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# Guided tissue regeneration of a left mandibular first molar tooth in a dog

## Guided tissue regeneration -tekniikka vasemman alaleuan ensimmäisessä molaarissa koiralla

### YHTEENVETO

*Kahden vuoden ikäinen jackrusselteriერიuros tuotiin läheteellä eläinlääkäriasemalle parodontiitin jatkohoitoon. Anestesiassa tehdyssä hampaiden tarkastuksessa ja röntgenkuvauksessa todettiin parodontiitin aiheuttamaa, vakavaa, hammasta ympäröivää kiinnityskudoskatoa: syventyneitä ientaskuja ja hammasta ympäröivää luukatoa erityisesti vasemman alaleuan neljännessä premolaarissa (P4) ja ensimmäisessä molaarissa (M1). Vasen ala-P4-hammas poistettiin ja tärkeemmän, ala-M1-hampaan vertikaalinen luukato hoidettiin niin sanotulla guided tissue regeneration -tekniikalla, jonka tarkoituksena on ohjata kudoksen paranemisprosessia ja auttaa uuden alveolaariluun muodostumista hampaan ympärille käyttäen hyväksi luusiirrännäistä ja kollageenikalvoa. Tekniikka vaatii eläinlääkäriltä erikoisosaamista ja omistajalta sitoutumista huolelliseen potilaan suubygieniaan kotona. Kontrollissa 6 kuukauden kuluttua syvä ientasku oli parantunut täysin ja hammasta ympäröivä luukato korjaantunut.*

### SUMMARY

*A 2-year-old male Jack Russell Terrier was referred to a clinic for evaluation and treatment of periodontal disease. Dental charting and intraoral radiographs revealed attachment loss, deep gingival pockets and alveolar bone loss especially at the left mandibular fourth premolar (P4) and first molar (M1). The left mandibular P4 was extracted, while the more important M1-tooth with vertical bone loss was treated by guided tissue regeneration using a bone allograft and collagen to modify the healing process of periodontal tissue and to allow new bone formation around the tooth. This technique requires special skills from the veterinarian, and the owner must be committed for excellent oral hygiene at home. The 6-month recheck revealed complete healing of the gingival pocket and alveolar bone.*

### INTRODUCTION

Periodontal disease is an inflammatory response to plaque-induced infection. It affects one or more of the periodontal tissues: the gingiva, periodontal ligament, cementum and alveolar bone. Optimal therapy includes the management of infection, control of the host-immune response and regeneration of diseased or lost portions of the periodontium.<sup>1</sup> In addition to conservative, non-surgical treatment consisting of tooth scaling and polishing, surgical exposure of deeper pockets offers access for debridement of root surface and surrounding periodontal lesions and enables modifying the

healing process of periodontal tissue. However, without home care, gingivitis will form again in days or weeks.<sup>2</sup> Uncontrolled periodontitis will eventually result in the loss of teeth and complex systemic inflammatory consequences.<sup>1</sup>

### CASE REPORT

In May 2011, a 2-year-old, 7 kg, male Jack Russell Terrier was referred to Anident Veterinary Clinic for evaluation and treatment of periodontal disease. The patient was vaccinated and otherwise healthy but had a history of intolerance to carprofen. The diet based on commercial dry food. The dog had

### YDINKOHDAT:

- Parodontiitin hoito pyrkii pysäyttämään taudin etenemisen, sillä hammasta ympäröivä luukato ei perinteisin menetelmin ole korjattavissa.
- Hammasta ympäröivää kiinnityskudoskatoa voidaan korjata guided tissue regeneration (GTR) -tekniikalla.
- GTR-tekniikalla pyritään muodostamaan kestävä, uutta hammasta ympäröivää luuta ja parhaassa tapauksessa parodontaaliligamenttia.
- GTR-tekniikka vaatii erikoisosaamista ja hyvän kotihoidon.

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occasional access to bones. No dental home care was provided.

### Physical examination

Results of complete physical examination were unremarkable during every visit. Additionally, results of preoperative laboratory tests, (CBC and biochemical profile) were within normal limits.

