INTRODUCTION

Root canal therapy (RCT) is the main approach of various treatments for irreversible pulpitis and periapical disease, and the success rate of RCT is vary from 50 to 95% 1,2. The failing reasons are related to the complexity and diversity of root canal system, the improper operation during treatment, the stimulation to periapical tissues, and the infection of microorganisms. It was reported that some kinds of microorganisms, such as E. faecalis, C. albicans, E. coli, S. aureus, could be detected from the root canal of failing treatment 3,4. In addition, the root canal sealer is designed to be confined to the root canal system, but it can be extruded from the root tip sometimes 5,6. Therefore, its biocompatibility is essential to periapical tissues. The key of the successful RCT is to eliminate the infection from the root canal, fill the root canal tightly and reduce the stimulation to the periapical tissues. However, it is quite difficult to meet all the conditions due to the defect of the material. Here we develop a novel root canal sealer (MZOE), in which zinc oxide eugenol (ZOE) were fabricated with polyhexamethylene guanidine (PHMG), and the PHMG’s concentration is 0.8, 1.0, 1.2 and 1.4%. Our investigation tested its physical properties, antibacterial effect to E. faecalis, C. albicans, E. coli, S. aureus, and cytotoxicity to human periodontal ligament fibroblasts (HPDLFs). The physical properties of the MZOE conformed to the ISO 6876:2001, and its antibacterial effect was stronger than ZOE (p<0.05), the RGR of HPDLFs was tested between 1 to 24%, belonging to moderate cytotoxicity. It was suggested that MZOE had good physical properties, high antibacterial effect, and moderate cytotoxicity.

Keywords: Polyhexamethylene guanidine, Zinc oxide eugenol, Antibacterial effect, Cytotoxicity

Physical and biological properties of a novel root canal sealer modified by polyhexamethylene guanidine

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The key of the root canal therapy is to eliminate the micro-organism infection, fill the root canal tightly and reduce the stimulation to the periapical tissues. However, it is quite difficult to meet all the conditions due to the defect of the material. Here we develop a novel root canal sealer (MZOE), in which zinc oxide eugenol (ZOE) were fabricated with polyhexamethylene guanidine (PHMG), and the PHMG’s concentration is 0.8, 1.0, 1.2 and 1.4%. Our investigation tested its physical properties, antibacterial effect to E. faecalis, C. albicans, E. coli, S. aureus, and cytotoxicity to human periodontal ligament fibroblasts (HPDLFs). The physical properties of the MZOE conformed to the ISO 6876:2001, and its antibacterial effect was stronger than ZOE (p<0.05), the RGR of HPDLFs was tested between 1 to 24%, belonging to moderate cytotoxicity. It was suggested that MZOE had good physical properties, high antibacterial effect, and moderate cytotoxicity.

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INTRODUCTION

Root canal therapy (RCT) is the main approach of various treatments for irreversible pulpitis and periapical disease, and the success rate of RCT is vary from 50 to 95% 1,2. The failing reasons are related to the complexity and diversity of root canal system, the improper operation during treatment, the stimulation to periapical tissues, and the infection of microorganisms. It was reported that some kinds of microorganisms, such as E. faecalis, C. albicans, E. coli, S. aureus, could be detected from the root canal of failing treatment 3,4. In addition, the root canal sealer is designed to be confined to the root canal system, but it can be extruded from the root tip sometimes 5,6. Therefore, its biocompatibility is essential to periapical tissues. The key of the successful RCT is to eliminate the infection from the root canal, fill the root canal tightly and reduce the stimulation to the periapical tissues. However, the traditional sealers such as epoxy resin sealer, gutta-percha and polymethylsilane sealer have shortcoming such as weak antibacterial activity, the stimulation to the periapical tissue, easy dissolution, curing shrinkage and so on 7. Nowadays, there are none of the sealers satisfying the ideal requirement completely. Thus, it is necessary to develop a novel root canal sealer, which not only conforms to the International Organization for Standardization (ISO 6876:2001), but also has ideal antibacterial activity and biocompatibility.

