Antimicrobial activity and physicochemical performance of a modified endodontic sealer

Atividade antimicrobiana e desempenho físico-químico de um cimento endodôntico modificado

Actividad antimicrobiana y rendimiento fisicoquímico de un sellador endodóntico modificado

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Abstract

Introduction: this study aimed to evaluate the antimicrobial and physicochemical properties of a commercial endodontic sealer modified by the addition of montmorillonite (MMT) nanoparticles loaded with two different drugs: chlorhexidine (CHX) or metronidazole (MET). Methods: 5 wt% MMT/CHX or MMT/MET nanoparticles were added to the sealer AH-Plus. The experimental materials were evaluated for drug release, antimicrobial activity, flow, flexural strength, and flexural modulus. Data were subjected to one-way ANOVA, Kruskal-Wallis, and Mann-Whitney tests. Results: The drug incorporation into MMT particles was 9% and 10% for CHX and MET, respectively. At 20 days after manipulation, 16.5% of the drug

was released by the sealer with MMT/MET and 0.4% by MMT/CHX. The addition of both nanoparticles decreased the flow of materials, but they were still in compliance with ISO 6876-2012. The conversion, flexural strength, and flexural modulus of MMT/MET (87%, 37 \pm 7 MPa, 2.3 GPa) and MMT/CHX (78%, 29 \pm 2 MPa, 2.7 GPa) were similar in both groups but lower than in the control group (100%, 54 \pm 7 MPa, 4.0 \pm 0.7 GPa). Both experimental materials were able to form an inhibition halo for *E. faecalis* bacteria (CHX: 4.8 \pm 1.4 and MET: 4.0 \pm 1.6 mm), whereas the control group did not inhibit the microorganism. Conclusion: both formulations proposed as endodontic sealer presented effective antimicrobial activity and acceptable flow. The addition of MMT/CHX and MMT/MET particles decreased the conversion and mechanical properties, but further studies are required to clarify the clinical relevance of these properties.

Keywords: AH-Plus; Antimicrobial activity; Chlorhexidine; Metronidazole.

Resumo

Introdução: o presente trabalho teve como objetivo avaliar as propriedades antimicrobianas e físico-químicas de um cimento endodôntico comercial modificado pela adição de nanopartículas de montmorilonita (MMT) carregadas com dois diferentes fármacos: clorexidina (CHX) ou metronidazol (MET). Métodos: 5% em peso de nanopartículas de MMT / CHX ou MMT / MET foram adicionadas ao selante AH-Plus. Os materiais experimentais foram avaliados quanto à liberação do fármaco, atividade antimicrobiana, fluxo, resistência à flexão e módulo de flexão. Os dados foram submetidos a One-way ANOVA, testes de Kruskal-Wallis e Mann-Whitney. Resultados: A incorporação do fármaco nas partículas de MMT foi de 9% e 10% para CHX e MET, respectivamente. Aos 20 dias após a manipulação, 16,5% do fármaco foi liberado pelo cimento com MMT / MET e 0,4% pelo com MMT / CHX. A adição de ambas as nanopartículas diminuiu o escoamento dos materiais, mas eles ainda estavam em conformidade com a ISO 6876-2012. A conversão, resistência à flexão e módulo de flexão de MMT / MET (87%, 37 ± 7 MPa, 2,3 GPa) e MMT / CHX (78%, 29 ± 2 MPa, 2,7 GPa) foram semelhantes em ambos os grupos, mas menores do que no grupo controle (100%, 54 \pm 7 MPa, 4,0 \pm 0,7 GPa). Ambos os materiais experimentais foram capazes de formar um halo de inibição para a bactéria E. faecalis (CHX: 4,8 ± 1,4 e MET: $4,0 \pm 1,6$ mm), enquanto o grupo controle não inibiu o microrganismo. Conclusão: ambas as formulações propostas como cimento endodôntico apresentaram efetiva atividade antimicrobiana e escoamento aceitável. A adição de partículas de MMT / CHX e MMT /

MET diminuiu a conversão e as propriedades mecânicas, entretanto mais estudos são necessários para esclarecer a relevância clínica dessas propriedades.

