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Research Article

A Comparative Evaluation of Antimicrobial and Five Physical Properties of Irreversible Hydrocolloid Incorporated with Silver Nanoparticles and Curcumin: An In-Vitro Study

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ABSTRACT

Background and Objectives: Dental impressions are widely used in dentistry and they come in contact with patient's saliva, blood and bacterial plaque. Therefore, disinfection is mandatory. The purpose of this study is to evaluate and compare antimicrobial property of irreversible hydrocolloid impression materials incorporated with silver nanoparticles and curcumin and its effect on five physical properties- gelation time, gel strength, permanent deformation, flow and dimensional accuracy. ***Materials and Methods:*** A total of 64 specimens, 32 for each brand and subgroups used (Zelgan and Tropicalgin) were mixed according to manufacturer's instruction. Silver nanoparticles and curcumin were added as antimicrobial agents. The samples were tested for antimicrobial activity against Staphylococcus aureus and Candida albicans and for five physical properties - gel strength, permanent deformation, flow, gelation time and dimensional accuracy. Statistical analysis was done with one-way ANOVA to compare within the group and Tukey post hoc to compare between the groups. ***Results:*** The results of silver nanoparticles in Zelagan irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(7mm) and S. aureus(17mm). The mean gelation time(126sec), permanent deformation(43.2%) increased whereas, and gel strength(1.4MPa) decreased and curcumin in Zelagan irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(18mm) and S. aureus(27mm). The mean gelation time(105sec) and gel strength(4.3MPa) and flow(17mm) increased whereas, permanent deformation(24%) decreased. Silver nanoparticles in Tropicalgin irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(15mm) and S. aureus(15mm). The mean gelation time(40sec), permanent deformation(28.6%), gel strength(5MPa) and flow(17sec) increased and curcumin in Tropicalgin irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(7mm) and S. aureus(22mm). The mean gel strength(4.9MPa) and flow(17.4mm) increased whereas, permanent deformation(24.6%) and gelation time(94sec). ***Interpretation and Conclusion:*** From this invitro study, we conclude that, the incorporation of silver nanoparticles and curcumin in irreversible hydrocolloid (Zelgan and Tropicalgin) showed statistically significant improvement in antimicrobial activity and all the physical properties. Zelgan incorporated with curcumin was the best combination.

Introduction

A dental impression is a negative replica of oral structures that is used to create a positive replica for use as a permanent record or in the fabrication of a dental restoration or prosthesis. Owing to its simplicity of mixing, the flexibility of the set impression, accuracy when properly handled and inexpensive cost, alginate is one of the most extensively used dental impression materials [1].

Dental impressions come in contact with patient's saliva, blood and bacterial plaque. The surface texture and hydrophilic nature of irreversible hydrocolloid impression material allow it to retain the maximum quantity of microbial pathogens not only on the surface but also deep within the substance during impression recording[2].

Disinfectants must be anti-microbially effective without compromising the dimensional correctness of the impression material and the subsequent gypsum cast, which is used to manufacture the denture. Irreversible hydrocolloids are disinfected using a disinfectant solution and either a spray or an immersion technique. Sodium hypochlorite, sodium metabisulphite, and biguanides, biguanides, iodine compounds (such as iodophors), quaternary ammonium salts, phenolics, and glutaraldehyde are some of the routinely used disinfectants[3].

Both spray and immersion methods, however, solely disinfect the impression on the surface. Furthermore, these processes may cause considerable dimensional changes in permanent hydrocolloid imprints

, resulting in detail loss. To overcome this, antimicrobial agents such as silver nanoparticles and curcumin can be incorporated in irreversible hydrocolloid[4].

Silver nanoparticles can continually release silver ions, which may be considered the mechanism of killing microbes. Owing to electrostatic attraction and affinity to sulfur proteins, silver ions can adhere to the cell wall and cytoplasmic membrane. The adhered ions can enhance the permeability of the cytoplasmic membrane and lead to disruption of the bacterial envelope[5].

