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To evaluate the dimensional accuracy and hardness of gypsum cast on repeated immersion in sodium hypochlorite and peroxygenic acid: An *in-vitro* study

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Abstract
Microbes, fungi, and viruses present in the dental atmosphere have been linked to debilitating and life threatening diseases. Transmission of infected materials from the clinic to the dental laboratory results in a high level of cross contamination. The objective of the study was to evaluate the dimensional accuracy and hardness of gypsum cast on repeated immersion in 0.525 percent Sodium hypochlorite and 1percent Peroxygenic acid. 72 specimens of type 3 dental stone were created. 24 specimens were immersed in slurry, 0.525 percent Sodium hypochlorite, and 1 percent Peroxygenic acid solutions for 30 minutes, then air dried for 24 hours. For testing purposes, this process was repeated seven times. The hardness of stone cylindrical specimens was evaluated using a Rockwell hardness testing equipment. The values obtained by Kruskal-Wallis and Mann-Whitney tests, which were employed for statistical analysis at $\alpha=0.05$, were used to evaluate dimensional accuracy and hardness. When compared to the 0.525 percent Sodium hypochlorite and slurry treatments, the specimens treated with 1 percent peroxygenic acid showed more dimensional change. Maximum hardness was achieved with 1 percent peroxygenic acid. Slurry & 0.525 percent sodium hypochlorite is dimensionally stable than 1 percent Peroxygenic acid. Hardness of type III dental stone samples disinfected by 1 percent Peroxygenic acid was the highest. Given the other benefits of virkon as a disinfectant agent, as well as the lowest reduction in gypsum mechanical strength, it can be recommended for the disinfection of stone casts in the clinic and laboratory.

Keywords: Disinfectants, dimensional accuracy, hardness

1. Introduction

A number of microbes, fungi, and viruses present in the dental atmosphere have been linked to debilitating and life threatening diseases. Direct physical interaction between the dental clinic and dental laboratory is inherent in the practice of general dentistry. Transmission of infected materials from the clinic to the dental laboratory results in a high level of avertable cross contamination. Every effort, therefore, must be made to avoid cross-contamination of these microorganisms and to prevent the potential transfer of disease in the dental setup.

Leung and Schonfeld indicated potential cross-infection between patients and dental staffs via contaminated dental casts [1]. Cross-contamination is possible due to the risk of transferring infectious agents from blood and saliva to the casts through Impression materials and other alternative appliances used in oral environments such as prostheses can be potential sources.

American Dental Association (ADA) recommends the impressions to be washed with water in order to cleanse them of blood, saliva and food debris then be sterilized and sent to the dental laboratory. Disinfection of impressions removes the microorganisms off their surface; dimensional changes can also take place due to chemical or physicochemical reactions between the set cast and therefore the disinfectant solutions.

Disinfection of stone casts is a vital measure for the control of cross contamination. The nature of dental casts is porous structured and extremely hydrophilic that alter deep penetration of microorganisms, rendering the surface disinfection techniques futile. The methods of disinfecting casts are arduous to control.

Although the addition of disinfectants like sodium hypochlorite and peroxygenic acid [VIRKON] appears capable of providing a disinfected cast, but information regarding surface hardness and dimensional changes of the resultants casts may be a concern. Infection control procedure must be attained in such a way that dental casts do not undergo dimensional changes and hardness.

Sodium hypochlorite is the most commonly used disinfectant due to inexpensive, availability and its potent antimicrobial properties. Tebrok *et al*. reported that application of 0.525% sodium hypochlorite solution was microbiologically effective within 30 min of application [2].

Peroxygenic acid [1% VIRKON] is a form of disinfectant that uses an acid peroxygen system and has excellent bactericidal properties against all major human pathogens like hepatitis B and HIV. This chemical agent has colour indicator properties, indicating that the solution should be discarded when the pink colour fades, and it has no irritating effect on the eyes or bare skin, indicating that there are no toxicity issues or chlorine generation, which is harmful to technicians in direct contact with waste materials. Virkon has proven to be effective in disinfecting imprints, burs, and toothbrushes.

