

Original Article

Impact of Different Chemical Agents on Rapid Chairside Disinfection and Surface Characteristics of Gutta-Percha Cones: An *In Vitro* Analysis

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The primary aim of this research was to evaluate and contrast the efficacy of five disinfecting agents: 3% sodium hypochlorite, 2% chlorhexidine, grape seed extract (*Vitisvinifera*), aloe vera juice (*Aloe barbadensis*) and normal saline - in the disinfection of gutta-percha (GP) cones contaminated with *E. faecalis*. A total of fifty GP cones (N = 50) were selected and deliberately contaminated through immersion in a culture of *E. faecalis*, which was inoculated in brain-heart infusion (BHI) broth at a regulated temperature of 37°C for a duration of 72 hours. After drying, the cones were categorized into five treatment groups, each comprising 10 samples. Each group was further subdivided into two time-based subgroups, representing disinfection intervals of 1 minute and 10 minutes. The cones were treated with their respective chemical disinfectants, air-dried and subsequently incubated in BHI broth at 37°C for an additional 72 hours. The turbidity of the broth and bacterial growth on chocolate agar media were monitored as indicators of bacterial presence. Scanning electron microscopy (SEM) was employed to examine any alterations in the surface texture of the GP cones post-treatment. The effectiveness of each disinfectant in inhibiting bacterial proliferation was evaluated employing the Pearson chi-square test to determine statistical significance. The sodium hypochlorite-treated group demonstrated a notably diminished level of *E. faecalis* growth on the GP cones at both the 1-minute and 10-minute intervals, with findings revealing statistical significance ($p < 0.05$). Grape seed extract yielded a moderate decrease in bacterial growth; however, aloe vera treatment did not produce a statistically significant effect on *E. faecalis* growth on the GP cones. Scanning electron microscopy (SEM) analysis of the GP cone surfaces illustrated varying degrees of pitting across the different groups. The 3% sodium hypochlorite group exhibited significant pitting, whereas the control and aloe vera groups presented minimal pitting. The 2% chlorhexidine (CHX) group displayed moderate pitting and mild pitting was noted in the grape seed extract group. Among the agents tested, sodium hypochlorite emerged as the most efficacious disinfectant for swift and comprehensive disinfection of GP.

Introduction

The primary objective of endodontic therapy is the thorough disinfection of the root canal system to prevent recurrent microbial infections. Achieving this disinfection is essential for eradicating resident microorganisms and minimizing external contamination during treatment. Adherence to strict aseptic protocols is fundamental in reducing microbial

load and ensuring successful and lasting outcomes in root canal therapy [1].

The intricate nature of root canal systems, coupled with the resilience of pathogens like *Enterococcus faecalis*, underscores the necessity of meticulous asepsis. Frequently isolated in failed root canal treatments, *E. faecalis* is known for its biofilm-forming capabilities and its ability to withstand harsh environmental conditions. *Enterococcus faecalis*

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is the most common bacterium associated with post-treatment infection of the root canal system, also, it is also known that this strain can survive in dentinal tubules for long periods [2]. Introducing contaminated materials, such as gutta-percha (GP) cones, into the root canal increases the risk of treatment failure. Therefore, endodontic materials must not only fulfil their intended purpose but also remain sterile to prevent the introduction of new pathogens during procedures [2,3].

GP cones, recognized as the gold standard for root canal obturation, are widely utilized due to their favorable physical and chemical attributes. These include biocompatibility, radiopacity, thermoplasticity, and dimensional stability, which collectively facilitate effective canal sealing and adaptability to complex anatomical structures. Despite being manufactured under sterile conditions, GP cones are prone to contamination during handling and clinical use. For instance, opening a package in the clinical environment exposes the cones to potential contaminants from aerosols, instruments, and surfaces. Consequently, chairside disinfection is indispensable to eliminate these contaminants and support successful treatment outcomes [4,5].

Selecting an appropriate disinfection protocol for GP cones involves addressing two primary concerns: the elimination of pathogens and the preservation of the cones' structural integrity. Due to their thermoplastic nature, GP cones are unsuitable for heat sterilisation, as they may cause deformation or damage. Consequently, chemical and natural disinfectants are often employed to decontaminate GP cones while maintaining their physical properties. However, the choice of disinfectant is critical, as certain agents may induce surface changes, such as pitting or roughness, which could compromise the cones' sealing efficacy and adherence within the root canal [6].