### Anesthesia and pain management

During every visit, the patient was premedicated with intramuscular medetomidin (Domitor vet 1 mg/ml, Orion Pharma) at 4 µg/kg and butorphanol (Butordol 10 mg/ml, Intervet International B.V.) at 0.2 mg/kg. Oxygen was administered by mask before and during induction of anesthesia with intravenous propofol (PropoClear 10 mg/ml, Pfizer) at 2 mg/kg and midazolam (Midazolam 5 mg/ml, Orion Pharma) at 0.2 mg/kg, followed by endotracheal intubation with a 7 mm cuffed tube. Anesthesia was maintained with 1.5–2.5 % sevoflurane (Sevoflo, Abbott Laboratories Ltd) with a ventilator (Merlin Small Animal Ventilator, Vetronic Services). Balanced isotonic electrolyte solution (Ringersteril, Baxter) was administered intravenously throughout the procedure at a rate of 10 ml/kg/h for the first hour and continued at a rate of 5 ml/kg/h throughout the procedure lasting more than one hour. Pain management included preoperative inferior alveolar nerve block with lidocain (Lidocain 20 mg/ml, Orion Pharma) 0.6 mg/kg and postoperative intravenous buprenorphine (Vetergesic 0.3 mg/ml, Reckitt-Benckiser Healthcare Limited) at 10 µg/kg during extractions and periodontal surgery.

### Oral examination and dental radiographs

The oral examination conducted to the conscious dog revealed generalized, moderate gingivitis

Surfaces and Directions	
Buccal	Surface of the tooth facing the vestibule or lips
Lingual	Surface of a mandibular tooth facing the tongue
Palatal	Surface of a maxillary tooth facing the palate
Mesial	Surface directed toward the first incisor
Distal	Surface opposite from the mesial surface
Apical	Direction towards the root apex
Coronal	Direction towards the crown of the tooth
Pinnat ja suunnat	
Bukkaalinen	Hampaan posken tai huulen puoleinen pinta
Linguaalinen	Alahampaan kielen puoleinen pinta
Palatinaalinen	Ylähampaan kitalaen puoleinen pinta
Mesiaalinen	Ensimmäisen inkisiivan puoleinen pinta
Distaalinen	Mesiaalisen vastakkainen pinta
Apikaalinen	Juuren kärjen suuntainen
Koronaalinen	Kruunun suuntainen

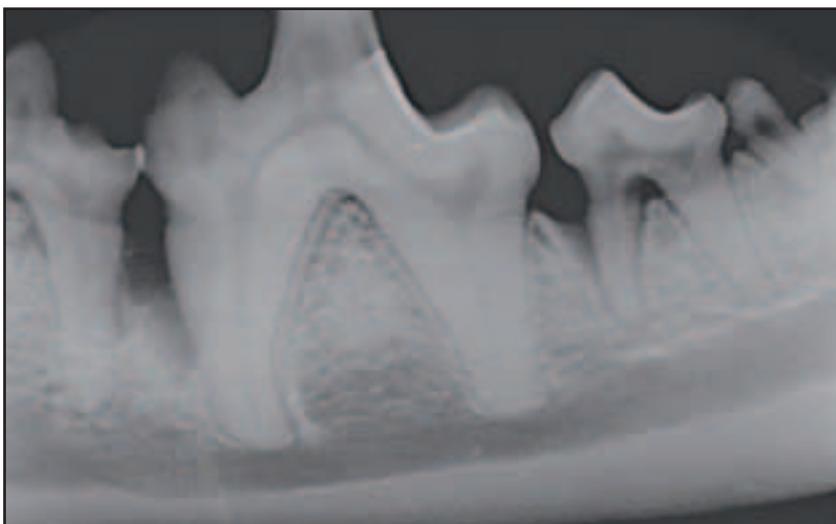
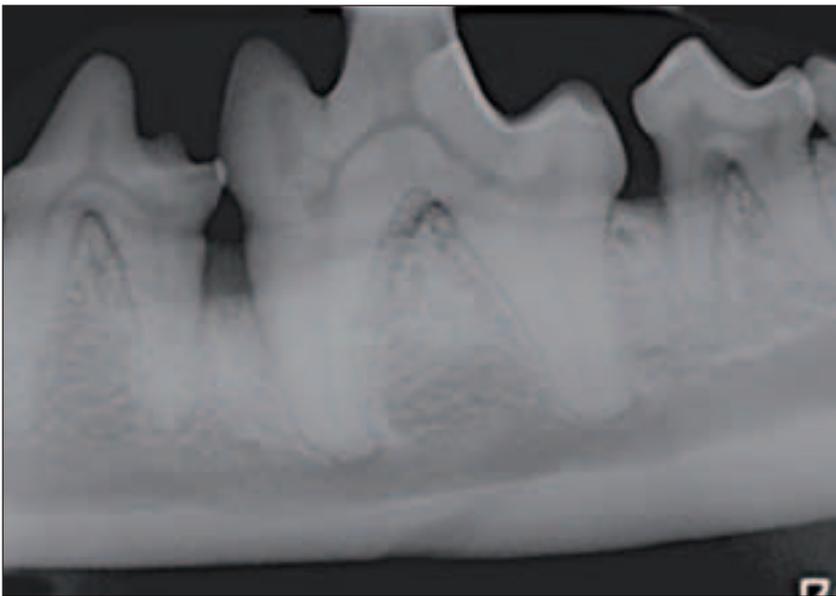


