Original Research
Pediatric Dentistry

Immunohistochemistry of resorption and inflammation factors in the periodontal ligament of human deciduous teeth

Bianca Zimmermann dos SANTOS[a]
Rafael Cypriano DUTRA[b]
Adair Roberto Soares dos SANTOS[c]
Maísa CASARIN[d]
Eliane Maria GOLDFEDER[e]
Vera Lucia BOSCO[f]
Mabel Mariela Rodriguez CORDEIRO[g]

[a]Universidade Franciscana, School of Dentistry, Postgraduate Programme in Maternal and Child Health, Santa Maria, RS, Brazil.

[b]Universidade Federal de Santa Catarina – UFSC, Department of Health Science, Araranguá, SC, Brazil.

[c]Universidade Federal de Santa Catarina – UFSC, Department of Physiological Sciences, Araranguá, SC, Brazil.

[d]Universidade Federal de Pelotas – UFPel, Department of Semiology and Clinic, Pelotas, RS, Brazil.

[e]Universidade Federal de Santa Catarina – UFSC, Centre for Biological Sciences, Department of Morphological Sciences, Florianópolis, SC, Brazil.

[f]Universidade Federal de Santa Catarina – UFSC, Centre of Health Sciences, Department of Dentistry, Florianópolis, SC, Brazil.

[g]Universidade Federal de Santa Catarina – UFSC, Postgraduate Programme in Dentistry, Department of Dentistry, Florianópolis, SC, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Bianca Zimmermann Santos
E-mail: biancazsantos@hotmail.com

https://doi.org/10.1590/1807-3107bor-2022.vol36.0056

Submitted: March 9, 2021
Accepted for publication: December 1, 2021
Last revision: January 26, 2021

Abstract: The understanding of the biological mechanisms involved in root resorption in deciduous teeth is important to the future development of preventive measures and treatments of this condition. The aim of the present study was to compare the expression and immunostaining of iNOS, MMP-9, OPG and RANKL in the periodontal ligament (PDL) of deciduous teeth with physiologic root resorption (GI), inflammatory pathological root resorption (GII) and permanent teeth (GIII), the negative control. Teeth in GI (n = 10), GII (n = 10) and (GIII) (n = 10) were submitted to immunohistochemical analysis to determine the expression of iNOS, MMP-9, OPG, and RANKL. The immunostaining was analysed by optical density. Statistical analysis included one-way ANOVA, followed by Student-Newman-Keuls post hoc test ($p < 0.05$). The results showed that iNOS, MMP-9 and RANKL expression in the PDL was higher in GII compared to GI and GIII ($p < 0.05$). Moreover, RANKL expression was higher in GI compared to GIII ($p < 0.001$), while OPG immunolabelling was lower in GII compared to GI and GIII ($p < 0.001$). The PDL of deciduous teeth bearing inflammatory processed exhibited upregulation of resorption-associated factors as well as enzymes related to tissue degradation which, in turn explains the exacerbation and greater susceptibility of those teeth to root resorption process.

Keywords: Tooth Resorption; Pediatric Dentistry; Root Resorption; Dentistry.

Introduction

Inflammatory root resorption is characterized by the gradual loss of dental tissue associated with persistent, progressive radiolucency of the adjacent alveolar bone.[1] Its prevalence in children is 16.3%,[2] and inflammatory deciduous root resorption is often associated with imbalances in the stomatognathic system, early loss of primary teeth, unerupted permanent successors, and coronal discoloration.[1,3] However, despite the severe consequences, inflammatory deciduous root resorption has not been considered a major concern in pediatric dentistry and in immunological studies.[2,4,5]

Previous studies have demonstrated that the mechanism of dental tissue resorption is similar to that observed in bone tissue.[5-9] The main biological difference between teeth and bones is the latter undergo constant physiologic renewal, whereas teeth only undergo physiologic resorption in the deciduous dentition,[10] and permanent teeth do not undergo resorption, except under pathologic conditions.

Osteoclasts are the cells responsible for bone resorption, while odontoclasts (cementoclasts and dentinoclasts), are responsible for physiologic and pathologic tooth resorption.[11] Cell differentiation and the function of odontoclasts in both physiological and inflammatory root resorption seem to be regulated by cytokines involved in the receptor activator of nuclear factor kappa-B ligand/receptor activator of nuclear factor kappa-B/osteoprotegerin (RANKL/RANK/OPG) pathway.[5,12] The activation of induced nitric oxide synthase (iNOS), is stimulated by pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and bacterial products.[13] It produces nitric oxide (NO), which acts in the induction of apoptosis and the inhibition of mitochondrial respiration and has a cytotoxic effect.[14] Previous research has shown a correlation between NO production and bone loss during the development of periodontal disease and periapical lesions.[15] Moreover, matrix metalloproteinase 9 (MMP-9), a proteolytic enzyme, has been shown to be associated with the progression of tooth resorption and tissue degradation.[16] However, no studies till date have evaluated the involvement of the processes in deciduous tooth resorption.

Studies have been carried out to understanding of the cellular and molecular mechanisms involved in the triggering and progression of tooth resorption.[5,10,12] However, questions remain, especially with regard to the deciduous teeth, which could help to develop preventive and treatment approaches. Research in this area may change the future of Dentistry, as the biomolecular knowledge of dental resorption gradually allows the development of biological therapies that control or prevent resorption, thus avoiding the loss of the affected tooth and its consequences. In this sense, long, invasive and costly treatments would not be necessary. Therefore, the aim of the present study was to compare the expression of iNOS, MMP-9, OPG and RANKL in the periodontal ligament (PDL) of deciduous teeth with physiologic root resorption, deciduous teeth with inflammatory pathological root resorption, and permanent teeth (no resorption).