Chemical disinfectants, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), alcohol, glutaraldehyde, hydrogen peroxide, and iodine compounds, are commonly used for sterilizing GP cones. NaOCl, in particular, is valued for its broad-spectrum antimicrobial activity and rapid bactericidal properties. Its mechanism involves oxidizing bacterial cell walls, denaturing proteins, and inhibiting microbial proliferation. Studies have consistently shown its effectiveness against pathogens like *E. faecalis*. However, higher concentrations and prolonged exposure to NaOCl may cause surface degradation of GP cones, including increased roughness and pitting, potentially affecting their performance [7,8].

CHX is another widely used disinfectant in endodontics. Known for its broad-spectrum antimicrobial activity and sustained efficacy, CHX acts by disrupting bacterial cell membranes, leading to cell death. While effective against a wide range of pathogens, CHX requires longer exposure times to achieve optimal results. Additionally, its impact on GP cones is less severe than that of NaOCl, but surface alterations can still occur [9].

Recently, there has been a growing interest in exploring natural alternatives to chemical disinfectants. Grape seed extract (GSE) and aloe vera juice, for example, have shown potential due to their antimicrobial properties and biocompatibility. GSE, derived from *Vitis vinifera*, contains proanthocyanidins, polyphenolic compounds with strong antimicrobial and antioxidant effects. These compounds disrupt bacterial cell walls, reducing microbial viability. Although promising, GSE's antimicrobial efficacy is generally lower than that of conventional disinfectants like NaOCl and CHX [10,11].

Aloe vera (*Aloe barbadensis*), known for its antibacterial, antifungal, and antiviral properties, contains bioactive components such as anthraquinones and saponins, which contribute to its antimicrobial action. Its non-toxic nature makes it an appealing choice for applications requiring high biocompatibility. However, studies examining its efficacy against resilient pathogens, including *E. faecalis*, have reported mixed results. This underscores the need for further research to enhance its disinfection potential [12,13].

The selection of a disinfectant for GP cones must balance microbial efficacy with material compatibility. While NaOCl and CHX are highly effective in eliminating pathogens, their potential to damage GP cones' surface integrity is a notable drawback. Conversely, natural agents such as GSE and aloe vera are gentler on the material but may not achieve the same level of microbial elimination. These trade-offs highlight the importance of developing tailored disinfection protocols that maximize

antimicrobial activity while preserving material properties [14].

Practical considerations, such as ease of application, contact time, and integration into clinical workflows, must also guide the disinfection process. Rapid chairside disinfection protocols are particularly advantageous, allowing clinicians to sterilize GP cones efficiently without interrupting the treatment process. Short-term exposure to NaOCl, for example, has demonstrated effective pathogen elimination within 1–10 minutes, making it a practical choice for clinical use. However, optimizing concentration and exposure time is vital to minimize adverse effects on material properties while ensuring complete disinfection [15–17].

The sterility of GP cones is a critical determinant of endodontic success, making the selection of effective disinfection protocols essential. While chemical disinfectants such as NaOCl and CHX remain the standard, natural alternatives like GSE and aloe vera offer a promising avenue for safer and more biocompatible solutions.

This study seeks to evaluate the antimicrobial efficacy of five disinfectants - 3% NaOCl, 2% CHX, GSE, aloe vera juice, and normal saline (control) - in sterilizing GP cones contaminated with *E. faecalis*. Additionally, the impact of these agents on the surface texture of GP cones will be assessed using scanning electron microscopy (SEM). By comprehensively analyzing disinfection efficacy and material integrity, this research aims to inform the development of optimized disinfection protocols, ultimately contributing to improved outcomes in endodontic practice.

Materials and Methods

No human participants were involved in this study. The research protocol received formal approval from the Institutional Ethics Committee of the Manipal Academy of Higher Education (MAHE), under approval number 21039. The study was conducted in collaboration between the Department of Microbiology and the Department of Conservative Dentistry and Endodontics, adhering strictly to ethical guidelines for *in vitro* experimental research.

Study design

An *in vitro* experimental framework was adopted to evaluate the disinfection efficacy of selected chemical agents on gutta-percha (GP) cones. The study involved a systematic process of contaminating the GP cones with a known endodontic pathogen, followed by their subsequent disinfection using various antimicrobial solutions.

