Original article

UDC: 616.314.16-007.23-07
doi:10.5633/amm.2021.0205

ULTRASTRUCTURAL ANALYSIS OF EXTERNAL APICAL ROOT RESORPTION

Aleksandar Mitić[1], Vladimir Mitić[2], Jelena Popović[1], Stefan Dačić[1], Radomir Barac[1], Kosta Todorović[3]

Primary teeth mineralized tissue resorption is considered physiological, while this process in persons with permanent dentition is obligatory pathological. External root resorption is the consequence of multinuclear clastic cell activity which starts at the root surface and spreads further into cementum all the way to the dentin. In normal conditions there is a dynamic balance between osteoblastic and osteoclastic cell activity that maintain a physiological state of root and bone. Due to the disbalance of cells activity, under the influence of different factors, root resorption occurs. The aim of the present study was to perform ultrastructural analysis of pathologically resorbed apical root cementum and dentin formed after traumatic occlusion. The study was conducted on 18 extracted teeth from male patients aging from 54 to 73 years with internal and external pathological root resorption. The resorbed root surface (dentine structure) was analyzed using scanning electron microscope. In all studied samples occlusal surface enamel cracks, as a consequence of traumatic occlusion, were found. Ultrastructural analysis of the dentine surface in the peripheral parts of the root revealed the smooth surface of the resorbed apical root surface, described as "eggshell", with clear demarcation line separating preserved from the resorbed dentine. Also, wavy multi-layered resorption with irregular structure could be seen. Based on the scanning electron microscopic analysis of the apical root dentin one can conclude that the main cause of the external pathological resorption of the apical root, occurring due to traumatic occlusion, is aseptic inflammation.

Acta Medica Medianae 2021;60(2):44-50.

Key words: *traumatic occlusion, external root resorption, SEM analysis*

[1]University of Niš, Faculty of Medicine, Department of Restorative Dentistry and Endodontics, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Pediatric and Preventive Dentistry and Orthodontics, Dental Clinic, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Maxilofacial and Oral Surgery, Niš, Serbia

Contact: Aleksandar Mitić
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: alek.mitic@yahoo.co.uk

Introduction

Tooth resorption represents a process, seen in different physiological and pathological conditions, which can lead to irreversible dentine, cementum and alveolar bone loss in both vital and nonvital teeth (1, 2). Primary teeth mineralized tissue resorption is considered physiological, while this process in persons with permanent dentition is obligatory pathological (3). This process depends on the interaction between different inflammatory, clastic and mineralized tissue cells. Besides a great number of cells (monocytes, macrophages, osteoclasts, etc.) that control this process, systemic and local factors (cytokines, prostaglandins, bacteria, etc.) are involved in this process as well (4).

Pathological resorption can be internal and external. Internal resorption is a pathological process that leads to mineralized tooth tissue loss due to tooth destruction from within, where this process starts from pulp and spreads towards the surface (5).

The etiology of external resorption has two stimulatory components (i) mechanical/chemical and (ii) infection/pressure that affect osteoclasts. External root resorption is the consequence of multinuclear clastic cell, localized in the periodontal ligament and bone, activity, which starts at the root surface and spreads further into cementum all the way to the dentin (6). The mineralized tissue of the permanent denture is hard to resorb since it is well protected by predentin and odontoblasts found in

root canals and cementoblasts found on the root surface. When predentin or precementum is mineralized and the mechanical damage of precementum occurs, osteoclastic cells migrate to the mineralized surface in order to resorb it (1, 7).

Based on the clinical and histological manifestations, there are numerous root resorption classifications, however the authors present the following external resorption classification as it is the most frequent one:

(i) superficial root resorption;
(ii) inflammatory resorption, called cervical/apical;
(iii) ankylosis;
(iv) replacement resorption and
(v) transient apical disorders (8).

According to Andersons classification from 1988, there are three types of external resorption: superficial, external ankylosis-resorption and external inflammatory resorption. Recently, Hulsman et al. also described three types of external resorption: progressive inflammatory, cervical (extra-canalicular invasive resorption) and replacement resorption (9).

External root resorption can be found in both vital and nonvital teeth and is most frequently revealed on routine radiographic image as an asymptomatic condition (1). Pathological resorptions can be diagnosed using radiographic images only when they are large, with lesion diameters of 2 mm and at least 1 mm separated from the superficial cortex. Numerous external resorptions are painless and pass unnoticed by patients until the pulpous or periodontal tissue gets inflamed (1). Deep cavity occurring during resorption can lead to heat tenderness of the tooth due to pulp vicinity.

