Original Article

Apoptosis in Physiological Root Resorption of Primary Teeth

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ABSTRACT

Background: During physiological root resorption of deciduous teeth, apoptotic cell death triggered by physiological processes might play a role in physiological root resorption in addition to collagen destruction. Little information has been obtained about the sequence of events and the mechanism responsible for the physiological death of pulp tissue cells. **Aim:** This study evaluated apoptotic cell death in the pulp tissue of deciduous teeth that showed various levels of physiological root resorption. The role of apoptosis in pulp tissue elimination during the physiological resorption of deciduous teeth was also examined. **Materials and Methods:** For orthodontic reasons, 12 healthy permanent teeth and the pulp of 34 healthy deciduous teeth showing signs of early and advanced root resorption were extracted. To detect apoptotic cells in the pulp tissue, the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) method and transmission electron microscopy (TEM) were used. The apoptotic index (AI) values of the study groups were determined using the TUNEL method. This technique required calculating the Apoptag positive(+) fibroblast cell ratio in accordance with the total number of cells. **Results:** No statistically significant differences were found for the AI values of each study group ($p>0.05$). Apoptosis was detected in the vascular endothelial cells, the mononuclear inflammatory cells, and the odontoblasts of the connective pulp tissue. In the pulp tissue, evaluated using TEM, various pulp cells were observed at distinct stages of apoptosis. **Conclusion:** The similarity between the AI values for both study groups suggested that in early and advanced stages of resorption, apoptosis may contribute to the regulation of the pulp cell population in a way that does not relate to the physiological process of deciduous teeth root resorption.

Keywords: *Apoptosis, deciduous teeth, dental pulp, physiological resorption*

Introduction

The mechanisms associated with the physiological root resorption of deciduous teeth have been extensively studied.[1,2] However, many questions regarding this process remain unanswered. Odontoclast cells, which are similar to osteoclast cells, play an essential role in the physiological resorption of deciduous dental hard tissue.[1-3] At present, little information has been obtained regarding the resorption mechanism of soft tissue, such as pulp and the periodontal ligament.[1,2,4] Existing studies suggest that apoptotic cell death triggered by physiological processes may play a role in physiological root resorption in addition to collagen destruction.[1,5] The exact sequence of events, however, and the mechanism responsible for the physiological death of pulp cells remains unclear.[2,6]

Apoptosis, which is a form of programmed cell death, helps to regulate physiological events in tissue kinetics.[7,8] The morphological features of apoptosis that occur without any inflammatory reactions include nuclear and cytoplasmic condensation, the endonucleolytic cleavage of DNA, and the fragmentation of cells into apoptotic bodies.[8,9] Throughout odontogenesis, the

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apoptotic mechanism participates from initial tooth formation until the completion of root development.[10,11] Previous studies suggest that apoptosis may play a role in pulp elimination due to the programmed mechanism of physiological root resorption concerning deciduous human teeth.[2]

In this study, we utilized morphological evidence of apoptosis, as well as *in situ* detection of cellular DNA fragmentation using terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) and transmission electron microscopy (TEM). This study was designed to evaluate apoptotic cell death in the pulp tissue of deciduous teeth with different stages of physiological root resorption. In addition, it sought to examine the role of apoptosis in pulp tissue elimination during the physiological resorption of deciduous human teeth.

Material and Methods

This study examined teeth extracted from 46 patients (aged 7–12 years) who had been referred to the Department of Pedodontics and advised to seek orthodontic treatment. Following patient consultations, the teeth were extracted using planned orthodontic serial extractions. Pulp tissue samples were obtained from 34 healthy deciduous teeth. In addition, 12 premolar teeth with near-completion apex formation were also obtained.

The medical histories of the participants revealed no systemic or allergy-related problems. Routine diagnostic tests were performed, periapical radiographs were taken, and informed consent was obtained from the parents or guardians for the inclusion of each participant. Following the ethical guidelines of the Medical Ethical Committee of Gazi University, oral consent was obtained from patients and the committee agreed that the extracted teeth could be used for scientific research purposes. In 2011, the government revised the ethical approval rules for experimental research (Legal Gazette, issue no: 28030).