Sample selection and preparation

Fifty gutta-percha cones (ISO size F1 ProTaper specification) were randomly selected from a single production batch manufactured by Dentsply Sirona (Maillefer, Petrópolis, RJ, Brazil). All selected cones were verified to be within their expiration date to ensure consistency in material properties.

The GP cones were subjected to a standardised contamination process. Initially, the cones were immersed in a concentrated culture of *Enterococcus faecalis* and incubated in Brain Heart Infusion (BHI) broth at 37°C for 72 hours. This procedure facilitated bacterial adherence and the formation of biofilms on the cone surfaces, simulating real-world clinical contamination scenarios.

Grouping and treatment protocol

The contaminated GP cones were randomly divided into five experimental groups (n=10 cones per group) based on the disinfectant employed. Each group was further subdivided into two subgroups to investigate the impact of contact time, with intervals of 1 minute and 10 minutes, respectively.

The treatment groups were designated as follows: Group I: GP cones treated with 3% sodium hypochlorite (NaOCl); Group II: GP cones treated with 2% chlorhexidine; Group III: GP cones treated with grape seed extract (GSE); Group IV: GP cones treated with aloe vera juice; and Group V: GP cones treated with physiological saline (control group).

After disinfection, each cone was carefully dried under sterile conditions and transferred to fresh BHI broth. The samples were incubated

at 37°C for another 72 hours to monitor potential microbial regrowth, serving as an indirect measure of disinfectant efficacy.

Disinfection efficacy assessment

Two distinct methods were employed to assess the antimicrobial efficacy of each treatment. *Bacterial Growth Analysis Using Chocolate Agar Plates*: After incubation, aliquots from each BHI broth culture were streaked onto chocolate agar plates and incubated further. The presence of bacterial colonies served as a qualitative indicator of residual contamination. *Turbidity Assessment in BHI Broth*: The visual turbidity of the BHI broth was assessed as a quantitative measure of bacterial presence. A clear broth indicated successful disinfection, while turbidity suggested bacterial survival or regrowth. These methods provided complementary data on the efficacy of each disinfectant.

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (SEM) was employed to evaluate the surface texture and structural integrity of the GP cones post-disinfection. This analysis identified potential surface alterations, such as textural irregularities or structural degradation, resulting from exposure to the disinfecting agents, particularly during prolonged contact periods.

Scoring and statistical analysis

Bacterial colony-forming units (CFUs) were quantified using the following scoring system: Score 0: No growth observed; Score 1: 10^1 - 10^2 CFU; Score 2: 10^3 - 10^4 CFU; Score 3: 10^4 - 10^5 CFU.

For turbidity evaluation, the scoring system was defined as; Score 0: No turbidity observed; and Score 1: Turbidity observed.

Statistical analysis was performed using SPSS software version 23.0 (IBM Corp., Armonk, NY, USA). Because the outcome variables (turbidity present/absent) were categorical and the sample size in each group was small ($n = 5$), Fisher's Exact Test was used to compare the antimicrobial efficacy among the five experimental groups. A two-sided p -value of <0.05 was considered statistically significant.

Results

The study evaluated the disinfection efficacy of various chemical agents on gutta-percha cones contaminated with “*Enterococcus faecalis*”, a pathogenic bacterium often found in endodontic infections. The results were analysed based on bacterial colony counts on chocolate agar and turbidity in Brain Heart Infusion (BHI) broth following treatment with each disinfectant at 1-minute and 10-minute exposure intervals. The detailed findings are summarised below, and the data are presented in tables 1 and 2. Values are presented as number (percentage). Fisher's Exact Test was used because of the small sample size. A p -value < 0.05 was considered statistically significant.

Sodium hypochlorite (NaOCl, 3%)

Sodium hypochlorite demonstrated the highest efficacy among the tested agents. In both the 1-minute and 10-minute subgroups, there was a complete absence of bacterial colonies on the chocolate agar plates, indicat-

ing that NaOCl achieved total disinfection of the gutta- cones at both exposure times. Additionally, there was no turbidity in the BHI broth in either subgroup, suggesting that “*E. faecalis*” growth was entirely inhibited. The effectiveness of NaOCl can be attributed to its potent oxidizing properties, which cause protein denaturation and cell lysis in bacterial cells. NaOCl thus outperformed all other agents in both rapidity and extent of disinfection.

