Periodontal impact of surgically induced dental lesions in mandibular osteodistraction: An animal study

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SUMMARY. Aim: The objective of the study was to evaluate the impact of dental lesions on the periodontium, in a canine model of mandibular osteodistraction. Material and methods: In six adult male Beagle dogs, an osteotomy was made between the right second lateral incisor and canine, and a distraction device placed. The roots adjacent to the osteotomy were deliberately damaged by the reciprocating saw and chisel, with preservation of the attached gingiva. The osteodistraction protocol used was: latency of 7 days, rate of distraction 1 mm per day, and rhythm once a day for 5 days. Vital staining was carried out with tetracycline, Xylenol Orange and Calcein Green. The dogs were sacrificed after 12 weeks of consolidation and the specimens were evaluated with light microscopy (native, polarized light, fluorescence, and after toluidin blue staining). Results: The periodontal ligament (PDL) regeneration was observed in the 2500 slices examined. Cementum and dentine lesions were repaired by cellular cementum. Loose dentine and cementum-dentine fragments were embedded in regenerated PDL and their surface repaired by cementum. By means of light microscopic examination and within the limited observation time, no degenerative pulpal changes were found, when the pulp canal was not entered. Extensive pulp exposure and destruction resulted in ingrowth of the PDL and bone-like tissue. In that case, cellular cementum also lined the dentine surface of the pulp canal. Conclusion: Although there was an extensive reparative response to the para-pulpal lesions, none of the changes observed showed evidence of a loss of functional integrity of the periodontium at the distraction site. The fate of the tooth with exposed pulp canal remains uncertain. © 2008 European Association for Cranio-Maxillofacial Surgery

Keywords: osteogenesis, distraction, mandible, periodontium, animal experimentation

INTRODUCTION

Symphyseal widening by means of distraction osteogenesis is a less popular treatment option for severe irregularity and crowding of anterior mandibular teeth due to a narrow anterior alveolar base. Indeed, an inter-dental osteotomy is required and besides its morbidity, permanent damage to the periodontium and teeth may occur. In animal experiments (Bell et al., 1997, 1999; Del Santo et al., 2000) and clinical investigations (Guerrero et al., 1997; Weil et al., 1997; Kewitt and Van Sickels, 1999; Cope et al., 2002) inter-dental callus formation and tooth movements were studied, and occasionally accidental damage to teeth and periodontal structures was reported. Mommaerts et al. (2005) described the periodontal status and tooth vitality changes during the distraction and consolidation phase more in depth in a clinical study. Also in this study, however, the surgeon tried to avoid damage to the dental structures.

The aim of the present study was to assess any repair mechanisms after deliberately induced lesions to teeth and periodontal tissues adjacent to the regenerate bone during transversal mandibular osteodistraction.

MATERIALS AND METHODS

The study project was approved by the Board of Health Defense and Animal Welfare of the Ministry of Agriculture and Fisheries of Portugal (#14.MAI 01 04603). Six purebred male adult Beagle dogs with a difference in age of less than 15 days, and weighing between 11 and 15 kg, were acquired from the Animal Experimentation and Production Service of the University of Granada, Spain, in accordance with the EC Rules. All were cleared for facial malformation and oral pathology. The surgeries took place at the “Estação Zootécnica Nacional”, Santana, Portugal, in the Institute of Biomedical Technology. The dogs were microchip tagged but for convenience of the reader, numbered 1—6 according to the chronology of the operations. The chronology of all procedures, including surgical procedures, dye injections, impressions, radiographs, periodontal probing, and the distraction parameters, is depicted in Fig. 1.
Anaesthesia

Preliminary maxillary canine crown amputation, osteodistraction surgery itself, and sacrifice of the animals were done with the following anaesthesia protocol. Thirty minutes before the surgical intervention, the animals were tranquillized with an intramuscular injection of 0.25 mg/kg acepromazine (Calmivete®, Vétoquinol, Lure, France). They were placed on the operating table in a lateral position. Anaesthesia induction was obtained with sodium thiopental (Pentothal®, Abbot Laboratories, Abbott Park, Illinois, USA) with a dosage of 15 mg/kg by intravenous bolus. After oro-tracheal intubation, the anesthetic state was maintained with 100% oxygen (2 l/m) and 1.5% halothane (Fluothane®, Abbott Laboratories, Abbott Park, Illinois, USA).

