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Evaluation of the efficacy of a novel antibiotic–steroid paste versus conventionally used intracanal antibiotic pastes and irrigating solutions against a 3-week-old biofilm of *Enterococcus faecalis*

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Abstract

Introduction: Intracanal medicaments have been used during endodontic regenerative procedures to eradicate endodontic pathogens.
Aims: The aim of our study was (1) to evaluate the antimicrobial efficacy of a novel antibiotic–steroid paste over the regularly used calcium hydroxide ($Ca(OH)_2$), double antibiotic paste (DAP), and modified triple antibiotic paste (M-TAP) and (2) to check the antimicrobial efficacy of irrigating solutions, Chlorhexidine (CHX), and sodium hypochlorite (NaOCl) against a 3-week-old *Enterococcus faecalis* (*E. faecalis*) biofilm.
Materials and Methods: A total of 112 human extracted teeth were contaminated with *E. faecalis* for a period of 21 days. A novel antibiotic–steroid paste, $Ca(OH)_2$, DAP, M-TAP, and a placebo were placed inside the canal, sealed, and incubated in an aerobic environment at 37°C. For irrigating solutions, each prepared sample was immersed in 1 ml of sterile saline for 1 min, followed by irrigating and immersion with 1.5% NaOCl and 2% CHX for 5 min. An antimicrobial assessment was performed at the end of 2 days and 7 days, with seven teeth from each group, for each time interval. Dentin debris collected was transferred to the respective medium for culture. After 24 h, colonies were counted using classical bacterial counting technique as colony-forming units.
Results: Statistical analysis revealed that the novel antibiotic–steroid paste showed a statistically insignificant difference when compared to DAP, which had the highest antimicrobial properties.
Conclusion: This novel functional paste has antimicrobial efficacy comparable with that of DAP.

Keywords: Antibiotic–steroid paste; double antibiotic paste; *Enterococcus faecalis*; irrigating solutions; regenerative endodontics

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Introduction

Regenerative endodontic procedures (REPs) have emerged as an alternative treatment for apexification in young necrotic pulp. The ultimate goal of the procedures is to regenerate the components of the pulp–dentin complex. Commonly observed are positive clinical outcomes and continued root development. There have been a few unwanted adverse reactions such as coronal staining. Clinicians face the challenge of debriding the large infected root canals in which microbial count control is extremely crucial. The canals with compromised and fragile underdeveloped dentinal walls are a contraindication for mechanical instrumentation and hence chemical debridement is the main form of disinfection in REPs. Choices of irrigants and medicaments must be made based on their antimicrobial efficacy and with the least harm to stem cells and growth factors present in the microenvironment. Therefore, clinicians must make evidence-based decisions on the various chemical and mechanical interventions on stem cells, scaffolds, and growth factors when maintaining the basic principles of disinfection.[1]

Irrigating solutions help in the removal of dentin chips, microorganisms, and tissue remnants, from the root canal system through a flushing mechanism, which prevents packing of the hard and soft tissue in the apical third of the root canal and also prevents extrusion of infected material into the periapical area. Sodium hypochlorite (NaOCl) is the most widely used agent for chemical debridement in endodontic procedures, including REPs.[2] It has several desirable characteristics including excellent bactericidal efficacy,[3],[4],[5] tissue dissolution capacity,[6],[7],[8] and effective lubrication for endodontic instruments.

However, use of hypochlorite as the final rinse following ethylenediaminetetraacetic acid (EDTA) rapidly produces severe erosion of the canal wall dentin and should be avoided. To overcome that another disinfecting solution of chlorhexidine(CHX) is used because of its extended antimicrobial activity.[9],[10],[11] CHX does not possess some of the undesired characteristics of NaOCl (bad smell and strong irritation to periapical tissues). However, CHX has no tissue-dissolving capability, and therefore, it cannot replace NaOCl.

Intracanal medicaments,most commonly calcium hydroxide has been used time and again to eradicate endodontic pathogens. Hermann in 1920 introduced calcium hydroxide,[12] which has been widely being used as an intracanal medicament since then. It possesses tissue-dissolving ability, antimicrobial activity, hard tissue formation, and inhibition of tooth resorption.

An antibiotic mixture composed of ciprofloxacin, metronidazole, and minocycline, known as triple antibiotic paste (TAP) or Hoshino's paste, has been the most widely used medicament. As minocycline caused coronal staining, it was replaced with clindamycin to form modified TAP (M-TAP)[13] or was deleted from the preparation to form a double antibiotic paste (DAP).

