DOI: 10.1111/j.1365-263X.2011.01132.x

INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

Primary teeth show less protecting factors against root resorption

MABEL M. R. CORDEIRO[1], BIANCA Z. SANTOS[2], JESSIE F. REYES-CARMONA[3] & CLAUDIA P. FIGUEIREDO[4]

[1]Department of Morphological Sciences, Biological Sciences Center, Federal University of Santa Catarina, Florianopolis, SC, Brazil, [2]PhD Program in Paediatric Dentistry, Federal University of Santa Catarina, Concentration Area Pediatric Dentistry, Florianopolis, SC, Brazil, [3]Department of Restorative Sciences, University of Costa Rica, San José, Costa Rica, and [4]Department of Physiological Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil

International Journal of Paediatric Dentistry 2011; 21: 361–368

Background. Physiological root resorption differentiates primary from permanent teeth. The understanding of what protects and regulates root resorption might help to develop therapies to its control.

Aim. To verify the presence and distribution of ECRM and the expression of CK14, OPG, TRAP and COX-2 in the periodontal ligament (PDL) of human primary and permanent teeth.

Design. Eight primary teeth undergoing physiological or pathological root resorption and 4 permanent teeth were immunohistochemically processed for CK14, TRAP, COX-2 and OPG expression.

Results. PDL from primary and permanent teeth showed similar morphological features; however, fewer ECRM clusters and higher immunoreactivity to CK14 were found in primary PDL. In permanent teeth, ECRM were distributed along the entire PDL tissue. Howship's lacunae were found only in primary teeth, associated with the presence of TRAP-positive cells and increase in COX-2 expression. OPG expression in primary PDL was detected in nonresorptive cervical areas and in lacunae showing reparative tissue. It was observed higher expression of OPG in all permanent teeth when compared to primary specimens.

Conclusions. It may be concluded that PDL from primary teeth shows less ECRM clusters and lower expression of OPG. These features may be associated with lower protection against root resorption in primary teeth.

Introduction

The main biological difference between bone and tooth is that bone suffers constant physiological renovation, whereas in tooth the only resorption that is physiological is the one that occurs in primary teeth. Although the protection of the tooth against resorption while the bone is being constantly renewed is a disquieting phenomenon, even more intriguing is the fact that the roots of primary teeth are eventually resorpted, whereas the roots of permanent teeth are not.[1] Root resorption and exfoliation of primary teeth are physiologic events that are not yet fully understood.[2]

Correspondence to:
Mabel M. R. Cordeiro, DDS, PhD, Post-Doc, Departamento de Ciências Morfológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Campus Universitário – Trindade, CEP: 88.040-900, Florianópolis, SC, Brazil. E-mail: mcordeiro@ccb.ufsc.br

Primary teeth undergo physiological root resorption that ultimately leads to their exfoliation. On the other hand, permanent teeth do not undergo resorption except in pathological conditions.[3] It is believed that the consistency between time and pattern of root resorption of the primary tooth and the subsequent eruption of the permanent germ are indicatives that these are interrelated genetically programmed events.[4] Nevertheless, the presence of a permanent germ is not a prerequisite, because even a primary tooth without a successor permanent tooth eventually resorpts.[1]

Several studies have looked at the mechanism of root resorption[5–14] and found that the process is similar to that of bone. Therefore, odontoclasts, cementoclasts and osteoclasts share the same characteristics, gene expression features and mineralized tissue-resorbing activity, being all differentiated from monocyte/macrophage-lineage cells.[7,10]

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

As tartrate-resistant acid phosphatase (TRAP) is an enzyme expressed in high amounts by bone-resorbing osteoclasts and inflammatory macrophages, it is probably the most reliable marker of clastic cell differentiation.

