Research Square

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Evaluation of the effectiveness of diclofenac when used in endodontic regeneration in comparison with calcium hydroxide and triple antibiotic paste: An in vitro study

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Abstract

Aims

This study assessed the biocompatibility and antibacterial activity of diclofenac sodium (DS) in comparison to calcium hydroxide $Ca(OH)_2$ and tripple antibiotic paste (TAP).

Methods

One hundred eight dentin discs and sixty-eight semicylindrical root dentin halves were prepared, sterilized and used for cell viability and antibacterial assessment, respectively. Human mesenchymal stem cells (hMSCs) viability was assessed by AlamarBlue and *Fusobacterium nucleatum* biofilms in dentinal tubules was assessed by confocal laser-scanning microscopy. The specimens were randomly devided into four groups: DS, $Ca(OH)_2$, TAP, no medicament.

Results

Statistical analysis was performed using two-way ANOVA for cell viability and one-way ANOVA for antibacterial activity. DS and TAP groups showed enhanced cell viability in comparison with control-disk and $Ca(OH)_2$ at day 1 and 7 (P = 0.00) but DS group showed significantly lower cell viability when compared to TAP group (P = 0.00). At Day 14, there was no significant differences between cell viabilities in all experimental groups whereas the control group had the outmost increase (P = 0.00). DS and TAP showed the highest percentage of dead cells 96.7% and 96.6%, respectively.

Conclusion

Our study showed that DS had a comparable results to TAP by means of cell viability of hMSCs and antibacterial activity against *F.nucleatum*.

Introduction

Regenerative endodontic procedures (REPs) are a biologically based procedure designed to replace damaged pulpal tissue, dentin and root structures with viable tissues to restore the normal function of a tooth [1]. It was mainly introduced for immature permanent teeth to maintain pulp vitality for continuous root development and apical closure [2]. Favorable outcomes were reported in term of apical healing and continued root development [3,4]. However, root canal disinfection is an essential step in REPs since infection prevents pulp tissue regeneration and may cause damage to stem cell in periapical tissue [5]

In the clinical consideration of REPs, the American Association of Endodontists recommends minimal instrumentation and the use of $Ca(OH)_2$ or TAP for root canal disinfection [6]. $Ca(OH)_2$ is the intracanal medicament of choice due to its antimicrobial and biological effects [7], although it's use for thorough disinfection is still controversial[8–10]. In addition, TAP is the available alternative and is composed of ciprofloxacin, metronidazole and minocycline. A 1 mg/ml of TAP has been recommended to avoid the toxic effects on the stem cells of the apical papilla [11], yet the concern of developing antibiotic resistance is still there [12,13]. Therefore, in recent years several studies reported the potential use of nonsteroidal anti-inflammatory drug (NSAID) for root canal disinfection [14–16]

Diclofenac Sodium, a potent anti-inflammatory medication, used to relieve pain and reduce swelling and inflammation in cases of rheumatoid arthritis, osteoarthritis and acute goat. It has been shown to have significant antibacterial effect against both gram-positive and gram-negative bacteria [17–19]. A recent study has reported that diclofenac sodium has a greater antibacterial activity against *Enterococcus faecalis* compared to TAP in a concentration dependent manner when used as an[15]. Furthermore, it has been shown to exert a more prominent antibacterial effect when compared to $Ca(OH)_2$ [14]. The available data advocate the possible use of diclofenac sodium as an intracanal medicament. To the best of our knowledge, the antibacterial effect, biocompatibility of diclofenac

in a paste form compared to $Ca(OH)_2$ and TAP remains unknown. The purpose of this *in vitro* study was to evaluate the antibacterial effect and cell viability of DS compared to $Ca(OH)_2$ and TAP.

Materials and Methods

Preparation of dentin specimens

Extracted human premolars were selected and used in the present study. Teeth were examined under an operating microscope (Zeiss, Oberkochen, Germany) to exclude any tooth with root caries, cracks, or fractures. Teeth with one, noncalcified canal were selected as confirmed by radiographs taken from buccolingual and mesiodistal views. Only teeth with canals of less than 20° curvature were included[20]. Teeth were stored in saline until the beginning of the experiment.

For cell viability assay, dentin discs (1 mm in thickness) from the middle third of each root were prepared (n = 108). The canal space was prepared to a standardized inner diameter of 1.5 mm with the use of a high-speed dental handpiece and diamond round dental bur (Komet dental, Lemgo, Germany) and constant water cooling. The outer diameter of the dentin disk was standardized to 4 mm using a metal circular template.