Steroids are of great importance in endodontics, mainly because they cause reduction of inflammation and pain.[14] It has also been seen that fluocinolone acetonide in low concentrations (0.1–10 mmol/L) is not cytotoxic on human dental pulp cells (HDPCs) and might have the potential to stimulate healing of inflamed dental pulp.[15] A combination of antibiotic (demethylchlortetracycline) and corticosteroid (triamcinolone) paste,[16] called Ledermix, has been very effective for the treatment of progressive root resorption and also the accompanying periodontal ligament inflammation in teeth which followed dental trauma.[17],[18]

In our study, we incorporated one of the active ingredients contained in Ledermix paste, triamcinolone acetonide, which is a potent anti-inflammatory corticoid into TAP, to replace minocycline which causes coronal discoloration. We called this novel mixture as antibiotic–steroid paste (metronidazole, ciprofloxacin, and triamcinolone acetonide).

Our null hypothesis states that the novel antibiotic steroid paste has no significant antibacterial effect against a 3-week-old *Enterococcus faecalis* biofilm.

Materials and Methods

Sample preparation

Samples were prepared in the Preclinical Lab of Department of Conservative and Endodontics, SDM College of Dental Sciences, Dharwad. A total of 112 freshly extracted single-rooted anterior teeth were collected. All the teeth were decoronated using a rotary diamond disk and standardized to 16 mm in length. The internal diameter of the coronal part of the root canal was prepared and standardized using Gates Glidden (GG) drill number 3 and biomechanical preparation till F3 ProTaper file. The formed debris was removed by treating the teeth with 17% EDTA (EndoClean, Vishal Dentocare Pvt. Ltd.) for 5 min, followed by 5% NaOCl (Vensons, Mumbai, India) for 5 min.

The teeth were then immersed in distilled water for 5 min to remove any remnants of irrigants and sterilized in an autoclave at 121°C for two cycles. The teeth were then immersed in 1 ml of brain heart infusion (BHI) broth in individual microcentrifuge tubes.

Contamination of the teeth

The test organism chosen for this study was *E. faecalis* as it is the most frequently isolated bacterial species from the root canals of endodontically failed teeth, and the contamination was carried out in Department of Microbiology, SDM College of Medical Science, Dharwad. Twenty-four-hour colonies of pure culture of *E. faecalis* were grown on BHI agar and were suspended in 5 ml BHI broth for *E. faecalis* and incubated for 24 h at 37°C. Bacterial growth changes in turbidity were compared with 0.5 McFarland standard against a ruled paper, which is comparable with a bacterial suspension of 1.5×10^8 colony-forming unit (CFU)/ml. A volume of 50 μl of inocula was transferred to presterilized individual microcentrifuge tubes containing 1 ml of broths and teeth. All the procedures were carried out in a biosafety cabinet. The purity of the culture was checked by subculturing 5 μl of the broth from the incubated teeth, on agar plates. Contamination of the teeth was carried out for a period of 21 days.

Antimicrobial assessment

At the end of 21st day, the teeth were irrigated with 5 ml of sterile saline to remove the incubation broth.

In each group, the teeth were assigned to the following eight groups with $n = 14$, with alpha error of 5% and power of test 90%. The groups were further subdivided into seven samples each for the assessment of antimicrobial efficacy on the 2nd and 7th day, respectively.

Irrigating solution

- Group I: NaOCl (1.5%) (Vensons, Mumbai, India)
- Group II: Chlorhexidine gluconate (2%) (Deor RC Chlor, Azure Lab Pvt. Ltd.)
- Group III: Saline (Otsuka Pharmaceutical Pvt. Ltd.).

Each prepared sample was immersed in 1 mL of sterile saline for 1 min to remove loosely attached planktonic bacteria followed by irrigating and immersion with 1.5% NaOCl and 2% CHX for 5 min on the 2nd and 7th day, respectively.

Intracanal medicaments

- Group I: Calcium hydroxide (Prodent Ratnagiri)
- Group II: DAP (Metronidazole 400 mg [Flagyl 400, Qubari Pharma] + ciprofloxacin 500 mg [Cipro 500, Unichem Laboratories Ltd.])
- Group III: M-TAP (metronidazole 400 mg + ciprofloxacin 500 mg + clindamycin 1% [Acnesol, Systopic Laboratories Ltd.])
- Group IV: Antibiotic–steroid paste (metronidazole 400 mg + ciprofloxacin 500 mg + triamcinolone acetonide 0.1% [Turbocort, Indoco Remedies Ltd.])
- Group V: Methylcellulose placebo paste (Ara Gel, Sunways India Pvt. Ltd.).