The erstwhile studies were not concerned with evaluation of the dimensional changes and hardness of stone casts disinfected by substituting gypsum mixing water with solutions of disinfectants like sodium hypochlorite and peroxygenic acid.

Accordingly, the objective of this invitro study was to quantitatively valuate their impact on hardness and dimensional changes of the set cast, by adding selected disinfectants like sodium hypochlorite and peroxygenic acid [VIRKON] to the dental stone. Sodium hypochlorite and Peroxygenic acid [VIRKON] solutions are utilized in completely different concentrations to disinfect the dental stones.

2. Materials and Methods

This study was conducted in Department of Prosthodontics of K.V.G. Dental College and Hospital, Sullia.

2.1 Materials

Materials used in this study are Polyvinyl siloxane material [Dentsply], Dental stone [Type III Gold stone], 0.525% Sodium hypochlorite, 1% Peroxygenic acid [Virkon], Slurry [supernatant solution of calcium sulfate in distilled water]

Table 1: Materials

SL No:	Materials	Composition	Manufacturer
1.	Dentsply Aquasil®	Vinyl terminated polydimethylsiloxane, crystalline quartz, fumed silica	Dentsply DeTrey GmbH Konstanz, Germany.
2.	Goldstone®	Calcium sulphate hemi-hydrate (CaSO2) 2. H_2O, Sodium Citrate,	Asian Chemicals, Rajkot, Gujarat, India.
3.	Emplura®	4% Sodium hypochlorite	Merck Life Science Pvt Ltd, Godrej one, Vikhroli, Mumbai, India.
4.	Virkon™	Triple Salt: Pottasium Monopersulphate/Pottasium Hydrogen Sulphate/Pottasium Sulphate, Sodium C10-13-alkylbenzenesulfonate.	Lanxess India Pvt Ltd by Trisis ventures, Himachal Pradesh, India.

2.2 Methods

A master metal die was used to make a specimen which is highly polished surface approximately 15 mm in diameter, on which two parallel lines were inscribed for the determination of dimensional stability. A collar was fabricated in metal for the test die to fit into. A space of 5 mm was provided between the test die and the collar for the elastomeric impression materials.

Grouping was done as follows

Group A: Control group (Slurry, a supernatant solution of calcium sulfate in distilled water)

Group B: 0.525% sodium hypochlorite

Group C: 1%Peroxygenic acid

For evaluating dimensional accuracy, samples are divided into groups as follows-

Group A_1: Dimensional accuracy of Type III dental stone in A sample

Group B_1: Dimensional accuracy of Type III dental stone in B sample

Group C_1: Dimensional accuracy of Type III dental stone in C sample

For evaluating hardness, samples are divided into groups as follows-

Group A_2: Hardness of Type III dental stone in A sample

Group B_2: Hardness of Type III dental stone in B sample

Group C_2: Hardness of Type III dental stone in C sample

Type III dental stone was subjected to repeated immersion in slurry and with two different disinfectant solutions, namely, 0.525% sodium hypochlorite and 1%peroxygenic acid.

A master metal die was used to make a specimen, which is highly polished surface approximately 15mm in diameter, on which two parallel lines were inscribed for the determination of dimensional stability.

A collar was fabricated in metal for the test die to fit into. A space of 5 mm was provided between the test die and the collar for the elastomeric impression material. The impression was made with polyvinyl siloxane impression material. (Fig 1)

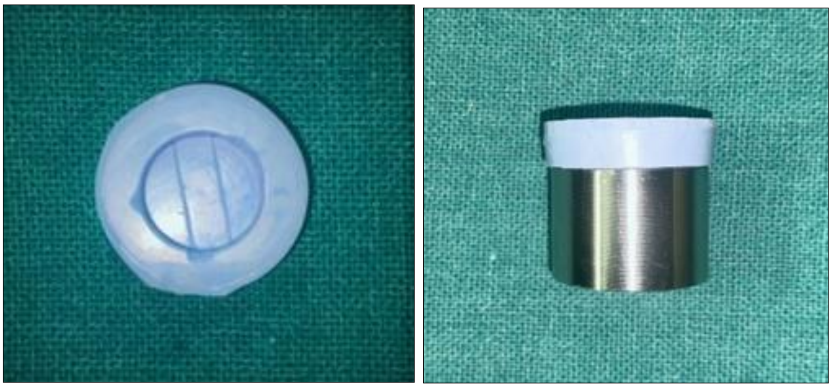


Fig 1: The Putty impression made with polyvinyl siloxane impression material.

