Original Article

An evaluation of antimicrobial potential of irreversible hydrocolloid impression material incorporated with chitosan

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Abstract

Aim: To evaluate the antimicrobial potential of irreversible hydrocolloid impression material manipulated using chitosan impregnated solution at various time intervals.

Setting and Design: Evaluative invivo study design.

Materials and Methods: Maxillary impressions made for 20 dentulous volunteers using irreversible hydrocolloid impression material manipulated using distilled water as control and using 1% chitosan impregnated solution as test group using stock metal trays with one-week interval. Bacterial samples were collected using dry sterile cotton swab in the mid-palatal region at the time intervals of 0, 10, 20 minutes. Bacterial swabs were inoculated on nutrient agar media and incubated at 37° C for 24 hours. Bacterial colonies were counted with the aid of colony counter.

Statistical Analysis Used: The resultant data was subjected to statistical analysis using repeated measures ANOVA and independent t test.

Results: Adding water soluble chitosan to irreversible hydrocolloid impression material resulted in superior antimicrobial activity. With the passage of time there was a significant decrease in the microbial colony count upto 10min ($p=0.016$). However, the rate of decrease of microbial colony count was statistically insignificant between the samples collected at 10 and 20 min.

Conclusion: Incorporation of water soluble chitosan to irreversible hydrocolloid impression material showed significant antimicrobial activity in 10 minutes.

Keywords: Antimicrobial and Antiviral agent, Cross infection, Dental impressions, Irreversible hydrocolloid impression material, Self-disinfecting alginates, Water-soluble chitosan

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INTRODUCTION

Irreversible hydrocolloid impression materials are being popularly used in dentistry for both diagnostic and definitive impression procedures.[1] While making the impression, they come in contact with the patient's saliva, blood, and plaque.[2,3] These contaminated impressions act as a medium for the transfer of microorganisms

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from patients to auxiliary dental staff and/or laboratory personnel placing them at a higher risk of cross-infection.[4]

Studies have shown that rinsing the irreversible hydrocolloid impressions with running tap water alone removes 40% of bacteria[5,6] and rinsing under the tap for 15 s reduced up to 90% of the contamination.[3] However, other researchers clearly emphasize on mere washing of impressions results in inadequate disinfection.[1] New researchers have shown that 67% of the impressions sent to the dental laboratories are infected by various microorganisms.[7,8] Taking this into account, effort should be made to eliminate the microorganisms and reduce the rate of infection transmission in dental laboratories.

International dental federation insists on disinfecting all impressions made from patients before sending them to laboratories.[8] American Dental Association has recommended high disinfection standards for dental equipment, including dental impressions to prevent cross-infection between members of the dental team. It has been suggested that impressions must be disinfected immediately after their removal from the mouth to reduce the risk of cross-infection.[9] The most common chemical disinfectants used are alcohols, aldehydes, chlorine combinations, phenols, biguanides, iodide combinations, and ammonium.[5]

The impressions are disinfected either by spraying of the disinfectant on the impression surface or by immersion of the impression in the disinfectant. The presently used disinfection techniques disinfect only the impression surface. Most of the surface disinfectants used are biological irritants. Inhalation of the disinfectant vapors may present health risks to the dental team.[10] Further, studies have proven that these disinfectant techniques may result in significant dimensional changes, loss of surface detail, deterioration in surface quality, and hardness of gypsum casts obtained from disinfected impressions.[11]

The difficulties associated with surface disinfection of irreversible hydrocolloid impression materials have resulted in the development of self-disinfecting irreversible hydrocolloid impression materials.[12] The self-disinfection of the impression material has shown to reduce the overall quantity of bacteria on the impression material.[10] One of the main advantages of self-disinfectant irreversible hydrocolloid impression material is that they are not only disinfected on the surface but also disinfected throughout the material as the disinfectant is distributed within the material.[11]

