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EFFECT OF CERTAIN DISINFECTANT SOLUTIONS INCORPORATED INTO GYPSUM CASTS ON CERTAIN PATHOGENS

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ABSTRACT
Dental casts come into direct contact with impression materials and other items that are contaminated by saliva and blood from the patient mouth leaving the cast susceptible to cross contamination, topical methods of disinfecting cast are difficult to control with immersion methods which are potentially destructive. Thus an additional method to control cross contamination between patients and laboratory in the liquid of dental plaster and dental stone is needed. This study was done to evaluate the antimicrobial efficiency of three recommended chemical disinfectants (chlorhexidine digluconate mouth wash, iodine and ethanol**)** incorporated into gypsum casts**.** Dental plaster (AL-Ahliya gypsum) specimens incorporated with three disinfectant solutions (chlorhexidine digluconate, iodine and ethanol) at different concentrations were prepared. Agar diffusion test was employed to assess the antimicrobial action of these disinfectants against *Steptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*. The data collected were analyzed with ANOVA test ($p<0.05$) and LSD test. The disinfectant solutions demonstrated antimicrobial activity against all the microorganisms tested with the exception of *C. albicans*. Clear microbial inhibition zones were observed at higher concentrations of the disinfectants used in this study. The disinfectant agents analyzed were effective against the bacterial pathogens tested (*S. aureus* and *S. mutans*) with the exception of *C. albicans.*

KEY WORDS: Chemical disinfectants, Gypsum products, CHX, iodine, ethyl alcohol.

INTRODUCTION

Gypsum products serve dental profession more adequately than any other materials. Several studies demonstrated that the usual operating procedures of the prosthodontic laboratory are a possible source of cross contamination between patients, technicians and dental personnel [1-4]. The dentists and health workers are exposed to wide variety of potentially dangerous microorganisms. This occupational potential for disease transmission becomes evident as most human microbial pathogens have been isolated from oral secretions because of repeated exposure to the micro organisms present in blood and saliva[1].The general routes for transmission of microbial agents in dental clinics are as follows:

1. Direct contact with infectious lesions or infected saliva or blood. Some pathogenic contaminants include bacteria such as *E. coli, S. aureus, P. aeruginosa, S. mutans* and yeast *C. albicans* have been isolated from the mouth[2,3].
2. Indirect transmission via transfer of micro organisms from a contaminated intermediate objects including impressions, impression trays and gypsum casts [4-7]. Dental gypsum i.e. calcium sulphate hemihydrates (CaSo4.1/2 H2O) is most widely used for cast formation[8,9]. Cast poured against contaminated impression have shown micro organisms therefore disinfection of cast is an important measure for the control of cross- contamination[10-12]. Several studies have been attempt to disinfect the gypsum cast by immersion or spraying the casts with disinfecting solutions[4,7,13,14]] also microwave energy has been suggested for disinfection of gypsum casts[15], some of these procedures resulted in adverse effect on the strength, hardness and roughness of gypsum materials. The aim of this study was to assess the antibacterial and antifungal activity of certain disinfectant solutions mixed with dental plaster.

MATERIALS & METHODS

The gypsum products used in this study are dental plaster (AL- Ahliya Co. for gypsum industries Ltd/ Baghdad)**.**
The disinfectant incorporated in the gypsum products are:

1. 0.12% chlorhexidine digluconate with 0.05% sodium fluoride (mouth wash).
2. 2% Povidone iodine.
3. 70% ethyl alcohol.

Preparation of the gypsum samples

100 gram of dried gypsum materials are weight using an accurate electronic digital balance and mixed with 45 ml of distilled water according to the manufacturer's recommendations, the mixture was poured in a bronze mold with dimensions of 10 mm in diameter and 5 mm depth then a glass plate was placed over the mold to remove the excess material. Separating medium was used (SND, China) to facilitate removal of gypsum specimens.
The CHX disinfectant was added in concentrations of 0.01%, 0.03% and 0.06%, while iodine was used in concentrations of 0.125%, 0.25% and 0.5%, also ethanol was used in concentrations 8.5%,15% and 35%. Four gypsum samples were prepared for each concentration of the disinfectant agent used.