Prior to fabricating each specimen, the surface of the master metal die was rinsed with distilled water and dried. Impression of test die was made in metal collar by placing polyvinyl siloxane material in the collar. Dental stone was mixed according to manufacturer's instructions. Spatulation was be done for 30sec and added to the impression in small increments, placed on a mechanical vibrator to prevent formation of air bubbles. The cylindrical specimens were allowed to set for 1h at ambient room temperature. Similarly, 72specimens were produced. (Fig 2).

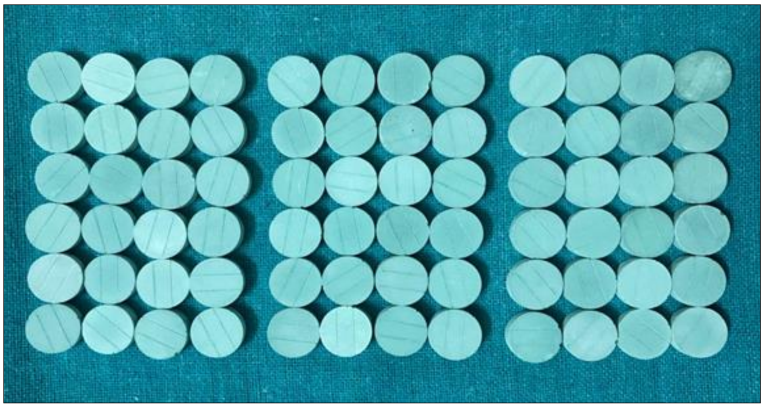


Fig 2: 72 Specimens of Type 3 stone casts

Three different solutions were tested for their effect on the dimensional accuracy and hardness of cylindrical test specimens:

Group A: Slurry, a supernatant solution of calcium sulfate in distilled water

Group B: Sodium hypochlorite (0.525%)

Group C: Peroxygenic acid (1%) [Virkon]

Slurry was prepared by placing clean, completely set dental stone pieces in a plastic container of distilled water and soaked for 48 h. The resultant supernatant solution was stored at room temperature and used for soaking the test cylindrical specimens.

The second solution is sodium hypochlorite diluted to 0.525% concentration with distilled water, which was made fresh daily to ensure efficacy.

The third solution used is 1%peroxygenic acid. An immersion bath of 500ml for disinfection of cylindrical specimens will be established.

24 cylindrical specimens of type III dental stone were immersed in each of the three solutions:

Group A: (control) slurry,

Group B: 0.525% sodium hypochlorite, and

Group C: 1%Peroxygenic acid.

Fig 3: Immersion of specimens in Slurry, 0.525% Sodium Hypochlorite & 1% Peroxygenic Acid

Immersion was done for 30 min at room temperature (fig – 3). After immersion, all cylindrical specimens were removed from their respective baths and allowed to dry for 24 h at room temperature. The process of immersing and drying at room temperature conditions was repeated 7 times, and immersion bath solutions are replaced for each cycle. The sequence of 7 cycles chosen as an average for the number of immersions in disinfectant solution necessary in the construction of complete and partial removable prostheses. The accuracy of linear dimensions of stone cylindrical specimens was examined by using profile projector and hardness was determined by using Rockwell hardness testing machine.

2.3 Statistical analysis

Results were plotted with a mean and standard deviation.
SPSS (Statistical Package for Social Sciences) version 20. [IBM SPASS statistics (IBM corp. Armonk, NY, USA released 2011)] was used to perform the statistical analysis
Kruskal-wallis test will be applied to check the statistical difference of hardness and dimensional accuracy among the groups with post-hoc Mann-whitney for pair-wise comparison.

