Original Article

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Dentistry Section

Comparative Evaluation of Contact Angle in Biosynthesised and Chemically Synthesised Silver Nanoparticle-based Root Canal Irrigants: An In-vitro Study

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ABSTRACT

Introduction: Irrigating solutions are vital in endodontics, serving to meticulously disinfect root canals, enzymatically dissolve pulpal remnants, and facilitate thorough flushing to eradicate microorganisms. This intricate process ensures optimal microbial control, tissue dissolution, and debris removal, contributing to enhanced endodontic outcomes and overall oral health in clinical practice.

Aim: To evaluate the contact angle of Trisodium Citrate (TSC) Silver Nanoparticles (AgNP) and Biosynthesised AgNPs when used in root canal irrigation.

Materials and Methods: This in-vitro study was conducted at White Lab, Department of Conservative Dentistry and Endodontics, Saveetha Dental College Saveetha Institute of Medical and Technical Sciences Saveetha University, Chennai, Tamilnadu, India, from January 2023 to August 2023. A total of 40 single-rooted dentine samples extracted from human teeth were utilised. Ethical clearance was duly obtained. The samples were categorised into four groups (n=10 each). The groups underwent distinct irrigation processes, including Biosynthesised AgNPs from Azadirachta Indica (Group-1), Chemically synthesised AgNPs from Trisodium citrate (Group-2), 5.25% Sodium Hypochlorite (Group-3), and Distilled Water (DW) as the control (Group-4). The contact angle of the tested irrigants was thoroughly evaluated in all the samples using an Ossila goniometer. The data were analysed using Statistical Package for Social Sciences (SPSS) 21.0. Statistical tests used were Analysis of Variance (ANOVA). A p-value of <0.05 was considered statistically significant.

Results: Group-1 and Group-2 showed significantly low contact angles (p=0.002) compared to the other irrigants like 5.25% Sodium Hypochlorite (NaOCl) and water used as control. There was no statistically significant difference among the tested irrigants, with TSC AgNPs and A. Indica AgNPs showing the best results followed by NaOCl and distilled water.

Conclusion: The AgNPs in prepared root canals can serve as a good alternative as an irrigating solution. Biosynthesised AgNPs are equivalent to Trisodium citrate AgNPs in regard to wettability.

Keywords: Azadirachta indica, Endodontics, Gram-positive bacteria, Sodium hypochlorite, Trisodium citrate

INTRODUCTION

Endodontic success is primarily measured by the achievement of a three-dimensional seal and the eradication of bacteria and bacterial by-products. In order to reach this goal, chemomechanical root canal preparation uses a variety of irrigants as required adjuncts to help remove microbes, necrotic tissue, biofilms, and debris from the root canal [1]. It is well known that endodontic infections are multimicrobial [2].

For complete root canal disinfection, it would be undesirable to use an antimicrobial agent that targets only certain types of bacteria (gram-positive or gram-negative microbes, or only aerobic microbes) [3]. Therefore, the irrigating solution, apart from being biocompatible, should have the ability to dissolve necrotic pulpal remnants, a broad antimicrobial spectrum, the ability to inactivate endotoxin, high efficacy against bacterial biofilms, and efficiency in smear layer removal formed during mechanical preparation [4]. This is especially important for teeth with complex internal anatomy [5].

Currently, all irrigants in use have certain limitations, and the search for the ideal root canal irrigant continues. The irrigating solution most widely used is sodium hypochlorite due to its ability to dissolve pulp tissues, effectiveness as a lubricant, and marked bactericidal activity [6].

Silver nanoparticles (AgNPs), a type of metallic nanoparticle, have drawn particular attention in scientific studies because they exhibit antibacterial qualities and biological activity against bacteria, fungus, and enveloped viruses. The release of cationic silver and its oxidative potential is primarily responsible for how AgNPs work. Due to their nanoscale dimensions, AgNPs have the capability to enter bacterial cell walls and induce alterations in the structure of the cell membrane.

The nanoscale size facilitates the denaturation of the cytoplasmic membrane, leading to the rupture of organelles and potentially causing cell lysis [7].

The synthesis of AgNPs using various microorganisms revealed that the formation of AgNPs can occur both inside and outside the cell. In extracellular synthesis, the process involves the presence of proteins and enzymes on the cell wall of bacteria, along with secreted proteins, facilitating the reduction of Ag+ to Ag0. This extracellular synthesis of AgNPs was observed in Gram-positive bacteria like Bacillus species (including B. pumilus, B. persicus, B. licheniformis, B. indicus, and B. cecembensis), Planomicrobium sp., Streptomyces sp., Rhodococcus sp., as well as in Gram negative bacteria such as Klebsiella pneumoniae, Escherichia coli, and Acinetobacter calcoaceticus [8,9].

