Majalah Kedokteran Gigi Indonesia
Vol 7 No 2 – August 2021
ISSN 2460-0164 (print), ISSN 2442-2576 (online)
Available online at https://jurnal.ugm.ac.id/mkgi
DOI: http://doi.org/10.22146/majkedgiind.66092

RESEARCH ARTICLES

Effect of two different materials in sealing vertical root fractures of intentional replantation on epithelial thickness of periradicular tissue

Raphael Tri Endra Untara*, Widjijono, Widya Asmara***, Diatri Nari Ratih*✉**

*Department of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
**Department of Biomaterial, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
***Department of Microbiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
*Jl Denta No 1 Sekip Utara, Yogyakarta, Indonesia; ✉ correspondence: diatri@ugm.ac.id

ABSTRACT

Vertical root fractures of intentional replantation need a material capable of binding the fragments of the fracture line tightly and encouraging the regeneration of periradicular tissue. One of the indicators that regeneration of periradicular tissue takes place is epithelial thickness. This study aimed to investigate the effect of mineral trioxide aggregate (MTA) and self-adhesive resin cement as the adhesive materials in sealing vertical root fractures of intentional replantation on the epithelial thickness of periradicular tissue. This study used 27 male New Zealand rabbits with the age range of 8-12 weeks. The mandibular incisor was extracted, and the tooth was cut from the cervical border to the 2/3 apical third. The samples were assigned randomly into three groups of 9 each, namely Group 1 with no application of any material in the fracture line (control group), Group 2 with MTA, and Group 3 with self-adhesive resin cement. All the teeth in all the groups were then inserted back into the socket. Each group was further divided into three subgroups based on the observation time, namely days 7, 14, and 21. Histological observations of the epithelial thickness were carried out under the light microscope (400x magnification). Data were analyzed using a two-way ANOVA and LSD post hoc test with a confidence level of 95%. The two-way ANOVA analysis showed that the materials used for sealing the fracture lines had a significant effect on the epithelial thickness ($p<0.05$), while the observation time did not affect the epithelial thickness ($p>0.05$). No interaction occurred between the material used and observation times ($p>0.05$). It can be concluded that MTA generated a greater epithelial thickness of periradicular tissue compared than self-adhesive resin cement in sealing vertical root fractures of intentional replantation.

Keywords: epithelial thickness; Intentional replantation; MTA; Regeneration of periradicular tissue; Self-adhesive resin cement

INTRODUCTION

Trauma to the teeth is one factor that induces fracture of hard tissue such as the crown along with the root or only the crown or the tooth root. Various factors can generate fracture, such as iatrogenic errors, accidents, and mastication. This fracture, which occurs not only in anterior but also in posterior teeth, causes loose or avulsed tooth.[1] Fracture of teeth can be categorized into vertical (longitudinal) and horizontal fractures. The majority of vertical fracture is caused by occlusal pressure and dental procedures. The vertical fracture occurs if the force within the root canal surpasses the strength of the dentin of the root. As a consequence of the fracture, many irritants may enter and inhabit the fracture gap and its adjacent canals, which in turn, bacteria and their products could then conceal in the fracture gap.[2,3] The inflammation of periodontal tissue will appear, and then connective tissue will grow into the fracture gap to the root canal. This condition may induce root surface resorption, which results in the extraction of the tooth.[4]

Intentional replantation is the most desirable treatment if vertical root fracture occurs since the tooth root remains embedded in its socket.[5] According to Becker,[6] intentional replantation is defined as extracting the tooth intentionally, followed by a root canal treatment entirely out of the socket, and finally, the tooth is returned to the same socket in one visit. A previous study reported that approximately 96.9% of 64 vertical fractures of posterior teeth achieved clinical success following two years of treatment using composite resin to seal the fracture gap, and intentional replantation

was performed.[4] Another study on the intentional replantation of vertical tooth fractures was also done by Unver et al,[5] which showed that the fracture gap of vertical root fracture was sealed by adhesive resin materials. After 36 months, the clinical evaluation demonstrated that the fractured tooth was asymptomatic and radiographically good, and its periodontal pocket depth and vertical bone loss reduced.

