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ORIGINAL RESEARCH

Efficacy of mixed diclofenac solutions against root canal biofilms

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Abstract

The objective of this research was to evaluate the efficacy of diclofenac sodium solutions, with or without cetrimide (CTR) added, against polymicrobial root canal biofilms grown in dentin specimens. The study groups were: (1) 5% diclofenac sodium (DCS); (2) 2.5% DCS; (3) 2.5% DCS + 0. 2% CTR; (4) 2.5% DCS + 0.4% CTR and (5) 0.9% saline solution (SS) as the control. After 5 min of solution contact with the biofilms, the antimicrobial activity was evaluated by means of the adenosine triphosphate (ATP) assay as well as confocal laser scanning microscopy (CLSM). Microbial quantification was indicated as the percentage reduction of relative light units (RLUs) for the ATP assay, the Log_{10} total biovolume and the viability percentage (green cells) for CLSM. Solutions of 2.5% DCS + 0.4% CTR and 5% DCS showed the highest antimicrobial efficacy. Cetrimide increased the antibiofilm activity of diclofenac sodium against endodontic biofilms.

KEYWORDS

antimicrobial activity, cetrimide, diclofenac, polymicrobial biofilm, root canal dentin

INTRODUCTION

The medicaments most extensively used to relieve acute and chronic pain from inflammatory conditions in a variety of musculoskeletal disorders [1-3], including the treatment of osteoarthritis of the temporomandibular joint are nonsteroidal anti-inflammatory drugs (NSAIDs).

Diclofenac sodium (DCS) is an NSAID that belongs to the phenylacetic acid family. It acts by inhibiting the activity of both cyclooxygenase enzymes, COX-1 and COX-2 and is considered one of the most efficient inhibitors of PGE2 production [4], an essential component of the inflammatory response. Its efficacy as a topical medication has been proven [5], and moreover, it appears to have few adverse effects when used for cutaneous application in

acute and chronic musculoskeletal conditions, especially at concentrations ≤5% [6].

In endodontics, effective pain management is sometimes challenging. The efficacy of DCS using different delivery routes for preventing post-endodontic pain has been studied with favourable results [7-9]. Likewise, studies have demonstrated the antimicrobial efficacy of DCS, considering it a non-antibiotic compound useful in resistant infections of various kinds [10-12].

Its effectiveness against *Enterococcus faecalis* is greater than calcium hydroxide [13], enhancing the activity of the latter when combined in a paste form as temporary intracanal medication [14]. A recent study has shown the efficacy of DCS solutions at 5% and 2.5% against *E. faecalis* biofilm [15], pointing to its utility as an alternative for the control of infection in teeth with apical periodontitis.

In turn, surfactants are substances that reduce the surface tension between two phases [16]. In this sense, one way to improve the antimicrobial efficacy of disinfectant solutions in root canal preparation is to use surface-active agents to increase the antimicrobial solution's wettability [17]. Thus, various surfactants incorporated into irrigating solutions for endodontic use have shown their efficacy against planktonic bacteria and microbial biofilms [18]. Cetrimide (CTR) is a surface-active agent with proven antifungal [19] and antibacterial activity [20, 21], and its topical use does not present toxicity at concentrations of up to 2% [22].

To date the antibiofilm effects of diclofenac sodium with the addition of surfactants is unknown. The null hypothesis of this study was that the antimicrobial effects of diclofenac sodium increase with the addition of cetrimide. Accordingly, the aim was to evaluate the efficacy of 5% DCS and 2.5% DCS solutions, with or without the addition of CTR, against polymicrobial biofilms grown on radicular dentin specimens.

MATERIALS AND METHODS

The protocol of the work (no. 1076 CEIH/2020) was approved by the Ethics Committee of the University of Granada, Spain.

Dentin specimen preparation

Forty-one freshly extracted single-rooted human teeth were selected and stored at 4°C in 0.2% thymol until use. The crowns were sectioned at the cemento- and enamel junction, and the middle and apical thirds of the root were discarded to obtain 80 dentin blocks that served as substrate for grown biofilm. Dentin specimens of 4 × 4 × 0.7 mm (width × length × height) were obtained by sectioning longitudinally the coronal portion of the roots into two halves. To create a flat surface, the outer cementum of each half was removed and the dentin root was polished with silicon carbide papers (220–800-grit). The smear layer created during specimen preparation was eliminated with 17% EDTA for 5 min. The specimens were then washed for 10 min with distilled water and sterilised at 121°C for 20 min. The sterility of the samples was proven by incubating the specimens in 5 mL of brain–heart infusion broth (BHI) (Scharlau Chemie SA) for 24 h at 37°C and verifying the absence of turbidity in the culture medium.