Microbiological procedures

Agar diffusion tests were used to assess the antibacterial and antifungal activities of the samples. The microorganisms selected for this study were *streptococcus mutans, Staphylococcus aureus* & *Candida albicans. S. mutans* was cultivated in brain heart infusion broth (BHI) and incubated for 24 hours at 37 ºC. The other organisms (*S. aureus and C. albicans*) were cultivated in Muller-Hinton broth (MHB) and incubated at 37ºC for 24 hr. *for S. aureus* and 48 hr. for *C. albicans*, the concentration of the inoculum was (10^7 CFU/ml) according to 0.5 Mcfarland Standard.

Preparation of the wells

Under sterile conditions, wells having the same dimensions as the prepared gypsum samples (measuring 10 mm in diameter and 5 mm in depth) were cut into BHI agar for *Streptococcus mutans and MH agar for Staphylococcus aureus* and *C. albicans* on plates previously inoculated with the appropriate microorganisms by using a sterile cork borer and the disinfected gypsum samples were placed in the wells.

After incubation the inoculated agar plates at 37ºC for 24h. and 48 hours, then the inhibition zones were measured around each sample with a nearly 0.1 mm, all measurements being recorded in millimeters, figure (1). The control plates containing gypsum samples with distilled water also were inoculated.

FIGURE1: the inhibition zones around gypsum samples containing different concentrations of iodine against *Streptococcus mutans*

RESULTS

Table (1) showed the descriptive mean and standard deviation of the anti microbial action of chlorhexidine against the tested microorganisms. Table (2) and table (3) demonstrate that chlorhexidine has a highly significant anti bacterial activity against the tested bacteria especially when used in high concentrations also the results exhibited that *Candida albicans* was the most resistant micro organism.

TABLE1: The antimicrobial effect of various concentrations of chlorhexidine (CHX) disinfectant

Bacterial pathogen	CHX % Diameter of inhibition zone in mm					
	(A)0.01%	±SD	(B)0.03%	± SD	(C)0.06%	± SD
Streptococcus mutans	13	1.000	15	1.000	17	1.000
Stapylococcus aureus	14	1.000	22	1.000	38	1.000
C.albicans	-		-		-	

- = no inhibition zone

TABLE 2: one-way analysis of variance for various concentrations of chlorhexidine (CHX) against the tested microorganisms

Bacterial pathogen	CHX % Diameter of inhibition zone in mm	
	F value	Sig.
Streptococcus mutans	12.000	0.008
Stapylococcus aureus	48.000	0.000
C.albicans	-	-

****highly significant, **P<0.01**

TABLE 3: LSD–test among tested microorganisms regarding the various concentrations of chlorhexidine

Disinfectant agent	Studied microorganisms		Mean difference	P value
chlohexidine	*Streptococcus mutans*	A&B	-2.000	0.050
		A&C	-4.000	0.003
		B&C	-2.000	0.050
	Staphylococcu saureus	A&B	-8.000	0.000
		A&C	-24.000	0.000
		B&C	-16.000	0.000

*Significant, P<0.05 **Highly significant, P<0.01

Table (4) showed the descriptive mean and standard deviation of the anti microbial action of povidone iodine against the tested micro organisms. Table (5) and table (6) showed a significantly increased anti microbial activity of povidone iodine associated with an increased concentration of the disinfectant, 0.5% has the highest efficacy against the tested bacteria while 0.125% has the least anti bacterial efficacy.

TABLE 4: The antimicrobial effect of various concentrations of povidone iodine

Bacterial pathogen	iodine % Diameter of inhibition zone in mm					
	(A)0.125%	±SD	(B)0.25%	±SD	(C)0.5%	±SD
Streptococcus mutans	17.5	0.764	19	1.527	20	1.000
Stapylococcus aureus	16	1.000	18	1.000	20	1.000
C.albicans	-		-		-	

- = no inhibition zone

TABLE 5: one-way analysis of variance for various concentrations of iodine against the tested microorganisms

Bacterial pathogen	iodine % Diameter of inhibition zone in mm	
	F value	Sig.
Streptococcus mutans	4.851	0.056
Stapylococcus aureus	12.000	0.008
C.albicans	-	-

TABLE 6: LSD–test among tested microorganisms regarding the various concentrations of iodine

Disinfectant agent	Studied microorganisms		Mean difference	P value
iodine	*Streptococcus mutans*	A&B	0.333	*0.733
		A&C	-2.333	0.046
		B&C	-2.666	0.029
	Staphylococcus aureus	A&B	-2.000	0.050
		A&C	-4.000	0.003
		B&C	-2.000	0.050

*Significant, P<0.05, **Highly significant, P<0.01

Table (7) showed the descriptive mean and standard deviation of the anti microbial action of ethanol against the tested micro organisms. Table (8) and table (9) revealed statistically significant difference among the various concentrations of ethanol, ethanol in a concentration of (35%) showed an increase in anti bacterial activity.