Applying the TUNEL method

The deciduous and permanent teeth were cleaned using a Polyod solution (7.5% iodine complex, Drogsan, Turkey), extracted under local anesthesia, and immediately collected. For the deciduous teeth, the degree of physiological root resorption was determined by measuring the root lengths after extraction. The distance between the enamel–cementum junction and the region of highest root resorption was measured using an orthodontic diagnostic ruler (Dentaurum 029-301, Germany). The measurement of root lengths was supported by previous research undertaken by Kramer and Ireland.[12] Subsequently, two longitudinal threads were formed by joining the occlusal and apical regions using an air turbine. Each tooth was then divided into two parts using a sterile surgical elevator placed in the inflicted groove. Using a sharp sterile excavator, the coronal and radicular pulp was carefully removed. The pulp tissue was then placed in a 10% formol solution at 4˚C for 24 hours and soaked in paraffin.

The teeth utilized in this study were divided into three groups as follows:

Group I: n = 14 healthy deciduous teeth (primary canine and 1st molar) in the early stages of physiological root resorption (resorption level 0–1/3 of root length);

Group II: n = 16 healthy deciduous teeth (primary canine and 1st molar) in the advanced stages of physiological root resorption (resorption level 1/3–2/3 of root length).

Group III: n = 10 healthy young permanent teeth (1st premolar) near apex completion (control group).

The TUNEL method and an *in situ* hybridization technique were applied using the ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Serologicals Corp, USA cat#S7101, lot#3G036) to detect apoptotic cells, based on fragmented DNA. Following the manufacturer's instructions, pulp sections were dewaxed and dehydrated so that they could undergo proteinase treatment prior to the TUNEL reaction. The TUNEL reaction process was carried out using terminal deoxynucleotidyl transferase to label DNA fragments that had been obtained by incubating each section at 37˚C for 90 minutes. The sections were then counterstained with %0,5 methyl green. Rodent mammary tissue, included in the ApopTag detection kit, was used as control tissue.

For each study group, the pulp tissue sections stained by the TUNEL method were then examined using a light microscope (Nikon Eclipse E600, Japan) and photographed (Nikon Coolpix 5000, Japan). In the pulp tissue samples, the fibroblast cells showed dark brown nuclear staining on the base of the pulp counterstained by light methyl green. These stains were considered ApopTag positive (+). Calculations were carried out by counting cells with positive staining and cells without staining using five microscopic fields at ×400 magnification and an ocular grid (10X10 mm). The apoptotic index (AI) values were determined for each group by calculating the ratio of ApopTag (+) cells in relation to the total number of cells.

Statistical analysis

The AI data for each study group displayed normal distribution confirmed by the Kolmogorov–Smirnov test. Pulp data for each study group were then

statistically evaluated using a one-way analysis of variance (ANOVA). The results indicated that a value of $P < 0.05$ was considered statistically significant.

Transmission electron microscopy

To produce separate evaluations for the coronal and radicular pulp tissue, the pulp tissue was removed and divided into two parts (coronal and radicular) using a scalpel, taking the reference level as the enamel–cementum junction. The pulp tissue was then immersed in 2.5% glutaraldehyde at 4˚C for 24 hours for primary fixation. The fixation procedure was then reinforced by immersing the pulp tissue samples in 1% osmium tetroxide for 60–90 minutes in a dark room. The tissue was then dehydrated in graded ethanol and embedded in Epon. Semi-thin sections were then taken from blocks formed using a pyramidome (LKB 11800, Germany) and stained in a 1% methylene blue-Azur II mixture with 1% borax. The semi-thin sections were examined under a light microscope (Leica DMR, Germany). To determine the appropriate specimen regions, 70 ηm ultrathin sections were obtained using Ultramicrotome (Leica Ultracut-R, Germany) and placed on copper grids to be contrasted with uranyl acetate and lead citrate. The coronal and radicular pulp connective tissue cells were then examined by TEM (JEOL-JEM 1011, Japan) at 80 kV and photographed.