Dentin specimen infection

Microbial samples were collected from infected root canals of single-rooted teeth of three volunteers with apical periodontitis, as described in a previous methodology [23]. Afterwards, the microbial samples were mixed in BHI and incubated in anaerobic conditions for 24 h at 37°C. The density of cells was balanced to a concentration of approximately 3.0×10^7 colony-forming units per millilitre in BHI broth, using a spectrophotometer.

The wells of microtiter plates were inoculated with 1.8 mL of sterile BHI and 200 μL of the microbial suspension. Seventy-eight dentin specimens were introduced in the wells and incubated at 37°C in an anaerobic atmosphere for 3 weeks [24]. The BHI was refreshed once a week. Throughout the experiments, four dentin specimens inoculated in sterile BHI were used as a sterility control.

Eight specimens were used to confirm the growth of the biofilms on dentin samples. The specimens were processed and observed with field-emission scanning electron microscopy (FESEM) Gemini [Carl Zeiss, Centre for Scientific Instrumentation (CIC), University of Granada, Spain].

Antimicrobial determination

Antimicrobial efficacy was determined by the ATP test (BacTiter-Glo; Promega) and CLSM evaluation.

Fifty infected specimens were washed with SS for 1 min and used for the ATP determination. They were randomly divided into five groups ($n = 10$/group) according to the solutions tested (Table 1): (1) 5% DCS; (2) 2.5% DCS; (3) 2.5% DCS + 0.2% CTR; (4) 2.5% DCS + 0.4% CTR; and (5) 0.9% SS.

The specimens were immersed in the antimicrobial solutions (120 μL) for 5 min. Next, the specimens were

placed in 200 µL of BHI in Eppendorf tubes, vortexed for 10 s and then sonicated for 10 min to dislodge the biofilms. The control group (0.9% SS) was not exposed to any antimicrobial solution. Afterwards, 100 µL of bacterial suspension was added to 100 µL BacTiter-Glo reagent in a 96-well white plate (Greiner) and the mixture was incubated for 5 min. A luminometer (GloMax; Promega) was used to measure the luminescence produced and was expressed as the percentage reduction of relative light units (RLUs) of the test specimens with respect to the control using the formula: (1 − [RLUs test/RLUs control]) × 100.

For CLSM evaluation, 20 infected dentin specimens ($n = 5$/group) were used to obtain 20 stacks/group for each solution used (Table 1). After washing the specimens with saline solution for 1 min they were submerged in the solutions for 5 min. The samples were rinsed anew with 0.9% SS and stained with a 1:1 mixture of Syto 9 and propidium iodide [PI] for 15 min, and cell viability was evaluated by means of the viability kit (LIVE/DEAD; BacLight; Invitrogen) [20]. They were then rinsed again with SS and mounted on a 60 L-Dish (Ibidi) with mounting oil (BacLight; Invitrogen), and they were observed in a CLSM (Leica TCS-SP5 II; Leica Microsystems). The absorption and emission wavelengths were 494/518 nm for Syto 9 and 536/617 nm for PI. A total of five microscopic volumes of 512 × 512 pixels were obtained from random areas from each specimen, using a 40× oil lens and 1-µm step size. Each picture represented an area of 387 × 387 µm. The scanning was carried out from the top surface of the biofilm to the dentin. Results were expressed as total biovolume and green percentage (cells with intact membrane).

The bioImage_L software was used for quantification purposes [25]. The Log_{10} total biovolume and the percentage of viable cells [green population/(green population + red population)] were calculated in each study group. Statistical analysis was performed by means of SPSS 23.0 (SPSS Inc.). The Log_{10} total biovolume followed a normal distribution by the Kolmogorov–Smirnov test. Data on the reduction percentage of RLUs and the green percentage were previously subjected to the Anscombe transformation. The Levene test showed significant differences of variances among groups for all variables tested. Global comparisons were performed using the ANOVA test with Welch's correction and the Games-Howell test for post-hoc comparison.

TABLE 1 Reduction percentage of RLUs, Log_{10} biovolume (μm^3) and green percentage, after 5 min contact of irrigating solutions on polymicrobial root canal biofilms. Mean (standard deviation).

