and chronified for 3 weeks. After that, full flaps were elevated and expanded polytetrafluoroethylene (e-PTFE) membranes were adapted, sutured and recovered by the flaps. Two weeks after surgery, two membranes on the same side were removed and the other membranes were removed four weeks after surgery. The dogs were euthanized at 12 weeks following placement of the e-PTFE membranes. P3 and P4 teeth as well as the second premolars (healthy control teeth) and their periodontal tissues were removed and histologically processed for Collagen Quantification (COLQ). The amount of type III collagen was higher in native bone compared to the regenerated area. For periodontal ligament, COLQ for type I collagen showed statistically significant differences (Tukey’s Multiple Comparison, p<0.05) between the regenerated groups and the control group. These differences were not found for type III COLQ. There are significant differences in collagen distribution among the regenerated, native and control tissues. Membrane removal 2 or 4 weeks postoperatively did not influence the collagen composition.

Key words: Periodontal regeneration, Collagen, Furcation defects, Histomorphometry

Introduction

Collagen is the main organic structural component of periodontal tissues (bone, cement and periodontal ligament), and the most abundant protein in the human body, making up about 30% of the total protein content. The cement and bone organic matrix is mainly composed by collagen (Nanci and Bosshardt, 2006), and the periodontal ligament is also constituted mainly by collagen. Therefore, the analysis of regenerated periodontal tissue collagen composition would be important to understand the periodontal regeneration process.

Collagen fibrils have a parallel organization into fibers and are birefringent to polarized light, due to the structure of the protein (Minor, 1980). Furthermore, collagen molecules are rich in basic groups that react with acid stains, like Sirius Red, which is a very acid, dark red stain, with a long molecule (Sweat et al., 1964). The birefringence of collagen fibers can be better visualized when they are analyzed by optical microscopy with polarized light (Constantine and Mowry, 1968).
Junqueira et al. (1979) described the Picrosirius Red (PSR) method, that is, Sirius Red + Picric Acid, in which the collagen molecules are even more evident. Furthermore, with PSR staining we can also differentiate collagen fiber density or size by means of a spectrum of color changes under polarized light (Kesler et al., 2000): in polarized light, type I collagen is yellow, orange or red, and type III is green (Junqueira et al., 1978).

Although this methodology was described three decades ago, its use in Dentistry is still restricted, mainly in Periodontology. Dayan et al. (1993) evaluated, in polarization microscopy, the picrosirius red-stained collagen from oxodipine-induced hyperplastic gingival of beagle dogs. The predominant collagen color in the male dogs treated with the drug was green-yellow, and in the control dogs (without treatment) or females treated with Oxidipine the main color was red-orange. According to the authors, these differences could indicate a gender variation in the genesis of gingival hyperplasia in dogs treated with calcium channel blockers.

Kamata and Kamoi (1989) analyzed the qualitative changes in gingival collagen fibers for experimental periodontitis in rats, evaluating the remodeling of type I and III collagens detected by the picrosirius-polarization method. The results showed, in areas with collagen degradation caused by periodontal disease, an increase in type III collagen proportion and a decrease in type I collagen. Some studies have used Sirius Red staining and polarized light microscopy to evaluate, in humans, tissue destruction caused by periodontal disease (Séguier et al., 2000, 2001; Ejeil et al., 2003a,b). The gingival biopsy analyses showed, both in gingivitis and periodontitis groups, a smaller percent area with collagen fibers than that observed in the healthy group, and this difference was statistically significant. Séguier et al. (2000) suggested that a quantitative evaluation of the area fraction occupied by gingival collagen fibers may reflect the clinical severity of the periodontal disease. Besides, Ejeil et al. (2003b), found a relationship between collagen destruction and the Interleukin-1, and Interleukin-4 cytokine presence in the gingival tissue.

The PSR methodology was also described for the analysis of tissue repair after periodontal treatment. Kesler et al. (2000) evaluated the differences in gingival fibers after periodontal plastic surgery using ER:YAG laser. In 10 patients, 6 treated for gingival hyperplasia and 4 for gingival melanotic pigmentations, the analysis of biopsies before and after surgery showed: pre-operatively, the predominance of the red-orange range color; in the first week postoperatively, the predominant color was in the green-yellow range; finally, 14 days after surgery, the collagen fibers acquired again the same color observed in the pre-operative period. The authors suggested that the PSR staining analysis of the gingival collagen fibers could be useful for the evaluation of gingival health.