Palavras-chave: AH-Plus; Actividade antimicrobiana; Clorexidina; Metronidazol.

Resumen

Introducción: este estudio tuvo como objetivo evaluar las propiedades antimicrobianas y fisicoquímicas de un sellador endodóntico comercial modificado por la adición de nanopartículas de montmorillonita (MMT) cargadas con dos fármacos diferentes: clorhexidina (CHX) o metronidazol (MET). Métodos: Se añadieron 5% en peso de nanopartículas de MMT / CHX o MMT / MET al sellador AH-Plus. Los materiales experimentales se evaluaron en cuanto a liberación de fármaco, actividad antimicrobiana, flujo, resistencia a la flexión y módulo de flexión. Los datos se sometieron a ANOVA de una vía, pruebas de Kruskal-Wallis y Mann-Whitney. Resultados: La incorporación del fármaco en las partículas de MMT fue del 9% y 10% para CHX y MET, respectivamente. A los 20 días después de la manipulación, el sellador liberó el 16,5% del fármaco con MMT / MET y el 0,4% con MMT / CHX. La adición de ambas las nanopartículas disminuyó el flujo de materiales, pero aún cumplían con la norma ISO 6876-2012. La conversión, la resistencia a la flexión y el módulo de flexión de MMT / MET (87%, 37 ± 7 MPa, 2,3 GPa) y MMT / CHX $(78\%, 29 \pm 2 \text{ MPa}, 2.7 \text{ GPa})$ fueron similares en ambos grupos pero menores que en el grupo de control (100%, 54 \pm 7 MPa, 4,0 \pm 0,7 GPa). Ambos materiales experimentales pudieron formar un halo de inhibición para la bacteria E. faecalis (CHX: 4.8 ± 1.4 y MET: 4.0 ± 1.6 mm), mientras que el grupo de control no inhibió el microorganismo. Conclusión: ambas formulaciones propuestas como sellador endodóntico presentaron actividad antimicrobiana efectiva y flujo aceptable. La adición de partículas MMT / CHX y MMT / MET disminuyó la conversión y las propiedades mecánicas, pero se requieren más estudios para aclarar la relevancia clínica de estas propiedades.

Palabras clave: AH-Plus; Actividad antimicrobiana; Clorhexidina; Metronidazol.

1. Introduction

Root canal infection is caused by several different microorganisms (Ercan, Dalli, Yavuz, & Özekinci, 2006). However, one of the main reasons for endodontic treatment failure is associated with the presence of resistant and facultative oral microorganisms, such as *Candida albicans, Enterococcus faecalis, Staphylococcus aureus*, and *Streptococcus* spp

(Stuart, Schwartz, Beeson, & Owatz, 2006). Among these, *Enterococcus faecalis* has been pointed out as the most prevalent microorganism in the infected canal and in retreated teeth with apical lesions, constituting about 24 to 77% of the present flora (AlShwaimi et al., 2016; Stuart et al., 2006). Furthermore, a strong relationship between *E. faecalis* and persistent intracanal infection has been observed (C. Zhang, Du, & Peng, 2015).