Solutions	RLUs % reduction	Log_{10} biovolume	Green %
5% DC	83.21 (2.21)[a]	4.52 (0.27)[a]	4.88 (6.38)[a]
2.5% DC	42.30 (18.28)[b]	4.44 (0.24)[a,b]	9.84 (7.33)[b]
2.5% DC + 0.2% CTR	75.54 (20.28)[a]	4.17 (0.27)[b,c]	6.68 (2.10)[b]
2.5% DC + 0.4% CTR	88.55 (7.15)[a]	3.96 (0.24)[c]	2.03 (3.10)[a]
0.9% Saline solution*	—	4.89 (0.38)[d]	82.70 (7.31)[c]

Note: Global comparison between groups determined by ANOVA test with Welch's correction ($p < 0.001$). The same superscript letter read vertically indicates differences that were not statistically significant according to the Games-Howell test.

*Values of RLUs control: mean (standard deviation): 835406 (32228).

RESULTS

The representative scanning electron microscope images of 3-week polymicrobial endodontic biofilms obtained by SEM are shown in Figure 1. The percentage of RLU reduction ranged between 42.80 and 88.55, respectively, for 2.5% DCS and 2.5% DCS + 0.4% CTR. There were no statistically significant differences between the 5% DCS and the 2.5% DCS groups with CTR (0.2% and 0.4%), whereas the 2.5% DCS group was statistically different from all groups.

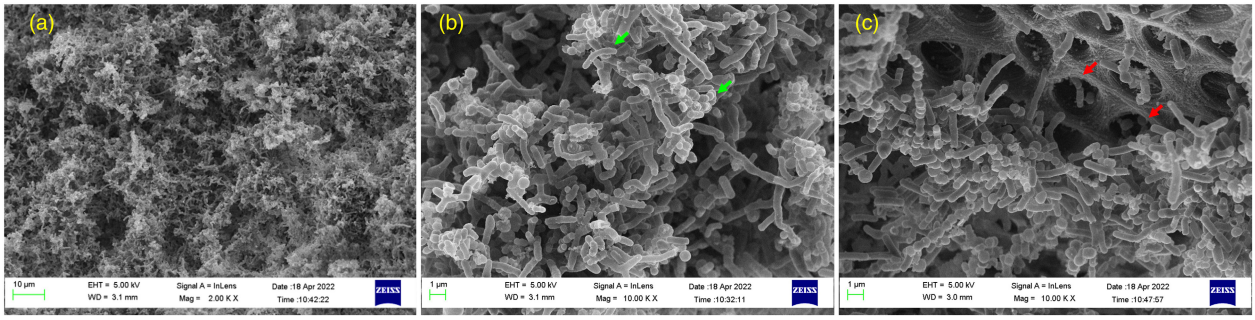

FIGURE 1 (a) Representative scanning electron microscope image of 3-week polymicrobial endodontic biofilms. (b) Note the layered structure of bacterial aggregates with green arrows showing different species and (c) their introduction into dentinal tubules with red arrows.

For antimicrobial quantification with CLSM, 100 operative fields (3-dimensional stacks) were evaluated. In all groups, the Log_{10} total biovolume showed significant differences with respect to the control (Table 1). In the control group cell viability was 82.70%. Concentrations of 2.5% DCS + 0.4% CTR and 5% DCS showed the greatest reduction in cell viability percentage (2.03% and 4.88%, respectively), giving statistically significant differences from 2.5% DCS and 2.5% DCS + 0.2% CTR, yet without significant differences between the two. Representative CLSM images of polymicrobial biofilms after treatment in the study groups are displayed in Figure 2.

DISCUSSION

Bacterial infection—organised as biofilms attached to root canal dentin—is the main cause of apical periodontitis [26]. The resistance to antibiotics is an important concern behind the search for new approaches to root canal disinfection, including the use of NSAIDs [27]. Several investigations report that NSAIDs have antimicrobial and antibiofilm effects against clinically relevant bacteria [10-13, 28]. Their topical application, as irrigating solutions or intracanal medication, is an alternative in root canal therapy that is gaining interest in terms

