ORIGINAL RESEARCH

Physicomechanical Properties and Antimicrobial Efficacy of an Auto-disinfecting Irreversible Hydrocolloid Impression Material Containing a Quaternary Ammonium Compound and Silver Nanoparticles

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Abstract

Aim: The study aims to evaluate the physicomechanical properties and antimicrobial efficacy of an irreversible hydrocolloid (IH) impression material modified with N, N, N-tetramethyl methanaminium chloride (MAC) and silver nanoparticles (AgNPs).

Methods: The study formulated IH groups containing different concentrations of MAC and AgNPs (0.5, 1.0, and 1.5 wt%) individually. These groups were tested for setting time (ST), tear strength (TS), and antimicrobial activity. The surface topography was also analyzed by a field emission scanning electron microscope (FESEM). The ST and TS were evaluated using the stopwatch test and angle tear test, respectively. The antimicrobial efficacy was assessed using colony-forming unit (CFU) counts postincubation with the IH samples. The data was obtained and statistically analyzed.

Results: Setting time and TS were not significantly affected by the incorporation of MAC or AgNPs up to 1.5 wt%, maintaining these properties within acceptable ranges as per ISO 21563:2021. Both agents demonstrated dose-dependent antimicrobial efficacy, with 1.5 wt% MAC showing statistically higher effectiveness ($p < 0.05$) against *Staphylococcus aureus* and *Candida albicans* compared to 1.5 wt% AgNPs. 1% AgNPs elicited an antifungal effect, and beyond which, there is no significant effect.

Conclusion: Incorporating MAC and AgNPs in IH enables effective antimicrobial activity while preserving the ST and TS, rendering the IH auto-disinfecting at up to 1.5 wt% concentration for both disinfectants.

Clinical Significance: This formulation of IH with MAC and AgNPs offers a viable solution for reducing infection risks in dental practice by its self-disinfecting property, not only disinfecting the surface but also the inner impression en masse without imbibition.

Keywords: Antimicrobial efficacy, Irreversible hydrocolloids, Quaternary ammonium compound, Setting time, Silver nanoparticles, Tear strength.

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Introduction

Impression-making is essential in prosthodontics, with irreversible hydrocolloid (IH) commonly used.[1] IH impressions can be contaminated by saliva or blood carrying infectious pathogens. Due to its porous and hydrophilic nature, IH retains microbes on and within the material.[2] These pathogens may transfer to gypsum models, posing risks to dental personnel. Thus, strict infection control is vital to prevent cross-contamination and ensure safety.[3] Spray and immersion methods are the IH's most widely proposed disinfection techniques. However, both techniques disinfect only the impression's surface.[4] Nevertheless, these techniques would significantly affect the dimensional stability by imbibition-syneresis phenomena and reproduction accuracy.[5–9] To prevent dimensional instability, the disinfection time of the IH should not be excessive. However, such a compromise may significantly reduce the disinfectant's efficacy. Deteriorated surface quality, increased surface roughness, and hardness are some problems associated with impression disinfection procedures, which are eventually reflected in the gypsum models.[10,11]

Auto-disinfecting irreversible hydrocolloids (ADIHs) with inherent disinfectants have been developed to circumvent and obviate the limitations of traditional disinfection techniques of conventional IHs. Several water-soluble biguanide disinfectants and ammonium-based disinfectants have been added to disinfect not only the IH impression surface but also the impression en masse.[4,12–14] Chlorhexidine and quaternary ammonium

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compounds (QACs) are broad-spectrum disinfectants that were found to exhibit good antimicrobial properties.[4,12,13,15] However, chlorhexidine ADIHs exhibit prolonged gelation time and cytotoxicity on human gingival fibroblasts.[4,14] Silver nanoparticles (AgNPs) in IHs are also an antimicrobial agent, conferring auto-disinfection ability.[16] *N, N, N*-tetramethyl methanaminium chloride [$(CH_3)_4N^+Cl^-$); MAC] is a basic, colorless, hygroscopic, water-soluble QAC possessing low-residue bactericidal activity. Notable alterations in the characteristics of ADIHs, including gel firmness, gelation duration, distortion, and the ability to replicate surface details, have been documented in the dental literature.[4,7,17] However, MAC's incorporation in the IHs as a disinfectant has never been attempted.

