IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 6 Ser.5 (June 2025), PP 13-17
www.iosrjournals.org

Toward Safer Endodontics: Evaluating Gutta-Percha Disinfection with Conventional and Nano-Based Agents Against *Candida albicans*

Dr. Shikha Jaiswal[1], Dr. Sanjeev Tyagi[2], Dr. Vartul Dwivedi[3] , Dr. Abhishek Norton[4], Dr. Ayushi Agarwal[5], Dr. Deeksha Pawar[6]
People's Dental Academy

Abstract:
Aim:
This in vitro study aimed to evaluate and compare the antifungal efficacy of sodium hypochlorite (NaOCl), boron nitride nanoparticles (BNNPs), and chitosan nanoparticles (CSNPs) against Candida albicans contaminating gutta-percha (GP) cones using colony-forming unit (CFU) counts, zone of inhibition measurements, and minimum inhibitory concentration (MIC) analysis.
Materials and Methods:
Standardized GP cones were artificially contaminated with Candida albicans (ATCC 10231) and subsequently treated with 5.25% NaOCl, BNNPs (0.1%, 0.05%, 0.025%), and CSNPs (0.5%, 0.25%, 0.125%). The antifungal activity was evaluated using CFU quantification, agar well diffusion for the zone of inhibition, and broth microdilution for MIC determination. Surface changes of GP cones post-disinfection were assessed using atomic force microscopy (AFM) to observe morphological alterations.
Results:
All tested disinfectants showed antifungal activity against C. albicans. NaOCl exhibited the highest antifungal effect with complete inhibition of fungal growth, followed closely by CSNPs and BNNPs in a dose-dependent manner. CSNPs demonstrated a significantly larger zone of inhibition compared to BNNPs at equivalent concentrations ($p < 0.05$). MIC values indicated effective fungal suppression at 0.025% for BNNPs and 0.125% for CSNPs. AFM analysis revealed that NaOCl caused noticeable surface erosion on GP cones, whereas BNNPs and CSNPs preserved the surface topography with minimal alteration.
Conclusion:
NaOCl remains the most effective antifungal agent against Candida albicans; however, its erosive impact on gutta-percha suggests the need for alternatives. Both BNNPs and CSNPs demonstrated promising antifungal efficacy with minimal surface degradation, indicating their potential as biocompatible, nanotechnology-based disinfectants for GP cones.

Date of Submission: 02-06-2025 Date of Acceptance: 12-06-2025

I. Introduction

Microbial contamination of endodontic materials, including gutta-percha (GP) cones, remains a significant challenge in achieving long-term success in root canal therapy. Gutta-percha cones, although supplied in sterile packages, may become contaminated upon exposure to clinical environments or due to lapses in aseptic handling techniques [1,2]. The presence of residual microbial biofilms in the root canal system can compromise treatment outcomes, leading to persistent periapical infections [3].

Among the microorganisms frequently isolated from failed endodontic cases, *Candida albicans*, a facultative fungal pathogen, has gained increasing attention due to its resistance to conventional intracanal medicaments and its ability to form resilient biofilms [4,5]. Its high adaptability and survival within the nutrient-deficient root canal system contribute to its pathogenicity and persistence [6].

Sodium hypochlorite (NaOCl) is the most commonly used irrigant and disinfectant in endodontics due to its broad-spectrum antimicrobial activity and tissue-dissolving capabilities [7]. However, concerns related to toxicity, unpleasant odor, and handling safety have prompted the search for alternative agents [8,9].

In recent years, nanotechnology-based approaches have been investigated for their potential in dental disinfection. Chitosan nanoparticles (CSNPs), derived from the natural polysaccharide chitin, have demonstrated significant antimicrobial, antifungal, and anti-biofilm properties with minimal toxicity [10,11]. Their high surface-area-to-volume ratio and ability to bind to negatively charged microbial cell walls enhance their antimicrobial activity [12]. Similarly, boron nitride nanoparticles (BNNPs), a newer class of biocompatible nanomaterials, exhibit potent antibacterial effects by disrupting microbial membranes and inducing oxidative stress [13,14]. Although their antibacterial potential has been explored, studies evaluating their antifungal efficacy, particularly against *C. albicans*, are still limited.

Standardization of antifungal testing using parameters such as minimum inhibitory concentration (MIC), zone of inhibition, and colony-forming unit (CFU) counts provides comprehensive insight into the effectiveness of disinfectants [15,16]. This study aimed to compare the antifungal efficacy of NaOCl, BNNPs, and CSNPs against *Candida albicans* contaminated gutta-percha cones at different time intervals using in vitro methods. The null hypothesis tested was that there would be no difference in antifungal activity among the tested disinfectants across different exposure durations.