Ideally, endodontic sealer should not only tightly seal the root canal system (Bouillaguet, Shaw, Barthelemy, Krejci, & Wataha, 2008), but also present antimicrobial activity towards the elimination of any residual microorganisms(AlShwaimi et al., 2016; Baer & Maki, 2010; H. Zhang, Shen, Ruse, & Haapasalo, 2009). Nowadays, there is a wide array of endodontic sealers in the market, but none of them offer prolonged antimicrobial activity. Resin sealers is the most widely used material in dental practice due to their excellent flow properties (Siqueira et al., 2000), effective mechanical retention, low solubility (Schwartz, 2006), low viscosity, low film thickness, low dimensional change (Lacey, Pitt Ford, Yuan, Sherriff, & Watson, 2006; Versiani et al., 2006), good apical sealing, and high radiopacity (Prullage, Urban, Schafer, & Dammaschke, 2016). However, the setting reaction of resinbased has the disadvantage of causing significant shrinkage (Y. K. Kim et al., 2010), which can result in the formation of a gap between the material and the walls of the canal, facilitating bacterial colonization. Therefore, the inclusion of an antimicrobial agent in endodontic sealers would serve for two purposes: prevention of bacterial proliferation into interfacial gaps and elimination of residual microorganisms from the root canal. Also, the antimicrobial agent should be gradually released to provide a long-term action.

Several materials have been proposed in the literature as drug delivery agents (Webster, Sundaram, & Byrne, 2013). Montmorillonite (MMT) is a ceramic material used in the pharmaceutical industry, the association of MTT and chlorhexidine (CHX) shows effective antimicrobial activity (Wu et al., 2013). Therefore, these nanoparticles seem to be a viable additive to incorporate antimicrobial agents into endodontic sealers.

Based on the above, the objectives of this study were (1) to develop experimental endodontic sealers, adding MTT nanoparticles loaded with CHX or metronidazole (MET) to the resin matrix of AH-Plus; (2) to evaluate the rate of drug release, the physicochemical properties, and also the antimicrobial activity of these materials. The null hypothesis was that the incorporation of MMT nanoparticles does not affect the tested variables.

2. Methodology

2.1 MMT nanoparticles

It was used MMT Cloisite 30B[®] nanoparticles with surface area of 750 m² g⁻¹. To synthesize MMT nanoparticles loaded with MET and CHX, 50 mg of each were added to an aqueous solution of 1M HCl (pH 5.0), kept under agitation for 2 h. 450 mg of MMT nanoparticles was added in small portions to the initial solution, agitated for another 2 h and centrifuged for 20 min at 4000 rpm. The pellet was dryed in an oven at 37°C for 48 h to obtain the particles. The thermal decomposition profiles of MMT nanoparticles, MET, CHX, MMT/MET and MMT/CHX were evaluated by thermogravimetric analyses (Metter Toledo TGA/SDT851). The samples were heated from 25 to 800°C, with a rate of 10°C/min, under nitrogen atmosphere. The amount of drug incorporated into the particles was estimated by residue analysis.

2.2 Experimental sealers

A commercial endodontic sealer (AH-Plus, Dentsply, Germany, composition in table 1), was used to receive the addition of 5 wt% MMT/MET or MMT/CHX particles. Both AH-Plus pastes were weighed in similar amounts and the particles were mixed into the pastes until homogenization. The control group consisted of AH-Plus without any nanoparticle. The samples had 5 mm in diameter and 1 mm in thickness for testing the degree of conversion, microbial inhibition, and drug release. 10x2x1mm³ bars were built for the three bending tests.

2.3 In vitro drug release

The specimens (n=5) were weighed and immersed in 5 mL of saline buffer solution (0.9%, pH 7.5 mM) and incubated at 37°C in a shaker under mechanical agitation of 80 rpm. Aliquots of 200 μ L were collected at 0, 1, 2, 3, and 4 h and every 24 h for 20 days (0 to 480 h). MET concentration was determined by UV analysis at 320 nm and CHX concentration was performed at 255 nm, and both were read in an i3x Spectramax microplate reader (Molecular Devices, USA).

2.4 Antimicrobial activity

The antibacterial activities of the materials were tested on *Enterococcus faecalis*. The agar diffusion technique was performed according to the CLSI (*Clinical and Laboratory Standards Institute*) protocol. The bacteria were cultured in TSB and a bacterial suspension with an optical density of 0.1 was prepared. Petri dishes with the TSB medium were inoculated with *E. faecalis* suspension and five specimens of the same group were placed in the same Petri dish. All the dishes were maintained at 37°C for 24 h. The inhibition halos were measured (in mm) using a digital caliper, considering the area without growth visible to the naked eye. The sample size was performed considering: power test 0.8; standard deviation 2.0; difference between the groups 0.5.

