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The Effect of Mineral Trioxide Aggregate and Other Endodontic Materials in Contact with Osteoblast-Like Cell

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ABSTRACT

Objective: The objective of the study was to compare the effect of mineral trioxide aggregate and other endodontic materials in contact with osteoblast-like cell over a time period of 20 days.

Materials and methods: Human PDL cells and osteoblasts were gathered, cultured and permitted to standardized protocols. The cell populations were characterized with the conforming surface markers following standardized processes. The specimens were produced with special concern to constant dimensions and volume in the different groups. Cell attachment and proliferation were assessed morphologically after Richardson staining and cell count was performed after 1d, 7d, 14d and 21d. All trials were done in triplets. The outcomes were statistically analyzed using the ANOVA- and Tukey-test ($p < 0.05$).

Results: Morphological examination showed good proliferation and cell attachment in both cements. A remarkable outcome was the prepared increasing and parallel alignment of the PDL cells in connection with MTA and particularly Biodentine. From 7d forward Biodentine indicated the highest quantity of PDL cells ($p < 0.05$). Biodentine and MTA caused in a significantly greater cell density in osteoblast and PDL cell culture. The other groups presented a lower PDL cell density from 7d and a lower osteoblast cell density from 14d when matched to control and cement samples ($p < 0.05$).

Conclusions: MTA and Biodentine showed a good biocompatibility in contact with the human osteoblasts and cells of the periodontal ligament. Regarding cell survival and proliferation particularly of PDL cells Biodentine showed good results and can be considered as a well-tolerated bioactive endodontic material.

Keywords: Composite resin, Mineral Trioxide Aggregate, Biodentine, Periodontal ligament cells, Osteoblasts.

INTRODUCTION

It is assessed that over 24 million endodontic procedures are implemented on an annual basis, with up to 5.5% of those procedures including endodontic apical surgery, perforation repair, and apexification treatment [1]. Endodontic surgery is performed to resolve inflammatory processes that cannot be effectively treated by conventional methods, which might be as a result of complex canal and/or apical anatomy and external inflammatory processes [2]. Surgical procedures can similarly be specified for the resolution of procedural misadventures, to include root perforation that may occur either through canal instrumentation or post-space preparation [2,3]. Surgical treatment regularly includes the placement of a material designed to seal the root canal contents from the periradicular tissues and repair root defects [2]. Comprehensibly, this material should demonstrate the ability to form a seal with dental tissues while similarly exhibiting biocompatible behavior with the periodontal tissues [3]. Throughout endodontic treatment a perforation of the root canal system might arise or a root-end surgery might be required. Both procedures cause in communication of the pulp chamber or the root canal system with the periodontium. For the best prognosis, these contact areas should be restored and sealed. Therefore, purpose of such filling is to obturate the root-end or a perforation cavity and to avoid micro leakage. A suitable filling material should be biocompatible; ensure a long-term three-dimensional sealing of all margins, preferably by a molecular bonding to the dentinal walls; be bacteriostatic, or not encourage bacterial growth; stable; insoluble; non-absorbable; not moisture-sensitive; easy to prepare and place; radiopaque and bioactive and induce regeneration of the periodontal ligament and bone [4-7].

However, for an effective endodontic treatment a high quality apical root canal filling or perforation repair is necessary. Earlier, several diverse materials similar to amalgam, glass ionomer cement, reinforced zinc oxide eugenol cements (IRM, Super-EBA), and composite resin

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were utilized to fill endodontic perforations or as root-end filling material [7,8]. Later, another bioactive calcium silicate bond, Biodentine (Septodont, St. Maur-des-Fossés, France), was propelled on the dental market. Biodentine comprises of a powder in a case and fluid in a pipette. As indicated by Camilleri et al. the powder comprises principally of SiO2 (16.90%), CaO (62.9%), ZrO2 (5.47%), and the fluid is made out of Na (15.8%), Mg (5.0%), Cl (34.7%), Ca (23.6%), and H2O (20.9%) [9].

Despite the fact that ProRoot MTA (Dentsply/Tulsa, Tulsa, OK, USA) seems, by all accounts, to be the favored material in the previously mentioned signs with numerous positive highlights, the concrete has a few disadvantages: the taking care of can be troublesome, the setting time is long, the utilization in the obvious crown zone may prompt tooth staining, the compressive and flexural quality is lower than dentine and it is very costly [10, 11]. In the current years Mineral Trioxide Aggregate (MTA), a refined Portland cement [12], was broadly tried for this propose and was found to gives unmistakably less cytotoxic impacts and better outcomes concerning material properties, biocompatibility, microleakage assurance, bioactivity and accordingly, clinical accomplishment than customary materials suggested for root-end fillings or puncturing repair. Because of its great biocompatibility, mechanical strength and regenerative effect on hard tissue and periodontium, ProRoot MTA is indicated as a kind of perspective material for root aperture repair and root-end obturation [13, 14].