TABLE 7: The antimicrobial effect various concentrations of ethanol

Bacterial pathogen	ethanol % Diameter of inhibition zone in mm					
	(A)8.5%	±SD	(B)15%	±SD	(C)35%	±SD
Streptococcus mutans	11.5	0.764	14	1.000	19	1.000
Stapylococcus aureus	13	1.000	15	1.000	20	1.000
C.albicans	-		-		-	

- = no inhibition zone

TABLE 8: one-way analysis of variance for various concentrations of ethanol against the tested microorganisms

Bacterial pathogen	ethanol % Diameter of inhibition zone in mm	
	F value	Sig.
Streptococcus mutans	48.903	0.000
Stapylococcus aureus	39.000	0.000
C.albicans	-	-

TABLE 9: LSD–test among tested microorganisms regarding the various concentrations of ethanol

Disinfectant agent	Studied microorganisms		Mean difference	P value
ethanol	*Streptococcus mutans*	A&B	-2.333	0.022
		A&C	-7.333	0.000
		B&C	-5.000	0.001
	Staphylococcus aureus	A&B	-2.000	0.050
		A&C	-7.000	0.000
		B&C	-5.000	0.001

*Significant, P<0.05
**Highly significant, P<0.01

DISCUSSION

Establishing the spectrum of activity of any antimicrobial agent is essential for improving infection control, the results revealed statistically significant differences in antibacterial effect among the different concentrations of disinfectant agents incorporated into gypsum products this could be related to the differences in solubility and diffusibility of the disinfectants in agar[2].Gypsum samples incorporated with chlorhexidine digluconate mouth wash exhibits significant antimicrobial activity (growth inhibition zone) against *Streptococcus mutans* and *Staphylococcus aureus* associated with an increased concentration of CHX disinfectant. Also chlorhexidine digluconate showed antibacterial activity even in low concentrations, this result disagree with Pereira[12], who demonstrate that 2% chlorhexidine solutions was effective against *Escherichia coli,Staphylo coccus aureus, Bacillus subtilis and Candida albicans.*

The antimicrobial action mechanism is explained as chlorhexidine is a positively-charged molecule that binds to the negatively-charged sites on the cell wall; it destabilizes the cell wall and interferes with osmosis.The bacterial uptake of the chlorhexidine is very rapid, typically working within 20 seconds. In low concentrations it affects the integrity of the cell wall. Once the cell wall is damaged, chlorhexidine then crosses into the cell itself and attacks the cytoplasmic membrane (inner membrane). Damage to the cytoplasm's delicate semipermeable membrane allows for leakage of components leading to cell death. In high concentrations, chlorhexidine causes the cytoplasm to congeal or solidify [19].

No antifungal activity of CHX was observed in the agar diffusion test this finding is in agreement with Rathore[20], this finding could be related to the resistant of this microbe which made it less susceptible to disinfection and also could be related to the low concentrations of CHX used in this study also our findings are in agreement with[10,21],who demonstrated that 2% chlorhexidine has antibacterial activity against all the tested microorganisms *Escherichia coli, Staphylo coccus aureus, Bacillus subtilis* with the exception of *C. albicans*. Iodine shows an obvious growth inhibition against *Streptococcus mutans* and *Staphylococcus aureus* when used in concentrations 0.125% and 0.25% while a great growth inhibition was observed against the tested bacteria in a concentration of 0.5% this finding is in agreement with that of [22]. Ethanol exhibits a clear bacterial growth inhibition especially when used in high concentrations against both *Streptococcus mutans* and *Staphylococcus aureus*, this finding is in agreement with [14] who found that 50% ethanol was needed to inhibit the growth of *S. aureus.* No inhibition zones were observed against *C. albicans* regarding the various disinfectants used in this study this finding disagree with [14].

CONCLUSION

Chlorhexidine, iodine and ethanol disinfectant agents revealed great antibacterial activities especially at the higher concentrations against *Streptococcus mutans* and *Staphylococcus aureus.* No antifungal growth inhibition was noticed with the selected concentrations of the disinfectants used.

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