3. Results

The aim of the present study was to evaluate the dimensional accuracy and hardness of gypsum cast on repeated immersion in sodium hypochlorite and peroxygenic acid [1% Vikon].
The statistical tests used for the analysis of the result were:

1. Descriptive statistics calculated by mean, standard deviation
2. Inferential statistics like Kruskal-wallis test

SPSS (Statistical Package for Social Sciences) version 20. [IBM SPASS statistics (IBM corp. Armonk, NY, USA released 2011)] was used to perform the statistical analysis. Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation for quantitative variables, frequency and proportions for qualitative variables. Inferential statistics like Kruskal-wallis test were applied to check the statistical difference of dimensional accuracy and hardness among the groups with Mann-whitney for pair-wise comparison. The level of significance was set at 5%.
The dimensional accuracy and hardness of type 3 stone casts were evaluated after immersing in slurry, disinfectants like 0.525% Sodium hypochlorite and 1% Peroxygenic acid [Virkon]. A total of 72 specimens in which 39 specimens for dimensional accuracy & 33 specimens for hardness. A master metal die was used to make a specimen which is highly polished surface approximately 15 mm in diameter, on which two parallel lines were inscribed for the determination of dimensional stability.
24 cylindrical specimens of type III dental stone were immersed in each of the three solutions:
Group A: (control) slurry,
Group B: 0.525% sodium hypochlorite, and
Group C: 1% Peroxygenic acid.

Immersion was done for 30 min at room temperature and all cylindrical specimens were removed from their respective baths and allowed to dry for 24 h at room temperature. This process was repeated 7 times. Profile projector for dimensional accuracy examined each specimen and hardness was determined by Rock well hardness testing machine at K.V.G College of engineering.
Table 2 and Table 4 indicates results of current study. The mean dimensional accuracy of control group A was 4.87, the mean dimensional accuracy of 0.525% Sodium hypochlorite group B was 4.81 and the mean dimensional accuracy of 1% peroxygenic acid group C was 4.62. The mean hardness of control group A was 21.45, the mean hardness of 0.525% Sodium hypochlorite group B was 15 andThe mean hardness of 1% peroxygenic acid group C was 21.45.
Pairwise comparison of dimensional accuracy and hardness was done and found to be highly statistically significant (Table 3) and (Table 5)

Table 2: Comparison of different groups for dimensional accuracy Kruskal-Wallis test

	Dimentional Accuracy				P Value
	Mean	**Standard Deviation**	**Median**	**N**	
Group A – Control Group	4.87	0.22	4.87	13	<0.01**
Group B – 0.525% Sodium Hypochlorite	4.81	0.07	4.83	13	
Group C – 1% Peroxygenic Acid	4.62	0.09	4.65	13	

Table 3: Pairwise comparisons using Mann-Whitney U test (Bonferroni)

	Group A – Control Group	Group B – 0.525% Sodium Hypochlorite
Group B – 0.525% Sodium Hypochlorite	1	-
Group C – 1% peroxygenic acis	<0.01**	<0.01**