The most important factor in enhancing the success of the treatment of vertical root fracture is the selected materials used to seal the fracture gap and the technique, hence, the fracture gap is sealed tightly, preventing any bacteria and their products from occupying the fracture gap.[6] Recently, self-adhesive resin cement has been popular in the clinic. This material needs only one step to seal the fracture gap and does not require pre-treatment on the tooth surface.[2] It contrasts with a previous study conducted by Liying et al,[7] which used dentin bonding and composite resin in a separate step to seal the fracture gap. Thus, self-adhesive resin cement is proposed to be utilized to seal vertical root fractures that will simplify the treatment.[8,9]

Thus far, mineral trioxide aggregate (MTA) is a material of choice to seal the fracture gap since it has many beneficial properties, such as biocompatibility, closure density, mechanical strength, and the healing capacity of periradicular tissue.[10,11] Even though the clinical success of employing composite resin in intentional replantation showed more than 90% after two years following the treatment,[7] it is still necessary to evaluate its success histologically to evaluate the regeneration of periradicular tissue, particularly the epithelial thickness. In addition, tooth extraction on intentional replantation could rupture the periodontal ligament, resulting in tooth socket injury.[12] This injury is a complicated process, which includes homeostasis and inflammation, proliferation, and maturation phases. In the proliferation phase, there will be an increase in the number of cells and wound healing factors; one of them is fibroblast proliferation. Fibroblasts will produce collagen that enables them to bind the wound and affect the re-epithelialization process.[13]

The periodontal healing phase consists of the inflammatory, proliferation (angiogenesis, epithelialization, and fibroplasia), and maturation.[14] Epithelialization is one of the parameters of successful wound healing perceived from increased epithelial thickness. This epithelialization is a crucial factor related to epithelial function, which forms the first barrier between the body and the environment.[15] It is not possible to undertake a histological study of vertical root fracture in the intentional replantation in humans because it involves the procedure of cutting the jaw to make histologic preparations. Thus, a histological observation can be carried out on experimental animals. One of the experimental animals that can be used is rabbits since they have physiological conditions similar to humans, specifically the gingival mucosa.[16]

However, only several studies are available in the literature that have evaluated MTA's effect and self-adhesive resin cement as the adhesive materials for vertical root fracture fragments on the epithelial thickness of periradicular tissue. Therefore, the purpose of this study was to investigate the effect of MTA and self-adhesive resin cement as the adhesive material for vertical root fracture fragments on the epithelial thickness of periradicular tissue.

MATERIALS AND METHODS

Ethical Clearance has been obtained from the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada number 00566/KKEP/FKG-UGM/ EC/ 2020. A total of 27 white male rabbits, New Zealand, aged 8-12 weeks with a bodyweight of 2500-3000 grams were used in this study.

All the rabbits were physically healthy with normal oral cavity conditions and no infection. Intramuscular anesthesia on the thigh with 10% xylazine ketamine 10 mg/kg body weight was performed on all the rabbits. Before surgery, oral prophylaxis was done with 10% povidone-iodine solution (Betadine, PT Mahakam Beta Farma, Jakarta, Indonesia). The gum tissue was separated from the tooth socket.

Majalah Kedokteran Gigi Indonesia. August 2021; 7(2): 88 – 94
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

Furthermore, the mandibular incisor was extracted with pliers. The crown was cut incisal half o prevent contact with the antagonist. To simulate the vertical fracture, the tooth was sectioned perpendicularly to the tooth axis from the CEJ edge in a labio-palatal direction using a 0.6 mm diameter of round-end diamond fissure bur (DDS lab, Tampa, FL, USA) along 2/3 of the root length, and leaving 1/3 of the apical tooth intact.[17] The pulp tissue was extirpated using barbed broach (Dentsply Sirona, Charlotte, NC, USA) and the teeth were then irrigated using sterile saline (0.9% NaCl, PT. Otsuka, Semarang, Indonesia). Group 1 as the control group received no application of any material in the fracture line; the tooth was inserted back into the socket (replantation). In group 2, MTA was applied to the the fracture line. In group 3, self-adhesive resin cement was applied to the fracture line, then polymerized using a LED light source (Woodpecker, China) for 20 seconds at both sides of the sections. Following the material application to the fracture line, the teeth in groups 2 and 3 were inserted back into the socket. The area of operation was smeared with 10% povidone-iodine antiseptic.