In this way, previous studies have shown that there is a relationship between periodontal disease and the distribution of collagen fibers, which could be studied by the Picrosirius Red staining analysis. However, to our knowledge, the method has not yet been applied to regenerated periodontal tissues, which could contribute to the understanding of the periodontal regeneration dynamic process.

**Materials and methods**

**Surgical procedures**

The research protocol was approved by the Institutions Ethical Committee for Animal Research (protocol number 04.1.316.53.4). Five young adult mongrel dogs, weighing approximately 15 kg, were selected for the study. The animals had intact crowns, without occlusal alterations, did not have buccal lesions or any fungal infections, and were in good general health.

The animals were not fed the night before surgery and were anesthetized with sodium thiopental (30 mg/kg, 500 mg of thiopental diluted in 20 ml of NaCl, resulting in a 25% solution). To simulate normal clinical routine, the systemic anesthesia was complemented with infiltration anesthetic.

Both supra- and subgingival scaling with Gracey curets (Hu-Friedy MFG. Co. Inc., Chicago, IL, USA) and ultrasonic instruments (Cavitron 3000, Dentisply Mfg. Co., York, PA, USA) were performed on the mandibular third (P3) and fourth (P4) premolars of both sides. One week later, full flaps were elevated from the distal side of the second premolar up to the mesial side of the mandibular first molar. All remaining soft tissues that adhered to the alveolar bone and to the root surface were removed. With a round bur, Class II furcation lesions, 5 mm in height and 2 mm in depth, were surgically created on the buccal surface of these premolars (Fig. 1). The bilateral osseous defects were filled with a rubber base impression material, in order to induce inflammation and to prevent spontaneous repair. After repositioning of the periodontal flaps, the wound was closed with absorbable sutures. Animals were fed only with water-softened dog food to prevent rupture of the suture. The sutures were removed 10 days postoperatively, and the impression material was left for 21 days. Following this period, the impression material was removed with curettes and the teeth were thoroughly scaled and root planed. In addition, plaque control was maintained by daily topical application of 0.12% chlorhexidine.

Two weeks after the removal of the impression material, full flaps were elevated and the remaining soft tissue that adhered to the alveolar bone and the root surface of the premolars were removed. Reference notches were made in the root at the base of the defect as a guideline for histological analysis, using a spherical 33½ diamond bur with abundant irrigation with saline solution. The e-PTFE membranes (Gore-Tex Periodontal Material, Flagstaff, AZ, USA) were sutured with e-PTFE sutures around the teeth, covering the buccal furcations,
and the flaps were sutured with the same sutures, completely covering the membranes (Fig. 2). From the evening prior to the surgery and every 4 days until the removal of all membranes, intramuscular injections with 20,000 IU penicillin and erythromycin (0.1 g/10 kg weight) were administered. In the days following surgery, the dogs were placed on a soft ration diet.

The two membranes at the same side were removed after 2 weeks whereas the two contralateral ones were only removed after 4 weeks, by careful elevation of the flap to gain access to the membranes, and resuturing afterwards. The designation of which side would have the membranes removed was randomly determined by a coin toss. The sutures were removed after 15 days. Plaque control was maintained by daily topical application of a 0.12% chlorhexidine solution and weekly by careful prophylaxis with ultrasonic points until the time of euthanasia. The mandibular second premolars of both sides were designated as healthy controls, and did not receive any surgical procedure. The latter teeth were submitted to the same plaque control procedures as those applied to P3 and P4.

Sample processing and histology

Euthanasia was performed by a thiopental overdose 12 weeks after membrane placement (Fig. 3). The mandibles were dissected, placed in 10% formalin, and decalcified for 3 months in multiple baths of 10% trichloroacetic acid. After decalcification, the blocks were immersed in paraffin, and semi-serial 7 µm wide histologic sections were made in a mesio-distal direction. Three non-serial histologic sections, located in the central portion of the furcation, were selected for each tooth (P2, P3 and P4). The paraffin was then removed from the selected sections using Xylene (Merck & Co. Inc., New Jersey, USA) and the sections were hydrated in a decreasing series of ethanol (5 minutes each: 100% - twice, 95%, 90%, 80%, 70%, 50%). Then, the slides were rinsed in distilled water and stained according to the following sequence: immersion for 20 minutes in a 0.1% Picrosirius Red solution - Direct Red 80 (Sigma Aldrich, St. Louis, USA) diluted in saturated picric acid solution; washing in running tap water for 3 minutes; washing in distilled water for 1 minute, twice; counterstaining in Harris Hematoxylin for 1 minute; washing in distilled water (1 minute), twice. After staining, the sections were dehydrated in an increasing series of ethanol (5 minutes for each ethanol concentration): 50%, 70%, 80%, 90%, 95%, 100%. Finally, the sections were cleared with Xylene (5 minutes, twice) and mounted with Permount (Sigma Aldrich, St. Louis, USA).