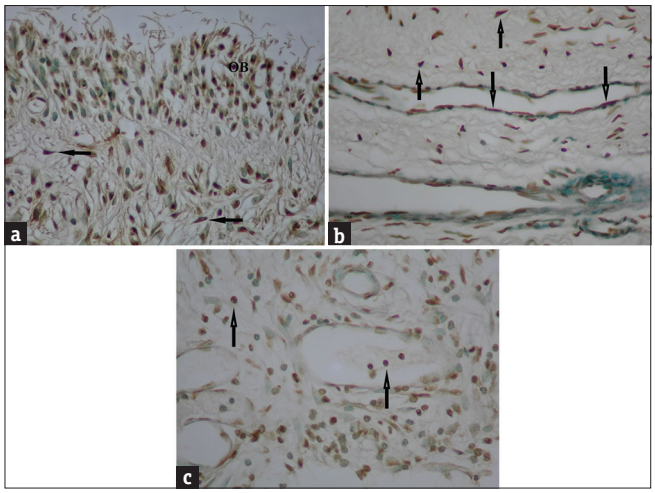

Figure 1: (a) ApopTag (+) fibroblast cells (←), odontoblast (OB) layer with moderate ApopTag positivity; deciduous dental pulp tissue sample, the advanced physiological root resorption stage (TUNEL method, ×400). (b) ApopTag (+) fibroblast cells (↑), ApopTag (+) vascular endothelial cells (←); young permanent dental pulp tissue (TUNEL method, ×400). (c) ApopTag (+) mononuclear inflammatory cells (↑); perivascular area, deciduous dental pulp tissue sample, the early physiological root resorption stage (TUNEL method, ×400)

Results

The TUNEL method

Five pulp tissue samples that displayed advanced root resorption were excluded from this study due to tissue loss caused by TUNEL method procedures and technical problems.

In the remaining samples, the ApopTag (+) cells appeared similar in the coronal and radicular pulp types, showing almost homogeneous distribution. Likewise, no staining difference was observed in the central and peripheral pulp cells.

The main cells that were evaluated were fibroblasts, which are the basic cells in pulp tissue [Figure 1a and b].

In four sections of the deciduous dental pulp tissue samples of Group I, apoptosis was observed in vascular endothelial cells (early-stage physiological root resorption phase). The same results were found in five

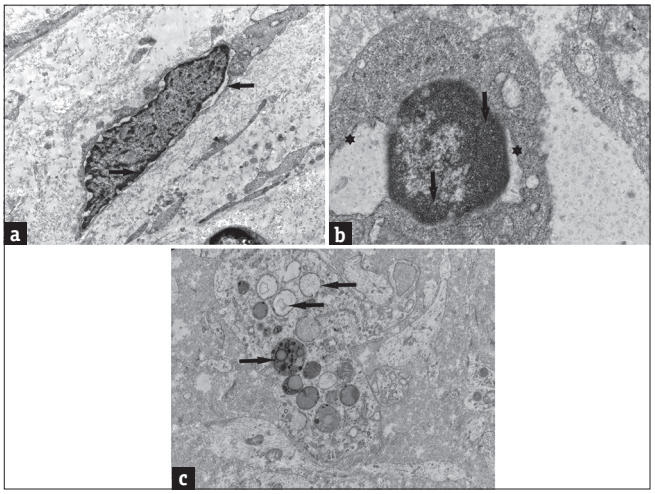

Figure 2: (a) Apoptotic fibroblast cell: perinuclear halo (←), limited chromatin condensation (→). Deciduous tooth: coronal pulp tissue, the early physiological root resorption stage (Uranyl acetate and lead citrate, original magnification ×10000). (b) Apoptotic fibroblast cell: late stage, expanded perinuclear cisterna (↓), characteristic chromatin condensation (*). Deciduous tooth: coronal pulp tissue, the advanced physiological root resorption stage (Uranyl acetate and lead citrate, original magnification ×30000). (c) Apoptotic macrophage cell: expanded rough endoplasmic reticulum (←), phagocytized apoptotic bodies (→), organelles. Deciduous tooth sample: coronal pulp tissue, the advanced physiological root resorption stage (Uranyl acetate and lead citrate, original magnification ×10000)