The IHs should have sufficient tear strength (TS) to resist tearing and elasticity, allowing untorn removal from crucial stomato-dental undercuts without permanent deformations.[18] Nevertheless, the material should either tear at or shortly after considerable strain to prevent the risk of the deformation going unnoticed by the dentist.[19] Therefore, maximum TS is mandated at the time of impression removal. Also, a precise dental impression is assertively governed by the setting time (ST) and smoothness of the impression's surface employing IH.[20] However, there is no documented literature concerning the TS, ST, topographical surface, and antimicrobial efficacy of IH incorporated with MAC. Hence, the present study aimed to evaluate the TS, ST, and antimicrobial efficacy of IH incorporated with MAC and AgNPs at 0.5, 1.0, and 1.5 wt% concentrations. The first null hypothesis was that the incorporation of MAC and AgNPs would not affect the TS and ST of IH. The second null hypothesis was that there would not be an auto-disinfection/antimicrobial effect in the IH incorporated with both disinfectants.

Materials and Methods

The ST was evaluated at the Prosthodontics Department, Vivekanandha Dental College for Women, Tiruchengode, India. The TS evaluation was conducted at the Manufacturing Department, Annamalai University, Chidambaram, India, and the FESEM evaluation was conducted at Sitra Chemistry Lab, Coimbatore, India. The *in vitro* antimicrobial study was conducted at Acme Progen Lab, Salem, India. The study duration was 18 months (November 2022–May 2023). The study was approved by the Institutional Review Board, Vivekanandha Dental College for Women (Approval No. VDCW/IEC/194/2019), Namakkal, Tamil Nadu, India.

Formulation of ADIH Powder

The chemicals to formulate IH with the weight percentage and the disinfectants employed in this research are tabulated in Table 1. All the ingredients for formulating IH powder were taken in the appropriate ratios and mixed in a fully automatic mortar mixer (30 rpm) for 30 minutes at 37 ± 1°C and stored in a sterile polycarbonate container for further use. The study groups were divided into groups Q and S, where MAC and AgNPs were substituted in the IH powder, respectively. The experimental subgroups were divided based on the disinfectant's concentrations at 0.5, 1.0, and 1.5 wt%. The subgroups were Q0.5, Q1.0, and Q1.5 based on the MAC concentrations; S0.5, S1.0, and S1.5 based on AgNPs concentrations. A common control subgroup for both groups was without disinfectants. The disinfectant and IH powder were blended in the automatic mortar mixer for 15 minutes and stored in separate labeled sterile containers. The labels were masked by opaque stickers with random alphabets to blind the investigator from the groups and subgroups, including the control, to prevent experimental bias. The randomly assigned alphabets for the groups and subgroups were used till the statistical analysis.

ST and Surface Topography

Four grams of impression material from each group (n = 70; i.e., 10 specimens per subgroup) was mixed with 9.5 mL distilled water in a semi-automated Alghamix II device (Zhermack, GmbH, Deutschland, Germany) for 15 s at 23 ± 1°C with 120 rev/min to standardize the manipulative variables. The mixed material was then dispensed in a cylindrical mold (30 ± 0.2 mm diameter; 16 ± 0.1 mm high). The ST was measured from start-mixing to when the material was no longer sticky to an indicator acrylic rod (100 mm length; 6 mm diameter) using a stopwatch at 10-second intervals. The ST test was conducted adhering to ISO 21563:2021 guidelines.[21] The ultrastructural surface topography of the specimens was analyzed by field emission scanning electron microscopy (FESEM) (Zeiss FESEM Sigma VP 03–04). From each subgroup, 5 × 5 × 3 mm^3 set material was cut from the cylindrical specimen for FESEM analysis at 5,000× magnification.