Turmeric, scientifically known as Curcuma longa L. and belonging to the Zingiberaceae family, is extensively cultivated in tropical regions of Asia. Also referred to as turmeric root or yellow root, it typically grows 3–5 feet tall with oblong leaves and yellow funnel-shaped flowers. Cultivation thrives in well-drained sandy or clay loam soils with a pH of 4.5–7.5 and good organic content, under temperatures of 20–35°C and an annual rainfall of 1500 mm[6]. This spice has a rich history in Asian cuisines and globally, known as Zard choobe in Persian. Turmeric enhances the flavor and color of dishes like rice, yogurt, and chicken, often used in combination with other spices. Beyond culinary uses, various communities, notably in China, India, Iran, and Indonesia, employ turmeric and its derivatives in traditional medicines for conditions such as dyslipidemia, stomach disorders, arthritis, and hepatic diseases. Curcumin, a polyphenol extracted from turmeric, contributes to its yellow color and comprises curcumin I, curcumin II, and curcumin III. With up to 8% curcumin content in the dehydrated root, turmeric also contains diferuloylmethane and volatile oils[7, 8].

Curcumin exhibits anti-inflammatory, antimicrobial, antitumoral, antioxidant, and hypolipidemic properties. It has been recognized for its potential in preventing cancer by countering inflammation and oxidation processes. Turmeric oil, containing essential fatty acids, demonstrates antifungal, antimutagenic, and antibacterial activities[9]. The medicinal attributes of turmeric, particularly curcumin, are illustrated in **Figure 1.**