Chlorhexidine (CHX, 2%)

Chlorhexidine showed a time-dependent efficacy. In the 1-minute subgroup, bacterial colonies were present on chocolate agar plates, indicating incomplete disinfection with brief exposure. However, in the 10-minute subgroup, chlorhexidine achieved complete bacterial inhibition, with zero colonies observed on agar plates and no turbidity in the BHI broth. This outcome reflects chlorhexidine's slower bacteriostatic mechanism, which primarily disrupts cell membranes and intracellular components. CHX was the second most effective agent, especially with extended exposure.

Grape seed extract (GSE)

Grape seed extract (*Vitis vinifera*), a natural agent rich in polyphenolic antioxidants, demonstrated moderate antibacterial activity. In the 1-minute subgroup, GSE-treated cones showed bacterial colonies on chocolate agar plates, indicating insufficient disinfection within this timeframe. Conversely, in the 10-minute subgroup, there was a notable reduction in bacterial growth, as evidenced by fewer colonies on agar plates and diminished turbidity in the BHI broth. The antimicrobial activity of GSE is likely due to its high polyphenol content, which disrupts bacterial structures and reduces viability. Despite its potential, GSE was less effective than both NaOCl and CHX, reflecting its limited capacity for complete sterilization under the tested conditions.

Aloe vera extract

Aloe vera (*Aloe barbadensis*), renowned for its soothing and antibacterial properties, demonstrated the least efficacy among the agents tested. In both the 1-minute and 10-minute subgroups, substantial bacterial colonies were observed on chocolate agar plates, accompanied by consistent turbidity in the BHI broth. These findings indicate that aloe vera is insufficient for achieving rapid or comprehensive disinfection of gutta-percha cones. While its biocompatibility is noteworthy, its minimal antimicrobial potency renders it unsuitable for clinical disinfection purposes in the context of endodontic therapy.

Scanning electron microscopy (SEM) analysis

The SEM analysis provided critical insights into the surface alterations of gutta-percha (GP) cones following exposure to the various disinfectants. Cones treated with 3% sodium hypochlorite exhibited the most significant surface damage, characterized by pronounced roughness and extensive pitting. In contrast, cones exposed to 2% chlorhexidine displayed moderate pitting and less severe surface irregularities compared to the NaOCl-treated group. GSE-treated cones showed only mild pitting, reflecting a gentle interaction with the material. Aloe vera-treated

Table 1: Results of bacterial growth (turbidity) after disinfection with different solutions at 1 min time interval

Group	Turbidity Present, n (%)	Turbidity Absent, n (%)	Total (n)
Saline	5 (100.0)	0 (0.0)	5
Aloe vera	5 (100.0)	0 (0.0)	5
Grape seed extract	4 (80.0)	1 (20.0)	5
Sodium hypochlorite	0 (0.0)	5 (100.0)	5
Chlorhexidine	2 (40.0)	3 (60.0)	5
Total	16 (64.0)	9 (36.0)	25

Fisher's Exact Test (two-sided): $p = 0.003$

Table 2: Results of bacterial growth (turbidity) after disinfection with different solutions at 10 min time interval

Group	Turbidity Present, n (%)	Turbidity Absent, n (%)	Total (n)
Saline	5 (100.0)	0 (0.0)	5
Aloe vera	5 (100.0)	0 (0.0)	5
Grape seed extract	3 (60.0)	2 (40.0)	5
Sodium hypochlorite	0 (0.0)	5 (100.0)	5
Chlorhexidine	0 (0.0)	5 (100.0)	5
Total	13 (52.0)	12 (48.0)	25

