Original Article

DOI: 10.7860/JCDR/2022/52919.16383

Dentistry Section

Efficacy of a Commercially Available Herbal Formulation for the Disinfection of Elastomeric Heavy Body Impression Materials

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ABSTRACT

Introduction: Cross infection refers to the transfer of harmful microorganisms from one person to another. Dental impressions are considered semi-critical as it directly comes in contact with patient's saliva and oral mucosa. Improper handling of these dental impressions can potentially have the risk of transmitting infections especially to the dentist. The role of herbal preparation in disinfecting the dental impressions is sparse in the literature.

Aim: To evaluate and compare the disinfection ability of a commercially available herbal formulation (HiOra®) with chlorhexidine digluconate solution (0.2%) and 1% sodium hypochlorite on dental impressions made using condensation silicone.

Materials and Methods: A cross-sectional study was conducted in Outpatient Department of Periodontology and Implantology at Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, between June 2019 and November 2019. A total of 60 maxillary and mandibular dental impressions using condensation silicone material were obtained from 30 systemically healthy volunteers. The impressions were divided into three groups and were subjected to three antimicrobial agents for 10 minutes: 1% Sodium Hypochlorite (group 1); 0.2% chlorhexidine digluconate (group 2); HiOra® (group 3), respectively. Following this, microbial culturing was performed to detect the presence of Streptococcal and Staphylococcus species in samples obtained both prior and after impression disinfection. One-way Analysis of Variance (ANOVA) was performed to assess the significance of the difference in microbial reduction between the groups.

Results: Antimicrobial efficacy was similar against both Streptococcus spp., and Staphylococcus spp., for the three disinfectants tested. No statistically significant difference in the microbial colony count reduction between the three disinfectants tested was observed (Streptococcus spp, p-value=0.064, Staphylococcus spp., p-value=0.337).

Conclusion: The herbal mouthwash was found to have an equivalent efficacy as chlorhexidine, sodium hypochlorite for disinfection of impressions made from condensation silicone.

Keywords: Chlorhexidine digluconate, Dental impressions, Herbal mouthwash, Microbial reduction, Sodium hypochlorite

INTRODUCTION

Disinfection is the destruction of pathogenic microorganisms or their toxins by direct exposure to chemical or physical agents. The various disinfectants are broadly classified into physical and chemical agents [1]. The ideal requisites for the disinfectant are: they should not be toxic even if absorbed into circulation, should have a broad spectrum of activity, speedy action, and should be stable [1].

Disinfection is an integral part of the dental practice, and one of the commonly overlooked areas of disinfection is dental impressions. Dental impressions are made with a wide variety of materials such as alginate, addition and condensation silicone materials, and polyethers [2]. The risk of spread of infection through impression has been highlighted in a study by Egusa H et al., [3]. The authors assessed alginate impression from 56 patients and concluded that patient derived dental impressions and casts were contaminated with pathogens like Streptococci spp., Staphylococci spp, Methicillin-Resistant *Staphylococcus Aureus* (MRSA), Candida spp., *P.aeroginosa*, which is responsible for cross infections. Hence, there is an absolute need for the disinfection of the dental impressions [4].

Several methods of disinfection of dental impressions have been used. These include the use of chemicals of specific concentrations such as hydrogen peroxide, sodium hypochlorite, aldehyde based agents, alcohols of high concentrations (>70% and above), chlorhexidine gluconate [5]. Physical methods such as the use of ultraviolet light and microwave have also been attempted for impression disinfection with varying degrees of success [5]. Though extremely efficient, these disinfectants can pose a risk to the dentist's health and the environment [6]. Hence, an alternative disinfectant should be used which is both effective and is not hazardous to the dentist and the environment.

HiOra® is an antimicrobial, antiseptic herbal mouthwash manufactured by the Himalaya drug company. Its main composition consists of Meswak (*Salvadora Persica*), Betel (*Nagavalli*) leaf, Belleric Myrobalan (*Bibhitaki*) [7]. It is commonly used for plaque control, to prevent halitosis, and combats common oral bacteria and fungi [8]. The above-mentioned herbal formulation has not been tested as a disinfectant for impression materials to date, and it may thus provide a viable eco-friendly alternative to disinfection of impression materials.

The aim of the present study was to evaluate and compare the disinfection ability of a commercially available herbal formulation (HiOra®) with chlorhexidine digluconate solution (0.2%) and 1% sodium hypochlorite (NaOCl) on dental impressions made using condensation silicone.