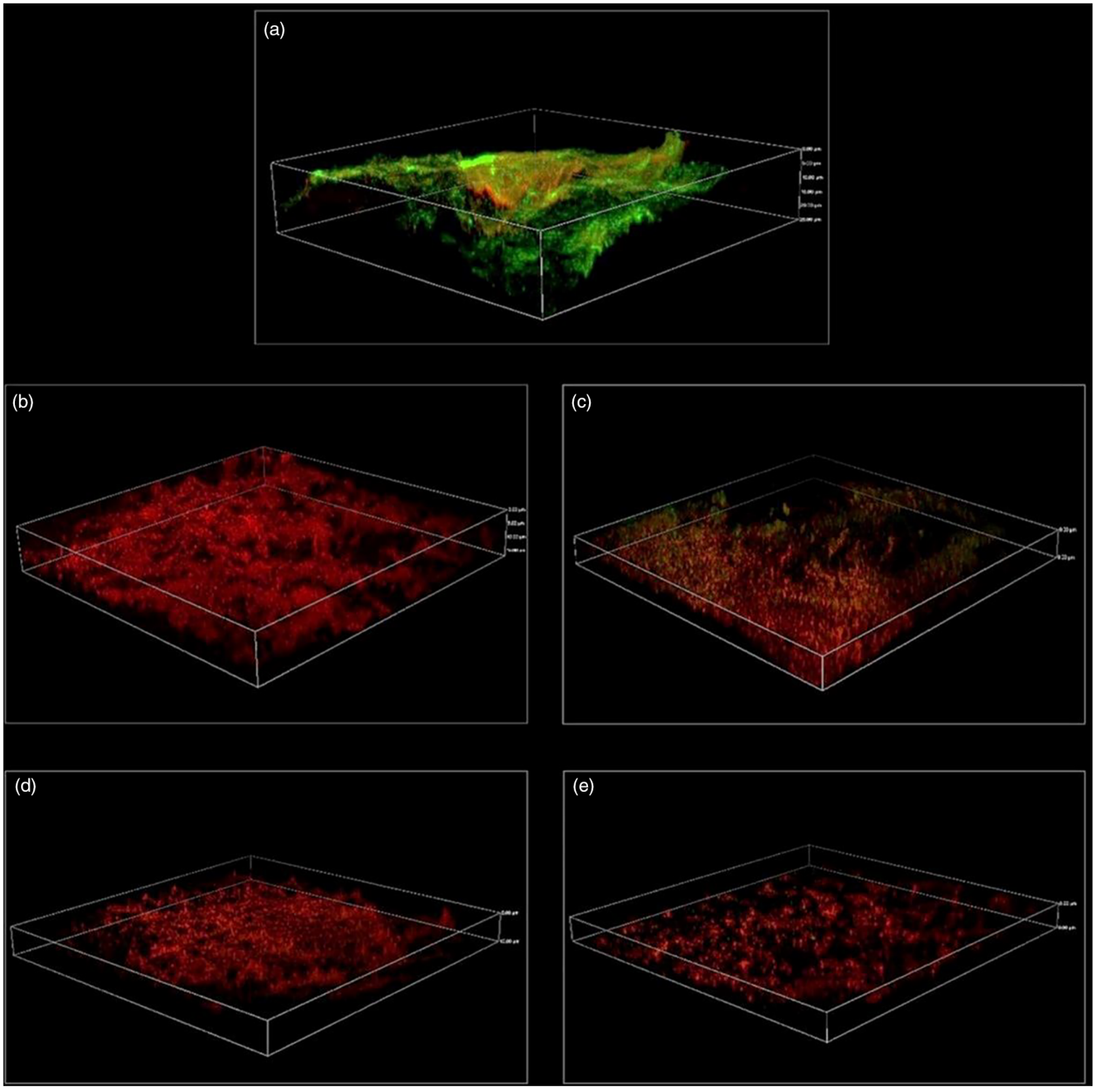

FIGURE 2 Representative confocal laser scanning microscopic images of polymicrobial biofilms in dentine samples after the different treatments: (a) positive control; (b) 5% diclofenac; (c) 2.5% diclofenac; (d) 2.5% diclofenac+0.2% cetrimide; (e) 2.5% diclofenac + 0.4% cetrimide.

of antibiofilm efficacy [14, 15]. On the other hand, quaternary ammonium salts could become an antimicrobial support and innovative means of managing bacterial resistance [29].