Periodontal pocket probing, together with the taking of impressions and radiographs, was done using 5 mg/kg of ketamine (Imalgène®, Merial, Toulouse, France) and 0.1 mg/kg of diazepam (Valium®, Roche, F. Hoffmann-La Roche Ltd., Basel, Switzerland) intramuscularly.

Surgery

Because the canines were in cross-bite, being a characteristic of the breed, the crowns of the upper canines were sectioned at the cemento-enamel junction followed by a conventional gutta-percha endodontic treatment. This was done as a preliminary procedure to avoid traumatic occlusion during mandibular expansion, since a malocclusion in the sense of a cross-bite tendency will occur during symphyseal widening.

After dissection, a horizontal buccal sulcus incision was made, approximately 3 cm in length, 5 mm caudally to the mucogingival margin, from canine to canine, in the lower jaw. Cranially and caudally based mucoperiosteal flaps exposed the osteotomy site on the right side. Care was taken not to disrupt the gingival fibre system. A transmandibular distractor (Mommaerts, 2001; TMD®, Surgi-Tec, Bruges, Belgium) was adapted to the bony surface, fixed with six bicortical screws, and temporarily removed. The TMD device is made entirely of pure titanium grade 2. It consists of two vertical footplates for fixation, each provided with an offset extension that pierces the vestibular incision. Two parallel distraction rods connect the intra-oral extensions, with an opposite thread running along each side. The device is activated with a screwdriver than can be inserted at either end of the rods. Every full turn equals 1 mm expansion.

With a reciprocating saw (Nouvag, Basle, Switzerland), the blade of which is 0.5 mm thick, a unilateral paramedian osteotomy of the mandible was carried out between the second lateral incisor and the canine tooth, in between the holes made for the osteosynthesis screws. A chisel was used to split the crestal bone between the roots, with preservation of the gingival cuff. Digital control maintained the integrity of the lingual mucoperiosteum. The space between the roots at the crestal level was so limited (Fig. 2) that root damage was inevitable. The distractor was finally fixed again and the wound sutured in a watertight fashion with 3-0 polyglactin 910 (Vicryl®, Ethicon, Neuilly, France). The antibiotic amoxicillin (7 mg/kg) (Clavamox®, Pfizer Animal Health, NY, USA) was administered intramuscularly every 24 h for three days. Dipirona and hioscine (Diplabiol®, Sabiol, Lisbon, Portugal) were administered intramuscularly for analgesia, at a dosage of 2 ml/day, during three days.

The dogs were sacrificed after 12 weeks of callus consolidation. After intravenous injection of thiopental, the common carotid arteries were bilaterally clamped with vessel loops, and injected with 120 ml formaldehyde in a 10% concentration. Hence, fixation of the tissues occurred in vivo and cardiac arrest within a few minutes. After the sacrifice, the anterior segment of the mandible was removed with an oscillating autopsy saw (Stryker Corporation, Kalamazoo, USA).

Distraction policy

The animals were kept on soft granulated diet with water ad libitum (Selection 7 – Evolution®, Royal Canin, Emargues, France) until the 8th week of the consolidation phase. Oral

Fig. 1 — Time frame of experiment, with indication of distraction parameters, injections for in vitro staining, taking of the impressions and occlusal radiographs, and periodontal pocket probings.
hygiene was ensured with a daily cleansing with a 20/100 brush (Pierre Fabre, Castres, France), and fluoride toothpaste (Elgidium, Pierre Fabre, Castres, France). Active distraction started on day 8, with a rate of 1 mm once a day, corresponding to a 360° rotation of both distractor rods. Anesthesia was not necessary. The activation period lasted for 5 days, the consolidation phase for 84 days.

Data acquisition

Periodontal pocket probing and bacterial plaque control were performed at the time of canine crown amputation, and at the time of animal sacrifice. Radiological evaluation was done at the time of maxillary crown amputation (day 8), at the time of the first activation, and after 2 weeks (day 27), 8 weeks (day 69) and 12 weeks (day 97) of consolidation. Impressions were made at the time of the preliminary procedure (for individual tray fabrication), at the time of the distraction surgery and the first activation, and after 8 weeks (day 69) of consolidation (Fig. 1).