Polyhexamethylene guanidine (PHMG) is an environmentally friendly high-molecular polymer with the characters of colorless, tasteless, solubility in water 8. It has a strong antibacterial activity and offers a broad antibacterial spectrum, and it has been widely used for many years as antiseptics in medicine and the food industry 9,10. Vitt et al. 9 found that the bacteria of caries and periodontal diseases were killed by PHMG in a short period. In addition, zinc oxide eugenol (ZOE) are the most common and conventional sealers used in endodontic treatment 11 which have good physical properties 12 but with weak antibacterial effect. The aim of this study is to fabricate the ZOE modified by PHMG, and to study its physical and biological properties in vitro.

MATERIALS AND METHODS

Preparation of MZOE

The pure glycerol (Zhi Yuan, Tianjin, China) was used as a medium to mediate PHMG (Sunny Chemical, Jiangsu, China) and eugenol (Zhi Yuan) soluble mutually according to glycerol and eugenol ratio of 1 to 9, and then mixed with zinc oxide powder (Zhi Yuan) at a ratio of 1 to 1. Finally, MZOE of 0.8, 1.0, 1.2 and 1.4% concentration was obtained. ZOE sealer was taken as the control group.

Preparation of MZOE extraction

The extraction was made by eluting the sealers in Dulbecco’s modified eagle medium (DMEM; Hyclone, Mairuibo, Beijing, China) using the surface area-to-volume ratio of approximately 1.25 cm²/mL between the surface of the samples and the volume of medium. The extraction vials were incubated at 37°C for 24, 48 or 72 h. The specimens were then discarded and the elute extracts were filtered by 0.22 μm pore size membranes. Control sample containing only culture medium were similarly treated.
Physical properties
The physical properties, including setting time, liquidity, film thickness, dimensional change, and solubility of the MZOE, were evaluated according to the International Organization for Standardization (ISO 6876:2001(E)).

Antimicrobial effect assay
The antimicrobial effect of MZOE was determined by agar diffusion test\(^{13}\). The bacteria of \(E.\ faecalis\) (ATCC29212), \(C.\ albicans\) (ATCC10231), \(E.\ coli\) (ATCC25922), \(S.\ aureus\) (ATCC25923) (kept in Bacterial species preservation center, Lanzhou University) were cultured with the brain heart infusion broth (BHI; Hai Bo, Qingdao, China). When the sterilized BHI agar broth cooled down to 45°C, it was divided into four groups with 100 mL medium for each group, and then the 10 μL bacteria suspension (1×10\(^6\) CFU/mL) was added into each group separately. The suspension and broth were mixed well and poured into 2×10 cm sterilized Petri plates (Shen Yuan, Shanghai, China) which had five sterilized Oxford cups (Shen Yuan, Shanghai, China) at equidistant points in agar plate to make five uniform cavities (4 mm in diameter, one for each test material). One hour later, the cavities were filled with well-mixed sealers. Then plates were incubated at 37°C for 24 h. The antimicrobial activity of MZOE was recorded by measuring the diameter of inhibition zone.

Cytotoxicity assay
Cell cultures of human periodontal ligament fibroblasts (HPDLFs) were established from PDL tissue harvested from the roots of healthy premolar teeth which extracted for orthodontic reasons from 18- to 20-year-old teenagers. All of the procedures were under an informed consent approved by the Human Studies Committee of Hospital of Stomatology Lanzhou University. The freshly extracted teeth were immediately placed in a sterile phosphate buffered saline (PBS) with penicillin (100 U/mL) and streptomycin (100 μg/mL) (Sangon biotech, Shanghai, China). Under sterile conditions, the PDL tissue was scraped from the one-third of the root surface with a sharp blade and cut into 0.5×0.5×0.5 mm patches. These tissue patches were placed in 25 cm\(^2\) tissue culture flask containing DMEM with 10% fetal bovine serum (FBS; Allbio, Beijing, China), penicillin (100 U/mL) and streptomycin (100 μg/mL) (Sangon biotech, Shanghai, China). The culture flasks were incubated at humidified incubator (5% CO\(_2\), ambient air, 37°C) for 2–4 weeks. The medium was replaced every 2 days until cell outgrowth was confluent. Then HPDLFs were trypsinized and reseeded in 25 cm\(^2\) tissue culture flask containing DMEM with 10% fetal bovine serum (FBS; Allbio, Beijing, China), penicillin (100 U/mL) and streptomycin (100 μg/mL) (Sangon biotech, Shanghai, China). After 24 h in 96-well microtiter plates (Lai Bo, Jiangsu, China), medium was changed with extraction 100 μL/well for 24 h. The cytotoxicity of MZOE (Table 1) was evaluated by incubating the cells with 20 μL/well of MTT dye (0.5 mg/mL) in PBS in humidified incubator (5% CO\(_2\), ambient air, 37°C) for 4 h. The optical density (OD) was measured by adding 150 μL/well dimethyl sulphoxide (DMSO; Suolaibao, Shanghai, China) at 570 nm using Microplate reader (Oral Research Laboratory, Lanzhou University). The relative growth rate (RGR) of cells was calculated with OD.

