Comparative Evaluation of the Antimicrobial Effect of Irreversible Hydrocolloid Impressions Following Surface Disinfection, Chitosan Incorporation, and Chlorhexidine Incorporation: An In Vivo Study

Dr. Anuradha Nemane[1], Dr. Jagruti R. Rajput[2] , Dr. Saloni Mistry[3], Dr. Parmeet Banga[4], Dr. Sheetal Chaudhary[5], Dr. Aditi Rane[6]

[1]Professor, Department of Prosthodontics and Crown and bridge, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

[2]Postgraduate student, Department of Prosthodontics, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

[3]Head of the department,Department of Prosthodontics and Crown and bridge, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

[4]Professor, Department of Prosthodontics and Crown and bridge, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

[5]Head of the department, Department of Oral Pathology and microbiology, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

[6] Postgraduate student, Department of Prosthodontics, Dr G D Pol Foundation Y M T Dental college and Hospital, Kharghar

Corresponding Author:
Dr. Jagruti R. Rajput[2]
Email id: rajputjagruti12345@gmail.com

Abstract

Background: Dental impressions are frequently contaminated with saliva, blood, and microorganisms immediately after removal from the oral cavity, thereby acting as potential sources of cross-infection among patients, clinicians, and dental laboratory personnel. Conventional chemical disinfection effectively reduces microbial contamination but may adversely affect the dimensional stability of irreversible hydrocolloid impression materials. Incorporation of antimicrobial agents directly into impression materials has emerged as a promising alternative to overcome these limitations. Chitosan and chlorhexidine have demonstrated broad-spectrum antimicrobial activity; however, comparative clinical evidence evaluating their effectiveness in irreversible hydrocolloid impression materials remains limited.

Aim: To comparatively evaluate the antimicrobial efficacy of irreversible hydrocolloid impressions following rinsing under running tap water, surface disinfection using 2% glutaraldehyde spray, incorporation of 1% chitosan, and incorporation of 1% chlorhexidine gluconate.

Materials and Methods: An in vivo comparative experimental study was conducted among twenty-two participants requiring prosthodontic treatment. Four maxillary irreversible hydrocolloid impressions were obtained from each participant at one-week intervals using four different disinfection protocols. Group A served as the control and impressions were rinsed under running tap water. Group B impressions were disinfected using freshly prepared 2% glutaraldehyde spray. Group C impressions were prepared using 1% chitosan solution as the mixing liquid, while Group D impressions were prepared using 1% chlorhexidine solution. Microbial samples were collected from impression surfaces after ten minutes and from corresponding gypsum casts immediately after pouring. Samples were inoculated on nutrient agar, incubated at 37°C for twenty-four hours, and colony-forming units (CFUs) were quantified. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test, with statistical significance established at $p<0.05$.

Results: Statistically significant differences were observed among all experimental groups on both impression surfaces ($F = 4715.81$, $p<0.001$) and gypsum casts ($F = 4462.39$, $p<0.001$). Chlorhexidine incorporation demonstrated the greatest reduction in microbial counts, followed by chitosan incorporation. Glutaraldehyde spray significantly reduced microbial contamination compared with conventional rinsing but was less effective than chlorhexidine incorporation. Similar findings were observed on corresponding gypsum casts, with the greatest proportional reduction in microbial transfer seen in the chlorhexidine (48.1%) and chitosan (40.6%) groups.

Conclusion: Incorporation of 1% chlorhexidine and 1% chitosan into irreversible hydrocolloid impression material significantly reduced microbial contamination of both impressions and gypsum casts. Chlorhexidine demonstrated the

highest antimicrobial efficacy, while chitosan represents a promising natural alternative for development of self-disinfecting impression materials.
Keywords: Alginate; Chlorhexidine; Chitosan; Dental impressions; Infection control; Irreversible hydrocolloid.
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Introduction

Cross-infection control is a fundamental aspect of contemporary dental practice, as dental procedures routinely expose clinicians, patients, and laboratory personnel to saliva, blood, and a wide range of oral microorganisms (1,2). Dental impressions are recognized as potential vehicles for microbial transmission because they are transferred directly from the patient's oral cavity to the dental laboratory, where they undergo repeated handling during cast fabrication and prosthesis construction (3). Consequently, inadequate disinfection of impression materials may contribute to cross-contamination among patients, clinicians, and dental technicians (4). International infection control guidelines therefore recommend that all dental impressions be appropriately disinfected before laboratory procedures to minimize the risk of disease transmission (5). Irreversible hydrocolloid (alginate) remains one of the most commonly used impression materials because of its ease of manipulation, hydrophilic nature, low cost, patient comfort, and satisfactory clinical accuracy (6). However, its porous structure readily retains saliva, blood, and microorganisms, making effective disinfection essential without compromising dimensional stability and surface detail reproduction (7,8).

