In vitro comparative evaluation of antifungal efficacy of three endodontic sealers with and without incorporation of chitosan nanoparticles against Candida albicans

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Abstract

Aim: The aim of this study was to compare the antifungal efficacy of three endodontic sealers (AH Plus, Apexit Plus, and MTA Fillapex) with and without the incorporation of chitosan nanoparticles against Candida albicans.

Materials and Methods: The present study was carried out by the Kirby–Bauer method. C. albicans were cultured in Sabouraud Dextrose Agar plates. Filter papers (n = 10) were placed in the cultured Petri dishes and the sealers were mixed according to the manufacturer’s instructions and placed on the top of the filter papers. Group division of sealers is as follows: Group I – AH Plus, Group II – Apexit Plus, and Group III – MTA Fillapex. Group IIC, Group IIC, and Group IIIC were the addition of 2% chitosan nanoparticles with respective sealers. Plates were incubated for 18 h, and the zone of inhibition was measured with a measuring scale and values (in millimeter) were recorded. Statistical analysis was done by one-way analysis of variance followed by post hoc multiple pair-wise comparisons.

Results: All the tested groups showed statistically significant difference (P < 0.05) from each other. Two percent chitosan-incorporated groups showed superior zone of inhibition compared to sealers used alone. Group IIC (16.35 ± 0.71 mm) had the highest zone of inhibition followed by Group I (13.8 ± 0.86 mm). For the remaining groups, the zone of inhibition was in the following order: Group IIC > Group II > Group IIIC > Group III.

Conclusion: AH Plus sealer mixed with 2% chitosan showed significantly higher antifungal property. Mixing of 2% chitosan with endodontic sealer provides an added advantage so that endodontic re-infections can be minimized and will be helpful in retreatment cases.

Keywords: Antifungal activity, Candida albicans, chitosan, endodontic sealers

INTRODUCTION

To achieve successful endodontic treatment, it is necessary to prevent and treat the periradicular inflammation by eliminating the microorganisms from the root canal systems.¹ Endodontic treatment is fairly predictable in nature with reported success rates up to 86%–98%.² Root canal system is a closed space or chamber with low oxygen concentration and serves as an incubator promoting the growth of microorganisms. One of the foremost causes of endodontic failure is persistent microbiological infection where fungi and Candida albicans have the major role than other microorganisms.³,⁴

Endodontic sealers are used to seal spaces between core filling material and root canal walls to obtain a fluid impervious seal, and their antibacterial properties provide added benefit of eliminating bacteria persisting within the...
root canals after cleaning and shaping procedures. Chitosan, a versatile hydrophilic polysaccharide, has properties such as antimicrobial activity; biocompatibility; fungistatic; hemostatic potential; noncarcinogenicity; and promotion of cell adhesion, proliferation, and differentiation. Due to its biocompatible and nontoxic characteristics, chitosan has recently gained more interest for application in dentistry.

Hence, the purpose of this study was to evaluate and compare the effects of an epoxy resin-based sealer (AH Plus), a calcium hydroxide-based sealer (Apexit Plus®), and an MTA-based sealer (MTA Fillapex) with and without the incorporation of 2% wt/vol. chitosan nanoparticle on the facultative anaerobic microorganism, C. albicans.

MATERIALS AND METHODS

Resin-based sealer: AH Plus (Dentsply DeTrey, Konstanz, Germany), calcium hydroxide-based sealer: Apexit Plus (Ivoclar Vivadent, Schaan, Liechtenstein), and MTA-based sealer: MTA Fillapex (Angelus, Londrina, Brazil) were selected for the study. Strains of C. albicans (ATCC 10231) were collected and cultured with brain–heart infusion (BHI) broth (HiMedia Laboratories Pvt. Limited, Mumbai, Maharashtra, India). Sabouraud Dextrose Agar (SDA) was used as a selective medium for the growth of Candida. Nystatin discs (HiMedia Laboratories Pvt. Limited, Mumbai, Maharashtra, India) were used as control disc for the experiment. The present study was carried out by the Kirby–Bauer Method, also called disc diffusion antibiotic sensitivity test. In this method, wafers containing antibiotics are placed on an agar plate with bacteria and incubated. If an antibiotic stops bacterial growth or kills the bacteria, an area around the wafer where the bacteria have not grown enough is visible. This is called zone of inhibition and compared to a database of zone standards to determine the susceptibility or resistance of the bacterium to antibiotics. This information can be used to choose appropriate antibiotics to combat a particular infection.