TS

The angle tear test method was employed to determine the TS. Right-angled specimens were prepared using an acrylic mold designed according to ISO 21563:2021 guidelines.[21] 70 specimens (n = 10 per each experimental subgroup; n = 10 for control) were fabricated on the spot of TS evaluation. Specimens were fabricated

Table 1: Chemicals and disinfectants

Chemicals for formulating IH powder in the research			
Material and function	*Weight% (Wt%)*	*CAS No.*	*Manufacturer/supplier*
Alginic acid; potassium salt (K^+–Alg); reactant	10	9005 - 36 - 1	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India
Alginic acid; sodium salt (Na^+–Alg); reactant	5	9005 - 38 - 3	
Calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$); reactant	16	10101 - 41 - 1	
SiO_2; filler	60	68855 - 54 - 9	
ZnO (<5 µm particle size, 99.9% pure); filler	4	1314 - 13 - 2	
Dipotassium hexafluoro titanate (K_2TiF_6); accelerator	3	16919 - 27 - 0	
Tetrasodium pyrophosphate; retarder	2	7722 - 88 - 5	
Disinfectants used in the research			
MAC; ≥98% purity		75 - 57 - 0	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India
AgNPs; 80–100 nm; 99.9% purity		7440 - 22 - 4	Nano Research Lab, Jharkhand, India

by a single investigator (powder–water ratio 1:2.375) in the Alghamix. The mixture was dispensed within the mold, and a clean glass tile was delicately compressed onto the mold for 5 minutes to eliminate superfluity and guarantee a consistent thickness of the specimen. The specimens were retrieved after setting and subjected to tear testing not less than 10 minutes from setting. On either end of a specimen, cloth strips were attached with α-methyl cyanoacrylate to form cloth loops encompassed within a custom-fabricated plastic specimen aligning tray. The cloth strip-specimen assembly was meticulously removed from the tray and aligned for tear testing with the cloth loops attached to the gripping mechanism of the upper and lower jaws of an Instron universal test machine (Model TTCM, Instron, Massachusetts, United States). The TS was tested at a cross-head speed of 500 mm min^{-1}. The TS was computed using the equation: TS = *F*/*d*, where *F* represents the peak tearing force (N), and *d* denotes the thickness (mm) of the specimen.[21]

Antimicrobial Efficacy

A total of 120 disc-shaped (15 mm diameter; 3 mm thick) specimens (*n* = 60/disinfectant; *n* = 30/microbe; *n* = 10/experimental subgroup) were prepared. An additional 20 control specimens were prepared commonly without disinfectants (*n* = 10/microbe). All the specimens were sterilized at 121°C and 15 Psi for 15 minutes in an autoclave. *C. albicans* (MTCC 183; *C. albicans*) in yeast extract peptone-dextrose medium and *S. aureus* (MTCC 87; *S. aureus*) in mannitol salt agar were incubated at 37°C for 24 h. The microbes' strains were available at the testing laboratory, Acme Progen Lab, Salem, Tamil Nadu. After 24 hours, the lawn culture suspension of *C. albicans* was transferred to 20 mL of yeast nitrogen base medium (YN_2) with 50 mM glucose and incubated for 21 hours at 37°C. After centrifuging (5000 gm for 5 minutes) and washing the cells twice in phosphate-buffered saline solution (PBS), the cells were again treated with 20 mL YN_2 with 100 mM glucose. The microbial suspensions were serially diluted and adjusted to 10^6 CFU/mL[22] by matching the turbidity of the 0.5 McFarland standard before testing. Antimicrobial activity was assessed using the direct contact test [Acme ProGen Biotech (India) Private Limited].

The disc specimens were positioned in three sterilized polystyrene 12-well culture plates (one specimen per well; three specimens per subgroup) and labeled appropriately. 3 mL of microbial suspension was added to each well containing the specimen and incubated for 60 minutes at 37°C. The specimens were recovered and transferred to another 12-well culture plate, where they were treated with PBS to remove the nonadhered cells, simulating the clinical washing of the impression. The adhered cells from both sides of the specimen were meticulously collected with sterile cotton swabs, streaked on the blood agar medium by the four-sector streak method,[23] and incubated aerobically for 24 hours at 37°C. The number of CFU was counted manually under transmitted light, and the CFU/mL was calculated using the following formula:

$$\text{CFU mL}^{-1} = \text{CFU} \times \frac{1}{\text{Final Dilution Factor (FDF)}}$$

Here, FDF is a product of sample dilution, total series dilution, and plating dilution factors.

