Immunohistochemical Localization of Osteoclastogenic Cell Mediators in Feline Tooth Resorption and Healthy Teeth

David Senn, Dr med vet; Peter Schwalder, Dr med vet; Philippe Roux, Dr med vet; Dieter D. Bosshardt, PhD; Michael H. Stoffel; Dr Med vet

Summary:
Tooth resorption is among the most common and most challenging problems in feline dentistry. It is a progressive disease eventually leading to tooth loss and often root replacement. The etiology of tooth resorption remains obscure and to date no effective therapeutic approach is known. The present study is aimed at assessing the reliability of radiographic imaging and addressing the possible involvement of receptor activator of NFκB (RANK), its ligand (RANKL), and osteoprotegerin (OPG) in the process of tooth resorption. Teeth from 8 cats were investigated by means of radiographs and paraffin sections followed by immunolabeling. Six cats were diagnosed with tooth resorption based on histopathologic and radiographic findings. Samples were classified according to a four-stage diagnostic system. Radiologic assessment of tooth resorption correlated very strongly with histopathologic findings. Tooth resorption was accompanied by a strong staining with all three antibodies used, especially with anti-RANK and anti-RANKL antibodies. The presence of OPG and RANKL at the resorption site is indicative of repair attempts by fibroblasts and stromal cells. These findings should be extended by further investigations in order to elucidate the pathophysiological processes underlying tooth resorption that might lead to prophylactic and/or therapeutic measures. J Vet Dent 27(2); 75 - 83, 2010

Introduction
In addition to periodontal disease and chronic gingivostomatitis, tooth resorption (previously termed feline odontoclastotic resorptive lesions, FORL) are among the most common and frustrating diagnostic findings in feline dentistry.1,2 Reported prevalence rates of tooth resorption range from about 30 to 70 %, depending on the composition of the population examined and on the diagnostic tools used to establish the diagnosis of tooth resorption.3,4 The prevalence increases with age.3 Tooth resorption is associated with pain, gingivitis, ankylosis, and impairment of the periodontal ligament (PDL). Eventually, tooth resorption leads to crown and often root loss.3,5,6 To date, extraction of affected teeth is the treatment of choice.

Tooth resorption appears as defects in cervical enamel, dentin, and cementum. The resorption of these tooth substances is not due to carious decay but results from odontoclastic activity.7,8 Odontoclasts are tartrate-resistant, acid phosphatase (TRAP)-positive, multinucleated giant cells that fulfill the same function in dental tissues as osteoclasts in bone tissue.9

Odontoclasts are smaller in size than osteoclasts, possess fewer nuclei (2-8), and produce smaller resorption lacunae.10 Recent investigations on the regulation of osteoclastogenesis have focused on three cytokine-like proteins (Fig. 1). These proteins belong to the tumor necrosis factor (TNF) superfamily.11 The first is the receptor activator of NFκB ligand (RANKL), a type II transmembrane polypeptide expressed in lymphoid tissues and on leucocytes, osteoblasts, lymphocytes, and stromal cells.20-22 Its natural binding site is receptor activator of NFκB (RANK), a type I membrane receptor that is expressed by dendritic cells, foreskin fibroblasts, some T-cells, and osteoclasts and their precursor cells.23,24 Binding of RANKL to RANK on osteoclast precursor cells induces their differentiation into mature, activated osteoclasts, which initiates bone resorption.25 The third protein is osteoprotegerin (OPG). It is secreted by osteoblasts and stromal cells in a soluble form as both a monomer and a dimer and acts as a decoy receptor for RANKL.25,26,27,28 Thus, OPG inhibits osteoclastogenesis by preventing RANK-RANKL interaction.25 Its expression is regulated by calcitropic hormones and cytokines.11

The cell mediators RANK, RANKL, and OPG play a pivotal role in the regulation of osteoclastogenesis and bone alteration. Yet, to our knowledge, only very few studies have addressed a possible involvement of these mediators in the development of tooth resorption.9 The goals of this study, therefore, were to assess the reliability of the radiographic assessment of tooth resorption and to clarify whether the interaction of RANK-RANKL-OPG plays a role in this disease.

Materials and Methods
Thirty-eight mandibular teeth (25 premolar and 13 molar teeth) were obtained from eight animals aged 3 to 19-years (average = 10.6-years, one cat of unknown age), which were patients at the Small Animal Clinic of the University of Berne. The animals used were obtained dead or were euthanized for reasons not related to tooth resorption. One or both mandibles of each animal were taken with a parallel technique in order to classify each tooth into one of four radiographic groups (RG). After dissection, the mandibles were sectioned between the third and fourth premolar teeth and between the fourth premolar and first
molar teeth, respectively. Samples were fixed individually in 4% paraformaldehyde in 0.1 M phosphate buffer for 12-hours at 4°C.