FIGURE 1 KUVA

*Phase 1 treatment: horizontal, 20% (2 mm), bone loss at the distal aspect of left mandibular P4 and vertical, 60% (7 mm), bone loss at the mesial aspect of left mandibular M1, extending mesially towards the distal aspect of left mandibular P4. Vertical, 70% (5 mm), bone loss is also present at the mesial aspect of left mandibular M2 with suspected external resorption, not detected on probing. Pulp stones are present in the pulp chamber of left mandibular M1.*

*Ensimmäisellä hoitokerralla vasemman mand-M1:n etupinnalla on horisontaalinen 20 %:n (2 mm) luukato ja vertikaalinen 60 %:n (7 mm) luukato, joka ulottuu vas. ala-P4:n takapinnalle. Vasemman ala-M2:n etupinnalla on vertikaalinen, 70 %:n (5 mm) luukato ja lievää ulkoista syöpymää, joka ei ollut tunnettavissa koettimella. Vasemman ala-M1:n pulpaontelossa on pulpakiviä.*



**FIGURE 2 KUVA**

*Six weeks after phase 1 treatment: horizontal 20% (2 mm) bone loss at the distal aspect of left mandibular P4, vertical 35% (4 mm) bone loss at the mesial aspect of left mandibular M1, extending mesially towards the distal aspect of left mandibular P4 and vertical, 35 % (3 mm) bone loss at the mesial aspect left mandibular M2.*

*Kuusi viikkoa ensimmäisen hoitokerran jälkeen vasemman ala-P4:n takapinnalla on horisontaalinen, 20 %:n (2 mm) luukato ja vasemman ala-M1:n etupinnalla vertikaalinen 35 %:n (4 mm) luukato, joka ulottuu vasemman ala-P4:n takapinnalle. Vasemman ala-M2:n etupinnalla on vertikaalinen, 35 %:n (3 mm) luukato.*



**FIGURE 3 KUVA**

*Six weeks after phase I treatment moderate gingival inflammation is present interdental to left mandibular P4-M1.*

*Kuusi viikkoa ensimmäisen hoitokerran jälkeen vasemman ala-P4-M1:n välissä on kohtalaista ientulehdusta.*

and plaque and calculus formation with extensive focal accumulation on the buccal surfaces of maxillary canines and fourth premolars and first molars.

Oral examination, dental charting and dental radiographs conducted while the dog was under anesthesia revealed severe periodontal disease (stage 4, i.e. more than 50% attachment loss) of the left mandibular P4 and all molars. Deep (9 mm) periodontal pocket was present at the distal root of the P4 and mesial root of the M1 with vertical bone loss, a so called three-walled infrabony pocket. The left mandibular M2 and M3 teeth had an almost complete loss of attachment with deep pockets.

Incidental findings included two pulp stones in the pulp chamber of left mandibular M1.