Chitosan is a natural, nontoxic biopolymer produced by the deacetylation of chitin.[13] Chitin is the main component of the cell walls of fungi and the exoskeleton of arthropods such as crustaceans (lobsters and shrimps).[14] It is a versatile hydrophilic polysaccharide with a potent antimicrobial and antiviral activity with broad spectrum and high killing rate and low toxicity toward mammalian cells.[13] Chitosan is being used in dentistry as an implant surface modifier, as a component in dental adhesives, dental composite resins, and in combination with dentifrices and mouthwashes to reduce plaque.[15]

After a thorough investigation in the literature, the use of chitosan as an antimicrobial agent in irreversible hydrocolloid impression materials has not been explored. The present study was formulated to evaluate the antimicrobial effect of mixing irreversible hydrocolloid impression material with chitosan impregnated solution as a method of disinfection and the sustainability of the antimicrobial potential of the irreversible hydrocolloid impression between the time interval of making the impression and pouring the cast.

MATERIALS AND METHODS

A pilot study was conducted to optimize the feasibility of manipulating irreversible hydrocolloid impression material (3M ESPE, Germany) using chitosan impregnated solution at varying concentrations. Chitosan impregnated solutions with concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.4%, 1.6%, 1.8%, and 2.0% were prepared by diluting water-soluble chitosan solution (Chitosan hydrochloride, Mahtani Chitosan Pvt. Ltd., India) in distilled water using magnetic stirrer. Each concentration of the chitosan impregnated solution was mixed with irreversible hydrocolloid impression material according to the manufacturer's instructions. It was observed that concentrations above 1.0% altered the smoothness of the mix. Hence, the concentration of 1% chitosan impregnated solution was used in the present study.

Twenty dentulous patients who were willing to participate were selected for the study. Ethical clearance was obtained from the institutional review board (IRB Ref No: IRB/VDC/MDS14 PROSTHO 6). The volunteering participants were selected following inclusion and exclusion criteria.

Inclusion criteria

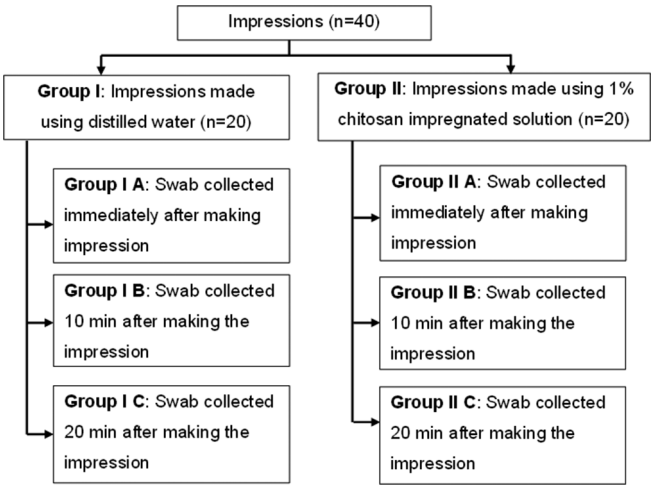
a. Age: above 20 years
b. Either sex
c. More than 8 teeth in each arch
d. Normal salivary flow rate.

Exclusion criteria

a. History of use of antibiotics or antimicrobials within 3 months
b. Use of antiseptic mouthwashes
c. Patients undergoing orthodontic treatment
d. Smokers and alcoholics
e. Presence of gingivitis or periodontitis
f. Presence of active carious lesions.

Crossover randomized control design was adopted. The layout of the design is shown in Flowchart 1

For each participant, two maxillary impressions were made: randomly the first impression was made using irreversible hydrocolloid impression material mixed either with only distilled water or 1% chitosan impregnated solution using sterile maxillary dentulous stock metal trays and for second impression *vice versa*. A time gap of 1 week was maintained between the two impressions for microbial recovery.