Contrasted with ProRoot MTA relatively little data about Biodentine is accessible. Utilized as root-end filling Biodentine demonstrated clinically a decent recovery after apicoectomy [15]. When contrasting its material attributes with set up tricalcium silicate bonds, Biodentine emerges by its more prominent compressive quality, in all probability caused by the low water/concrete apportion of the blend. The material was found less permeable and denser than MTA; the antacid pH of Biodentine is practically identical to different bonds.Examinations of the microleakage uncovered that tracer dispersion between dental material and dentin dividers was altogether lessened in Biodentine tests contrasted with glass ionomer concrete and MTA. The shading steadiness of Biodentine permits its machine in tastefully vulnerable territories [16].

On the other hand, the choice of a repair material is basic since biocompatibility and fixing capacity are accounted for to affect the forecast of conclusion or apicoectomy. The biocompatibility of endodontic materials is fundamental on the grounds that amid application the materials/ cements may get immediate contact to the encompassing bone or the periodontium for a delayed timeframe. Periodontal ligament (PDL) fibroblasts with specific capacities are in charge of the arrangement and support of PDL fiber connections and repair, redesigning, and recovery of the neighboring alveolar bone and cementum [17]. PDL cells are in charge of ordinary upkeep and recovery of the PDL. Notwithstanding PDL fibroblasts, cells from the encompassing alveolar bone are probably going to assume an imperative part in the repair and recovery of periradicular tissue [18]. PDL cells are normally conformed to the root-end or aperture filling materials [19]. Osteoblasts and PDL cells are the essential cells in charge of rigid excisional twisted recuperating after periradicular surgery.

Materials and methods

The following materials were counted in this study: ProRoot MTA (Dentsply/Tulsa, Tulsa, OK, USA), Biodentine as other calcium silicate cement (Septodont, Saint-Maur-des-Fossés, France), an amalgam (Oralloy Magicap S; Coltène/Whaledent, Altstätten, Switzerland) and a light-curing composite resin (Estelite Σ Quick; Tokuyama Dental, Tokyo, Japan).

From all materials, samples were produced with a defined diameter of 5 mm and a height of 2 mm. All materials were handled strictly according to manufacturer recommendations. The samples were prepared with consideration of their specific curing processes: while Biodentine sets for 12 minutes, MTA sets for four hours and amalgam for 24 hours. The composite samples were light cured in layers (incremental technique).

Primary osteoblasts were harvested from bone chips collected through modelling mandibular osteotomies or the surgical removal of lower wisdom teeth. The bone particles were cultured in MM0 medium (High Growth Enhancement Medium; MP Biomedicals, Eschwege Germany) with fetal bovine serum, Penicillin (10.000U/ml), Streptomycin (10.000 µg/ml) and Amphotericin B 250 µg/ml (Biochrom, Berlin, Germany). After 10 days dexamethasone (Merck, Darmstadt, Germany; 0.02% in phosphate buffered saline (PBS Dulbecco, Biochrom, Berlin, Germany) was added to the medium.

The proliferation of the osteoblasts and the PDL cells was considered and assessed referring to the determined absolute cell counts in the occurrence of the four materials in addition to their absence. The cell colonies were evaluated based on the

morphological analysis of the cell size, the morphology of the nucleus and the proliferation. All outcomes were statistically analyzed using the ANOVA and Tukey test ($p < 0.05$).

The study was done after approval of ethical board of King Abdulaziz university.

Results

One day after the utilization of the human osteoblasts onto the material examples the cell amount was essentially diminished in all groups contrasted with the untreated control group ($p < 0.05$). In the next days it wound up noticeably clear that the light cured composite resin tests negatively affected the osteoblasts. The quantity of cells dropped extensively. In every single other groups the measure of cell expanded in one week. After that time the Biodentine gather indicated essentially a greater number of osteoblasts than all other gathering ($p < 0.05$), though after 14 d altogether more cells could be distinguished in the ProRoot MTA gathering ($p < 0.05$). After 21 d a slight diminishment in the cell sum was unmistakable in all gatherings. Be that as it may, Biodentine and ProRoot MTA demonstrated essentially a larger number of cells than every single other gathering. Effectively after 7 d Biodentine demonstrated a critical higher amount of cells contrasted with the control gathering, while in the amalgam aggregate the measure of cells was noteworthy lower contrasted with the control amass at all days ($p < 0.05$) (Figure 1).