The dispersion of AgNPs is known to be cytotoxic-free and biocompatible, although its antibacterial efficacy has been proven [10]. However, in the past, no study has evaluated the effect of AgNPs on dentin surface characteristics.

In the past, dentinal surface treatment caused changes in the chemical and structural characteristics of dentin, thereby affecting its wettability with various substances [6]. Wettability, in terms of contact angle, is the angle of intersection between the tangent to the plane solid surface on which it rests and a liquid drop [9]. Wettability and surface-free energy have an inverse relationship with the contact angle. In comparison to a larger contact angle, a surface with a lower contact angle has superior wettability. Additionally, the measurement of the contact angle also indicates the adsorption and spreading of liquids [8,11]. Greater adherence to the dentin surface is achieved with lower contact angles [12].

Chemical solutions must possess a significant amount of wetness (for endodontic usage), which improves their antimicrobial activity and solvent capability in non instrumented areas of the root canal system [13]. The present study was conducted to assess the contact angle among Biosynthesised AgNPs (AgNPs synthesised from Azadirachta Indica/Neem) and Trisodium citrate AgNPs (chemically synthesised AgNPs), NaOCl, and distilled water to calculate their wettability.

MATERIALS AND METHODS

This In-vitro study was conducted at White Lab, Department of Conservative Dentistry and Endodontics, Saveetha Dental College Saveetha Institute of Medical and Technical Sciences Saveetha University, Chennai, Tamil Nadu, India, from January 2023 to August 2023. The study utilised a longitudinal design to investigate the wettability of various irrigants on human dentin specimens. A total of 40 single-rooted human teeth, extracted due to periodontal conditions or orthodontic reasons, were utilised. Ethical clearance was obtained from the relevant ethical committee with the number SDC/Ph.D./07/18/49. Informed consent was obtained from all participants or donors of the extracted teeth.

Sample size: The sample size calculation was based on the availability of suitable teeth. Thus, the sample size determined was 40 in each group for four groups to achieve a margin of error of 10% and a confidence level of 95%.

The teeth were placed at 20°C in a sterile phosphate buffer solution with a 1% antibiotic-antimycotic solution (Corning Life Sciences, Tewksbury, MA, USA). At the time of experimentation, any debris or soft tissue was removed and evaluated under a dissecting microscope to avoid teeth with cracks or hypoplastic defects.

Inclusion criteria: Inclusion criteria involved the selection of single-rooted human teeth extracted for periodontal conditions or orthodontic reasons.

Exclusion criteria: Exclusion criteria included teeth with cracks, hypoplastic defects, or any observable debris or soft tissue. The final sample consisted of 40 teeth meeting these criteria.

Study Procedure

Specimen preparation and parameters studied: The teeth were longitudinally sectioned in a bucco-lingual direction, acquiring two halves for dentin specimen preparation (4×4×1 mm) [Table/Fig-1].

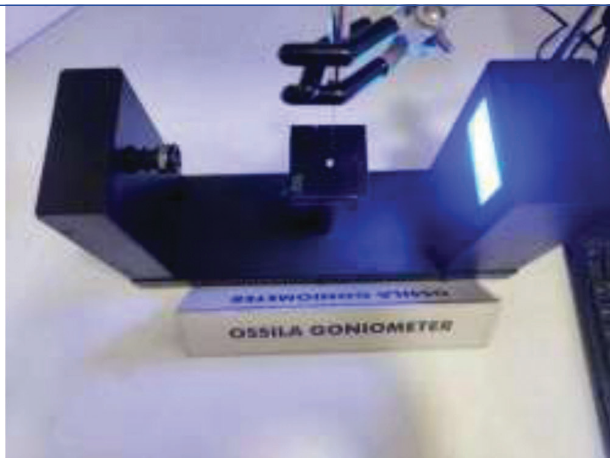
[Table/Fig-1]: Illustrates the bucco-lingual longitudinal sectioning of teeth, yielding two halves for dentin speciemen preparation.