In order to approximate clinical reality, this study used a polymicrobial root canal biofilm to determine the efficacy of the antimicrobial solutions. Concentrations of 5% DCS and 2.5% DCS were selected in view of their previously demonstrated efficacy on *E. faecalis* biofilm [15]. CTR was the surfactant of choice given its antimicrobial effectiveness and residual activity in dentin specimens [30] and in root canals [31]. Additionally, when combined with chelating and antimicrobial irrigating solutions it can enhance the antibiofilm activity of the mixture [32, 33].

The results obtained show the antimicrobial potential of 5% DCS and 2.5% DCS + CTR solutions on polymicrobial biofilm from necrotic root canals. The ATP assay confirmed significant differences between experimental groups. The highest RLU reduction was seen for 2.5% DCS + 0.4% CTR and 5% DCS (88% and 82%, respectively) followed by the combination of 2.5% DCS + 0.2% CTR (75%), without significant differences between these solutions; differences were significant with respect to the 2.5% DCS solution (42%), however. The greater diversity of microorganisms in the biofilm might have required a higher concentration of DCS for their reduction, as evidenced by the lower efficiency obtained with a concentration of 2.5% DCS. The amphipathic nature of CTR permits the surface tension reduction of solutions [34], facilitating penetration into the dentinal tubules and providing residual antibacterial activity [30]. The use of CTR against a polymicrobial mature biofilm has been shown to increase the antimicrobial activity of antiseptic solutions in human dentin [35].

The outcomes of the reduction percentage of RLUs appear to coincide with the percentage of viable cells (green %) by the CLSM evaluation for the 2.5% DCS + 0.4% CTR and 5% DCS groups, which showed the lowest viability values. Although the total biovolume was scarcely reduced, the reduction was somewhat greater for the solutions combined with CTR. This finding could be due to biofilm destabilisation when the surfactant interacts with the extracellular polymeric substance and might explain the minor biovolume obtained for both CTR-combined solutions [36].

In this study, the null hypothesis was accepted, given that both 2.5% DCS + CTR solutions demonstrated efficacy similar to 5% DCS solutions on polymicrobial biofilms obtained from necrotic root canals. The addition of CTR reduced by half the effective antimicrobial concentration of diclofenac sodium in mixed solutions. In addition, CTR could lend substantivity to the combination and increase disinfection over time [31, 32]. The biological compatibility of the DCS and CTR association is determined by the aggregation property of the tensioactive agent. Surfactant molecules are organised into micelles, which in aqueous solution show hydrophobic tails directed towards the centre and hydrophilic heads towards the outside. In this way, micellar partitioning can increase the bioavailability of a drug, providing a more sustained release pattern and protecting it against metabolisation and degradation [37]. Cationic surfactants improve the binding efficiency and sustained release of NSAIDs [38].

The purpose of the NSAID + CTR association is to maintain disinfection over time when used as final irrigation solution in root canal treatment and increase the effectiveness of disinfection between sessions when used as intracanal medication. A recent study evaluated the antimicrobial potential of new diclofenac-based hydrogels and triantibiotic and diantibiotic hydrogels and compared their efficacy with calcium hydroxide paste [39]. A 5% DCS hydrogel showed, in dentin specimens and in root canals, statistically significant differences with respect to all the other materials tested. In this sense, a previous investigation showed the effectiveness of a calcium hydroxide paste with 0.2% CTR as intracanal medication, for 2 and 7 days, in simulated open apex root canals contaminated with *E. faecalis biofilms* [40]. Future research could investigate the antibiofilm effectiveness of mixed DCS + CTR hydrogels.

The possible introduction in endodontic protocols of DCS + CTR is meant to enhance disinfection in root canal treatments and, additionally, postoperative pain reduction [7-9]. Moreover, their use in regenerative procedures in a gel form could contribute to reducing antibiotic resistance [40]. Further studies are needed to determine the cytotoxicity of mixed NSAIDs compounds and to address their usefulness as final irrigating solutions or intracanal medications in endodontic therapy.

CONCLUSION

Solutions of 2.5% DCS + 0.4% CTR and 5% DCS showed the highest antimicrobial efficacy. Cetrimide increases the antibiofilm activity of diclofenac against polymicrobial root canal biofilms.