Statistical Analysis

The data interpretation was conducted utilizing the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., 29.0). Shapiro–Wilk and Kolmogorov–Smirnov normality tests showed a statistically insignificant difference in the data distribution ($p > 0.05$). Consequently, based on the data obtained, one-way analysis of variance (ANOVA) coupled with *post hoc* Tukey–Kramer multiple comparison analyses was utilized to assess differences among and between the subgroups. Furthermore, the independent *t*-test was executed to evaluate the subgroups of identical concentrations across the different groups. $p < 0.05$ was considered statistically significant.

Results

ST and Surface Topography

The mean [± standard deviation (SD)] ST of the control and experimental subgroups is depicted in Figure 1. Concerning groups Q and S, statistically insignificant differences were found among the subgroups (Q: $p = 0.7932$; S: $p = 0.6709$). Increasing the MAC and AgNPs concentration to 1.5 wt% in IH did not affect the ST negatively. Multiple comparisons between the subgroups of both groups and intergroup comparisons between the same concentration showed imperceptible variations ($p > 0.05$). Concerning FESEM analysis, in all the subgroups, the fillers appeared as cylindrical perforated sticks, where the maximum and minimum lengths of the fillers were 16.84 and 5.28 µm, respectively, with a mean length of 9.65 µm. The maximum and minimum diameters of the fillers were 14.04 and 3.21 µm, with a mean diameter of 8.55 µm. Additionally, in group S, AgNPs were nanospherical with a diameter between 80 and 100 nm, interspersed in the matrix. The mean AgNP diameter from the FESEM micrographs was 88 nm. Figures 2A to C depict the FESEM images of the control, S1.5, and Q1.5, respectively. Therefore, it is inferred that the IH with MAC and AgNPs did not affect the ST when compared with the neat IH.

TS

The mean [± SD] TS of the control and experimental subgroups is depicted in Figure 3. Concerning groups Q and S, statistically imperceptible variations were observed among the subgroups (Q: $p = 0.7195$; S: $p = 0.9055$). Increasing the MAC and AgNPs concentration to 1.5 wt% in IH did not affect the TS negatively. Multiple comparisons between the subgroups of both groups

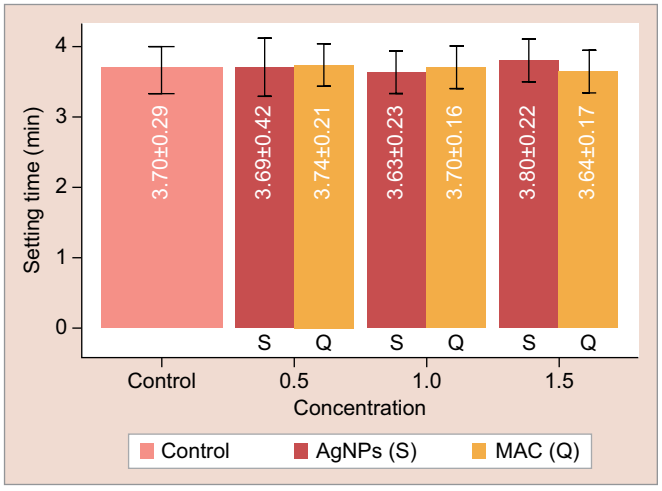
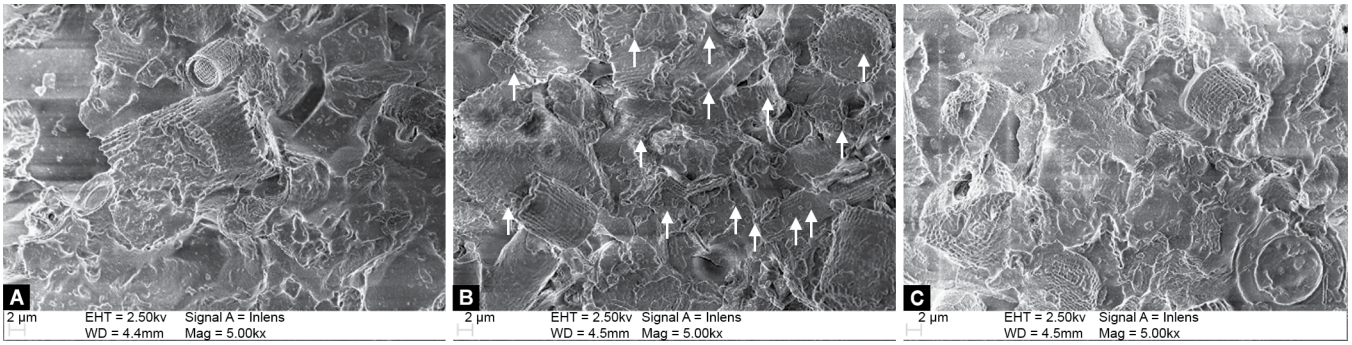