Collagen quantification

For detection and quantification of types I and III collagen, the slides stained by PSR were analyzed and photographed with a light microscope (Olympus BX50, Olympus Corporation, Tokyo, Japan) using polarized light, by an experienced single examiner. The Image ProPlus 4.5 software (Media Cybernetics, Bethesda, USA) was used for the collagen quantification of the captured images. Bone and periodontal ligament were analyzed. For each tissue, five regions, representing the experimental groups of this study, were determined. For the bone tissue, the experimental regions/groups were: 1) Native bone 2 weeks - bone tissue located apically to the root notch, in a tooth in which the membrane remained in position for 2 weeks. It is the bone in the furcation region apically to the regenerate area; 2) Regenerated bone 2 weeks - bone tissue located coronally to the root notch, in a tooth in which the membrane remained in position for 2 weeks; 3) Native bone 4 weeks - bone tissue located apically to the root notch, in a tooth in which the membrane remained in position for 4 weeks. It is the bone of the furcation region apically to the regenerate area; 4) Regenerated bone 4 weeks - bone tissue located coronally to the root notch, in a tooth in which the membrane remained in position for 4 weeks; 5) Control bone - bone tissue of the P2 tooth, furcation region.

The periodontal ligament groups/regions were: 1) Native ligament 2 weeks - periodontal ligament located apically to the root notch, in a tooth in which the membrane remained in position for 2 weeks. It is the periodontal ligament of the furcation region apically to the regenerate area; 2) Regenerated ligament 2 weeks - periodontal ligament tissue located coronally to the root notch, in a tooth in which the membrane remained in position for 2 weeks; 3) Native ligament 4 weeks - periodontal ligament located apically to the root notch, in a tooth in which the membrane remained in position for 4 weeks. It is the periodontal ligament of the furcation region apically to the regenerate area; 4) Regenerated ligament 4 weeks - periodontal ligament tissue located coronally to the root notch, in a tooth in which the membrane remained in position for 4 weeks; 5) Control ligament - periodontal ligament of the P2 tooth, furcation region. Figures 4 and 5 (Mallory tricromic staining) shows a representation of the grids in the regions where the collagen quantification was done.

Three slides per tooth and two fields per slide were evaluated. As cited before, in polarized light, type I collagen has a red-orange-yellow color, and type III collagen has a green color. So, for each one of the bone and periodontal ligament groups, types I and III collagen were quantified. For this, grids were used: the quantification values were expressed in percentages, as the ratio between the collagen area and the total area of the grid. In each slide, two grids were measured per group, for bone and periodontal ligament, and the average value was calculated.

Statistical analysis

Statistical analysis of the data was comparative and non-parametric. The experimental unity was the dog (n=5). For both tissues evaluated (bone and periodontal ligament), in each one of the parameters analyzed (type I
Collagen percentage and type III collagen percentage) the five experimental groups (native 2 weeks, regenerated 2 weeks, native 4 weeks, regenerated 4 weeks and control) were compared. Analysis of variance (ANOVA) was used to evaluate the existence of statistically significant differences among groups (p>0.05). If this difference existed, the groups were tested again, in pairs, using the Tukey test, to determine which groups were statistically different one from the other (p<0.05).

Results

Clinical findings

All surgical procedures were well tolerated by the animals and no postoperative complications were observed. However, one animal from each group exhibited gingival recession with exposure of the e-PTFE collar of the membrane. In these dogs, two teeth were excluded due to purulent exudates.

Collagen Quantification

Figure 6A-E shows fields of bone tissue from the 5 experimental groups stained with PSR, under polarized light. Collagen Types I and III percentage averages, standard deviations and medians for bone tissue are summarized in Table 1. Figure 7 (A to E) shows fields of periodontal ligament from the 5 experimental groups stained with PSR, under polarized light. Collagen Types I and III percentage averages, standard deviations and medians for periodontal ligament are summarized in Table 2.