For antibacterial assay, semicylindrical root dentin halves were prepared according to the protocol described by Alsubait et al. 2019 [21]. Briefly, a root dentin block with a length of 4 mm were horizontally sectioned from each tooth. The root canals inside the blocks were enlarged with a complete pass of Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), sizes 2, 3, 4, 5, and 6, at 300 rpm under water cooling to achieve a diameter of 1.5 mm. Each cylindrical dentin block were sectioned into 2 semicylindrical halves using the diamond saw (n = 68). For specimen standardization, the cementum was removed from the outer surfaces of the semicylindrical halves using 600-grit fine-grain sandpaper (Buehler, Lake Bluff, IL). The size of each specimen is 4 × 4 × 2 mm (width × length × height).

In all dentin specimens, the smear layer was removed by immersing the dentin sections in 5.25% NaOCl for 4 min and 17% ethylenediamine tetraacetic acid (EDTA) (Vista Dental Products, Racine, WI, USA) for 1 min in an ultrasonic bath. Sodium thiosulfate was used for 1 min to inactivate NaOCl. Finally, all sections were rinsed in sterile water for 1 min and placed in closed containers containing phosphate buffered saline (PBS) and sterilized using gamma irradiation with dose of 25 Kilo Gray in the Gamma Radiation [22]. Two dentin discs and 2 root dentin blocks were then incubated in 5 ml brain heart infusion broth for 24 hours at 37°C to ensure no bacterial contamination.

Dentin Infection with Fusobacterium nucleatum Biofilms

Under laminar flow, *F. nucleatum* was inoculated in cooked meat medium and fastidious anaerobe broth. The *F. nucleatum* suspension was then spread onto an agar plate (Fastidious Anaerobe Agar, Lab M). The plates were incubated anaerobically at 37oC for 24 hours. The colonies were diluted in fresh FAB and incubated to match the turbidity equivalent to a 0.5 McFarland standard, corresponding to an optical density of 0.08–0.1 absorbance at 600 nm in a spectrophotometer.

The sterilized root dentin blocks were placed in 24 well tissue culture plates (Nunc; Thermo Scientific, Darmstadt, Denmark), inoculated with 2.0 mL *F. nucleatum* suspension, and incubated under anaerobic conditions for 21 days at 37oC. Fresh FAB was replaced every 72 hours to remove dead cells and ensure bacterial viability. The monoculture of the strains was confirmed by gram staining and colony morphology and further confirmed with the Vitek device (bioMerieux, Inc, Hazelwood, MO). After incubation, the specimens were aseptically removed from the wells and gently rinsed with sterile phosphate- buffered saline for 1 minute to remove loosely attached bacteria. Two dentin slices were randomly selected from each group and examined using a confocal laser scanning microscope (Nikon C21 system; Nikon Instruments Inc, Melville, NY) to verify the viability, thickness, and homogeneity of the biofilm.

Placement of test material

Calcium hydroxide ($Ca(OH)_2$) was used in a commercially available form (Ultracal; Ultradent, South Jordan, UT). Triple antibiotic paste (TAP) was prepared by mixing equal portions of metronidazole (Eczacibasıbası, Istanbul, Turkey), ciprofloxacin (Bayer, Leverkusen, Germany), and minocycline (Sanovel, Istanbul, Turkey) with sterile distilled water (powder/liquid ratio of 3:1). Diclofenac

Sodium paste (DS) was prepared by mixing diclofenac sodium (Diclomol, Win Medicare) with sterile distilled water (powder/liquid ratio of 3:1).

The dentin specimens were randomly divided into four groups according to the intracanal medicament, as follows: $Ca(OH)_2$, TAP, DS, and no medicament (control). Intracanal medicaments were applied at the lumen of the discs for the experimental groups or placed inside the blocks using a disposable 3-mL syringe. A scalpel was used to remove the excess material. All specimens were placed at 37°C in 100% relative humidity for 28 days.

Cell culture

Immortalized human bone marrow-derived mesenchymal stem cells (hMSCs), from Stem Cell Unit, Department of Anatomy, College of Medicine, King Saud University and King Khalid University Hospital, were used in the present study(23). The cells were grown in growth medium and maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Cell viability assessment

After 28 days, the medicaments were removed from the lumen of the discs using 2.5% NaOCl, 17% EDTA, and sterile saline. Cells (5 $x10^4$ cells/well) were seeded on each dentin disc surface and incubated for 1, 7, and 14 days by changing the culture medium three times a week. After each time period, the culture medium was removed and the cells were incubated with AlamarBlue agent (AbD Serotec, Kidlington, UK) for 4 h. The fluorescence signal from the samples was measured at an excitation wavelength of 530 nm and an emission wavelength of 590 nm using a SpectraMax M5/M5e Multimode Plate Reader (Molecular Devices/BioTek, Winooski, VT, USA), and the data was obtained using SoftMax Pro Microplate Data Acquisition and Analysis Software (Molecular Devices/BioTek).