Osteoclasts are multinuclear cells, originating from multipotent hematopoietic stem cells, involved in bone resorption. Their origin is much closer to immune then to connective tissue cells (2). Using specific receptors osteoclast adhere to root bone and cementum, thus forming an isolated area in which they secrete proteolytic enzymes, that degrade protein matrix, and acids that "meltdown" the mineral composition of the bone and other structures. Under physiological conditions, when there are no traumatic and/or pathological tooth changes, there is a dynamic balance between osteoblastic and osteoclastic activity that maintains the root bone homeostasis. Different factors can disturb this balance leading to root resorption (10).

Osteoclast polarity is regulated by their actin cytoskeleton and in contact with the mineralized extracellular matrix, this cytoskeleton forms a zone without cellular organelles within the cell (clear zone), allowing the cell to form a direct contact with cementum via cell membrane. The clear zone is surrounded by a numerous finger-like cell membrane invaginations (podosomes) that are known as a ruffled border. Bellow this border the resorption process is occurring, where the resorbed surface within the clear zone, isolated from the extracellular surface, is forming acidic microenvironment for mineralized tissue resorption (8).

Multinuclear clastic cells responsible for the bone and root resorption do not possess the receptors for direct binding of parathormone (PTH), thus the clast cell stimulation by PTH is indirect. Both PTH and PTH related protein (PTHrP) bind to osteoblasts and increase the receptor activator of nuclear factor-kB ligand (RANKL) expression, which further binds to a RANK receptor of osteoclast precursors, activating these cells to fuse and form osteoclasts (2, 8).

The multinuclear clastic cells of the periodontal ligament also express RANKL that binds the same way as described previously. The intracellular signal pathway includes TRAF6 that activates protein complex NF-B, protein kinase c-Src and JNK-cell cycle regulators. If the OPG would block RANKL, clastic cells would undergo apoptosis rather than their activation. The relation RANKL-RANK is mandatory for clastic cell survival (8).

Osteoprotegerin (OPG) is a secreted glycoprotein belonging to TNF receptor family and has different biological functions including tissue remodulation. OPG is a powerful competitive inhibitor of osteoclastic bone resorption (11).

Recently, it was discovered that besides proinflammatory cytokines IL-1 and IL-2, TNF-α significantly contributes to the development of osteoclasts and multinuclear cells with dentine resorption potential (1). According to Komine et al., human TNF-α significantly stimulates mononuclear preosteoclast cells (POC) in the presence of conditioned osteoblast cell medium and contributes to hematopoietic cell differentiation to POC. The TNF-α induce POC to form multinuclear cells, which further express dentin resorbing potential. Extremely low levels of TNF-α in POC increase calcitonin receptor cathepsin K mRNA. Both RANKL and TNF-α effects on osteoclast development are inhibited by OPG.

Transformation of macrophage-like clastic cells leads to the formation of multinuclear clastic cells that are almost identical to osteoclasts. Several mediators produced by various cells can be involved in the development of multinuclear cells from their precursors. These multinuclear resorbing cell activators/stimulators include PTH, PTHrP, IL-1, IL-6, IL-11, PDGF, 1α,25-dihydroxy vitamin D3, glucocorticoids, and substance P, while on the other hand calcitonin, estrogen, interferon, IL-4, IL-8, IL-10, IL-18, and corticosteroids are known to inhibit osteoclast/odontoclast cells (8).

Cytokines (IL-1, IL-6, and TNF-α as proinflammatory ones) bind to pattern recognition receptors (PRR) and via secondary messengers, e.g. tyrosine-kinase, activate cells to start their clastic activity.

The aim of the present study was to perform ultrastructural analysis of pathologically resorbed apical root cementum and dentin formed after traumatic occlusion by using scanning electron microscope.

Materials and methods

The study was conducted on 18 extracted teeth from male patients, aged 54 to 73 years, with internal and external pathological root resorption

(Figure 1). The diagnosis was made according to the radiographic images (type and process localization), where in all studied patients with external root resorption the abrasion of the occlusal surface due traumatic occlusion was found.

