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Efficacy of Chlorhexidine in Reduction of Microbial Contamination in Commercially Available Alginate Materials – In-Vitro Study

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Efficacy of Chlorhexidine in Reduction of Microbial Contamination in Commercially Available Alginate Materials – In-Vitro Study

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Results: A total of 24 out of 36 samples inoculated on various media showed growth, with an average contamination frequency of 67%. Bacillus species was highest in all three brands, followed by aerobic spore bearing bacilli and coagulase negative staphylococcus. Pseudomonas species was detected in two brands. All dilutions at 20μg/ml or above were clear of turbidity at 0.02% concentration, 10μg/ml or above were clear of turbidity at 0.05% concentration and all dilutions were clear of turbidity at 0.1% concentration.

Conclusion: The level of contamination with microorganisms in commercially available alginate materials is high. 0.1% Chlorhexidine solution mixed in alginate can be effective alternative for disinfection impression material.

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I. Introduction

Dental practitioners, patients, and laboratory personnel are subject to notable risks with respect to infectious diseases, which can be spread by saliva or blood from contaminated impression material, particularly irreversible hydrocolloid impression material like alginates (Wang et al, 2007).A growing concern regarding the control of cross-infection in dentistry can be seen in literature. Laird and Davenport have stated that, it is often impossible to sterilize dental materials contaminated during manipulation in the mouth. Dental impressions are one such kind where the sterilization is not possible and can therefore act as a means of transmitting infectious agents from patients to those who handle them subsequently. In order to prevent the transmission of these infectious agents, effective infection control procedures should be exercised by all dental health care personnel (Ghani F, Hobkirk JA and Wilson M, 1990).

Saliva and blood can be a source of high concentrations of potentially infective pathogens that can cause Common cold, Herpes, Hepatitis B virus (HBV), Pneumonia, Tuberculosis, and are the suspected mode of transmission of Acquired Immune Deficiency Syndrome (AIDS). The dental clinics have responsibility for infection control and if not practiced, a cycle of cross-contamination may occur, thereby exposing dental health care personnel and patients to infection.

American Dental Association (ADA, 1985) proposed guidelines to limit cross-contamination during dental clinical procedures like impression disinfection. But unfortunately enough information regarding infection control and sterility of materials received by the dentist from the manufacturer, is not readily available. Further study in this area is warranted because of the increasing number of subjects who were immunocompromised due to disease process, chemotherapy or elderly individuals and are, thus very prone to normal or opportunistic infections.

The increasing emphasis on infection control has created interest in the possible hazard of the clinical use of commercial available alginate impression material. The need of prevention of transmission of microorganisms from the patient's oral fluids to the dental health care personnel via impression materials has been studied previously(Wang et al, 2007; Ghani F, Hobkirk JA and Wilson M, 1990).Previous studies have demonstrated microbial contamination in commercially available alginate impression material (Rice CD, Dykstra MA and Feil PH, 1992; Casemiro LA et al, 2007; Jafari AA et al, 2012).

Several methods of disinfection for alginate impression materials were proposed. Spray and immersion methods are the two most widely used techniques in clinical practice. However, these conventional strategies present several disadvantages like loss of surface detail and dimensional inaccuracy of the impression (Wood PR, 1992). Due to the difficulties

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with the disinfection of alginate impression materials, self-disinfecting alginate impression materials were developed. Studies have shown that this technique demonstrated better dimensional stability than spray and immersion techniques, and saved disinfection time (Touyz LZ and Rosen M, 1991; Rosen M and Touyz LZ, 1991; Poulos JG and Anton off LR, 1997).

Therefore, the need of hour was to develop a self-disinfecting irreversible hydrocolloid impression material with broad spectrum antimicrobial agent in order to provide a blanket cover against the spread of infection while recording the impression and handling. Hence, we aimed to evaluate the prevalence of contamination, minimum inhibitory concentration (MIC) of chlorhexidine against the isolated microbes and also to assess the surface activity of chlorhexidine in three commercially available alginate impression materials.