Antibacterial effect

Dentin blocks were stained with the fluorescent LIVE/DEAD BacLight Bacterial Viability stain (Molecular Probes, Eugene, OR) and examined by CLSM. The medicament was removed from dentin walls by rinsing it in sterile phosphate-buffered saline for 1 min. The fluorescent LIVE/DEAD BacLight Bacterial Viability stain kit was used following the manufacturer's instructions. The mounted specimens were observed by CLSM through a 10/0.49 NA air immersion objective. For standardization, four corners of each dentin disc were scanned. The fluorescence signal of live bacteria were obtained by illuminating the samples with a 488 nm laser, and the emission was recorded through a 520–540 nm bandpass filter (green channel). Excitation by a 568 nm laser and emission through a 600–630 nm filter were used to detect the fluorescence of dead bacteria (red channel). The mean numbers of both live and dead bacteria were calculated using the ImageJ software (Wayne Rasband, NIH, USA) by automated calculation of any ovoid cell with 0.5–1 μm in diameter. The percentage of dead cells was calculated.

Statistical Analysis

The results of the Kolmogorov-Smirnov and Shapiro–Wilk normality test revealed that the data were normally distributed. The means of the differences in the cell viability after exposure to different materials were compared by two-way ANOVA and the means of differences in the antibacterial effect were compared by one-way ANOVA. But the equality of variance was not satisfied (levene's test < 0.05). So, Dunnet T3 post hoc test was used for multiple comparisons. Data were expressed as the mean ± the standard error of the mean. The level of significance was set at 0.05. Statistical analysis was performed using the SPSS statistical software for Windows (version 23; SPSS Inc., Chicago IL, USA).

Results

Cell viability

Cells in the DS and TAP groups showed a significant increase in the proliferative capacity in comparison with control-disk and $Ca(OH)_2$ groups at day 1 and 7 (P = 0.00). However, at day 7, DS group showed significantly lower cell viability when compared to TAP

group (P = 0.00) (Fig. 1). At day 14, there were no significant differences between cell viabilities in all experimental groups whereas the control group had the outmost increase (P = 0.00).

All experimental groups had a significant increase in cell viability at day 7 compared to day 1 (P=.00). However, there was no significant difference in the control group between day 1 and 7 (P = 0.00). The TAP group continued to have a significant increase in the viability at day 14 compared to day 1 (P = 0.00) but the difference was not significant compared to day 7 (P = 0.00). The $Ca(OH)_2$ and DS groups showed a decrease in cell viability at day 14 compared to day 7 (P = 0.00). The control group has a significant increase in cell viability at day 14 compared to day 1 and 7 (P = 0.00) (Fig. 1).

Antibacterial effect

The CLSM images confirmed the presence of the homogenous *F. nucleatum* biofilms on the dentin surfaces after a 3 weeks incubation period. The CLSM images verified the presence of *F. nucleatum* biofilms during the experimental period in the control group (Fig. 2). The CLSM images exposed to DS and TAP showed the highest number of dead cells by 96.7 and 96.6%, respectively. The $Ca(OH)_2$ showed lower number of dead cells 76.8% followed by the control group which was nearly 0%. There was no significant difference in the antibacterial effect between DS and TAP (P = 0.596) but there was a significant difference between $Ca(OH)_2$ and DS (P = 0.00) and $Ca(OH)_2$ and TAP (P = 0.00). All three experimental groups killed bacteria significantly more than the control group (P = 0.00).

Discussion

Disinfection is a critical clinical challenge. The persistence of infection in root canal system can prevents pulp tissue regeneration and may have detrimental effect on stem cell in periapical tissue[24]. NSAIDs is a new alternative approach to disinfection of root canal. And an ideal disinfection material must ensure biocompatibility. In this study, we compared the antibacterial effect and biocompatibility of DS paste to $Ca(OH)_2$ and TAP. Considering that the AAE guideline have suggested the use $Ca(OH)_2$ and low concentration of TAP as intracanal medicaments for disinfection [6], DS was compared to them. To mimic the dental micro-environmental niche, the materials were used indirectly with dentin discs for biocompatibility and directly with semicylindrical root dentin for antibacterial effects.

hMScs are adult stem cells that differentiate into odontoblast-like cells under the stimulus, and they are capable of regenerating pulp-like tissue [12,25]Thus, hMSCs are essential for REPs. Concerns about the safety of stem cells have led researchers to explore different intra-canal medicaments with lesser concentrations [26,27]. NSAIDs, such as DS, although there extensive use in regenerative medicine the exact mechanism of action is not entirely known. The anti-inflammatory, antipyretic, and analgesic actions are attributed to the inhibition of postaglandin synthesis by inhibiting cyclooxygenase [28]. NSAIDs have a conflicting evidence in the literature regarding stem cell viability.