Fig. 1: STs of the subgroups



Figs 2A to C: FESEM images of the specimens: (A) Control group; (B) S1.5 group (white arrows point to the AgNPs); (C) Q1.5 group

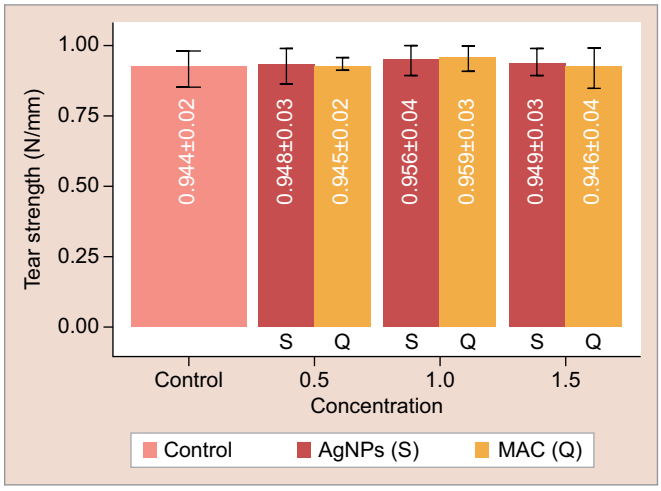


Fig. 3: TS of the subgroups

and intergroup comparisons between the same concentration presented insignificant variations ($p > 0.05$). Therefore, it is inferred that the IH with MAC and AgNPs did not affect the TS when compared with the neat IH.

Antimicrobial Efficacy

The mean [± standard deviation (SD)] CFU of the subgroups for *S. aureus* and *C. albicans* concerning both disinfectants are depicted in Figures 4 and 5, respectively. Concerning *S. aureus* with AgNPs (S), statistically discernible variations were found among and between the subgroups ($p < 0.05$). Concerning *S. aureus* with MAC (Q), a perceptible variation was appreciable among the mean CFUs of the subgroups ($p < 0.05$). Upon multiple comparisons, there were statistically significant differences between the common control group and the three subgroups (Q0.5, Q1.0, Q1.5; $p < 0.05$). The comparisons Q0.5–Q1.0 ($p = 0.9999$), Q0.5–Q1.5 ($p = 0.9998$), and Q1.0–Q1.5 ($p = 1$) did not demonstrate statistical differences. Concerning *C. albicans* with AgNPs (S), a notable variation was

