Modification of Col Shape and Interproximal Area after Periodontal Surgery Associated with the Restorative Alveolar Interface (RAI) Technique. Histological Study in Dogs

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The modification of the Col shape and position by the restorative alveolar interface technique (RAI) was studied in the interproximal areas between the mandibular first molars and fourth premolars of 10 dogs. Full thickness flaps were raised to expose the interproximal root surface and alveolar bone crest. The RAI procedure was performed only on the experimental sides and the control areas were the opposite side of the same animal. The animals were sacrificed at zero hour, 7, 14, 21 and 28 days for histological analyses. Approximately 6.0-µm-thick sections were made in buccolingual and mesiodistal directions and stained with hematoxylin-eosin and Mallory for light microscopy analysis. A satisfactory healing process was observed up to the 14th and 21st days which showed a modified Col shape. At this time, an inflammatory reaction developed affecting the evolution of the healing. The surgery had probably created conditions for the installation of an inflammatory process resulting from the modified anatomy of the interdental area.

Key Words: RAI, Col, periodontal surgery.

INTRODUCTION

A healthy coexistence between dental restorations and their surrounding periodontal structures must be the objective of conscientious professionals. This ideal inter-relation in the interproximal region is at times difficult to achieve. When unfavorable situations exist, such as contact area between teeth, proximity between roots, configuration of the interproximal gingiva, cleaning difficulties, or location of restoration margins, this healthy coexistence is impossible to be completely achieved. All treatments involving the interproximal region must consider it as a whole and take into account the teeth and the gingival tissue, as well as the inter-relation between them.

The subgingival location of restorations causes alterations in the deeper periodontal tissues due to bacterial plaque retention in the setting area (1-4). Margins of crowns should be placed in the most accessible position for proper hygiene and as far as possible from the gingival sulcus base (5).

Restorative procedures should not be carried out in the presence of periodontal disease nor should they cause irritation to the sub-sulcular anatomic structures (2). The biological junction of the junctional epithelium to the teeth as well as that of the supra-alveolar connective tissue must be preserved (6). When teeth are too close, the interproximal areas present non-keratinous epithelium and are more permeable to bacterial toxins. Whenever a restoration is placed in such an area, all efforts must be made to make it more resistant and healthier. Adequate preparation of the tooth must...
increase the interproximal space, thus permitting the keratinization of the epithelium and creating conditions for the effective control of bacterial plaque (7).

To make such procedures possible and meeting the previously cited requirements, Ross and Garguilo (8) described the RAI (restorative alveolar interface) technique for the modification of the interproximal area, which could create the desired ideal conditions for the restoration of the tooth structure and maintenance of periodontal health.

This technique is usually used for root separation in multiradicular teeth, concavity reduction, correction of excessively close roots and in the idealization of the tooth-alveolus relationship, thus improving the emergence profile for future restoration (3,9,10).

The possible modifications of Col shape and position as well as those of the interproximal tissues are analyzed after RAI surgery.

**MATERIAL AND METHODS**

Ten healthy adult male dogs of undefined breeds, weighing approximately 10 kg, with intact maxillary and mandibular teeth without malocclusion were used.

**Surgical Procedures**

The animals were kept em jejum for 12 h prior to surgery. They were sedated with iv thionembutal (2.5% solution; Abbott, São Paulo, SP) through the cephalic vein. The animals were kept on spontaneous respiration and intubated with an endotracheal tube.

The proximal region between the mandibular first molars and the fourth premolars, whose contact areas were crowded and therefore appropriate for the RAI technique, were used. In the experimental areas (right side), mucoperiosteal flaps were made to expose the interproximal areas and root scaling and planning, osteotomy-osteoplasty and the RAI technique were performed. The osteotomy and osteoplasty procedures were carried out with chisels to reduce the interproximal bone crest by approximately 1.0 mm.

The RAI procedure was performed on each of the experimental sites with long conic diamond high-speed drills with abundant irrigation, as recommended by Ross and Garguilo (8). The movement in the root buccolinguinal proximal direction and parallel to the long axis of the teeth created a straight emergence profile in relation to the bone tissue. Root divergence was eliminated in the cervical-occlusal direction, thus increasing the interproximal embrasure. A sulcus was made with a #½ spherical drill on the interproximal surfaces of the roots to establish a reference for histological analysis. The flap was sutured back to its original position (4.0 silk thread, Ethicon Johnson & Johnson, São José dos Campos, SP). The same surgical procedures were performed in the control areas (left side) of the same animals. The height of the interproximal bone septum was reduced and its shape was modified, thus marking the root. RAI procedure was not carried out.