The results of this study showed a delayed increase in MSC viability in the control 3D cultures from day 1 to 7, with a significant rise by day 14. This may be explained by that MSCs often requires an adaptation period when transitioning from a 2D to 3D culture environment to support better cell-cell and cell-matrix interactions, leading to increased cell viability and proliferation at later stages, such as day 14 [29–31]

It was demostrated that DS enhanced cell proliferative capacity at day 1 and 7 but had a signficant decrease at day 14 compared to control. The initial enhancement and subsequent decrease in viability of DS is observed in various studies on different cell types and conditions. DS can initially support cell viability by reducing inflammation and modulating cellular pathways [32]. Over time, DS can become cytotoxic, particularly at higher concentrations. Studies have shown that DS significantly reduces the viability of hMSCs and other cell types, such as fibroblasts, when used at higher concentrations or over extended periods [33].This cytotoxicity is linked to increased reactive oxygen species (ROS) production and alterations in apoptosis-related gene expression [33]

Moreover, the results of this study showed that DS and TAP were less cytotoxic than $Ca(OH)_2$ at all time points. Previous reports showed different results and this may be explained by the different concentrations used and exposure durations [34,35].

In the second part of the study, we evaluated the antibacterial proprieties of TAP, $Ca(OH)_2$, and DS 28 days after infecting dentin semicylinders with *F. nucleatum* in a clinical reality approach. *F. nucleatum* is a gram-negative anaerobe and is the most prevalent

cultivable bacteria from root canals [36,37]. It is frequently connected with endodontic infections precisely pulp necrosis and apical periodontitis [38]. In addition, *F. nucleatum* is among the species that is most found in post medications samples[39]Biofilm was generated as they are more resistant to antibacterial effect and closely resemble their nature in endodontic infections [40]

In agreement with previous studies [14,41,42], DS and TAP showed enhanced antibacterial activity when compared to $Ca(OH)_2$. The NSAID have significantly more pronounced antibacterial activity in comparison with Ca(OH)2. An in vitro study by Salem-Milani et al.14 was the first to comparatively evaluate anti-bacterial efficiency of ibuprofen, diclofenac and Ca(OH)2, and also to recommend the use of NSAIDs as intracanal medicaments, utilizing the agar diffusion test, the authors revealed the anti-bacterial properties of NSAIDs (ibuprofen, diclofenac) against *E. faecalis*, whereas Ca(OH)2 failed at the same.

Moreover, DS and TAP did not have significant difference. An in vitro study showed that the DS has a antibacterial concentration-dependent effect when compared to antibiotic mixtures [15]. The DS was found to cause greater reduction of viable bacteria biofilm at higher concentrations [15]However, as a paste, it was difficult to adjust the concentration and the diffusion and it required elemination, unlike the solution form. Moreover, a recent study demonstrated that the diclofenac-based hydrogels had supperior antimicrobial efficacy against polymicrobial biofilms when compared to triantibiotic and diantibiotic hydrogels[42]. This may be attributed to the hydrogel, as the antimicrobial action will be more dispersed and consistent. Moreover, the exact mechanism of the antibacterial activity of DS is unclear. However, studies have proposed inhibition of bacterial DNA synthesis[43] or impairment of membrane activity[17] as possible underlying mechanisms.

The purpose of using DS as intracanal medicament is to increase the effectiveness of disinfection betweens sessions. Recent studies shown that diclofenac sodium has supperior antimicrobial effect [14,41,42]. However, the results of this study did not support that. Further research involving different concentrations of DS against complex bacterial biofilms related to primary infections could be conducted.

This study is the first to evaluate the biocompabtibility and antibacterial properties of DS compared to TAP and $Ca(OH)_2$ for regenerative endodontic purposes. However, A limitation of the current study, the materials were utilized in a ready to use form. The minimum inhibitory concentration and minimum cytotoxic concentration for DS were not determined. As the prolonged use or higher concentrations of DS can lead to decreased viability due to cytotoxicity effects and apoptotic induction. This dual effect necessitates careful consideration of dosage and duration in therapeutic applications. Another limitation is the use of single bacterial biofilm to study the efficacy of antimicrobial materials. As endodontic disease is a biofilm-mediated infection.

In conclusion, our study showed that DS had a comparable results to TAP by means of cell viability of hMSCs and antibacterial activity against F.nucleatum. DS can be a potential root canal disinfection material. As the use of NSAIDs have a major advantage as the anti-inflammatory topical action could reduce the intraappointment and/or the postoperative pain. Further research is necessary to assess the cytotoxicity and antibacterial activity in a concentration dependent manner.