The medicament was weighed using a digital weighing machine and taken in 1:1 ratio, mixed with saline, and was placed inside the canal using a Lentulo spiral, and all the teeth were sealed with temporary restorative material and incubated in an aerobic environment at 37°C.

An antimicrobial assessment was performed at the end of 2 days and 7 days, with seven teeth from each group, for each time interval. The teeth were washed with 5 ml of sterile saline to remove the medicament. The dentin debris which was collected was harvested at the depth of 200 μm using GG drill number 4 and collected in 1 ml of phosphate buffer saline solution. Diluted solution was transferred to their respective medium for culture. Plates were then incubated for 24 h at 37°C. After 24 h, colonies of bacteria were counted using classical bacterial counting technique and they were counted as number of CFU (Collins *et al*., 2004). The number of CFU between 2nd and 7th day was compared for all the groups.

Statistical analysis

The statistical package SPSS, Statistical Package for the Social Sciences, version 4, SPSS Inc., Chicago, IL, USA. was used for statistical analysis. The data were analyzed with dependent *t*-test to check the differences in microbial inhibition between the groups ($P < 0.05$). The Kruskal–Wallis analysis of variance (ANOVA) was used to check the differences in growth at different time intervals within the groups ($P < 0.05$).

Results

A total of 112 samples were included in the study.

Normality of microbial inhibition was carried out in five intracanal medicament groups and three irrigating

solutions group at the 2nd and 7th day by Kolmogorov–Smirnov test, and it showed that it followed a normal distribution. Therefore, parametric tests were applied.

The mean values of placebo group, Ca(OH) 2 group, M-TAP group, DAP group, and antibiotic–steroid paste group on the 2nd and 7th day were 422.86 and 396.43, 17.57 and 9.14, 11.86 and 8.57, 5.14 and 3.57, and 5.86 and 4.00, respectively [Figure 1].

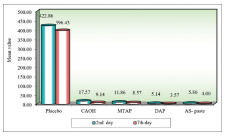

Figure 1: Comparison of 2nd and 7th day with microbial inhibition scores in five groups of intracanal medicaments

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The mean values of NaOCl group, CHX group, and saline group on the 2nd and 7th day were 106.43 and 90.00, 143.57 and 117.86, and 347.14 and 317.14, respectively [Figure 2].

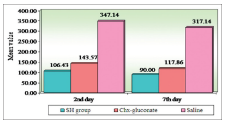

Figure 2: Comparison of 2nd and 7th day with microbial inhibition scores in three groups of irrigating solutions

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One-way ANOVA was used to compare the microbial inhibition in five groups of pastes and three irrigating groups.

A significant difference was observed on the 2nd and 7th day in between the groups and within the groups of intracanal medicaments and irrigating solutions.

Dependent *t*-test was used to compare the microbial inhibition in five groups of pastes and three irrigating groups. The percentage of change for $Ca(OH)_2$ group, M-TAP group, DAP group, and antibiotic–steroid paste group on the 2nd and 7th day was 6.25, 47.97, 27.71, 30.56, and 31.71, respectively, which was found to be statistically significant.

The percentage of change for the 2nd and 7th day in NaOCl group, CHX group, and saline group was 15.44, 17.91, and 8.64, respectively, which was found to be statistically significant.

Discussion

Regeneration of the pulpal tissue of an infected immature tooth with apical periodontitis had once been thought to be impossible. However, if a suitable environment could be achieved, that is, absence of intracanal infection and presence of a scaffold conducive to tissue ingrowth, regeneration of pulp might take place. The key factor for the success of this process is disinfection of the root canal system, because tissue growth will halt at the level where bacteria are found.[19],[20]

In our study, 1.5% NaOCl provided complete eradication of *E. faecalis* biofilm. This finding agrees with a recent study,[21] but partially disagrees with other studies[22],[23] that found significant antibiofilm effects of 1%–1.5% NaOCl without complete elimination of the biofilm. This disagreement might be justified by the shorter NaOCl exposure time(1-3mins) used in those studies[22],[23] in comparison with the 5-minute exposure time used in our study which is in accordance to the clinical recommendation of REPs.[2],[24]

The findings of our study indicated that the 5-min biofilm exposure to 2% CHX irrigants provided an antibiofilm effect against 3-week-old *E. faecalis*, but not as effective as the group of NaOCl. Recent *in vitro* studies suggested that 2% CHX had unfavorable effects on survival[25] and attachment[26] of stem cells from apical and dental pulp, respectively.