2.5 Degree of conversion (DC)

The specimens (n=5) were analyzed by Fourier transformed infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) in a spectrometer (Vertex 70, Bruker Optik GmbH, Germany). The sample size was performed considering: power test 0.8; standard deviation 2.0; difference between the groups 0.5. The spectra were obtained by the co-addition of 16 scans with a 4 cm⁻¹ resolution. The degree of conversion at 1, 4, 24, 168, and 336 h after manipulation was calculated by the relation between the peak area related to epoxy resin (916 cm⁻¹) and the reference peak area (1,183 cm⁻¹) in both cured and uncured materials, according to the formula:

$$DC = 1 - \left(\frac{\frac{A_{cured}^{916}}{A_{cured}^{1183}}}{\frac{A_{uncured}^{916}}{A_{uncured}^{1183}}}\right)$$

2.6 Flow

The flow of the sealer was measured according to ISO 6876-2012 (n=3). 0.5 mL of the sealer was dispensed onto a glass plate. A 20g plate was positioned above the sealer 3 min after manipulation and a load of 100g (9.8N) was applied up to 10 min after manipulation. The load was removed, and the diameter of the circle obtained was measured with a digital

caliper. The sample size was performed considering: power test 0.8; standard deviation 2.0; difference between the groups 7 mm (20% of the control flow).

2.7 Flexural strength

The three-point bending test was performed after 14 days dry storage at 37°C. The specimens were tested in a universal testing machine (Instron 5565, Canton, MA, USA), at a speed of 0.5 mm/min and span of 8 mm. Flexural strength (FS) was calculated according to the formula: $FS = \frac{(3xLxD)}{(2xWxh^2)}$, and the flexural modulus (FM) was calculated using data from the linear portion of the load x displacement graph, according to the formula: $E = \frac{(LxD^3)}{(4xWxh^3xd)}$, where L is the load recorded at the fracture time, D is the span, W is the specimen's width, h is the specimen's height, and d is the specimen's displacement. The sample size was performed considering: power test 0.8; standard deviation 6.0; difference between the groups 10.

2.8 Statistical Analysis

Data were checked for normality and homoscedasticity. Flow and flexural modulus data were subjected to one-way ANOVA and Tukey's test. Microbial inhibition data were analyzed by the Mann-Whitney test, and flexural strength and conversion data were assessed by the Kruskal-Wallis test. A 95% global level of significance was adopted (α =0.05).

3. Results

Thermogravimetric analysis of MMT nanoparticles, MET, and CHX, either associated or pure, are presented in Figure 1. Three loss mass events were observed for pure MMT nanoparticles, with a residue of 75%. Just one loss mass event was observed for MET between 160°C and 250°C with 4% of residue. Three loss mass events were also observed for CHX with 14% of residue. The MMT/MET thermogram shows two main loss mass events with a residue of 65%. The residue analysis estimates that the percentage of MET incorporated into MMT particles was around 10%. Two loss mass events were observed in the MMT/CHX material with a residue of 9% lower than MMT, indicating that the percentage of CHX was around 9%.

The *in vitro* release profile in terms of MET and CHX release by the sealers are shown in Figure 2. MET cumulative release was around 16.5%, whereas CHX maximum release corresponded to 0.4% at the end of 20 days of observation.

Degrees of conversion of the experimental sealers are presented in Figure 3. The addition of both particles, MMT/MET and MMT/CHX, significantly decreased the conversion of the material 14 days after manipulation, compared to the control group. Such reduction was more pronounced when the MMT/CHX was incorporated to the resin.