Declarations

Data Availability Statement:

The data that support the findings of this study are available from the cross-ponding author upon reasonable request.

Ethics Declarations:

The authors declare no conflicts of interest.

Author contributions:

SAS: Conceptualization, Methodology, Investigation, Formal analysis, Writing original draft, Visualization. AAM: Investigation, Data curation, writing review and editing. NAK: Conceptualization, Resources, Supervision. WM: Formal analysis, Software, Validation, writing review and editing. TS: Conceptualization, Supervision, writing review and editing, Project administration

References

1. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod. 2007;33(4):377–90. Available from: https://pubmed.ncbi.nlm.nih.gov/17368324/
2. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod. 2004;30(4):196–200. Available from: https://pubmed.ncbi.nlm.nih.gov/15085044/
3. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod. 2009;35(10):1343–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19801227/
4. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. J Endod. 2012;38(10):1330–6. Available from: https://pubmed.ncbi.nlm.nih.gov/22980172/
5. Aurora AB, Olson EN. Immune modulation of stem cells and regeneration. Cell Stem Cell. 2014;15(1):14–25. Available from: https://pubmed.ncbi.nlm.nih.gov/24996166/
6. AAE Clinical Considerations for a Regenerative Procedure.
7. Desai S, Chandler N. Calcium hydroxide-based root canal sealers: a review. J Endod. 2009;35(4):475–80. Available from: https://pubmed.ncbi.nlm.nih.gov/19345790/
8. Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod. 2007;33(2):114–8. Available from: https://pubmed.ncbi.nlm.nih.gov/17258626/
9. Zandi H, Rodrigues RCV, Kristoffersen AK, Enersen M, Mdala I, Ørstavik D, et al. Antibacterial Effectiveness of 2 Root Canal Irrigants in Root-filled Teeth with Infection: A Randomized Clinical Trial. J Endod. 2016;42(9):1307–13. Available from: https://pubmed.ncbi.nlm.nih.gov/27452293/
10. Arruda MEF, Neves MAS, Diogenes A, Mdala I, Guilherme BPS, Siqueira JF, et al. Infection Control in Teeth with Apical Periodontitis Using a Triple Antibiotic Solution or Calcium Hydroxide with Chlorhexidine: A Randomized Clinical Trial. J Endod. 2018;44(10):1474–9. Available from: https://pubmed.ncbi.nlm.nih.gov/30144986/
11. Althumairy RI, Teixeira FB, Diogenes A. Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. J Endod. 2014;40(4):521–5. Available from: https://pubmed.ncbi.nlm.nih.gov/24666903/
12. Huang GTJ, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res. 2009;88(9):792–806. Available from: https://pubmed.ncbi.nlm.nih.gov/19767575/
13. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod. 2013;39(3):319–26. Available from: https://pubmed.ncbi.nlm.nih.gov/23402501/
14. 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. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3666061/
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. Available from: https://pubmed.ncbi.nlm.nih.gov/33864882/
16. Ferrer-Luque CM, Solana C, Aguado B, Ruiz-Linares M. Antimicrobial Activity and Cytotoxicity of Nonsteroidal Anti-Inflammatory Drugs against Endodontic Biofilms. Antibiotics. 2023;12(3):450. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10044545/
17. 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. Available from: https://pubmed.ncbi.nlm.nih.gov/17644318/
18. Dutta NK, Mazumdar K, Dastidar SG, Park JH. Activity of diclofenac used alone and in combination with streptomycin against Mycobacterium tuberculosis in mice. Int J Antimicrob Agents. 2007;30(4):336–40. Available from: https://pubmed.ncbi.nlm.nih.gov/17644321/