In the current study, antibiotic–steroid paste, calcium hydroxide, DAP, M-TAP, and methylcellulose placebo paste were compared to evaluate the antimicrobial efficacy of each against a 3-week-old biofilm of *E. faecalis*. The microbial inhibition was highest with DAP, followed by antibiotic–steroid paste, M-TAP, and least with calcium hydroxide.

It was hypothesized that DAP exerts significant residual antibiofilm effects regardless of the concentration used or application time, which might be the reason why it had the highest antimicrobial efficacy against *E. faecalis* when compared to the other groups.[27]

M-TAP showed a statistically significant difference in the antimicrobial efficacy when compared to the control group, but the antimicrobial efficacy was lesser than DAP and antibiotic–steroid paste.

It is stated that dentin pretreated with $Ca(OH)_2$ did not demonstrate any notable residual antibiofilm effect regardless of the treatment time, which might be the reason why it had least antimicrobial efficacy when compared

to the other groups in our study.[28]

Antibiotic–steroid paste which was used for the first time in our study showed a statistically significant difference in the antimicrobial efficacy when compared to the control group. The microbial inhibition was comparable with that of DAP. As this is a novel combination, there have been no previous studies done to potentiate our claim of antimicrobial efficacy. Another concern with the use of steroids is the cytotoxicity to the underlying stem cells.

A study was done to check the cytotoxicity and proliferation of fluocinolone acetonide on HDPCs and their results demonstrated that fluocinolone acetonide was not only nontoxic to HDPCS, but it had a stimulatory effect on cell proliferation, fibronectin, and type I collagen synthesis.[15]

In another study, it was seen that diclofenac and triamcinolone acetonide impaired tenocytic differentiation and promoted adipocytic differentiation of mesenchymal stem cells and might alter mesenchymal stem cell differentiation in a nonfavorable way regarding tendon regeneration.[29] There are very few studies regarding the cytotoxic effects of steroids on mesenchymal stem cells.

In the current study, the antimicrobial effect against of *E. faecalis* (commonly used as a control strain) was investigated. It is well known that some strains of *E. faecalis* have high biofilm formation capacity and can be resistant to various types of antibiotics.[30] Therefore, future studies should investigate the antimicrobial efficacy of antibiotic steroid paste against different clinically isolated strains of *E. faecalis*.

Conclusion

In our study, we found that 1.5% NaOCl and DAP had the highest antimicrobial properties in their respective groups and the novel antibiotic–steroid paste had antimicrobial efficacy similar to that of DAP.

This study provides an insight into the potential for creating better intracanal medicaments which can overcome the disadvantages of conventionally used medicaments. Furthermore, this study opens new opportunities for the use of this novel antibiotic–steroid paste and other combinations as well with improved properties for use in daily clinical practice.

The null hypothesis that the novel antibiotic–steroid paste used in our study has no significant antibacterial effects against the 3-week-old *E. faecalis* biofilm was rejected. These results are obtained within the experimental conditions; thus, many more *in vitro* and *in vivo* studies are to be conducted using this newer combination of antibiotic–steroid paste.

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I affirm that I have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements, or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript,nor have any such arrangements existed in the past 3 years. Any other potential conflict of interest is disclosed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Diogenes AR, Ruparel NB, Teixeira FB, Hargreaves KM. Translational science in disinfection for regenerative endodontics. J Endod 2014;40:S52-7.
2. Diogenes A, Henry MA, Teixeira FB, Kenneth M. Hargreaves. An update on clinical regenerative endodontics. Endod Top 2013;28:2-23.
3. Harrison JW, Wagner GW, Henry CA. Comparison of the antimicrobial effectiveness of regular and fresh scent Clorox. J Endod 1990;16:328-30.
4. Vianna ME, Horz HP, Gomes BP, Conrads G. *In vivo* evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. Int Endod J 2006;39:484-92.
5. Martinho FC, Gomes BP. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. J Endod 2008;34:268-72.

6. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60-4.