Osteoprotegerin (OPG), receptor activator of the nuclear factor kappa B (RANK) and its ligand (RANKL) are the key proteins that regulate bone metabolism and osteoclastic biology.[8,15–18] Their coordinated expressions seem to be related to bone remodelling and root resorption.[19] The role of RANKL is to promote the formation, fusion, differentiation, activation and survival of clastic cells, thus increasing resorption. The main action of OPG is to inhibit clastic differentiation, thus inhibiting the action and stimulating apoptosis of these cells.[19] The biological effects of RANKL occur when it binds to RANK, while OPG acts as a soluble antagonist receptor, neutralizing and preventing RANK-RANKL interaction.[19,20]

Pro-inflammatory cytokines, such as IL-1 and TNF-α, regulate RANKL and OPG expressions enhancing the process of resorption.[10,19,20] As these cytokines are mainly involved in the activation of several genes related to the inflammatory process, including COX-2, this molecule may be used to investigate the participation of an inflammatory response in pathological root resorption.

Epithelial cell rests of Malassez (ECRM) are fragments of the epithelial sheath of Hertwig and form a network in the periodontal ligament (PDL) that separates tooth (internal organ) from the alveolar bone (skeleton). It is believed that these epithelial rests are involved in PDL space maintenance and protection against root resorption.[21–23] A specific marker for epithelial cells is the cytokeratin 14 (CK14), and thus, it may be used to detect ECRM within PDL.

Although literature presents several studies regarding the process of root resorption, the possible mechanisms or structures involved in the protection against clastic activity are still unclear. Thus, the aim of this study was to describe the presence and distribution of ECRM and odontoclasts/cementoclasts evaluated through CK14 and TRAP markers, respectively, as well as the expression of OPG and COX-2, in the PDL of primary and permanent human teeth.

Material and methods

Eight primary molars, being four healthy teeth undergoing physiological root resorption and four with periradicular lesion and pathological root resorption as consequences of caries decay, were collected from 5- to 8-year-old patients, attending Pediatric Dentistry Clinic of the University. Four healthy third molars completely erupted and with complete root formation were obtained from 20- to 25-year-old patients, attending Dental Surgery Clinic. As it is not possible to determine whether the root resorption process is already established, even when no macroscopic and/or radiographic signs are visualized, all primary teeth were used to study root resorption, only differentiating physiologic from pathologic resorption, whereas permanent teeth were used as negative controls for root resorption. All teeth were extracted for reasons unrelated to this study.

Research project was approved by the Ethics Committee for Research with Human Beings of the University, and teeth were collected after donation and informed consent obtained from the patient or his legal guardian.

Teeth could not have history of gum disease, trauma, orthodontic or endodontic treatment, and patients must not have systemic diseases that would influence hard tissue resorption such as abnormal function of glands. Primary teeth, also, should have at least half of the root remaining and healthy teeth could not show signs of decay, demineralization or any other loss of crown structure.

Histological procedures

For haematoxylin–eosin and immunohistochemistry staining, teeth were fixed in 10% buffered formalin at 4°C for 24 h and demineralized with 10% EDTA pH 7.2 at room temperature until the dentin offered no resistance to cutting. Crowns were then separated from the roots and the latter were transversally

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

sectioned to obtain two fragments, one cervical and one apical. These fragments were then processed for histology. Sections of 3-mm thickness were cut and prepared on conventional glass slides. The histological sections were stained with haematoxylin–eosin or kept unstained for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed using the following anti-human primary antibodies and respective dilution ratios: mouse monoclonal anti-tartrate-resistant acid phosphatase (TRAP) (1 : 100; Novocastra, Newcastle, UK), rabbit polyclonal anti-cyclooxygenase-2 (COX-2) (1 : 200, Cell Signaling Technology, Danvers, MA, USA), mouse monoclonal anti-cytokeratin 14 (CK14) (1 : 300; Novocastra) and goat polyclonal anti-osteoprotegerin (OPG) (1 : 50; Santa Cruz Technology, Santa Cruz, CA, USA). High-temperature antigen retrieval was applied by immersing the slides in a water bath at 95–98°C in 10 mmol/L trisodium citrate buffer (pH 6.0) for 45 min. After overnight incubation with primary antibodies at 4°C, the slides were washed with PBS and incubated with the ready-to-use secondary antibody EnVision Plus (DakoCytomation EnVision Doublestain System, Carpinteria, CA, USA) for 1 h at room temperature. Visualization was completed using 3,30-diaminobenzidine (DAB) (DakoCytomation) and counterstained with Harris' haematoxylin solution. As controls, sections were incubated with an isotype-matched nonspecific immunoglobulin G (Santa Cruz Technology).