Conventional disinfection of alginate impressions is generally achieved by immersion or spray techniques using disinfectants such as glutaraldehyde, sodium hypochlorite, chlorhexidine, iodophors, and hydrogen peroxide (9). Although these methods effectively reduce microbial contamination, prolonged immersion may alter dimensional stability because of imbibition and syneresis, whereas spray disinfection depends on complete surface coverage and operator compliance (10). These limitations have encouraged the development of self-disinfecting impression materials in which antimicrobial agents are incorporated directly into the impression material during manipulation, thereby eliminating an additional chairside disinfection step while maintaining continuous antimicrobial activity throughout impression making and laboratory handling (11,12).

Among the various antimicrobial agents investigated, chlorhexidine gluconate has been widely regarded as the gold standard because of its broad-spectrum antimicrobial activity, substantivity, and favorable safety profile (13). Chitosan, a naturally occurring biopolymer obtained from chitin, has also attracted considerable attention because of its excellent biocompatibility, biodegradability, and broad-spectrum antimicrobial properties (14). Previous investigations have suggested that incorporation of chlorhexidine or chitosan into irreversible hydrocolloid impression materials significantly reduces microbial contamination while preserving acceptable physical properties, in keeping with the broader development of self-disinfecting dental materials (15). However, direct clinical comparisons between these self-disinfecting approaches and conventional surface disinfection remain limited, particularly regarding microbial transfer from impressions to gypsum casts. Therefore, the present in vivo study was undertaken to comparatively evaluate the antimicrobial efficacy of conventional tap water rinsing, 2% glutaraldehyde spray disinfection, incorporation of 1% chitosan, and incorporation of 1% chlorhexidine into irreversible hydrocolloid impression material.

Materials and Methods

This in vivo comparative experimental study was conducted in the Department of Prosthodontics in collaboration with the Department of Microbiology after obtaining approval from the Institutional Ethics Committee **[YMTIX/IEC/OGT/2023/177]**. The study was designed to compare the antimicrobial efficacy of irreversible hydrocolloid impressions following four different disinfection protocols: conventional tap water rinsing, 2% glutaraldehyde surface spray disinfection, incorporation of 1% chitosan, and incorporation of 1% chlorhexidine gluconate into the impression material. The study protocol adhered to the ethical principles of the Declaration of Helsinki, and written informed consent was obtained from all participants before enrolment. Participant confidentiality was maintained throughout the study.

A total of 22 healthy participants aged between 18 and 60 years who required maxillary diagnostic impressions for prosthodontic treatment were recruited using purposive sampling. Individuals with systemic diseases affecting oral microbial flora, active oral infections, current antibiotic, antifungal, or

antiviral therapy, xerostomia, salivary gland disorders, immunocompromised conditions, known allergy to impression materials, or unwillingness to participate were excluded from the study. Each participant underwent four separate impression procedures at one-week intervals, resulting in a total of 88 maxillary irreversible hydrocolloid impressions for microbiological evaluation.

Sample Size

Twenty-two participants were recruited using purposive sampling, each contributing four impressions under a repeated-measures design, yielding 88 impressions (22 per group) for analysis. A retrospective (post hoc) analysis of the recorded data confirmed that this sample size was adequate: for the impression-surface CFU counts, the one-way ANOVA effect size was very large (partial $\eta^2 \approx 0.99$; Cohen's $f \approx 13.0$), corresponding to an achieved power exceeding 0.99 at $\alpha = 0.05$. By comparison, a conventionally 'medium' effect ($f = 0.25$) would require approximately 178 participants per group, and a 'large' effect ($f = 0.40$) approximately 72 per group, to achieve 80% power in a four-group one-way ANOVA; the pronounced antimicrobial separation between groups in the present study meant that a considerably smaller sample was sufficient to detect statistically and clinically meaningful differences.