Materials and methods

Twenty deciduous teeth were obtained from five-to-nine-years-old patients treated at the Paediatric Dentistry Clinics of Universidade Federal de Santa Catarina (UFSC) (Brazil). These were divided into two groups, GI and GII, consisting of 10 healthy teeth with physiologic resorption and 10 teeth with peri-radicular lesions and inflammatory pathologic resorption, respectively. Additionally, a negative control group (GIII) consisting of 10 healthy fully erupted third molars with complete root formation obtained from patients aged 20–25 years and treated at the Dental Surgery Clinics of the UFSC was also created. All teeth were extracted for reasons unrelated to the present study.

This study received approval from the Human Research Ethics Committee of the UFSC (Brazil) (under process number 113/09). The material was obtained following patient's agreement to donate the teeth and the signing of a statement of informed consent by the patients or legal guardians.

The inclusion criteria were as follows: a) for all teeth – no history and clinical detection of periodontal disease, trauma, root canal or orthodontic treatment; b) related to patients – no systemic disease that could affect the resorption process, such as diabetes, thyroid conditions or other hormonal conditions; c) for deciduous teeth – at least half of the root structure remaining; and d) for healthy teeth (GI and GIII) – no signs of de-mineralization due to caries or any loss of crown structure.

Preparation for histological analysis

Following the extraction, macroscopic analysis was performed through direct visual inspection, observing the morphological characteristics of the

external surface of the roots and adhered tissues. Immediately after the extracted and the visual inspection, the teeth were fixed in a buffered 4% paraformaldehyde solution for 24 hours at 4°C and then decalcified with 10% EDTA solution, pH 7.2, at room temperature, with the solution changed daily. Following decalcification, cross-sectional cuts were used to separate the roots from the crows and create two root fragments (cervical and apical), for histological processing, embedment in paraffin, and sectioning on a microtome (McBain Instruments, Chatsworth, USA). The slices (thickness: 3 to 4μm) were then mounted on positively charged slides prepared by immersion in a 5% silane solution (3-aminopropyltriethoxysilane; Sigma-Aldrich, São Paulo, SP, Brazil) in acetone (v/v), which were kept in an oven at approximately 50°C for one hour to allow fixation of the histological sections. The sections were de-paraffinized through consecutive immersions in xylene and rehydrated in decreasing concentrations of alcohol (100%, 90%, and 70%). One section from each sample was stained with hematoxylin-eosin (HE), while and the others were reserved for the immunohistochemical analysis. The analysis of localization of resorptive areas on root surface, morphologic and microscopic characteristics of periodontal tissue, presence of clastic cell and Howship lacunae were initially analysed in HE. The HE-stained slides were analyzed by light microscopy, with 20x, 40x, 100x and 400x magnifications.

Immunohistochemical reaction

Upon completion of aforementioned procedures, endogenous peroxidase was blocked with a 1.5% hydrogen peroxide solution in absolute methanol (v/v) for 20 minutes (min) to avoid any non-specific reactions. The sections were washed in distilled water and submitted to antigen reactivation to recover the antigen sites covered by fixation and the embedment of the tissue in formalin and paraffin. The slides were immersed in citrate buffer (0.01 M, pH 6.0) for 40 min in water bath at a temperature of 95°C to 98°C, immediately removed and kept at room temperature for 20 min and washed in distilled water and phosphate buffer solution (PBS).

Immunodetection of the proteins of interest was performed using the following antibodies: anti-, USA), anti-metalloproteinase 9 (MMP-9,1:500; Abcam, Cambridge, MA, USA), anti−osteoprotegerin (OPG,1:50; Santa Cruz Biotechnology, Santa Cruz, USA) and anti-RANKL (1:100; Santa Cruz Biotechnology, Santa Cruz, USA). The solution containing the antibodies was placed on the tissue sections and the slides were kept in a humidity chamber for 12 to 18 hours at 2°C to 8°C. The sections were then washed with PBS and the histological slides were incubated in a humidity chamber for 60 min at room temperature with the biotinylated secondary antibody [goat anti-IgG, rabbit anti-IgG, or mouse anti-IgG (Santa Cruz, Dallas, USA)], which was selected based on the primary antibody (Dako Cytomation, Carpinteria, USA).

The sections were washed in PBS, incubated with streptavidin-biotin-peroxidase (Dako Cytomation, Carpinteria, USA) for 40 min and washed again with PBS. Immunodetection was completed using a chromogen solution containing 0.03% 3,3′-diaminobenzidine (3, 3′, 4, 4′-tetraaminobiphenyl tetrahydrochloride) (DAB, Dako Cytomation, Carpinteria, USA) and hydrogen peroxide (Sigma Chemical Co., St. Louis, USA). Counterstaining was performed with Harris' hematoxylin solution and the samples were dehydrated using increasing concentrations of alcohol (70%, 80%, 90% and 100%), cleared in xylene and mounted in a permanent mounting medium (Entellan, Merck, Frankfurt, Darmstadt, Germany). The expression of iNOS, MMP-9, RANKL and OPG was extracellular.

Microscopic analysis

The results were documented using a digital camera (Sight DS-5ML1) coupled to an Eclipse 50i light microscope (Nikon, Melville, USA). Adjustments for the acquisition of the images were the same for all groups, considering the same marker. The slides were observed under 20X, 40X, 100X and 400X magnification to allow localization of resorptive areas on the root surface; morphologic characteristics of periodontal tissues; presence of clastic cells and Howship lacunae; expression of iNOS, MMP-9, OPG and RANKL and their spatial expression in

the PDL. Analysis of the antigen-antibody reaction, was carried out using 10 images were obtained from the histological sections of 10 teeth per group. The selected histological slides were those that showed areas of greater expression of the OPG, RANKL, iNOS, and MMP-9, and the images were analyzed using the NIH ImageJ 1.36b program (National Institutes of Health, USA). Brown coloration at the site of the antibody labelling was indicative of a positive reaction. OPG, RANKL, iNOS and MMP-9, levels were analyzed at a magnification of 400x by determining the total pixel intensity and expressing these as optical density (OD). All the analyses were performed by a single trained and calibrated researcher with experience in histological analysis.