The prepared SDA was poured into 12 Petri dishes of 4 mm depth with the help of sterile pipettes, stored in a refrigerator at 4°C, and used within 1 week of preparation. With a sterile loop, morphologically similar colonies of C. albicans were touched, and the growth was transferred to a sterile test tube containing 1.5 ml of BHI Broth and incubated (Model: EIE201, EIE Instruments Pvt. Ltd., Ahmedabad, Gujarat, India) at 37°C for 2–4 h to produce fungal suspension of moderate turbidity. The fungal suspension density was standardized by comparing the BHI broth at a density equivalent to the barium sulfate standard of 0.5 McFarland units (equivalent to 1.5 × 10⁸ colony-forming unit per milliliter). A sterile cotton swab was dipped inside the fungal suspension and streaked in three directions on the entire surface of the plate. After the inoculum dried, 60 sterile filter papers (Sigma–Aldrich, Germany) of 6-mm diameter (10 filter papers/group) were placed in 12 cultured plates (5 filter papers/plate). The sealer was mixed according to the manufacturer’s instructions, and 100 µl (0.1 ml) of each sealer was taken with the help of a micropipette and placed on the sterile filter paper disc.

Antifungal activities of three endodontic sealers with and without incorporation of chitosan nanoparticles were tested. 2% wt/vol chitosan (i-CHESS, Mumbai, Maharashtra, India) was used, and 1 drop of chitosan was mixed manually along with the sealer with the help of a plastic spatula in a circular motion. Groups were divided as follows: Group I – AH Plus Sealer, Group II – Apexit Plus Sealer, and Group III – MTA Fillapex Sealer. Groups IC, IIC, and IIIC were the addition of 2% wt/vol. chitosan nanoparticles with respective sealers. Nystatin discs (antifungal agent) were used as control for all the groups. Petri dishes containing sealer impregnated discs and microorganism C. albicans were incubated at 37°C for 18 h to assess the zone of inhibition. Vernier caliper was used to measure the zone of inhibition produced by different groups and the values were recorded to the nearest millimeter.

Statistical analysis

IBM SPSS statistics 24.0 SPSS South Asia (P) Ltd, Bengaluru, Karnataka, India, (www.spss.co.in) was used for the following statistical procedures: (i) comparison of the size of zone of inhibition of each group with control following one-sample t-test procedure and (ii) comparison of group means of the size of zone of inhibition using one-way analysis of variance followed by post hoc multiple comparison. The test of significance difference value was taken as \( P < 0.05 \).

RESULTS

The mean sizes of zones for each group were compared against the control (nystatin), which is a known antifungal agent. Table 1 represents comparison of the mean size of zones of inhibition among the six groups. The multiple comparisons of pair-wise group means of size of zones are presented in Table 2. All the groups showed a statistically significant difference \( (P = 0.000) \) from each other. The highest zone of inhibition was seen in Group IC (AH Plus + chitosan).

DISCUSSION

During endodontic therapy, residual microorganisms resisting chemomechanical procedures may cause failure of treatment. Presence of C. albicans in the root canals may be associated with therapy-resistant periapical pathosis. In the present study, C. albicans was chosen because they
The agar diffusion test or direct contact test measures the bacteriostatic and bactericidal effect of different endodontic sealers and root canal filling materials. It also measures the effect of direct and close contact between the test microorganism and the material to be tested on microbial viability, regardless of the diffusibility and solubility of the antimicrobial components. Therefore, this test was considered a standard to evaluate the antimicrobial activity of the tested samples.

Different root canal sealers and chitosan selected to assess the antifungal activity with or without the addition of chitosan nanoparticles induce a clinical situation inside the root canal system and also enable the assessment of antifungal activity inside the dentinal tubules against C. albicans. Nystatin showed a zone of inhibition of approximately 18 mm for all groups. Ramachandra et al. have demonstrated that AH Plus sealer showed greater zone of inhibition for C. albicans than Enterococcus faecalis and Escherichia coli, whereas some studies concluded that AH Plus sealer had no antifungal action.