\[
\text{RGR} = \left( \frac{\text{Experiment group OD}}{\text{Control group OD}} \right) \times 100\%
\]

Statistical analysis
Statistical analysis was performed using commercially available software SPSS19.0. Values were expressed as the mean and standard deviation for each group, and were statistically performed using the Levene test for homogeneity of variances. As the variances were not different among the groups (p>0.05), the mean comparison is one-way ANOVA, and the multiple comparison between groups is performed by LSD-t method. As the variances were different among the groups, the mean comparison is Welch test, and the multiple comparison between groups is performed by T2 method. The significance level was p<0.05.

RESULTS
Physical properties of MZOE
Assessment of the physical properties revealed that the liquidity of MZOE was less than control group and the film thickness was thicker than control group (p<0.05, Figs. 1B, C); there were no difference between the MZOE groups (p>0.05). As expected, the setting time decreased with the PHMG concentration increase (Fig. 1A). The dimensional change after curing is enlarged in contrast to the control group (Fig. 1D). It is decreased with the PHMG concentration; the dimensional change after curing of MZOE with PHMG concentration of 1.4% was significantly different from 0.8 and 1.0% (p<0.05). The solubility of MZOE was greater than control group (p<0.05), and it increased with the PHMG concentration.

| RGR | CTS | Result evaluation |
|-----|-----|-------------------|
| 100 | 0   | Noncytotoxicity   |
| 75–99 | I   | Slight cytotoxicity |
| 50–74 | II  | Moderate cytotoxicity |
| 25–49 | III | Severe cytotoxicity |
| 1–24 | IV  |                   |
| 0   | V   |                   |
Fig. 1 Physical properties of MZOE. 
A) Line graph of the setting time of MZOE with 0.8, 1.0, 1.2 and 1.4% concentration and control group. B) Histogram distribution of the liquidity shows that MZOE was less than control group ($p < 0.05$), while there were no difference between the experimental groups ($p > 0.05$). C) Histogram distribution of the film thickness shows that MZOE was thicker than control group ($p < 0.05$). D) The dimensional change after curing of MZOE with 0.8, 1.0, 1.2 and 1.4% concentration and control group. E) The solubility of MZOE increased linearly with MZOE concentration.

The solubility of MZOE with PHMG of 0.8% concentration was larger than the other group ($p < 0.05$), and the solubility of MZOE with PHMG of 1.4% concentration was significantly different from 1.0 and 1.2% ($p < 0.05$). All the physical properties were in accordance with ISO standard.

Antibacterial effects of MZOE
The 1–4 groups represent PHMG concentrations of 0.8, 1.0, 1.2 and 1.4%. Results illustrated bigger diameter of inhibition zone treated by MZOE, indicating an excellent antibacterial activity (Fig. 2A). The diameter of inhibition zone of MZOE to *S. aureu*, *E. faecalis*, *E. coli*, *C. albicans* was greater than control group ($p < 0.01$), there was no significant difference between the MZOE
Fig. 2 Antibacterial effects of MZOE.

A) Representative inhibition zone of MZOE and control group. The concentration of Blank group is 0%, and the 1–4 group represents MZOE with concentrations of 0.8, 1.0, 1.2 and 1.4% respectively. B–E) Histogram distribution of the diameter of inhibition zone. There were four species of bacteria, *S. aureus* (B), *E. faecalis* (C), *E. coli* (D) and *C. albicans* (E).