Specimens were rinsed with phosphate buffered saline (PBS) and demineralized with Labonord DC3 for 10 to 12-days. Thereafter, teeth were embedded in paraffin according to standard protocols and 3-µm thick transverse sections were obtained and mounted on APES-coated glass slides. Sections were dewaxed with xylene and rehydrated through a descending series of ethanol ending with distilled water. Selected sections were stained with hematoxylin-eosin (H&E).

All incubation steps were carried out at room temperature using immunostaining chambers. Sections were incubated overnight with one of the primary polyclonal antibodies at a dilution of 1:50 in PBS containing 5% normal goat serum (NGS) and 0.5% casein. Sections were rinsed for 10-minutes with PBS. The primary antibodies were a rabbit anti-RANK-antibody, rabbit anti-RANKL-antibody, and a rabbit anti-OPG-antibody. As a secondary antibody, a goat anti-rabbit IgG conjugated to a peroxidase-labeled dextran polymer in Tris-HCl buffer was added for 30-minutes. After another two washes with PBS followed by two washes with dH2O, samples were incubated with chromogen (2.5 ml imidazole buffer, 100 µl DAB and three drops of 3% H2O2 in 22.5 ml H2O) for 15-minutes. Specimens were then washed twice with H2O, dehydrated through an ascending series of ethanol ending with xylene, mounted in Entellani and examined in a Zeiss Axioskop 2 equipped with a digital AxioCam HR and the corresponding Axio Vision II LM software.

As antibodies with known specificity for feline tissue were missing, commercially available products were used after thorough validation on tissues of known reactivity. Control experiments included omission of primary antibody and omission of both the primary as well as the secondary antibody. In addition, a rabbit anti-calcitonin antibody, a rabbit anti-CS3 antibody (E. coli surface antigen), and a mouse anti-CFA/I antibody (E. coli colonization factor antigen) were used as irrelevant substitutes for pertinent primary antibodies. Positive controls were carried out on bone samples of arthritic canine elbow joints. This protocol had previously been validated with reference to mouse bone.

Tooth resorption was classified according to a four-stage system based on histopathologic and radiographic findings. Teeth without resorptive lesions detectable by radiographs were assigned to radiographic group 1 (RG1). Group 2 (RG2) included all teeth with resorptions extending into cementum, dentin, or the pulp chamber. Lesions in this group showed areas of radiolucency with well-preserved PDL. Teeth exhibiting one or more of the following lesions were assigned to group 3 (RG3): dentoalveolar ankylosis, irregularities of the root surface, indistinct radiopacity, radiopacity with washy and spongy patterns in the area of the PDL. In teeth attributed to group 4 (RG4), the crown was lost and only root remnants, so-called “ghost roots,” were detectable. In these cases, exaggerated alveolar bone was present at the site of the missing crown. Occasionally, however, the alveolar bone had regressed (Fig. 2). When teeth were completely absent radiographically, they were considered to be missing for other reasons than tooth resorption and were assigned to RG1.

Figure 1
Illustration showing the immunohistochemistry activity of antibodies evaluated.

- **RANK**: Receptor Activator of Nuclear Factor-B is expressed on osteoclast precursor cells, mature osteoclasts, and chondrocytes.
- **RANKL**: RANK-Ligand is found on stromal cells, osteoblasts, osteocytes, osteoclasts, and endothelial cells.
- **OPG**: Osteoprotegerin is secreted by osteoblasts and stromal cells and it binds to RANKL.

Figure 2
Radiographs of the left and right mandibles of two 16-year-old cats. The fourth premolar tooth (308) is assigned to RG1, showing no signs of tooth resorption (arrow). Only ghost-roots remain from resorption (white arrowheads) designating the third premolar tooth (307) to RG4 (A). The fourth premolar tooth (408) shows a characteristic resorptive lesion of RG2 (circled area). The periodontal ligament space is no longer detectable (black arrowheads) designating the first molar tooth (409) to RG2 and RG3 (B).
Three to 5 full size transverse sections were used for histopathologic classification but no complete sets of serial sections were available. Teeth without signs of resorption (intact enamel, dentin, cementum and PDL) were assigned to histological group 1 (HG1) [Fig. 3]. Teeth with resorptive lesions of various depth and size were attributed to HG2. Group 3 (HG3) were cases in which resorptive activity and attempts of bone formation were concomitantly observed, with normal root dentin being adjacent to newly formed bone-like material. Group 4 (HG4) included teeth without crowns but that had root remnants covered by normal or hyperplastic gingival tissue.