The periodontal pockets were measured with a calibrated probe (HU-Friedy®, Chicago, Illinois, USA). For all eight front teeth, pockets were measured at six surfaces; buccal, buccomesial (BM), buccodistal (BD), lingu al, linguomesial, and linguodistal. Bacterial plaque was controlled with erythrosine (Dentoplastique®, Pierre Fabre, France). Plaque was assessed according to the Plaque Index (Pl.I; Löe and SILNESS, 1963) and also scored on 6 surfaces of all eight front teeth. Occlusal films (Kodak Ektaspeed®, Eastman Kodak, Rochester, USA) were fitted into a PVC frame for standardization purposes. This device comprised a custom-made cylinder adapted to the X-ray tube (Oralix 65, Philips®, Eindhoven, the Netherlands). Impressions were made with polyvinyl siloxane (Express®, 3M, Minneapolis, USA). Models were made of acrylic (Orthosin-Uni, Schütz Dental, Rosbach, Germany). The intercanine distance, the distance between the second lateral incisors at their distal surfaces, and the distance between the adjacent surfaces of the canine and second lateral incisor related to the osteotomy line were measured with a digital caliper (Mitutoyo CD-15DC, Kanagawa, Japan) (Fig. 3).

Histology protocol

A vital staining protocol was used as recommended by RAHM (1999). OXYTETRACYCLIN (Pfizer AG, Zurich, Switzerland) in a dosage of 25 mg/kg, diluted in 10 ml of a 0.9% saline solution, was injected subcutaneously three times, with a two-day interval, covering the activation period (days 8, 10, 12) (Fig. 1). Xylenol Orange (Fluka AG, Buchs, Switzerland), at a dose of 90 mg/kg, diluted in a 9% distilled water solution, was injected subcutaneously in the first part of the consolidation phase (days 27, 29, 31). Calcein Green (Fluka AG, Buchs, Switzerland) at a dosage of 5 mg/kg of a 0.5% distilled water solution was injected in the second part of the consolidation period (days 69, 71, 73).

After sacrifice, the marked mandibular specimens were placed in appropriate containers with a 4% formalin solution and sent to the University of Aachen, Germany, for histological preparation and processing. After gradual dehydration by ethanol wash, the specimens were infiltrated with methacrylate. The histological preparation method was the cutting—grinding EXAKT/Donath technique (Donath and Brenner, 1982). The slices had intervals of 800—900 μm and were 70—90 μm thick. The specimens were evaluated by light microscopy (native, polarized light, fluorescence, and after toluidin blue staining) with magnifications 0.8×, 1.6×, 1.8×, 2.5×, 5×, 10× and 20×.

Quantification and statistical analysis were not performed because of the small number of animals, which was in turn because of moral restrictions. Hence, the analysis is descriptive.

RESULTS

Specific tooth movements during the activation and early consolidation phase are listed in Table 1. The “walking
between the two sides were not statistically analyzed. Because of the size of the sample, differences pocket depths at the experimental side were slightly in-
tion (10 mm) and delayed consolidation as a result. The
tized its oral vestibule with pathological pocket forma-
from the calculations because it had accidentally trauma-
did not add valuable information. Dog 5 was excluded

### Table 1

The difference in the distances (mm) depicted in Fig. 3, between the first day of activation (day 8) and 8 weeks of consolidation (day 69), indicative for the “walking tooth phenomenon” occurring in the activation and first half of the consolidation phase.

<table>
<thead>
<tr>
<th></th>
<th>Intercanine distance (1)</th>
<th>Distance between the lateral surfaces of the 2nd lateral incisors (2)</th>
<th>Distance between the proximal surfaces of the canine and 2nd lateral incisor adjacent to the distraction gap (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>1.7</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Dog 2</td>
<td>4</td>
<td>4.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Dog 3</td>
<td>3.6</td>
<td>1.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Dog 4</td>
<td>4.4</td>
<td>1.2</td>
<td>5</td>
</tr>
<tr>
<td>Dog 5</td>
<td>0.2</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Dog 6</td>
<td>1.5</td>
<td>2.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Depth in millimeter.

tooth phenomenon” can be deducted from the increase in distance 2, being the distance between the distal surfaces of the second lateral incisors (Fig. 3). On average, the distance over the six incisors increased by 2.2 mm (standard deviation [SD] 1.1). The changes in the inter-canine distance (distance 1) did not correlate with the width of the distraction gap measured at the dental level (distance 3).

The differences in pocket depths of the teeth adjacent to the distraction gap and the contralateral control teeth are listed in Table 2. The probings of the other areas did not add valuable information. Dog 5 was excluded from the calculations because it had accidentally traumatized its oral vestibule with pathological pocket formation (10 mm) and delayed consolidation as a result. The pocket depths at the experimental side were slightly increased. Because of the size of the sample, differences between the two sides were not statistically analyzed.

### Table 2

Differences in BM and BD pocket depth, measured at the teeth adjacent to the distraction gap at the experimental side, and at the contralateral teeth at the control side, between the preoperative situation and the situation at sacrifice.