Table 1: Statistical results for apoptotic index

Groups	*n*	Mean AI value	Min. AI value	Max. AI value	Std. Deviation	Std. Error
Group I	14	0.2568	0.0465	0.4288	0.1176	0.0314
Group II	11	0.2681	0.0366	0.4502	0.1199	0.0366
Group III	10	0.2403	0.0594	0.5581	0.1784	0.0564

P>0.05

sections from Group II (advanced physiological root resorption) and four sections from Group III (young permanent dental pulp tissue) [Figure 1b].

Minimal to moderate mononuclear inflammatory cells were observed in or around the vessels in three sample sections from Group I (the early resorption stage), four sample sections in Group II (the advanced resorption stage), and one sample section in Group III (young permanent dental pulp tissue). Apoptosis was detected in inflammatory cells in one sample of the young permanent dental pulp tissue, three samples of early-stage resorption, and one sample of advanced resorption [Figure 1c].

The mean apoptotic index values of the pulp tissue samples were similar across all groups [Table 1]. The mean AI values for deciduous teeth with advanced resorption increased in comparison with the other two groups. However, when all groups were compared, no significant difference was found ($P > 0.05$).

Transmission electron microscopy

A limited number of apoptotic fibroblast cells at different stages of apoptosis were detected in the pulp tissue samples using TEM [Figure 2a and b]. The appearance of chromatin condensation was evident in apoptotic cells within the samples of the pulp tissue. No difference was observed between the coronal and radicular pulp tissue samples in terms of apoptotic cell distribution. A limited presence of apoptotic bodies was observed in the deciduous and permanent dental pulp tissue samples. The presence of apoptotic bodies was thought to contribute to the characteristics of apoptosis.

A small number of mononuclear inflammatory cells and apoptotic vascular endothelial cells were also found in the pulp tissue samples across all three groups [Figure 2c].

Discussion

During physiological root resorption, cementum and dentin are eliminated by odontoclasts (specialized cells that contribute to the resorption of dental hard tissue).[1-3] Domon *et al*.[13] detected odontoclast fragments of various sizes, which suggested that apoptosis occurred during the physiological resorption of deciduous human teeth. Existing studies have primarily focused on the elimination of hard root structures; however, the mechanisms responsible for the physiological death and resorption of soft tissue, such as the periodontal ligament and dental pulp, have not yet been studied sufficiently.[1,2,6,13,14] Rodrigues *et al*.[9] investigated the elimination of pulp cells during physiological root resorption in deciduous human teeth. Using AI values, a morphometric analysis indicated that the presence of apoptosis in the pulp of deciduous teeth with advanced-stage resorption was more intense than in the pulp of young permanent teeth. In turn, this suggested that apoptosis was involved in pulp cell elimination in physiological resorption. Existing studies on apoptosis often relate to changes in the deciduous dental pulp during physiological root resorption. At present, few quantitative evaluations exist for apoptotic cells in pulp tissue during resorption.

Accordingly, the turnover rate of pulp cells alongside the occurrence and meaning of physiological pulp cell death must be clarified. Studies evaluating the apoptotic mechanism in pulp tissue cells are limited. It was stated that apoptosis helped to regulate the pulp cell population, including aged odontoblasts, which complete their life cycle in healthy pulp tissue.[8,15] Vermelin *et al*.[15] observed apoptosis in various pulp cells and pulp fibroblasts using the TUNEL method. They reported that, within the permanent dental pulp of healthy humans and rats, apoptosis occurred in the incisal and occlusal parts of the pulp cells, rather than the apical part. Apoptotic cells in healthy pulp tissue were observed in the periphery and the subodontoblastic layer, rather than the odontoblastic layer. In this study, apoptotic cells displayed an almost homogeneous distribution in both the coronal and radicular pulp samples across all three study groups. However, when the groups of the current study were revised, the apoptotic pulp cells showed no difference or specific localization in terms of distribution within the peripheral and central pulp types.