Microscopic analyses

Images of the sections were acquired using a digital camera (Sight DS-5ML1; Nikon, Melville, NY, USA) connected to a light microscope (Eclipse 50i; Nikon) under 20×, 40× and 100× magnifications, observing: morphological characteristics of PDL, areas of resorption, presence and localization of ECRM and clastic cells, and expression of COX-2 and OPG and its spatial distribution.

Results

Morphological characteristics of PDL tissues

Macroscopically, no differences were found between groups regarding the morphology of the tissue attached to the roots after tooth extraction.

Microscopically, some areas of the PDL of primary and permanent teeth showed similar histological characteristics (Fig. 1a–c). Howship's lacunae were however found in all the primary teeth. In the physiologic root resorption group, Howship's lacunae were associated with a PDL still organized (Fig. 1d) or showing signs of tissue repair (Fig. 1e), whereas in the pathologic resorption group, these lacunae were always associated with the presence of clastic cells and a completely unorganized or absent soft tissue (Fig. 1f).

Presence and localization of the epithelial cell rests of Malassez (ECRM)

ECRM were distributed in intermediate areas of PDL or close to the root cement. These cells were organized as round, oval or elongated clusters (Fig. 1g).

A difference in quantity and intensity of immunoreaction to CK14 was observed between primary and permanent teeth. In primary teeth, the clusters were present in less quantity and in areas without root resorption; also they showed a darker brown colour (Fig. 1h,i). In permanent teeth, ECRM were found in more quantity, localized closer to the cement surface as if they were forming a belt (Fig. 1j,k). Immunoreaction was less intense, showing a light brown colour (Fig. 1j,k).

Root resorption and inflammation

TRAP-positive clastic cells (cementoclasts and odontoclasts) showing several morphologies and numbers of nuclei were found within Howship's lacunae in root cement or dentin tissues (Fig. 2a), or even a little farther away from the external root surface (Fig. 2b), in areas with no resorption, in all the primary teeth. In teeth with inflammatory resorption,

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

resorpting lacunae were found also in dentin of the root canal walls, with TRAP-positive cells present not only inside the Howship's lacunae (odontoclasts) but also in the pulp tissue (active macrophages) (Fig. 2c).

COX-2 expression was detected in the same pattern of TRAP immunoreaction. Therefore, COX-2 expression was more intense in Howship's lacunae and in the PDL of primary teeth undergoing pathologic root resorption (Fig. 2d), but it was also present in specimens with physiologic resorption (Fig. 2e).

Resorptive areas or TRAP-positive clastic cells were not found in permanent teeth. Some areas of the PDL of these teeth however showed a very light immunoreaction of COX-2, consistent with a constitutive expression (Fig. 2f).

Osteoprotegerin expression

Expression of OPG was detected in permanent teeth (Fig. 2g,h) and in primary specimens only in cervical areas (Fig. 2i,j). In one physiologic root resorption tooth, it was observed OPG immunoreaction in cells associated with a repaired Howship's lacuna (Fig. 2k), also in a cervical area. OPG expression was not found in apical and intermediate root areas in any of the primary teeth (Fig. 2l).

Discussion

Root resorption is fundamental for exfoliation of primary teeth and later eruption of permanent successors, and it is believed to start immediately after the primary roots are completely formed.[4] On the other hand, pathologic resorptions have a significant frequency, characterized as consequences or complications of conditions such as dental trauma and periradicular lesions, being a common cause of tooth loss.