#### **Treatment plan**

The treatment plan included a general complete periodontal debridement. For the left mandibular M2 and M3, the only viable treatment plan was extraction. The treatment options for the P4 and M1 included open periodontal debridement (flap surgery to expose and debride the infrabony pocket), guided tissue regeneration (GTR) with bone allograft and collagen membrane, or extraction of both teeth. After discussion with the owner, a GTR of the M1 and extraction of the P4 were selected. Extraction of the P4 would allow a surgical exposure and bone augmentation of the three-walled defect of the mesial root of M1. The owner was committed to meticulous home oral hygiene and the necessary rechecks.

The treatment was staged. During the first visit (phase I therapy), the treatment plan consisted of scaling and periodontal debridement with an ultrasonic scaler (Satelec P5, Newtron XS Led) and a curette (XP Gracey G11, American Eagle Instruments). Meticulous

**FIGURE 4 KUVA**

*After elevation of a triangle flap showing infrabony, three-walled pocket with granulation tissue.*

*Kolmion muotoisen ienkielekkeen avaamisen jälkeen on nähtävissä luun sisäinen, kolmeseinäinen tasku täynnä granulaatiokudosta.*

**FIGURE 5 KUVA**

*After debridement of the three-walled pocket.*

*Luun sisäinen, kolmeseinäinen tasku kyretoinnin jälkeen.*

home care with daily brushing and chlorhexidine gel (Dentisept, Dr. E. Graeub AG) for a minimum of 2 weeks was provided.<sup>2,4</sup> During the second visit, (phase II therapy) the treatment plan was GTR of left mandibular M1 and extraction of left mandibular P4, M2 and M3.

#### Treatment

During the phase I treatment, a pharyngeal pack was placed to

prevent aspiration of blood, water and debris, and the oral cavity rinsed with 0.12 % chlorhexidine gluconate solution (Chlorhexidine Gluconate Oral Rinse 0,25%, University Pharmacy). Following

ultrasonic scaling and supra- and subgingival periodontal debridement, the teeth were polished with pumice (Prophylactic paste, Orbis, Plandent) using a prophy angle in a low-speed handpiece.<sup>1</sup>

Since the owners were unable to return at the recommended 2–3 week interval,<sup>2,4</sup> they presented the patient for the phase II treatment 6 weeks later.

During induction, the patient received preoperative intravenous ampicillin (A-pen, Orion Pharma) at 20 mg/kg. The pharynx was packed, oral cavity rinsed and the teeth scaled and polished as before. A buccal triangle, full-thickness mucogingival flap with a mesial releasing incision interdental to the left mandibular P3 and P4 was created, continuing intrasulcularly to the distal aspect of left mandibular M3. Two 5-0 polyglytone (Caprosyn 5-0, P-13, Covidien) stay sutures were placed: one at the corner and another one at the mid-edge of the flap. Stay sutures provide access to the surgical area with minimal trauma from tissue handling. The flap was kept moist throughout the procedure with saline. In order to extract the left mandibular P4, M2 and M3, a partial buccal alveolectomy was performed with

**FIGURE 6 KUVA**

*The placement of the membrane (Extra-cellular matrix membrane, ACell Vet).*

*Kalvo on paikallaan.*

**FIGURE 7 KUVA**

*Flap closure with single, interrupted sutures.*

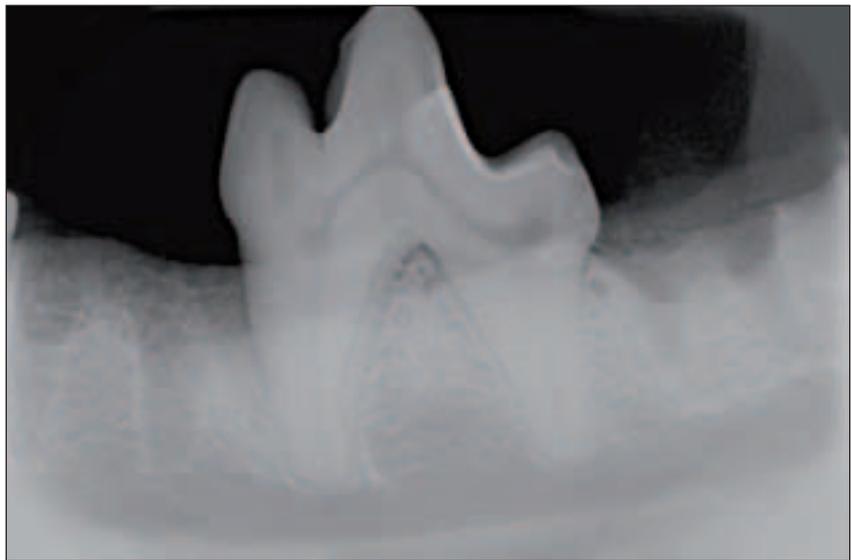
*Ienkieleke on suljettu yksittäisillä ompeleilla.*