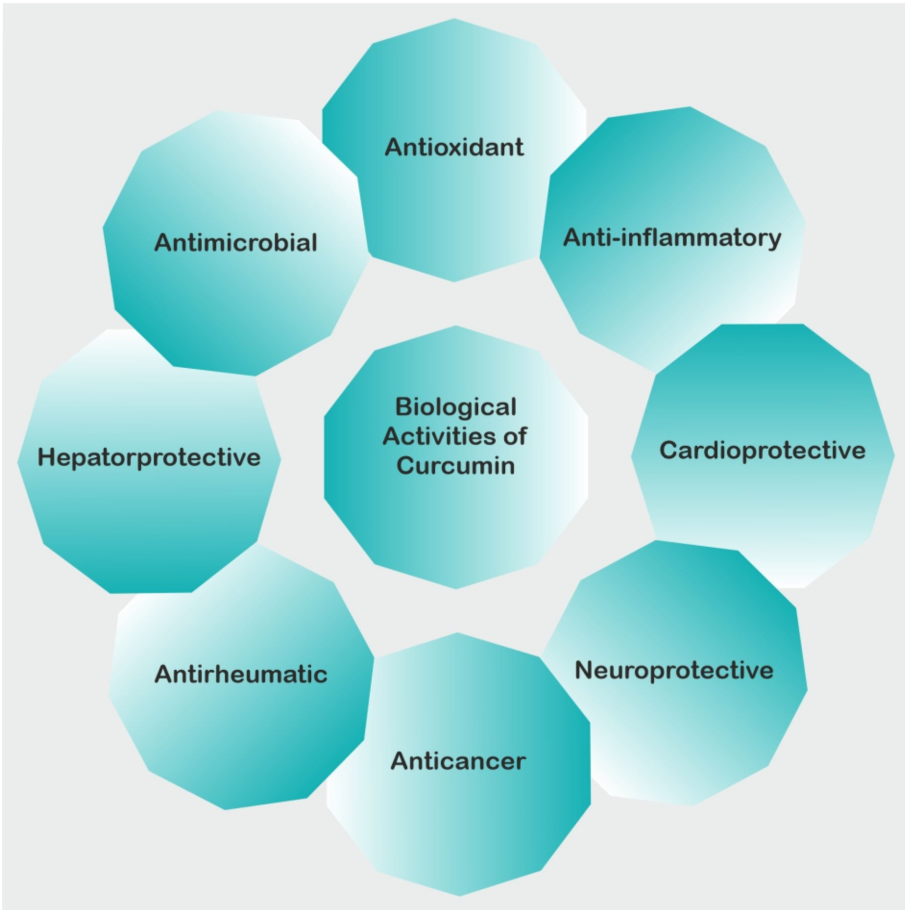

Figure 1: Different pharmacological activity of curcumin

In 1949, Schraufstatter and colleagues were pioneers in reporting the antibacterial properties of curcumin. Over the last seven decades, numerous studies have investigated curcumin's broad-spectrum inhibitory effects against both Gram-negative and Gram-positive bacteria. The list includes A. baumannii, E. faecalis, K. pneumoniae, P. aeruginosa, Bacillus subtilis (B. subtilis), Staphylococcus epidermidis, Bacillus cereus (B. cereus), Listeria innocua, Streptococcus pyogenes, S. aureus, Helicobacter pylori (H. pylori), Escherichia coli (E. coli), Salmonella enterica serotype Typhimurium, and Streptococcus mutans[10, 11].

Crucially, curcumin has demonstrated substantial antibacterial activity against multidrug-resistant (MDR) isolates, such as polymyxin-resistant K. pneumoniae and methicillin-resistant Staphylococcus aureus (MRSA). Recent research by Batista de Andrade Neto *et al.* revealed minimum inhibitory concentration (MIC) values for curcumin against clinical MRSA isolates ranging from 125 to 500 µg/-

mL [12].Another study by Yasbolaghi Sharahi et al. reported MICs of curcumin against MDR A. baumannii, P. aeruginosa, and K. pneumoniae in the range of 128–512 µg/mL. Importantly, variations in reported MICs among different research groups may be attributed to differences in curcumin solubility in various vehicles (e.g., water, DMSO, and ethanol), MIC test methodologies, the impact of the vehicle on the bacterial outer membrane, and the purity of the curcumin used in the studies[13-15].

Nanotechnology, an advanced technology with potential applications in various fields. Diverse nanotechniques, including nanoparticles and nanoemulsions, have been employed to bolster antioxidant and antibacterial properties [16]. Curcumin's limited water solubility, approximately 11 ng/mL, leads to poor bioavailability when orally consumed. Furthermore, its rapid degradation results in low concentrations in the bloodstream and organs, posing challenges in achieving effective concentrations for treating bacterial infections in organs like the liver and lungs [17, 18]. To address these issues, scientists have devised various nanocarriers for curcumin, including lipid-based formulations such as liposomes, solid lipid nanoparticles, nanostructured lipid carriers, and nano-emulsions, as well as biopolymers like nanocomposites, polymeric nanoparticles, hydrogels, and polymeric micelles **(Figure 2).** Other techniques include spray-dried nano-formulations, nanofibers, and miscellaneous types like curcumin nanocrystals, quantum dots, and graphene oxide[19-21].