Besides the aesthetic factor, tooth loss leads to alterations in mastication, breathing,

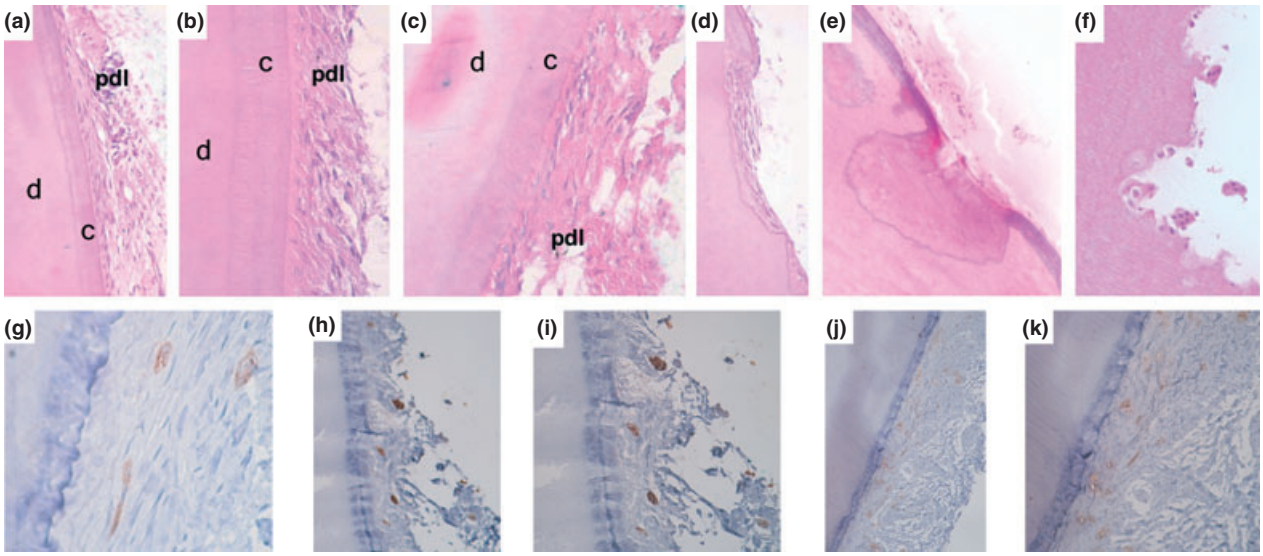

Fig. 1. Microscopic features of PDL from human primary and permanent teeth, and presence and localization of ECRM. (a) PDL of a primary molar with physiological root resorption. (b) PDL of a primary molar with inflammatory pathological root resorption. (c) PDL of a permanent molar. (d) Howship's lacuna on a root surface of a primary molar undergoing physiologic resorption showing PDL still organized. (e) Howship's lacuna on a root surface of a primary molar with physiologic resorption, filled with mineralized tissue characterizing tissue repair. (f) Howship's lacuna on a root surface of a primary molar with pathologic resorption, showing absence of an organized PDL. (g) ECRM clusters organized in different shapes. 200×. (h, i) ECRM of a primary tooth with physiological resorption, showing few clusters, arranged in the middle of the soft tissue and darker brown coloured, 200× and 400×, respectively. (j, k) ECRM of a permanent molar, showing more clusters, arranged closer to the cement on the root surface and lighter brown coloured, 200× and 400×, respectively. d, dentin; c, cement; pdl, periodontal ligament. H&E staining from a to f, at 200× magnification. Immunohistochemistry for CK14 from g to k.