Figure 1. Radiographic image of a tooth with external root resorption

The resorbed root surface (dentine structure) was analyzed using JEOL-JSM-5300 scanning electron microscope (SEM) by a single researcher.

Sample preparation included teeth preservation in sterile saline at 4 ^{O}C, without any fixative. Occlusal surfaces (2-3 mm thick) of the tooth crowns were circularly cut by the thinnest diamond borer. The roots were cut transversely using shafts in order to separate the apical part of the root. In order to eliminate superficial debris, generated by cutting, the samples were rinsed with distilled water and dried using a compressed air. First, the occlusal sur-face was separated using separating forceps, followed by transversal separation of the roots (apex third) down the cut gutters. Each sample was mounted on a special holder and covered with gold in a vacuum evaporator before they were analyzed under SEM.

Results

Results obtained using SEM are shown in Figures 2-7. In all investigated samples superficial occlusal enamel cracks, as a consequence of traumatic occlusions, were found (Figure 2). Ultrastructural analysis of the dentine root surface appeared as smooth surface of the resorbed apex, described as "eggshell" (Figure 3). At the root peripheral parts, a clear demarcation line separating the preserved from the resorbed dentin could be seen (Figure 4). The irregularity of resorbed dentine surface can be seen in Figures 6 and 7. At the level of the middle and apical third (proximal side) of the root, cellular cementum was observed (Figure 5).

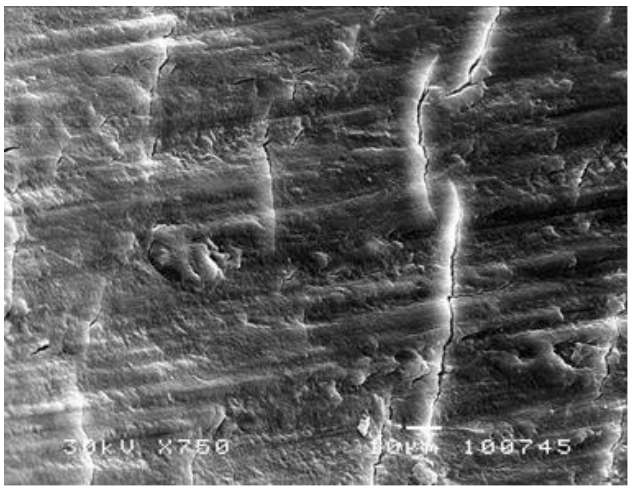

Figure 2. Occlusal enamel surface with cracks due to traumatic occlusion

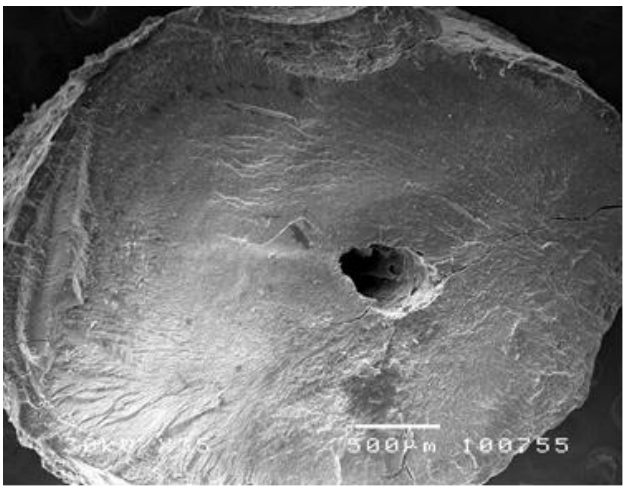

Figure 3. External resorption of root apex with broad, irregular and funnel-like apical foramen