The animals received 1200 IU penicillin and 500 mg streptomycin (Laboratório Fort Dodge, Campinas, SP) for 8 days. The healing process was evaluated weekly and the sutures were removed after 7 days. The teeth were sprayed 3 times a week with 0.12% chlorhexidine until the day the animals were sacrificed.

**Histological Technique**

The animals were sacrificed at zero hour, 7, 14, 21 and 28 days and separated into two groups. Five of them were used for buccal-lingual sections and the other five for mesiodistal sections. Their mandibles were dissected, immediately fixed and decalcified. The blocks containing the samples were dehydrated, embedded in paraffin and 6.0-µm thick sections were obtained, which provided an integral view of the interproximal areas. The slides were stained with hematoxylin/eosin and Mallory’s trichrome stain and examined with light microscopy.

**Morphometric Technique - Karyometry**

The nuclear measurements of the basal, squamous and granular cells in the epithelium of the interproximal gingival papilla (control), as well as those of the epithelium in healing regions (14th and 21st days), were calculated according to Sala et al. (11). The longest (D) and the shortest axis (d) were measured in the drawing of each nucleus in order to estimate the following nuclear parameters: mean geometric diameter, ratio D/d, volume (V), area (A), ratio V/A, perimeter, shape factor, contour index and eccentricity. Comparison of the results for the experimental and the control groups was made by the nonparametric Mann-Whitney test (12).
RESULTS

Zero Hour - Control and Experimental Groups

The control and experimental areas had similar aspects at zero hour. The surgical procedure that affected periodontal tissues was distinguished only by the larger wearing of the tooth surface on the experimental side, which resulted from the RAI technique. The general aspect of the injury showed two gingival margins, buccal and lingual, with whole oral epithelium and connective tissue. In the incision area, cut fibers and open blood vessels were observed. In the mesial-distal plane there was a clot which thoroughly covered the exposed bone surface and filled the notches and the space between the gingival margins. This is typical of periodontal surgery with a total flap.

Seven Days - Control

Suture removal and handling of the part during histological processing caused the separation of the flap margins. The marginal gingiva epithelium was normal and showed migration in order to cover the exposed connective tissue. It was slightly concave in the interproximal area. There was a large clot in the

Figure 1. A: Seven days – control. Initial healing phase. B: Seven days – experimental. Oral mucosa epithelium migration towards the Col. C: Fourteen days – control. Mesiodistal plane: the normality patterns of the Col tissues were present. D: Fourteen days – experimental. The Col was less accentuated and had simple epithelium.

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inner central area alternating with regions of granulation tissue that covered the bone tissue in its initial healing phase (Figure 1A).

**Seven Days - Experimental**

The interproximal tissues were more organized than those in the control area. The division between the original tissue of the buccal and lingual flaps and the young tissue in the central area was more evident. The typical oral mucosa epithelium was more advanced with differentiated migration towards the central Col in order to keep its lining and superficial protection function (Figure 1B). The Col region did not yet have lining epithelium either in the control or in the experimental area. The connective tissue was exposed, showing new organization (granulation tissue), dilated and congested vessels and many inflammatory cells. The entire region was edematous.

**Fourteen Days - Control**

Healing developed satisfactorily and the epithelium of the Col area was recovered with less extensive stratification and concavity. The connective tissue had a slight inflammatory reaction that is consistent with the interproximal Col under normal conditions. It was integrated to the buccal and lingual flaps, which dem-
onstrates that the transition from granulation tissue to connective tissue was complete. The bone septum medullary spaces were recovered.

The normality patterns were present in the mesiodistal plane. In the papilla central areas, the epithelial tissue was not very thick, uniform, with few circumvolutions, rectilinear in its basal portion and covered young connective tissue that was not very dense and had slightly increased vascularization (Figure 1C).

Fourteen days - experimental

Despite some similarity to the control side, the quality of the epithelial and connective tissues suggests that healing was slightly more advanced. The subepithelial connective tissue was denser and more fibrous. The Col central portion was less accentuated and had simple epithelium. A difference between the quality of the connective tissue and that of the epithelium in the flap area was still observed and identified the young tissues formed during this period. This connective tissue was fibrous in the gingival papillae and organization fibers could already be observed in the healing region. There were inflammatory cells and edematous regions (Figure 1D). The surface of the bone crest was more uniform, with less communication with the medullary spaces. A periosteum and supra-alveolar transepithelial fibers were being formed.

