Abstract: The aim of the present investigation was to evaluate and compare the antifungal activity of sodium hypochlorite (NaOCl), silver nanoparticles (Ag-NP), and zinc nanoparticles (ZnO-NP) against candidal biofilms. Twenty-two single-rooted premolars were decoronated to a root length of 12 mm. Shaping of the canals was done with ProTaper size F2 files. The specimens were inserted into vials containing 2 mL of the Sabouraud broth and then incubated for 14 days. From \( n = 22 \), one sample was subject to SEM evaluation, and from one more sample, dentinal shavings were taken on a Sabouraud dextrose agar plate to confirm the presence of candidal biofilms. The remaining 20 samples were divided (\( n = 5 \)) and irrigated as follows. Group 1: saline; Group 2: 5.25% NaOCl; Group 3: 0.02% Ag-NPs; Group 4: 0.02% Zn-NPs. The CFUs (colony forming units) were determined per group. SEM imaging of one sample per group was undertaken to correlate the microbiological results. Statistical analysis was conducted using Kruskal–Wallis, with \( p < 0.05 \) kept as the significant level. NaOCl reduced the colony counts to a maximum, which was of statistical significance, followed by Ag-NP and Zn-NP. Saline showed the least antimicrobial efficacy. NaOCl was the most effective irrigant against candidal biofilms. Ag-NPs and ZnO-NPs reduced the fungal load but failed to eradicate the biofilm completely.

Keywords: nanoparticles; endodontics; zinc nanoparticles; silver nanoparticle; Candida albicans

1. Introduction

The primary causative agents for the development of diseases of pulpal and peri-apical origin are microorganisms and their metabolic by-products. A significant contributor to endodontic treatment failure is the persistence and prevalence of microorganisms that exhibit resistance to conventional root canal therapy. Furthermore, endodontic infections are predominantly biofilm-mediated, emphasizing the importance of disease management through the elimination of endodontic biofilms [1]. Failures in endodontic treatment may stem from the challenges associated with eliminating the biofilm mode of growth in microorganisms. These microorganisms organize themselves into a three-dimensional, structured community, creating fluid channels for the transport of substrates and waste products, as well as for the signaling of molecules. The complexity of addressing this organized biofilm community contributes to the difficulties in achieving successful outcomes in endodontic therapy [2].

Due to the anatomic complexities in the root canal configuration, there are often uninstrumented portions in the prepared root canal space, which harbors microorganisms.
Hence, in conjunction with biomechanical preparation, chemical irrigants are used to disinfect the remote areas of the root canal [3]. However, at times, the endodontic biofilm is resistant to numerous irrigants, such as saline, hydrogen peroxide, MTAD, and citric acid [4,5]. Sodium hypochlorite, due to its good antimicrobial activity and tissue dissolution ability, is extensively used in endodontics. However, it is highly toxic to tissues; hence, a more biocompatible irrigant is always being researched [6].

The distinctive features of nanoparticles, including their exceptionally small size, expansive surface area, and heightened chemical action, have opened up new possibilities for the treatment and prevention of oral diseases. Nanoparticle-based irrigant delivery has been a promising method to eliminate microorganisms [7]. The polycationic/polyanionic nature of nanoparticulates demonstrates greater antibacterial activity with larger surface area and charge density, which would result in better communication with the bacterial cell. The antibacterial effectiveness of nanoparticles is significantly influenced by their size, with smaller particles demonstrating greater antibacterial activity in comparison to larger ones on a macroscopic level [5–7].

Antimicrobial properties were imparted to dental materials through the incorporation of silver and zinc nanoparticles. Favorable results regarding antimicrobial activity and antibiofilm effects were noted upon incorporating silver nanoparticles and zinc oxide nanoparticles into different dental materials [8]. During endodontic therapy, silver nanoparticles are employed for disinfecting root canals, serving dual roles as an intracanal dressing and an irrigant. Likewise, solutions containing ZnO-NP were applied to discourage the adherence of E. faecalis to dentinal walls and to disrupt the biofilm structure [9].