This study aimed to quantitatively examine pulp fibroblasts in terms of the apoptotic mechanism of pulp tissue in deciduous teeth at different stages of physiological root resorption. In addition, it examined the role of apoptosis in deciduous dental pulp tissue during physiological resorption.

When the TUNEL method results were evaluated, apoptosis was detected in pulp fibroblasts. Apoptosis was also detected in other pulp tissue sample cells in deciduous teeth with early and advanced physiological root resorption, as well as young permanent teeth. The AI values were calculated, based on where fibroblast cells were found to be similar across all groups. The number of apoptotic cells did not increase in terms of the physiological root resorption in deciduous teeth. A limited number of existing studies[9] have evaluated apoptosis in the pulp tissue of deciduous teeth. However, no research determining apoptotic cells quantitatively in the pulp tissue of deciduous teeth, in collaboration with the early and advanced resorption stages, was found. Rodrigues *et al*.[9] investigated the

elimination of pulp cells in human deciduous teeth in the late stage of physiological root resorption and conducted a morphometric analysis using AI values to show that apoptosis in the pulp of deciduous teeth was more intense than in the pulp of young permanent teeth. The TUNEL labeling method was more intense and diffused in the pulp of deciduous teeth compared with the pulp of permanent teeth. The same study also provided evidence that cellular DNA fragmentation occurred in an internucleosomal manner by the ladder pattern in genomic DNA electrophoresis. These results supported that apoptosis had been involved in pulp cell elimination in physiological root resorption in human deciduous teeth. Contrastingly, our results suggested that AI values reflected similarity at the early (resorption level 0–1/3 of the root length) and advanced (resorption level 1/3–2/3 of the root length) stages of physiological resorption and showed no difference to the AI values of young permanent dental pulp tissue samples. For the TUNEL technique, there were no statistically significant differences between the AI values of the study groups ($P > 0.05$). Considering this, it was assumed that the regulation of the dental pulp cell population continued at the early and advanced stages of physiological root resorption in deciduous teeth. This process did not differ from the homeostasis of the pulp cells of young permanent teeth, in which physiological resorption did not take place. This indicated that the apoptotic mechanism was not involved in the physiological resorption of deciduous dental pulp tissue until the latest stages of root resorption.

In some studies, apoptosis was also observed in perivascular endothelial cells and mononuclear cells nearby or inside the vessels, as well as fibroblasts and odontoblasts in the pulp tissue.[8,15] The findings of our study were also consistent with these results. Mononuclear inflammatory cells and vascular endothelial cells were also detected as ApopTag (+) in some pulp tissue samples of our study groups. It appeared that apoptosis was a mechanism involved in the regulation of different types of cells in the pulp connective tissue.

The presence of various pulp cells in different stages of apoptosis was detected in our study groups by TEM. The typical chromatin condensation in apoptotic cells in the pulp tissue and apoptotic bodies was observed, similar to a study conducted by Vermelin *et al*.,[15] who evaluated apoptosis in pulp tissue using the TUNEL method together with TEM. In our study, the density of apoptotic cells detected by the TUNEL method was considerably higher than the apoptotic cells evaluated via TEM. The low presence of apoptotic cells detected by TEM was attributed to the possibility of detecting late nuclear and cytoplasmic morphological changes using this technique, and the inability to morphologically detect early biochemical changes. Contrastingly, the TUNEL method enabled the early biochemical diagnosis of cells that had undergone DNA fragmentation before apoptotic morphological findings occurred, as well as cells that did not morphologically reflect classical apoptotic cell characteristics.[16] In the present study, the TUNEL method was applied by showing sensitivity to points; for example, as noted, using a positive control tissue sample, two experienced pathologists identified the cell type leading to apoptosis, and via the evaluation of apoptotic cells in at least five microscopic areas in the specimen sections. In addition, the study's TEM results supported the findings obtained using the TUNEL technique by providing support for the presence of apoptotic cells in the pulp tissue of deciduous teeth at different resorption stages, as well as in young permanent teeth, and by evidencing a similar apoptotic pulp-cell density in all of the study groups.