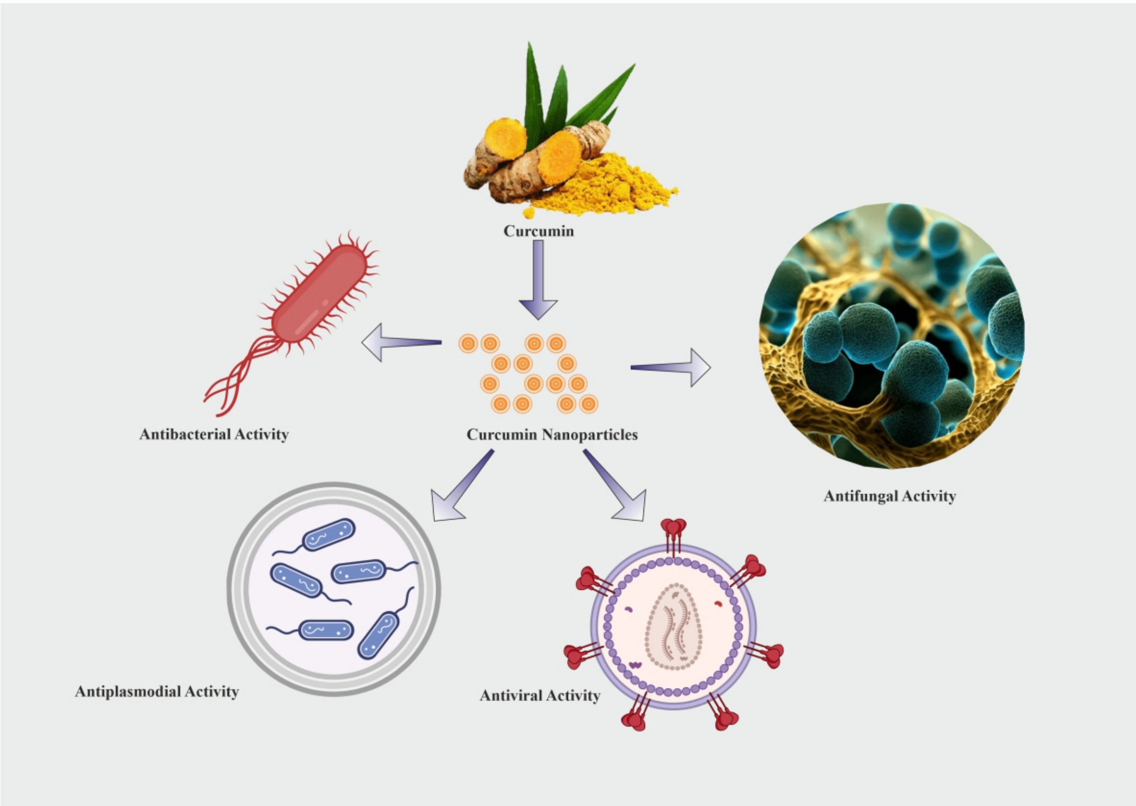

Figure 2: Anti-bacterial activity of curcumin nanoparticles

Combinations of curcumin with other antibacterial agents effective against bacteria, particularly in cancer therapy, have also been developed. Notably, despite their improved antibacterial effects observed in vitro and animal experiments due to enhanced solubility and biocompatibility, there has been no clinical trial to test the effectiveness of these various nano-formulations of curcumin [14, 22]. Therefore, additional clinical trials are still needed. In addition to nano-formulations, various new formulations like inclusion technology, solid dispersion technology, microspheres, and microcapsules have been developed to enhance curcumin's solubility and bioavailability. For instance, Yaday *et al.* demonstrated that cyclodextrin (CD) complexes of curcumin could increase its solubility in water by over 100 times compared to curcumin alone. However, like nano-formulations, these new curcumin formulations are currently in the laboratory research stage, lac-king necessary clinical studies[23, 24].

Dental impressions play a crucial role in dentistry, frequently coming into contact with a patient's saliva, blood, and bacterial plaque. As a result, disinfection is imperative [25]. This study aims to assess and compare the antimicrobial efficacy of irreversible hydrocolloid impression materials integrated with silver nanoparticles and curcumin. Additionally, the research seeks to investigate the impact of these additives on five key physical properties: gelation time, gel strength, permanent deformation, flow characteristics, and dimensional accuracy.

Materials and Methods

The two commercial irreversible hydrocolloids studied were a Zelgan and Tropicalgin. Silver nanoparticles(0.001wt%) and curcumin(0.1wt%) were added to these irreversible hydrocolloids, and their properties were evaluated.