Table 2 shows the physical and antimicrobial properties of the sealers evaluated. Control group did not show antimicrobial activity, whereas both experimental groups showed similar inhibition halo for *E. faecalis*. MMT/MET and MMT/CHX incorporation decreased flow, flexural strength, and flexural modulus; however, there were no significant differences between the experimental sealers.

4. Discussion

The null hypothesis was partially accepted, and though the experimental materials showed remarkable antimicrobial activity when compared to the control group and flowability in compliance with ISO 6876-1012, both showed a decrease in degree of conversion and mechanical properties.

The efficiency of drug incorporation into MMT particles was verified by thermogravimetric analysis and around 9% of CHX and 10% of MET could be incorporated into MMT. Instead of adding the drugs directly to the resin matrix of the sealer, as in other studies (Bailon-Sanchez, Baca, Ruiz-Linares, & Ferrer-Luque, 2014; Hoelscher, Bahcall, & Maki, 2006), MMT nanoparticles were used as carrier for gradual drug release, once antimicrobial activity for a short period would not be enough to guarantee the success of the treatment of resistant infections. In the proposed system, the materials were able to release the antimicrobial for 15 days, as presented in Figure 2. However, in both cases, the drug release rates were low, and the quantifications performed revealed sub-therapeutic levels of the drugs being released over time. Although CHX showed a lower release rate than MET (Figure 2), both materials were able to generate statistically similar inhibition halos for *Enterococcus faecalis* and the control group does not show any inhibition halo. This information provides evidence that any biocidal activity would be a result of direct contact, such materials may lead to cell death whether via chemical or physical disruption cell morphology, or even the combination of both (Hasan, Crawford, & Ivanova, 2013). This specific type of material

offers as advantage, the possibility to fight infections or control pathogenic microbial growth locally rather than at systemic levels, which significantly contributes to diminish antimicrobial resistance and avoid undesirable specific and non-specific adverse reactions or side effects, which is not achieved otherwise (Elbourne, Crawford, & Ivanova, 2017).

To date, the antimicrobial effect of CHX in the endodontic field has been consolidated by its use as intracanal irrigation agent at a concentration of 0.2 to 2% (Ghivari, Bhattacharya, Bhat, & Pujar, 2017; Mohammadi, Jafarzadeh, & Shalavi, 2014; Pinheiro et al., 2018). A few studies in which CHX was incorporated into sealers revealed an effective antimicrobial activity against *Enterococcus faecalis, S mutans, S salivarius, Staphylococcus aureus, and Lactobacillus casei*, using concentrations of 1 to 20% in sealers with several compositions (Bailon-Sanchez et al., 2014; Collares et al., 2018; Nambu, 1984). Just one study evaluated the incorporation of 10% MET into an endodontic sealer composed of zinc oxide and eugenol, but the study did not observe any difference from the control group in antimicrobial activity against *E. faecalis (Hoelscher et al., 2006)*. This is at odds with the findings of the present study and it is possibly due to some antimicrobial effect of eugenol, also present in the control group (Thosar, Chandak, Bhat, & Basak, 2018).

It should be underscored that most studies on antibacterial activity studies were developed immediately after sealer manipulation (Cobankara, Altinoz, Ergani, Kav, & Belli, 2004; Morgental et al., 2011; Wainstein et al., 2016), but after the complete set of the materials, the antimicrobial effect is absent, even in bioceramic materials in which a high pH plays a bactericidal role (Wainstein et al., 2016). The present study evaluated antimicrobial activity 48 h after manipulation of the materials; therefore, this reinforces that the antimicrobial effects occurred via contact only. The negative biocidal action featured by the control resin excludes a possible contribution of the resin itself or its monomers in terms of biocidal action. In recent studies, on the antimicrobial activity of AH-Plus, a higher bactericidal effect was observed when the material was freshly manipulated and presented a elevate pH, and no antimicrobial activity was observed after 24 h or 7 days after manipulation (Kapralos, Koutroulis, Orstavik, Sunde, & Rukke, 2018),(Pawinska, Szczurko, Kierklo, & Sidun, 2017). It should be highlighted that an antimicrobial effect of less than 24 h in the treatment of resistant infections is not enough to eliminate periapical and intraradicular infection.