Afterwards, the rabbits were euthanized using an injection of ketamine xylazine at a dose of 10 mg/kg body weight, and their jaw specimens were taken according to the observation time, namely 7, 14, and 21 days. Then the jaw in the incisor region was dissected, and the specimens for Hematoxylin Eosin (H&E) examination were made from the root cleavage site to the mucosal outer margin. Hematoxylin Eosin staining was performed using an Automatic Staining Machine (Thermoscientific, Waltham, MA, USA). The sequences of H&E staining started with a deparaffinization process, which was performed by inserting the preparations into xylol three times for 5 minutes each. Then the specimens were gradually rehydrated with alcohol concentrations of 100%, 95%, 80%, and 70% for 2 minutes, respectively. The remaining alcohol was removed by placing the specimen slides in distilled water. Hematoxylin staining was done by immersing the specimens in a hematoxylin solution for 7 minutes to obtain a blue color on the cell nucleus and then washing them by inserting the specimen slides in distilled water for 7 minutes to remove any remaining staining. Eosin staining was done to provide a red color as a contrast to the cytoplasm. Counterstain was performed using eosin solution for ½-1 minute, then rinsed by adding them to distilled water to remove the remaining eosin solution, and dehydrated with 70%, 80%, 95%, and 100% alcohol for 4 minutes, respectively. The specimens were immersed in xylol 1, 2, and 3 solutions for 2 minutes each to provide tissue transparency.

The histological preparations of the epithelial thickness were observed under a light microscope (Olympus, Japan) along with OptiLab with a magnification of 400X. The examination was done by measuring the epithelial thickness from the basal stratum to the stratum corneum in µm using image raster software (versions: 4.0) in three selected fields of view, and then the results were averaged. The data obtained were analyzed using two-way ANOVA followed by the LSD (Least Significant Difference) post-hoc test at a significance level of 95%.

RESULTS

Table 1 shows that the epithelial thickness of periradicular tissue after intentional replantation of vertical tooth fractures was the highest in the control group with an observation time of 21 days (533.438 + 151.3 um). Conversely, the lowest epithelial thickness was observed in the self-adhesive resin cement application on the fracture line group with an observation time of 14 days (188.617 + 48.9 um).

Figure 1 reveals that based on the observation time, the mean epithelial thickness in the control group increased from day 7 to day 21. In contrast, the mean epithelial thickness using MTA and self-adhesive resin cement decreased from day 7 to day 14, then increased on day 21. However, MTA produced greater epithelial thickness than self-adhesive resin cement on all days of observation.

The two-way ANOVA analysis showed that observation time had no effect on the epithelial thickness ($p>0.05$). In contrast, a significant

Table 1. The mean and standard deviation (SD) of the epithelial thickness observed on days 7, 14, and 21 following intentional replantation of vertical root fractures with the application of MTA and self-adhesive resin cement (in um)

No	Group	n	Day 7	Day 14	Day 21
1	Group 1	3	277.89 ± 102	453.39 ± 5.2	533.44 ± 151.3
2	Group 2	3	443.71 ± 168.7	351.65 ± 31.8	449.42 ± 140.4
3	Group 3	3	256.45 ± 54.6	188.62 ± 48.9	324.43 ± 89.8

Note:

- Group 1 (Control): teeth were split 2/3 in an apical direction and then replanted without application of any material
- Group 2: teeth were split 2/3 in an apical direction, then MTA was applied to the fracture line; finally, the tooth was replanted
- Group 3: teeth were split 2/3 in an apical direction, then self-adhesive resin cement was applied to the fracture line ; finally, the tooth was replanted.