Data were expressed as mean ± standard error of the mean (SEM) of 10 teeth per group. The statistical analysis was performed with one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls *post hoc* test. The level of significance was set at 5% ($P<0.05$). The statistical analysis was carried out using the Graphpad Prism 4 program (GraphPad Software Inc., San Diego, USA).

Results

Morphologic characteristics of periodontal tissues

No significant macroscopic differences were detected among the different groups regarding the morphology of the tissue adhered to the dental roots following extraction (Figure 1A-C).

Microscopic analysis of the periodontal tissues showed similar histological characteristics in areas without resorption in the deciduous and permanent teeth (Figure 2A-C). However, all specimens from deciduous teeth exhibited areas of resorption (Howship lacunae). These areas were associated with an organised PDL (Figure 2D) or a ligament with signs of repair (Figure 2E) in GI, whereas the lacunae in GII contained more clastic cells and completely disorganized or absent tissue (Figure 2F).

Expression of iNOS

iNOS labelling was observed in the areas of root resorption in the apical fragments in GI, but with a lesser degree than that found in GII (Figure 3A).

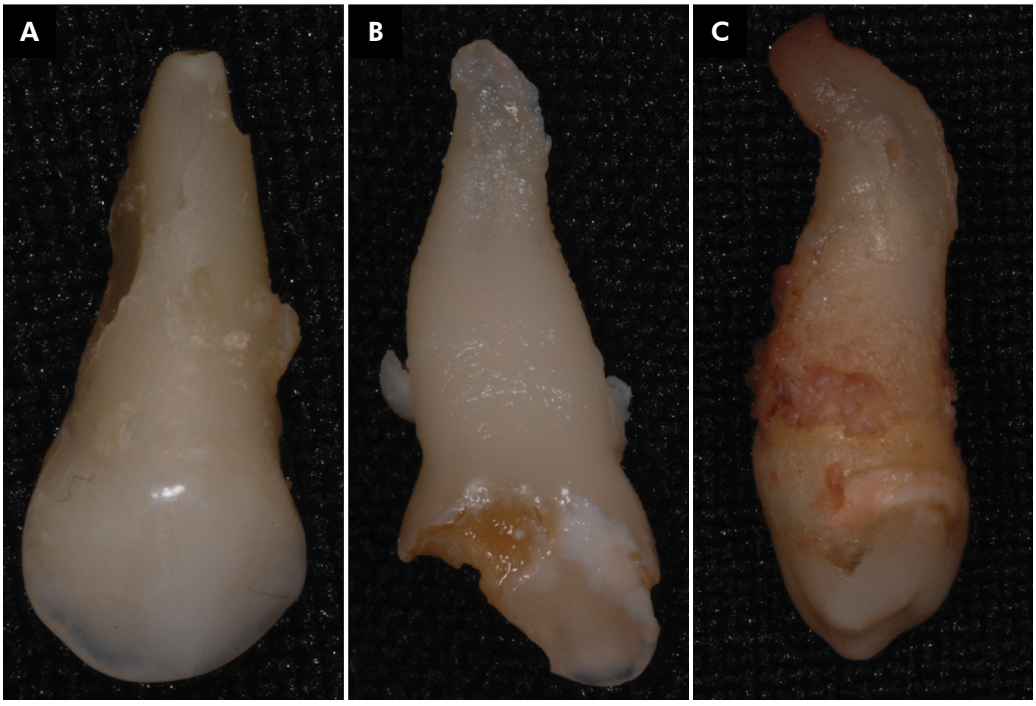

Figure 1. Macroscopic image of group I (deciduous teeth with physiologic root resorption), group II (deciduous teeth with inflammatory pathological root resorption, and group III (permanent teeth, control group) in the buccal site.