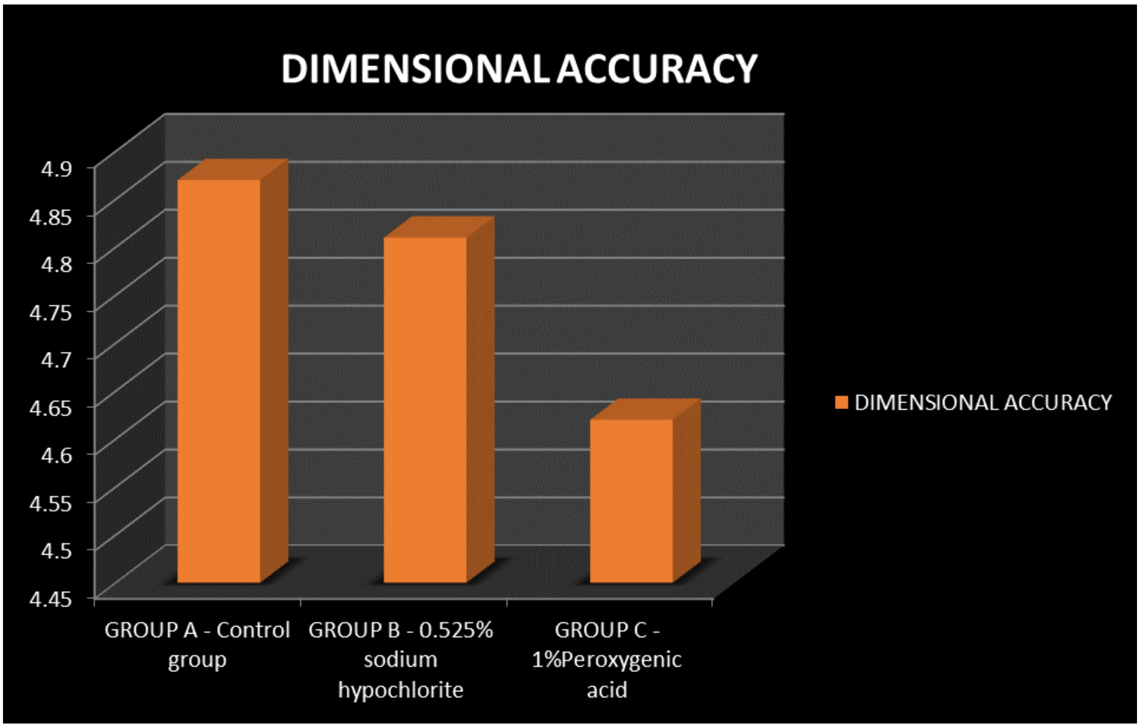
Table 4: Comparison of different groups for HARDNESS VALUES: Kruskal-Wallis test

	Hardness Values				P Value
	Mean	**Standard Deviation**	**Median**	**N**	
GROUP A – Control Group	21.45	0.01	21.5	11	<0.01**
Group B – 0.525% sodium hypochlorite	15	3.13	16	11	
Group C – 1% Peroxygenic Acid	21.45	1.29	22	11	

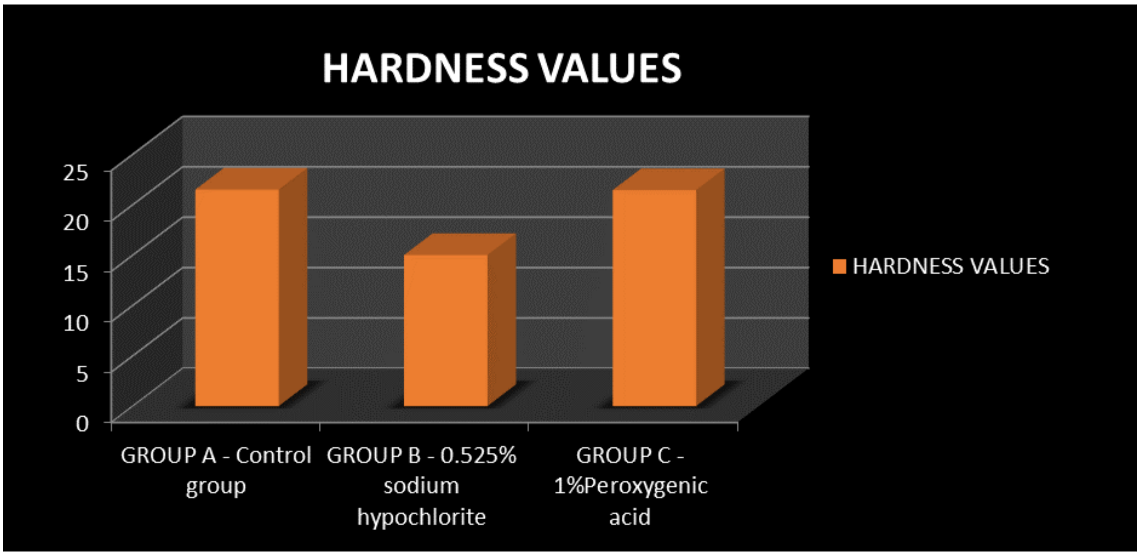
Table 5: Pairwise comparisons using Mann-Whitney U test (bonferroni)