The incorporation of both particles into AH-Plus resulted in a decrease in flowability; however, albeit lower, the flow of experimental sealers still complied with ISO 6876-2012

standards, which determines final diameter of the material disk of at least 17 mm. The reduction in flowability can be explained by the hydrogen bond between the nitrogen from and chlorhexidine acetate molecules and the hydrogen from the resin matrix of AH-Plus. The hydrogen bonds decrease the mobility of monomers and consequently decrease the propagation rate of the reaction, resulting in a lower final degree of conversion (Dickens, Stansbury, Choi, & Floyd, 2003; Sideridou, Tserki, & Papanastasiou, 2002). The high conversion (close to 100%) observed for the unmodified AH-Plus is in line with what was described in other studies (Baldi et al., 2012; H. R. Kim, Kim, & Kwon, 2017). MMT/CHX shows lowest conversion (78%), and though its conversion was 20% lower than that of the control group, it is similar to or even higher than that of other experimental sealers (de Souza, Branco Leitune, Bohn, Werner Samuel, & Collares, 2015; Lee, Wang, Fang, Hsieh, & Lin, 2011).

The experimental materials presented a decrease in flexural modulus and flexural strength, if compared with the control group. Two main factors can help to explain these findings: the lower conversion of these materials, compared with the control group, and absence of a chemical bond between MMT particles and the polymer matrix, with loss of continuity. However, the flexural strength of these experimental materials is higher than the ones of mineral trioxide aggregate sealer (Ballal, Sona, & Tay, 2017), of gutta-percha, and higher than or like Resilon (Grande et al., 2007). Also, the flexural modulus was 10 times higher than gutta-percha or Resilon (Grande et al., 2007), corroborating the real potential of application of these experimental materials.

5. Final Considerations

Based on the findings described above and considering the limitations of this study, it can be concluded that both experimental formulations proposed as endodontic sealers presented effective antimicrobial activity and flow in accordance with ISO 6876-2012. The addition of MMT/CHX and MMT/MET particles decreases the conversion and mechanical properties, when compared with control materials, but further studies are needed to evaluate the clinical effect of these changes.

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Disclosures

The authors deny any conflicts of interest related to this study.

References

AlShwaimi, E., Bogari, D., Ajaj, R., Al-Shahrani, S., Almas, K., & Majeed, A. (2016). In Vitro Antimicrobial Effectiveness of Root Canal Sealers against Enterococcus faecalis: A Systematic Review. *J Endod*, *42*(11), 1588-1597.

Baer, J., & Maki, J. S. (2010). In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. *J Endod*, *36*(7), 1170-1173.

Bailon-Sanchez, M. E., Baca, P., Ruiz-Linares, M., & Ferrer-Luque, C. M. (2014). Antibacterial and anti-biofilm activity of AH plus with chlorhexidine and cetrimide. *J Endod*, *40*(7), 977-981.

Baldi, J. V., Bernardes, R. A., Duarte, M. A., Ordinola-Zapata, R., Cavenago, B. C., Moraes, J. C., & de Moraes, I. G. (2012). Variability of physicochemical properties of an epoxy resin sealer taken from different parts of the same tube. *Int Endod J*, *45*(10), 915-920.

Ballal, N. V., Sona, M., & Tay, F. R. (2017). Effects of smear layer removal agents on the physical properties and microstructure of mineral trioxide aggregate cement. *J Dent*, *66*, 32-36.

Bouillaguet, S., Shaw, L., Barthelemy, J., Krejci, I., & Wataha, J. C. (2008). Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow and Epiphany. *Int Endod J*, *41*(3),

Cobankara, F. K., Altinoz, H. C., Ergani, O., Kav, K., & Belli, S. (2004). In vitro antibacterial activities of root-canal sealers by using two different methods. *J Endod*, *30*(1), 57-60.