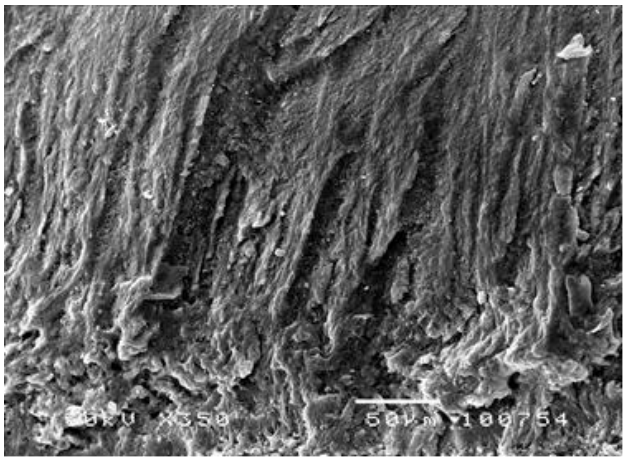

Figure 4. Demarcation border between resorbed and preserved peripheral dentine

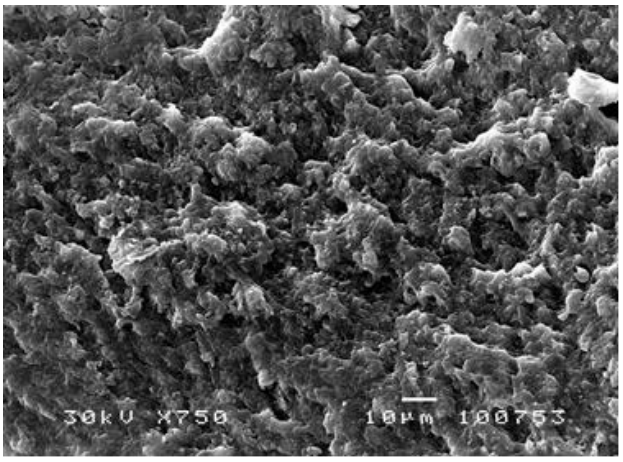

Figure 5. Cellular cementum at the approximal side of the root at the level of the apical and mid third

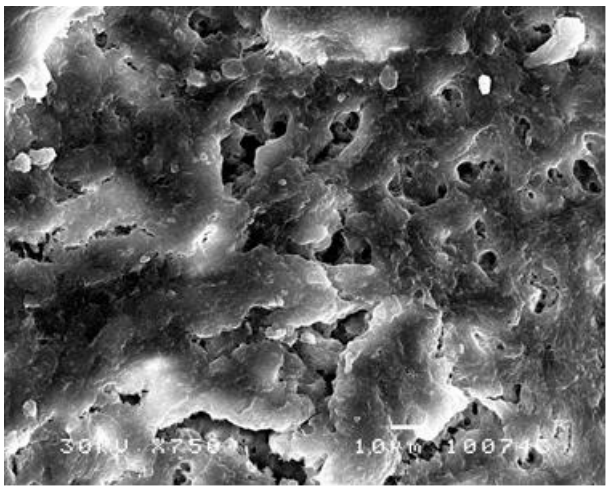

Figure 6. Resorbed irregularly structured dentine surface near apical foramen

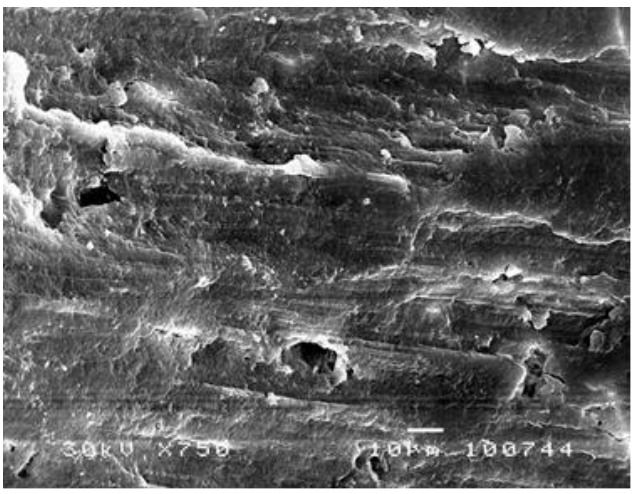

Figure 7. Irregular structure of resorbed dentine surface at the peripheral parts of root apex

Discussion

Osteoclasts and other cells (e.g. macrophages, monocytes and osteocytes) have the ability of mineralized tissue resorption. They from erosions, Howship's lacunae, by liberating demineralizing agents and enzymes, allowing phagocytes to ingest the degraded products (13). In some of the lacunae examined on the teeth in the present study dentine tubules could be seen, which points to the fact that the process is affecting the dentin as well. Substances that are liberated from the surrounding tissue cells, such as osteoclast activating factor, macrophage chemotactic factor and prostaglandins are intensifying the process of mineralized tissue resorption (5).

Different causes of root resorption are attributed to pressure, inflammation, neoplastic processes and some systemic conditions (1). Clastic cells in different physiological and pathological conditions have different potential for root resorption and the variations in the type of resorption can be expected. Active external resorption can be found in patients two months after orthodontic treatment. The areas of resorption could be seen on the entire surface and lead to the loss of normal smooth surface appearance.