The Analysis of Variance test was applied to evaluate if there were statistically significant differences among groups (p<0.05). The ANOVA results showed statistically significant differences, in bone, for Types I

<table>
<thead>
<tr>
<th>Table 1. Collagen Types I and III percentage averages, standard deviations and medians for bone tissue in the five experimental groups.</th>
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<tbody>
<tr>
<td><strong>Type I Collagen</strong></td>
</tr>
<tr>
<td>Average ± SD</td>
</tr>
<tr>
<td>NatBone2Week</td>
</tr>
<tr>
<td>RegBone2Week</td>
</tr>
<tr>
<td>NatBone4Week</td>
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<tr>
<td>RegBone4Week</td>
</tr>
<tr>
<td>ControlBone</td>
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NatBone2Week: Group of native bone with 2 weeks of membrane maintenance; RegBone2Week: Group of regenerated bone with 2 weeks of membrane maintenance; NatBone4Week: Group of native bone with 4 weeks of membrane maintenance; RegBone4Week: Group of regenerated bone with 4 weeks of membrane maintenance; ControlBone: Group of healthy bone, area without surgery.
were statistically significant differences between the control group (which expressed the highest values for type I collagen quantification) and all other groups (Table 3). Regarding type III collagen, the comparison between groups showed that the regenerated groups were significantly different from the respective native groups. The regenerated bone, both with 2 or 4 weeks with the membrane, showed a substantially lower quantity of type III collagen. Furthermore, the native groups had higher averages than the control group, although only in the native group with 2 weeks with the membrane was this difference statistically significant (Table 4).

Concerning the periodontal ligament, the Type I collagen quantification showed statistically significant differences between the regenerated groups (both with 2 or 4 weeks of membrane maintenance) and the control group (Table 5).

Qualitatively evaluating the collagen organization/disorganization in bone, the control group was the most

### Table 2. Collagen Types I and III percentage averages, standard deviations and medians for periodontal ligament in the five experimental groups.

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<tr>
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<th>Type I Collagen</th>
<th>Type III Collagen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Average ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>NatLig2Week</td>
<td>22.02±4.72</td>
<td>22.01</td>
</tr>
<tr>
<td>RegLig2Week</td>
<td>19.34±5.62</td>
<td>17.78</td>
</tr>
<tr>
<td>NatLig4Week</td>
<td>23.40±6.08</td>
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<tr>
<td>RegLig4Week</td>
<td>15.98±2.51</td>
<td>16.85</td>
</tr>
<tr>
<td>ControlLigament</td>
<td>32.11±9.78</td>
<td>34.49</td>
</tr>
</tbody>
</table>

Nat Lig2 Week: Group of native periodontal ligament with 2 weeks of membrane maintenance; Reg Lig2 Week: Group of regenerated periodontal ligament with 2 weeks of membrane maintenance; Nat Lig4 Week: Group of native periodontal ligament with 4 weeks of membrane maintenance; Reg Lig4 Week: Group of regenerated periodontal ligament with 4 weeks of membrane maintenance; ControlLigament: Group of healthy periodontal ligament, area without surgery.
Fig. 6. Bone tissue with PSR staining and polarized microscopy, showing types I and III collagen in the five experimental groups. 
A. Native bone, two weeks of membrane maintenance. B. Regenerated bone, two weeks of membrane maintenance. C. Native bone, four weeks of membrane maintenance. D. Regenerated bone, four weeks of membrane maintenance. E. Control bone. m: marrow bone; b: woven bone. x 40
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Fig. 7. Periodontal ligament with PSR staining and polarized microscopy, showing types I and III collagen in the five experimental groups. A. Native periodontal ligament, two weeks of membrane maintenance. B. Regenerated periodontal ligament, two weeks of membrane maintenance. C. Native periodontal ligament, four weeks of membrane maintenance. D. Regenerated periodontal ligament, four weeks of membrane maintenance. E. Control periodontal ligament. b: woven bone; pl: periodontal ligament; r: root. x 40
organized, showing concentric bundles of collagen fibers corresponding to the osteon bone structures (Fig. 6E), and the least organized was the regenerated group in which the membrane was removed after 2 weeks (Fig. 6B). The native groups (membrane removal after 2 and 4 weeks) showed an organized structure (Fig 6A,C), and the regenerated group in which the membrane was removed after 4 weeks (Fig. 6D) presented more organized fibers than the regenerated group where the membrane was removed earlier. In the periodontal ligament from the control group, the collagen was more organized and presented compact bundles of fibers (Figure 7E); in the native groups (Fig. 7A,C), fibers were less compact than in the control group, and in the regenerated groups (Fig. 7B,D) the fibers were sparse and more diffused.