AUTHOR CONTRIBUTIONS

All authors have contributed significantly, and all authors are in agreement with the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

1. Smith SR, Deshpande BR, Collins JE, Katz JN, Losina E. Comparative pain reduction of oral non-steroidal anti-inflammatory drugs and opioids for knee osteoarthritis: systematic analytic review. Osteoarthr Cartil. 2016;24(6):962–72.
2. Derry S, Conaghan P, Da Silva JA, Wiffen PJ, Moore RA. Topical NSAIDs for chronic musculoskeletal pain in adults. Cochrane Database Syst Rev. 2016;4:CD007400.
3. Derwich M, Mitus-Kenig M, Pawlowska E. Orally administered NSAIDs-general characteristics and usage in the treatment of temporomandibular joint osteoarthritis—a narrative review. Pharmaceuticals (Basel). 2021;14(3):219.
4. Altman R, Bosch B, Brune K, Patrignani P, Young C. Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology. Drugs. 2015;75(8):859–77.
5. Tieppo Francio V, Davani S, Towery C, Brown TL. Oral versus topical diclofenac sodium in the treatment of osteoarthritis. J Pain Palliat Care Pharmacother. 2017;31(2):113–20.
6. Taylor RS, Fotopoulos G, Maibach H. Safety profile of topical diclofenac: a meta-analysis of blinded, randomized, controlled trials in musculoskeletal conditions. Curr Med Res Opin. 2011;27(3):605–22.
7. Metri M, Hegde S, Bhandi S. Effect of pretreatment diclofenac sodium on postendodontic pain: a randomised controlled trial. J Conserv Dent. 2016;19(1):7–10.
8. Jenarthanan S, Subbarao C. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: a randomized controlled clinical trial. J Conserv Dent. 2018;21(3):297–301.
9. Mangal S, Mathew S, Murthy BVS, Hegde S, Dinesh K, Ramesh P. The efficacy of transdermal and oral diclofenac for post-endodontic pain control: a randomised controlled trial. Indian J Dent Res. 2020;31(1):53–6.
10. Dastidar SG, Ganguly K, Chaudhuri K, Chakrabarty AN. The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int J Antimicrob Agents. 2000;14(3):249–51.
11. Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, et al. Potential management of resistant microbial infections with a novel non-antibiotic: the anti-inflammatory drug diclofenac sodium. Int J Antimicrob Agents. 2007;30(3):242–9.
12. Mazumdar K, Dastidar SG, Park JH, Dutta NK. The anti-inflammatory non-antibiotic helper compound diclofenac: an antibacterial drug target. Eur J Clin Microbiol Infect Dis. 2009;28(8):881–91.
13. Salem-Milani A, Balaei-Gajan E, Rahimi S, Moosavi Z, Abdollahi A, Zakeri-Milani P, et al. Antibacterial effect of diclofenac sodium on *Enterococcus faecalis*. J Dent (Tehran). 2013;10(1):16–22.
14. de Freitas RP, Greatti VR, Alcalde MP, Cavenago BC, Vivan RR, Duarte MA, et al. Effect of the association of nonsteroidal anti-inflammatory and antibiotic drugs on antibiofilm activity and pH of calcium hydroxide pastes. J Endod. 2017;43(1):131–4.
15. Ferrer-Luque CM, Baca P, Solana C, Rodríguez-Archilla A, Arias-Moliz MT, Ruiz-Linares M. Antibiofilm activity of diclofenac and antibiotic solutions in endodontic therapy. J Endod. 2021;47(7):1138–43.
16. Rosen MJ, Kunjappu JT. Surface Tension and Interfacial Phenomena. 4th ed. New York: John Wiley & Sons; 2012.
17. Taşman F, Cehreli ZC, Oğan C, Etikan I. Surface tension of root canal irrigants. J Endod. 2000;26(10):586–7.
18. Mohammadi Z, Shalavi S, Giardino L, Palazzi F. Effect of surfactants on the efficacy of root canal Irrigants: a review. N Y State Dent J. 2017;83(3):37–42.
19. Vieira DB, Carmona-Ribeiro AM. Cationic lipids and surfactants as antifungal agents: mode of action. J Antimicrob Chemother. 2006;58(4):760–7.
20. Ruiz-Linares M, Ferrer-Luque CM, Arias-Moliz T, de Castro P, Aguado B, Baca P. Antimicrobial activity of alexidine, chlorhexidine and cetrimide against *Streptococcus mutans* biofilm. Ann Clin Microbiol Antimicrob. 2014;20(3):41.
21. Arias-Moliz MT, Ferrer-Luque CM, González-Rodríguez MP, Valderrama MJ, Baca P. Eradication of *Enterococcus faecalis* biofilms by cetrimide and chlorhexidine. J Endod. 2010;36(1):87–90.
22. Cloete TE, Jacobs L, Brözel VS. The chemical control of biofouling in industrial water systems. Biodegradation. 1998;9(1):23–37.
23. Siqueira JF Jr, Rôças IN. A critical analysis of research methods and experimental models to study the root canal microbiome. Int Endod J. 2022;55(Suppl 1):46–71.
24. Ruiz-Linares M, Solana C, Baca P, Arias-Moliz MT, Ferrer-Luque CM. Antibiofilm potential over time of a tricalcium silicate material and its association with sodium diclofenac. Clin Oral Investig. 2022;26(3):2661–9.
25. Chávez de Paz LE. Image analysis software based on color segmentation for characterization of viability and physiological activity of biofilms. Appl Environ Microbiol. 2009;75(6):1734–9.
26. Siqueira JF Jr, Rôças IN. Community as the unit of pathogenicity: an emerging concept as to the microbial pathogenesis of apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(6):870–8.
27. Leão C, Borges A, Simões M. NSAIDs as a drug repurposing strategy for biofilm control. Antibiotics. 2020;9(9):591.
28. Karataş E, Baltacı MÖ, Uluköylü E, Adıgüzel A. Antibacterial effectiveness of calcium hydroxide alone or in combination with ibuprofen and ciprofloxacin in teeth with asymptomatic

