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ORIGINAL RESEARCH

Comparison of Antimicrobial Activities and Compressive Strength of Alginate Impression Materials following Disinfection Procedure

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ABSTRACT

Aim: This study investigated the effectiveness of disinfecting solution when incorporated into alginate powder instead of water against some microorganisms and on compressive strength of alginate.

Materials and methods: For measuring antimicrobial activity of alginate, 60 alginate specimens were prepared and divided into two groups: One with water incorporated in the mix (control) and the other with 0.2% chlorhexidine digluconate incorporated in the mix instead of water. The tested microorganisms were: Gram +ve cocci, Gram –ve bacilli and yeast (each group 10 samples). For measuring compressive strength, 20 specimens of alginate were divided into two groups: One with water incorporated in the mix (control) and the other with chlorhexidine incorporated in the mix.

Results: The statistical analysis of antimicrobial efficacy of alginate was performed with Mann-Whitney U-test, which revealed very high significant difference when comparing among groups ($p < 0.000$). Student t-test analyzed the compressive strength data which revealed nonsignificant difference between groups ($p > 0.05$).

Conclusion: The incorporation of disinfecting agents into impression materials could serve an important role in dental laboratory infection control and it had no adverse effect on compressive strength of the hydrocolloid alginate.

Clinical significance: The risk of transmitting pathogenic microorganisms to dental laboratories via impression has been considered a topic of importance for a number of years.

Keywords: Hydrocolloid alginate, Antimicrobial of alginate, Chlorhexidine digluconate, Compressive strength.

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INTRODUCTION

Irreversible hydrocolloids are responsible for the highest retention of bacteria due to its hydrophilic nature.[1,2] Antimicrobial agents are applied to irreversible hydrocolloid impression in the following ways: Sprays, which do not completely expose the contaminated surface to the antimicrobial agent, immersion: Which are not considered ideal as it changes physical dimensions,[3] incorporation of the agent to the impression material solution instead of water.[4] Some mechanical properties that can determine success or failure with an impression material are strain in compression, elastic recovery and compressive strength.[5]

MATERIALS AND METHODS

Part I: Preparation of Alginate Specimens for Antimicrobial Test

The antibacterial activity of alginate was assessed by disk diffusion test. Alginate impression (Millienium, Lascod, SpA, Laboratory Scientific, Firenze, Italy, Batch no. 0159311) was manipulated in accordance with manufacturer's recommended powder to liquid mixing ratio. For 9 gm of powder, 20 ml of distilled water or 0.2% of chlorhexidine digluconate (corsodyl mouthwash, GSK, GlaxoSmithKline consumer Healthcare, Brentford TW8 9GS, UK; Fig. 1) was added and mixed for 45 seconds. The mixture was poured in polytetrafluoroethylene mold having 10 holes (each hole 7 mm diameter × 4 mm height), where they remained there for the gelation time (2 minutes). Then the samples were stored in sterile Petri dishes until inoculation.

Fig. 1: Alginate impression material and chlorhexidine digluconate

Specimens' Grouping

The specimens were divided according to the type of mixing solution into two groups:

A: Alginate specimens with water incorporated in the mix

B: Alginate specimens with chlorhexidine digluconate incorporated in the mix.

Each group will be subdivided into three subgroups according to type of microorganism inoculated with (each group 10 specimens):

A1: Inoculated with *Candida albicans*

A2: Inoculated with *Staphylococcus aureus*

A3: Inoculated with *Pseudomonas aeruginosa*

B1: Inoculated with *Candida albicans*

B2: Inoculated with *Staphylococcus aureus*

B3: Inoculated with *Pseudomonas aeruginosa*

Microbiological Testing

Disk diffusion test method is used to investigate the release of disinfectant solution from alginate specimens.[6] *Candida albicans* ATCC 10231 yeasts, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were cultivated in Mueller-Hinton Broth and incubated for 24 hours. The microorganisms were supplied by the Pathological Analysis Department at the College of Health and Medical Technologies, Baghdad, Iraq. In each Petri dish, five specimens were placed equidistantly from each other. Subsequently, 15 ml of Mueller-Hinton Broth seeded with the indicator microorganism (10^6 cfu/ml) were poured over the specimens. The Petri dishes remained at room temperature for 60 minutes for the antimicrobial agent to diffuse. Then they were incubated aerobically at 37°C for 24 hours.