MATERIALS AND METHODS

A cross-sectional study was conducted in Outpatient Department of Periodontology and Implantology at Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, between June 2019 and November 2019. Informed consent was obtained prior to enrolment in the study. The Ethics Committee approval was obtained prior to the start of the study (CSP/17/JUN/59/198). The study was conducted in line with the ethical principles established by the Helsiniki Declaration (2013).

Sample size calculation: Sample size calculation was done based on the observations of Contreras GF et al., 2016 [9]. Assuming power (β) of 80% and alpha value of 5% and a sample size of 18 per group was needed for establishing the .difference between the groups. A total of 20 samples were selected for each of the group.

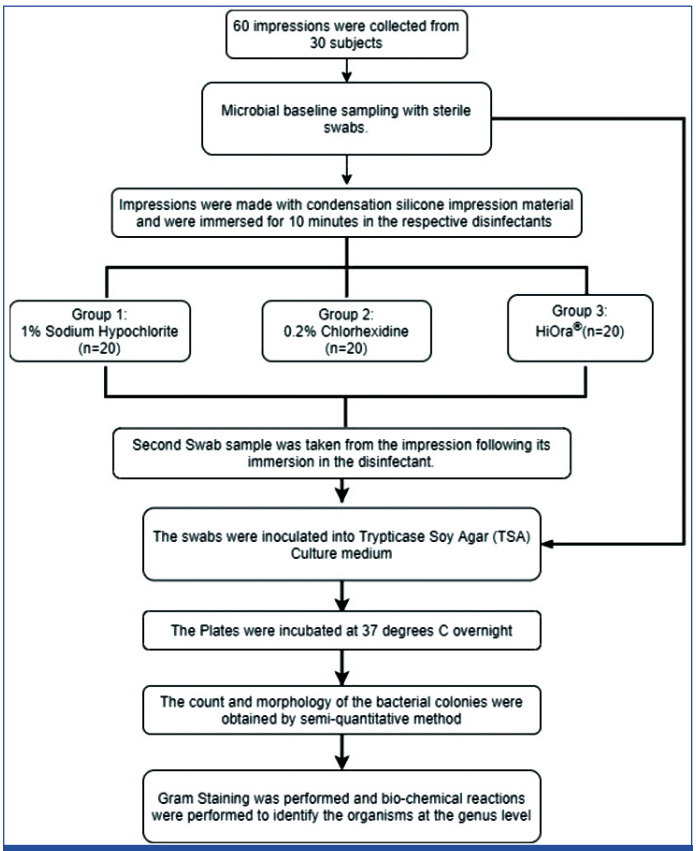
Inclusion and Exclusion criteria: Systemically healthy individuals (both males and females) in the age range of 20-35 years, who were non smokers were included in the study. Individuals with extensive prosthodontic or orthodontic appliances, individuals with existing periodontal disease and patients taking long term medication were excluded from the study.

Study Procedure

A total of 60 impressions were taken from both maxillary and mandibular arches of the 30 patients (one each from maxilla and mandible of each individual). The study consisted of immersing the impressions made of condensation silicone into three different disinfection agents as given below:

- Group 1: Immersion of 20 condensation silicone impression in 60 mL of 1% sodium hypochlorite (NaOCl) for 10 minutes.
- Group 2: Immersion of 20 condensation silicone impression in 60 mL of 0.2% chlorhexidine digluconate solution for 10 minutes.
- Group 3: Immersion of 20 condensation silicone impression in 60 mL of the Herbal formulation (HiOra®) for 10 minutes.

The methodology involved the following clinical and laboratory protocols [Table/Fig-1].



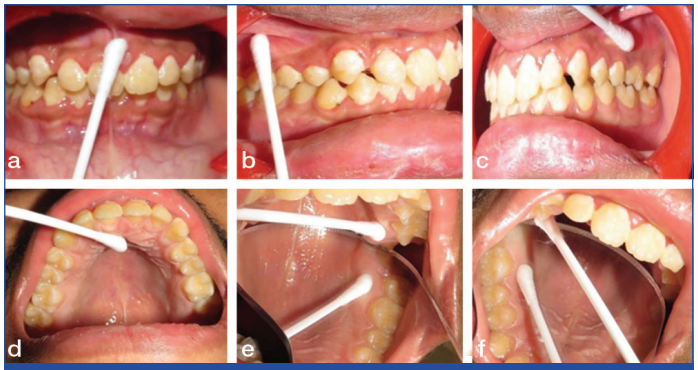
[Table/Fig-1]: Methods followed in this study.