II. Materials and Methods

Twelve sealed packets of three different routinely used commercially available alginate impression materials were purchased from the local vendor. The materials tested were Tropicalgin(Zhermack), Microflex (Septodent) and Zelgan (Dentsply) henceforth, referred as Brand A, B and C.

The packaging was examined to ensure that it had not been tampered. Each packet was shaken well before opening it. The packets were opened, under a laminar flow chamber and three 0.1 gram samples were aseptically obtained from each packet. First sample of 0.1 gram was inoculated in 20ml of glucose broth; the second sample was inoculated into 20ml of thioglycolate broth, and vortexed for 10 seconds to disperse the irreversible hydrocolloid. The third sample was inoculated aseptically into 20ml sabouraud's dextrose agar tubes which were incubated at 22°C and 37°C for seven days, for the isolation of fungus. Ten sabouraud's dextrose agar tubes were inoculated with Candida albicans as positive control. Ten sham inoculated sabouraud's dextrose agar tubes served as negative control. The isolated fungus was identified by standard procedures (Moore GS and Jaciow DM, 1979).Ten sham inoculated glucose broth tubes and equal number of sham inoculated thioglycolate broth tubes served as negative controls. Ten glucose broth tubes and thioglycolate broth tubes were each challenged with Acinobacter and Bacteroidsfragilis respectively to serve as positive controls. All media were incubated at 37°C for 18 to 24 hours and later subcultures were done on blood agar. The isolated bacterial growth was identified by using standard microbiological techniques (Bailey and Scott, 1994; Mackie and McCartney, 1989).

In this study Pour plate technique (Bailey and Scott, 1994) was used for colony counting. Five allocates of 10mg each were made under aseptic conditions from each container, and each of these allocates were spread evenly on the base of an agar plate using a sterile spatula. The inoculums was covered completely with agar medium and allowed to set. The plates were incubated for 24 hours and colony counts were done following standard technique (Miles AA, Misra SS and Irwin JO; 1938).

The minimum inhibitory concentration (MIC) of chlorhexidine against Bacillus species, Aerobic spore bearing bacilli and Coagulase negative staphylococcus which were isolated from the alginate powder, was determined by using Broth dilution method (Bailey and Scott, 1994).A constant volume (1ml) of different concentrations of the chlorhexidine was incorporated into 5ml of glucose broth in test tubes to get the final concentrations of (80μg/ml, 40μg/ml, 20μg/ml, 10μg/ml, 5μg/ml and 1μg/ml while 0μg/ml served as a control) to which 1ml of the isolated standardized bacterial suspension was added. The efficacy of chlorhexidine was also tested against the standard strains (Escherichia coli, ATCC No: 25922; Staphylococcus aureus, ATCC No: 25923; Pseudomonas aeruginosa, ATCC No: 27853) (Robert Cruickshank, 1992).Inhibition of bacterial growth was determined by noting the turbidity in broth solution after overnight incubation.

Surface activity of chlorhexidine in alginate mix was assessed using the Agar well technique(Bailey and Scott, 1994). The powder of alginate impression material was spatulated in standard proportions with distilled water containing varying concentrations of chlorhexidine (0%, 0.01%, 0.02%, 0.05%, and 0.1%) for 30 seconds and poured into petridish such that the prepared blocks had a flat and smooth surface. The mixing was done according to the powder/liquid ratio (10g/23ml) recommended by the manufacturer.

The Specimens were divided into five groups in each test: specimens mixed with (0.01%, 0.02%, 0.05%, and 0.1%) of chlorhexidine solution served as test groups and specimens mixed with distilled water (0%) of chlorhexidine served as control group. Group allocations were consistent for all tests. Then impression disks, 6mm in diameter by 2mm thickness, were prepared. After that, wells of the same size as the impression disk were cut into nutrient agar plates previously inoculated with the appropriate microorganisms under sterile conditions. On each agar plate, five wells were cut and specimen was selected from each of four test groups (0.01%, 0.02%, 0.05%, and 0.1%) and put into four of the agar wells respectively. The control group (0%) was placed in the fifth well of each plate. Three independent assays were performed for each microorganism (n=3).Finally, all plates were incubated in the appropriate aerobic environment for 24 to 48 hours at 37°C. After incubation, clear zones or inhibitory areas were observed in the culture plates around the specimens and measured to

evaluate the antibacterial effect.Standard strains of the following microorganisms were used: Staphylococcus aureus (ATCC No.25923), Pseudomonas aeruginosa (ATCC NO.27853), Escherichia coli (ATCC No.25922) and Candida albicans.