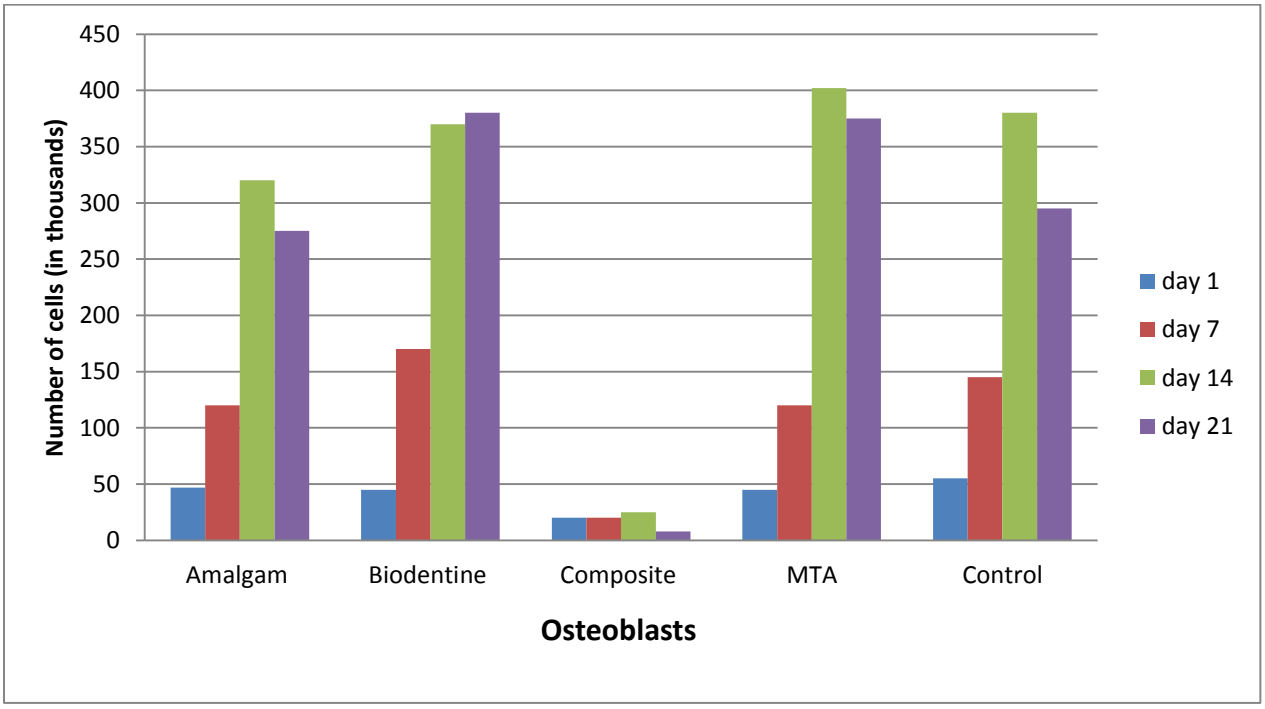

Figure 1: Amount of human osteoblasts after contact to different endodontic restorative materials up to 21 d.

The use of the material samples to PDL cells had not that effect on the cell amount than it has on the osteoblasts following one day. In the amalgam and in the ProRoot MTA group the cell amount was statistically not significantly the same as the control group ($p > 0.05$). Comparable to the osteoblast culture the composite resin showed a strong negative effect on PDL cells ($p < 0.05$). Thus, the amount of cells dropped considerably. Also comparable to the osteoblasts the amount of PDL cells clearly increased in all other groups after one day. Nevertheless, after 8 d and 20 d in contact with amalgam and ProRoot MTA the amount of cells was significantly lower compared to the control group ($p < 0.05$) while from 7 d forward Biodentine presented a significantly higher quantity of PDL cells matched to all other groups ($p < 0.05$). After 14 d a difference in the cell number could not be detected amid the controls and ProRoot MTA ($p > 0.05$) (Figure 2).