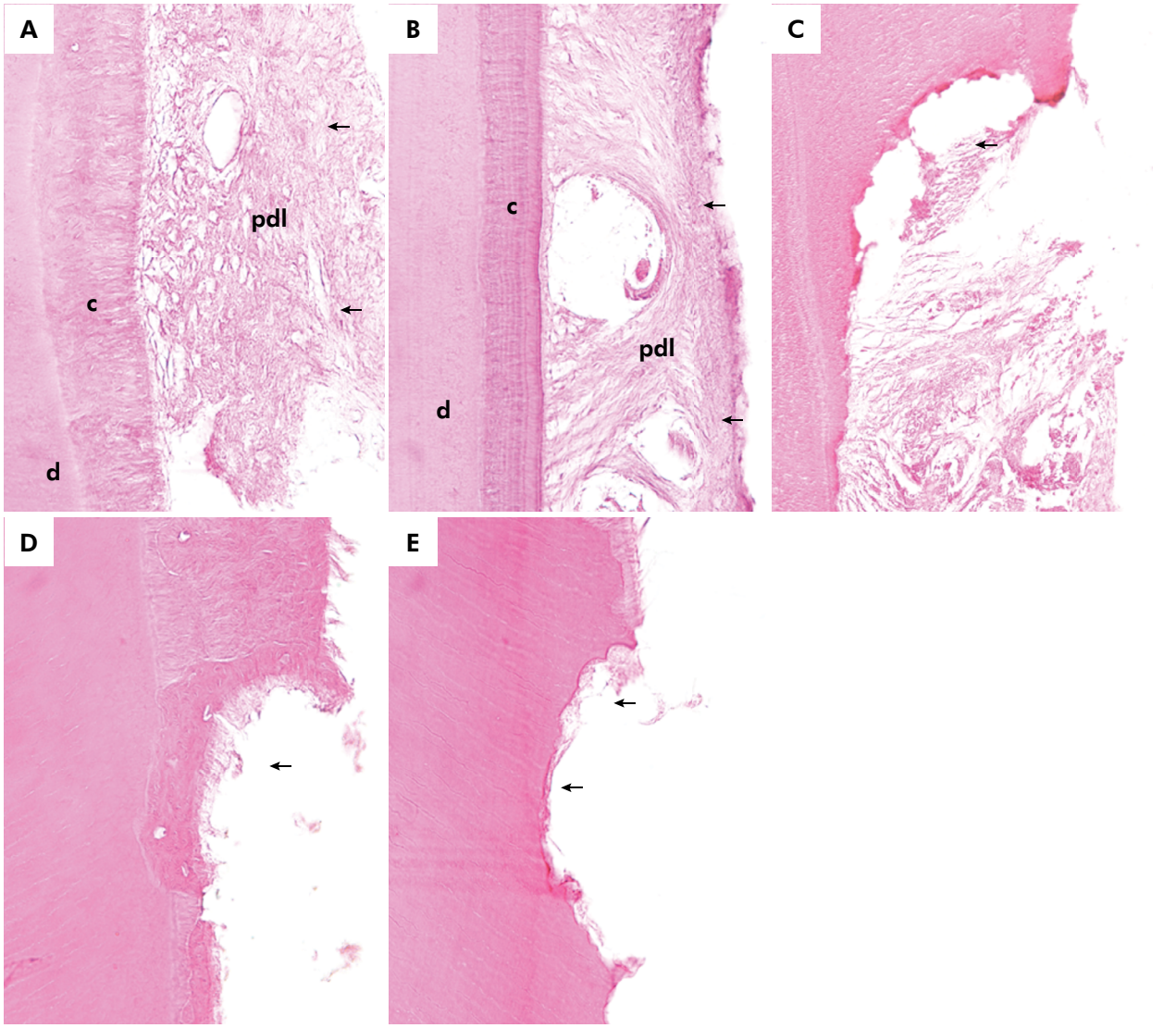

Figure 2. Photomicrography of PDL of human deciduous and permanent teeth; (A) PDL of deciduous molar with physiologic root resorption (magnification: 40x); (B) PDL of deciduous molar with inflammatory pathologic root resorption (magnification: 40x); (C) PDL of permanent molar (magnification: 40x); (D) Howship lacuna on root surface of deciduous molar with physiologic root resorption associated to organised PDL (magnification: 20x); (E) Howship lacuna on root surface of deciduous molar with physiologic root resorption associated to PDL with signs of repair (magnification: 40x); (F) Howship lacuna on root surface of deciduous molar with inflammatory pathologic root resorption, completely disorganised or with absent periodontal tissue (magnification: 20x); d: dentine; c: cementum; pdl: periodontal ligament; HE staining.

The greatest expression of iNOS was seen in the Howship lacunae in the PDL in GII (Figure 3B). Very mild iNOS expression was found in GIII, denoting a normal constitutive status of the enzyme (Figure 3C). The OD graph (Figure 3D) demonstrates that iNOS immunolabelling in the PDL of GII was significantly greater than that found in GI ($p < 0.05$) and GIII ($p < 0.05$) with no significant differences between the latter two groups.

Expression of MMP-9

MMP-9 expression followed the same immunolabelling pattern as that of iNOS, with much less intensive labelling observed in the Howship lacunae and PDL in GI (Figure 3E) in comparison to GII (Figure 3F) and very mild MMP-9 expression in the periodontal tissue in GIII (Figure 3G). The OD graph (Figure 3H) demonstrates that MMP-9 expression in the PDL was significantly greater in

