Study Protocol

Comparative Evaluation of Antimicrobial Activity of Irreversible Hydrocolloid Impression Material Incorporated With Various Disinfectants – An In-Vitro Study.

ABSTRACT:

Background: Blood and saliva can easily contaminate the dental impressions. It contains variety of bacteria, fungi, and viruses which causes cross infection to health personnel & environment. Therefore, sterilizing the impression material is the best way to avoid disease transmission. Spraying and Immersion technique are conventional methods of disinfection of irreversible hydrocolloid impression material but in both of these techniques impression is disinfected only on the surface. Self-disinfecting impression are disinfected throughout the impression material. This study categorized various disinfectants into Chemical, Herbal & Physical. Nanoparticles and herbal disinfectants are mixed with irreversible hydrocolloid impression material, which will suggest its antimicrobial activity. **Aim & Objectives:** To evaluate and compare antimicrobial-activity of irreversible hydrocolloid impression materials incorporated with various disinfectants. **Methodology:** Commercially existing irreversible hydrocolloid impression materials will be used in the study. The disinfectants used will be chlorhexidine, silver nanoparticles and cinnamon. The selected disinfectants will be added into the irreversible hydrocolloid at the manipulation stage and their antimicrobial activity will be evaluated using the "Kirby Bauer Disk Diffusion Method" against the selected microorganisms. **Expected Outcome:** Differential Antimicrobial activity of Chlorhexidine, Silver Nanoparticle and Cinnamon when added to irreversible hydrocolloid for disinfection of impressions. **Conclusion:** Addition of various disinfectants in irreversible hydrocolloid may imbibe the antimicrobial activity within the impression materialand with the comparative investigations, more potent disinfectant can be applicable for clinical use as per findings of the present study.

Keywords: Disinfection of impressions, irreversible hydrocolloid impression material, chlorhexidine, silver nanoparticles, cinnamon.

INTRODUCTION:

Various tools or equipment utilized in the stomatognathic system and prosthetic oral devices that are soiled or imprint are capable of causing of cross-infection. Impressions of oral cavity can simply become soiled with a patient's secretion such as saliva and blood. They comprise of pathogens such as numerous bacteria, fungi and viruses. Some of them can remain active for prolonged time outside their hosts. It is not possible to govern all the probable patients that are infected from their medical history. The uniform set of disinfection criteria and techniques should be utilized on every patient.[1]

Laboratory technicians and auxiliaries are at a higher risk for cross-infection[2]. Therefore, impressions should be efficiently sterilized before transporting it to the laboratory. This exercise of sterilization would be the reason of alteration in dimensions that happen in the impressions. Numerous disinfectants namely sodium metabisulphite, sodium hypochlorite, iodine compounds, biguanides, quaternary ammonium salts, 2% glutaraldehyde[3], povidone iodine powder are routinely used. Only one disinfectant could not be designated as a "universal disinfectant", so it is impervious to choose a disinfectant having broader anti-microbial property which doesn't disturb the details of the record[3].

In clinical practice alginate is routinely used for recording the impressions of the patients. Due to its properties such as hydrophilicity and the surface texture of impression material, recording of impression allows maximum retention of the pathogenic microbes on the surface and within the material.[4]

To disinfect hydrocolloid (irreversible) guiding principle is recognized by the American Dental Association (ADA) such as impression disinfection with the help of disinfectant solution by spray or an immersion technique[1]. However, both the procedures disinfect only on the surface of the impression. Further, it may result in significant changes in the dimensions due to its inherent properties like syneresis and imbibition leading to loss of accurate details. Worsening in the surface quality as well as the strength of the cast attained from dental gypsum material that has been disinfected is also extensively documented.[4] "Spraying technique" which is achieved with reduced time of contact may decrease the effectiveness of disinfection, main component being the porosity of impression material being used this causes the microorganisms to survive in the material. In addition to that it can have several deleterious effects like irritation of eyes and skin. Hence, they are harmful to both health personnel and the environment.[4]

To report the shortcomings linked with traditional method of disinfection of irreversible hydrocolloids, if the material has the properties of self-disinfection, it will be possible to disinfect throughout and not just on the surface. In some studies, self-disinfection carried out with addition of chlorhexidine, silver nanoparticle[5] which showed positive effect to inhibit the microbial count.[6]