Flowchart 1: Layout of the study design

Table 1: Comparison of the mean colony-forming unit within the samples obtained from Group I using repeated-measure ANOVA test

	Number of samples (*n*)	Mean	SD	*F*	Significance
Group I A	20	1001.0500	104.71690	0.123	0.885
Group I B	20	996.4000	128.30859		
Group I C	20	989.8500	60.42331		

SD: Standard deviation

Table 2: Comparison of the mean colony-forming unit within the samples obtained from Group II using repeated-measure ANOVA test

	Number of samples (*n*)	Mean	SD	*F*	Significance
Group II A	20	898.0000	81.16520	5.738	0.012*
Group II B	20	845.3500	81.48056		
Group II C	20	844.8500	73.59081		

*$P \leq 0.005$ was considered significant. SD: Standard deviation

After removing the impression from the oral cavity, the impressions were rinsed for 15 s using running distilled water to remove the excess microbial load. Microbial samples were collected using dry sterile cotton swabs

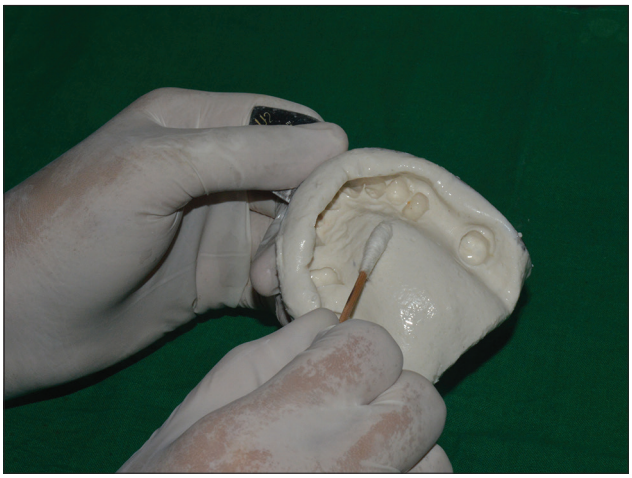


Figure 1: Swab taken from the mid-palate region

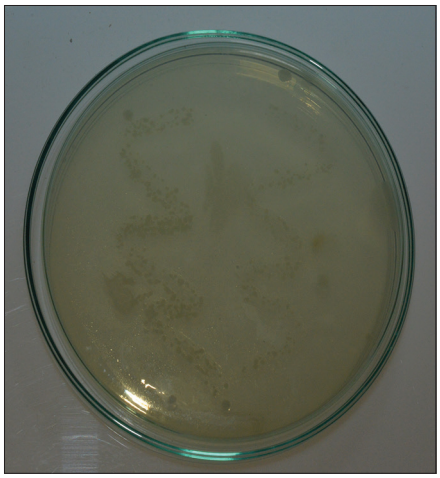


Figure 2: Streaked nutrient agar culture plate

Figure 3: Colony counter

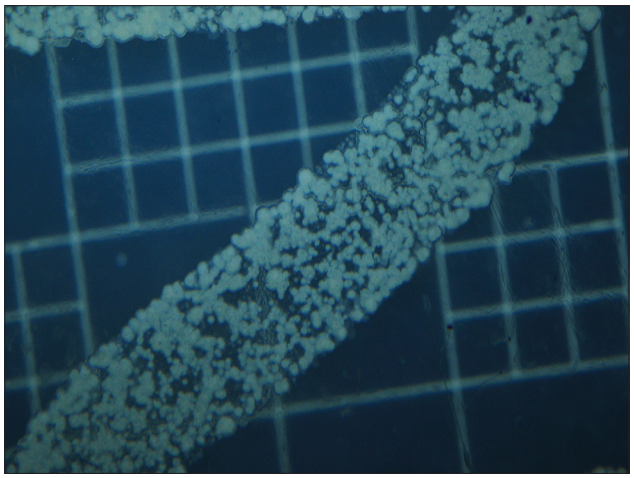


Figure 4: Colony-forming units

Figure 5: Colony-forming unit from Group I A

Figure 6: Colony-forming unit from Group I B

Figure 7: Colony-forming unit from Group I C

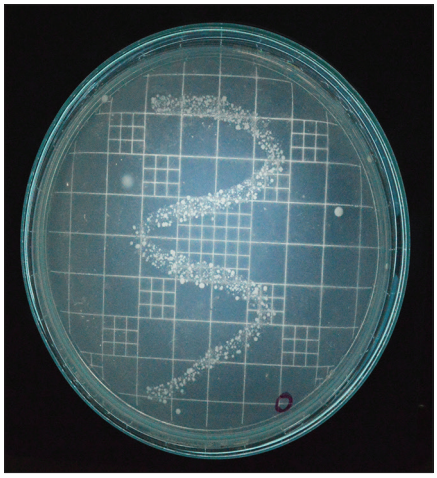


Figure 8: Colony-forming unit from Group II A

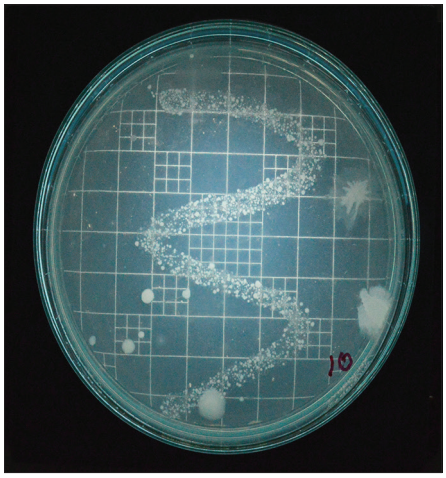


Figure 9: Colony-forming unit from Group II B

for all the impressions with randomization in the area of collection in the left, mid, and right palatal regions at the time intervals of 0, 10, and 20 min, respectively [Figure 1]. Between the time intervals, the impressions were stored in sealed noncoated plastic bags (ziplock covers). These swabs were inoculated on nutrient agar media and incubated at 37°C for 24 h [Figure 2]. The agar media was observed for colony-forming units (CFU). The CFU