Participants were allocated to four experimental groups according to the disinfection protocol employed. In Group A (Control), impressions were prepared according to the manufacturer's instructions and rinsed under running tap water for approximately 30 seconds before microbiological sampling. Group B impressions were prepared conventionally and disinfected using freshly prepared 2% glutaraldehyde spray, ensuring complete coverage of the impression surface, followed by storage in a sterile polyethylene bag for 10 minutes. In Group C, 1% chitosan solution was used as the mixing liquid instead of distilled water during manipulation of the irreversible hydrocolloid impression material, thereby producing a self-disinfecting impression. Similarly, in Group D, 1% chlorhexidine gluconate solution replaced distilled water during mixing to produce chlorhexidine-incorporated self-disinfecting impressions. No additional surface disinfection was performed for Groups C and D because the antimicrobial agents were incorporated directly into the impression material.

All impressions were made by a single calibrated operator to eliminate operator-related variability. Appropriate maxillary stock trays were selected for each participant, and tray adhesive was uniformly applied before impression making. The impression material was manipulated according to the manufacturer's recommended powder-to-liquid ratio, except in the experimental groups where the designated antimicrobial solutions replaced distilled water. After complete setting, impressions were carefully removed, inspected for defects, and repeated if any distortion or voids were detected. Following the assigned disinfection protocol, all impressions were allowed to remain undisturbed for 10 minutes before microbiological sampling.

Microbiological sampling was performed under strict aseptic conditions using sterile cotton swabs moistened with sterile saline. The entire tissue surface of each impression was swabbed using standardized pressure and stroke length, after which the swabs were immediately transferred to sterile transport tubes and transported to the microbiology laboratory. Samples were inoculated onto nutrient agar plates and incubated aerobically at 37°C for 24 hours. Following incubation, bacterial growth was quantified using a digital colony counter, and microbial contamination was expressed as colony-forming units per milliliter (CFU/mL).

Immediately after microbiological sampling, all impressions were poured using freshly mixed Type III dental stone according to the manufacturer's recommendations. After complete setting, gypsum casts were carefully separated from the impressions, and standardized areas of each cast were sampled using sterile saline-moistened swabs. The swabs were cultured on nutrient agar under identical incubation conditions, and colony counts were recorded to evaluate microbial transfer from the impression surface to the corresponding gypsum cast.

The primary outcome measure was the reduction in microbial colony-forming units present on the impression surfaces, whereas the secondary outcome measure was the reduction in microbial transfer from impressions to gypsum casts. To minimize experimental bias, all procedures were standardized with respect to operator, impression material, tray design, mixing technique, room temperature, setting time, incubation period, microbiological culture conditions, gypsum product, and colony-count evaluation. Universal infection-control precautions were maintained throughout the study, including the use of personal protective equipment and appropriate biomedical waste disposal procedures.

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics Version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were summarized using frequencies and percentages where appropriate. The normality of the data distribution was assessed using the Shapiro–Wilk test. Intergroup comparisons

of mean colony-forming unit (CFU) counts among the four experimental groups were performed using one-way analysis of variance (ANOVA). When statistically significant differences were observed, Tukey's post hoc multiple comparison test was applied to identify pairwise differences between groups, with 95% confidence intervals calculated for each pairwise mean difference. A two-tailed p value of <0.05 was considered statistically significant for all analyses.

Results

A total of 22 participants completed the study, and 88 maxillary irreversible hydrocolloid impressions were evaluated without any dropouts or protocol deviations. Microbiological assessment was performed on both the impression surfaces (Subgroup I) and their corresponding gypsum casts (Subgroup II).

Table 1. Descriptive statistics of microbial counts (CFU) on impression surfaces 10 minutes after impression-making (Subgroup I), by group (n = 22 per group)

Group	Mean (CFU)	SD	SE	Minimum	Maximum
Group A (Control)	1127.73	33.82	7.21	1000	1170
Group B (2% Glutaraldehyde spray)	853.00	18.30	3.90	830	907
Group C (1% Chitosan)	408.50	26.58	5.67	340	450
Group D (1% Chlorhexidine)	372.32	17.12	3.65	347*	402

**The minimum value for Group D was recorded as 47.0 CFU in the source data table but is inconsistent with the underlying per-participant values (range 347–402); 347.0 CFU is used here as the value supported by the raw data.*

Table 2. One-way ANOVA — intergroup comparison of microbial counts on impression surfaces (Subgroup I)

Source	df	F	p value	Significance
Between groups	3, 84	4715.81	<0.001	Significant**

***Significant at p<0.05.*

Table 3. Tukey's post hoc pairwise comparisons — impression surfaces (Subgroup I)

Group (I)	Group (J)	Mean Difference	95% CI (Lower, Upper)	p value
Group A	Group B	274.73	255.06, 294.40	<0.001**
Group A	Group C	719.23	699.56, 738.90	<0.001**
Group A	Group D	755.41	735.74, 775.08	<0.001**
Group B	Group C	444.50	424.83, 464.17	<0.001**
Group B	Group D	480.68	461.01, 500.35	<0.001**
Group C	Group D	36.18	16.51, 55.85	<0.001**