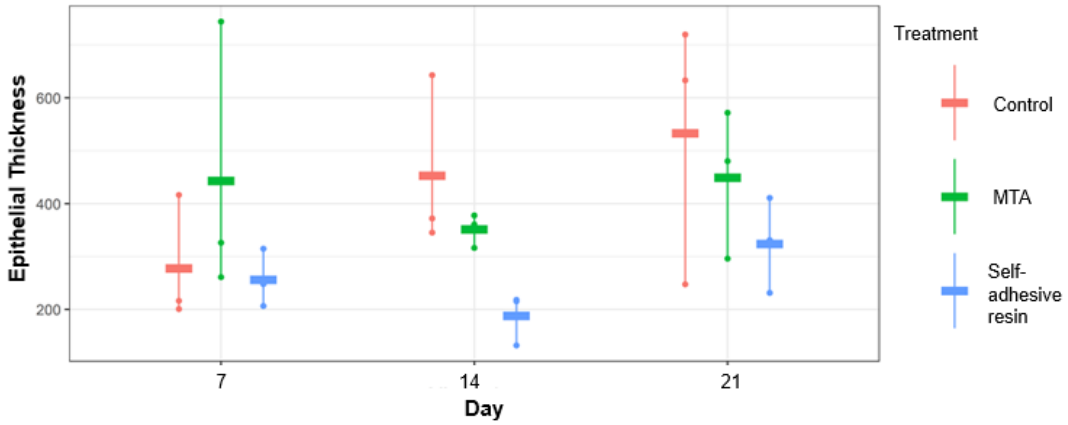

Figure 1. The graph of mean epithelial thickness on days 7, 14, and 21

- The point on the vertical line is the observed value (epithelial thickness)
- The vertical line shows one group and shows the width (range) of observations per group per day
- The thick horizontal line is the per-group mean per day.

Table 2. Analysis using two-way ANOVA of the epithelial thickness observed on days 7, 14, and 21 after intentional replantation of vertical root fractures with the application of MTA and self-adhesive resin cement

Parameter	df	Sum of squares	Average of squares	F	p-value
Observation time	1	54201.68	54201.68	2.483	0.127
Material used	2	157173.77	78586.89	3.600	0.040*
Interaction	2	50737.52	25368.76	1.162	0.337

*) Significant difference

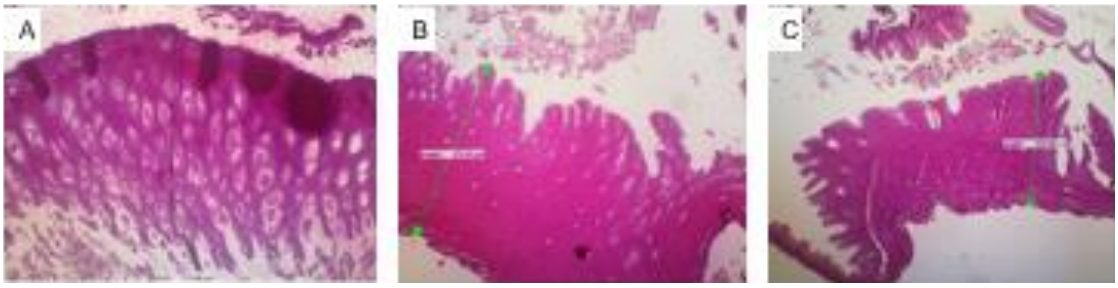

Figure 2. Histological images exhibiting the epithelial thickness on day 21 of the control group (A), MTA (B), and self-adhesive resin (C) (H&E stain at 400x magnification)

Majalah Kedokteran Gigi Indonesia. August 2021; 7(2): 88 – 94
ISSN 2460-0164 (print)
ISSN 2442-2576 (online)

difference was found in the material used on the epithelial thickness ($p<0.05$). No interaction occurred between observation time and the material used on the epithelial thickness ($p> 0.05$) (Table 2).

The representative specimens of the histological images of the epithelial thickness are revealed in Figure 2. It can be seen that on day 21, the control group obtained the highest epithelial thickness compared to the other groups, namely MTA and self-adhesive resin cement.

DISCUSSION

The indicator to observe the regeneration of the periradicular tissue in this study was the epithelial thickness formed during the observation period, namely days 7, 14, and 21 following the intentional replantation. The epithelium was used as an indicator since epithelialization is one of the parameters of wound healing, which can be revealed from an increased epithelial thickness. Furthermore, this epithelialization is crucial for the epithelial function that performs as the first barrier between the body and the environment.[18]

The results of the study based on the observation time demonstrated that on day 21, the epithelial thickness of the control group was the highest compared to the MTA and self-adhesive resin cement groups. This phenomenon is possibly due to no foreign bodies entered the tooth socket, resulting in normal wound healing. It can be seen that the epithelial thickness was the greatest in the control group than the other two groups.[19] This condition occurred because the tooth roots had a similar composition as the hard tissue in the body, hence, no reaction of the body tissues resisted against the tooth replanted into its socket.[20]