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

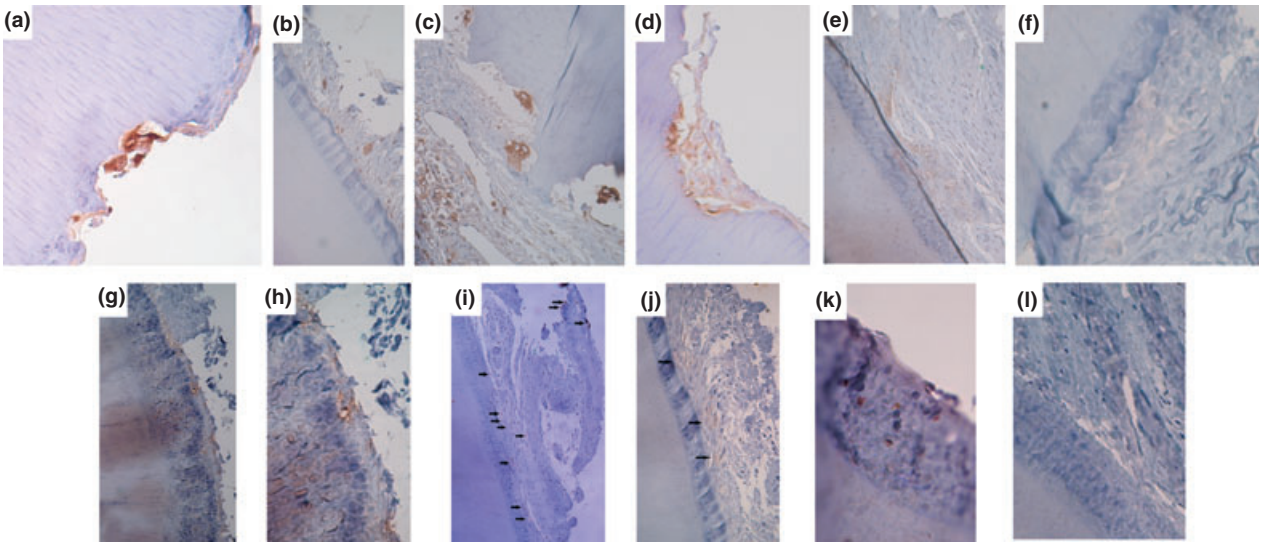

Fig. 2. Root resorption, inflammation and OPG expression. (a) Clastic cells in a Howship's lacuna of a primary molar undergoing physiologic resorption, *TRAP*, 400×. (b) Presence of active macrophages in the periodontal ligament (PDL) of a primary molar with physiologic resorption, in an area with no root resorption, *TRAP*, 400×. (c) Presence of resorption in the pulp cavity wall associated with the presence of clastic cells and active macrophages in the connective tissue of a primary molar with pathologic root resorption, T*RAP*, 400×. (d) COX-2 expression associated with clastic cells in a Howship's lacuna of a primary molar with pathologic resorption. *COX-2*, 400×. (e) COX-2 expression in the cervical area of the PDL of a primary molar with physiologic resorption, *COX-2*, 400×. (f) Permanent molar PDL showing no Howship's lacunae or signs of inflammation, *COX-2*, 400×. (g, h) OPG expression close to the cement surface in a permanent tooth, *OPG*, 400× and 1000×, respectively. (i, j) PDL of primary teeth with physiologic (i) and pathologic (j) root resorption showing OPG expression related to cells from areas with no resorption (*black arrows*), *OPG*, 400×. (k) OPG expression in the tissue inside a repaired Howship's lacuna in the cervical region of a physiological resorpted primary tooth, *OPG*, 1000×. (l) No expression of OPG was observed in medial and apical areas of primary teeth, *OPG*, 400×.

phonation and swallowing.[24,25] Premature loss of primary teeth may have long-term harmful effects, such as problems of space in the dental arch, problems in the eruption of the successor tooth, alterations in tongue's posture, among others.[26]

In this study, it was possible to observe that the macroscopic and microscopic morphology of the PDL of primary teeth is similar to that of permanent ones, as previously described by Bille *et al.*[27] It however shows some structural and molecular differences that may be involved in a higher susceptibility of root resorption in primary teeth.

ECRM are usually considered to be residual cells from the tooth development stage and, thus, not taken into consideration in the analysis of a periodontal tissue. Ohshima *et al.*[28] however characterized the pattern of cytokine expression in ECRM isolated from the PDL of three teeth. They found significant amounts of cytokines, chemokines, growth factors and other related proteins, demonstrating that those cells, unlike previously thought, actively participate in the homeostasis of PDL. Although it is thought that these epithelial cells are in a quiescent state inside the tissue, they can proliferate under specific conditions, such as periradicular inflammation, and form the capsule of periapical cysts.