In the early phases of the orthodontic treatment, this causes premolar buccal leaning of the crown and with its apex leaning lingually producing zones of tension and compression on opposite sides of the crown and root. In the present study, the

resorption process observed under SEM was not focused on that part. There are numerous cases of root resorption, except in advanced phase, seen as a consequence of inflammation due to orthodontists' therapy (13). In the present study, all premolar resorption was limited to the small cementum surface, probably due to the short time period of tooth exposure to mechanical force. The size and location of the resorption were not uniform in the studied sample, thus one cannot conclude that the local factors are playing a key role in root resorption caused by orthodontic treatment. It is very important to know that even in these phases of orthodontic treatment, the cementum resorption is occurring, although these processes are not always visible on radiographic images. Inflammatory resorption of the apical parts of the root can be seen in teeth with apical periodontitis and is probably more frequent than reported (15-17). Ferlini found, in his microscopic analysis, that in teeth with chronic apical periodontitis resorption is occurring in the foramen region (18). In most of the cases root resorption can be detected on radiographic images, however, in everyday clinical practice, these images are not sufficiently precise for establishing the diagnosis of resorption defects as a consequence of apical periodontitis.

In the present study, the formed lacunae are spreading on the teeth surface, rather than affecting deeper parts (layers). In some lacunae, dentine tubules could be seen, which implicates that the process is affecting dentin as well. Only in the cases of severe resorption, changes in the foramen contours, which appeared irregular, were seen (Figure 2). In accordance with the results of the present study, Rosa Neto et al., found apical root surface to be irregular, eroded, with cementum-dentine resorption (19).

External root resorption can be seen as root contour irregularity located in the neck of the tooth (cervical resorption) at any level of external root surface, or at the apex followed by surrounding bone resorption (19). Root apex is susceptible to resorption due to anato-morphological variations in cementum-dentine connection structure, as well as due to a number and Sharpey's fibers attachments. The mentioned structures with some others as well, form a barrier that prevents the clastic cell activity (21). In the present study, the apical region appeared funnel-shaped, which is in accordance with previous research (22). According to the present findings, the resorption areas are localized at the apex with clear demarcation line which separates healthy from the affected area, pointing to the fact that pressure which creates lesions can be a significant factor in this process (Figure 3).

Different epidemiological research related to root resorption is allowing us to see a bigger picture and enable us to perform adequate and "timely" diagnosis, and treatment of the tooth with external root resorption (23).

Resorption areas are unreachable for the chemical-mechanical treatment, thus they remain the resident places for different microorganism (24, 25). This information should be beared in mind when treating root canals since the presence of external root resorption can be the cause of therapeutic failure.

For the treatment of external resorption 2% solution of chlorhexidine and calcium hydroxide powder are used, due to their relatively low cytotoxic potential and stable antibacterial properties. The usage of sodium hypochlorite is not recommendable since in the case of root resorption it can pass through apical foramen and cause periapical irritation (23). Chlorhexidine increases dentin pH, inhibits acidic hydrolase, arriving from osteoclasts, activity in periodontal tissue and at the same time inhibits alkaline phosphates. It also acts as antiseptic with a prolonged activity which increases antibacterial activity of calcium hydroxide (23).

External root resorption is a process that involves the activity of multinuclear clastic cells originating from periodontal ligaments. The process is spreading from the surface, in a form of infiltration, into a cementum and dentin at different root levels (2).

The most commonly affected teeth are upper molars and incisors, which is explained by the fact that these teeth are under greater occlusal pressure, especially when the occlusion is not adequate. Additionally, in these teeth, crown abrasion as a consequence of occlusal pressure could be seen (8). The present study revealed that the teeth affected by external apical root resorption were secondary mandibular premolars, which is not in agreement with the previous findings. However, such conclusion related to the frequency of the affected teeth, in this case, is not possible due to small sample size.

The literature data related to RANKL-RANK-OPG system response to traumatic occlusion, which could be observed as a mechanical stress that would trigger inflammatory mechanism within the pulp, are scarce. It is known that aseptic inflammatory response is the organisms' response to any mechanical stress including the traumatic occlusion (13). Having in mind that there are no data related to this topic, one can assume, but not claim, that the external root resorption is the consequence of stress caused by traumatic occlusion.