Manipulation of Material

Manipulation of irreversible hydrocolloid impression material that are incorporated with silver nanoparticles and curcumin was done by mixing powder (gm) and water(ml) in the ratio of 4:9 for 45 seconds and the temperature of water used was 370 C and was assessed for antimicrobial activity and five physical properties which includes gelation time, gel strength, permanent deformation, flow and dimensional stability.

Method for Assessing Antimicrobial Activity

Antimicrobial activity was assessed using the Kirby Bauer disk diffusion method. Disks of 3 mm thickness and 8 mm diameter were made by placing experimental groups. Disks were placed on Mueller Hinton agar plates containing lawn cultures of Staphylococcus aureus and Candida albicans and were incubated at 37 degrees centigrade for 24 hours. The lawn cultures of microbes to be tested were made with a yeast suspension matching the turbidity of a 0.5 Mc Farland standard. To test anticandidal activity, Mueller Hinton agar plates were incorporated with 2% glucose. After 24 hours, antimicrobial activity was evaluated by measuring the zones of inhibition in milli-meter **(Figure 3).**

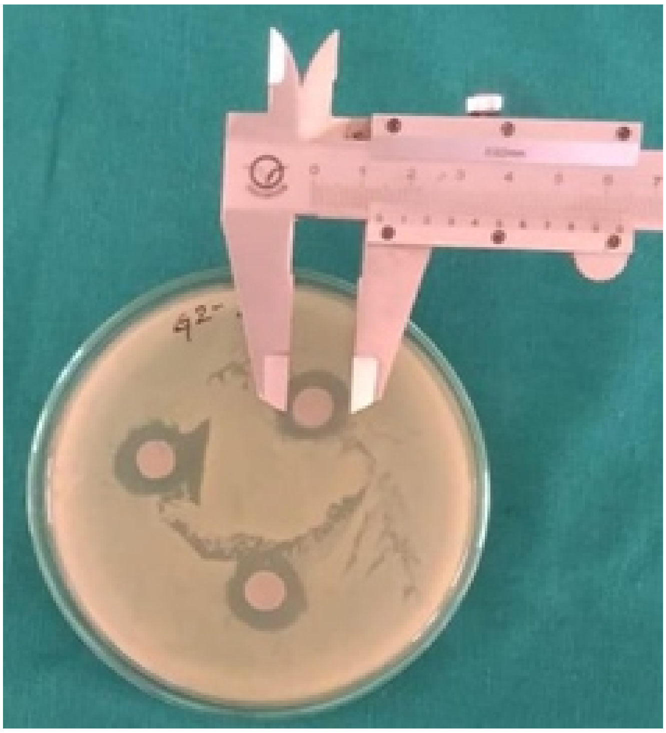

Figure 3: Measuring zone of inhibition

Method for Assessing Flow

Flow was determined as described by wang et al3. 0.5 ml of material was injected onto a glass plate, another glass plate was placed on top and a load of 14.7N (1.5kg) was applied for 5 seconds. Diameter of impression formed was measured at 3 different areas and average diameter was considered as flow **(Figure 4).**