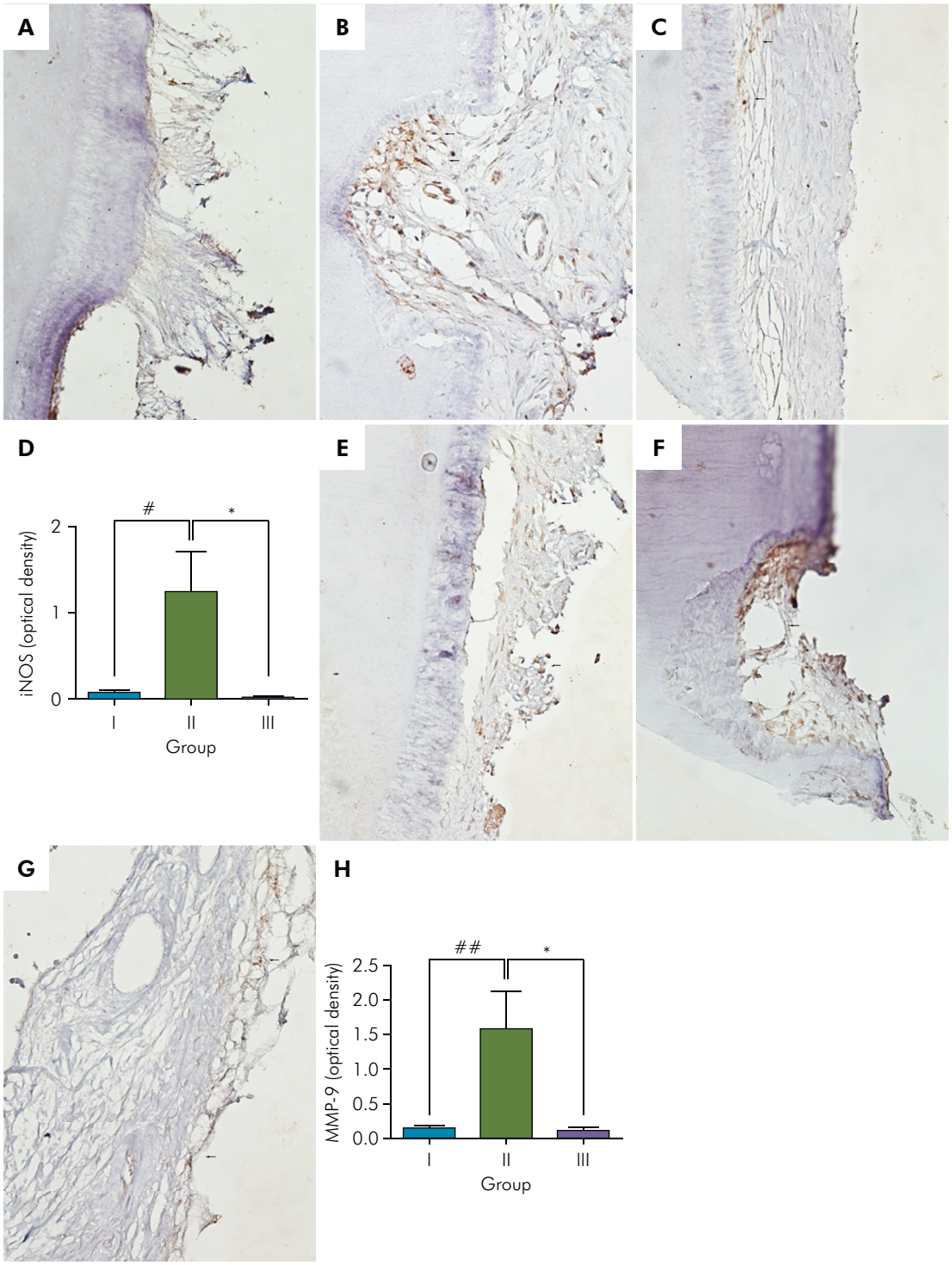

Figure 3. Expression of iNOS and MMP-9 in PDL of human deciduous and permanent teeth; (A) moderate expression of iNOS in area of root resorption in apical fragment in Group I (magnification: 400x); (B) Strong expression if iNOS in Howship lacunae in the PDL in Group II (magnification: 400x); (C) mild expression of iNOS in PDL in Group III (magnification: 400x); (D) comparison of iNOS expression in periodontal tissue in different groups; (E) expression of MMP-9 in PDL of cervical fragment in Group I much less intense than (F) in Howship lacunae in cementum and dentine of apical fragment in Group II (magnification: 400x); (G) mild labelling of MMP-9 in PDL in Group III (magnification: 400x); (H) comparison of MMP-9 expression in periodontal tissue in different groups; data expressed as mean ± SEM of 10 teeth/group; #p < 0.05 and ##p < 0.001 versus Group I and *P<0.05 versus Group II (one-way ANOVA, followed by Student-Newmann-Keuls post hoc test); immunohistochemical analysis from A to C and E to G.

GII in comparison to GI ($p < 0.001$) and GIII ($p < 0.05$) with no significant difference found between the latter two groups.

Expression of OPG

Lower OPG expression was observed in the PDL of the apical fragments in comparison to the cervical fragments in GI (Figure 4A-B). Minimal OPG labelling was found in the fragments in GII (Figure 4C), regardless of the region analyzed (cervical or apical). The greatest labelling of this protein was detected in the PDL in GIII (Figure 4D). The OD graph (Figure 4E) demonstrates that OPG expression in the PDL was considerably lower in GII in comparison to GI ($p < 0.001$) and GIII ($p < 0.001$), with no statistically significant differences between these two latter groups.

Expression of RANKL

RANKL expression was detected in the Howship lacunae in both groups of deciduous teeth (Figure 4F-H), with greater labelling being observed in the apical fragments of GII (Figure 4G-H). Minimal RANKL expression was detected in some areas of the PDL in GIII (Figure 4I). The OD graph (Figure 4J) showed that RANKL expression in the ligament was significantly greater in GII in comparison to GI ($p < 0.05$) and GIII ($p < 0.001$). Moreover, a significant difference was found between the latter two groups, with greater expression in GI ($p < 0.001$).

Discussion

The results of the present study demonstrate that the macroscopic and microscopic morphology of the PDL in areas without resorption is quite similar in deciduous and permanent teeth. However, the PDL of deciduous teeth bearing inflammatory process exhibit upregulation of resorption-associated factors as well as enzymes related to tissue degradation.

While the root resorption process in deciduous teeth is a physiological mechanism that is important for the replacement of deciduous dentition with permanent teeth, pathologic root resorption in both deciduous and permanent teeth, especially when caused by caries, can cause irreversible harm to dental tissues and even tooth loss.[1,3,4] Therefore, studies investigating these processes can contribute to the establishment of new, more effective clinical conduct for the control and treatment of pathologic root resorption.

The onset and progression of resorption processes in both deciduous and permanent teeth are orchestrated by cellular and molecular mediators that are not fully understood and act in the local microenvironment, modulating the tissue response. Such mediators include iNOS, the RANK/RANKL/OPG pathway and metalloproteinases.[4,12,13,16]

The effect of iNOS induction, in bony tissue is complex, affecting the recruitment, proliferation, differentiation, activity and survival of osteoclasts and osteoblasts. Previous studies have demonstrated that high concentrations of NO may induce apoptosis of osteoclasts and osteoblasts, exerting an inhibitory effect on the expression of RANKL.[17,18] However, in the present study, the group of deciduous teeth affected by inflammatory pathologic resorption exhibited greater iNOS and RANKL expression compared to the other groups. A possible explanation for this is the fact that the highly cytotoxic NO produced in a large quantity and for a prolonged period of time by iNOS triggered the acceleration of the death of cells that protect the root, as cementoblasts and odontoblasts, in this group.[15] Thus, although NO may cause the death of odontoclasts, it also facilitates the installation of bone remodelling units composed of clastic cells as it causes the destruction of root protecting cells.