Fisher's Exact Test (two-sided): $p < 0.001$

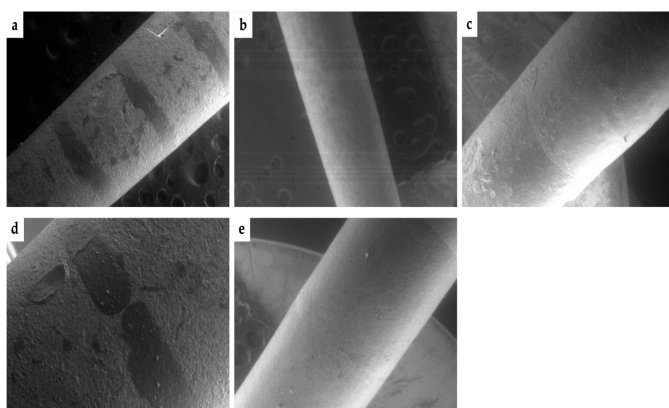


Figure 1: Scanning Electron Microscopy (SEM) Images of Gutta-Percha Cones After Treatment with Various Disinfectants: (a) Sodium Hypochlorite (3%): SEM image showing pronounced surface alterations, including significant pitting and increased roughness, indicating chemical interaction with the gutta-percha material. (b) Normal Saline (Control): SEM image of untreated gutta-percha cones, exhibiting a smooth surface with no visible alterations, serving as the baseline for comparison. (c) Grape Seed Extract: SEM image revealing mild surface pitting and slight irregularities, demonstrating a gentler interaction compared to chemical agents. (d) Chlorhexidine (2%): SEM image showing moderate surface pitting and roughness, indicating a balance between microbial efficacy and preservation of surface integrity. (e) Aloe Vera Juice: SEM image illustrating minimal surface changes with negligible pitting, reflecting its mild impact on gutta-percha material

cones exhibited minimal surface changes, with negligible roughness and pitting. Similarly, the control group, which was not subjected to any disinfecting agent, maintained a nearly unaltered surface, demonstrating no significant morphological changes (figure 1).

Statistical analysis revealed that sodium hypochlorite demonstrated a significantly greater reduction in bacterial presence compared to the other agents across both tested exposure times ($p < 0.05$). While chlorhexidine also exhibited notable antimicrobial efficacy, its effectiveness was pronounced only with extended exposure durations. Grape

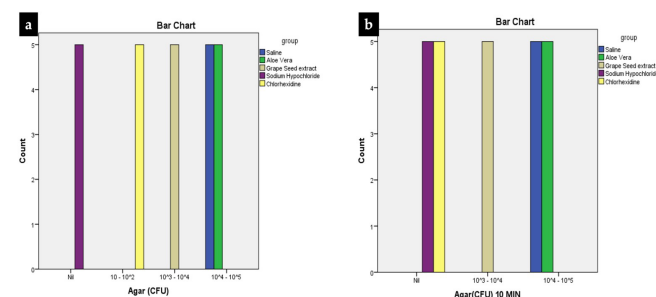


Figure 2: Bacterial Growth Analysis (Colony-Forming Units) on Chocolate Agar Plates Post-Treatment. (a) The 3% sodium hypochlorite group exhibits no CFUs across both time intervals, indicating complete disinfection, whereas the saline group shows significant bacterial growth, serving as the control. (b) Bacterial Growth Analysis (Colony-Forming Units) on Chocolate Agar Plates Post 10 minutes

seed extract showed moderate antibacterial activity, whereas aloe vera displayed minimal impact under the given conditions. The observed significant differences among all groups - ranked in decreasing order of efficacy as sodium hypochlorite, chlorhexidine, grape seed extract, and aloe vera - underscore the variability in disinfection potential between these agents.

Bacterial growth assessments, quantified using colony-forming units (CFUs) on chocolate agar plates, and turbidity measurements in brain heart infusion (BHI) broth post-treatment, are depicted in figures 2 and 3, respectively. These results highlight the differential capabilities of each disinfectant in eliminating *E. faecalis* contamination.

In summary, sodium hypochlorite emerged as the most effective disinfectant for achieving rapid and thorough sterilisation of gutta-percha cones contaminated with *E. faecalis*. Chlorhexidine, while effective, required prolonged exposure to achieve comparable results, positioning it as the second-most effective agent within this study's parameters.

Discussion

The primary objective of endodontic treatment is to achieve a sterile environment within the pulp space, to prevent microbial reinfection and ensure long-term therapeutic success. Contamination of gutta-percha (GP) cones poses a significant risk, particularly at the point of initial packaging and subsequent handling during clinical procedures [18]. Research highlighted that the highest risk of gutta-percha cone contamination occurs during the initial unsealing of new packs in the clinical setting, underscoring the critical importance of strict aseptic protocols to preserve sterility [12]. Given that obturation is the final step in endodontic treatment, introducing contaminated GP cones into the canal can jeopardise the outcome and lead to treatment failure. Thus, maintaining the sterility of GP cones throughout the procedure is imperative, with rapid-sterilisation techniques serving as efficient, practical, and cost-effective solutions for chairside use [19,12].