In the MTA and self-adhesive resin cement groups, the epithelial thickness decreased from day 7 to day 14 but increased on day 21. This indicated that the wound healing process on the intentional replantation did not occur mormally.[21] This condition might be due to the utilization of the two materials to seal the fracture line, namely MTA and self-adhesive resin cement, which were considered foreign bodies by the body tissues. As a consequence, the epithelial thickness decreased on day 14. The increase in the epithelial thickness on day 21 might be due to the neutralization effect of the foreign bodies by the body tissues.[20,21]

The epithelial thickness of the MTA group was higher than that of the self-adhesive resin cement group. This proved that MTA has high biocompatibility, which can be well tolerated by body tissue compared to self-adhesive resin cement.[10,11] These results are in accordance with a study conducted by Lillo et al[22] and Solanki et al,[23] which reported the biocompatibility of MTA in human cells. In addition, another study reported that the 12-week observation of animal tissue *in vitro* and *in vivo* exhibited normal cell growth and no infection.[24]

On the contrary to MTA, the fact that self-adhesive resin may have a detrimental effect on periodontal tissue is due to cytotoxic constituents influencing the regeneration of wounded tissue because of intentional replantation.[25] The components contained in self-adhesive resin cement that are believed to be cytotoxic are TEGMA and fluoride.[9] Furthermore, the LED light cure unit, which was used in this study to polymerize the resin cement, might also cause a negative effect because this light source may not completely polymerize the resin cement.[8] Subsequently, free monomer components, such as Bis-GMA, UDMA, TEGDMA camphorquinone, and HEMA, may be found. These free monomers have the potential to cause pulp injury and prevent a proper tissue healing process.[26]

CONCLUSION

Based on the results of this study, it can be concluded that MTA resulted in a greater epithelial thickness of periradicular tissue compared to self-adhesive resin cement in sealing the vertical root fractures of the intentional replantation. Therefore, using MTA rather than self-adhesive resin cement in the clinic is recommended, although further study is still needed.