Candida albicans, the most frequently identified fungus in endodontic infections, was initially reported by Grossman to be present in root canals at a prevalence ranging from 1% to 17% [10]. Candida exhibited elevated antimicrobial resistance within biofilms compared to its planktonic counterparts. Biofilms represent an altered form of microbial growth that enables survival in challenging environments. Even with meticulous cleaning and irrigation in standard endodontic procedures, research indicates the ongoing presence of Candida in infections resistant to conventional methods [11,12]. The challenges in successfully treating root canal infections involving C. albicans can be linked to the diverse nature of biofilms, which contributes to their resistance to treatment and capacity for regrowth within the root canals [13].

However, with the unavailability of an irrigant with all desirable properties that is effective against candida biofilms, the search for an ideal irrigant still continues. There are no known previous studies that have tested the antifungal efficacy of silver and zinc nanoparticles against candida biofilms. Hence, considering the promising results of antimicrobial efficacy of nanoparticle-based irrigation, the present investigation evaluated and compared the antifungal activity of NaOCl and solutions of Ag-NPs and Zn-NPs against a candida biofilm in root canals.

2. Methodology

This in vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Manipal, in collaboration with the Department of Microbiology, Kasturba Medical College, Manipal. Ethical clearance was obtained from the Institutional Ethical Committee (IEC: 620/2019).

2.1. Specimen Preparation

A total of 22 single-rooted extracted mandibular premolar teeth were acquired, cleaned, and preserved in sterile saline until undergoing testing. The included teeth were intact, free from decay, and extracted either as a result of periodontal disease or for orthodontic reasons. The samples were decoronated below the cementoenamel junction (CEJ) using a diamond disk. Root length was standardized at 12 mm apicocoronally. A #10 K file was used till it was just seen at the apical end. Working length determination and the cleaning and shaping of the teeth were conducted using a ProTaper file, and the canals were enlarged up...
to F2 (Dentsply Maillefer, Tulsa, OK, USA). During biomechanical preparation (BMP), the canal was disinfected with 5.25% sodium hypochlorite, and 17% EDTA (PreverstDenpro) was used to eliminate the smear layer. The external surfaces and the apical foramen of all samples were coated with two layers of epoxy adhesive (Araldite, Ciba Geigy AS, Rio de Janeiro, Brazil) to make it impermeable. A solution of sodium thiosulphate was used to neutralize the effects of NaOCl. All teeth were then subjected to sterilization (Autoclave: 121 degrees centigrade for 15 min).

2.2. Inoculation of Specimen in the Sabouraud Dextrose Broth

The prepared samples were placed in separate vials, each containing 2 mL of Sabouraud’s broth, and incubated at 37 °C for a period of 14 days. To ensure the organism’s viability, the broth was renewed every 3 days. To confirm biofilm formation, two sections of a prepared specimen were analyzed via scanning electron microscopy (SEM).

2.3. Colony Counting

Following a 14-day incubation period, dentinal shavings were extracted from a single prepared sample and placed on a Sabouraud dextrose agar plate to verify the presence of *C. albicans*.

The samples were divided into 4 groups (n = 5):
- Group 1: Biofilm contaminated tooth irrigated with saline.
- Group 2: Biofilm contaminated tooth irrigated with 5.25% NaOCl.
- Group 3: Biofilm contaminated tooth irrigated with 0.02% Ag-NPs.
- Group 4: Biofilm contaminated tooth irrigated with 0.02% Zn-NPs.

The Ag and Zn NPs used in the study were colloidal Ag-NPs (Purity: 99%) (20–30 nm, spherical configuration; Nano research lab, Jharkhand, India) and colloidal Zn-NPs (Purity: 99%) (30–50 nm, spherical configuration; Nano research lab, Jharkhand, India). The vehicle used for both the colloidal solutions of Ag-NPs and Zn-NPs was deionized water.

All samples underwent irrigation with 10 mL of the respective irrigant for a duration of 3 min. The irrigation needle (26 gauge, Unolok Syringe with needle, Hindustan Syringes, New Delhi, India) was positioned 1 mm short of the working length. Dentin shavings were eliminated from the samples using a #25 Hedström file (H file, Dentsply Maillefer, USA) through 10 vertical strokes and gathered on a sterile Sabouraud dextrose agar plate. After that, the agar plates were incubated at 37 °C for 48 h, and subsequently, the determination of colony-forming units (CFUs) was determined per plate. Moreover, a singular sample from each group underwent SEM analysis to corroborate the microbiological findings.