In this study, we performed immunohistochemistry with CK14 antibody, a specific marker of epithelial cells, to facilitate their localization within the tissue. It was observed a higher intensity of CK14 immunoreaction in ECRM from primary teeth which, associated with the finding of a higher expression of COX-2 in these same specimens, might imply that the presence of pro-inflammatory factors activates in some way the epithelial cells to express more proteins, including cytokines and growth factors, as described by Götz *et al.*[29] and Ohshima *et al.*[28]

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

It is believed that the molecular control of defence reactions is mediated by pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α)[30] that trigger a cascade of events activating or inhibiting enzymes and transcriptional factors.[31] IL-1 and TNF-α activate NF-κB and phospho-c-jun (AP-1)[31] that promote the transcription of several genes involved in the inflammatory process, including COX-2.[32]

It is speculated that pro-inflammatory cytokines, such as IL-1 and TNF-α, regulate the balance between RANKL and OPG, to increase the expression of RANKL and reducing OPG.[10,19] Uchiyama *et al.*[20] found in their study that OPG could not completely inhibit the differentiation into osteoclasts, suggesting that RANKL-independent TNFα signals were partially involved in the differentiation. A similar pattern was observed in this study, as primary teeth showed higher expression of COX-2 and lower expression of OPG, mainly in pathological root resorption specimens, associated with active resorption demonstrated by the presence of TRAP-positive clastic cells. The more intense immunoreaction to COX-2 and the presence of clastic cells and active macrophages may imply that pro-inflammatory cytokines would be present in the PDL, stimulating down-regulation of OPG and up-regulation of RANKL, and activating resorption of dental tissues.

TRAP-positive mononuclear and multinucleated odontoclasts are observed in the dental pulp, leading to resorption cavities in dentin, but no TRAP-positive cells are detected before resorption.[7,20] As apoptosis has an important role in the elimination of pulp cells during the physiological root resorption of human primary teeth,[33] it can be speculated that the finding of a high amount of active macrophages within the remaining pulp tissue of teeth undergoing pathologic root resorption, in this study, may indicate not only phagocytosis related to the presence of an inflammatory response combined with mineralized tissue resorption activity, but also the participation of those phagocytic cells in removing apoptotic bodies during pulp tissue elimination in an active and rapid resorption process as it is an inflammatory root resorption of human primary teeth.

Studies have investigated the expression of RANKL and its role during physiological root resorption through *in vitro* characterization[8,11,13] or evaluated the immunohistochemical RANK and RANKL expression in human primary teeth.[10] *In vitro* studies using molecular methods of detection and total tissue samples are not able to determine the cellular sources of the mRNA or the specific protein investigated. Thus, immunohistochemical analyses allow verifying not only the cellular source but also the spatial localization of the protein within the tissue. Negative results must however be cautiously interpreted, because the protein may be expressed in quantities below the detection capability of the method.

Although the small number of specimens might be a limitation of this study, to our knowledge, this is the first study showing proteins associated with root resorption and factors reported as protective, in human PDL. The findings of this study may therefore give an initial overview of the differences in the PDL of primary teeth, associated or not with inflammation, compared to permanent teeth. The understanding of what protects, controls and regulates root resorption might lead to manipulate biology, making it possible to maintain a primary tooth as long as it is necessary, or even prevent root resorption and consequent loss of a permanent tooth after episodes of trauma.

It may be concluded that the PDL of primary teeth has less clusters of ECRM and lower expression of OPG, features that may be associated with a lower protection against root resorption when compared to permanent teeth. The importance of root resorption control, both in primary and in permanent teeth, brings out the need of further studies to understand the meaning and the physiology of these features, with a view to future clinical therapeutic applicability.