For *S. aureus*, the biggest diameter of inhibition zone was MZOE of 1.2% (42.18 mm), which represented a 1.28-fold increase compared to the control group (Fig. 2B). For *E. faecalis*, the biggest diameter of inhibition zone was MZOE of 0.8% (41.52 mm), which represented a 1.21-fold increase compared to the control group (Fig. 2C). For *E. coli*, the biggest diameter of inhibition zone was MZOE of 1.4% (46.24 mm), which
Fig. 3  Assessment of the cytotoxicity of MZOE. 
A) Representative morphology of HPDLFs treated by the extraction of MZOE with 0.8, 1.0, 1.2 and 1.4% concentration and control group. B) Line graph of the RGR treated by the extraction of MZOE for 24 h (blue line), 48 h (red line), and 72 h (black line).

represented a 1.29-fold increase compared to the control group (Fig. 2D). For C. albicans, the biggest diameter of inhibition zone of MZOE was 44.04 mm, which was 1.32 times to the control group (Fig. 2E). Overall, MZOE exhibited high antibacterial activity which was 1–2 times higher than those treated by traditional ZOE.

Cytotoxicity of MZOE
Through calculating RGR, we assessed cytotoxicity of MZOE. As expected, HPDLFs treated with MZOE of 0.8% concentration were the long spindle and polygonal morphology similar to the ZOE group but worse than the blank control group (Fig. 3A). However, the normal morphology of HPDLFs disappeared, and part of cells floated in the medium; and even karyopyknosis took place in the ZOE and MZOE group, which indicated the materials had a significant inhibitory effect on the growth of PDLFs. The RGR of ZOE and MZOE was between 1 and 24%, which decreased as the increase of PHMG concentrations and time. These dates indicated that the cytotoxicity level of ZOE and MZOE were IV, which were moderate cytotoxicity (Fig. 3B).

DISCUSSION
The ultimate goal of RCT is to eliminate the microorganism thoroughly, while avoiding reinfection via good three-dimensional root canal obturation\(^\text{14}\). After mechanical instrumentation, irrigation, or medication, we used root canal sealers to prevent periapical periodontitis\(^\text{15}\). However, it was reported that, microorganisms existed in the lateral canals and dentinal tubules still cannot be cleaned thoroughly, even after rigid chemo-mechanical preparation\(^\text{16,17}\). In addition, it was frequently observed that the root canal sealers were extruded over the apical constriction. Therefore, these sealers should have good biocompatibility\(^\text{18,19}\). It is necessary to invent a novel root canal sealer with stronger antibacterial effect, and low stimulation to the periapical tissues.

PHMG can meet the requirement of root canal sealer due to its colorless, tasteless, and strong bactericidal effect. In addition, ZOE based sealers as one of the conventional sealers had undergone a lot of modifications and different commercial products. Thus, in this study, we modified ZOE for antimicrobial effect to fabricate a novel root canal sealer. The antibacterial effect of MZOE was stronger than ZOE significantly, that indicated it conformed to the strong antibacterial requirement. PHMG is a quite new compound so people know a little about its properties, potency, and effects. Studies have shown that PHMG in solutions has antifungal and antibacterial activity\(^\text{20-22}\). Feng et al.\(^\text{20}\) and Zhou et al.\(^\text{21}\) demonstrate that PHMG affects permeability of the cell membrane, and mechanically damages the cell membrane so as to the leakage of the cell contents to the external environment and the cell death. In the case of the antibacterial effect of PHMG on gram-negative bacteria such as E. coli, PHMG is absorbed by cell envelope, rapidly enters the cell and perturbs the cytoplasmic membrane, damages membrane and intracellular structures of bacteria. Furthermore, PHMG increases the permeability of the cytoplasmic membrane and leads to the formation of local pores. As a result, the intracellular component leakage occurs and cell inactivation follows, which is speculated that PHMG
could bind to the phospholipid of cell membrane and lead to the formation of the local pores, and that the cells could lose their viability21. In the case of the antibacterial effect of PHMG on the gram-positive bacteria such as *E. faecalis* and *S. aureus*, however, physicochemical interaction between the polymer molecule and the bacterial envelope is the main mechanism. The cell envelope of gram-positive bacteria is composed of peptidoglycan and teichoic acid that can be readily traversed by high molecular-weight substances20, 25. So, these polymers can readily traverse the cell membrane and interact with the negatively charged phospholipids, which may damage the cytoplasmic membrane and lead to the bacterial death22. On the antifungal effect, PHMG causes distortion and concave collapses of hyphae, also breaks down the plasma membranes severely which results in the intracellular components leakage and the cellular death20. Choi et al.24 demonstrated that exposed to PHMG, *C. albicans* cell shrank and the phospholipid area reduced in the plasma membrane which made the membrane more unstable; When yeast cells underwent membrane depolarization, K+ efflux systems were activated in response to changes in membrane potential, and the consecutive loss of cytoplasmic K+ led to cell death. However, the antibacterial effect of MZOE in *vivo* is not clear because it is still in the experimental research stage and not used in clinical practice. Kaplan et al.25 stated that the most effective antimicrobial sealers contain eugenol and formaldehyde. ZOE, the main component of MZOE, contains eugenol that indicates it has a certain antibacterial effect20. In addition, Zhou et al.22 showed the activity of PHMG against antibiotics-resistant strains isolated clinically. Therefore, MZOE is modified by adding antibacterial agent PHMG to ZOE, and has a stronger and more extensive antibacterial effect than ZOE and PHMG. Thereafter, it is clinically useful. However, further studies are needed to assess clinical efficacy of MZOE in details.