were counted with the aid of the colony counter "(LAPIZ Digital Colony Counter) [Figures 3-10]. The resultant data collected were subjected to statistical analysis using SPSS Version 20.0 software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp., USA) applying repeated-measures ANOVA and independent *t*-tests.

RESULTS

The mean CFU of samples observed at 0, 10, and 20 min did not show any significant variations in Group I ($P = 0.885$) [Table 1] however showed a significant decrease in Group II ($P = 0.012$) [Table 2]. The decrease in mean CFU was more significant from 0 to 10 min ($P = 0.016$) and from 0 to 20 min ($P = 0.014$). The decrease was not significant from 10 to 20 min ($P = 1.000$) [Table 3]. Comparison of mean CFU obtained at 0, 10, and 20 min showed a significant decrease between Group I and Group II ($P = 0.001$, $P = 0.000$, and $P = 0.000$, respectively) [Table 4].

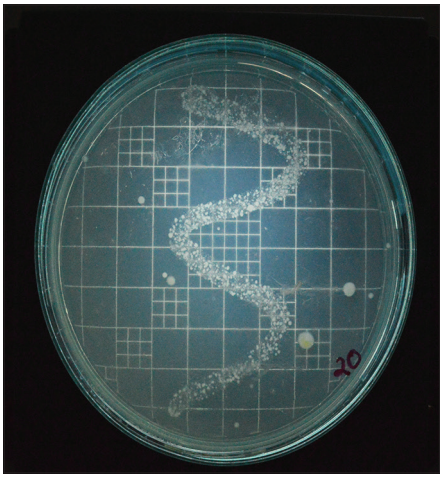


Figure 10: Colony-forming unit from Group II C

DISCUSSION

The irreversible hydrocolloid impressions produced significantly higher levels of contamination (6.11×10^7 microorganisms) than polyvinyl siloxane impressions (8.26×10^5 microorganisms) and polyether impressions (1.25×10^6 microorganisms) from the same individual. The hydrophilic nature and porous structure of irreversible hydrocolloid impression materials lead to maximum retention of microorganisms both on the surface and within the material.[6] To make self-disinfecting irreversible hydrocolloid impression material, the disinfectant can be impregnated into the material by incorporation of the agent either to the impression material powder or mixing of the impression material with antimicrobial agent impregnated water.[12] In this process, the disinfectant is distributed throughout the material; hence, there would be an internal disinfection throughout the material and not just on the external surface as would normally occur. It would also prevent any deposition and survival of microorganisms on the surface as well as in the pores of the set material.