Collares, F. M., Leitune, V. C. B., Portella, F. F., Santos, P. D., Balbinot, G. S., Dos Santos, L. A., Samuel, S. M. W. (2018). Methacrylate-based root canal sealer containing chlorexidine and alpha-tricalcium phosphate. *J Biomed Mater Res B Appl Biomater*, *106*(4), 1439-1443.

de Souza, M. O., Branco Leitune, V. C., Bohn, P. V., Werner Samuel, S. M., & Collares, F. M. (2015). Physical-mechanical properties of Bis-EMA based root canal sealer with different fillers addition. *J Conserv Dent*, *18*(3), 227-231.

Dickens, S. H., Stansbury, J. W., Choi, K. M., & Floyd, C. J. E. (2003). Photopolymerization Kinetics of Methacrylate Dental Resins. *Macromolecules*, *36*, 6046-6053.

Elbourne, A., Crawford, R. J., & Ivanova, E. P. (2017). Nano-structured antimicrobial surfaces: From nature to synthetic analogues. *J Colloid Interface Sci, 508*, 603-616.

Ercan, E., Dalli, M., Yavuz, İ., & Özekinci, T. (2006). Investigation of Microorganisms in Infected Dental Root Canals. *Biotechnology & Biotechnological Equipment*, 20(2), 166-172.

Ghivari, S. B., Bhattacharya, H., Bhat, K. G., & Pujar, M. A. (2017). Antimicrobial activity of root canal irrigants against biofilm forming pathogens- An in vitro study. *J Conserv Dent*, 20(3), 147-151.

Grande, N. M., Plotino, G., Lavorgna, L., Ioppolo, P., Bedini, R., Pameijer, C. H., & Somma, F. (2007). Influence of different root canal-filling materials on the mechanical properties of root canal dentin. *J Endod*, *33*(7), 859-863.

Hasan, J., Crawford, R. J., & Ivanova, E. P. (2013). Antibacterial surfaces: the quest for a new generation of biomaterials. *Trends Biotechnol*, *31*(5), 295-304.

Hoelscher, A. A., Bahcall, J. K., & Maki, J. S. (2006). In vitro evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against Enterococcus faecalis. *J Endod*, *32*(2), 145-147.

Kapralos, V., Koutroulis, A., Orstavik, D., Sunde, P. T., & Rukke, H. V. (2018). Antibacterial Activity of Endodontic Sealers against Planktonic Bacteria and Bacteria in Biofilms. *J Endod*, *44*(1), 149-154.

Kim, H. R., Kim, Y. K., & Kwon, T. Y. (2017). Post space preparation timing of root canals sealed with AH Plus sealer. *Restor Dent Endod*, *42*(1), 27-33.

Kim, Y. K., Grandini, S., Ames, J. M., Gu, L. S., Kim, S. K., Pashley, D. H., . . . Tay, F. R. (2010). Critical review on methacrylate resin-based root canal sealers. *J Endod*, *36*(3), 383-399.

Lacey, S., Pitt Ford, T. R., Yuan, X. F., Sherriff, M., & Watson, T. (2006). The effect of temperature on viscosity of root canal sealers. *Int Endod J*, *39*(11), 860-866.

Lee, B. S., Wang, C. Y., Fang, Y. Y., Hsieh, K. H., & Lin, C. P. (2011). A novel urethane acrylate-based root canal sealer with improved degree of conversion, cytotoxicity, bond strengths, solubility, and dimensional stability. *J Endod*, *37*(2), 246-249.

Mohammadi, Z., Jafarzadeh, H., & Shalavi, S. (2014). Antimicrobial efficacy of chlorhexidine as a root canal irrigant: a literature review. *J Oral Sci*, *56*(2), 99-103.

Morgental, R. D., Vier-Pelisser, F. V., Oliveira, S. D., Antunes, F. C., Cogo, D. M., & Kopper, P. M. (2011). Antibacterial activity of two MTA-based root canal sealers. *Int Endod J*, *44*(12), 1128-1133.