Intergroup comparison of the mean CFU counts on impression surfaces demonstrated a statistically significant difference among the four experimental groups (Table 2). Group D (1% chlorhexidine) showed the lowest mean CFU count, followed by Group C (1% chitosan), Group B (2% glutaraldehyde spray), and Group A (control), confirming that antimicrobial incorporation was more effective than either conventional spray disinfection or simple rinsing. Tukey's post hoc analysis (Table 3) confirmed statistically significant pairwise differences between every pair of groups (p<0.001 for all comparisons), including the smaller but significant difference between chitosan and chlorhexidine (mean difference 36.18 CFU).

Table 4. Descriptive statistics of microbial counts (CFU) on resultant gypsum casts (Subgroup II), by group (n = 22 per group)

Group	Mean (CFU)	SD	SE	Minimum	Maximum
Group A (Control)	1100.59	36.67	7.82	960	1156
Group B (2% Glutaraldehyde spray)	797.09	16.91	3.61	760	846
Group C (1% Chitosan)	242.86	23.32	4.97	202	280
Group D (1% Chlorhexidine)	193.27	40.58	8.65	110	255

Table 5. One-way ANOVA — intergroup comparison of microbial counts on gypsum casts (Subgroup II)

Source	df	F	p value	Significance
Between groups	3, 84	4462.39	<0.001	Significant**

***Significant at p<0.05.*

Table 6. Tukey's post hoc pairwise comparisons — gypsum casts (Subgroup II)

Group (I)	Group (J)	Mean Difference	95% CI (Lower, Upper)	p value
Group A	Group B	303.50	279.07, 327.93	<0.001**
Group A	Group C	857.73	833.30, 882.15	<0.001**
Group A	Group D	907.32	882.89, 931.75	<0.001**
Group B	Group C	554.23	529.80, 578.65	<0.001**
Group B	Group D	603.82	579.39, 628.25	<0.001**
Group C	Group D	49.59	25.16, 74.02	<0.001**

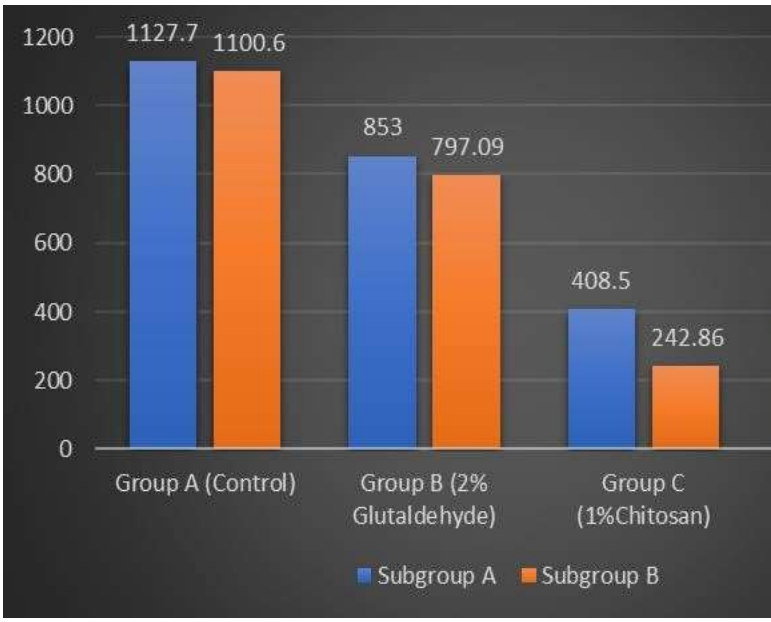
A similar pattern was observed on the resultant gypsum casts (Tables 4–6). Group D again showed the lowest microbial contamination, followed by Group C, while Groups B and A showed progressively higher counts, confirming that the antimicrobial effect on the impression surface was reflected in the corresponding cast.