apical periodontitis: a randomized controlled clinical study. Int Endod J. 2020;53(6):742–53.
29. Jennings MC, Minbiole KP, Wuest WM. Quaternary ammonium compounds: an antimicrobial mainstay and platform for innovation to address bacterial resistance. ACS Infect Dis. 2015;1(7):288–303.
30. Baca P, Junco P, Arias-Moliz MT, Castillo F, Rodríguez-Archilla A, Ferrer-Luque CM. Antimicrobial substantivity over time of chlorhexidine and cetrimide. J Endod. 2012;38(7):927–30.
31. Ferrer-Luque CM, Teresa Arias-Moliz M, Ruiz-Linares M, Elena Martínez García M, Baca P. Residual activity of cetrimide and chlorhexidine on *Enterococcus faecalis*-infected root canals. Int J Oral Sci. 2014;6(1):46–9.
32. Ferrer-Luque CM, Conde-Ortiz A, Arias-Moliz MT, Valderrama MJ, Baca P. Residual activity of chelating agents and their combinations with cetrimide on root canals infected with *Enterococcus faecalis*. J Endod. 2012;38(6):826–8.
33. Solana C, Ruiz-Linares M, Baca P, Valderrama MJ, Arias-Moliz MT, Ferrer-Luque CM. Antibiofilm activity of sodium hypochlorite and alkaline tetrasodium EDTA solutions. J Endod. 2017;43(12):2093–6.
34. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. J Endod. 2012;38(7):948–53.
35. Ruiz-Linares M, Aguado-Pérez B, Baca P, Arias-Moliz MT, Ferrer-Luque CM. Efficacy of antimicrobial solutions against polymicrobial root canal biofilm. Int Endod J. 2017;50(1):77–83.
36. Simões M, Pereira MO, Vieira MJ. Effect of mechanical stress on biofilms challenged by different chemicals. Water Res. 2005;39(20):5142–52.
37. Croy SR, Kwon GS. Polymeric micelles for drug delivery. Curr Pharm Des. 2006;12(36):4669–84.
38. Gupta RD, Raghav N. Differential effect of surfactants tetra-n-butyl ammonium bromide and N-Cetyl-N, N, N-trimethyl ammonium bromide bound to nano-cellulose on binding and sustained release of some non-steroidal anti-inflammatory drugs. Int J Biol Macromol. 2020;1(164):2745–52.
39. Ruiz-Linares M, Monroy-Rojas JF, Solana C, Baca P, Aguado B, Soriano-Lerma A, et al. Antimicrobial potential of new diclofenac hydrogels for disinfection in regenerative endodontics: an in vitro and ex vivo study. Int Endod J. 2023;56(1):103–17.
40. Valverde ME, Baca P, Ceballos L, Fuentes MV, Ruiz-Linares M, Ferrer-Luque CM. Antibacterial efficacy of several intracanal medicaments for endodontic therapy. Dent Mater J. 2017;36(3):319–24.

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