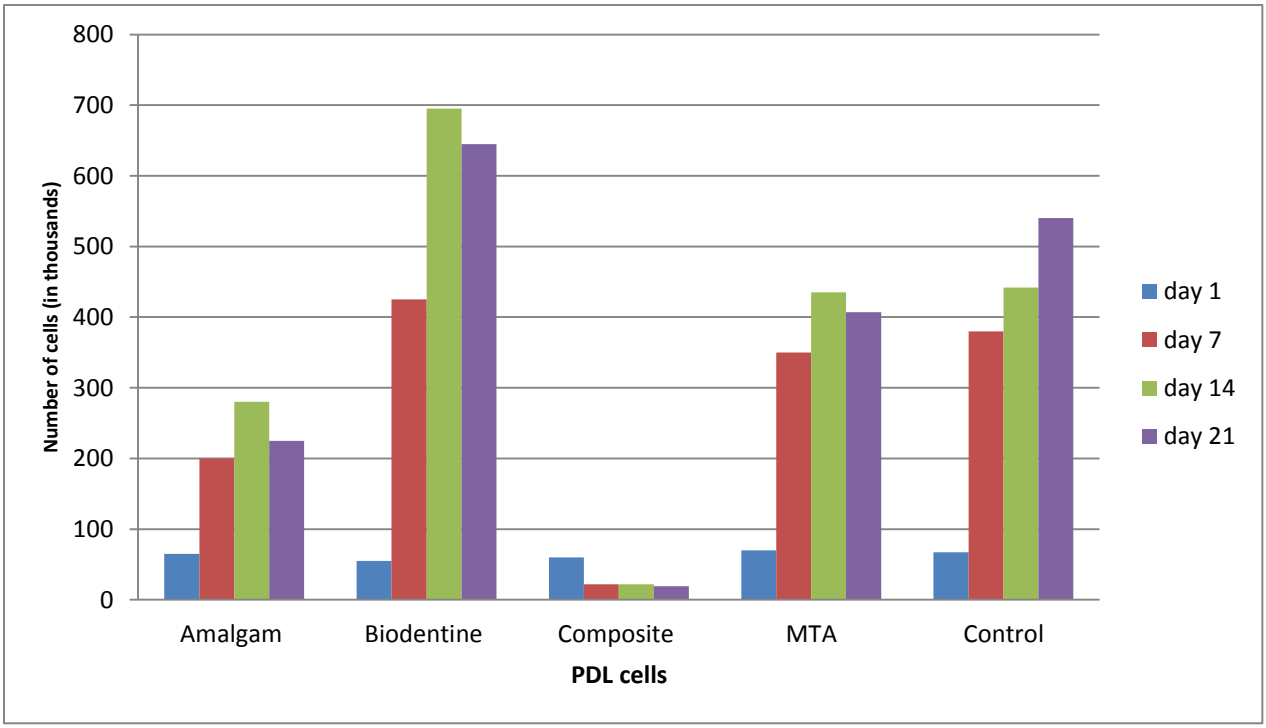

Figure 2: Amount of PDL cells after contact to different endodontic restorative materials up to 21 d.

DISCUSSION

The best mending result subsequent to filling the resected root trench or the aperture would be reconstruction of a typical connection contraption with sound bone, periodontal tendon, and cementum [20]. Consequently, a definitive objective of treatment of root holes or root-end surgery is to keep up or re-set up the harmed connection device [21]. Harm of the PDL will have adverse effects on healing following endodontic surgery, hard recovery and may prompt an unfavorable result of treatment. Thus, to assess the biocompatibility and bioactivity of another calcium silicate cement (Biodentine) in contrast with ProRoot MTA human osteoblasts and PDL cells were decided for this ex vivo examine. The outcomes from investigations of these cells are positive to those from other cell lines (e.g. osteosarcoma cells) or cells of animal root origin because their response concerning cell connection and mineralization might be diverse to human osteoblasts or PDL cells [18]. Recently, MTA has been widely analyzed in dental science and various cytotoxicity and cell attachment examinations with different cell cultures demonstrated better outcomes with MTA in contrast to many other dental materials [14]. Currently, the utilization of MTA may be assumed as the best quality level for the closure of perforations defects or as root-end filling against which other materials should be tried. The great biocompatibility and bioactivity of MTA on PDL cells and osteoblasts are affirmed in the current study and are in completely understanding with the present knowledge about MTA [13-14].

While MTA is extremely very much researched additionally on human cell lines [13] to the best of our insight until today no information are distributed concerning the impact of Biodentine to human osteoblasts or PDL cells. Just Zhou et al. looked at Biodentine and MTA in coordinate contact on human gingival fibroblasts. The two bonds demonstrated no huge contrasts in cell viabilities. The cells joined to and spread over both material surfaces [22].