Nambu, T. (1984). Study on antibacterial root canal sealer containing chlorhexidine dihydrochloride. II. Investigation of antibacterial activity and follow-up study on clinical usage. *Dent Mater J*, *3*(2), 288-311.

Pawinska, M., Szczurko, G., Kierklo, A., & Sidun, J. (2017). A laboratory study evaluating the pH of various modern root canal filling materials. *Adv Clin Exp Med*, *26*(3), 387-392.

Pinheiro, S. L., da Silva, C. C., da Silva, L. A., Cicotti, M. P., Bueno, C., Fontana, C. E., de Campos, F. U. (2018). Antimicrobial efficacy of 2.5% sodium hypochlorite, 2% chlorhexidine, and ozonated water as irrigants in mesiobuccal root canals with severe curvature of mandibular molars. *Eur J Dent, 12*(1), 94-99.

Prullage, R. K., Urban, K., Schafer, E., & Dammaschke, T. (2016). Material Properties of a Tricalcium Silicate-containing, a Mineral Trioxide Aggregate-containing, and an Epoxy Resin-based Root Canal Sealer. *J Endod*, *42*(12), 1784-1788.

Schwartz, R. S. (2006). Adhesive dentistry and endodontics. Part 2: bonding in the root canal system-the promise and the problems: a review. *J Endod*, *32*(12), 1125-1134.

Sideridou, I., Tserki, V., & Papanastasiou, G. (2002). Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins. *Biomaterials*, 23(8), 1819-1829.

Siqueira, J. F., Jr., Favieri, A., Gahyva, S. M., Moraes, S. R., Lima, K. C., & Lopes, H. P. (2000). Antimicrobial activity and flow rate of newer and established root canal sealers. *J Endod*, *26*(5), 274-277.

Stuart, C. H., Schwartz, S. A., Beeson, T. J., & Owatz, C. B. (2006). Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *J Endod*, *32*(2), 93-98.

Thosar, N. R., Chandak, M., Bhat, M., & Basak, S. (2018). Evaluation of Antimicrobial Activity of Two Endodontic Sealers: Zinc Oxide with Thyme Oil and Zinc Oxide Eugenol against Root Canal Microorganisms- An in vitro Study. *Int J Clin Pediatr Dent*, *11*(2), 79-82.

Versiani, M. A., Carvalho-Junior, J. R., Padilha, M. I., Lacey, S., Pascon, E. A., & Sousa-Neto, M. D. (2006). A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. *Int Endod J*, *39*(6), 464-471.

Wainstein, M., Morgental, R. D., Waltrick, S. B., Oliveira, S. D., Vier-Pelisser, F. V., Figueiredo, J. A., Scarparo, R. K. (2016). In vitro antibacterial activity of a silicone-based endodontic sealer and two conventional sealers. *Braz Oral Res, 30*, e18.

Webster, D. M., Sundaram, P., & Byrne, M. E. (2013). Injectable nanomaterials for drug delivery: carriers, targeting moieties, and therapeutics. *Eur J Pharm Biopharm*, 84(1), 1-20.

Wu, Y., Zhou, N., Li, W., Gu, H., Fan, Y., & Yuan, J. (2013). Long-term and controlled release of chlorhexidine-copper(II) from organically modified montmorillonite (OMMT) nanocomposites. *Mater Sci Eng C Mater Biol Appl, 33*(2), 752-757.

Zhang, C., Du, J., & Peng, Z. (2015). Correlation between Enterococcus faecalis and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. *J Endod*, *41*(8), 1207-1213.

Zhang, H., Shen, Y., Ruse, N. D., & Haapasalo, M. (2009). Antibacterial Activity of Endodontic Sealers by Modified Direct Contact Test Against Enterococcus faecalis. *Journal of Endodontics*, *35*(7), 1051-1055.

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