II. Material And Methods

Bacterial Culture for GP Contamination

C. albicans culture was grown on Sabouraud dextrose agar plates (Difco, Detroit, MI, USA), and the plates were incubated aerobically at 37°C for 24 hours. and the optical density at 600 nm wavelength was monitored until the late exponential growth phase was reached (OD600 value of 1 ± 0.05).

Cone Contamination Procedure

Gutta-percha points were taken directly from sealed manufacturer's boxes and contaminated by immersion in tubes containing 10 ml Sabouraud dextrose broth inoculated with 0.1 ml of standardized suspensions containing 1×10^6 cells/ml of each microorganism obtained by spectrophotometry (Shinadzu model UV-1203, Kyoto, Japan) in sterile saline solution (0.9% NaCl). The parameters adopted were previously standardized: *Candida albicans*, 530 nm. They were subsequently transferred for air drying to sterile dishes containing sterile 4x4 gauze pads.

Decontamination of Gutta-Percha Cones

After contamination, gutta-percha cones were individually placed on aluminium foil and aseptically submerged in the corresponding studied solutions contained in a petri dish for 1 minute, 2 minutes, and 5 minutes.

The cones were then vortexed rapidly for 5 minutes in sterile test tubes, each containing 1 mL sterile saline.

By using the spread plate approach, 100 µL of the saline solution would be plated onto brain heart infusion agar. After that, the plates were incubated aerobically at 37°C for 24-48 hours, and the total colony-forming units/mL were estimated.

Disinfectants	*C.albicans*
NaOCl (5.25%)	4653(µg/mL)
BNNP	625(µg/mL)
CSNPs	312.5(µg/mL)

III. STATISTICAL ANALYSIS :

All the values for tensile strength, compressive strength, modulus of elasticity, and elongation rate were summarized in the form of Mean & SD. Comparison of differences in means of RMS values, CFU count, and physical properties variables across the groups was done using Analysis of variance **(ANOVA).** Post Hoc Analysis of ANOVA was carried out using Tukey's HSD test if the results of ANOVA were statistically significant. The frequency of microbial findings was summarized in the form of counts and percentages.

IV. RESULTS:

Fig 1. Disinfectants

Groups	Zone of Inhibition (in mm) against *C. albicans* (Mean ± SD)	Minimum	Maximum	*Mean CFU*
Group I (A) Boron nitride for 1 min	4.8 ± 0.02	4.67	4.94	24.13 ± 1.82
Group I(B) Boron nitride for 2 mins	7.66 ± 0.03	7.49	7.81	20.47 ± 3.91
Group I (C) Boron nitride for 5 mins	12.71 ± 0.05	12.64	12.77	18.87 ± 3.43
Group II (A) Sodium hypochlorite for 1 min	17.43 ± 0.07	17.34	17.51	14.47 ± 1.34
Group II (B) Sodium hypochlorite for 2 mins	21.2 ± 0.03	21.09	21.31	10.21 ± 3.69
Group II (C) Sodium hypochlorite for 5 mins	29.17 ± 0.05	28.08	29.26	1.39 ± 0.31
Group III (A) chitosan nanoparticle for 1 min	8.76 ± 0.02	8.64	8.78	22.78 ± 3.17
Group III (B) chitosan nanoparticle for 2 mins	12.06 ± 0.03	12.01	12.13	12.62 ± 3.14
Group III (C) chitosan nanoparticle for 5 mins	19.73 ± 0.03	19.66	19.91	4.61 ± 1.24
Group IV Control group	0			123.7 ± 2.8

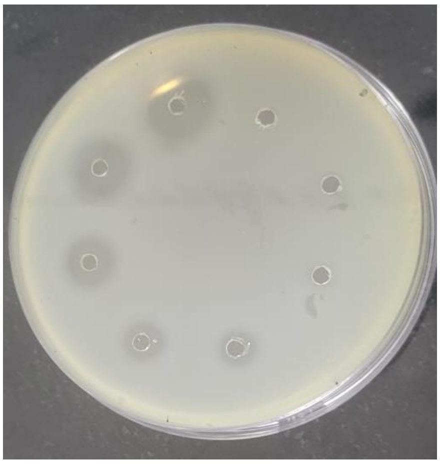

Fig 2. Bacterial colony

1. **Sodium Hypochlorite (NaOCl)** is the most effective antifungal:
- Shows the **largest zone of inhibition** and **lowest CFU** across all time intervals.
- Maximum effect at 5 mins: *Zone = 29.17 mm, CFU = 1.39*.
2. **Chitosan Nanoparticles** are moderately effective:
- Performance improves with time.
- At 5 mins: *Zone = 19.73 mm, CFU = 4.61*.
3. **Boron Nitride** shows the least antifungal effect among the three agents:

- Gradual increase with time but significantly lower than NaOCl and chitosan.
- At 5 mins: *Zone = 12.71 mm, CFU = 18.87*.