Even herbal products like Neem, Aloe vera, Clove, Cinnamon has proved their antimicrobial effect against various strains of microorganisms. In the field of Ayurveda amongst the various known herb, cinnamon is used as a health improvising agent to treat many diseases. There were some studies in which antimicrobial activities of cinnamon were evaluated[2]. Cinnamaldehyde, the major component of cinnamon, possesses antimicrobial effect.[7] It being used for ages for medicinal purposes and also could be used successfully in the dental field as well. The important benefit of the use of cinnamon is being a natural product. So, it exhibits minimal side-effects. It is readily available, cheap and most important is that it is completely biodegradable and environment or eco-friendly[4]. In spite this, use of cinnamon as an antimicrobial substance in impression materials hasn't been studied.

Therefore, the purpose of this study is to evaluate and compare various disinfectants as categorized into Chemical, Nanoparticle and cinnamon mixed with irreversible hydrocolloid impression material which will suggest its antimicrobial activity which has not been studied till date.

AIM:

To evaluate and compare antimicrobial-activity of irreversible hydrocolloid impression material incorporated with various disinfectants and the subsequent changes that can be observed in dimensional stability of the material.

OBJECTIVES:

1. To measure the Minimal Inhibitory Concentration [MIC] of Chlorhexidine, Silver nanoparticles and Cinnamon against St. Aureus and Candida Albicans.
2. To evaluate the Antimicrobial-activity of Chlorhexidine, Silver nanoparticles and Cinnamon mixed with irreversible hydrocolloid impression material against St. Aureus.
3. To evaluate the Antimicrobial-activity of Chlorhexidine, Silver nanoparticles and Cinnamon mixed with irreversible hydrocolloid impression material against Candida Albicans.
4. To Compare the Antimicrobial-activity of Chlorhexidine, Silver nanoparticles and Cinnamon added in irreversible hydrocolloid impression material against St. Aureus.
5. To Compare the Antimicrobial-activity of Chlorhexidine, Silver nanoparticles and Cinnamon added in irreversible hydrocolloid impression material against Candida Albicans.

MATERIAL AND METHODS:

Sources of Data

- It is an in-vitro experimental study, which will be carried out at Department of Prosthodontics and Crown & Bridge, Sharad Pawar Dental College, Sawangi (Meghe), DMIMS DU, Wardha. Institutional ethical clearance and informed consent were obtained from all the subjects before the commencement of the study (ref no. DMIMS(DU)/IEC/2020-21/9394)

Statistical Analysis

The data will be analysed by using descriptive and inferential statistics using one way ANOVA test and the analysis will be done using SPSS 24.0 version and level of significance $p < 0.05$.

Sample size calculated using:

Sample size for difference between two means

$$n = \frac{(Z_\alpha + Z_\beta)^2 \left[\frac{{\delta_1}^2 + {\delta_2}^2}{K}\right]}{\Delta^2}$$

Where,

$Z\alpha$ is the level of significance at 5% i.e. 95% confidence interval=1.96

$Z\beta$ is the power of test=80%=0.84

δ1=SD of antimicrobial activity of C. albicans in Zelgan =1.73

δ_2=SD of antimicrobial activity of C. albicans in Tropicalgin=0.58

K = 1

Δ=difference in mean =8-5.67=2.33

$$n = \frac{(1.96 + 0.84)^2 \left[\frac{1.73^2 + 0.58^2}{1}\right]}{2.33^2}$$

=7.70

=8 samples needed in each group.