Although GP cones are packaged under sterile conditions, contamination from environmental aerosols, inadequate storage, and improper handling remains a considerable concern. Studies indicate microbial contamination in approximately 5–19% of newly opened GP boxes [1,5,20]. The microbial load during packaging is typically low; however, clinical handling increases the risk of contamination due to exposure to the operatory environment [14]. Given the thermoplastic nature of GP cones, sterilisation using heat-based methods is impractical due to the risk of compromising their structural integrity [21]. Consequently, chemical disinfectants, many of which are routinely employed in endodontic irrigation, have emerged as the preferred approach for decontaminating GP cones [22-24].

Among these chemical agents, sodium hypochlorite (NaOCl) is widely regarded as one of the most effective endodontic irrigants, owing to its

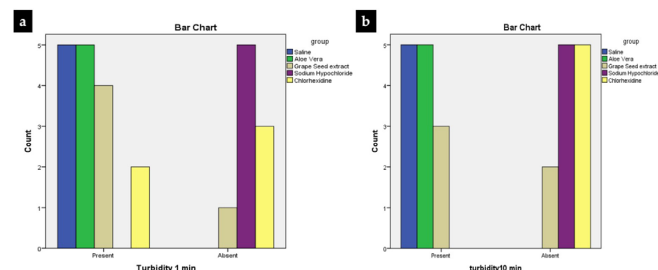


Figure 3: Turbidity Assessment in Brain Heart Infusion (BHI) Broth Post-Treatment. Clear broth indicates effective bacterial inhibition, while turbidity signifies bacterial growth. The sodium hypochlorite group maintained clear broth in both time intervals, confirming its efficacy, whereas the aloe vera and control groups displayed consistent turbidity, indicating limited antimicrobial activity. (a) post 1 minute and (b) 10 minutes

potent antimicrobial properties and broad-spectrum efficacy. Typically applied in concentrations ranging from 1% to 5.25%, NaOCl's bactericidal activity is attributed to its strong oxidative properties and alkaline pH. These factors work synergistically to disrupt bacterial cell walls, denature proteins, and induce cell lysis. Its rapid and comprehensive antimicrobial effects, particularly against resilient pathogens such as *Enterococcus faecalis*, have solidified its position as the disinfectant of choice for GP cone decontamination. Numerous studies have endorsed its efficacy, emphasizing its ability to eliminate persistent microorganisms efficiently [5-7,25].

In this study, NaOCl demonstrated superior disinfection capability, achieving complete bacterial inhibition within both 1-minute and 10-minute exposure intervals. These results align closely with findings reported by Rozendo et al. (2022) and Yadav et al. (2016), further validating NaOCl's antimicrobial effectiveness through its oxidative mechanism. Notably, scanning electron microscopy (SEM) analysis revealed that treatment of GP cones with NaOCl resulted in the formation of a cubic crystalline framework on their surfaces, likely as a precipitate [25,26]. Such surface changes have been previously documented, where similar structures were observed following short-term (1-minute) disinfection protocols. Prolonged exposure to NaOCl (10 minutes) was associated with more pronounced surface irregularities, including the formation of sodium chloride crystals on GP cones [27].

Chlorhexidine (CHX), another widely adopted endodontic irrigant, exhibits bactericidal and sporicidal effects, coupled with substantive antimicrobial action, making it a valuable alternative for root canal disinfection. CHX disrupts bacterial cell membranes and inhibits intracellular processes, though it requires extended exposure for optimal effect. The current study observed that CHX demonstrated limited efficacy within the 1-minute interval, but substantial bacterial reduction after 10 minutes of contact with the GP cones, highlighting its slower bacteriostatic mechanism. These findings align with Ramos-Meléndez et al. (2015), who emphasized CHX's need for prolonged exposure to achieve significant disinfection. However, they differ from Sequeira et al. [28], who reported reduced effectiveness over time, indicating potential variability in antimicrobial efficacy based on study conditions and CHX concentration.