a small round carbide bur (FG 801-010, Meisinger). Two-rooted P4 and M2 were sectioned with a #701 fissure bur (Jet Brand) in a high-speed handpiece. All roots were extracted completely with luxation.

The three-walled pocket of mesial root of the M1 was debrided carefully. Undesired granulation and epithelial and connective soft tissue was removed with a curette (XP Gracey G11, American Eagle Instruments) and an ultrasonic scaler (Satelec P5, Newtron XS Led) with a subgingival tip.<sup>4</sup> Alveolar bone walls and root cementum were exposed. The sharp edges of the alveolar bone at the extraction sites and infrabony pocket were smoothed using a small, round diamond bur (FG 801-010, Meisinger) in a high-speed handpiece. Additionally, the edges of the infrabony pocket were contoured with a sharp curette and periodontal chisel (Ochsenbein chisel # 1, Hu-Friedy) to improve the site architecture for the barrier placement and flap closure.<sup>8</sup> The alveolar bone and root surface were flushed with a Ringer's solution (Ringersteril, Baxter).<sup>1,6,7</sup> Bone allograft material (Osteoallograft Periomix, Veterinary Transplant Services) was mixed with a small amount of the patient's blood and placed in the osseous defect to the level of the alveolar margin. The membrane (Extracellular matrix membrane, ACell Vet) was placed to cover the defect and 2–3 mm area around it and sutured tightly around M1 with a sling suture. The flap margins were trimmed and the periosteum of the flap incised to allow a tension-free closure of the surgical site using simple, interrupted sutures of 5-0 polyglytone.<sup>8-10</sup> Dental radiographs were obtained after surgery.

#### **Post-operative care**

After phase 1 periodontal treatment, the patient was discharged



**FIGURE 8 KUVA**

*Allograft placement to the level of alveolar margin at the mesial aspect of left mandibular M1. Because allograft is made of 50% demineralized bone, it is not as dense as the bone.*

*Luusiirre ulottuu alveolaariluun rajalle vasemman ala-M1:n etupinnalla. Koska luusiirteestä 50 % on demineralisoitua luuta, se ei ole yhtä röntgentiivistä kuin luu.*

and the owner instructed to give chlorhexidine gel once a day interdentally to the area of the left mandibular P4-M1. After the GTR, oral tramadol (Tramal 50 mg, Grünenthal GmbH) at 3 mg/kg every 4 hours for 3–5 days and amoxicillin-clavulanic acid (Amoxiclav 200 mg/50 mg, Norbrook Laboratories Ltd) twice daily at 20 mg/kg for 14 days were prescribed. The owner was instructed to give soft food for 3–5 days and to avoid giving hard food for 2 weeks. Chlorhexidine mouth rinse (Hexarince, Virbac) twice a day for 2 weeks was prescribed. The owner was advised to brush the dog's teeth avoiding the surgical sites.

#### **Follow-up**

Two weeks after the GTR and extractions, oral examination of the conscious dog showed good healing of the surgery site. The owner was instructed to continue tooth brushing and the use the chlorhexidine gel once a day for

one week in a month. Six months after surgery, the patient returned for a second follow-up. It was doing well. Oral examination and dental radiographs revealed 1–2 mm pockets and almost complete healing of the vertical bone at the mesial root of the left mandibular M1. The owner was instructed to continue daily homecare and return periodontal treatment and dental radiographs annually.