Results

Teeth required extended demineralization times which led to loss of structural detail that compromised cellular analysis.

Although no complete sets of serial sections were available, full-size transverse sections enabled a comprehensive assessment of the teeth and periodontal ligament.

Sites of tooth resorption were characterized by numerous lacunae with the smallest measuring 15-µm in diameter. They merged occasionally to a size of 200-µm, and, in many cases, they contained amorphous material that completely filled up the lesion. This content was composed of erythrocytes, cellular debris, and amorphous material that could not be identified specifically. In contrast, larger lesions were often infiltrated by granulation tissue that was rich in fibroblasts, fibrocytes, and blood vessels (not shown). Some areas showed an accumulation of cells with a loss of tissue architecture only. In lesions close to the alveolar margin, the inflamed or hyperplastic gingiva communicated with the lacuna and its contents (Fig. 4). Out of 15 teeth showing histological signs of TR, the dental pulp was exposed in two cases.

When pertinent primary antibodies were substituted with irrelevant immunoglobulins, background labelling was virtually absent. This observation indicated that the protocols used were highly specific in detecting RANK, RANKL and OPG in feline...
teeth. However, weak immunostaining of epithelial tissue, blood vessels, and dental calculus was observed. This moderate cross-reactivity in specific locations did not interfere with interpretation of results as corresponding structures were clearly distinct from the regions and tissues of interest.

In HG 1, immunohistochemistry did not unveil any lesions in teeth that had been radiographically and histologically classified as being negative for tooth resorption (Fig. 3). Dental calculus, Malassez’s rests in the PDL, connective tissue, gingiva, and the content of blood vessels were slightly immunopositive for RANK in healthy teeth. RANKL yielded a stronger staining of the tissues mentioned above compared to RANK. In contrast, staining for OPG was virtually absent in healthy teeth.

In HG 2 lesions, the amorphous material attached to the surface of the resorption lacunae was distinctly immunopositive for RANK and RANKL, and slightly positive for OPG (Fig. 4). Malassez’s rests stained intensely for RANKL. Immunostaining was particularly strong towards the edge facing the resorptive front. In the granulation tissue, several cells close to the resorption front were immunopositive for RANKL and OPG. This signal was stronger than for erythrocytes, which were immunopositive in healthy teeth as well. The areas of the granulation tissue were devoid of any signal.

In HG 3, sequestered fragments of dentin were encircled by granulation tissue or newly formed bone-like tissue (Fig. 5). The periodontal space in such lesions was partly lost due to ankylosis between alveolar bone and root surfaces. Lesions assigned to HG 3 showed an indistinct staining pattern in accordance with their inconsistent organization. In areas with active resorption fronts and granulation tissue, immunopositive results were similar to that found in group 2. In other areas, where cancellous bone had already been formed, no immunostaining was observed at all.

In HG 4, former tooth roots were remodeled into loosely organized cancellous bone (Fig. 6). No periodontal space was left and the shape of the former root could only be estimated from the structural differences between mature and newly
formed bone. In some cases, the medullary cavity seemed to be enlarged at the expense of boney tissue. No explicit immunostaining was observed except in the hyperplastic gingival tissue. Immature bone-like material and the remaining components of original dentin were unstained.

When evaluating results in controls, immunostaining was negative when primary antibodies were omitted. Similarly, immunostaining was absent when rabbit anti-CS3 antibody, rabbit anti-calcitonin, and mouse anti-CFA/I antibodies were used as irrelevant substitutes for corresponding primary antibodies. Positive control experiments with RANK, RANKL, and OPG were carried out on bones of arthritic canine elbow joints and yielded specific staining patterns as reported in an earlier study.33

Two of 8 cats showed no radiographic or histopathologic signs of tooth resorption. The age of the 2 cats without lesions was 3-years in one case, and unknown in the other. Cats that showed lesions of group 4 were 16-years or older, whereas lesions of group 2 were found in cats of 8-years or younger except for one cat (16-years) that showed lesions consistent with groups 2, 3, and 4.