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

What this paper adds

- This paper contributes new information about the differences between PDL tissues of primary and permanent teeth, which is poorly understood and studied in Paediatric Dentistry.
- The findings of this study give an initial overview of the differences in the PDL of primary teeth, associated or not with inflammation, compared to permanent teeth.
- It highlights a concept that is of interest in the better understanding of resorptive factors between primary and permanent teeth.

Why this paper is important to paediatric dentists

- Paediatric dentists should be aware of the differences between PDL tissues from primary and permanent teeth and how these differences may influence root resorption process.
- It may help paediatric dentists to understand why a primary tooth without a successor permanent tooth eventually resorpts.

Acknowledgements

The authors would like to acknowledge the Federal University of Santa Catarina for invaluable support in this study.

References

1 Harokopakis-Hajishengallis E. Physiologic root resorption in primary teeth: molecular and histological events. *J Oral Sci* 2007; **49**(1): 1–12.
2 Monteiro J, Day P, Duggal M, Morgan C, Rodd H. Pulpal status of human primary teeth with physiological root resorption. *Int J Paediatr Dent* 2009; **19**(1): 16–25.
3 Gunraj MN. Dental root resorption. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **88**(6): 647–653.
4 Wise GE, Frazier-Bowers S, D'Souza RN. Cellular, molecular, and genetic determinants of tooth eruption. *Crit Rev Oral Biol Med* 2002; **13**(4): 323–334.
5 Hammarström L, Lindskog S. General morphological aspects of resorption of teeth and alveolar bone. *Int J Endod* 1985; **18**(2): 93–108.
6 Sasaki T, Watanabe C, Shimizu T, Debari K, Segawa K. Possible role of cementoblasts in the resorption organ of human deciduous teeth during root resorption. *J Periodontal Res* 1990; **25**(3): 143–151.
7 Sahara N, Toyoki A, Ashizawa Y, Deguchi T, Suzuki K. Cytodifferentiation of the odontoclast prior to the shedding of human deciduous teeth: an ultrastructural and cytochemical study. *Anat Rec* 1996; **244**(1): 33–49.
8 Hasegawa T, Kikuiri T, Takeyama S *et al.* Human periodontal ligament cells derived from deciduous teeth induce osteoclastogenesis in vitro. *Tissue Cell* 2002; **34**(1): 44–51.
9 Linsuwanont B, Takagi Y, Ohya K, Shimokawa H. Expression of matrix metalloproteinase-9 mRNA and protein during deciduous tooth resorption in bovine odontoclasts. *Bone* 2002; **31**(4): 472–476.
10 Lossdörfer S, Götz W, Jäger A. Immunohistochemical localization of receptor activator of nuclear factor kappa B (RANK) and its ligand (RANKL) in human deciduous teeth. *Calcif Tissue Int* 2002; **71**(1): 45–52.
11 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; **111**(4): 346–352.
12 Lee A, Schneider G, Finkelstein M, Southard T. Root resorption: the possible role of extracellular matrix proteins. *Am J Orthod Dentofacial Orthop* 2004; **126**(2): 173–177.
13 Fukushima H, Jimi E, Kajiya H, Motokawa W, Okabe K. Parathyroid-hormone-related protein induces expression of receptor activator of NF-κB ligand in human periodontal ligament cells via a cAMP/Protein kinase A-independent pathway. *J Dent Res* 2005; **84**(4): 329–334.
14 Low E, Zoellner H, Kharbanda OP, Darendeliler MA. Expression of mRNA for osteoprotegerin and receptor activator of nuclear factor kappa B ligand (RANKL) during root resorption induced by the application of heavy orthodontic forces on rat molars. *Am J Orthod Dentofacial Orthop* 2005; **128**(4): 497–503.
15 Kaku M, Uoshima K, Yamashita Y, Miura H. Investigation of periodontal ligament reaction upon excessive occlusal load-osteopontin induction among periodontal ligament cells. *J Periodontal Res* 2005; **40**(1): 59–66.
16 Kanzaki H, Chiba M, Arai K *et al.* Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. *Gene Ther* 2006; **13**(8): 678–685.
17 Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res Ther* 2007; **9**(Suppl 1): S1.
18 Walker CG, Ito Y, Dangaria S, Luan X, Diekwisch TGH. RANKL, osteopontin, and osteoclast homeostasis in a hyper-occlusion mouse model. *Eur J Oral Sci* 2008; **116**(4): 312–318.
19 Tyrovola JB, Spyropoulos MN, Makou M, Perrea D. Root resorption and the OPG/RANKL/RANK system: a mini review. *J Oral Sci* 2008; **50**(4): 367–376.
20 Uchiyama M, Nakamichi Y, Nakamura M *et al.* Dental pulp and periodontal ligament cells support osteoclastic differentiation. *J Dent Res* 2009; **88**(7): 609–614.
21 Zeichner-David M, Oishi K, Su Z *et al.* Role of Hertwig's epithelial root sheath cells in tooth root development. *Dev Dyn* 2003; **228**(4): 651–663.