III. Results

A total of 24 out of 36 samples inoculated on various media showed growth, with an average contamination frequency of 67%. The three brands showed wide variation in contamination frequency, contamination average of Brand A (Tropicalgin), Brand B (Microflex) and Brand C (Zelgan) were 83, 67 and 50% respectively (Table 1).

There was an average of 0.733 colonies in each 10mg sample placed on nutrient agar plates for the three brands, yielding an average contamination of approximately 73.3 colony forming units (CFUs) per gram of materials. Brand C showed the lowest concentration of organisms at 40 CFUs per gram, followed by Brand B, 80 CFUs per gram and Brand A being the highest (100) CFUs per gram.

Among the various aerobic bacteria isolated, Bacillus species contamination was observed with maximum frequency in all three brands of alginate powder, followed by aerobic spore bearing bacilli and coagulase negative staphylococcus. Pseudomonas species was detected in two brands which is a known pathogen to cause nosocomial infections. However, there were no anaerobic bacteria isolated from any of the three brands of alginate impression material. Among the fungi isolated Aspergillus fumigatus and Aspergillus Niger were observed to be the commonest contaminants followed by Mucor and Rhizopus (Table 2).

In our study, the lowest dilution of chlorhexidine which inhibited the bacterial growth of three test strains of bacteria (Bacillus species, aerobic spore bearing bacilli and coagulase negative Staphylococcus) isolated from the irreversible hydrocolloid impression material and against the standard strains was observed to be 20μg /ml at 0.02% concentration,10μg/ml at 0.05% concentration .

All dilutions at 20μg/ml or above were clear of turbidity at 0.02% concentration, 10μg/ml or above were clear of turbidity at 0.05% concentration and all dilutions were clear of turbidity at 0.1% concentration, indicating inhibition of bacterial growth on overnight incubation and were confirmed by subcultures on blood agar.

The dilutions of (0μg/ml, 1μg/ml, 5μg/ml and 10μg/ml) at 0.02% concentration and dilutions of (0μg/ml, 1μg/ml, 5μg/ml) at 0.05% concentration showed turbidity of broth solution, indicating bacterial growth after overnight incubation at 37°C,which was confirmed later by subcultures on blood agar plates.

Well-defined zones of inhibited growth became apparent after this incubation period and allowed for consistent measuring of inhibitory fields. The results demonstrated that zones of growth inhibition around all the test specimens were observed on all plates. No zones of inhibited growth were observed around the control wells on all agar plates. ANOVA test revealed that the inhibition zones tested became significantly larger ($p<0.001$) for each microorganism when the concentrations of chlorhexidine solution were raised from 0.01% to 0.1% (Table 3).

IV. Discussion

Our study showed 67% of contamination from three different brands of commercially available alginate impression material. This was comparable to the findings of Rice et al, who reported a 60% contamination from four commercial brands of irreversible hydrocolloid(Rice CD, Dykstra MA and Feil PH, 1992). However, Jafari et al reported overall 75% bacterial contamination with wide range of bacteria and fungi which are known to cause opportunistic infections (Jafari et al, 2012).Similarly, in our study the isolated organisms were avirulent members of environmental and normal skin flora, which might have a pathogenic potential in immunocompromised patients. The organisms isolated in our study were aerobic spore bearing bacilli, Bacillus species, coagulase negative Staphylococcus, Pseudomonas species, Aspergillus species, Mucor and Rhizopus. However, no anaerobic bacteria were isolated in our study which was contrary to the findings of Rice and co-workers, who reported 6% contamination with anaerobic bacteria(Rice CD, Dykstra MA and Feil PH, 1992; Rice CD et al, 1991).