## **DISCUSSION**

### ***Periodontal disease and loss of alveolar bone***

Typical to periodontal destruction is collagenolysis with loss of epithelial attachment, followed by loosening of periodontal ligament. Alveolar bone is progressively destroyed at advancing stages of periodontal disease. Horizontal bone loss is the most common type of bone loss. The bone is reduced in height, but the bone margin remains approximately



**FIGURE 9 KUVA**

*Surgery site 6 months after the operation: 1 mm periodontal pocket is located on the mesial, buccal, lingual and distal aspect of left mandibular M1. Bleeding occurred after probing at the mesial aspect of left mandibular M1.*

*Kuusi kuukautta operaation jälkeen vasemman ala-M1:n etupinnalla, posken puoleisella pinnalla, kielen puoleisella pinnalla ja takapinnalla on 1 mm:n ientasku. Tutkimuksen jälkeen ilmeni lievää verenvuotoa vasemman ala-M1:n etupinnalla.*



**FIGURE 10 KUVA**

*Surgery site 6 months after the operation after probing: 1 mm periodontal pocket was located on the mesial, buccal, lingual and distal aspect of left mandibular M1.*

*Kuusi kuukautta operaation jälkeen vasemman ala-M1:n etupinnalla, posken puoleisella pinnalla, kielen puoleisella pinnalla ja takapinnalla on 1 mm:n ientasku.*

perpendicular to the tooth surface and parallel to the original alveolar margin. Vertical bone loss is the more aggressive form and occurs in an oblique direction, thus forming an infrabony pocket. Vertical bone loss may be one, two or three-walled, referring to the number of bony walls. In our case, the interdental occurring three-walled defect had a mesial, buccal and lingual wall. Vertical defects can generally be seen on dental radiographs. However, radiographs give a two-dimensional view of a three-dimensional object. For diagnosis we need to rely also on periodontal probing, especially at buccal and lingual/palatal aspect of the tooth.<sup>3,11</sup>

In dogs, common locations for vertical bone loss are palatal to maxillary canine teeth and interproximal to the mandibular first and second molar.<sup>1</sup> Although tooth

extraction and proper flap closure resolve the disease, the canines and mandibular first molars are good candidates for advanced periodontal treatment, in an attempt to preserve these strategically important and functionally and structurally valuable teeth.<sup>8</sup> In general, the morphology of the defect determines the treatment technique. Three-walled defects, as in this case, can often be treated with reconstructive periodontal surgery.<sup>9</sup>

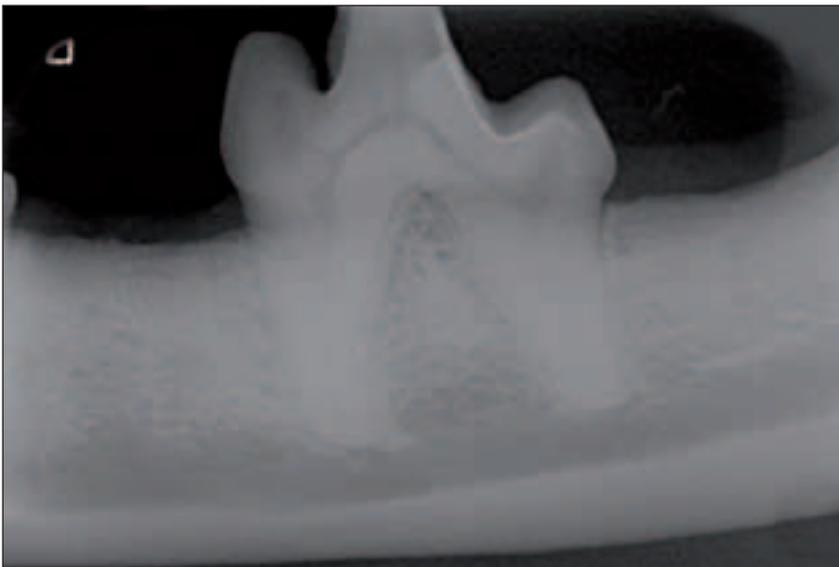
#### **Healing of the periodontal pocket**

After curettage and debridement, a blood clot immediately forms in the defect and starts the healing process. Cells from four tissues enter the clot: gingival epithelium, gingival connective tissue, alveolar bone and periodontal ligament. Only when periodontal

ligament cells arrive and proliferate coronally, new formation of cementum and periodontal ligament can occur and restore a healthy attachment. If bone cells are allowed to fill the defect, the most likely outcome is the formation of new alveolar bone that attaches directly to the cementum without necessarily forming a new periodontal ligament.<sup>12,13</sup>