Study Design

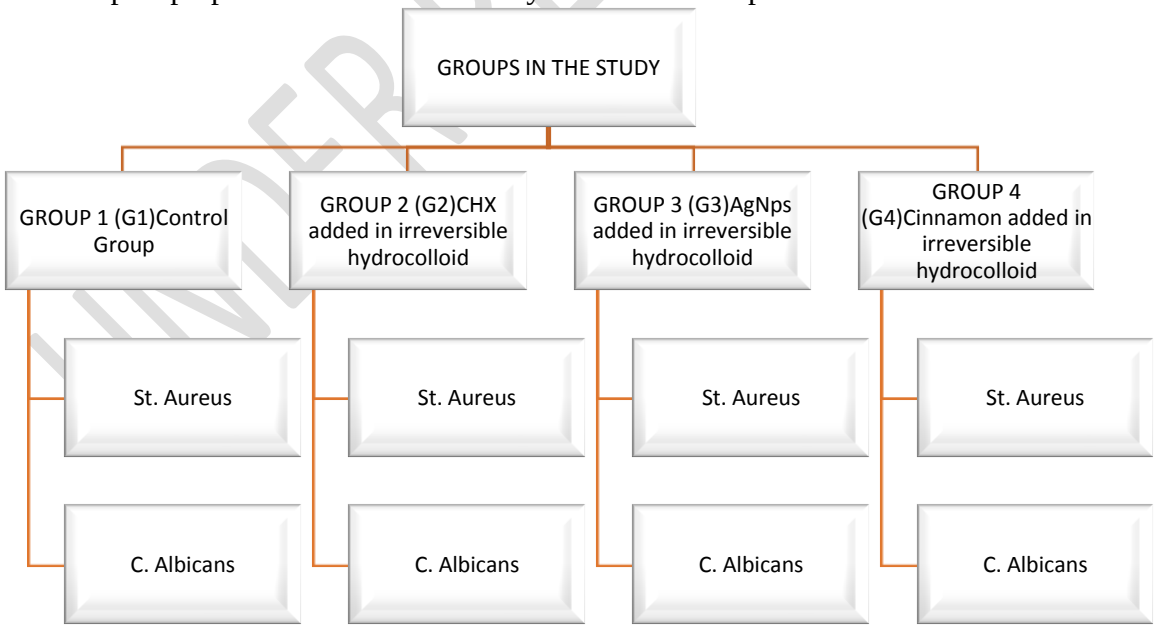
In this experimental study

Total No. of Groups=8

Total Sample Size: n=64

GROUPS –

G1- Samples prepared with Irreversible hydrocolloid
G2- Samples prepared with Irreversible hydrocolloid incorporated with Chlorhexidine (CHX)
G3- Samples prepared with Irreversible hydrocolloid incorporated with Silver nanoparticles (AgNps)
G4- Samples prepared with Irreversible hydrocolloid incorporated with Cinnamon



Materials used in study

Materials:
For fabrication of samples:

1. Zelgan Plus, Irreversible hydrocolloid impression material-Dust- free alginate
2. Disinfectants:

a. Chlorhexidine (chlorhexidine solution containing 2% chlorhexidine gluconate)
b. Silver nano particles
c. Cinnamon extract

For microbiological analysis:

1. Standard St. Aureus Strain (ATCC 25923)
2. Standard Candida Albicans Strain (ATCC 24433)
3. Mueller Hinton Agar plates
4. Sabouraud medium plates
5. Nutrient Agar culture plates
6. Glass tubes
7. Sterile gauze
8. 90mm Petri dish

Instruments:

1. Rubber bowl, Spatula
2. Polyvinyl chloride mould, glass slab

Equipment's:

1. Incubator
2. Centrifugation machine
3. Autoclave

Methodology:

1.DETERMINATION OF MINIMAL INHIBITORY CONCENTRATION:

The antimicrobial efficacy of Chlorhexidine, Silver Nano particles (Ag-NPs) and Cinnamon will be examined using the standard broth dilution method. The MIC will be determined using serial two-fold dilutions of Chlorhexidine, Silver nanoparticles and Cinnamon with adjusted concentrations of St. Aureus and C. Albicans. The MIC will be noted by the visual turbidity of the tubes both before and after incubation.

2.FABRICATION OF SAMPLES

- Preweighed irreversible hydrocolloid powder will be mixed with chlorhexidine, silver nanoparticles and cinnamon separately dispensed into a container.
- Irreversible hydrocolloids without disinfectant will be tested as control groups.
- Specimens of impression material with or without disinfectant will be prepared by mixing volume of water which is premeasured with the powder as recommended by the manufacturer.
- Then the polyvinyl chloride mould will be poured with the material which is of 16 mm height and 30 mm of internal diameter when placed on a glass slab. Just after when the mould is filled, 2nd mould which is having 19mm of height and 15 mm of internal diameter will be enforced in the impression material mixture which is in the previous mould until it extrudes onto the top. Then, the excess material will to be removed with the help of a flat

glass plate by pressing on the top of the 2nd mould and left until it sets; then the specimens will be tested upon retrieval.