<table>
<thead>
<tr>
<th>Pocket depth</th>
<th>Experimental side</th>
<th>Control side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canine</td>
<td>2nd Lateral</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>BD</td>
</tr>
<tr>
<td>Mean</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>SD</td>
<td>0.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The PI at time of crown amputation was on average 1, to increase to 1.83 at time of sacrifice (Table 3). The difference was significant (P > 0.001 — paired t-test) but there was no correlation (Pearson Product Moment Correlation).

Occlusal radiographs (Fig. 4) were useful to correlate the type of dental lesion with the findings of the histology slices. Callus formation was evident in all occlusal radiographs already after the second week of consolidation (day 27), except in those of Dog 5, in which there was no expansion.

The axial histology slices were grouped according to the apical, middle and coronal levels of the second lateral incisor, and viewed with different magnifications, lighting and staining (a total of 2500 pictures). In all the out-fractured root fragments embedded in the distraction regenerate, signs of resorption and of deposition of mineralized material with an important cellular component could be observed. Fibrous tissue resembling periodontal ligament (PDL) was surrounding the fragments, with fibres running parallel to the fragment’s surface. There were no signs of inflammation or of foreign body reaction (Fig. 5). A pattern of regeneration was observed in the PDL adjacent to the sectioned root surface. Although there were some inconsistent aspects, e.g. localized signs of ankylosis alternating with others of scar tissue formation, the dominant appearance was that of bundles of collagen fibres with an altered orientation almost coincidental with the direction of the distraction forces. These aspects were more evident in the polarized light pictures (Fig. 6). In none of the sectioned areas could inflammation be observed, except in Dog 5. However, magnifications of up to 10× are not detailed enough to make a definitive statement.

A fluorescent green line (compatible with Calcein Green fixation) could be consistently seen covering tooth zones with saw cut lesions (Fig. 7). The intensity of the fixation had considerable variation, being almost nonexistent (but still present) in Dog 5 with the laceration. Correlation of the native histology with the fluorescence revealed that this green line most probably represented a layer of regenerated cementum at different levels of intensity. The presence of bone-like tissue is observed in the pulp canal after an oblique lower third root section (Fig. 8). This tissue is formed by several small keratinized structures that are present not only in the open zone of the pulp canal but also in the contiguous upper slices (inside the pulp chamber). The fluorescence reveals a high degree of fixation. Those could

### Table 3

Plaque index of each dog for each tooth surface, averaging all 8 mandibular front teeth. The index was calculated at the time of canine crown amputation (“initial”) and at the moment of sacrifice (“final”).

<table>
<thead>
<tr>
<th></th>
<th>Buccal</th>
<th>Lingual</th>
<th>BM</th>
<th>BD</th>
<th>Linguomesi al</th>
<th>Linguodistal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>DOG 1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DOG 2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DOG 3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DOG 4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>DOG 5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DOG 6</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Loe and Silness (1963), PI.
be bone fragments dislocated by the saw at the moment of the osteotomy although its presence in the upper slices is not congruous with this vision.

The fact that Dog 5 had a traumatic gingival disruption induced by a convulsion (apparently caused by the subcutaneous injection of Xylenol Orange) turned out to be a key issue in this study. The presence of bacterial plaque on the surface of the coronal thirds of the roots adjacent to the osteotomy had induced inflammation in the surrounding soft tissues. Regeneration of periodontal structures was almost nonexistent (Fig. 9). The fluorescence observed on the root surfaces may represent sites of resorption or perhaps simply be a site of mechanical matrix injury.

Whereas all alveolar crests in the uninjured animals had maintained their original height after consolidation, there was significant resorption down to the level of the second lateral incisor apex in Dog 5.

When osteosynthesis screws extended into the root canal, the pulp remained viable (Fig. 10a). The screw thread may transplant bone fragments into the root canal. This bone may stay viable and undergo extensive remodelling (Fig. 10a). The periodontium reacted similarly to tangential trauma from a screw thread as to a clean cut from a saw or chisel; periodontal-like tissue encompassed the root surface (Fig. 10b) which showed cementum regeneration under polarized light.

DISCUSSION

The changes in the intercanine distance (distance 1) do not reflect the width of the distraction gap measured at the dental level (distance 3), because the distraction had an important antero-posterior vector and the unknown degree of mandibular segment tilting will have had an influence as well. The tendency for anterior teeth to close the gap mesially is a known phenomenon in anterior mandibular osteodistraction with midline osteotomy (“walking tooth phenomenon”; Weil et al., 1997). In the present experiment, the histological section showed that only the lateral incisors tended to close the gap by distal movement, probably because the dog canine has such a long, firmly anchored root.