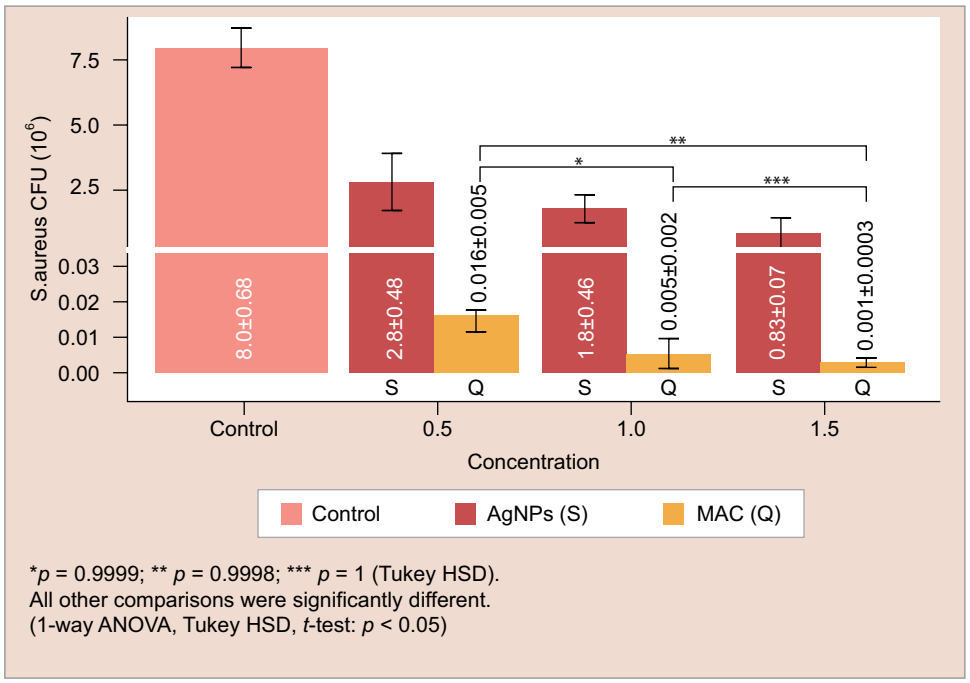


Fig. 4: CFUs concerning *S. aureus*

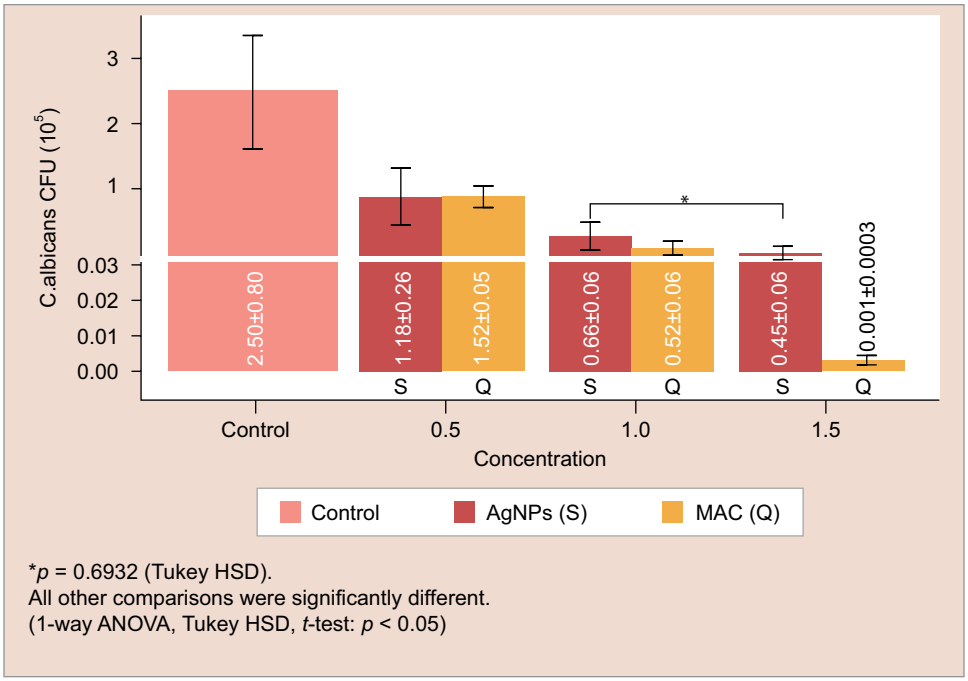


Fig. 5: CFUs concerning *C. albicans*

evident among the mean CFUs of the subgroups ($p < 0.05$). Upon multiple comparisons, there were statistically perceptible variations between the subgroups ($p < 0.05$) except for the S1.0–S1.5 comparison ($p = 0.6932$). Concerning *C. albicans* with MAC (Q), appreciable variances were observed among and between the subgroups ($p < 0.05$). Against *S. aureus*, MAC is more efficacious than AgNPs in all three concentrations ($p < 0.05$). Against *C. albicans*, MAC is more efficacious than AgNPs at 1.0 wt% and 1.5 wt% concentrations ($p < 0.05$). At 0.5 wt%, AgNP was more efficacious than the MAC ($p < 0.05$). Therefore, from the results, it is inferred that the MAC- and AgNPs-incorporated IH exhibited concentration-specific antimicrobial activity against *S. aureus* and *C. albicans*.

Discussion

The present study found that incorporating AgNPs or MAC disinfectants did not affect the ST of IH, thus supporting the first null hypothesis. This may be due to the chemical inertness of the disinfectants with IH components. ST is vital for clinical efficiency and patient comfort.[24] According to the American Dental Association (ADA), IH is classified into type I (1–2 minutes) and type II (2–4.5 minutes) based on ST.[25] ISO 21563:2021 allows a 20% deviation from the manufacturer's ST.[21] In this study, ST remained within the normal set limits. Factors influencing IH's ST include water-powder ratio, mixing time, mixing temperature,[26] water temperature,[27] trisodium phosphate content, and disinfectant used.[28] Selvaraj *et al.* found that the ST increased significantly when the IH was combined with a hypochlorous solution.[29] Nallamuthu *et al.* demonstrated decreased ST by adding magnesium oxide in IH due to its crosslinking ability, besides antimicrobial properties.[30] Gingupalli *et al.* found an increase in the ST in the Tropicalgin commercial brand and no significant change in the ST in the Zelgan brand incorporated with AgNPs.[16]