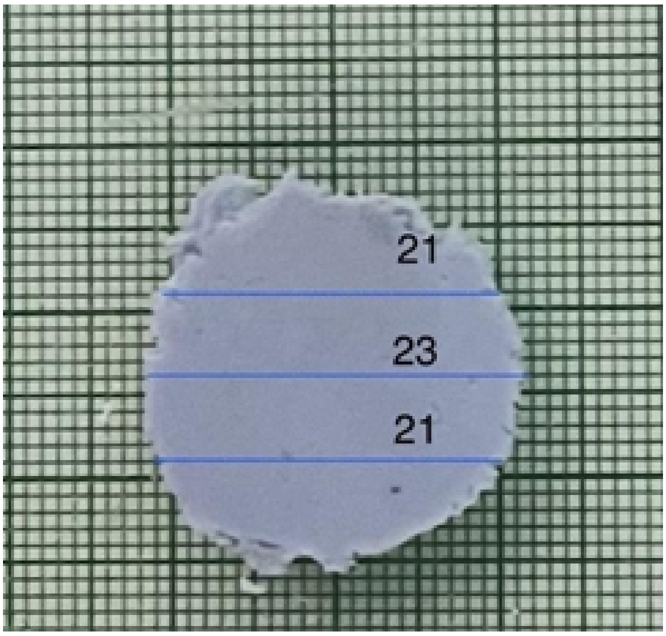

Figure 4: Measurement of flow

Method for Assessing Gel Strength

Gel strength was measured using universal testing machine. The mould was of cylindrical shape with 8 x 13 mm in dimension. The impression material was poured into the mould placed on a glass plate, and the excess was squeezed out by placing a second glass plate on the top of the mould. The thickness of the specimens was determined with a standard thickness gauge for testing rubber. After 45 seconds of mixing the samples with water of 37 degrees centigrade in the ratio of 4:9, the material was inserted into the mould and was allowed to set. After 5.15 mins the material was placed on bottom plate of universal testing machine and was stressed at a rate of 10 mm/min. Gel strength was calculated as maximum compressive load reported during the test divided by area of material **(Figure 3).** (Gel strength = maximum compressive load /area of material).

Figure 5: Measurement of gel strength and permanent deformation using universal testing Machine

Method for Assessing Dimensional Accuracy

For evaluation of dimensional accuracy, impressions were made on maxillary dentulous cast. These impressions were poured using dental stone. 3 points were marked on the casts (one on mesio-incisal angle of right central incisor and one each on mesiopalatal cusp of first maxillary molar) and distance between these points was measured using vernier caliper (cast poured after 5 minutes of impression making) **(Figure 6).**

Figure 6: Dimensional accuracy measurement using vernier calliper

Method for Assessing Gelation Time

Gelation time was measured using indentation method as described by lemon et al.6 After 45 seconds of mixing samples with water of 37 degrees centigrade in the ratio of 4:9, polymethylmethacrylate rod was placed in contact with the sur--face of the material and was withdrawn immediately and wiped clean. This was repeated at every 5 seconds interval until impression material no longer adhered to the rod. The time from the start of mix till the material no longer adhered to the rod was considered as gelation time. The significant difference between the control group, group incorporated with silver nanoparticles and the group incorporated with curcumin were compared for antimicrobial activity and physical properties.

Results

The results revealed that the incorporation of silver nanoparticles in Zelgan irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(7mm) and S. aureus(17mm). The mean gelation time (126sec), permanent deformation (43.2%) increased and gel strength(1.4MPa) decreased whereas, flow(16.18mm) and dimensional accuracy(40mm) did not alter. Curcumin in Zelgan irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(18mm) and S. aureus (27mm). The mean gelation time (105sec) and gel strength (4.3MPa) and flow (17mm) increased and permanent deformation (24%) decreased whereas, dimensional accuracy(40mm) did not alter. Zelgan when incorporated with curcumin showed superior properties as compared to that incorporated with silver nanoparticles.The results revealed that the incorporation of silver nanoparticles in Tropicalgin irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(15mm) and S. aureus(15mm). The mean gelation time(40sec), permanent deformation (28.6%), gel strength(5MPa) and flow(17sec) increased whereas, dimensional accuracy(40mm) did not alter. Curcumin in Tropicalgin irreversible hydrocolloid showed increase in antimicrobial activity against C. albicans(7mm) and S. aureus(22mm). The mean gel strength(4.9MPa) and flow(17.4mm) increased whereas, permanent deformation (24.6%) and gelation time(94sec) whereas, dimensional accuracy(40mm) did not alter. Tropicalgin when incorporated with curcumin showed superior properties as compared to that incorporated with silver nanoparticles **(Table 1).**