#### **Phases of periodontal treatment**

In human dentistry, advanced periodontal therapy has six stages: preliminary phase, nonsurgical, phase I therapy with scaling and closed root planing, evaluation of response to the nonsurgical phase, surgical phase II therapy, restorative phase III therapy and maintenance phase IV therapy.<sup>4</sup> Thus, the decision about treating loss of attachment is delayed for a



**FIGURE 11 KUVA**

*Six months after surgery: new bone formation at the mesial aspect of left mandibular M1.*

*6 kuukautta operaation jälkeen vasemman ala-M1:n etupinnalla on uutta luun muodostusta.*

couple of weeks, until the effect of scaling and closed root planing or periodontal debridement is clear. In veterinary dentistry, the need of anesthesia, time and patient access are limiting factors. Therefore, phases must often be combined.<sup>1,16</sup>

At phase II, the vertical bone loss of the left mandibular M1 of our patient had reduced a lot due to through closed periodontal debridement. The owner had taken good care of the teeth since phase I procedure. Due to the owner's commitment to the treatment plan, the prognosis of further treatment of the left mandibular M1 was considered good.

### **Guided tissue regeneration**

The ideal outcome of periodontal surgery is the elimination of the pocket and reconstruction of the periodontal tissue. After periodontal debridement, epithelial cells are the fastest to migrate and regrow in the defect. While epithelial attachment is better than none, it is weak and disrupts easily. There-

fore, a stronger attachment with new bone formation and even periodontal ligament is desirable. The placement of a bone graft and membrane barrier prevents the migration of gingival epithelial cells into the healing periodontal pocket. Osteoblasts and periodontal ligament fibroblasts have a chance to repopulate the pocket and form periodontal ligament, cementum and new bone.

Barriers used in GTR are either synthetic resorbable, synthetic non-resorbable and naturally derived absorbable, which we used. This extracellular matrix membrane (ECM) consists of 90% of collagen and also glycosaminoglycans and growth factors, which support the growth and differentiation of many cell types. Although there are no published data of ECM used for oral surgical applications in veterinary patients, there are promising results of the use of ECM in other surgical applications.<sup>17-20</sup>

Defect-filling grafts promote

new bone growth.<sup>21,22</sup> Graft materials are classified as autografts (from the same individual), allografts (from same species), xenografts (from other species) and non-bone graft materials, and evaluated based on their osteogenetic, osteoinductive or osteoconductive potential. Osteogenesis refers to the formation of new bone by the cells contained in the graft, whereas osteoinduction is a chemical process, by which the molecules contained in the graft (for instance bone morphogenic protein) convert the neighboring cells into osteoblasts.<sup>9</sup>

Periodontal regeneration, including that of new bone, periodontal ligament and cementum, occurs more predictably with autografts and demineralized freeze-dried bone allografts than with other materials.<sup>23</sup> We chose the latter to avoid trauma to the donor and to achieve a favorable outcome. The material (Osteoallograft Periomix, Veterinary Transplant Services) contains osteoinductive demineralized bone matrix and cancellous bone chips that serve as an osteoconductive scaffold.

In this case, home care after GTR included meticulous daily tooth brushing, antiseptic oral rinsing and a 7-day course of systemic antibiotic therapy. While the use of systemic antibiotics is controversial,<sup>3,24</sup> oral hygiene after GTR is mandatory.<sup>1,8</sup> Moreover, owners' long-term commitment for daily homecare and continued removal of plaque is critical.<sup>1,8</sup>

In humans, GTR provides predictable reconstruction of the periodontium for the treatment of infrabony pockets, when performed adequately.<sup>25,26</sup> Additionally, several studies have shown good results with GTR in dogs.<sup>27-29</sup> In our case, GTR resulted in regeneration of alveolar bone into the defect surrounding the mesial root of the left mandibular M1, as confirmed radiographically 6

months after surgery. Only histological examination would reveal if new periodontal ligament was present. This is not possible in a clinical case. Currently, no veterinary clinical studies of GTR with histologically confirmed results are available.

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