Radiologic examination of 38 teeth yielded 18 teeth (47 %) with at least one lesion. Two teeth showed group 2 as well as group 3 signs based on radiographic and histopathologic results and were recorded in both groups. Because two teeth disintegrated after demineralization, histologic sections were obtained from 36 teeth only. Of these teeth, 15 (42 %) exhibited one or more lesions in different stages of resorption. As sectioning required extensive demineralization, loss of structural detail was unavoidable. Therefore, assessment of cellular details was often difficult. Only 1 tooth showing a group 3 lesion based on histopathologic examination had a negative radiographic analysis. Whereas, 3 teeth that were radiographically positive for tooth resorption had no histopathologic signs of resorption. However, since complete sets of serial sections through whole teeth were not

---

**Figure 5**

Immunohistochemical micrographs of the same premolar tooth as shown in Figure 4 showing the disorganized architecture of a HG3-type lesion. The PDL is barely identifiable [H & E]. Staining for RANK reveals some areas with an immunopositive signal, especially in the more apical parts of the root (outlined area) [B]. There is no distinct signal (C and D) for RANKL and OPG [Original magnification = 2.5X].

---
available, false-negative histopathologic results may have occurred.

The covariance test revealed radiologic assessment of tooth resorption and histopathologic findings to be strongly correlated (correlation coefficient = 0.86) [Tables 1 and 2]. Radiographic and histopathologic signs of tooth resorption matched exactly in 32 of 38 teeth (84%). In particular, all teeth from RG4 were also assessed as group 4 lesions based on histopathologic assessment. Two teeth could not be evaluated due to disintegration artifact.

Discussion

Bone alteration by osteoclasts and osteoblasts is regulated by a number of signaling mediators including RANK, RANKL, and OPG that play a pivotal role.\textsuperscript{36-38} In contrast to the numerous studies addressing the impact of these molecules on structural adaptation of osseous tissue to strain, little information is available regarding resorption and repair of tooth substances and attachment of teeth. However, mice genetically deficient in RANK or RANKL not only developed severe osteopetrosis but also suffered from abnormal tooth eruption secondary to complete deficiency in osteoclast development.\textsuperscript{39} The present study was aimed at addressing a possible involvement of these mediators in tooth resorption by investigating their distribution in normal teeth and in tooth resorption using immunohistochemistry methods.

Together with maxillary premolar teeth, the mandibular premolar and molar teeth are commonly affected by tooth resorption and were the teeth investigated in this study.\textsuperscript{2,4,5,30,35,40,41} The diagnosis of tooth resorption relies on clinical and radiologic findings. The latter are essential since the prevalence of tooth resorption is greatly underestimated when based on oral examination without dental radiography.\textsuperscript{1,4,42} The assessment of the left (307) and right (407) third mandibular premolar teeth allows the overall tooth resorption status to be predicted correctly in 93% of cats.\textsuperscript{35} Animals examined in our study were sampled

---

**Figure 6**

Immunohistochemical micrographs of a premolar tooth where the crown is absent in HG4. Newly formed cancellous bone is covered by hyperplastic gingiva (A) [H & E]. Only the gingival epithelium has immunostaining [Original magnification = 2.5X].
without taking their dental status into account. Yet, post-mortem radiographs of split mandibles and light microscopy of single teeth indicated that 75% of cats studied in a small sample were affected by tooth resorption. This is in the upper range of data published previously on the prevalence of tooth resorption in living cats but this difference may be related to the sample size.3,4 Despite the fact that radiographs were taken in one projection only, our findings indicate that the combination of radiographs and histopathologic examination provide a sensitive tool to reliably identify tooth resorption post-mortem.

Distribution of RANK, RANKL and OPG was assessed by means of immunohistochemistry on paraffin sections of demineralized tissue. Distinction of four different groups of tooth resorption as suggested in the present study complies with previously published criteria.7,34 The American Veterinary Dentistry College (AVDC) Nomenclature Committee differs on the assumption that tooth resorption is a progressive condition (http://www.avdc.org/Nomenclature-current.pdf) and suggests a 5-stage classification system. In our investigation, AVDC stages 1 to 3 were considered together in RG 2 and HG 2, respectively. These early and closely related phases did not seem sufficiently different to justify their distinction within the context of the present study. AVDC stage 4 is similar to our groups 3 and AVDC stage 5 matches our groups 4. Radiologic findings reported herewith are in agreement with previous studies.35,40

Identification of the periodontal ligament (PDL) and distinction of tooth resorption from dentoalveolar ankylosis are problematic especially in aged cats. Additional radiographic findings such as radiolucent areas or irregularities of the tooth surface must be taken into account in order to support the diagnosis of tooth resorption.35 Yet, in this study, congruence between radiographic and histopathologic assessment was very good. Of the 4 teeth for which classification did not match, severity of tooth resorption was overestimated on radiographs in 3 cases and underestimated in 1 tooth. Because complete sets of serial sections through the whole tooth were not available, single lesions may have been under diagnosed using microscopy compared with radiographic assessment. These findings corroborate the high reliability of radiographs in establishing the diagnosis of tooth resorption.