Each flattened or cut (pulpal) side root half was used as a dentine sample after standardisation to dimensions of 4×4×1 mm. After that, the exposed dentin surface of the root segments was horizontally immersed in autopolymerising acrylic resin (Acrostone, Dent Product, Egypt), leaving the dentin surface exposed. The dentinal surface of the mounted specimens was ground smooth and flat under distilled water using a series of carbide abrasive papers in ascending grades of 500, 800, 1,000, and 1,200 grit (Bigo, Dent Product, Germany). Finally, the surface was polished to obtain a glossy, smooth, mirror-like surface with a 0.1-mm alumina suspension on a rotary felt disc (Microdont LDA, Brazil). The samples were randomly assigned according to the irrigation solution used, as described in [Table/Fig-2] (n=10/group).

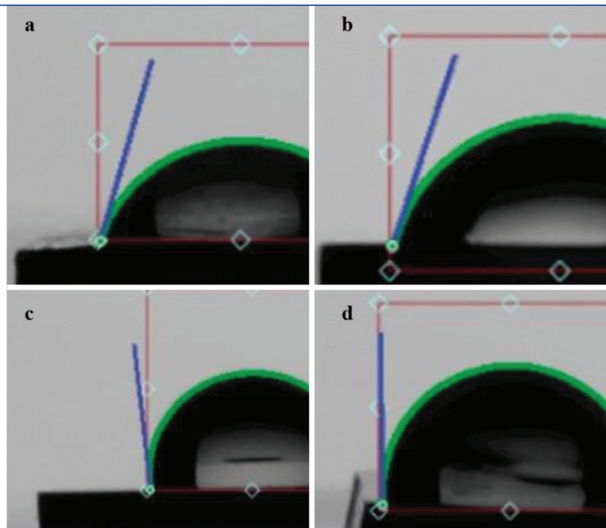
Group-1	Biosynthesised AgNPs (Biosynthesised AgNPs synthesised from Azadirachta Indica/Neem)
Group-2	Trisodium citrate Silver Nanoparticle (chemically synthesised AgNPs)
Group-3	5.25% NaOCl
Group-4	Distilled water (as a control)

[Table/Fig-2]: Sampling of the groups.

Contact angle measurement: The contact angle of the irrigant was determined using an Ossila goniometer on a prepared dentine slab of 4×4×1 mm. After placing the irrigant droplet on the surface of the dentine slab, measurements were taken to determine the contact angle, which was captured using a digital camera attached to a goniometer, as shown in [Table/Fig-3], using the tangent of the angle between the solid surface and the droplet. Images of the droplets were immediately analysed to provide the values of the contact angle, as shown in [Table/Fig-4a-d].



[Table/Fig-3]: Ossila Goniometer for measuring contact angle.



[Table/Fig-4]: (a) Images of the drop analysed to provide the values of contact angle; (b) Group-1 (Biosynthesised AgNPs); b) Group-2 (Trisodium citrate AgNP); (c) Group-3 (5.25% NaOCl); (d) Group-4 (Distilled water).

STATISTICAL ANALYSIS

Statistical analysis was performed using parametric ANOVA (one-way analysis of variance) to identify significant differences between the irrigant solutions. Tukey's post-hoc analysis was employed to discern specific variables that differed significantly. A significance level of $p<0.05$ was established for all analyses.

RESULTS

The average contact angle values for the various irrigants are displayed in [Table/Fig-5]. The variance analysis (ANOVA) revealed that among the tested irrigants, there is no statistically significant difference, although Group-1 and Group-2 showed lower contact angles when compared with other groups. As the comparison was not found to be statistically significant, a posthoc pair-wise comparison was not required.

In the field of endodontics, silver nanoparticles (AgNPs) demonstrate diverse applications. They can be integrated into irrigants and medicaments, serving as a multi-purpose solution to prevent biofilm regrowth. AgNPs exhibit antibacterial effects, making them potential candidates for root canal disinfection, although some studies report varied efficacies. Additionally, AgNPs enhance the properties of root canal sealers, medicaments, Mineral Trioxide Aggregate (MTA), and fibre posts, contributing to improved endodontic outcomes. Their application extends to endodontic surgery, where they enhance physicochemical properties and biocompatibility. Notably, AgNPs may play a role in mitigating postoperative pain in endodontic procedures. These advancements highlight the evolving landscape of endodontics, incorporating nanotechnology for enhanced therapeutic outcomes [17].

The current study aimed to evaluate the contact angle of irrigants (Trisodium citrate AgNPs, Biosynthesised AgNPs, NaOCl, DW) and dentin to determine wettability. Wettability refers to the tendency of a given fluid to spread or adhere to a solid surface. The spreading is linked to the extension of the projected area, while wetness is correlated with the contact angle [1]. This property is crucial for chemical solutions to penetrate the dentinal tubules, including main and lateral canals, and is largely dependent on surface tension [17]. In the present study, AgNP and TCS exhibited the highest wettability, followed by NaOCl and distilled water [18].