Although the presence of iNOS inhibitors is known to lead to a reduction in the intensity of the inflammatory processes[19] involved in periapical lesions, periodontal disease and inflammatory pathologic root resorption. On the other hand, the presence of iNOS inducers, such as IFN-γ, is associated with inflammatory processes and resorption in dental tissue.[9,20,21] Therefore, NO can stimulate inflammatory and clastic activity and may be considered necessary for the adequate function of osteoclasts and osteoblasts.[22]

TNF-α and IL-1 regulate the balance between RANKL and OPG by stimulating the expression of the former and reducing the expression of the latter[23] and can stimulate the expression of iNOS.[24] Therefore,

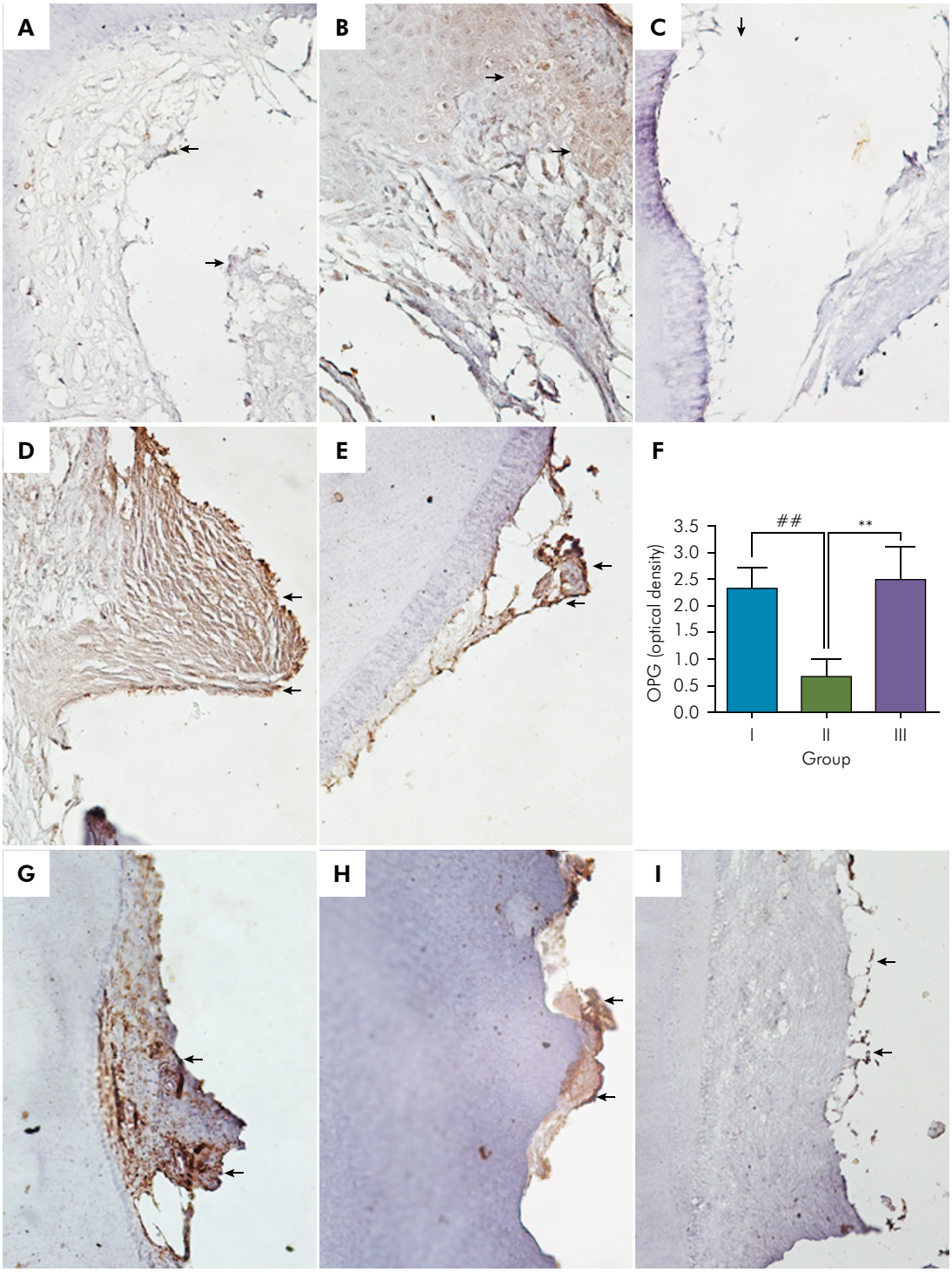

Figure 4. Expression of OPG and RANKL in PDL of human deciduous and permanent teeth; (A) labelling of OPG in PDL of areas of resorption of root surface in apical fragment and (B) cervical fragment in Group I revealing greater expression in cervical fragment (magnification: 400x); (C) absence of OPG expression in PDL in Group II (magnification: 400x); (D) strong expression of OPG in PDL in Group III (magnification: 400x); (E) graph comparing optical density in OPG expression among groups; (F) clear expression of RANKL in PDL of apical fragment in Group I, but to lesser degree in comparison to (G-H) resorption area of apical fragment in Group II (magnification: 400x); (I) minimal RANKL expression in PDL of permanent molar (magnification: 400x); (J) comparison of RANKL expression in periodontal tissue in different groups; data expressed as mean ± SEM of 10 teeth/group; #$p < 0.05$ and ##$p < 0.001$ versus Group I; **$p < 0.001$ versus Group II; $\Delta\Delta P < 0.001$ versus Group III (one-way ANOVA, followed by Student-Newmann-Keuls post hoc tests; immunohistochemical analysis from A to D and F to I.

although studies are underway to determine the precise participation of NO in inflammatory processes in dental tissues, its mechanism of action is still not fully understood.[14,19,24]

RANKL is known to play an important role in permanent tooth resorption through differentiation and activation of clastic cells[2,5,25] binding to its RANK receptor, and greater RANKL expression is related to mineral loss in bone and teeth.[5-8,12,23,26] Evidence shows that OPG may be a limiting factor to resorption[1,2,5,8] due to its capacity to bind to RANKL, thereby impeding the RANK-RANKL binding. In the present study, significantly greater RANKL expression and lesser OPG expression was found in the deciduous teeth with inflammatory pathologic root resorption, which is likely related to the greater tissue destruction and loss in these teeth and is consistent with the greater number of Howship lacunae in comparison to the deciduous teeth with physiologic resorption and healthy permanent teeth. Moreover, the significantly greater expression of RANKL in the deciduous teeth with physiologic root resorption in comparison to the permanent teeth may be explained because healthy permanent teeth do not undergo root resorption.