Step 1: The subject's mouth was dried using suction, and a baseline sample was obtained using a sterile swab from six regions in either the maxilla or the mandible (anterior labial region, anterior lingual region, left posterior buccal, left posterior palatal/lingual region, right posterior buccal region, right posterior palatal/lingual region) [Table/Fig-2a-f].

Step 2: Maxillary and Mandibular Impressions were obtained from the patient using Condensation silicone putty (Zhemarck Zetaplus) [Table/Fig-3a]. Number of impressions obtained were 60.

Step 3: Twenty impressions each were immersed in 60 mL of 1% sodium hypochlorite, 0.2% chlorhexidine gluconate, and herbal formulation for 10 minutes each [10].

Step 4: Postimmersion swab was taken from the dental impression made from condensation silicone after the completion of the immersion period [Table/Fig-3b].

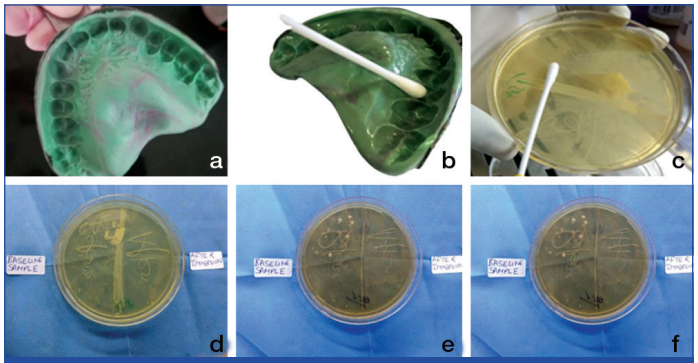


[Table/Fig-2]: Sample collection from: a) Anterior Labial Region; b) Left Posterior Buccal; c) Right Posterior Buccal Region; d) Anterior Lingual Region; e) Right Posterior Palatal Region; f) Left Posterior Palatal Region.

Step 5: The collected swabs were transported to the Microbiology laboratory for further processing without any delay. In case of any delay, the swabs were stored in ice packs for a maximum of not more than one hour. In the laboratory, the swabs were inoculated by streaking on Trypticase Soy Agar plates [Table/Fig-3c].

Step 6: The plates were incubated overnight at 37°C.

Step 7: After overnight incubation, the plates were observed for any growth. If growth was present, the morphology and the colony count (Semi-quantitative) were recorded [Table/Fig-3d-f]. *Staphlococcus aureus* appeared as white opaque colonies whereas *Streptococcus* appeared as tiny transluscnt colonies.



[Table/Fig-3]: a) Maxillary putty impression made; b) Following disinfection swab taken from the impression; c) Inoculation of the swab on the culture plates; d) Bacterial count during baseline sampling vs postimmersion in 1% Sodium Hypochlorite; e) Bacterial count during baseline sampling vs postimmersion in 0.2% Chlorhexidine; f) Bacterial count during baseline sampling vs postimmersion with Hiora®.

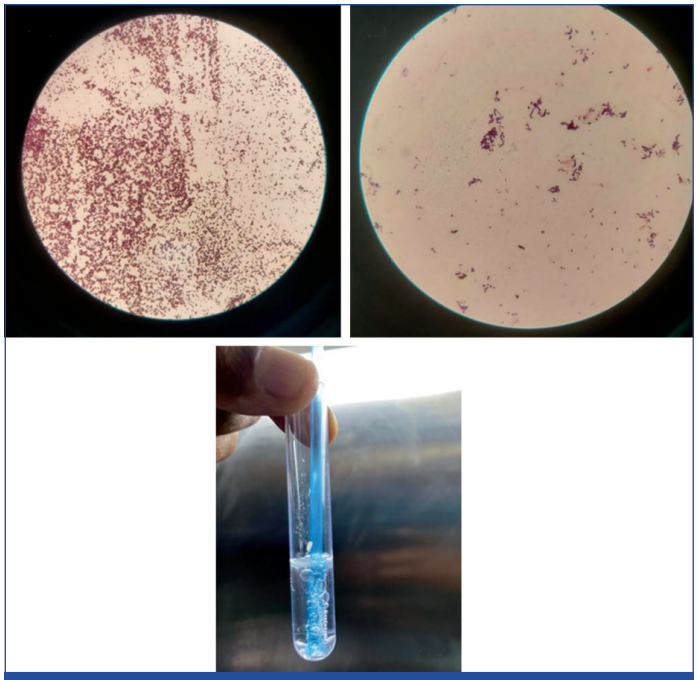
The Staphylococcus spp. or Streptococcus spp. group was identified by subjecting the colony growth to a gram stain. The Staphylococcus spp. appeared purple and was arranged in groups and clusters, whereas the Streptococcus species were arranged in pairs and chains [11]. Further confirmation was done by doing a catalase test which was positive for Staphylococcus spp. and negative for Streptococcus spp. [Table/Fig-4a-c] [11].