Table 1: Physical properly and anti microbial activity of silver nano-particles

	Group 1a (z-control)	Group 1b (z-Ag)	Group 1c(z-curcumin)	Group2a (t-control)	Group 2b(t-Ag)	Group 2c (t-curcumin)
Gel strength (MPa)	2.264	1.796	4.392	4.225	4.495	5.697
	2.313	1.098	4.275	6.524	7.175	4.45
	2.287	1.347	4.373	4.374	4.985	4.973
Permanent deformation (%)	42.467	51.217	27.44	24.939	29.339	26.191
	43.723	34.961	21.852	31.818	28.08	21.817
	43.295	43.529	23.646	28.378	28.519	25.054
Gelation time (seconds)	100	125	105	135	140	90
	95	130	105	130	140	95
	95	125	105	135	140	95

Flow (mm)	16.36	16.16	17	15.66	16.33	17.33
	16.0	16.16	17	16	18.83	17.66
	16.25	16.28	17	15.83	17.88	17.585
Dimensional accuracy (mm)	40	40.1	40	40.1	40.1	40
	40	40.1	40	39.8	39.8	40.1
	40	40.1	40	39.95	39.95	40.05
Antimicrobial activity (mm) – S. Aureus	0	17	26	0	14	20
	0	17	25	0	15	21
	0	17	29	0	15	25
Antimicrobial activity (mm) – C. Albicans	0	7	18	0	15	7
	0	8	18	0	15	7
	0	7	18	0	15	7

Discussion

In dental clinical settings, the disinfection of impressions offers an efficient barrier against cross infection. Irreversible hydrocolloids have hydrophilic properties and porous structure that maximize the retention of microorganisms on the surface and within the substance. Since irreversible hydrocolloids lack strong antibacterial activity on their own, typical immersion or spray cleaning methods only treat the impression surface. However, the disinfectant integrated into the irreversible hydrocolloid offers a thorough disinfection of the substance. Silver nanoparticles in particular are increasingly being employed in numerous biomedical applications as antibacterial agents[26].

In the present study, silver nanoparticles and curcumin were incorporated into the irreversible hydrocolloid and antimicrobial activity was measured using disk diffusion method. In both Zelgan and Tropicalgin irreversible hydrocolloid impression material incorporation of silver nanoparticle and curcumin had significant increase in antimicrobial activity against S. Aureus and C. Albicans [27]. This may be due to the antimicrobial activity of silver ions. In addition to being able to release silver ions, silver nanoparticles can themselves kill bacteria. Silver nanoparticles can accumulate in the pits that form on the cell wall after they anchor to the cell surface. The accumulated silver nanoparticles can cause cell membrane denaturation. Silver nanoparticles also have the ability to penetrate bacterial cell walls and subsequently change the structure of the cell membrane, because of their nanoscale size [28, 29]. The denaturation of cytoplasmic membrane can rupture organelles, and even result in cell lysis. In addition, silver nanoparticles can be involved in bacterial signal transduction. Bacterial signal transduction is affected by phosphorylation of protein substrates, and nanoparticles can dephosphorylate tyrosine residues on the peptide substrates. Disruption of the signal transduction can lead to cell apoptosis and termination of cell multiplication[5, 30].

Flow of irreversible hydrocolloid impression material allows it to record all the finer details. Upon mixing the irreversible hydrocolloid with mixing liquid, it forms a sol which is fluid and as the gelation occurs its fluidity gradually decreases. The impression material should flow freely and wet the tissue as it is being placed to achieve adaptation, and then resist flow away from the intended surface areas [31]. Flow refers to time dependent plastic deformation and it is directly related to gelation time of material and inversely related to the viscosity of the material. Measuring flow of the impression material is essential as the maximum flow is expected for impression material during insertion as it allows excellent registration of details of the mouth[32].

Minimum flow or maximum viscosity is expected for an ideal material during removal to confine the material within the tray and to resist possible distortion outside the mouth. Flow depends mainly on time and temperature. In both experimental groups incorporation of silver nanoparticle had significant increase in flow. This may be due to increase in gelation time as flow is directly related to gelation time of the material. Flow of Tropicalgin is higher compared to that of Zelgan impression material[33].