In permanent teeth, *previous in vivo* studies have demonstrated that RANKL is a mediator of alveolar bone loss in periodontal disease[2,5,25] as well as tooth resorption caused by orthodontic forces.[8,26,27] OPG has proven quite effective at blocking RANKL activity and reducing bone loss in induced periodontal disease and tooth resorption caused by orthodontic forces.[2,5,8,26,27] Evaluation of the activity of RANKL antagonists in the inhibition of bone resorption and tissue inflammation in experimentally induced periodontitis showed that the treatment of the condition with OPG was not limited to the inflammatory process and also induced a significant increase in fibroblasts in the PDL, possibly by inhibiting cell apoptosis.[8]

Investigating the expression of RANKL and OPG in cells of the PDL of human deciduous teeth with and without physiologic resorption and healthy permanent teeth, researchers report greater RANKL and lesser OPG expression in the teeth with resorption in comparison to the other groups.[5] Other researcher report a difference in OPG expression in the PDL of deciduous teeth with physiologic or inflammatory root resorption and healthy teeth, with greater expression in the group of permanent teeth, but the protein was also expressed in areas without evident resorption in the ligament of the cervical region of deciduous tooth roots.[10] It is suggested that the most apical region has greater clastic activity than the cervical one. However, few studies describe the clastic activity in primary teeth, for this reason the present study divided the roots (cervical and apical) into two sections.

The differentiation of clastic cells seems to be regulated by the expression of RANKL and OPG.[27] Therefore, the RANK/RANKL/OPG pathway plays a crucial role in the physiopathology of bone and tooth resorption, specifically acting on the mediation of alveolar bone loss and mineral loss from dental tissue. It has also been proposed that approaches that impede the binding between RANK and RANKL could prevent the destruction of dental and bone tissues, thus impeding their future loss.[28]

This is the first study to determine the expression of MMP-9 in the periodontal tissue of human deciduous teeth exhibiting physiologic and inflammatory pathologic root resorption. When activated, clastic cells release specific substances that solubilize the mineral matrix of dental roots, and it is hypothesized that MMP-9 is one of them, since it is associated with tissue degradation. This hypothesis is supported by the findings of the present study, as significantly greater MMP-9 expression was found in the group of deciduous teeth with inflammatory pathologic root resorption, which exhibited greater tissue damage. Moreover, these teeth exhibited a greater expression of both iNOS and RANKL expression, which are associated with clastic activity. In fact, some authors suggest that the expression of MMP-9 is increased in odontoclasts during the root resorption process.[16]

In qualitative terms, greater RANKL, iNOS and MMP9 labelling was generally found in the apical fragments of the deciduous teeth, likely because this is the region in which dental tissue destruction and loss begins in the resorption process. It is suggested that the apical region showed greatest clastic activity. Moreover, the fact that OPG acts as a protective factor against resorption processes explains its greater expression in the cervical fragments of the

deciduous teeth (which are more intact) and in both fragments of the permanent teeth. In conclusion, the PDL of deciduous teeth with associated inflammatory process displays a greater expression of factors involved in clastic differentiation and enzymes related to tissue degradation, which translates to an exacerbation of and greater susceptibility to root resorption process. The PDL of deciduous teeth with physiologic resorption also display the labelling of molecules of clastic activity and tissue destruction, but the expression of these molecules appears to be more controlled.

This study present limitations. This observational study was carried out based on the collection of samples and immunohistochemical marking of some molecules related to the root resorption process, however there was no emphasis on a specific signaling pathway. However, it should be noted that this was not the objective of the study, and the preliminary analysis of some molecules are fundamental to know the physiological and pathological inflammatory root resorption process. Comparison of the apical and cervical fragments was not realized in different groups, as wells as, the comparison of the apical and cervical fragments within the same group, were only analysed in qualitative terms. Another limitation is that there was no group that studied root resorption by replacement.

Conclusion

The PDL of deciduous teeth with associated inflammatory process displays a greater expression of factors involved in clastic differentiation and enzymes related to tissue degradation, which translates to an exacerbation of and greater susceptibility to root resorption process. Future studies should evaluate root resorption by replacement and the molecular pathways involved in the resorption process.

Acknowledgments

This study was supported by the Brazilian fostering agency Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC – T.O. 6335/2011-5).

References

1. Cardoso M, Rocha MJ. Identification of factors associated with pathological root resorption in traumatized primary teeth. Dent Traumatol. 2008 Jun;24(3):343-9. https://doi.org/10.1111/j.1600-9657.2007.00554.x
2. Vieira-Andrade RG, Drumond CL, Alves LP, Marques LS, Ramos-Jorge ML. Inflammatory root resorption in primary molars: prevalence and associated factors. Braz Oral Res. 2012 Jul-Aug;26(4):335-40. https://doi.org/10.1590/S1806-83242012000400009
3. Fuss Z, Tsesis I, Lin S. Root resorption: diagnosis, classification and treatment choices based on stimulation factors. Dent Traumatol. 2003 Aug;19(4):175-82. https://doi.org/10.1034/j.1600-9657.2003.00192.x
4. Qassem A, Goettems M, Torriani DD, Pappen FG. Radicular maturity level of primary teeth and its association with trauma sequelae. Dent Traumatol. 2014 Jun;30(3):227-31. https://doi.org/10.1111/edt.12072
5. Fukushima H, Kajiya H, Takada K, Okamoto F, Okabe K. Expression and role of RANKL in periodontal ligament cells during physiological root-resorption in human deciduous teeth. Eur J Oral Sci. 2003 Aug;111(4):346-52. https://doi.org/10.1034/j.1600-0722.2003.00051.x
6. Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M Jr, Kostenuik PJ, et al. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. J Periodontol. 2007 Jul;78(7):1300-8. https://doi.org/10.1902/jop.2007.070073
7. Uchiyama M, Nakamichi Y, Nakamura M, Kinugawa S, Yamada H, Udagawa N, et al. Dental pulp and periodontal ligament cells support osteoclastic differentiation. J Dent Res. 2009 Jul;88(7):609-14. https://doi.org/10.1177/0022034509340008
8. Yuan H, Gupte R, Zelkha S, Amar S. Receptor activator of nuclear factor kappa B ligand antagonists inhibit tissue inflammation and bone loss in experimental periodontitis. J Clin Periodontol. 2011 Nov;38(11):1029-36. https://doi.org/10.1111/j.1600-051X.2011.01780.x
9. Ribeiro FV, Mendonça AC, Santos VR, Bastos MF, Figueiredo LC, Duarte PM. Cytokines and bone-related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. J Periodontol. 2011 Aug;82(8):1187-96. https://doi.org/10.1902/jop.2011.100643
10. Cordeiro MM, Santos BZ, Reyes-Carmona JF, Figueiredo CP. Primary teeth show less protecting factors against root resorption. Int J Paediatr Dent. 2011 Sep;21(5):361-8. https://doi.org/10.1111/j.1365-263X.2011.01132.x
11. Väänänen K. Mechanism of osteoclast mediated bone resorption—rationale for the design of new therapeutics. Adv Drug Deliv Rev. 2005 May;57(7):959-71. https://doi.org/10.1016/j.addr.2004.12.018