When contrasting Biodentine and its characteristic properties with other settled dental materials as Super EBA or glass ionomer concrete one has no response to much exploratory information in current literature. In an examination by 2012 Al-Hiyasat et al. who watched the nature of cell connection to different root-end filling materials and inferred that the best cell connection of fibroblasts can be seen on the surface of MTA, though Super EBA surfaces did not pull in cell adherence in all likelihood because of the spilling of eugenol into the dentinal tubules. Unwashed glass ionomer concrete surfaces did not instigate cell connection either [23]; these discoveries bolster our information concerning the organic impact of MTA. In an immediate examination of Biodentine and glass ionomer concrete as dentine substitution material by Camilleri in 2013, glass ionomer bonds demonstrated more physical and concoction solidness and prompted fundamentally

less microleakage when connected under composite rebuilding efforts in a sandwich system. The sign and application for every material must be very much considered [24].

The outcomes of the current examination underline that Biodentine - adjacent to MTA - can be known as a bioactive cement by up-regulating osteoblasts and PDL cells action. Biodentine can be considered as a material that may incite periodontal recovery as well as repair. Biodentine has great properties in regards to biologic reaction of the cells inside the periodontium which were assessed in this investigation. Just in contact to Biodentine the PDL cells developed in a moment cell layer crossway to the first. What's more, from 7 days ahead Biodentine demonstrated the measurably noteworthy most astounding amount of PDL cells. Biodentine is basically made out of tri-and dicalcium silicate. Late research in drug unmistakably demonstrated that the expansion of tricalcium silicate to calcium phosphate bone bonds enhances the bioactivity of those materials on osteoblast or osteoblast like cells [25]. This might be identified with the arrival of silicon (Si) from calcium silicate bonds. It is notable that Si positively affects bone digestion and upgrades the rate of new bone development when discharged from bioactive materials in vivo [26, 27]. These discoveries propose that the arrival of Si from calcium silicate concretes may give extra in vivo bioactivity of these materials. Contrasted with MTA in guide contact to human PDL cells amalgam was fundamentally more cytotoxic inside 1 d and the PDL cell thickness was bring down after 4 d [28]. After 72 h guide contact to amalgam the quantity of osteoblast-like cells was fundamentally lower than after contact to MTA. Though the quantity of the cells in the MTA gather was fundamentally lower than that in the untreated control assemble [29].

The outcome of this study demonstrated that the negative effect amalgam on human PDL cells and osteoblasts was not as particular as imagined. However, in light of the fact that the amount of PDL cells and osteoblasts were significantly less than in the control group at all days, amalgam demonstrated no bioactivity and thus can't be suggested as filling material in endodontic surgery. Just constrained reports about the impact of composite saps on cells related with bone arrangement and periodontal repair are accessible [30]. Consequently, ex vivo ponders like the present? are of some significance. It might be finished up from the after effects of the present investigation that the immediate contact of PDL cells and osetoblasts to composite sap ought to be kept away from in light of the fact that cell multiplication is stifled.

CONCLUSION

ProRoot MTA and Biodentine demonstrated no cytotoxicity and a decent biocompatibility in coordinate contact with osteoblasts and PDL cells. Concerning cell survival and multiplication especially of PDL cells Biodentine demonstrated great outcomes and can be considered as a very much endured endodontic material with stimulatory bioactive properties. Amalgam and particularly composite resin may not give a proper situation to osteoblasts and PDL cells.