19. Mazumdar K, Dastidar SG, Park JH, Dutta NK. The anti-inflammatory non-antibiotic helper compound diclofenac: An antibacterial drug target. European Journal of Clinical Microbiology and Infectious Diseases. 2009;28(8):881–91. Available from: https://www.researchgate.net/publication/24366541_The_anti-inflammatory_non-antibiotic_helper_compound_diclofenac_An_antibacterial_drug_target
20. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971;32(2):271–5. Available from: https://pubmed.ncbi.nlm.nih.gov/5284110/
21. Alsubait S, Albader S, Alajlan N, Alkhunaini N, Niazy A, Almahdy A. Comparison of the antibacterial activity of calcium silicate- and epoxy resin-based endodontic sealers against Enterococcus faecalis biofilms: a confocal laser-scanning microscopy analysis. Odontology. 2019;107(4):513–20. Available from: https://pubmed.ncbi.nlm.nih.gov/30927150/
22. Whitel JM, Goodis HE, Marshall S, Marshall GW. Sterilization of Teeth by Gamma Radiation. 1994;73(9):1560–7.
23. Huang GTJ, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res. 2009;88(9):792–806. Available from: https://pubmed.ncbi.nlm.nih.gov/19767575/
24. Aurora AB, Olson EN. Immune Modulation of Stem Cells and Regeneration. Cell Stem Cell. 2014 Jul 3;15(1):14–25.
25. Murakami M, Hayashi Y, Iohara K, Osako Y, Hirose Y, Nakashima M. Trophic Effects and Regenerative Potential of Mobilized Mesenchymal Stem Cells From Bone Marrow and Adipose Tissue as Alternative Cell Sources for Pulp/Dentin Regeneration. Cell Transplant. 2015;24(9):1753–65. Available from: https://pubmed.ncbi.nlm.nih.gov/25199044/
26. Ruparel NB, Teixeira FB, Ferraz CCR, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod. 2012;38(10):1372–5. Available from: https://pubmed.ncbi.nlm.nih.gov/22980180/
27. Chuensombat S, Khemaleelakul S, Chattipakorn S, Srisuwan T. Cytotoxic effects and antibacterial efficacy of a 3-antibiotic combination: an in vitro study. J Endod. 2013;39(6):813–9. Available from: https://pubmed.ncbi.nlm.nih.gov/23683284/
28. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis. 2018;9(1):143–50. Available from: https://pubmed.ncbi.nlm.nih.gov/29392089/
29. Frith JE, Thomson B, Genever PG. Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential. Tissue Eng Part C Methods. 2010;16(4):735–49. Available from: https://pubmed.ncbi.nlm.nih.gov/19811095/
30. Tao Q, Wu C, Li X, Chen W, Sun K, Zhang P, et al. Three-dimensional bio-printed constructs consisting of human umbilical-derived mesenchymal stem cells promote cell viability, proliferation, and differentiation in vitro. Cell Mol Biol. 2020;66(2):165–71. Available from: https://cellmolbiol.org/index.php/CMB/article/view/3263
31. Li J, Zhang J, Ye H, Wang Q, Ouyang Y, Luo Y, et al. Pulmonary decellularized extracellular matrix (dECM) modified polyethylene terephthalate three-dimensional cell carriers regulate the proliferation and paracrine activity of mesenchymal stem cells. Front Bioeng Biotechnol. 2023;11. Available from: https://pubmed.ncbi.nlm.nih.gov/38260733/
32. Sidney LE, Heathman TRJ, Britchford ER, Abed A, Rahman CV, Buttery LDK. Investigation of localized delivery of diclofenac sodium from poly(D,L-lactic acid-co-glycolic acid)/poly(ethylene glycol) scaffolds using an in vitro osteoblast inflammation model. Tissue Eng Part A. 2015;21(1–2):362–73. Available from: https://pubmed.ncbi.nlm.nih.gov/25104438/
33. Adamičková A, Kyselovic J, Adamička M, Chomaničová N, Valášková S, Šalingová B, et al. Effects of Ibuprofen and Diclofenac Pre-Treatment on Viability and Apoptosis Processes in Human Dental Pulp Stem Cells. Medicina (Kaunas). 2024;60(5). Available from: https://pubmed.ncbi.nlm.nih.gov/38792973/
34. Labban N, Yassen GH, Windsor LJ, Platt JA. The direct cytotoxic effects of medicaments used in endodontic regeneration on human dental pulp cells. Dent Traumatol. 2014;30(6):429–34. Available from: https://pubmed.ncbi.nlm.nih.gov/24645753/
35. Sowmya MR, Ajitha P, Pradeep S. Evaluation of Cytotoxicity of Diclofenac Sodium on L929 Fibroblasts - An in vitro Study. J Pharm Res Int. 2020 Aug 26:171–7. Available from: https://www.researchgate.net/publication/343993380_Evaluation_of_Cytotoxicity_of_Diclofenac_Sodium_on_L929_Fibroblasts_-_An_in_vitro_Study
36. Narayanan LI, Vaishnavi C. Endodontic microbiology. J Conserv Dent. 2010;13(4):233. Available from: https://pubmed.ncbi.nlm.nih.gov/21217951/
37. Fouad AF. Endodontic Microbiology and Pathobiology: Current State of Knowledge. Dent Clin North Am. 2017;61(1):1–15. Available from: https://pubmed.ncbi.nlm.nih.gov/27912812/