Table 7. Comparison of microbial counts between impression surface (Subgroup I) and resultant gypsum cast (Subgroup II), with percentage reduction in microbial transfer

Group	Subgroup I (Impression, CFU)	Subgroup II (Cast, CFU)	Mean Difference (CFU)	% Reduction
Group A (Control)	1127.73	1100.59	27.14	2.4%
Group B (2% Glutaraldehyde)	853.00	797.09	55.91	6.6%
Group C (1% Chitosan)	408.50	242.86	165.64	40.6%
Group D (1% Chlorhexidine)	372.32	193.27	179.05	48.1%

Table 7 summarizes the reduction in microbial transfer from impression to cast across groups. The antimicrobial-incorporated groups showed the greatest proportional reduction in microbial transfer (Group D: 48.1%; Group C: 40.6%), compared with modest reductions in Group B (6.6%) and Group A (2.4%), indicating that incorporation-based disinfection limits microbial carryover into the laboratory more effectively than surface disinfection or rinsing alone.

Figure 1. Comparison of mean microbial counts (CFU) on the impression surface (Subgroup A) and resultant gypsum cast (Subgroup B) across the four experimental groups



Discussion

The present in vivo study demonstrated a clear efficacy gradient among the four disinfection protocols evaluated: 1% chlorhexidine incorporation produced the greatest reduction in microbial contamination, followed by 1% chitosan incorporation, 2% glutaraldehyde spray, and conventional tap-water rinsing, in that order, both on the impression surface and on the resultant gypsum cast. This gradient is consistent with the recognized vulnerability of dental impressions as vehicles for cross-infection between the operatory and the laboratory, and with international infection-control recommendations for minimizing cross-transmission in healthcare settings (16,17,21).

Irreversible hydrocolloid remains the most widely used impression material in prosthodontics because of its ease of manipulation, hydrophilicity, and cost-effectiveness, but its porous surface readily retains saliva, blood, and viable microorganisms even after rinsing (4,5). The markedly higher CFU counts recorded in Group A confirm that tap-water rinsing removes only loosely adherent debris and cannot substitute for a validated disinfection or self-disinfecting protocol (1).

Spray disinfection with 2% glutaraldehyde produced a significant reduction relative to the control, consistent with its broad-spectrum action through cross-linking of microbial proteins and nucleic acids; however, its efficacy remained inferior to both self-disinfecting groups, likely reflecting dependence on complete surface coverage, adequate contact time, and operator technique (9,10).

Chlorhexidine gluconate, incorporated directly into the impression material, produced the greatest

antimicrobial effect, in keeping with its established substantivity, broad antimicrobial spectrum, and sustained release throughout impression-making and laboratory handling (2,13). Chitosan incorporation also achieved a substantial reduction, attributable to electrostatic interaction between its protonated amino groups and negatively charged microbial cell membranes, which disrupts the membrane and causes leakage of intracellular contents (18,19). Its marginally lower efficacy compared with chlorhexidine may reflect variability in molecular weight, degree of deacetylation, and diffusion characteristics within the hydrocolloid matrix (20).

Because both antimicrobial agents were incorporated at the mixing stage, no additional chairside disinfection step was required, reducing operator dependence and the risk of incomplete disinfection (7). This is clinically relevant given that contaminated impressions are a recognized source of microbial transfer to gypsum casts and, subsequently, to laboratory personnel (8); the parallel reduction observed on the resultant casts in the present study supports incorporation-based approaches as a practical means of limiting this transfer.

Although the present study focused on antimicrobial efficacy, the clinical acceptability of self-disinfecting impression materials also depends on preservation of dimensional stability and surface-detail reproduction. Previous reports indicate that chlorhexidine and chitosan can be incorporated at low concentrations without clinically significant compromise of these properties, although the present study did not independently verify this (6).

This study was limited by its single-centre design, evaluation of a single fixed concentration of each agent, and microbial assessment at a single post-impression time point; long-term antimicrobial persistence, effects on dimensional stability during storage, and activity against a broader range of oral microorganisms were not assessed. The sample size, while shown post hoc to be more than adequate for the large effect observed here, was determined pragmatically rather than through a documented a priori power calculation (see Sample Size, Methods). Larger multicentric trials evaluating a range of agent concentrations are recommended before self-disinfecting alginate can be recommended for routine clinical use (14).

Conclusion

Within the limitations of this in vivo study, incorporation of 1% chlorhexidine and 1% chitosan into irreversible hydrocolloid impression material significantly reduced microbial contamination of both impression surfaces and the corresponding gypsum casts compared with conventional tap water rinsing and 2% glutaraldehyde spray disinfection. Among the tested protocols, 1% chlorhexidine incorporation demonstrated the highest antimicrobial efficacy, while 1% chitosan emerged as an effective natural alternative. These findings support incorporation-based self-disinfection as a practical strategy for reducing cross-contamination in both clinical and dental laboratory settings; further large-scale, multicentric studies are recommended to validate these findings before routine clinical implementation.

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