According to Leyhausen et al.27 the ideal root canal sealer not only need strong antibacterial effect but also good physical properties. The liquidity of MZOE decreased with the increase of PHMG concentration, the result may be related to the viscosity of the sealers after PHMG added. As the data showed the diameter of MZOE was not less than 20 mm, which accorded with ISO standard. It indicated that MZOE had good liquidity and it could enter the collateral root canal. However, we need combine with the micro-leakage detection to evaluate the sealing property of MZOE28. The film thickness decreased with the increase of PHMG concentration, but it was not over 50 μm, which accorded with ISO standard. It was reported that the adhesion of the periodontal pack was reduced when the concentration of PHMG was greater than 0.5%. Therefore, the addition of PHMG may reduce the bonding force between the material molecules, which leaded to the decrease of film thickness under the same load. It is also necessary to have low solubility and opportune dimensional change after curing to ensure the good apical sealing. If the material shrinkage is large or easy to dissolve, it must cause the apical micro-leakage. In contrast, if the expansibility is large, it can cause the disintegration of dental tissue. The solubility of MZOE conforms to the ISO standard, and the expansion increased gradually with the increase of PHMG concentration, which contributed to apical sealing. Proper setting time is necessary for clinical operation, and it is not easy to lead to apex overfilled. The setting time of MZOE is in accordance with the ISO standard, which achieved our purpose of modification.

The root canal sealer contacted with periapical tissue through the apical foramen for a long time. So it should not only have good physical properties and strong antibacterial effect, but also have good biocompatibility29. In this study, the cytotoxicity grade of MZOE to HPDLFs was IV, which showed a moderate cell toxicity. The results are similar to Brackett et al.30 research that ZOE has severe cytotoxicity to HPDLFs. ZOE led to severe inflammatory response in periapical tissue, which may relate to the stimulation of phenol, aldehyde and zinc freed from ZOE. It demonstrated that free eugenol was the main source of the cytotoxicity of ZOE, and the toxicity depended on the content of eugenol31, 32. The mechanism of cytotoxicity of PHMG to HPDLFs are not fully clear yet. Marchi et al.33 and Elmore et al.34 demonstrated PHMG conferred cellular toxicity through the intracellular reactive oxygen species (ROS) and alteration of gene expression. ROS has powerful oxidizing capability which leads to molecular products with active oxidation, which induces destruction of cellular structures, including DNA, proteins, lipids and cell membranes. Many genes related to apoptosis, cell damage, cell cycle, and fibrosis were greatly up-regulated (such as GADD45B, BAX, and SERPINE1) while genes to self-defense functions and oxidation were down-regulated (such as GSR and GPX2). Thus, changes in gene expression relevant to the progression of cell death included genes related to apoptosis, autophagy, fibrosis, and cell cycle. The generation of ROS and the reduction of cellular defense-related antioxidant genes might be one of the reasons for PHMG-induced cytotoxicity35.