Mature deciduous dental pulp without clinically physiological root resorption had similar histophysiological properties to that of the young permanent dental pulp samples.[17,18] Alexander[19] stated that the pulp histology of deciduous teeth was similar to that of permanent first premolars, even in cases where resorption reached 50%–75% of the root length. Young permanent first premolars that had been extracted for orthodontic reasons were used to evaluate the apoptotic mechanism as the control group in this study.

Studies on the presence of specific enzyme activity and mediators in the pulp and periodontal tissue of deciduous teeth with physiological root resorption revealed the possible role of these tissue types in physiological resorption.[19-21] Eronat *et al*.[20] examined the changes in pulp tissue in the early and late stages of deciduous root resorption using the Ag-NOR (silver-binding nucleolar organizer region) staining technique and revealed the proliferative activities of the cells involved. In that study, the increase in the mean number of Ag-NOR staining per nucleus with the progression of the resorption process in deciduous teeth suggested that metabolic cell activity in the early stages of resorption was enhanced and increased as the process continued, evidencing the importance and the role of pulp tissue in the resorption process. Yıldırım *et al*.[6] suggested that dental pulp may have cytokine-producing cells that could mediate a monocyte–macrophage lineage to form the osteo/odontoclasts that are involved in human deciduous tooth resorption. Such findings appear to indicate a role of pulp tissue in physiological root resorption and support for studies focused on the cellular population in the dental pulp.

Apoptosis is a physiological mechanism of cellularity control that regulates the size of tissue in an inverse mitotic state.[7] Dental pulp, similar to most living tissue, is constantly renewed. Apoptotic cells were identified in central pulp fibroblasts in healthy human premolars.[8] Accordingly, the occurrence of apoptosis is anticipated in permanent dental pulp as part of a regular turnover process. Rodrigues *et al*.[9] stated that the lower AI, the weaker labeling using the TUNEL method, and the weaker internucleosomal DNA fragmentation in genomic electrophoresis in their study resulted from a regular turnover of the immature permanent dental pulp. Healthy immature third molars with an indication for extraction were collected and processed for histological examination in that study. Third molars may not be an appropriate control group model due to the absence of the occlusal forces as some molars are impacted. Contrastingly, the current authors found similar AI values and similar labeling using the TUNEL reaction in young permanent first premolar tooth pulp samples, compared with deciduous tooth pulp. Teeth are under the influence of thermal factors and occlusal forces in the oral environment throughout their life cycle. We may assume that, as a result of damage, these chronically low-level forces and factors may harm pulp cells and eliminate them via the apoptotic mechanism to preserve the functionality of tissue, without damaging the neighboring cells and without causing inflammation. A specific rate of turnover occurred in the pulp fibroblasts in the cell-rich layer, particularly when apoptotic cell death occurred.[22]

Despite an insufficient number of studies on apoptosis and its role in physiological root resorption, the results of the present study appeared to indicate that deciduous teeth in the early and advanced root resorption stages did not display a distinct apoptotic mechanism in young permanent dental pulp tissue. Apoptosis in deciduous dental pulp tissue at the early and advanced resorption stages may, however, occur, regardless of root resorption of the deciduous tooth, which is a physiological and programmed event. Up to the latest stages of physiological resorption, there appears to be no apoptotic mechanism involved in the elimination of pulp during the root resorption process, and apoptosis is likely to be involved in the regulation of the fibroblast, odontoblast, vascular endothelial, and mononuclear inflammatory cell population in pulpal connective tissue. It was noted that mature deciduous dental pulp tissue in the period without physiological root resorption had similar characteristics to young permanent dental pulp tissue, and even at the early and advanced stages of resorption, no structural differences were found[17-19,23]; the pulp retained the structure necessary for pain perception, healing, and repair potential.[14] In the present study, similar AI values for deciduous dental pulp tissue at the early and advanced stages may indicate that, during physiological root resorption, the pulp complex preserved its structure and continued its functions up to the final stages of resorption.