7. Harrison JW, Hand RE. The effect of dilution and organic matter on the anti-bacterial property of 5.25% sodium hypochlorite. J Endod 1981;7:128-32.

8. Yang SF, Rivera EM, Baumgardner KR, Walton RE, Stanford C. Anaerobic tissue-dissolving abilities of calcium hydroxide and sodium hypochlorite. J Endod 1995;21:613-6.

9. Russell AD. Activity of biocides against mycobacteria. Soc Appl Bacteriol Symp Ser 1996;25:87S-101S.

10. Shaker LA, Dancer BN, Russell AD, Furr JR. Emergence and development of chlorhexidine resistance during sporulation of Bacillus subtilis 168. FEMS Microbiollett 1988;51:73-6.

11. Russell AD, Day MJ. Antibacterial activity of chlorhexidine. J Hosp Infect 1993;25:229-38.

12. Hermann BW. Calcium Hydroxidals Mittelzurn, Behandeln und Fullen von Wurzelkanalen [thesis]. Germany: University of Würzburg; 1920.

13. Garcia-Godoy F, Murray PE. Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. Dent Traumatol 2012;28:33-41.

14. Ehrmann EH. The effect of triamcinolone with tetracycline on the dental pulp and apical periodontium. J Prosthet Dent 1965;15:144-52.

15. Muincharern W, Louwakul P, Pavasant P, Lertchirakarn V. Effect of fluocinolone acetonide on human dental pulp cells: Cytotoxicity, proliferation, and extracellular matrix formation. J Endod 2011;37:181-4.

16. Pierce A, Lindskog S. The effect of an antibiotic/corticosteroid paste on inflammatory root resorption *in vivo*. Oral Surg Oral Med Oral Pathol 1987;64:216-20.

17. Abbott PV. Medicaments: Aids to success in endodontics. Part 1. A review of the literature. Aust Dent J 1990;35:438-48.

18. Thong YL, Messer HH, Siar CH, Saw LH. Periodontal response to two intracanal medicaments in replanted monkey incisors. Dent Traumatol 2001;17:254-9.

19. Myers WC, Fountain SB. Dental pulp regeneration aided by blood and blood substitutes after experimentally induced periapical infection. Oral Surg Oral Med Oral Pathol 1974;37:441-50.

20. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 2004;30:196-200.

21. Ordinola-Zapata R, Bramante CM, Cavenago B, Graeff MS, Gomes de Moraes I, Marciano M, *et al*. Antimicrobial effect of endodontic solutions used as final irrigants on a dentine biofilm model. Int Endod J 2012;45:162-8.

22. Stojicic S, Shen Y, Haapasalo M. Effect of the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent on the susceptibility of biofilm bacteria to antibacterial agents. J Endod 2013;39:473-7.

23. Ma J, Tong Z, Ling J, Liu H, Wei X. The effects of sodium hypochlorite and chlorhexidine irrigants on the antibacterial activities of alkaline media against *Enterococcus faecalis*. Arch Oral Biol 2015;60:1075-81.

24. American Association of Endodontists. Considerations for Regenerative Procedures. Available from: http://www.aae.org/clinical-resources/regenerative-endodontics/considerations-for-regenerative-procedures.aspx. [Last accessed on 2015 Jul 13].

25. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, *et al*. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011;37:1109-15.

26. Ring KC, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. J Endod 2008;34:1474-9.

27. Jenks DB, Ehrlich Y, Spolnik K, Gregory RL, Yassen GH. Residual antibiofilm effects of various concentrations of double antibiotic paste used during regenerative endodontics after different application times. Arch Oral Biol 2016;70:88-93.

28. Tagelsir A, Yassen GH, Gomez GF, Gregory RL. Effect of Antimicrobials Used in Regenerative Endodontic Procedures on 3-week-old *Enterococcus faecalis* Biofilm. J Endod 2016;42:258-62.

29. Fredriksson M, Li Y, Stålman A, Haldosén LA, Felländer-Tsai L. Diclofenac and triamcinolone acetonide impair tenocytic differentiation and promote adipocytic differentiation of mesenchymal stem cells. J Orthop

Surg Res 2013;8:30.

30. Al-Ahmad A, Ameen H, Pelz K, Karygianni L, Wittmer A, Anderson AC, *et al*. Antibiotic resistance and capacity for biofilm formation of different bacteria isolated from endodontic infections associated with root-filled teeth. J Endod 2014;40:223-30.

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Figures

[Figure 1], [Figure 2]