	Group A – Control Group	Group B – 0.525% Sodium Hypochlorite
Group B – 0.525% Sodium Hypochlorite	<0.01**	-
Group C – 1% Peroxygenic Acid	1	<0.01**

Comparison of different groups for dimensional accuracy (Graph 1) Group A shows maximum representation of graph, which indicates the highest value, and Group C showed the least values. (Graph 2) indicates hardness values Group A and Group C showed same significant values and Group B showed the least values



Graph 1: Comparison of different groups for dimensional accuracy



Graph 2: Comparison of different groups for hardness values

4. Discussion

Bacterial contamination and transmission during dental operations are unavoidable due to contact with blood and saliva. Cross contamination is a major issue in the field of prosthodontics. Bacterial colonies are transferred to casts when impressions are poured with dental stone. As a result, effective impression disinfection is required to prevent infection transmission from the clinic to the laboratory [3].

Microwave radiation in dentistry has been widely accepted for sterilizing, polymerizing acrylic resins, removing wax from moulds, shortening the dough stage of denture base acrylic resins, and for drying various kinds of gypsum products [4].

Some reports showed that certain microorganisms could be recovered from a gypsum cast when it is poured against a contaminated impression [5]. Several approaches to the sterilization and disinfection of either impressions or gypsum casts have been attempted, and most of them were microbiologically effective; The ADA recommendations for disinfecting impressions include "spraying or immersing in any compatible disinfecting product. however, some alterations in the physical properties of the materials were also detected [6].

On the other hand, disinfectants should not change the properties of impression materials, as these changes will be transferred to the casts and cause inaccuracy in their physical properties. Dental casts should have a high mechanical strength and be resistant to fracture and abrasion in order to be clinically useful.

An alternative method of using chemical disinfectant solutions as mixing-water substitutes was suggested [7]. Others used both gum arabic and calcium hydroxide additives to modify the hemihydrate powder before mixing with aqueous solutions of chemical disinfectants, and the results showed an improvement of strength and hardness of the resultant disinfected materials without affecting other surface properties [8].

The microbiological efficiency of the disinfectants like sodium hypochlorite and Peroxygenic acid [Virkon] used in this study has been established previously. Both sodium hypochlorite and Peroxygenic [Virkon] caused a great reduction in colony counts [9].

Mansfield and White investigated the antimicrobial properties of sodium hypochlorite and reported that this agent reduced the bacterial count in experimental stone casts to that of negative controls in one hour [10].

Gasparini *et al*. studied the effect of Virkon on different microorganisms and demonstrated its significant effectiveness against a wide spectrum of bacteria, hepatitis virus and bacterial spores [11].

Moslehifard *et al*. evaluated the effect of 0.525% hypochlorite and 1% Virkon on mechanical properties of dental gypsum casts such as compressive and tensile strengths. They found that Virkon disinfectant only slightly decreased the mechanical strength [9].

Sodium hypochlorite and Peroxygenic axid [Virkon] solutions were used in different concentrations to disinfect the dental stones. The properties such as dimensional stability and hardness of gypsum immersed in disinfectants solutions like sodium hypochlorite and Peroxygenic acid have not been characterized.

This present study is to evaluate the dimensional accuracy and hardness after repeated immersions disinfectants like 0.525% Sodium hypochlorite and 1% peroxygenic acid.

A total of 72 specimens were made of type 3 dental stone. Twenty-four specimens were immersed in slurry, a supernatant solution of calcium sulfate in distilled water (control casts), 24 specimens in 0.525% sodium hypochlorite, and 24 specimens in 1% Peroxygenic acid solutions for 30 min and air dried for 24 h. This process was repeated for 7 times for testing. According to a study by Stern *et al.* seven cycles of disinfection are required for the fabrication of complete or removable partial dentures [12]. To assess the dimensional stability of type 3 dental stone casts ADA specification No. 25 prescribes a stainless steel die with two parallel lines inscribed on it.

The stone casts were examined for dimensional stability measured with a profile projector of 1µm accuracy for entirety of the parallel lines. The Rockwell hardness machine is designed to measure hardness.

Our study reported that the values obtained by dimensional stability and hardness testing for all the three were statistically evaluated by kruskall wallis and Mann- Whitney tests which were used for statistical analysis at $\alpha=0.05$.