Discussion

In previous studies, e-PTFE membranes have demonstrated a satisfactory clinical performance, being capable of inhibiting the down growth of epithelium, to promote the isolation of the gingival connective tissue and to protect the blood clot (Schallhorn and McClain, 1988; Machtei et al., 1993; Cafruny et al., 1988; Becker et al., 1988; Scantlebury, 1993; Macedo et al., 2006). Non-absorbable membranes are usually removed after 4 to 6 weeks, and longer periods did not present additional benefits (Cafruny et al., 1988). A previous study in dogs has shown that the maintenance of e-PTFE membranes for only 2 weeks was capable of promoting periodontal regeneration in mandibular class II furcation lesions (Macedo et al., 2006).

Collagen is the main organic structural component of periodontal tissues. Collagen types I (predominantly) and III are found in periodontal tissues. In this way, analysis of regenerated and healthy periodontal tissue collagen composition would be important to understand the periodontal regeneration process. The aim of this study was to evaluate, in dogs, the type I and III collagen composition of regenerated bone and periodontal ligament, with two periods of membrane maintenance (2 and 4 weeks), comparing it to the periodontal tissues of healthy teeth.

The five evaluation areas of this study representing the five experimental groups, both for the analysis of bone and periodontal ligament, were: Native area-2 weeks; Regenerated area-2 weeks; Native area-4 weeks; regenerated area-4 weeks; and Control area. The regenerated areas represent the areas where the periodontal disease was induced, and in which new tissues formed as a result of the regenerative therapy. The native areas represent the tissues in the furcation region apically to the tissues destroyed by the induced periodontal disease. The control areas represent the healthy tissues of the control tooth (P2) that did not receive any surgical procedures. The aim of this experimental model was to allow the following comparisons: a) comparison between the regenerated area with 4 weeks of membrane maintenance and the regenerated area with 2 weeks of membrane maintenance, analyzing the influence of the early removal of the membrane in the collagen composition of the regenerated bone and periodontal ligament; b) comparison between the native area with 4 weeks of membrane maintenance and the native area with 2 weeks of membrane maintenance, analyzing the influence of the early removal of the membrane in the collagen composition of the areas apically to the defect; c) comparison between the regenerated areas (with 2 or 4 weeks of membrane maintenance) and the respective native area, analyzing if there were differences in the collagen composition between the regenerated tissues and the tissues apically to the regenerated area; d) comparison between the regenerated area (with 2 or 4 weeks of membrane maintenance) and the control area, analyzing the similarity in the collagen composition of the regenerated tissues compared to healthy tissues; and e) comparison between the native area (with 2 or 4 weeks of membrane maintenance) and the control area, analyzing whether regeneration affects the collagen composition of the tissues apically to the regenerated area.

Concerning bone tissue, the ANOVA test for types I and III collagen quantifications showed statistically significant differences among groups. Tables 3 and 4 show, respectively, the comparisons between groups, in pairs, for types I and III collagen. For type I collagen,
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There were statistically significant differences between the control group (which showed the highest values) and all the other groups. Li et al. (1997), have quantified, by immunohistochemistry, the values of types I and III collagen in human bone, and have found for type I collagen an average of 30.5%, which is similar to the average found in this study (28.76%) for the control group. Ivanovski et al. (2000), quantified, by immunohistochemistry, collagen type I in regenerated bone after GTR in semi-circumferential defects around mandibular bicuspids of four dogs, which were sacrificed 4 weeks after the placement of the e-PTFE membranes. The results showed a higher amount of type I collagen when compared to type III collagen for all groups (Table 1). There were no differences between the collagen composition of the regenerated bone and the pre-existent alveolar bone adjacent to the regenerated area (which could be compared to the native bone in the present study) - these results are in agreement with our study.