STATISTICAL ANALYSIS

The statistical analysis for the categorical data obtained on comparing the antimicrobial efficacy of the chlorhexidine and HiOra® were done using one-way Analysis of Variance (ANOVA). All the statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS for Windows, Version 11.0. Chicago, IL). A p-value <0.05 was considered statistically significant.

RESULTS

The mean age of the study population was 30±2.8 years. There were 18 females and 12 males included in the study. The demographic data is summarised in [Table/Fig-5]. The growth of the Staphylococcus and Streptococcus species in culture plates streaked using the swabs collected at baseline (from patient mouth) showed considerable variation and this data is summarised in [Table/Fig-6a].



[Table/Fig-4]: a) Staphylococcus spp. appearing purple in Gram's stain and arranged in clusters; b) Streptococcus spp. appearing purple in Gram's stain and arranged in chains; c) Positive catalase test for Staphylococcus spp.

The antimicrobial efficacy of 1% sodium hypochlorite against Streptococcus spp. was 99.99% and against Staphylococcus spp. was 99.83%. The antimicrobial efficacy of 0.2% chlorhexidine digluconate solution against Streptococcus spp. was 100.00% and against Staphylococcus spp was 99.93%. The antimicrobial efficacy of HiOra® against Streptococcus spp. was 99.97% and against Staphylococcus spp. was 99.98% [Table/Fig-6b].

Variables	1% Sodium hypochlorite (Group 1)	0.2% Chlorhexidine (Group 2)	HiOra® (Group 3)
Total number of impressions (N=60)	n=20	n=20	n=20
Upper arch (No. of impressions)	10	10	10
Lower arch (No. of impressions)	10	10	10

[Table/Fig-5]: Demographic data of the study population.

Groups and no. of swabs in each group	Agar plates with colonies of Staphylococcus observed	Agar plates with colonies of Streptococcus observed	Agar plates with colonies of Staphylococcus and Streptococcus observed	No growth seen
Group 1	-	7	12	1
Group 2	-	-	16	4
Group 3	-	1	14	5

[Table/Fig-6a]: Distribution of the colonies of Staphylococcus and Streptococcus in agar plates streaked with pre-disinfection intra oral swabs of groups 1, 2, 3 in the study (n=20 in each group).

Category	Mean	Standard deviation
Streptococcus species		
Group 1	99.99	0.22
Group 2	100.00	0.00
Group 3	99.97	0.04
Staphylococcus species		
Group 1	99.83	0.38
Group 2	99.93	0.24
Group 3	99.98	0.03

[Table/Fig-6b]: Mean percentage reduction in CFU counts of Staphylococcus and Streptococcus species after disinfection of the impression with sodium hypochlorite 1% (Group 1), 0.2% chlorhexidine (Group 2) and Herbal formulation- HiOra® (Group 3).

One-way ANOVA was done to determine the significance of the difference in the antimicrobial efficacy between the groups for the two bacteria evaluated. No significant difference in the efficacy could be determined between the antimicrobial agents (groups 1,2,3) tested for Streptococcus spp. (p-value=0.064) and Staphylococcus spp. (p-value=0.337) [Table/Fig-7].

Category	Sum of squares	Degree of freedom (df)	Mean square	F-value	p-value
Streptococcus species					
Between group	0.004	2	0.002	2.910	0.064
Within group	0.033	47	0.001	-	-
Total	0.037	49	-	-	-
Staphylococcus species					
Between group	0.150	2	0.075	1.117	0.337
Within group	2.618	39	0.067	-	-
Total	2.768	41	-	-	-

[Table/Fig-7]: Testing of significance of the intergroup differences by ANOVA. p-value <0.05 was considered as statistically significant

DISCUSSION

Disinfection and dentistry complement each other, and disinfection has been around since the inception of dentistry. Disinfection is practiced by various methods in dentistry (e.g., autoclaving of dental/surgical instruments, incineration of used cotton) [12].

Disinfection of dental impressions can be done by two different techniques: immersion and spray techniques. The immersion method is considered the gold standard amongst the two as the immersion method allows uniform distribution of the disinfectant over the surface of the impression material [2]. A condensation silicone impression material was chosen due to its hydrophobic nature which allows for immersion into the disinfectant without significant dimensional change or alteration of surface properties [13].