2.4. Statistical Analysis

The data were analyzed utilizing SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) with a significance level set at *p* < 0.05. Descriptive statistics were employed to determine the mean and standard deviation of the respective groups. The Kruskal–Wallis test was applied to ascertain the statistical significance among the groups.

3. Results

NaOCl reduced the colony counts to 102 CFU’s/mL (colony forming units per milliliter) from an initial inoculation of 108 CFU’s/mL. In comparison with other groups, there was a statistically significant reduction in colony counts in the NaOCl group (2.1462 ± 0.0818) (*p* < 0.0001). Saline (0.9% weight/volume) showed the least-effective antimicrobial efficacy (5.2263 ± 0.0329) when compared to NaOCl (2.1462 ± 0.0818) silver nanoparticles (4.9401 ± 0.39743) and zinc nanoparticles (3.9129 ± 0.0300) (*p* < 0.0001) (Table 1).

There was a marked reduction in colony counts between Ag and Zn NPs. Between Zn- and Ag NPs, Zn-NPs showed a lower number of colony counts (3.9129 ± 0.0300) compared to Ag-NPs (4.9401 ± 0.39743) (Figures 1 and 2). There was a significant difference between the negative control (5.22 ± 0.03) followed by the Ag-NP group (4.94 ± 0.39) and the Zn-NP group (3.91 ± 0.03) (3.25 ± 1.16). The lowest number of colonies was reported in the
positive control group, where the irrigant was NaOCl (2.18 ± 1.12). Inter-group analysis via post hoc test revealed that a significant difference in the reduction of colony-forming units exists, except in Group 1, which is the negative control saline, and Group 3, which is Ag-NP (p = 0.148) (Figure 3).

Table 1. Values in colony-forming units—Kruskal–Wallis test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (±SD)</th>
<th>Group 1 Saline Negative Control</th>
<th>Group 2 NaOCl</th>
<th>Group 3 Silver Nano Particles</th>
<th>Group 4 Zinc Nano Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>MEAN</td>
<td>5.2263</td>
<td>2.1462</td>
<td>4.9401</td>
<td>3.9129</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.0329</td>
<td>0.0818</td>
<td>0.39743</td>
<td>0.0300</td>
</tr>
<tr>
<td></td>
<td>1st quartile</td>
<td>5.2148</td>
<td>2.0791</td>
<td>5.07918</td>
<td>3.9030</td>
</tr>
<tr>
<td></td>
<td>3rd quartile</td>
<td>5.2552</td>
<td>2.2041</td>
<td>5.13033</td>
<td>3.9294</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>0.0404</td>
<td>0.1249</td>
<td>0.05115</td>
<td>0.0263</td>
</tr>
<tr>
<td></td>
<td>p-Value *</td>
<td>0.00047</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Indicates statistically significant.

Figure 1. Sabouraud dextrose agar plate showing Candidal colonies post-irrigation.

Figure 2. Scanning electron microscopy images showing Candida growth confirming microbiological results.

Figure 3. Bar graph depicting the amount of reduction in bacterial colonies.
4. Discussion

The challenges in achieving successful endodontic treatment associated with *C. albicans* may stem from the diverse attributes of biofilms, leading to increased resistance to treatment and the capacity to regenerate within root canals. Biofilm phenotypes developed on surfaces with less intricate topography have the capacity to exhibit variable resilience to standard endodontic irrigation protocols [13]. *C. albicans*, a pleomorphic dentinophilic organism, was reported to be the most prevalent pathogenic fungus isolated from the oral cavity but the least investigated [14].

Within the scope of this investigation, sodium hypochlorite demonstrated a notably higher degree of efficacy in eliminating Candida growth, and this difference reached statistical significance when compared to other experimental groups. This finding is supported by previous studies [15,16]. The hindrance of cell metabolism arises from the reaction between Cl and the NH2 group resulting in the formation of chloramines. This process interferes with fungal cell functions. Chlorine, known for its potent oxidizing properties, exhibits antifungal effects by inhibiting enzymes released by the fungus. This inhibition directs the enzymes towards an irreversible oxidation of their essential sulphhydryl groups. Moreover, upon contact with organic material, sodium hypochlorite (NaOCl) initiates simultaneous and synergistic saponification and neutralization reactions, resulting in the liquefaction of organic tissue. The antibacterial and dissolving effects of NaOCl, attributed to its oxidizing properties and reactivity with organic matter, make it a suitable root canal irrigant with anti-biofilm properties [17]. In this study, NaOCl was chosen as the control due to its established efficacy as the most effective irrigant [18–20].