Until a few years ago, it was a believed that type III collagen was present only in embryonal bone (Lukinmaa and Waltimo, 1992). Keene et al. (1991), showed, in a study in humans, that the remodeling bone also has type III collagen. Furthermore, the presence of type III collagen is not dependent on age, because it was found in a 30 week fetus and subjects with 80 years. In the present study, the multiple comparison between groups for type III collagen composition in bone showed that the regenerated groups were significantly different from the respective native groups. The regenerated bone, either with 2 or 4 weeks of membrane maintenance, showed a substantially lower quantification for type III collagen. Besides this, the native groups had higher averages than the control group, although only in the native group with 2 weeks of membrane maintenance were these differences statistically significant. These results also corroborate the findings of Keene et al. (1991), which stated that type III collagen is located in specific fibers, capable of mediating the insertion of tendons, ligaments and peristem around calcified bone. In our study, type III collagen quantification was lower in the regenerated bone than in the control bone (not significantly different), indicating that the attachment apparatus was still in formation and maturation; on the other hand, in native bone, the increased amount of type III collagen, compared to control bone, could indicate a functional adaptation that occurred during the period of time in which this bone had to increase its participation in supporting the teeth - starting with defect creation and during the subsequent regenerative process. Furthermore, Besides the higher biomechanical need, probably there was also an increased metabolic demand, as the neighboring tissues function as the major donor of cells and nutrients to the regenerating tissues.

In the periodontal ligament, collagen quantification showed fewer statistically significant differences than the ones found in bone. Nevertheless, the results also allowed us to formulate some hypotheses regarding the regenerative process in the furcation area. Type I collagen quantification in the periodontal ligament showed (Table 5) statistically significant differences between the regenerated groups (either with 2 or 4 weeks of membrane maintenance) and the control group. On the other hand, for type III collagen, the ANOVA test did not show statistically significant differences among the groups. Webb et al. (1998), evaluated collagen composition in the periodontium of developing rats, using mono and polyclonal immunolabelling, and observed that types I and III collagen were found in the periodontal ligament, and type III predominated where collagen fibres were inserted into the alveolar bone and cementum. Wang et al. (1980), studying type III collagen distribution in sheep periodontal ligament, related a positive association between this type of collagen and the presence of blood vessels. These findings could explain the higher numerical values (although without statistically significant differences) of type III collagen found in the native groups of periodontal ligament - these where the areas that had an increase in functional demand after the defect creation, either physically, supporting the teeth, or as a source of cells for furcation regeneration. On the other hand, Kamata and Kamoi (1989), described the quantitative changes (evaluated by PSR staining and polarized light microscopy) in the collagen fibers of the periodontium of rats, in which periodontal disease had been induced. According to the authors, in areas with collagen destruction there was a relative increase in type III collagen, caused by the reduction of type I collagen - which would be a balance in the quantitative changes between collagen types I and III, involving tissue destruction and formation. This type of balance was also observed in this study, in the Native groups, when compared to the Control groups.

The regeneration process of the tissues lost due to periodontal disease is complex and delicate. It requires the neoformation of bone, periodontal ligament and cementum, and to be successful, a series of requirements must be followed. The surgical procedures and the biomaterials used for periodontal regeneration have been extensively improved in the last two decades, but there are still important biological limitations which compromise the full regeneration of some types of defects. The understanding of the healing process of periodontal tissues could contribute to the development of more precise and adequate mechanisms, techniques and materials.

Although the clinical and histological outcomes of GTR have been extensively documented, the biological events occurring during barrier-mediated periodontal wound healing are still relatively unknown (Ivanovski et al., 2000). The analysis of the collagen composition of periodontal tissues is one of the parameters that should be analyzed in future studies. The collagen composition of healthy, contaminated and regenerated cementum, as well as the evaluation of the amount of different collagen types along the regeneration process are new possibilities for future investigations.

This study has shown that some differences between
regenerated and healthy tissues could be visualized by mean of a specific staining method. Therefore, the main results for collagen quantification in bone and periodontal ligament in this study seem to point to: a) the collagen composition of regenerated bone and periodontal ligament was not affected by the e-PTFE membrane maintenance, 2 or 4 weeks; b) regarding type I collagen, there were significant differences between the regenerated and control groups, possibly indicating that the regenerative process was still not finished 12 weeks after membrane placement, or that the new tissue has a different collagen composition than the normal tissue; c) the native bone was influenced by the higher functional demand generated by the periodontal defect created in the furcation area, and changed its collagen composition. In conclusion, there is an influence of periodontal regeneration process in the distribution of collagen fibers in bone and periodontal ligament, which was shown by the Picrosirius Red staining analysis.

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