- With the help of scalpel that has been sterilized, disks will be sliced from the specimen of irreversible hydrocolloid of 3 mm in thickness and positioned on "Mueller Hinton agar plates". Antimicrobial activity will be checked by using the "Kirby Bauer disk diffusion" method. The Kirby-Bauer antimicrobial disk diffusion procedure is used with **Mueller Hinton Agar plates**. It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on an agar surface, resulting in diffusion of the antimicrobial into the surrounding medium[10].

3.ZONE OF INHIBITION TEST:

- The *St. aureus* strain will be placed on Nutrient Agar incubated at 37°C for 24hrs and *C. albicans* strain will be placed on "Sabouraud' Dextrose Agar" (SDA) incubated at 37°C for 48 hrs. After this period, a suspension of *St. aureus* and *C. albicans* will be prepared in sterile saline solution.
- With the help of sterile swab, the suspension of pure culture will be spread uniformly over sterile Mueller Hinton Agar plate.
- At the centre of agar plate, Alginate disc containing disinfectant will be placed.
- The Muller Hinton Agar plate is incubated at 37°C for 18-24 hours.
- When the Antimicrobial agent leaches from the disks into the agar, it reveals a growth-inhibiting effect, called zone of inhibition (clear zone) that appears around the experimental sample.
- The existing antimicrobial action dictates the size of zone of inhibition in the sample.

EXPECTED OUTCOME:

Significant difference in the Antimicrobial activity of Chlorhexidine, Silver Nanoparticle and Cinnamon when added to irreversible hydrocolloid impression material.

DISCUSSION:

The study is to evaluate and compare various disinfectants as categorized into Chemical, Nanoparticle and cinnamon mixed with irreversible hydrocolloid impression material which will suggest its antimicrobial activity which has not been studied till date. When alginate without disinfection was wholly unsuccessful against five test bacteria, it was completely effective when combined with quaternary ammonium, and chlorhexidine had a cidal effect against all test germs[1].
Denise et al. (1998) studied the disinfection of alginate impression by adding disinfectant in it.

Wang et al. (2007) in their experimental study mixed irreversible hydrocolloid with various concentration of chlorhexidine solution and concluded that, chlorhexidine self-disinfecting impression material showed antibacterial activity without any change in the physical properties.[2]

Ginjupalli K. et al. (2016) evaluated physical properties and the antimicrobial-activity of hydrocolloid impression materials added with "silver nanoparticles" concluded that superior antimicrobial action of alginate by adding silver nanoparticle to it without adversely affecting their physical properties.[4]

Qing Liu et al. (2017) in their review article on anti-bacterial and anti-fungal activities of spices mentioned in detail effects of various spices. The article mentioned the antibacterial and antifungal effects of cinnamon extract on 10 bacterial species and 7 fungi species.[7]

In experimental systems using Escherichia coli and Staphylococcus, cinnamon essential oil (EO) showed significant antibacterial action against foodborne spoilage and pathogenic microorganisms. The minimum inhibitory concentration (MIC) of cinnamon EO was the same for both bacteria (1.0 mg/ml), while the minimal bactericide concentrations (MBC) for E. coli and Staphylococcus aureus were 4.0 mg/ml and 2.0 mg/ml, respectively. Cinnamaldehyde was the main ingredient in cinnamon EO, according to GC–MS analyses (92.40 percent).

Silver has a long history of use as a broad-spectrum antibacterial agent, and it's commonly used to treat skin ulcers, burns, and eye infections. 31 Silver nanoparticles have been used in a variety of biomedical applications since the emergence of nanotechnology. Despite this, there has been no research on the use of silver nanoparticles as an antibacterial agent in impression materials.

The goal of this in vitro study was to assess the antibacterial activity and characteristics of two commercially available irreversible hydrocolloid imprint materials that had silver nanoparticles added to them.

CONCLUSION:

Addition of various disinfectants in irreversible hydrocolloid impression material may enhance the antimicrobial property of the material and with the comparative investigations, more potent disinfectant can be applicable for clinical use after clinical trial.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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