Crestal bone loss has been observed with immediate expansion (Dorfman and Turvey, 1979), and with immediate expansion of 1 mm followed by a latency phase of 5 days to 2 weeks prior to further expansion. The authors suggested that it was caused by radicular surface exposure, resulting from inter-dental osteotomies in narrow inter-dental spaces (Bell et al., 1997, 1999). When
a true latency period of 5—7 days was respected, crestal bone loss was not observed and the attached gingiva maintained its preoperative level (Guerrero et al., 1997; Kewitt and Van Sickels, 1999; Niederhagen et al., 1999; Mommaerts et al., 2005). These findings, and the ones from the present experiment, suggest that keeping the junctional epithelium attached, or allowing its reattachment, may be more important than keeping bone on the roots. The periodontal response can though be greatly influenced by the preoperative periodontal status and maintenance of good oral hygiene habits in the postoperative period (Kewitt and Van Sickels, 1999; Mommaerts et al., 2005).

Andreasen et al. (1990) defined periodontal healing by “a newly formed PDL space surrounding the entire root periphery.” In this investigation, not yet-well organized connective tissue between the distraction callus and the damaged root surface was observed. All exposed tooth surfaces, being cementum, dentine (at the external and pulpal surface), or dental particles, were lined by a tissue indistinctive from normal PDL.
Bell observed by the distraction forces. This phenomenon has also been seen on each of the teeth that moved. However, some degree of root resorption occurs with any type of orthodontic tooth movement and approximately 75% is repaired by cellular cementum (Cope et al., 1999; Roberts-Harry and Sandy, 2004). Some basal level of root surface turnover may be normal in these dogs (Verna and Melsen, 2003).

The PDL of the teeth contiguous with the distraction gap showed a widening and an altered orientation of the collagen fibres that coincided with the direction of the distraction forces. This phenomenon has also been observed by Bell et al. (1997) and is identical to what is observed during orthodontic tooth movement in nondistraction bone (Verna and Melsen, 2003).

Bone tissue with osteocytes and osteoblasts has been observed in replanted and autotransplanted immature teeth whether the original pulp tissue was removed (Claus et al., 2004), or not (Skoglund and Tronstad, 1981). The amount of tubular hard tissue these authors found was very small and did not occupy large parts of the cavity or walls. Interestingly, this bone-like tissue, formed centrally in the pulp was in some teeth connected to the alveolar bone through the open apical foramen (Skoglund and Tronstad, 1981).

An interesting finding was that tooth fragments, whether small after being cut off tangentially, or large following extensive pulp exposure, were not expelled by an inflammatory reaction. On the contrary, until the end of the experiment, the reparation tissues surrounding or extending into these fragments resembled a normal periodontal apparatus, with oriented bundles of collagen, cellular cementum and supportive cell rich woven bone.

Tangential root damage from screws has been examined by Asschericks et al. (2005), also in Beagle dogs, with fluorescence and toluidin staining techniques. Their results corroborate with the present findings; tooth preservation is not jeopardized by tangential titanium screw lacerations as can occur during maxillo-facial trauma or orthognathic surgery, absolute anchorage for orthodontics or osteodistraction procedures.

CONCLUSIONS

Deliberate surgical injury to the teeth and surrounding periodontium was examined histologically and radiographically in Beagle dogs receiving transverse mandibular osteodistraction surgery. Cementum and dentine lesions were repaired by cellular cementum, and the damaged root surfaces surrounded by PDL-like collagen bundles. Loose dentine and cementum—dentine fragments were consistently embedded in regenerated periodontium and their surface repaired by cementum. The pulp remained viable when damaged by osteosynthesis screws. Extensive pulpal evisceration resulted in ingrowth of the PDL and cell rich woven bone. The dentine surface of the pulp canal was also lined by cellular cementum. These severely damaged teeth survived as giant fragments. Loss of attached gingiva in an inadvertently traumatized dog resulted in 10 mm pathological pocket formation with histological signs of inflammation and no bone formation. In none of the dogs was the distraction callus jeopardized by the root lesions, except in the traumatized dog.

Future research should focus on ultramicroscopic examination of the regenerative tissue in the periodontium, and on the phenomena occurring in the pulpal structures. The increased use of bone borne devices in modern orthodontics emphasizes its importance.

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