Groups	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum	p-value
				Lower bound	Upper bound			
Group-1	29.25	4.77	1.69	24.23	34.23	21.87	34.76	0.063
Group-2	29.85	4.87	1.99	24.73	34.97	21.97	34.76	
Group-3	32.36	2.03	0.83	32.22	36.49	31.15	37.27	
Group-4	31.92	5.55	1.13	29.27	33.96	20.55	40.28	

[Table/Fig-5]: Different groups showing contact angles of different irrigant solutions with dentin.

DISCUSSION

After instrumentation, irrigation is the best approach for eliminating dentin debris and tissue fragments. It has been suggested to employ a variety of root canal irrigants [13]. The influence of mechanical cleaning, the decrease of friction, and temperature management are all significant foundations for irrigation; nonetheless, the tasks of dissolving tissue (organic and inorganic) and eliminating pathogens are of utmost importance. The dentin/restoration interface could be altered when the mechanical characteristics of the dentin change [14].

Anterior teeth were selected for the present In-vitro study for the ease of longitudinally separating single-rooted teeth and exposing the root dentin surfaces for testing. Acrylic resin was used to provide proper support and a base for further testing. The sections made were embedded in it, and to prevent desiccation of the teeth, sectioning was done under water-cooling. The sections were then ground and polished for an even and smooth surface.

The wettability of irrigants is determined by the contact angle, which is measured by directly assessing the angle tangent to the liquid droplet and the solid surface of the substrate. Experimental values of the contact angle can be determined this way [15,16].

If the contact angle value is less than 90°, the liquid (sealer) wets the substrate; if it is more than 90°, it is considered non wetting. A contact angle of zero represents complete wetting. Therefore, contact angle and wettability provide an inverse measure, meaning that the lower the contact angle, the better the wettability [17].

Factors that can alter the contact angle include surface tension on the liquid side and the hydrophobic effect on the solid side, caused by changes in porosity due to surface energy and roughness effects based on alterations in the surface molecules' 3-dimensional structure. Consequently, polishing of the dentin surface was performed to reduce the influence of roughness on the surface energy of the root dentinal wall, thereby affecting the contact angle measurement [18].

In the present study, no substances were added to the selected irrigants; they were tested in their pure form. Surface-active agents can influence the surface tension of irrigants, thereby enhancing their wettability on a surface. Moreover, temperature and pH levels can impact the surface tension of solutions. Altering the temperature or pH can also affect the surface tension levels [19,20].

Due to the increased surface area that enables better contact between ions and microbial cells, nanoparticles demonstrate enhanced antibacterial activity [19]. The properties of nanoparticles differ from those of their corresponding bulk material due to the increased surface-to-volume ratio [21], affecting their biocompatibility and cytotoxicity, which are significantly lower compared to conventional compounds [22]. Despite AgNPs exhibiting superior wettability, studies indicate that their antifungal and antimicrobial activity surpasses that of sodium hypochlorite [23,24]. The study results revealed that the highest contact angle was observed with hypochlorite. AgNPs Group-1 and 2 showed no significant difference between them, with biosynthesised AgNPs displaying a low contact angle and better wettability. These findings align with Abbaszadegan A et al., who noted that AgNP irrigant could potentially reach the apical portions of root canals due to its lower viscosity and influence the physiochemical properties of dentin by reducing its surface wettability [25].

For an ideal irrigant solution, good wettability should be coupled with effective antimicrobial activity. Trisodium citrate AgNPs and Biosynthesised AgNPs can be considered as suitable irrigants due to their ability to enhance wettability on the dentin surface, a crucial aspect of endodontic therapy, in addition to their antibacterial effects and minimal cytotoxicity. Therefore, Trisodium citrate AgNP (chemically synthesised AgNPs) and Biosynthesised AgNPs (AgNPs synthesised from Azadirachta Indica/Neem) utilised in the current study were found to be superior to Hypochlorite.