Concerns on time needed for a complete disinfection, alteration of the properties of the impression materials following immersion have been well studied in the literature [12]. Silva SMLM da and Salvador MCG reported on the influence of the dimensional stability of the condensation silicone impression material following immersion for 10-20 minutes in 1% sodium hypochlorite and 2% glutaraldehyde [14]. The authors reported no significant differences in the dimensional stability following this method. Shetty S et al., reported on wettability changes of polyether impression material after immersing in four different chemical disinfectant solutions (2% glutaraldehyde, 5% NaOCl, 0.05% iodophor, 5.25% phenol) for two time periods of 10 minutes and 30 minutes, respectively [15]. The authors concluded 0.05% iodophor was an effective disinfectant without affecting wettability of the polyether impression material [15]. A 10 minute immersion time was also found to be effective for disinfection of the impressions made [15].

A more recent study by Azevedo MJ et al., evaluated the efficacy of 3% hydrogen peroxide, MD 250 (Durr Dental), 1% sodium hypochlorite and 5.25% sodium hypochlorite in impression disinfection by immersion technique for 10 minutes duration [16]. In addition, the dimensional stability of addition silicone impression materials was also evaluated. The authors reported no alteration in the dimensions of the impression made and a 99.9% reduction of the microbial load as assessed by the colony forming unit count.

The herbal formulation HiOra® is an antimicrobial, antiseptic herbal mouthwash manufactured by the Himalaya drug company [7]. The present study evaluated the efficacy of a commercially available herbal formulation, chlorhexidine gluconate 0.2% and sodium hypochlorite 1% for impression disinfection by the immersion method (10 minutes duration). Antimicrobial efficacy was evaluated against two common oral species *Staphylococcus aureus* and

Streptococcus viridans. Trypticase Soy agar was used to selectively grow the two species from the sample collected intraorally using the sterile swab. The herbal formulation was found to be equally efficacious as the chlorhexidine and 1% sodium hypochlorite in reducing the microbial load (>99%) as assessed by CFU count after plating in agar plates. The present study is possibly the first to evaluate the usefulness of this herbal formulation HiOra® as a disinfectant of impression materials.

Literature on the dimensional changes of the impression after immersion in the disinfectants has reported varied outcomes. Studies by several authors reported no significant changes in the dimensional stability of the impressions made with elastomeric impression materials [14-16]. However, a scanning electron microscopic evaluation of the surface changes in impressions made using four different elastomeric impression material which were disinfected with 0.525% NaOCl, 0.3% benzalkonium chloride and ozone revealed a wrinkling of the surface for short disinfection times of 2-5 minutes irrespective of the disinfectant and technique and significant surface degradation with exposure of the silicone crystals following long exposure periods of 30 minutes [17]. In contrast, Mc Cabe JF and Storer R reported silicone based impressions has the least dimensional change when immersed in 2% alkaline glutaraldehyde, 4% formalin, and 1% sodium hypochlorite for 16 hours [18].

A recent systematic review by AlZain S, evaluated the studies published on the different disinfectants used and their influence on properties of the impression made with different impression materials [5]. The authors selected data from 70 studies for evaluation and concluded there were variations in the outcomes reported (especially in dimensional stability, surface changes, wettability), which was attributable to the variations in the immersion times used, the difference in the methods of evaluation of the outcomes, the difference in the concentrations of the disinfectants used and also differences in the materials used to make the impressions. The authors further suggested that manufacturers of the dental impression materials propose specific disinfectants to be used with particular protocols which help maintain the material properties along with achieving an adequate disinfection.

Limitation(s)

One of the limitations of the present study was that, this study evaluated only the disinfection efficacy of the herbal formulation and not its influence on dimensional stability of the impressions made. In addition, pre-disinfection swab had been obtained from the patient's mouth and the post disinfection swab has been obtained from the impression material.

CONCLUSION(S)

In conclusion, the herbal formulation was found to have an equivalent efficacy as 0.2% chlorhexidine gluconate and sodium hypochlorite 1%, for disinfection of impressions made from condensation silicone. Further studies are needed to assess any dimensional changes of the impression following disinfection with this herbal formulation.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 21, 2021
- Manual Googling: Nov 27, 2021
- iThenticate Software: Mar 05, 2022 (7%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: Summer Research Fellowship, Sri Ramachandra Institute of Higher Education and Research (10,000/which will be given for research support).
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 18, 2021**
Date of Peer Review: **Nov 29, 2021**
Date of Acceptance: **Feb 21, 2022**
Date of Publishing: **May 01, 2022**