The mean of dimensional stability of Slurry (Group A) sample was 4.87, Sodium hypochlorite (group B) was 4.81 and that of Peroxygenic acid (Group C) was 4.62. In this study, gypsum specimens disinfected with peroxygenic showed more mean dimensional change compared to sodium hypochlorite and slurry. The lowest dimensional stability was shown by Peroxygenic acid. The findings of this study are in general agreement with previous studies.

The mean of Hardness of group a Slurry (Group A) sample was 21.5, 0.525% sodium hypochlorite (Group B) was 15 and that of 1% Peroxygenic acid (Group C) was 21.4. Both the groups such as Slurry (Group A) and 1% Peroxygenic acid (Group C) show same hardness. Sodium hypochlorite (Group B) showed lesser values as compared to Slurry (Group A) and Peroxygenic acid (Group C).

The decreased hardness in gypsum specimens immersed in disinfectant solutions may have been a result of a reaction between disinfectant and stone. This concentrated residual disinfectant may have reacted with gypsum to produce decreased hardness.

Based on the argument given, it is seen that the mechanical strength difference between gypsum disinfected in all types of solutions are not statistically significant. However, virkon has the highest strength value.

Based on the low toxicity and good environmental compatibility of Virkon [1% Peroxygenic acid], it may be used as an antimicrobial agent for disinfection of dental stone casts as non-critical items. Considering other advantages of Virkon [1% Peroxygenic acid] as a disinfectant as well as minimal reduction in the mechanical strength of gypsum, it may be preferred for the disinfection of stone casts in the clinical and laboratory settings.

Moslehifard *et al*. evaluated the effect of 0.525% hypochlorite and Virkon [1% Peroxygenic acid] on hardness of dental gypsum casts. They found that the formation of micropores is responsible for the reduction of hardness, which is the least in the dental stones disinfected with Virkon [1% Peroxygenic acid] [14].

Sarma and Neiman assessed the effect of 0.525% sodium hypochlorite on the mechanical properties of die stones and reported the least changes in their physical properties. There are advantages to choosing sodium hypochlorite disinfectant solution, given the low cost of the product. However, because of the poor stability of a sodium hypochlorite solution over time, it has to be made fresh daily to ensure the necessary efficiency [3].

Bass *et al* compared the effect of immersion disinfection of completely set stone casts in a mixture of 0.525% sodium hypochlorite with slurry (supernatant solution of calcium sulfate in distilled water), and slurry water alone, using 30- or 60-minute intervals. The authors reported no difference in the quality of the surface of the cast between immersion in sodium hypochlorite with slurry and slurry water alone [13].

Limitations of the study are exact simulation of the oral environment is difficult in an *in vitro* study. Therefore, the present study results would have been affected. Also these results cannot be absolutely extrapolated across other brands of related materials because of the possibility of minor changes in chemistry, causing significantly different responses. Furthur proper manipulation of specimens poured with type 3 dental stone also depends on clinician skills.

5. Conclusion

The present study was carried out to evaluate to evaluate the changes in dimensional accuracy and hardness of the dental casts as a result of repeated disinfection in 0.525% sodium hypochlorite and 1% peroxygenic acid solutions. Within the limitations of the study, the following conclusions could be drawn:

1. Stone casts immersed in 0.525% sodium hypochlorite and 1% peroxygenic acid solutions showed significant difference in dimensional accuracy compared to stone casts in slurry.
2. Stone casts immersed in 1% peroxygenic acid solutions showed significant reduction in dimensional accuracy when compared to stone casts in Slurry and 0.525% sodium hypochlorite

3. Stone casts immersed in 0.525% sodium hypochlorite showed reduction in hardness compared to stone casts in slurry and 1% peroxygenic acid.
4. Stone casts immersed in Slurry and 1% peroxygenic acid solutions showed no significant difference in hardness.

By this study, we can conclude that 0.525% sodium hypochlorite is dimensionally stable than 1% Peroxygenic acid and recorded 1% Peroxygenic acid has higher surface hardness compared to 0.525% sodium hypochlorite

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