Measurement of Zones of Inhibition

Each plate was examined after incubation. The diameter of zones of inhibition was measured with a ruler, which was placed on the back of inverted Petri dish that was illuminated with reflected light located few inches above a black, non-reflecting background. The zone margin is the area where no obvious growth was visible. The inhibition zone which was measured in millimeters and was interpreted using interpretation criteria scale[3,6] (Table 1).

Table 1: Interpretation criteria of antimicrobial effect[3,6]

Scale	*Agar*
1.	Agar layer above the samples shows the same growth of the test bacteria as that of the surrounding agar.
2.	On the agar layer above the samples, a few colonies are observed. Inhibition of growth is comparable to that of the surrounding area.
3.	No colonies observed on the agar above the sample.
4.	There is a definite zone of inhibition around the sample no larger than 2.0 mm.
5.	A zone of inhibition of 2.0 to 5.0 mm has developed around the sample.
6.	A zone of inhibition of 5.0 to 10.0 mm has developed around the sample.
7.	A zone of inhibition of more than 10.0 mm has developed around the sample.

Part II: Preparation of Alginate Specimens for Compressive Strength Test

Cylinder-shaped alginate specimens were prepared according to ADA specifications no. 18 (1992),[7] using the manipulating procedure outlined in part I.

Specimens' Grouping

The alginate specimens were divided according to the type of mixing solution into two groups (10 specimens for each group):

C1: Alginate specimens with water incorporated in the mix

C2: Alginate specimens with chlorhexidine digluconate incorporated in the mix.

Compressive Strength Test

Each specimen was placed in the compressive strength testing apparatus. The specimen was loaded continuously and as uniformly as possible to give an average rate of load of 100 ± 20 N min^{-1} until fracture. The compressive strength was expressed in MPa using the following formula:

$$\text{Compressive strength} = 4F/\pi d^2$$

RESULTS

Table 2 presents the average inhibition halos (mm) around the specimens and the interpretation in scale (sc). All the

Table 2: Results of antimicrobial activity in millimeters (mm) and interpretation criteria scale (sc)

Groups	*mm*	*Scale*
A1	0.0	1
B1	5.0	6
A2	0.0	1
B2	1.0	4
A3	0.0	1
B3	0.0	3

tested groups showed larger inhibition of the microorganisms, while in the control group, the agar layer above the samples showed the same growth of the test bacteria as that of the surrounding agar. Statistical analysis was performed using the Mann-Whitney U-test and the results revealed that, there was very high statistically significant difference among groups ($p < 0.000$), and there was no statistically significant difference among the control groups ($p > 0.05$). Mean and standard deviation (SD) of compressive strength values are presented in Table 3. Student t-test was performed between groups C1 and C2. Table 3 revealed nonsignificant difference between both groups ($p > 0.05$). Figure 2 showed bar charts of means and SD for both groups.

Table 3: Mean and SD for compressive strength values expressed in MPa. Student t-test compared between groups C1 and C2