**CONCLUSIONS**

In this study, we explored the physical and biological properties of MZOE with PHMG concentration of 0.8, 1.0, 1.2 and 1.4%. The results indicated that it had good physical properties, strong antibacterial effect, and moderate cytotoxicity. If applying MZOE to clinical practice, a series of pre-clinical studies need to be done, such as the sealing effect observed by scanning electron microscope. In conclusion, if MZOE is applied to clinical, it should be careful of overfilling, so as to ensure the success of RCT.

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REFERENCES

1) Cheung GS, Chan TK. Long-term survival of primary root canal treatment carried out in a dental teaching hospital. Int Endod J 2003; 36: 117-128.

2) Ng YL, Mann V, Rahbarran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature: Part 2. Influence of clinical factors. Int Endod J 2008; 41: 6-31.

3) Lin LM, Rosenberg PA, Lin J. Do procedural errors cause endodontic treatment failure? J Am Dent Assoc 2005; 136: 187-193.

4) Miyagak DC, de Carvalho EM, Rokazza CR, Chavasca JK, Levorato GL. In vitro evaluation of the antimicrobial activity of endodontic sealers. Braz Oral Res 2006; 20: 303-306.

5) Dahl JE. Toxicity of endodontic filling materials. Endod Topics 2010; 12: 39-43.

6) da Silva PT, Pappen FG, Souza EM, Dias JE, Bonetti Filho I, Carlos IZ, et al. Cytotoxicity evaluation of four endodontic sealers. Braz Dent J 2008; 19: 228-231.

7) Gesi A, Raffelli O, Goracci C, Pashley DH, Ferrucci M. Interfacial strength of Resilon and gutta-percha to intraradicular dentin. J Endod 2005; 31: 809-813.

8) Rosin M, Welk A, Bernhardt O, Ruhnau M, Pitten FA, Kocher EI, et al. Antimicrobial activity of polyhexamethylene biguanide mouthrinse on bacterial counts and plaque. J Clin Periodontal 2001; 28: 1121-1126.

9) Vitt A, Sofrata A, Slizen V, Sugars RV, Gustafsson A, Gudkova JA. Microbial leakage of MTA, Portland cement, Sealapex and zinc oxide-eugenol as root-end filling materials. Med Oral Patol Oral Cir Bucal 2005; 10: 115-124.

10) Barkova NP, Bogachuk GP. Quantum-mechanical characteristics and toxicity of guanidine-containing antiseptics. Gig Sanit 1995; 4: 38-42.

11) Metzger Z, Bassarani B, Goodid HE. Instruments, Materials, Devices. Pathways of the pulp 2011; 10: 236-238.

12) Estrela C, Estrada-Bernabé PF, de Almeida-Decurcio D, Almeida-Silva J, Rodrigues-Araújo-Estrela C, Pol-Figueiredo JA. Microbial leakage of MTA. Portland cement, Sealapex and zinc oxide-eugenol as root-end filling materials. Med Oral Patol Oral Cir Bucal 2011; 16: e118-e124.

13) Asgary S, Kamrani FA. Antibacterial effects of five different root canal sealing materials. J Oral Sci 2008; 50: 469-474.

14) Gjorgievsk E, Apostolska S, Dimkov A, Nicholson JW, Kafandzieva A. Incorporation of antimicrobial agents can be used to enhance the antibacterial effect of endodontic sealers. Dent Mater 2013; 29: e29-34.

15) Pizzo G, Giammanco GM, Cumbo E, Nicolosi G, Gallina G. In vitro antibacterial activity of endodontic sealers. J Dent 2006; 34: 35-40.

16) Möller AJ, Fabricius L, Dahlén G, Sundqvist G, Happonen RP. Apical periodontitis development and bacterial response to endodontic treatment. Experimental root canal infections in monkeys with selected bacterial strains. Eur J Oral Sci 2004; 112: 207-215.

17) Sundqvist G, Figdor D. Life as an endodontic pathogen: Ecological differences between the untreated and root-filled root canals. Endod Topics 2010; 6: 3-28.

18) Yamaguchi K, Matsunaga T, Hayashi Y. Gross extrusion of endodontic obturation materials into the maxillary sinus: a case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104: 131-134.

19) Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004; 15: 348-381.