In a previous study, adding 2 wt% zinc oxide (ZnO) nanoparticles to Zelgan significantly increased ST, while lower concentrations had no notable effect. For Tropicalgin, ST increased at 0.5–1 wt% but decreased at 2–5 wt%.[1] Copper oxide NPs reduced Zelgan's ST at 5 wt%, with minimal effects at lower doses; Tropicalgin showed a slight ST increase up to 2 wt% but a marked rise at 5 wt%. These findings highlight how antimicrobial concentration influences ST.[1] In the current study, a maximum of 1.5 wt% was used. ST is also affected by room temperature, with higher temperatures reducing ST.[27] To ensure consistency in the present research, all variables were standardized using an automated mixer.

The inorganic filler particles depicted in the FESEM imagery in the present research resemble the cell walls of algae categorized within the division Chrysophyta and the class Bacillariophyceae. These organisms, commonly acknowledged as diatoms, are mainly unicellular, though they may also exist in chains or as colonies.[31] The classification of diatoms hinges on the configuration and embellishment of their cell walls, referred to as frustules.[31] Without the filler, the resulting gel lacks proper firmness and has a tacky surface covered in an exudate caused by syneresis.[26] Filler frustules' inhalation from IH unequivocally presents a significant risk to dental professionals over extended exposure. This danger is primarily linked to the filler's diameter, not composition, with frustules <3 µm and longer than 20 µm being particularly obnoxious.[32] Research by Woody *et al.*[33] demonstrated that 10–15% of aerosolized frustules from two commercial IHs fall within this critical size range. In the present research, the mean diameter and length of the fillers were 8.55 and 9.65 µm, which are unlikely to contribute to respiratory pathosis.