Groups	*Mean ± SD*
C1	0.59 ± 0.02
C2	0.60 ± 0.01

Nonsignificant at $p > 0.05$

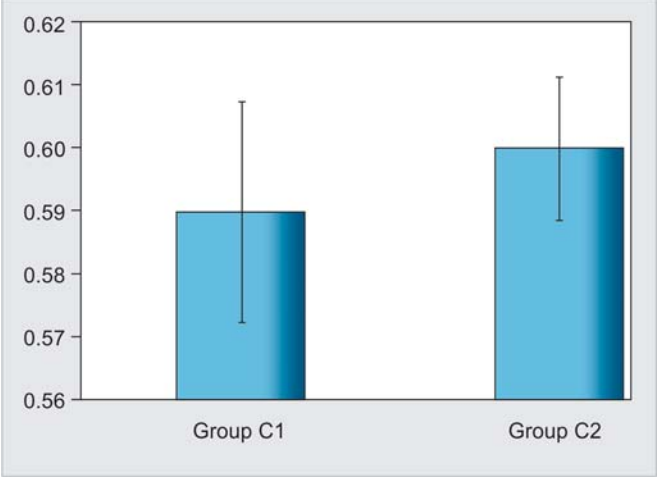

Fig. 2: Mean and SD of compressive strength of groups C1 and C2 in MPa

DISCUSSION

Impression materials are believed to carry various microorganisms from the oral cavity due to direct contact with saliva and possibly blood.[8] Spray and immersion disinfections are the two most widely used techniques in clinical practice. Although disinfection by immersion or spraying could be effective in reducing the chances of cross-infection, compliance by dental offices/clinics has been uneven.[9] This study incorporated an antimicrobial agent as replacement of the water used in the mixture. It allows internal disinfection, which eliminates microorganisms that are incorporated into the irreversible hydrocolloid during impression-makings as well as during the immediate pouring of the cast.[3]

When an impression is removed from the mouth, the material must withstand the forces produced. The accuracy of an impression material is related to strain in compression, elastic recovery, compressive strength and tear energy.[5] The microorganisms in this study are pathogenic bacteria which are used as indicators for the effectiveness of disinfection procedure.[9] Chlorhexidine digluconate is cationic bisbiguanide has broad-spectrum efficacy against gram +ve and gram –ve bacteria, some viruses and fungi, but little effective against spores.[3]

In this study, all the three microorganisms were sensitive to chlorhexidine in different degrees depending on the type of microorganism. This result in agreement with those of Touyz and Rosen,[10] Rosen and Touyz,[11] and Cserna.[12] Such studies recommended the use of 0.2% of chlorhexidine digluconate as water substitute for the alginate.

The results of the present study agree with,[13] a study found the use of chlorhexidine as impression disinfectant is a good measure for reduction of contamination and cross infection. The most sensitive microorganism was *Candida albicans*. This coincide with those of Flangan,[14] and with Casemiro,[3] such studies stated that *Candida albicans* are sensitive to chlorhexidine. This in agreement with,[15] a study found that 0.2% chlorhexidine has the ability to completely kill *Candida albicans* fungi.

The second microorganism sensitive to chlorhexidine was *Staphylococcus aureus*. This result is in agreement with,[16] a study found that the incorporation of 0.25% of chlorhexidine into alginate remarkably inhibited the growth of gram +ve cocci. This could be attributed to the fact that chlorhexidine is strongly adsorbed onto the negative group found on the surface of gram +ve bacteria and the bacteria lose their ability to adhere to the surface of material.[17] This result is in agreement with Flangan,[14] Castillo,[18] Casemiro[3] and Wang.[9]

Chlorhexidine has an affinity for bacteria because of an interaction between the positively charged chlorhexidine molecule and negatively charged groups on the bacterial cell wall and thus permits the agent to penetrate into the cytoplasm, leading to potassium leakage and causing death of microorganism.[6,18,19]

The least antibacterial activity of chlorhexidine digluconate was observed against *Pseudomonas aeruginosa* and this might be due to the fact that chlorhexidine is moderately active against *Pseudomonas aeruginosa*, which is considered to have resistance to antibacterial agents.[20] This explained by,[15] a study found that gram –ve bacteria has tolerance or resistance to antimicrobial agent higher than gram +ve bacteria and might explain the decrease in the activity of chlorhexidine against *Pseudomonas aeruginosa* when compared with *Staphylococcus aureus*. This result coincide with Casemiro[3] and Wang.[9]

However, this result conflict with,[14] a study which observed that chlorhexidine killed all the gram –ve bacilli. This difference in the observation may be attributed to the differences in the methodology employed in both studies. In the present study, alginate specimens were prepared by mixing powder with chlorhexidine while in[14] saline solutions containing standardized concentrations of tested microbes were used to mix the alginate.

In this study, there was no significant difference in compressive strength values between control group and the group with chlorhexidine incorporated. According to the knowledge of the author, there are no previous studies which investigated the effect of chlorhexidine incorporation into alginate mix on the compressive strength of alginate. In the present study, chlorhexidine has no adverse effect on the compressive strength of irreversible hydrocolloid impression material.

CONCLUSION

The incorporation of disinfecting agents into impression materials could serve an important role in dental laboratory infection control and it had no adverse effect on compressive strength of the hydrocolloid alginate.

CLINICAL SIGNIFICANCE

The risk of transmitting pathogenic microorganisms to dental laboratories via impression and other items received from dental clinics has been considered a topic of importance for a number of years.

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