A limitation of this study was its comparative absence of samples in the greater than two-thirds root resorption subgroup, compared to the other three groups. Future research should aim to include more deciduous teeth in the final stages of resorption. In the final stages of physiological root resorption, where apoptosis is considered to be present, mediator expression and metabolic activity in the cellular population of the pulp tissue of deciduous teeth should also be evaluated through additional studies to obtain precise results quantitatively. Additionally, studies should focus on the involvement of apoptosis in pulp tissue elimination during the final stages of physiological root resorption.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ten Cate AR, Anderson RD. An ultrastructural study of tooth resorption in the kitten. J Dent Res 1986;65:1087–93.
2. Harokopakis-Hajishengallis E. Physiologic root resorption in primary teeth: Molecular and histological events. J Oral Sci 2007;49:1–12.
3. Sasaki T, Shimizu T, Watanabe C, Hiyoshi Y. Cellular roles in physiological root resorption of deciduous teeth in the cat. J Dent Res 1990;69;67–74.
4. Sahara N, Okafuji N, Toyoki A, Ashizawa Y, Yagasaki H, Deguchi T, *et al*. A histological study of the exfoliation of human deciduous teeth. J Dent Res 1993;72:634–40.
5. Bille M-L, Thomsen B, Kjær I. Apoptosis in the human periodontal membrane evaluated in primary and permanent teeth. Acta Odontolog Scand 2011;69:385–8.
6. Yildirim S, Yapar M, Sermet U, Sener K, Kubar A. The role of dental pulp cells in resorption of deciduous teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:113–20.
7. Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239–57.
8. Franquin JC, Remusat M, Abou Hashieh I, Dejou J. Immunocytochemical detection of apoptosis in human odontoblasts. Eur J Oral Sci 1998;106:384–7.
9. Rodrigues LV, Vasconcelos AC, Campos PA, Brant JM. Apoptosis in pulp elimination during physiological root

resorption in human primary teeth. Braz Dent J 2009;20:179–85.
10. Matalova E, Tucker AS, Sharpe PT. Death in the life of a tooth. J Dent Res 2004;83:11–6.
11. Satchell PG, Gutmann JL, Witherspoon DE. Apoptosis: An introduction for the endodontist. Int Endod J 2003;36:237–45.
12. Kramer WS, Ireland RL. Measurements of the primary teeth. J Dent Child 1959;26:252–61.
13. Domon T, Taniguchi Y, Inoue K, Ushijima N, Taishi Y, Hiramatsu A, *et al*. Apoptosis of odontoclasts under physiological root resorption of human deciduous teeth. Cell Tissue Res 2008;331:423–33.
14. Monterio J, Day P, Duggal M, Rodd H. Pulpal status of human primary teeth with physiological root resorption. Int J Paediatr Dent 2009;19:16–25.
15. Vermelin L, Lecolle S, Septier D, Lasfargues JJ, Goldberg M. Apoptosis in human and rat dental pulp. Eur J Oral Sci 1996;104:547–53.
16. Sanders EJ, Wride MA. Ultrastructural identification of apoptotic nuclei using the TUNEL technique. Histochem J 1996;28:275–81.
17. Dard M, Kerebel LM, Kerebel B. A transmission electron microscope study of fibroblast changes in human deciduous tooth pulp. Arch Oral Biol 1989;34:223–8.
18. Fox AG, Heeley JD. Histological study of pulps of human primary teeth. Arch Oral Biol 1980;25:103–10.
19. Alexander SA. Collagenolytic activity from human deciduous pulps. J Endod 1981;7:418–20.
20. Eronat C, Eronat N, Aktug M. Histological investigation of physiologically resorbing primary teeth using Ag-NOR staining method. Int J Paediatr Dent 2002;12:207–14.
21. 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;111:346–52.
22. Ten Cate AR. Oral histology development, structure, and function. 8th ed. St. Louis, Baltimore, Boston: Mosby; 1998.
23. Sari S, Aras S, Gunhan O. The effect of physiological root resorption on the histological structure of primary tooth pulp. J Clin Pediatr Dent 1999;23:221–5.