In the present study, AH Plus sealer showed the highest antifungal activity among all the sealers tested. The antifungal activity of AH Plus sealer is because of bisphenol-A-diglycidylether component, which is also responsible for the antibacterial action of AH Plus. Leonardo et al. have reported that bisphenol-A-diglycidylether releases formaldehyde during polymerization.

In this study, the antifungal activity of sealer along with 2% wt/vol. chitosan was higher than that of sealers alone. This shows that chitosan has greater antifungal property and the sealer property is not altered by the addition of chitosan. The antifungal action of chitosan is due to its polycationic nature, which interacts with the negatively charged surface of bacteria, alters bacterial cell permeability, resulting in the leakage of the intracellular component of bacteria, causing cell death. The action of chitosan can also be explained by the mechanism of chitosan molecules penetrating into the nuclei of the microorganism, binding with the microbial DNA, leading to inhibition of mRNA, which subsequently stops the process of protein synthesis. In this mechanism, the bacterial cell wall is composed of multilayers of cross-linked murein, and the chitosan molecule is believed to be able to pass through the multilayered murein and reach the plasma membrane. Chitosan has excellent metal binding capacities, where the amine group (NH\(^{+}\)) in chitosan molecules is responsible for the uptake of metal cations by chelation. The antibacterial property of zinc oxide eugenol-based sealer was also improved with the incorporation of chitosan nanoparticle, and the combination of triple antibiotic paste and calcium hydroxide with chitosan produced superior result as compared to the intracanal medicaments combined with saline.

**CONCLUSION**

Under the limitations of this study, root canal sealers mixed with 2% wt/vol. chitosan nanoparticles showed superior antifungal efficacy than the sealer used alone. AH Plus sealer mixed with chitosan showed significantly higher antifungal property as compared to Apexit Plus and MTA Fillapex. Mixing of chitosan along with endodontic sealer provides an added advantage so that post-endodontic infections can be minimized and will be helpful in retreatment cases as well.

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**Conflicts of interest**

There are no conflicts of interest.

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**Table 1: Comparison of mean size (zone of inhibition) among groups with control**

| Group | Mean difference (mm) | SE | P |
|-------|----------------------|----|---|
| Group I | 6.5 | 0.32 | 0.000 |
| Group II | 10 | 0.32 | 0.000 |
| Group III | -2.55 | 0.32 | 0.000 |
| Group IIC | 4.18 | 0.32 | 0.000 |
| Group IIC | 8 | 0.32 | 0.000 |
| Group IIIC | 3.5 | 0.32 | 0.000 |
| Group IIC | -9.05 | 0.32 | 0.000 |
| Group IIC | -2.32 | 0.32 | 0.000 |
| Group IIC | 1.5 | 0.32 | 0.000 |
| Group IIC | -12.55 | 0.32 | 0.000 |
| Group IIC | -5.82 | 0.32 | 0.000 |
| Group IIC | -2 | 0.32 | 0.000 |
| Group IIC | 6.73 | 0.32 | 0.000 |
| Group IIC | 10.55 | 0.32 | 0.000 |
| Group IIC | 3.82 | 0.32 | 0.000 |

**Table 2: Multiple pair-wise comparisons of group mean size (zone of inhibition)**

| Group | Mean difference (mm) | SE | P |
|-------|----------------------|----|---|
| Group I | 6.5 | 0.32 | 0.000 |
| Group II | 10 | 0.32 | 0.000 |
| Group III | -2.55 | 0.32 | 0.000 |
| Group IIC | 4.18 | 0.32 | 0.000 |
| Group IIC | 8 | 0.32 | 0.000 |
| Group IIIC | 3.5 | 0.32 | 0.000 |
| Group IIC | -9.05 | 0.32 | 0.000 |
| Group IIC | -2.32 | 0.32 | 0.000 |
| Group IIC | 1.5 | 0.32 | 0.000 |
| Group IIC | -12.55 | 0.32 | 0.000 |
| Group IIC | -5.82 | 0.32 | 0.000 |
| Group IIC | -2 | 0.32 | 0.000 |
| Group IIC | 6.73 | 0.32 | 0.000 |
| Group IIC | 10.55 | 0.32 | 0.000 |
| Group IIC | 3.82 | 0.32 | 0.000 |

SE: Standard error.
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