Gel strength is defined as a measure of the ability of a colloidal dispersion to develop and retain a gel form based on its resistance to shear. Gel strength mainly depends on the flow of the material during impression making. In both Zelgan and Tropicalgin irreversible hydrocolloid impression material incorporation of silver nanoparticle had significant increase in gel strength. This may be due to the surface energy of this nanoparticle that caused a reinforcement of the cross-linked matrix[34].

Permanent deformation in irreversible hydrocolloid impression materials is related to the crosslinking density. Higher cross-linking density upon gelation imparts superior strength and elasticity to the gel. In ADA tests for permanent deformation, typical alginates undergo slightly more permanent deformation (1.5%). The amount of distortion will depend on the amount of deformation and length of time the impression is held in the deformed state. Larger deformations and longer holding times will increase the permanent deformation. Clinically, this translates into using relatively large thicknesses of impression material, so that distortions during withdrawal will yield small strains [35]. Also, the impression should be removed quickly to minimize the time in which the impression is distorted. Silver nanoparticles had more effect on permanent deformation compared to curcumin. Kishore in 2015 had done a similar study and found an increase in the permanent deformation with the incorporation of silver nanoparticles in both Zelgan and Tropicalgin. Compared to Zelgan irreversible hydrocolloid permanent deformation of Tropicalgin was lesser. Therefore, Tropicalgin is better impression material in terms of deformation[36].

Dimensional stability of impression controls the accuracy of the cast and thereby the prosthesis made based on the master cast. Therefore, it is mandatory to ensure the impression made is dimensionally stable. Volumetric changes may occur in hydrocolloid impressions due to imbibition of water or any other liquid in which the impression may come in contact before pouring [37]. Similarly, syneresis, the loss of water exudates from the surface of the impression may occur if the impression is kept in a dry atmosphere before pouring. Both Syneresis and imbibition are not recommended for impression materials as it leads to dimensional changes [38]. Thus, the present study evaluated the extent of dimensional changes occurred as a result of test criteria, fortunately, the results obtained was positive and no significant dimensional changes were observed. The value of experimental groups of silver nanoparticles and curcumin incorporated into irreversible hydrocolloid as compared to control group showed no significant difference between them at $p = 0.46$[39].

Gelation time refers to the formation and crosslinking of calcium alginate chains. The gelation time of irreversible hydrocolloids was found to increase with the increasing concentration of silver nanoparticles [40]. The value of experimental groups of silver nanoparticles and curcumin incorporated into Zelgan irreversible hydrocolloid as compared to control group showed increase in gelation time. The value of experimental groups of silver nanoparticles and curcumin incorporated into Tropicalgin irreversible hydrocolloid as compared to control group showed increase in gelation time. Similar study was done by Kishore Ginjupalli in 2015, he found that the gelation time of irreversible hydrocolloids was found to increase with the increasing concentration of silver nanoparticles[41, 42]. The increase in gelation time indicates a delay in the formation of calcium alginate gel. A similar increase in gelation time was observed with the incorporation of disinfectant into the irreversible hydrocolloid.

Conclusion

From this *in-vitro* study, we conclude that, the incorporation of silver nanoparticles and curcumin in irreversible hydrocolloid (Zelgan and Tropicalgin) showed improved antimicrobial activity. The incorporation of silver nanoparticles and curcumin in irreversible hydrocolloid showed increase in its gelation time, flow and gel strength whereas, decrease in permanent deformation. All of these properties have a positive impact on irreversible hydrocolloid impression making. Zelgan incorporated with curcumin has superior properties followed by Tropicalgin incorporated with curcumin followed by Zelgan incorporated with silver nanoparticles followed by Tropicalgin incorporated with silver nanoparticles.

Conflict of Interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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Ethical Clearance

This work has been approved by institutional ethical committee.

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