Silver nanoparticles (Ag-NPs) exhibit superior activity compared to NaOCl as they can penetrate dentinal tubules without being inactivated by organic tissues [19,20]. Our study aimed to test the efficacy of the nanoparticles against candida biofilms. Our study partially aligns with the findings of previous research where bacterial counts were only reduced to levels below detection. However, the strains used in this study are those of fungi, unlike the bacterial strains and mixed flora used previously.

Silver nanoparticles (Ag-NPs) interfere with microbial cell target sites such as the plasma membrane, enzymes, and plasmids, restricting the fungus’s capacity to develop resistance. While our study observed a reduction in fungal load with silver nanoparticles, complete eradication of growth was not achieved [8]. The influence of ZnO-NP in our study was remarkably noteworthy. Similar to the effect observed with Ag-NP, the antifungal influence of ZnO-NP can be ascribed to the disruption and disarray of the Candida cell wall induced by the smaller particles of ZnO-NP employed in this investigation. The interaction between ZnO-NP and the Candida cell may have altered cell wall permeability, leading to the release of proteins and other constituents, ultimately resulting in cell death [9,21,22]. In vitro, ZnO-NP exhibited inhibitory effects on biofilms of Candida isolates, such as those found on urinary catheters [23–25].

The incomplete elimination of the biofilm following Ag-Np and ZnO-Np irrigation may be due to the robust resistance of the biofilm matrix. In the organized state of a biofilm, fungi exhibit distinct phenotypic and physiological characteristics compared to planktonic forms [19,20], demanding higher concentrations of fungicides for efficacy [22]. Additionally, the extracellular matrix generated by fungal cells as biofilms mature may serve as a chemical barrier [26], hindering the penetration of nanoparticles through Candida cells [27].

The concentrations of the irrigants (Ag-NP and ZnO-NP) were established according to prior investigations. Earlier studies indicated that the application of 0.02% silver nanoparticle gel as a medicament notably disrupted the structural integrity of biofilms, leading to the lowest count of viable *E. faecalis* cells post-treatment [28]. According to previous studies, 5.25% sodium hypochlorite was the most potent among the three concentrations (0.5%, 2.5%, 5.25%) [29,30].

The present findings emphasized that treating the root canal surface with sodium hypochlorite and nanoparticles like ZnO-NP and Ag-NP markedly impeded Candida
adherence to the root canal. This inhibition, in turn, would function to hinder its recolonization and the development of biofilms. It was hypothesized that the decrease in load of the candida colonies (CFU count) would imply that the biofilm structure was disrupted and, in turn, a decrease in its adherence to the dentinal wall. This was evident following the use of both the control group and the experimental irrigants. Further investigations are warranted to determine the persistence of the antifungal effect induced by nanoparticulates on root canal dentin [31,32]. In summary, this study emphasizes the potential advantages of NaOCl, Ag-NP, and ZnO-NP in preventing fungal recolonization in root canals and improving the antifungal properties of root canal sealers. Nevertheless, additional investigations are imperative before advocating the use of antifungal nanoparticles within root canals in vivo to prevent biofilm formation.

5. Conclusions

Among the irrigants tested, NaOCl demonstrated the highest efficacy in eradicating C. albicans, while Ag-NP was found to be the least efficacious in eliminating C. albicans. The potential of NaOCl, Ag-NP, and ZnO-NP to decrease fungal load within canals and prevent their recolonization was a novel finding of the present investigation.

Author Contributions: Conceptualization, V.R. and S.K.; methodology, V.R., S.K. and N.S.; software, N.S.; validation, N.S. and S.K.; formal analysis, N.S., K.S. and PS.; investigation, V.R.; resources, S.K.; data curation, N.S. and S.K.; writing—original draft preparation, N.S., K.S. and P.S.; writing—review and editing, N.S. and S.K.; supervision, S.K. and P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Manipal College of Dental Sciences, Manipal.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data used are made available in the present work.

Conflicts of Interest: The authors declare no conflicts of interest.

References


Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.