Whether the different tooth resorption groups reflect progressive development or are associated with specific locations remains unclear.7,32 In our study, cats older than 6-years showed more lesions of groups 3 and 4, whereas in younger cats the overall tooth resorption status was milder and resorptive lesions of group 2 were more common. However, lesions of

<table>
<thead>
<tr>
<th>Cat</th>
<th>Age</th>
<th>Mandib. Tooth</th>
<th>Radiography</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unknown</td>
<td>PM 3 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 3 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td>2</td>
<td>6-years</td>
<td>PM 3 right</td>
<td>RG 3</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 2</td>
<td>HG 1</td>
</tr>
<tr>
<td>3</td>
<td>3-years</td>
<td>PM 3 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td>4</td>
<td>16-years</td>
<td>PM 3 right</td>
<td>RG 4</td>
<td>HG 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 3</td>
<td>HG 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 1</td>
<td>disintegrated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 3 left</td>
<td>RG 4</td>
<td>HG 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td>5</td>
<td>4-years</td>
<td>PM 3 right</td>
<td>RG 3</td>
<td>disintegrated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 1</td>
<td>HG 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 2</td>
<td>HG 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 3 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 left</td>
<td>RG 2</td>
<td>HG 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td>6</td>
<td>16-years</td>
<td>PM 4 right</td>
<td>RG 2 + 3</td>
<td>HG 2 + 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 2 + 3</td>
<td>HG 2 + 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 3 left</td>
<td>RG 4</td>
<td>HG 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 left</td>
<td>RG 3</td>
<td>HG 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 left</td>
<td>RG 3</td>
<td>HG 3</td>
</tr>
<tr>
<td>7</td>
<td>19-years</td>
<td>PM 3 right</td>
<td>RG 3</td>
<td>HG 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 3</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 3 left</td>
<td>RG 4</td>
<td>HG 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 left</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 left</td>
<td>RG 3</td>
<td>HG 1</td>
</tr>
<tr>
<td>8</td>
<td>8-years</td>
<td>PM 3 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM 4 right</td>
<td>RG 1</td>
<td>HG 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1 right</td>
<td>RG 2</td>
<td>HG 2</td>
</tr>
</tbody>
</table>

PM = premolar tooth; M = molar tooth; RG = radiographic group; HG = histopathologic group; bold lettering = radiographic and histopathologic findings were not congruent.

<table>
<thead>
<tr>
<th>Radiographic Signs n (%)</th>
<th>Histopathologic Signs n (%)</th>
<th>Number of Congruent Matches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>20 (53)</td>
<td>21 (58)</td>
</tr>
<tr>
<td>Group 2</td>
<td>6 (16)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Group 3</td>
<td>10 (26)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Group 4</td>
<td>4 (11)</td>
<td>4 (11)</td>
</tr>
</tbody>
</table>

RG = radiographic signs; HG = histopathologic signs
group 2 were usually observed at or coronal to the alveolar margin, whereas lesions of group 3 were most often noted apical to the cementoenamel junction. This observation is consistent with a previous study that reported 87.5 % of all resorptive lesions begin apical to the alveolar margin.1 It is also consistent with another study that showed resorptive lesions tend to be located in the apical third of canine tooth roots, whereas they are most commonly observed in the coronal third of the root in premolar and molar teeth.46 Lesions located near the alveolar margin were associated with a hyperplastic but non-inflammatory gingival reaction that was absent from lesions in more apical locations. This is in agreement with the contention that gingivitis and periodontitis are secondary to the exposure of dentin or the pulp to the oral cavity and that they result from bacterial colonization.7 Tooth resorption does not necessarily result in ankylosis. However, resorption in aseptic locations such as the cementoenamel junction and apical to it may be followed by replacement of cementum and dentin by bone and thus lead to ankylosis.7,12 As this reaction pattern results in group 4 lesions, a strong connection between specific locations and corresponding groups of tooth resorption is to be expected. This contention, however, does not preclude these locations from being affected preferentially in older cats.