Twenty-one days - control

The central epithelial tissue was thin and continuous. The connective tissue was more fibrous in deeper areas as well as in those close to the bone septum, whose surface was relatively plane and showed recovery activity in normal development. The most superficial cellular epithelial layers showed slight scaling mixed with slight exudate, suggesting that the epithelium was undergoing the beginning of a degeneration process due to inflammation (Figure 2A).

Twenty-one days - experimental

The tissue characteristics of the experimental side were similar to the control side. The Col epithelial tissue was discontinuous and was thinner in some areas and more stratified in others. The underlying connective tissue was more cellular and more vascularized than in deeper areas. The external surface of the epithelium was frayed in some sections where only the cell membranes were visible. The healing connective tissue in the central area was less edematous; however, the number of inflammatory cells was increasing. On the whole, this region appeared to be more degenerated than the control side and the epithelium was formed by looser cells. In these areas, the surface of the epithelial tissue remained continuous; however, superficial cellular scaling was accentuated (Figure 2B).

Twenty-eight days - control

The epithelium of the buccal and lingual papillae was normal with stratified squamous keratotic epithelium characteristics. Towards the Col, epithelial destruction and connective disorganization were generalized. The gingiva had an intense inflammatory process. The Col epithelium, which was previously recovered, was almost entirely eliminated, and was discontinuous and exposed a connective tissue layer in which an inflammatory process predominated. The absence of lining epithelial tissue led to the assumption that ulceration or necrotizing gingivitis was taking place because of the characteristic fibrinous pseudomembrane (Figure 2C).

Twenty-eight days - experimental

The general aspect was similar to the control side. There was destruction of the connective tissue by an intense inflammatory process. The oral and lingual epithelia were normal, thick and had the characteristic epithelial projections of inflamed areas. Epithelium, however, was absent in the Col area (Figure 2D). The main characteristics of the inflammatory process and the destruction of the Col epithelium suggest that although the inflammatory reaction was more restrained, it was intense enough to revert the healing recovery process that had satisfactorily developed until the second week. The general aspect of the healing region was that of an exposed injury with degenerated and loose tissue, whose epithelium had recently been extracted, probably by food mastication, since there had not been enough time for the formation of the fibrinous pseudomembrane.

Morphometric Results

The morphometric results are reported in Table 1.
DISCUSSION

The reduction of dental tissues during crown preparation must be compatible with the necessary thickness of the restorative material in use, thus leading to a correct emergence profile between the restoration and the root portion that emerges from the alveolus. The space created must be large enough to accommodate the gingival tissues at the same time that it allows interdental hygiene.

The RAI technique described by Ross and Garguilo (8) aims at modifying the interproximal embrasure through root recontour by making it larger and thus altering the root emergence profile in relation to the alveolus. It prevents the onset of interproximal periodontal disease in the presence of prosthetic restorations.

We observed the development of constant healing until the 14th day when interdental tissue with thick connective tissue, normal vascularization and stratified epithelium undergoing keratinization were formed. This evolution, marked by slight alterations, continued until the 21st day. Col shape was modified, became slightly concave and showed a smaller distance between its margins.

After this period, there was a complete inversion in the conditions with the onset of an intense inflammatory process and the resulting destruction of the histological aspect that had been previously observed, probably due to the interference of external factors (bacterial plaque and food impaction). The inflammatory process was typical of gingivitis and the increased interdental space in the experimental area, where the RAI technique had been performed, may have permitted a larger accumulation of food and bacterial plaque as well as trauma due to food impaction, which caused a more accentuated inflammatory response.

After 28 days, the inflammatory process compromised the healing of the soft interproximal tissues in both the control and experimental sides. The epithelial tissue was disorganized and interrupted, which left the subjacent connective tissue exposed to the external environment.

Because there are no other studies similar to ours, it is not possible to compare results in order to confirm or contradict them. We must admit that in experimental studies in animals the interproximal region is more subject to food retention, and needs intense hygienic care. Since the animals cannot carry out cleaning, the total absence of hygiene causes consequences as those observed in this study. The healing process of the interdental bone tissue did not suffer alterations as had occurred with the epithelium and the suprajacent connective tissue, thus with satisfactory development with increasingly more accentuated organization in the medullary spaces.

Unpredicted factors resulting from the anatomic characteristics, such as: experimental study in an inter-

Table 1. Karyometry.