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

22 Ohshima M, Tokunaga K, Sato S, Maeno M, Otsuka K. Laminin- and fibronectin-like molecules produced by periodontal ligament fibroblasts under serum-free culture are potent chemoattractants for gingival epithelial cells. *J Periodontal Res* 2003; **38**(2): 175–181.
23 Shinmura Y, Tsuchiya S, Hata KI, Honda MJ. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. *J Cell Physiol* 2008; **217**(3): 728–738.
24 Furkim AM, Mattana A. Fisiologia da deglutição orofaríngea. In: Ferreira LP, Befi-Lopes DM. (eds). *Tratado de Fonoaudiologia*. Limongi SCO, São Paulo: Roca, 2004: 212–218.
25 Marchesan IQ. Alterações de fala de origem musculoesquelética. In: Ferreira LP, Befi-Lopes DM. (eds). *Tratado de Fonoaudiologia*. Limongi SCO, São Paulo: Roca, 2004: 292–303.
26 Cordeiro MMR, Rocha MJC. The effects of periradicular inflammation and infection on a primary tooth and permanent successor. *J Clin Pediatr Dent* 2005; **29**(3): 193–200.
27 Bille ML, Nolting D, Kjær I. Immunohistochemical studies of the periodontal membrane in primary teeth. *Acta Odontol Scand* 2009; **67**(6): 382–387.
28 Ohshima M, Yamaguchi Y, Micke P, Abiko Y, Otsuka K. In vitro characterization of the cytokine profile of the epithelial cell rests of Malassez. *J Periodontol* 2008; **79**(5): 912–919.
29 Götz W, Lossdörfer S, Krüger U, Braumann B, Jäger A. Immunohistochemical localization of insulin-like growth factor-II and its binding protein-6 in human epithelial cells of Malassez. *Eur J Oral Sci* 2003; **111**(1): 26–33.
30 Huang TH, Yang CC, Ding SJ, Yeng M, Kao CT, Chou MY. Inflammatory cytokines reaction elicited by root-end filling materials. *J Biomed Mater Res B Appl Biomater* 2005; **73**(1): 123–128.
31 Roitt I, Brostoff J, Male D. *Imunologia*, 6th edn. São Paulo: Manole, 2002.
32 Lee DH, Kim NR, Lim BS, Lee YK, Hwang KK, Yang HC. Effects of root canal sealers on lipopolysaccharide-induced expression of cyclooxygenase-2 mRNA in murine macrophage cells. *J Endod* 2007; **33**(11): 1329–1333.
33 Rodrigues LV, Vasconcelos AC, Campos PA, Brant JMC. Apoptosis in pulp elimination during physiological root resorption in human primary teeth. *Braz Dent J* 2009; **20**(3): 179–185.

© 2011 The Authors
International Journal of Paediatric Dentistry © 2011 BSPD, IAPD and Blackwell Publishing Ltd

Copyright of International Journal of Paediatric Dentistry is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.