The disturbance in pulp circulation arriving from a traumatic occlusion initiates immunological mechanisms and causes clast cell differentiation, where the entire immunological mechanism is under the control of proinflammatory cytokines (4). Proinflammatory cytokines are binding to pattern recognition receptors (PRR), which include TRAF6 that activates protein complex NF-B, protein kinase c-Src and JNK-cell cycle regulators. If the OPG would block RANKL clastic cells would undergo apoptosis rather than their activation. The relation RANKL-RANK is mandatory for clastic cell survival (8).

Conclusion

In our population, external root resorption is not a very common clinical phenomenon and the data relating to its prevalence, accurate and fast diagnosis are a predisposition for successful endodontic treatment. Based on the scanning electron microscopic analysis of the apical root dentin, one can conclude that the main cause of the external

pathological resorption of the apical root, occurring due to traumatic occlusion, has immunopathogenic background. Aseptic pulp inflammation that is a consequence of mechanical stress, an initial factor in pro-inflammatory cytokine production (IL-1, IL-6, and TNF-α), leads to tissue resorption at various parts of the root.

References

1. Bartok RI, Văideanu T, Dimitriu B, Vârlan CM, Suciu I, Podoleanu D. External radicular resorption: Selected cases and review of the literature. J Med Life 2012;5: 145-8. [PubMed]
2. Felippe WT, Ruschel MF, Santos Felippe G, Pozzobon MH, Santos Felippe MC. SEM evaluation of the apical external root surface of teeth with chronic periapical lesion. Aust Endod J 2009;35:153-7. [CrossRef] [PubMed]
3. Sreeja R, Minal C, Madhuri T, Swati P, Vijay W. A scanning electron microscopic study of the patterns of external root resorption under different conditions. J Appl Oral Sci 2009;17:481-6. [CrossRef] [PubMed]
4. de Silveira PF, Boessio Vizzotto M, Montagner F, Dias da Silveira HL, Dias da Silveira HE. Development of a new in vitro methodology to simulate internal root resorption. J Endod 2016;42:978-83. [CrossRef] [PubMed]
5. Bouysson M, FH. Lepp, C. Zerossi, editors. Resorptions dentaires et biologie osseuse, Sciences et Lettres: Liege; 1965.
6. Andreasen JO. External root resorption: its implication in dental traumatology, paedodontics, periodontics, orthodontics and endodotics. Int Endod J 1985;18: 109-18. [CrossRef] [PubMed]
7. Saoud TMA, Mistry S, Kahler B, Sigurdsson A, Lin LM. Regenerative endodontic procedures for traumatized teeth after horizontal root fracture, avulsion, and perforating root resorption. J Endod 2016;42:1476-82. [CrossRef] [PubMed]
8. Ne RF, Witherspoon D, Gutmann JL. Root resorption. Quintessence Int 1999;30:9-25. [PubMed]
9. Hulsmann M, Schafer E. Resorption. In: Hulsmann M, Schafer E. editors. Problems in endodontics, eiology, diagnosis and treatment, London: Quintessence; 2009, p. 421-34.
10. Yusof WZ, Ghazali MN. Multiple external root resorption, JADA 1989;118:453-55. [CrossRef] [PubMed]
11. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309-19. [CrossRef] [PubMed]
12. Komine M, Kukita A, Kukita T, Ogata Y, Hotokebuchi T, Kohashia O. Tumor necrosis factor-α cooperates with receptor activator of nuclear factor κB ligand in generation of osteoclasts in stromal cell-depleted rat bone marrow cell culture. Bone 2001;28:474-83. [CrossRef] [PubMed]
13. Gunraj MN. Dental root resorption. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:647-53. [CrossRef] [PubMed]
14. Trope M, Chivian N. Traumatic injuries. In: Choen S, Burns RC. editors. Pathways of the pulp 7th ed., Maryland Heights: Mosby Inc; 1998. p. 572-82.
15. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3: 86-90. [CrossRef] [PubMed]
16. Hammarström L, Lindskog S. General morphological aspects of resorption of teeth and alveolar bone. Int Endod J 1985;18:93-9. [CrossRef] [PubMed]
17. Ehnevid H, Jansson L, Lindskog S, Weintraub A, Blomlof L. Endodontic pathogens: propagation of infection through patent dentinal tubules in traumatized monkey teeth. Endod Dent Traumatol 1995;11:229-34. [CrossRef] [PubMed]
18. Ferlini J. Estudo radiográfico e microscópico das reabsorções radiculares na presença de periodontites apicais crônicas (microscopia ótica e eletrônica de varredura). PhD thesis, Universidade de São Paulo, Bauru, 1999.
19. Rosa Neto JJ, Esberard RM, Consolaro A. Estudo em microscopia eletrônica de varredura do ápice radicular e do limite apical de obturação em dentes portadores de lesão periapical crônica. Rev Dent Press Biol Oral 2002;1:18-28.
20. Bergmans L, Van Cleynenbreugel J, Verbeken E, Wevers M, Van Meerbeek B, Lambrechts P. Cervical external root resorption in vital teeth. X-ray microfocustomographical and histopathological case study. J Clin Periodontol 2002;29:580-5. [CrossRef] [PubMed]
21. Opačić V, Živković S. Učestalost eksternih resorpcija na korenu zuba, Srpski arhiv 2004;132:152-6. [CrossRef] [PubMed]
22. Malueg LA, Wilcox LR, Johnson W. Examination of external apical root resorption with scanning electron microscopy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:89-93. [CrossRef] [PubMed]
23. Opačić-Galić V, Živković S. Endodotska terapija patoloških resorpcija korena zuba. Stom Glas S 2004;51: 130-5. [CrossRef]
24. Lomçali G, Sen BH, Çankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. Dent Traumatol 1996; 12:70-6. [CrossRef] [PubMed]
25. Leonardo MR, Rossi MA, Silva LAB, Ito Y, Bonifácio KC. SEM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002;28:815-8. [CrossRef] [PubMed]