<table>
<thead>
<tr>
<th>Nuclear parameters</th>
<th>Basal cells</th>
<th></th>
<th>Squamous cells</th>
<th></th>
<th>Granular cells</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Gp</td>
<td>14 d</td>
<td>21 d</td>
<td>Gp</td>
<td>14 d</td>
<td>21 d</td>
</tr>
<tr>
<td>Greater diameter D (µm)</td>
<td>5.41</td>
<td>4.97</td>
<td>6.52*</td>
<td>6.98</td>
<td>5.92</td>
<td>6.70</td>
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<tr>
<td>Smaller diameter D (µm)</td>
<td>3.66</td>
<td>4.08*</td>
<td>5.10*</td>
<td>4.56</td>
<td>4.56</td>
<td>5.76**</td>
</tr>
<tr>
<td>Mean geometric diameter (µm)</td>
<td>4.45</td>
<td>4.50</td>
<td>5.75*</td>
<td>5.64</td>
<td>5.19</td>
<td>6.20</td>
</tr>
<tr>
<td>Ratio D/d</td>
<td>1.48</td>
<td>1.21**</td>
<td>1.28**</td>
<td>1.53</td>
<td>1.32</td>
<td>1.16*</td>
</tr>
<tr>
<td>Volume (µm³)</td>
<td>46.50</td>
<td>48.73</td>
<td>100.41*</td>
<td>97.12</td>
<td>74.93</td>
<td>125.68</td>
</tr>
<tr>
<td>Area (µm²)</td>
<td>15.63</td>
<td>16.07</td>
<td>26.13*</td>
<td>75.32</td>
<td>21.38</td>
<td>30.32</td>
</tr>
<tr>
<td>Ratio V/A</td>
<td>2.98</td>
<td>3.02</td>
<td>3.86*</td>
<td>2.98</td>
<td>3.02</td>
<td>3.86*</td>
</tr>
<tr>
<td>Perimeter (µm)</td>
<td>10.91</td>
<td>10.73</td>
<td>13.81*</td>
<td>13.93</td>
<td>12.64</td>
<td>13.84</td>
</tr>
<tr>
<td>Shape factor</td>
<td>1.64</td>
<td>1.74**</td>
<td>1.72**</td>
<td>1.62</td>
<td>2.11</td>
<td>2.05</td>
</tr>
<tr>
<td>Contour index</td>
<td>2.77</td>
<td>2.69*</td>
<td>2.71**</td>
<td>2.78</td>
<td>2.74</td>
<td>2.54</td>
</tr>
<tr>
<td>Eccentricity</td>
<td>0.74</td>
<td>0.55**</td>
<td>0.60**</td>
<td>0.76</td>
<td>0.62</td>
<td>0.50*</td>
</tr>
</tbody>
</table>

Gp: Gingival papilla
Mann-Whitney test: *statistically significant (α = 0.01), **statistically significant (α = 0.05).
proximal area, interdental space opened by the RAI technique and the embrasure vertically increased by osteotomy, would have caused the formation of bacterial plaque, food retention and trauma due to food impaction, thus fraying the upper layers of the epithelium and partially or totally shattering it in the last two periods observed (21 and 28 days).

We suggest that in order to clinically achieve the Col transformation into a saddle with keratinization of the interproximal epithelium, the space between the teeth during application of the RAI technique should be increased even more. A more aggressive osteotomy would be necessary to reach a region where the roots might be more divergent, permitting an apical replacement of the flaps with larger margin approximation and the resulting reduction of the distance to be occupied by the interproximal gingival tissue.

The Col morphology found after 14 and 21 days indicates that the objective of modifying it can be achieved through surgical techniques. The projection of a healing development without interference suggests that in longer periods with greater tissue maturation the ideal shape would certainly be achieved.

RESUMO


Foi avaliada a modificação da forma e posição do Col das áreas interproximais entre os primeiros molares e quartos pré-molares inferiores de 10 cães, utilizando-se a técnica de IAR – Interface Alvéolo Restauração. Realizou-se retalhos de espessura total para exposição da superfície radicular interproximal e da crista óssea alveolar. Os procedimentos de IAR foram realizados apenas nos lados experimentais e as áreas controle foram os lados opostos dos mesmos animais. Os animais foram sacrificados a zero hora, 7, 14, 21 e 28 dias para análises histológicas. Secções de aproximadamente 6.0 µm de espessura foram obtidas no sentido buco-lingual e méso-distal, coradas com hematoxilina-eosina e Mallory para análise em microscopia óptica. Uma cicatrização satisfatória foi observada entre o 14º e 21º dia, evidenciando uma modificação na forma do Col. A partir desse período, desenvolveu-se uma reação inflamatória afetando a evolução da cicatrização. A cirurgia provavelmente criou condições para a instalação de um processo inflamatório, resultante da modificação da anatomia da área interdental.

Unitermos: IAR (interface alvéolo restauração), col, cirurgia periodontal.

REFERENCES


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