REFERENCES

1. **Nash KD, Brown J and Hicks ML(2002):** Private practicing endodontists: production of endodontic services and implications for workforce policy**. J Endod**.,28:699–705.
2. **Chong BS(2004):** Managing endodontic failure in practice. Chicago: Quintessence Publishing Co., Ltd.,Pp. 123–47.
3. **Lee YL, Lee BS, Lin FH, Lin AY, Lan WH and Lin CP(2004):** Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. **Biomaterials**,25:787–93.
4. **Pitt Ford TR(1994):** Surgical treatment of apical periodontitis. In: Ørstavik D, Pitt Ford TR, editors. Essential endodontology. Oxford: Blackwell, pp. 278–308.
5. **Carr GB(1994):** Surgical endodontics. In: Cohen S, Burns RC, editors. Pathways of the pulp. 6. St. Louis: Mosby, pp. 531–567.
6. **Stropko JJ(2009):**Micro-surgical endodontics. In: Castellucci A, editor. Endodontics Vol III. Florence: Edizioni Odontoiatriche Il Tridente, pp. 1118–1125.
7. **Rud J, Rud V and Munksgaard EC(2001):** Periapical healing of mandibular molars after root-end sealing with dentin-bonded resin composite. Int Endod J., 34:285–292.
8. **Bodrumlu E(2008):** Biocompatibility of retrograde root filling materials: A review. Aust Endod J.,34:30–35.
9. **Camilleri J, Kralj P, Veber M and Sinagra E(2012):** Characterization and analyses of acid-extractable and leached trace elements in dental cements. Int Endod J., 45:737–743.
10. **Belobrov I and Parashos P(2011):** Treatment of tooth discoloration after the use of white mineral trioxide aggregate. J Endod.,37:1017–1020.
11. **Karabucak B, Li D, Lim J and Iqbal M (2005):**Vital pulp therapy with mineral trioxide aggregate. Dent Traumatol.,21:240–243.
12. **Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV and Pitt F (2005):** The constitution of mineral trioxide aggregate. Dent Mater.,21:297–303.
13. **Parirokh M and Torabinejad M(2010):** Mineral trioxide aggregate: a comprehensive literature

review - Part III: Clinical applications, drawbacks, and mechanism of action. J Endod.,36:400–413.

14. **Parirokh M and Torabinejad M(2010):** Mineral trioxide aggregate: a comprehensive literature review - Part I: chemical, physical, and antibacterial properties. J Endod.,36:16–27.
15. **Dammaschke T(2012):** Root-end filling with a new bioactive cement. Inside Dent.,8:86–88.
16. **Rajasekharan S, Martens LC, Cauwels R and Verbeeck R (2014):** Biodentine™ material characteristics and clinical applications: a review of the literature. Eur Arch Paediatr Dent.,15:147–158.
17. **Boyko GA, Melcher AH and Brunette DM(1981):** Formation of new periodontal ligament by periodontal ligament cells implanted in vivo after culture in vitro. A preliminary study of transplanted roots in the dog. J Periodontal Res.,16:73–88.
18. **Al-Rabeah E, Perinpanayagam H and MacFarland D(2006):** Human alveolar bone cells interacts with ProRoot and tooth-colored MTA. J Endod.,32:872–875.
19. **Balto H and Al-Nazhan S(2003):** Attachment of human periodontal ligament fibroblasts to 3 different root-end filling materials: Scanning electron microscope observation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 95:222–227.
20. **Harrison JW and Jurosky KA(1992):** Wound healing in the tissues of the periodontium following periradicular surgery. III. The osseous excisional wound. J Endod.,18:76–81.
21. **Camp MA, Jeansonne BG and Lallier T(2003):** Adhesion of human fibroblasts to root-end-filling materials. J Endod.,29:602–607.
22. **Tai KW and Chang YC(2000):** Cytotoxicity evaluation of perforation repair materials on human periodontal ligament cells in vitro. J Endod.,26:395–397.
23. **Al-Hiyasat AS, Al-Sa'Eed OR and Darmani H(2012):** Quality of cellular attachment to various root-end filling materials. J Appl Oral Sci.,20:82–88.
24. **Camilleri J(2013):** Investigation of Biodentine as dentine replacement material. J Dent.,41:600–610.
25. **Zhao Q, Qian J, Zhou H, Yuan Y and Mao Y, Liu C(2010):** In vitro osteoblast-like and endothelial cells' response to calcium silicate / calcium phosphate cement. Biomed Mater.,5:1–9.
26. **Morejón-Alonso L, Ferreira OJB, Carrodeguas RG and dos Santos LA(2012):** Bioactive composite bone cement based on α-tricalcium phosphate/tricalcium silicate. J Biomed Mater Res B.,100B:94–102.
27. **Carlisle EM(1970):** Silicon: a possible factor in bone calcification. Science, 167:279–280.
28. **Keiser K, Johnson CC and Tipton DA(2000):** Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod.,26:288–291.
29. **Hakki SS, Bozkurt SB, Ozcopur B, Purali N and Belli S (2012):**Periodontal ligament fibroblast response to root perforation restored with different materials – a laboratory study. Int End J.,45:240–248.
30. **Pelliccioni GA, Ciapetti G, Cenni E, Granhi D, Nanni M, Pagani S and Giunti A(2004):** Evaluation of osteoblast-like cell response to ProRoot MTA (mineral trioxide aggregate) cement. J Mater Sci Mater Med.,15:167–173.