Originalni rad **UDC: 616.314.16-007.23-07**
doi:10.5633/amm.2021.0205

ULTRASTRUKTURNA ANALIZA EKSTERNE RESORPCIJE APEKSA KORENA ZUBA

Aleksandar Mitić[1], Vladimir Mitić[2], Jelena Popović[1], Stefan Dačić[1], Radomir Barac[1], Kosta Todorović[3]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za bolesti zuba i endodonciju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za preventivnu i dečiju stomatologiju i ortopediju vilica, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za maksilofacijalnu i oralnu hirurgiju, Niš, Srbija

Kontakt: Aleksandar Mitić
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: alek.mitic@yahoo.co.uk

Resorpcija mineralizovanog tkiva mlečnih zuba smatra se fiziološkim procesom, dok je ovaj proces kod osoba sa stalnom denticijom uvek patološki. Eksterna resorpcija korena posledica je multinuklearne aktivnosti klastnih ćelija, koja započinje na površini korena i širi se dalje u cement sve do dentina. U normalnim uslovima postoji dinamička ravnoteža između aktivnosti ćelija osteoblasta i osteoklasta, koje održavaju fiziološko stanje korena i kosti. Zbog dis-balansa aktivnosti ćelija, pod uticajem različitih faktora, dolazi do resorpcije korena. Cilj ovog rada je ultrastrukturna analiza patološki resorbovanog cementa i dentina na apeksu korena zuba, koji su posledica traumatske okluzije. Studija je sprovedena na 18 izvađenih zuba muškaraca starih od 54 do 73 godine, sa internom i eksternom patološkom resorpcijom korena zuba. Resorbovana površina korena zuba (dentinska struktura) analizirana je na skening elektronskom mikroskopu. Kod svih posmatranih uzoraka uočene su naprsline na okluzalnoj površini gleđi, kao posledica traumatske okluzije. Ultrastrukturnom analizom dentinske površine u perifernim delovima korena, uočena je glatka površina resorbovanog apeksa, slična "ljusci jajeta". Na periferiji korena zuba uočljiva je jasna demarkaciona linija, koja odvaja očuvani od resorbovanog dentina. Uočava se i slojevita, talasasta resorpcija iregularne strukture. Na osnovu analize skening elektronske mikroskopije dentina na apeksu korena zuba, može se zaključiti da značajnu ulogu u etiologiji patološke eksterne resorpcije apeksa korena zuba, pod dejstvom traumatske okluzije, ima aseptična inflamacija.

Acta Medica Medianae 2021;60(2):44-50.

Ključne reči: *traumaska okluzija, eksterna resorpcija korena, SEM analiza*