12. Kanzaki H, Chiba M, Arai K, Takahashi I, Haruyama N, Nishimura M, et al. Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. Gene Ther. 2006 Apr;13(8):678-85. https://doi.org/10.1038/sj.gt.3302707
13. Brennan PA, Thomas GJ, Langdon JD. The role of nitric oxide in oral diseases. Arch Oral Biol. 2003 Feb;48(2):93-100. https://doi.org/10.1016/S0003-9969(02)00183-8
14. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science. 2003 Feb;299(5608):896-9. https://doi.org/10.1126/science.1079368
15. Silva MJ, Sousa LM, Lara VP, Cardoso FP, Júnior GM, Totola AH, et al. The role of iNOS and PHOX in periapical bone resorption. J Dent Res. 2011 Apr;90(4):495-500. https://doi.org/10.1177/0022034510391792
16. Tsuchiya M, Akiba Y, Takahashi I, Sasano Y, Kashiwazaki J, Tsuchiya S, et al. Comparison of expression patterns of cathepsin K and MMP-9 in odontoclasts and osteoclasts in physiological root resorption in the rat molar. Arch Histol Cytol. 2008 Sep;71(2):89-100. https://doi.org/10.1679/aohc.71.89
17. Fan X, Roy E, Zhu L, Murphy TC, Ackert-Bicknell C, Hart CM, et al. Nitric oxide regulates receptor activator of nuclear factor-kappaB ligand and osteoprotegerin expression in bone marrow stromal cells. Endocrinology. 2004 Feb;145(2):751-9. https://doi.org/10.1210/en.2003-0726
18. Rahnert J, Fan X, Case N, Murphy TC, Grassi F, Sen B, et al. The role of nitric oxide in the mechanical repression of RANKL in bone stromal cells. Bone. 2008 Jul;43(1):48-54. https://doi.org/10.1016/j.bone.2008.03.006
19. Farhad AR, Razavi S, Jahadi S, Saatchi M. Use of aminoguanidine, a selective inducible nitric oxide synthase inhibitor, to evaluate the role of nitric oxide in periapical inflammation. J Oral Sci. 2011 Jun;53(2):225-30. https://doi.org/10.2334/josnusd.53.225
20. Boyce BF, Li P, Yao Z, Zhang Q, Badell IR, Schwarz EM, et al. TNF-alpha and pathologic bone resorption. Keio J Med. 2005 Sep;54(3):127-31. https://doi.org/10.2302/kjm.54.127
21. Pi SH, Jeong GS, Oh HW, Kim YS, Pae HO, Chung HT, et al. Heme oxygenase-1 mediates nicotine- and lipopolysaccharide-induced expression of cyclooxygenase-2 and inducible nitric oxide synthase in human periodontal ligament cells. J Periodontal Res. 2010 Apr;45(2):177-83. https://doi.org/10.1111/j.1600-0765.2009.01215.x
22. Cuzzocrea S, Mazzon E, Dugo L, Genovese T, Di Paola R, Ruggeri Z, et al. Inducible nitric oxide synthase mediates bone loss in ovariectomized mice. Endocrinology. 2003 Mar;144(3):1098-107. https://doi.org/10.1210/en.2002-220597
23. Tyrovola JB, Spyropoulos MN, Makou M, Perrea D. Root resorption and the OPG/RANKL/RANK system: a mini review. J Oral Sci. 2008 Dec;50(4):367-76. https://doi.org/10.2334/josnusd.50.367
24. Uitto VJ, Overall CM, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. Periodontol 2000. 2003;31(1):77-104. https://doi.org/10.1034/j.1600-0757.2003.03106.x
25. Lin SK, Kok SH, Lin LD, Wang CC, Kuo MY, Lin CT, et al. Nitric oxide promotes the progression of periapical lesion via inducing macrophage and osteoblast apoptosis. Oral Microbiol Immunol. 2007 Feb;22(1):24-9. https://doi.org/10.1111/j.1399-302X.2007.00316.x
26. Cochran DL. Inflammation and bone loss in periodontal disease. J Periodontol. 2008 Aug;79(8 Suppl):1569-76. https://doi.org/10.1902/jop.2008.080233
27. Zhou J, Feng G, Zhou W, Ren A, Wu Y, Zhang D, et al. Expression of osteoprotegerin and receptor activator of nuclear factor κB ligand in root resorption induced by heavy force in rats. J Orofac Orthop. 2011 Nov;72(6):457-68. https://doi.org/10.1007/s00056-011-0050-3
28. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009 Aug;361(8):756-65. https://doi.org/10.1056/NEJMoa0809493