REFERENCES

1. Benoist FL, Seck A, Benoist HM. 42-Month follow-up of double root fracture of a lateral

mandibular incisor. Eur Endod J. 2017; 2(1): 1-5. doi: 10.5152/eej.2017.17021
2. Khasnis SA, Kidiyoor KH, Patil AB, Kenganal SB. Vertical root fractures and their management. J Conserv Dent. 2014; 17(2): 103–110. doi: 10.4103/0972-0707.128034
3. Dhawan A, Gupta S, Mittal R. Vertical root fractures: An update review. J Rest Dent. 2014; 2(3): 107-113.
4. Kumar ARP, Shemesh H, Jothilatha S, Vijayabharathi R, Jayalakshmi S, Kishen A. Diagnosis of vertical root fractures in restored endodontically treated teeth: A time-dependent retrospective cohort study. J Endod. 2016; 42(8): 1175-1180. doi: 10.1016/j.joen.2016.04.012
5. Unver S, Onay EO, Ungor M. Intentional re-plantation of a vertically fractured tooth repaired with an adhesive resin. Int Endod J. 2011; 44(11): 1069–1078. doi: 10.1111/j.1365-2591.2011.01922.x
6. Becker BD. Intentional replantation techniques: a critical review. J Endod. 2018; 44(1): 14-21. doi: 10.1016/j.joen.2017.08.002.
7. Liying Y, Beiyun X, Bin W, Qing C, Fang YR, Yamamoto K, Inoue M, Suwa F. Clinical research on treatment of vertically fractured posterior teeth by intentional replantation using dentin bonding and composite resin. J Osaka Dent Univ. 2004; 38(2): 101-107. doi: 10.18905/jodu.38.2_101
8. Kamalak H, Taghizadehghalehjoughi A, Kamalak A. The biocompatibility of resin composite materials on different stem cells. Biomedical Research. 2019; 30(1): 32-41. doi:10.35841/biomedicalresearch.30-18-1162
9. Maño EP, Algarra RM, Fawzy A, Leitune VCB, Collares FM, Feitosa V, Sauro S. In vitro bonding performance of modern self-adhesive resin cements and conventional resin-modified glass ionomer cements to prosthetic substrates. Appl Sci. 2020; 10(22): 1-15. doi: 10.3390/app10228157
10. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review-part III: Clinical applications, drawbacks, and mechanism of action. J Endod. 2010; 36(3): 400-413. doi: 10.1016/j.joen.2009.09.009
11. Nagmode PS, Satpute AB, Patel AV, Ladhe PL. The effect of mineral trioxide aggregate on the periapical tissues after unintentional extrusion beyond the apical foramen. Case Reports in Dentistry. 2016; 2016: 1-5. doi: 10.1155/2016/3590680
12. Maddaluno L, Urwyler C, Werner S. Fibroblast growth factors: Key players in regeneration and tissue repair. Development. 2017; 144(22): 4047-4060. doi: 10.1242/dev.152587
13. Addis R, Cruciani S, Santaniello S, Bellu E, Sarais G, Ventura C, Maioli M, Pintore G. Fibroblast proliferation and migration in wound healing by phytochemicals: evidence for a novel synergic outcome. Int J Med Sci. 2020; 17(8): 1030-1042. doi:10.7150/ijms.43986
14. Shah R, Domah F, Shah N, Domah J. Surgical wound healing in the oral cavity: a review. Dental Update. 2020; 47(2): 135-143. doi: 10.12968/denu.2020.47.2.135
15. Paul J, D'Lima PJ, Thomas S, Karim S, Thomas P, Bindhu PK, Paul B, Salma R. Histological evaluation of epithelial thickness, microvasculature and number of mast cells in the gingiva of smokers and non-smokers with chronic periodontitis. Journal of Clinical and Diagnostic Research. 2017; 11(11): ZC18-ZC21. doi: 10.7860/JCDR/2017/29112.10886
16. Szabo Z, Bradley K, Cahalane AK. Rabbit soft tissue surgery. Vet Clin North Am Exot Anim Pract. 2016; 19(1): 159–188. doi: 10.1016/j.cvex.2015.08.007
17. Marouane O, Turki A, Oualha L, Douki N. Tooth replantation: an update. Med Buccale Chir Buccale. 2017; 23(2): 103-110. doi: 10.1051/mbcb/2016056
18. Prabhu P, Julius A, Elumalai M, Prabhu MN. Wound healing in periodontic. Biosciences, Biotechnology Research Asia. 2014; 11(2): 791-796. doi: 10.13005/bbra/1339.
19. Meilawaty Z, Chriestedy R, Recky F. Cassava leaf flavonoid extract on enhancing the gingival epithelium thickness of lipopolysaccharide-

induced rats. Journal of International Dental and Medical Research. 2020; 13(3): 909-914.
20. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff R, Tomi-Canic M. Epithelialization in wound healing: a comprehensive review. Adv Wound Care. 2014; 3(7): 445-464. doi: 10.1089/wound.2013.0473
21. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009; 37(5): 1528-1542. doi: 10.1177/147323000903700531
22. Lillo C, Quesada B, Lorente A. Biocompatibility of regular and white MTA and a grey portland cement in human dental pulp cells. Open Journal of Dentistry and Oral Medicine. 2018; 6(2): 1-5. doi: 10.13189/ojdom.2018.060202
23. Solanki NP, Venkappa KK, Shah NC. Biocompatibility and sealing ability of mineral trioxide aggregate and biodentine as root-end filling material: a systematic review. J Conserv Dent. 2018; 21(1): 10-15. doi: 10.4103/JCD.JCD_45_17
24. Suprastiwi E. Material bioaaktif dalam ruang lingkup perawatan konservasi gigi. Jakarta: Departemen Ilmu Konservasi Gigi FKG UI; 2018. 39-50.
25. Upadhyay A, Pillai S, Khayambashi P, Sabri H, Lee KT, Tarar M, Zhou S, Harb I, Tran SD. Biomimetic aspects of oral and dentofacial regeneration. Biomimetics. 2020; 5(4): 51. doi: 10.3390/biomimetics5040051.
26. Şişmanoğlu S, Demirci M, Schweikl H, Ozen-Eroglu G, Cetin-Aktas S, Kuruca S, Tuncer S, Tekce N. Cytotoxic effects of different self-adhesive resin cements: cell viability and induction of apoptosis. J Adv Prosthodont. 2020; 12(2): 89-99. doi: 10.4047/jap.2020.12.2.89