The present study is consistent with Abbas Abbaszadegan's findings, suggesting that AgNP irrigant exhibits high surface tension comparable to distilled water [25]. Another study by Vaiyshnavi W et al., revealed that in Subgroup C, where 0.2% AgNPs were incorporated, there was a consistent improvement in contact angle values compared to Subgroup A (0% Ag nanoparticles) and Subgroup B (0.05% Ag nanoparticles) throughout the storage periods. This

S. No.	Author/year/country	Type of study	Irrigants	Contact angle/surface tension	Results/inference
1.	Present study	Comparative experimental study	Trisodium Citrate AgNPs, Biosynthesised AgNPs, NaOCl, and distilled water.	The study focused on measuring the contact angle and surface tension properties of the irrigants to assess their wettability and dentinal tubule penetration potential.	Trisodium Citrate AgNPs and Biosynthesised AgNPs showed superior wettability. AgNPs had better wettability than NaOCl. Biosynthesised AgNPs were optimal due to low contact angle.
2.	Abbaszadegan A et al., 2019 Iran [25]	Clinical study	17% EDTA, 2% Chlorhexidine (CHX) 5.25% NaOCl, AgNPs at 5.7×10^{-8}	Water contact angles were determined using the sessile drop technique, while surface tension was measured utilising the Wilhelmy plate technique.	Significant variations in dynamic viscosity, water contact angles, and surface tension among irrigants with distinct impacts on dentin characteristics.
3.	Vaiyshnavi W et al., 2022 India [26]	Experimental design	Subgroup A-12 samples-0% Ag nanoparticles (control group) • Subgroup B-12 samples-0.05% of Ag nanoparticles • Subgroup C-12 samples-0.2% of Ag nanoparticles	Wettability which can be influenced by surface tension properties.	Wettability increased over time with subgroup C displaying the highest, followed by B and A. Subgroup C showed the most significant antifungal activity. Flexural strength was highest in subgroup C.
4.	Garbacz H et al., 2018 Poland [27]	Experimental study	Grain size of titanium and Silver Nanoparticles (AgNP)	Contact angle measurements reveal nanocrystalline titanium's hydrophilic nature, attributed to distinct oxide layers compared to microcrystalline titanium.	Nanocrystalline titanium displays an alkaline surface with higher Lewis base components, enhancing protein interaction and potentially favoring cell adhesion. These findings suggest nanocrystalline titanium's potential for positive biological responses.
5.	Farshad M et al., 2016 Iran [28]	Prospective study	17% EDTA, 2% CHX, 5.25% NaOCl, ImSNP (synthesised), and distilled water as control	Dentin surface roughness evaluated by Atomic Force Microscopy (AFM).	Dentin surface roughness significantly increased in all tested groups compared to the control (distilled water). ImSNP-treated dentin showed significantly lower roughness compared to CHX-treated dentin. However, no significant differences were observed between ImSNP and NaOCl or EDTA-treated dentin.

[Table/Fig-6]: Previous studies in comparison with the present study [25-28].

indicates a significant enhancement in wettability with the increased concentration of AgNPs, highlighting their potential for improving root canal irrigants [26]. A study conducted by Garbacz H et al., exploring the impact of particle size and shapes on the wetting characteristics of metallic micropowders, revealed an inversely proportional relationship. It showed that smaller particle sizes were correlated with elevated contact angle values [27].

The study revealed that ImSNP increased dentin roughness similar to NaOCl and Ethylenediaminetetraacetic Acid (EDTA) but less than CHX. This finding suggests ImSNP's potential influence on bacterial adhesion and root canal dentin surface properties [Table/Fig-6] [25-28]. To enhance the evidence-based nature of this discussion, tables comparing the present study with relevant published reports, including the latest articles, will be incorporated.

The present study examines the wettability of various root canal irrigants, including Trisodium citrate AgNPs, Biosynthesised AgNPs, NaOCl, and distilled water, assessing their contact angles with dentin. The research highlights the superior wettability of AgNPs, particularly Trisodium citrate AgNPs and Biosynthesised AgNPs, surpassing NaOCl and distilled water. Despite the high surface tension observed in AgNP irrigant, potentially limiting dentinal tubule penetration, its favourable wettability, antibacterial effects, and minimal cytotoxicity position it as a promising substitute. The findings expose a gap in the literature, prompting further exploration of the interplay between AgNPs' chemical characteristics, antibacterial abilities, and their impact on sealant adherence in the intricate root canal system. Future research should address these nuances, contributing to a comprehensive understanding of their clinical implications.

Limitation(s)

Future research is required to examine the antibacterial abilities of AgNPs in conjunction with the various characteristics that affect their activity. The size of the particles and the duration of contact are apparent to be significant variables. Due to the complicated anatomy of the root canal system, this could not always be the case in clinical settings. Therefore, more research is required to determine how much these chemical changes may impact sealants' ability to adhere to the surfaces they are applied.

CONCLUSION(S)

Within the parameters of the present investigation, it can be claimed that endodontic irrigation with AgNPs showed maximum wettability. Biosynthesised AgNPs thus can serve as a good alternative as an irrigating solution.

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