In the present research, incorporating either disinfectant did not affect the TS. Hence, the first null hypothesis was accepted. The plausible reason could be the noncrosslinking nature of the AgNPs and MAC with the IH polymer. There were no established guidelines or designated values for the TS of IH in either ISO standard 1563 or specification No. 18 of American National Standards Institute (ANSI)/ADA until the ISO 21563:2021 guidelines, where the minimum acceptable TS for IH was stipulated to 0.38 N/mm.[21] In the current research, the TS of all the subgroups was well above the stipulated value and was within the acceptable documented range (0.4–1.2 N/mm) in the literature.[30,34] Nallamuthu *et al.* found that adding

magnesium oxide in experimental IH dramatically enhanced the TS due to its crosslinking ability with IH polymer.[30] Singer and Bourauel incorporated chlorhexidine, silver nitrate, and plant-extracted AgNPs in the IH powder and found that the chlorhexidine and plant-extracted AgNPs increased the TS, and silver nitrate had no effect on the same.[35] Modifying the water–powder ratio, mixing technique, and filler (type and concentration) alters the characteristics of the gel, ST, TS, and elasticity recovery.[36] In IH, alginic acid and diatomaceous earth (SiO_2) are the key to mechanical strength: Guluronic acid yields strong, brittle gels, while mannuronic acid forms softer, elastic ones.[34] The high TS across subgroups may stem from a high guluronan–mannuronan ratio, with diatom fillers further enhancing TS. TS was measured using 4 mm-thick V-shaped specimens, matching the clinically recommended 4–6 mm thickness for alginate impressions, ensuring clinical relevance.[37]

In the present research, MAC showed antimicrobial effects equal to AgNPs against both microorganisms in IH, leading to rejection of the second null hypothesis. As a QAC, MAC inhibits fungal growth by retarding conidium germination, reducing spore germination, and suppressing hyphal elongation.[38] QACs also act as antibacterial agents by binding to negatively charged bacterial cell membranes, causing potassium leakage and cell death.[39] Previous studies have shown that incorporating metal oxide nanoparticles into IH offers potent self-disinfecting properties. Such incorporation does not adversely affect the physicomechanical characteristics of IH.[1] AgNPs exhibit remarkable antimicrobial effects primarily due to silver ions (Ag^+), which target and inhibit bacterial enzymes containing thiol groups, disrupting DNA replication. Research has shown that AgNPs can alter bacterial cell membrane structures[40] and hinder cell wall synthesis, obstructing essential bacterial metabolism.[41] They also induce pits on gram-negative bacteria, increasing surface area and permeability, ultimately leading to cell death.[42] Previous research has shown that using a colloidal solution of AgNPs results in a uniform distribution that minimizes aggregation, enhancing antimicrobial effectiveness.[41–43] In powdered form, smaller particles tend to cluster, reducing their antibacterial potential. Gingupalli *et al*. found no antimicrobial activity of AgNPs in 10–20, 30–50, and 50–80 nm sizes due to this agglomeration. Conversely, AgNPs sized 80–100 nm exhibited notable antimicrobial activity, likely due to less aggregation, which allows more silver to be available for effective action.[44] Hence, in the present research, 80–100 nm AgNPs were employed.

This first research to evaluate MAC in IH directly compares it with AgNPs at multiple clinically relevant concentrations under ISO 21563:2021 standards. Strong sample sizes, rigorous microbiological protocols, FESEM-based insights, and clinically relevant specimen design enhance validity and applicability, while transparent methods, blinded labeling, and robust statistics ensure reproducibility. Limitations include the lack of assessment of dimensional accuracy, gypsum compatibility, gel strength, detail reproduction, and biocompatibility. Further research is needed on efficacy against highly transmissible viruses such as hepatitis, herpes, and HIV. The use of a standardized mold does not replicate oral tissue dynamics; future studies should model saliva interaction to better evaluate IH flow and accuracy.

Conclusion

Within the research constraints, it can be concluded that the incorporation of MAC and AgNPs successfully endowed the IH with auto-disinfecting properties. This novel ADIH formulation demonstrated effective self-sterilizing capability without compromising the material's essential physical characteristics. Notably, the inclusion of MAC and AgNPs did not adversely influence the ST or TS of the material, ensuring usability in dental practice. In addition to its stable mechanical behavior, the ADIH displayed excellent antimicrobial efficacy against *C. albicans* and *S. aureus*. This antimicrobial effect was sustained up to a concentration of 1.5 wt% of each disinfectant.

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