The water-soluble and easily dispersible antimicrobial agents that have been added to irreversible hydrocolloid impression materials include chlorhexidine, quaternary ammonium salts, didecyldimethylammonium, sodium fluoride, and silver nanoparticles. However, irreversible hydrocolloid impression materials containing chlorhexidine have been found to exhibit longer gelation time and higher concentrations of chlorhexidine are cytotoxic to human fibroblasts. Some investigators have reported significant changes in the properties of self-disinfectant irreversible hydrocolloids such as gel strength, gelation time, and permanent deformation. The reproducibility of surface detail of self-disinfectant irreversible hydrocolloids was significantly reduced upon pouring with gypsum products.[16]

Table 3: Pairwise comparison of the mean differences in the colony forming units obtained from Group II using repeated measure ANOVA test

Specimens		Mean difference	SE	Significance	95% CI	
					Lower bound	Upper bound
Group II A	Group II B	52.650	16.735	0.016*	8.719	96.581
Group II A	Group II C	53.150	16.528	0.014*	9.761	96.539
Group II B	Group II C	0.500	13.480	1.000	−34.888	35.888

*$P \leq 0.005$ was considered significant. SE: Standard error, CI: Confidence interval

Table 4: Comparison of mean differences in colony forming units between Group I and Group II using independent *t*-test

Samples	*t*	df	Significance	Mean difference	SE	95% CI	
						Lower bound	Upper bound
Group A	−3.478	35.775	0.001*	−103.0500	29.62551	−163.14646	−42.95354
Group B	−4.444	32.181	0.000*	−151.0500	33.98689	−220.26379	−81.83621
Group C	−6.810	36.613	0.000*	−145.0000	21.29153	−188.15613	−101.84387

*$P \leq 0.005$ was considered significant. SE: Standard error, CI: Confidence interval

Although several disinfectants have been used to make self-disinfecting irreversible hydrocolloid impression material, no standard material has been devised so far.

Chitosan is a naturally acquired polysaccharide that is prepared by the deacetylation of chitin. Chitin is mainly obtained from crab and shrimp shells.[17] Chemically, chitosan is a poly β (1,4) glucosamine.[13] Chitosan is regarded as nontoxic, biocompatible, biodegradable, and is antibacterial in nature. Chitosan is used in a variety of fields such as wastewater treatment, agriculture, food, paper industry, cosmetics, and medicine for its antibacterial effect. Chitosans are well known for their hemostatic, fungistatic, antibacterial, antitumor, anticholesteremic and immunoadjuvant characteristics. In dentistry, chitosan is used as antimicrobial and antibacterial agent. It is used as a component in dentifrices and mouthwashes, dental adhesives and composite resins. However, the use of chitosan as a disinfectant has not been explored. Electrodeposition of chitosan in combination with calcium phosphate on the Ti_6Al_4V implants significantly improved the biocompatibility with no adverse effects on the other properties of implants.[15]

A pilot study was done to optimize the feasibility of manipulating irreversible hydrocolloid impression material using chitosan impregnated solution at varying concentrations up to 2%. Concentrations above 1% showed to cause changes in the consistency of the mix. Hence, in the present study, a 1% chitosan impregnated solution was used to manipulate the irreversible hydrocolloid impression material.

In the present study, microbiological effect of the modified alginate with disinfectant was done following the procedure described by Haralur *et al*.[1] in which the microbial swabs were collected using a dry sterile cotton swab from the impressions in the mid-palatal region. The microbial swabs were inoculated in agar media and incubated, and later, the microbial colonies were counted using the colony counter.

In the clinical scenario, the dentist has to transfer the impression to the laboratory to pour the cast. There may be about 10 min to 20 min time lapse between making of the impression and pouring of the cast. Irreversible hydrocolloid impressions should generally be poured immediately or within 12 min when stored in 100% humidity at room temperature.[18] To evaluate the potential of chitosan after the usual time lapse of 10 min, in the present study, the microbial CFUs were estimated at the time intervals of 0, 10, and 20 min to evaluate the passage of time (aging) on the rate of microbial death.