38. Gomes BPFA, Bronzato JD, Almeida-Gomes RF, Pinheiro ET, Sousa ELR, Jacinto RC. Identification of Fusobacterium nucleatum in primary and secondary endodontic infections and its association with clinical features by using two different methods. Clin Oral Investig. 2021;25(11):6249–58. Available from: https://pubmed.ncbi.nlm.nih.gov/33844080/
39. Sakamoto M, Siqueira JF, Rôças IN, Benno Y. Bacterial reduction and persistence after endodontic treatment procedures. Oral Microbiol Immunol. 2007;22(1):19–23. Available from: https://pubmed.ncbi.nlm.nih.gov/17241166/
40. Upadya MH, Kishen A. Influence of bacterial growth modes on the susceptibility to light-activated disinfection. Int Endod J. 2010;43(11):978–87. Available from: https://pubmed.ncbi.nlm.nih.gov/20722757/
41. Mehta S, Verma P, Tikku AP, Chandra A, Bains R, Banerjee G. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. Eur J Dent. 2017;11(1):53. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5379835/
42. 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. Available from: https://pubmed.ncbi.nlm.nih.gov/36169591/
43. 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. Available from: https://pubmed.ncbi.nlm.nih.gov/10773497/

Figures

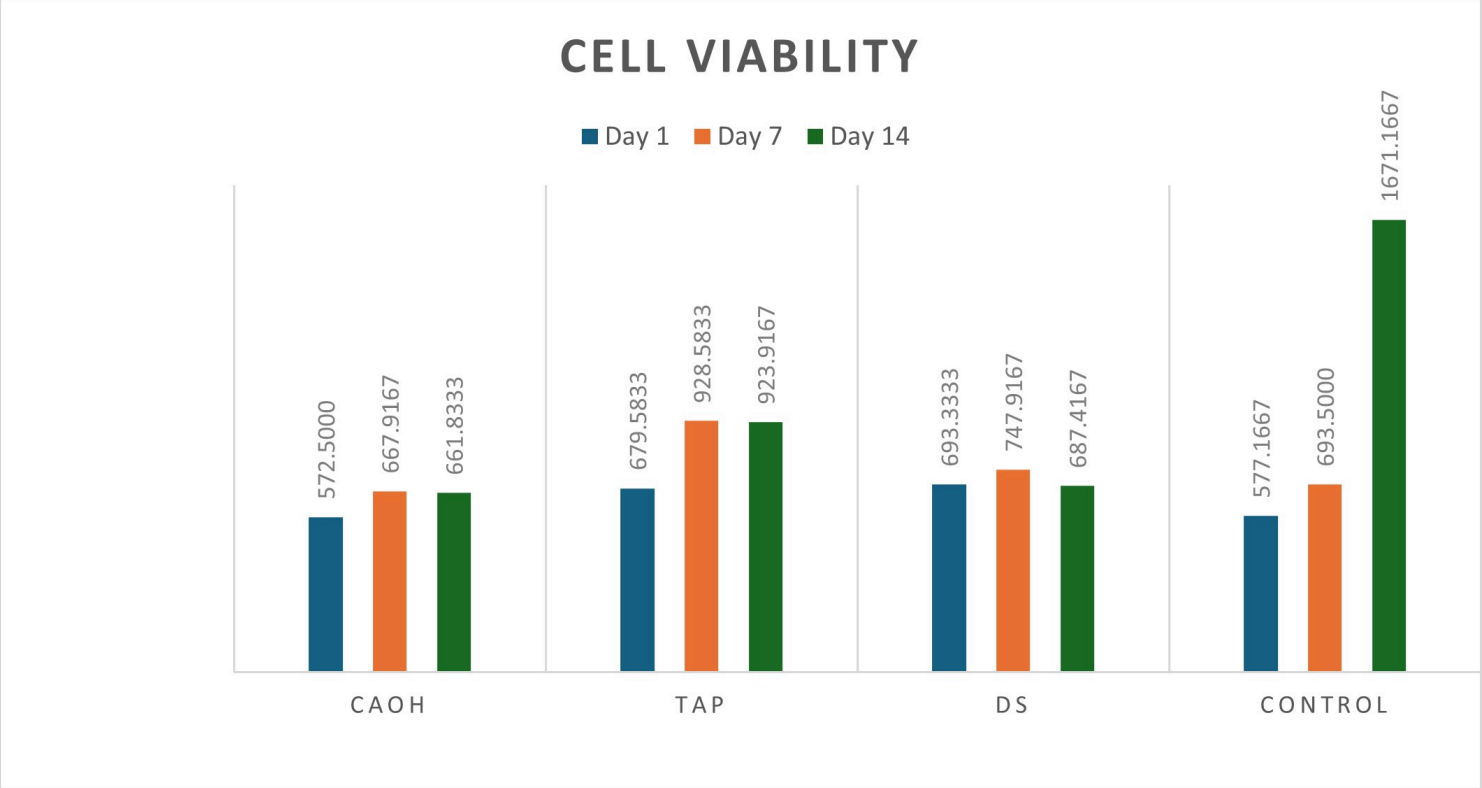


Figure 1

Cell viability effect of the Calcium hydroxide, Triple antibiotic paste, and Diclofenac sodium after 1, 7, and 14 days of exposure.