The level of contamination was demonstrated by the number of colony forming units (CFUs) from the positive samples. The average quantity of irreversible hydrocolloid powder used in routine clinical use is approximately 15gms or about 150 times larger than the tested material in this study. This would yield a much higher concentration of organisms per clinical sample of material. The CFU's (40-100 CFUs/gm) in our study were in corroboration with the study by Rice and co-workers et al who found that the concentration of organisms varied from 12-82 CFUs/gm of contaminated sample (Rice CDet al, 1991).

It was observed that 0.1% of chlorhexidine was sufficient to inhibit the growth of most of the isolated organisms from the alginate powder and the three standard tested bacteria. The MIC determined in this study was against a limited bacterial species so it needs further evaluation against a wider spectrum of organisms. Lower concentration of the disinfectant would be more acceptable to the patient and was shown to be effective in previous study than the routinely used concentrations of 0.2% (Tanaka et al.,1994).

Flanagan et al, tested the antibacterial effects with chlorhexidine or quarternary ammonium compounds (Flanagan et al, 1998). The results revealed that the quarternary ammonium containing alginates were completely effective against all five test microorganisms. The alginate containing chlorhexidine killed all the gram-negative bacilli and majority (95-99%) of the gram-positive cocci and yeast. Previous studies have also reported that irreversible hydrocolloids with chlorhexidine and quaternary ammonium were effective in reducing surface growth (Rice, Dykstra andFeil, 1992; Cserna A et al, 1994; Tobias, Browne and Wilson, 1989).Based on the findings from our study, we conclude that materials like alginate can be contaminated with bacteria and fungi during various phases of manufacturing and packing. So it is necessary to maintain aseptic conditions at the manufacturing level. Techniques like gamma ray irradiation should be employed to sterilize the alginate material. Interim audits of the various batches of alginates should be done by the manufacturer to ensure aseptic conditions in the packets. Guidelines should be made for clinicians and dental auxiliary personnel who handle the powder for mixing. Wherever possible, individual packets of alginate powder for each impression shall be supplied by the manufacturer to prevent cross contamination in dental clinics. Further studies are needed to evaluate the possibility of incorporating chlorhexidine into alginate powder so that only water can be used as previously. Studies are also needed to evaluate patient acceptability with incorporated disinfectants. 0.1% Chlorhexidine solution for mixing alginate can be a suitable alternative for disinfection of impression material.

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Table 1 : Frequency of contamination in three types of alginate impression material

Brand	Positive in Culture (n=12)
A	10(83%)
B	8(67%)
C	6(50%)

Table 2 : Growth in Thioglycolate broth & Glucose broth in three brands of alginate impression material

Type of Bacteria	Average (%)	Brand A	Brand B	Brand C
Coagulase negative Staphylococcus	19.44	4	2	1
Aerobic spore bearing Bacilli	30.56	6	3	2
Bacillus species	36.11	6	4	3
Pseudomonas species	5.56	1	1	0
Escherichia coli	8.33	2	1	0
Aspergillusfumigatus	25	4	3	2
Aspergillus	13.89	3	2	0
Mucor	11.11	2	1	1
Rhizopus	5.56	1	1	0

Table 3 : Mean diameter of inhibition zone (mm) and SD for each bacterial species and group

Species	0.01%	0.02%	0.05%	0.1%	p-value
Staphylococcus aureus	12±0.9	14.5±0.98	18.5±1.1	21.4±1.2	<0.001
Pseudomonas aeruginosa	10.7±1.2	14.3±0.78	16.3±0.84	19.4±1.2	<0.001
Escherichia coli	11.4±1.4	13.5±1.1	16.0±1.43	18.1±1.51	<0.001
Candida albicans	12.9±1.1	19.3±1.2	27.0±2.3	30.1±1.32	<0.001

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