In this study, grape seed extract (GSE) showed moderate antibacterial activity, which can be attributed to its polyphenolic compounds, especially proanthocyanidins, that have been noted to exert cell wall-disrupting effects on microorganisms [29]. At the 1-minute interval, GSE displayed limited efficacy; however, bacterial reduction was noted after 10 minutes, supporting findings by Umer et al. (2018), who identified GSE as a moderately effective agent in disinfection [30]. While GSE holds promise due to its biocompatibility and lower toxicity, its application in rapid disinfection protocols remains limited, particularly in cases where complete sterility is essential.

Aloe vera demonstrated the lowest antimicrobial efficacy among the agents tested. Despite its historical use in medicine, aloe vera's antimicrobial activity appears insufficient for reliable GP cone sterilisation, as evidenced by considerable bacterial survival even after 10 minutes of exposure.

These results align with the findings of a meta-analysis (Tariq R et al. 2023), who reported that aloe vera's antimicrobial effects were comparatively lesser compared to sodium hypochlorite and chlorhexidine against *E. Faecalis*, questioning its suitability for rapid and thorough disinfection in endodontic settings [31]. Aloe vera's limited efficacy could be due to its slower antimicrobial activity, which may not be sufficient for rapid disinfection against the robust cell walls of *E. faecalis* [32].

Alterations in the surface topography of gutta-percha were noted with all tested disinfectants, manifesting as crystal deposits, granular formations, or surface irregularities. Multiple studies have documented the deposition of chloride crystals on the surface of gutta-percha, which can negatively influence its mechanical properties. Introducing such crystals into the root canal system might hinder the sealing efficiency achieved during the filling process [1,11]. Valois et al. observed that exposure to 5.25% sodium hypochlorite (NaOCl) led to surface degradation of gutta-percha cones, resulting in increased surface irregularities and reduced elasticity. Such surface defects could contribute to leakage by creating

extensive interfacial gaps, thereby diminishing the adhesion or bond strength between gutta-percha cones and endodontic sealers [33].

These observations are consistent with the findings of Grecca et al. (2011), who reported significant surface irregularities on gutta-percha cones following treatment with a 2.5% sodium hypochlorite (NaOCl) solution for 10 minutes. These irregularities were attributed to the presence of residual sodium chloride crystals, likely formed as a result of chemical interactions between the disinfectant and the gutta-percha material [34]. Similarly, Tilakchand et al. (2014) documented pronounced topographic alterations at the tips of gutta-percha cones after exposure to a 5.25% NaOCl solution, further highlighting the impact of disinfectant concentration on material integrity [21]. In a related study, Short et al. (2003) utilised scanning electron microscopy (SEM) to examine treated gutta-percha cones and identified significant structural changes in the deeper layers of affected areas. These changes were hypothesised to result from the partial lysis of specific components within the gutta-percha matrix [1].

The findings underscore the delicate balance required in endodontic practice between achieving effective microbial disinfection and preserving the structural integrity of gutta-percha cones. Sodium hypochlorite emerged as a highly effective disinfectant, capable of rapid and comprehensive microbial eradication. However, its use was associated with notable alterations to the surface topography of gutta-percha. Chlorhexidine demonstrated considerable antimicrobial efficacy as well, though it required extended contact times to produce outcomes comparable to NaOCl. In contrast, natural disinfectants such as grape seed extract exhibited moderate antimicrobial effectiveness, while aloe vera showed limited activity, suggesting their potential roles as supplementary agents rather than primary disinfectants in clinical protocols.

These findings emphasize the critical importance of implementing stringent disinfection practices in endodontics, particularly given the susceptibility of gutta-percha cones to microbial contamination and the complex microbial environment of endodontic biofilms. The choice of disinfectant must be carefully considered to balance the need for effective antimicrobial action with the preservation of the material properties essential for achieving optimal sealing and adhesion in root canal therapy.

Conclusion

In summary, sodium hypochlorite (NaOCl) has emerged as the most effective agent for gutta-percha disinfection, demonstrating rapid and comprehensive antibacterial action. Chlorhexidine (CHX) offers a valuable alternative, particularly in scenarios where extended exposure durations are practical. While natural disinfectants present an appealing option due to their biocompatibility, further advancements are required to enhance their efficacy and establish a more prominent role in clinical settings. Future investigations should aim to encompass a wider range of microbial species and integrate *in vivo* studies to provide a more thorough evaluation of the clinical applicability and long-term outcomes associated with these disinfection protocols.

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