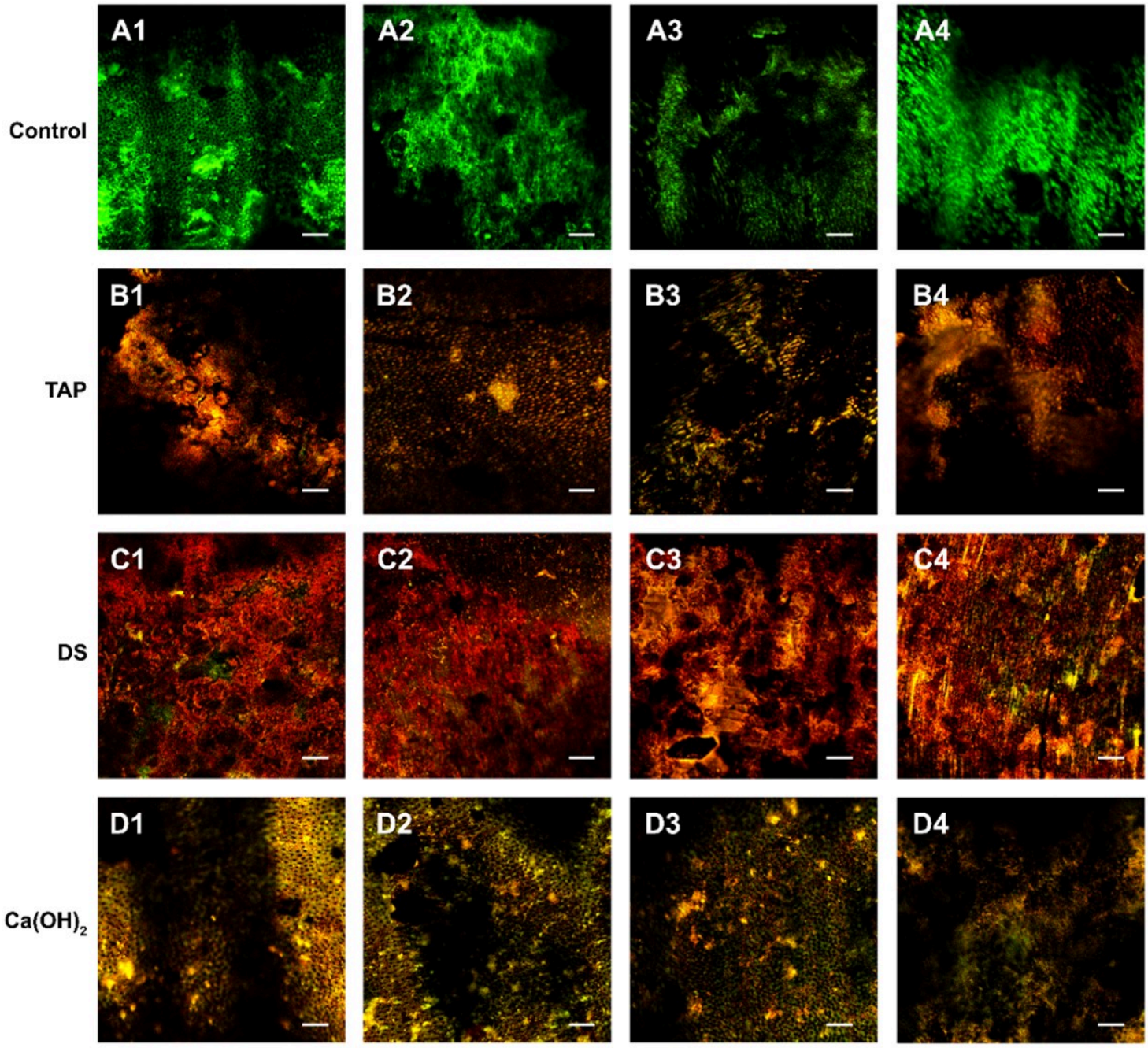


Figure 2

Confocal laser-scanning microscopic images of *F. Nucleatum* biofilms exposed to No material (**A1-4**), Triple Antibiotic paste (**B1-4**), Diclofenac Sodium (**C1-4**) or Calcium hydroxide (**D1-4**). Scale bar = 30 μm.