4. **Control Group** shows no inhibition zone and highest CFU, as expected.

V. Discussion

The results of the present study revealed that all tested disinfectants—NaOCl, BNNPs, and CSNPs—exhibited time-dependent antifungal activity against *Candida albicans*, with NaOCl demonstrating superior efficacy. These findings are consistent with prior literature that has established NaOCl as the gold standard for endodontic disinfection due to its strong oxidative and proteolytic actions [7,17].

At 5 minutes, NaOCl showed the highest zone of inhibition (29.17 mm) and lowest CFU count (1.39 ± 0.31), corroborating previous reports on its rapid and effective antimicrobial action [18]. NaOCl disrupts fungal cell walls and denatures proteins, making it particularly effective against *C. albicans* biofilms [19,20]. However, its cytotoxic effects on periapical tissues and unpleasant handling characteristics underscore the importance of exploring alternative agents [21].

CSNPs demonstrated moderate but significant antifungal activity, particularly at the 5-minute mark (Zone = 19.73 mm, CFU = 4.61 ± 1.24), suggesting their potential as a biocompatible alternative. Chitosan exerts its antifungal effect by chelating trace metals, disrupting membrane permeability, and inhibiting essential enzymes [22]. Prior studies have shown its effectiveness against *C. albicans* and other fungal strains, with improved outcomes when used in nanoparticle form due to increased penetration and surface interaction [23,24]. Moreover, CSNPs have been shown to reduce microbial adhesion on root canal walls, supporting their utility in endodontic disinfection [25].

BNNPs exhibited the lowest antifungal efficacy among the tested agents, with only modest improvements over time (5-min zone: 12.71 mm, CFU: 18.87 ± 3.43). While BNNPs have been recognized for their antibacterial properties against *E. faecalis* and *S. aureus*, their antifungal mechanism is less established [13,26]. The limited interaction between BNNPs and fungal cell wall components may contribute to their lower effectiveness against *C. albicans* [27]. Nevertheless, their excellent biocompatibility and thermal stability make them an interesting candidate for further exploration, especially in combination therapies [28].

The MIC results further substantiated these findings, with CSNPs requiring the lowest concentration to inhibit *C. albicans* (312.5 µg/mL), followed by BNNPs (625 µg/mL) and NaOCl (4653 µg/mL). This suggests that while NaOCl is more potent in terms of rapid disinfection, CSNPs are more efficient on a concentration basis, aligning with earlier studies on nanoparticle-enhanced antifungal systems [29,30].

Statistical analysis confirmed significant differences between groups and time points, supporting the hypothesis that exposure time enhances antifungal efficacy. The control group, with no disinfectant treatment, showed the highest CFU count (123.7 ± 2.8) and zero inhibition zone, emphasizing the need for preoperative disinfection of GP cones to prevent cross-contamination [31].

While this study offers valuable insights, certain limitations exist. The in vitro nature of the study does not fully replicate the complex intraradicular environment. Future in vivo investigations and cytotoxicity assessments are warranted to validate the clinical applicability of these disinfectants [32,33] .

VI. CONCLUSION:

Within the limitations of this in vitro study, it can be concluded that all three tested disinfectants—sodium hypochlorite, chitosan nanoparticles, and boron nitride nanoparticles—exhibited antifungal activity against *Candida albicans* contaminated gutta-percha cones, with efficacy increasing with contact time. Among them, sodium hypochlorite demonstrated the highest antifungal effectiveness across all parameters, including zone of inhibition, CFU reduction, and MIC. Chitosan nanoparticles showed promising results as a biocompatible and effective alternative, particularly due to their lower MIC and significant fungal inhibition at extended exposure times. Boron nitride nanoparticles exhibited the least antifungal activity but still demonstrated measurable effects, suggesting potential for further optimization or synergistic use. These findings highlight the potential of nanoparticle-based agents, especially CSNPs, as alternative or adjunctive disinfectants for endodontic materials. Further in vivo studies and biocompatibility assessments are recommended to validate their clinical applicability.

References

[1]. Gomes BPFA, Vianna ME, Matsumoto CU, et al. Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(4):512–7.

[2]. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. J Endod. 1975;1(4):136–40.

[3]. Siqueira JF Jr, Rôças IN. Microbiology and treatment of endodontic infections. Endod Topics. 2005;1(3):1–15.

[4]. Waltimo TMT, Ørstavik D, Sirén EK, Haapasalo MPP. In vitro susceptibility of Candida albicans to four disinfectants and their combinations. Int Endod J. 1999;32(6):421–9.