20) Feng L, Wu F, Li J, Jiang Y, Duan X. Antifungal activities of polyhexamethylene biguanide and polyhexamethylene guanidine against the citrus sour rot pathogen geotrichum citri-auroantis in vitro, and in vivo. Postharvest Biol Tec 2011; 61: 160-164.

21) Zhou ZX, Wei DF, Guan Y, Zheng AN, Zhong JI. Damage of Escherichia coli membrane by bactericidal agent polyhexamethylene guanidine hydrochloride: micrographic evidences. J Appl Microbiol 2010; 108: 898-907.

22) Zhou Z, Wei D, Guan Y, Zheng A, Zhong JI. Extensive in vitro, activity of guanidine hydrochloride polymer analogs against antibiotics-resistant clinically isolated strains. Mat Sci Eng C 2012; 31:1836-1843.

23) Russell AD. Similarities and differences in the responses of microorganisms to biocides. J Antimicrob Chemother 2003; 52: 750-763.

24) Choi H, Kim KJ, Lee DG. Antifungal activity of the cationic antimicrobial polymer-polyhexamethylene guanidine hydrochloride and its mode of action. Fungal Biol 2017; 121: 53-60.

25) Kaplan AE, Pecca M, Gonzalez MI, Macchi RL, Molgatini SL. Antimicrobial effect of six endodontic sealers: An in vitro evaluation. Endod Dent Traumatol 1999; 15: 42-45.

26) Pupo J, Biral RR, Benatti O, Ave A, Valdighi L. Antimicrobial effects of endodontic filling cements on microorganisms from root canal. Oral Surg Oral Med Oral Pathol 1983; 55: 622-627.

27) Bodrumlu E, Semiz M. Antibacterial activity of a new endodontic sealer against Enterococcus faecalis. J Can Dent Assoc 2006; 72: 637.

28) Leyhausen G, Heil J, Reifferscheid G, Waldmann P, Geurtsen W. Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH Plus. J Endod 1999; 25: 109-113.

29) Wang J, Zuo Y, Zhao M, Jiang J, Man Y, Wu J, et al. Physicochemical and biological properties of a novel injectable polyurethane system for root canal filling. Int J Nanomedicine 2015; 10: 697-709.

30) Brackett MG, Messer RL, Lockwood PE, Bryan TE, Lewis JB, Bouillaguet S, et al. Cytotoxic response of three cell lines exposed in vitro to dental endodontic sealers. J Biomed Mater Res B Appl Biomater 2010; 95: 380-386.

31) Hashieh IA, Pommel L, Camps J. Concentration of eugenol apically released from zinc oxide-eugenol-based sealers. J Endod 1999; 25: 627.

32) Leyhausen G, Heil J, Reifferscheid G, Waldmann P, Geurtsen W. Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH Plus. J Endod 1999; 25: 109-113.

33) Elmore S. Apoptosis: a review of programmed cell death. Pathways of the pulp 2011; 10: 236-238.

34) Elmore S. Apoptosis: a review of programmed cell death. Pathways of the pulp 2011; 10: 236-238.

35) Hashieh IA, Pommel L, Camps J. Concentration of eugenol apically released from zinc oxide-eugenol-based sealers. J Endod 1999; 25: 627.

36) Leyhausen G, Heil J, Reifferscheid G, Waldmann P, Geurtsen W. Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH Plus. J Endod 1999; 25: 109-113.

37) Hashieh IA, Pommel L, Camps J. Concentration of eugenol apically released from zinc oxide-eugenol-based sealers. J Endod 1999; 25: 627.

38) Ho YC, Huang FM, Chang YC. Mechanisms of cytotoxicity of eugenol in human osteoblastic cells in vitro. Int Endod J 2006; 39: 389-393.

39) Marchi S, Giorgi C, Suski JM, Agnoletto C, Bononi A, Bonora M, et al. Mitochondria-ros crosstalk in the control of cell death and aging. J Signal Transduct 2012; 329635.

40) Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007; 35: 495-516.

41) Huang FF, Zerin T, Podder B, Song HY, Kim YS. Cytotoxicity and gene expression profiling of polyhexamethylene guanidine hydrochloride in human alveolar A549 cells. Toxicol In Vitro 2014; 28: 684-692.