Intragroup comparison of the mean CFU obtained in Group I did not demonstrate any marked antimicrobial abilities. Although the CFU obtained from the irreversible hydrocolloid impressions decreased with increasing time intervals, the decrease was not significant. Decreasing amounts of free environmental water within the setting irreversible hydrocolloid impression material may be responsible for the fall in the number of viable microbial cells.

Intragroup comparison of the mean CFU obtained in Group II appears to possess rapid and marked antimicrobial abilities. Irreversible hydrocolloid impression material incorporated with 1% chitosan impregnated solution showed a significant increase in antimicrobial activity at 10 min and 20 min. However, the increase was insignificant between 10 min and 20 min. Hence, the optimum disinfection can be attained at 10 min.

The postulated mechanism of action of chitosan may consist of enzyme inactivation, chelation of essential metal ions, and formation of polyelectrolyte complexes with bacterial surface compounds.[17,19] An ionic interaction between the cations due to the amino groups of chitosan and anionic parts of bacterial cell wall, such as phospholipids and carboxylic acids, has also been proposed as the mechanism for the antimicrobial activity of chitosan.[19]

Chitosan has also been reported to exhibit antiviral activity. It has been suggested that chitosan can inhibit the replication of bacteriophages by several mechanisms: it can (a) decrease the viability of cultured bacterial cells, (b) neutralize the infectivity of mature phage particles in the inoculum and/or daughter phage particles, and (c) block the replication of the virulent phage.[20] N-carboxymethylchitosan N, O-sulfate, a polysaccharide derived from N-carboxymethyl chitosan by sulfation modification, could prevent HIV-1 infection by inhibiting viral adsorption to the CD4 receptor and reverse transcription of the viral genome.

Intergroup comparison of the mean CFU obtained from unsupplemented and supplemented irreversible hydrocolloid impressions has demonstrated that there was a significant decrease in the CFU obtained from supplemented irreversible hydrocolloid impressions (P = 0.001, 0.000, and 0.000 at 0, 10, and 20 min, respectively). Hence, 1% chitosan impregnated solution can be used as a water substitute to increase the antimicrobial potential of irreversible hydrocolloid impression material.

Irreversible hydrocolloid can be used in preliminary impressions, provisional crown-and-bridge impressions, study models, opposing dentition impressions, orthodontic

models, sports mouth guards, bleaching trays, and final impressions for indirect restorations when the preparation margins are chamfer.[21] The material of choice for making the impressions for edentulous arches is impression compound, zinc oxide–eugenol paste or elastomeric impression materials. Hence, in the present study, the targeted population were dentulous patients. Investigations and researches revealed that a number of pathogenic microorganisms which are present in the mouth when the patient was dentate were harbored in the oral cavity even when they are in an edentulous state.[22] Hence, the efficacy of 1% chitosan solution as a disinfectant might be similar in dentulous and edentulous population.

Limitations

1. In the present study, only the antimicrobial activity of the irreversible hydrocolloid impression material incorporated with 1% chitosan impregnated solution was tested. There is further scope to investigate the physical and mechanical properties of the irreversible hydrocolloid impression material incorporated with a 1% chitosan impregnated solution to substantiate its usage in clinical practice
2. The samples were collected only from the maxillary arch. Further evaluation can be done by collecting the samples from the mandibular arch.

Clinical significance

Adding water-soluble chitosan to irreversible hydrocolloid impression material will provide a significant antimicrobial activity to the impression material in 10 min causing self-disinfection of the impression, thus reducing the transfer of microorganisms from patients to the dental team.

CONCLUSION

The following conclusions can be drawn:

- 1% chitosan solution was the optimum concentration to use for the manipulation of irreversible hydrocolloid impression material without any changes in the handling properties of the material
- Incorporation of water-soluble chitosan can significantly increase the antimicrobial potential of irreversible hydrocolloid impression material
- Antimicrobial activity was found to increase up to 10 min and later increase was not significant. Hence, the optimum disinfection can be attained in 10 min.

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Conflicts of interest

There are no conflicts of interest.

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