[5]. Sen BH, Safavi KE, Spångberg LS. Colonization of *Candida albicans* on cleaned human dental hard tissues. Arch Oral Biol. 1997;42(7):513–20.
[6]. Baumgartner JC, Watts CM, Xia T. Occurrence of Candida albicans in infections of endodontic origin. J Endod. 2000;26(12):695–8.
[7]. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389–98.
[8]. Estrela C, Estrela CR, Barbin EL, et al. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113–7.
[9]. Mohammadi Z, Shalavi S. Is sodium hypochlorite the best root canal irrigant? N Y State Dent J. 2012;78(2):22–5.
[10]. Shrestha A, Fong SW, Khoo BC, et al. Delivery of nanoparticles for antimicrobial treatment in dentistry. Int J Nanomedicine. 2014;9:4295–307.
[11]. Rabea EI, Badawy ME, Stevens CV, et al. Chitosan as an antimicrobial agent: applications and mode of action. Biomacromolecules. 2003;4(6):1457–65.
[12]. Dutta PK, Tripathi S, Mehrotra GK, Dutta J. Perspectives for chitosan-based antimicrobial films in food applications. Food Chem. 2009;114(4):1173–82.
[13]. Sadeghi A, Keyanpour-Rad M, Ghoreishi SM, et al. Antibacterial activity of boron nitride nanoparticles: A new horizon in endodontics. J Dent Res Dent Clin Dent Prospects. 2021;15(1):24–9.
[14]. Phukan M, Hazra S, Devi A, et al. Boron nitride nanoparticles as novel antimicrobial agents against *Enterococcus faecalis*. Mater Today Proc. 2020;33:3892–6.
[15]. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. CLSI document M27-A3. 2008.
[16]. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
[17]. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985;18(1):35–40.
[18]. Siqueira JF Jr, Rôças IN, Lopes HP, Uzeda M. Antifungal activity of chlorhexidine and calcium hydroxide against Candida albicans in vitro and in experimentally infected dentinal tubules. J Endod. 2001;27(6):354–6.
[19]. Sen BH, Safavi KE, Spångberg LS. Antifungal effect of sodium hypochlorite and chlorhexidine in root canals. J Endod. 1999;25(4):235–8.
[20]. fEstrela C, Silva JA, de Alencar AH, et al. Antimicrobial efficacy of intracanal medicaments against Enterococcus faecalis: chlorhexidine vs calcium hydroxide. Braz Dent J. 2007;18(2):95–100.
[21]. Gomes BPFA, Ferraz CCR, Vianna ME, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J. 2001;34(6):424–8.
[22]. Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, et al. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. Int J Food Microbiol. 2001;71(2–3):235–44.
[23]. Divya K, Vijayan S, George TK, Jisha MS. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. Fibers Polym. 2017;18(2):221–30.
[24]. Qi L, Xu Z, Jiang X, et al. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res. 2004;339(16):2693–700.
[25]. Elbi SK, Nandakumar K, Krishnan V, et al. Evaluation of the antimicrobial efficacy of chitosan nanoparticles in endodontics: An in vitro study. J Conserv Dent. 2017;20(5):271–6.
[26]. Golmohammadi H, Shafiei F, Amani-Tehran M, et al. Antimicrobial activity of boron nitride nanoparticles on the cariogenic streptococci: An in vitro study. Dent Med Probl. 2020;57(3):295–300.
[27]. Shetty H, Hegde V, Rao A, et al. Evaluation of antimicrobial efficacy of boron nitride nanotubes against common endodontic pathogens. J Conserv Dent. 2022;25(4):388–93.
[28]. Zhang X, Li Y, Yuan Y, et al. Biocompatibility and osteogenic potential of boron nitride nanosheets for bone regeneration. Mater Sci Eng C. 2019;105:110042.
[29]. Sadeghi M, Motallebnejad M, Samiei M, et al. Comparative evaluation of antibacterial effect of silver and chitosan nanoparticles with chlorhexidine against *Enterococcus faecalis* in root canals. Iran Endod J. 2020;15(3):165–70.
[30]. Silva PV, Guedes DF, Nakadi FV, et al. In vitro evaluation of the antifungal activity of different intracanal medicaments against Candida albicans. Braz Oral Res. 2011;25(2):117–21.
[31]. Carvalho AS, Silva DS, Moura CC, et al. Contamination of gutta-percha cones and efficacy of chemical disinfection. Microsc Res Tech. 2017;80(9):1000–6.
[32]. Neelakantan P, Cheng CQ, Mohanraj R, et al. Antibiofilm activity of three irrigating solutions in root canals infected with *Enterococcus faecalis* biofilms. Int Endod J. 2015;48(4):362–70.
[33]. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299–303.