Teeth undergoing resorption usually displayed a strong staining with all three antibodies used, especially with anti-RANK and anti-RANKL antibodies, reflecting a high activity in alteration of tooth substances. In some group 2 teeth, a very strong immunoreactivity for RANK was observed at active resorption sites in the amorphous material filling the resorptive lacunae at the margin facing the resorption front. This is where odontoclasts are expected to be located. However, odontoclasts were identified in only 1 of 5 highly active resorptive lesions (Fig. 4). This may be due in part to loss of structural detail during demineralization. Notwithstanding technical restrictions, we consider the low incidence of odontoclasts to reflect that resorption may not be maintained at a constantly high level but rather occurs in intermittent waves. Similar staining patterns were observed for both RANKL and OPG. This is indicative of the presence of fibroblasts and stromal cells. Moreover, secretion of OPG by odontoblasts may contribute to the signal. In addition, self-activation of odontoclasts via RANKL as reported for osteoclasts may be considered as for osteoclasts as reported for osteoclasts should be considered.11

The observation that RANK, RANKL and OPG were absent from some areas in lesions of group 3 is consistent with histopathologic findings of several studies that reported many teeth with resorption as in group 3 displayed signs of resorption and repair.7,10 Consequently, areas with high activity of resorption and/or repair showed some activity for all three proteins. The fact that no staining was observed in lesions of group 4 is compatible with the belief that neither resorption nor repair processes occur in these lesions.

Involvement of the RANK, RANKL and OPG system has been demonstrated in hard tissue alteration under both physiologic and pathologic conditions including the hard tissue resorption during shedding of human teeth.11,24,43 Furthermore, production of OPG and RANKL by odontoblasts and pulp cell lines in mice in vitro and in vivo has been reported.25 This indicates that mechanisms involved in bone and tooth alteration may be closely related. Yet, tooth resorption is not associated with bone resorption in hyperparathyroidism.8 However, the observation that levels of OPG mRNA are significantly higher in mature teeth than in alveolar bone provides a rationale for the observation that teeth do not undergo resorption and alteration when systemic parathyroid hormone levels are high.7 Thus, subtle and most likely local differences in regulation mechanisms seem to account for the uncoupling of tooth and bone alteration. Similarly, species differences in local factors such as mechanical forces acting during mastication might preferentially promote degeneration of the PDL in cats and secondarily initiate tooth resorption as has been suggested by several authors.8,47 In addition, high dietary vitamin D, low oxygen, and high pH all have been suggested to be involved in tooth resorption.46-48

The present study provides evidence for the occurrence of RANK, RANKL, and OPG in feline tooth resorption. Further investigations are recommended in order to elucidate how the results reported here may contribute to understanding the pathophysiological processes underlying feline tooth resorption and subsequent prophylactic and/or therapeutic measures.

References

Author Information
From the Division of Small Animal Surgery, and the Division of Veterinary Anatomy (Stoffel), University of Berne Veterinary School, POB 846, CH-3001 Bern, Switzerland; and, Department of Oral Surgery and Stomatology and Department of Periodontology (Bosshardt), University of Berne, School of Dental Medicine, Freiburgstrasse 7, CH-3010 Bern, Switzerland. Email: david.senn@kkh.unibe.ch

Acknowledgements
This work was kindly supported by the Fleitmann Foundation, by Provet AG (Lyssach, Switzerland), and by a generous gift of a private donor, Mrs. M. E., to Prof. Dr. P. Schawalder.

The present study provides evidence for the occurrence of RANK, RANKL, and OPG in feline tooth resorption. Further investigations are recommended in order to elucidate how the results reported here may contribute to understanding the pathophysiological processes underlying feline tooth resorption and subsequent prophylactic and/or therapeutic measures.

Philips Oralix 65S, 65kV, 32ms, Philips Healthcare Europe, DA Best, The Netherlands
Verridal, Blonay, Switzerland
3-Aminopropyltriethoxysilane, Sigma, Buchs, Switzerland
CoverplateTM System; Thermo Shandon, Zug, Switzerland
H-300, Santa Cruz Biotecology, Inc, Santa Cruz, California
FL-317, Santa Cruz Biotecology, Inc, Santa Cruz, California
H-249, Santa Cruz Biotecology, Inc, Santa Cruz, California
EnVision™ ready-to-use; DAKO, Zug, Switzerland
Merck, Dietikon, Switzerland
Carl Zeiss AG, Feldbach, Switzerland
Donated by Berna Biotech AG, Bern, Switzerland


