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The Disinfection Efficiency of Heat Cure Poly Methyl Methacrylate Mixed With Antimicrobial Agent

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Abstract: *many dental applications are produced from acrylic resins based on heat –cured poly methyl methacrylate (PMMA).The aim of this study was to improve the hydrophobicity and reduce adherence of heat cure acrylic resin by addition of organic micro structured antibacterial agents.Organic micro structured anti microbial siwak powder was added to acrylic resin denture base in 3 different concentrations (G2:3%,G3:5% and G4:7%) ,G1:0% represent the control group devoid of siwak addition. The effect of organic additive on bacterial adherence to acrylic resin specimens was evaluated by using viable bacterial counting method, Kruskal- Wallis H test and Mann-Whitney U tests were used for statistical analysis. Siwak powder in 3% concentration combined to acrylic resin displayed minimal antibacterial effect against oral flora in comparison to other experimental groups, control group did not show any microbial inhibitory effect. Highly significant statistical difference between the experimental groups (3%,5% and 7% siwak addition) and the control group was observed concerning the antimicrobial efficiency (p < 0.05).This study demonstrate that organic structured additive siwak is suitable mean for making hybrid materials with antibacterial properties for dentistry applications.*

Keywords: *PMMA, antibacterial agents, acrylic resin additive.*

I. Introduction

The heat cure denture base resins are extensively used for their good properties [(1)] .However, one of the major problems that patients and dentists commonly faced using these removable acrylic appliances is their potential for plaque accumulation due to surface porosities and food adherence configuration which in turn increase bacterial activity of cariogenic oral flora [(2,3)] .

The human mouth is an ideal environment of the presence and growth of microorganisms. It provides a source of water and nutrients as well as a moderate temperature. Resident bacteria of the mouth adhere to the teeth surfaces and gingiva to resist mechanical flushing from the oral cavity [(4)] .

Dental impressions and stone models become contaminated with saliva, bacterial plaque and they participated in cross contamination when dental technician receive and handle these impressions and stone models in dental laboratories . The construction of dental prosthodontics , such as dentures and crowns also carries the risk of cross contamination , a wide range of disinfecting agents has been used for chemical disinfection e.g. alkaline gluteraldehyde, sodium hypochlorite and enzymatic solutions[(5,6)] .

However, previous studies have explained changes in physical and mechanical properties of denture base resin caused by immersion in chemical disinfectants [(7)] .

Several methods were recommended to minimize or prevent dental caries one of these methods was mixing of antibacterial agents within dental materials [(8)] .The antibacterial materials which have to be incorporated were nano sized silver particles and chlorhexidine powder [(9,10)] .

Several studies showed that siwak has antimycotic and antibacterial effects.A preliminary gross analysis of siwak demonstrated that it contains components that have a variety of pharmacological effects, the chemical compositions of siwak are as follow [(11,12)]:

1. Alkaloid, Trimethylamine, which have antibacterial and gingival stimulating effects.
2. Fluoride is an anticaries, protect the teeth from cariogenic bacteria.
3. Chlorides , posses antibacterial activity.
4. High amounts of calcium, phosphorous and silica.
5. Sulphur ($SO4^{-2}$) has antibacterial property.

The aim of the present study was to introduce PMMA containing antibacterial agent and to evaluate the antibacterial efficiency of this hybrid acrylic resin .

II. Materials And Methods

The specimens fit this test were in form of small rectangular bars having the dimensions of (20,10,10 mm). PMMA (Ivoclar Vivadent AG/ Liechtenstein) was mixed with siwak powder(75 µg) in different proportions 3%, 5% and 7% .

Sticks of siwak were removed from it's package and left to dry in the hot air oven at 37ºC for 24hr.Each stick was cut using sharp knife to small pieces ,they were ground using sharp commercially food grinder(IND. Co. LTD,Japan) which was cleaned before use by deionized distilled water then left to dry. Siwak powder was collected and kept in a closed glass container till use.

The control specimens were constructed first, the heat polymerizing acrylic resin specimens were constructed by mixing PMMA powder with MMA liquid at mixing ratio of 23.4 g powder:10 ml liquid according to the manufacture's recommendations .The resin was packed into the stone mold then the flasks were pressed together in hydraulic press for 5 minutes and processed, the processing was done by immersing the clamped flasks in cold water bath up to 100°C and then boil for 45 minutes , after that the flask was allowed to cool slowly at room temperature for 30 minutes followed by complete cooling of the flask with tap water for 15 minutes before deflasking.The acrylic resin specimens were removed from the flask, manually buffed under water and finished using sand paper with continuous water cooling , polishing was finished by using the rag wheel and dental pumice.

The positive control (G1:0% siwak) and experimental groups (G2:3%,G3:5% andG4:7%) were immersed in natural saliva of healthy adult female person aged 45 years for 5 minutes while the negative control immersed in de ionized distilled water only , the samples were incubated for 24 hours. The acrylic samples were swabbed by cotton swab at 1 cm^2 surface area for the control and experimental groups , inoculated in brain heart infusion agar then incubated at 37º aerobically for 24 hours. Counting the number of viable bacterial colonies which are adhered on the surface of acrylic resin specimens was done by colony count system: no. of colony × dilution (Cruick shank,1973) .

III. Results

The antimicrobial effect of acrylic resin mixed with various concentrations of siwak (3%,5% and 7%) in addition to control group (0% siwak) was demonstrated as the mean viable bacterial cells after 24 hrs incubation time ,3% siwak addition showed minimal viable bacterial count among the experimental groups, table(2).

Table1. Testing the normality of distribution

Groups	Kolmogorov-Smirnov test	d.f.	p-value
G1:0% S	0.349	5	0.046 (S)
G2:3% S	0.301	5	0.156 (NS)
G3:5% S	0.349	5	0.046 (S)
G4:7% S	0.473	5	0.001 (HS)

d.f. : degree of freedom

S: siwak powder

The negative control group (acrylic resin sample devoid of s powder and immersed in deionized distilled water) did not reveal any microbial inhibitory effect against the oral flora.

Groups 1, 3 and 4 showed significant difference, so they were not normally distributed. The comparison will be done by applying the Kruskal-Wallis H test.

Table 2. Descriptive statistics and groups' difference using Kruskal-Wallis H test.

Groups	Descriptive statistics						Comparison		
	N	Median	Mean	S.D	Min.	Max.	X^2	d.f.	p-value
G1:0% S	5	3000000	3600000	894427.191	3000000	5000000	16.965	3	0.001 (HS)
G2:3% S	5	10000	18400	15899.68553	2000	40000			
G3:5% S	5	100	160	89.4427191	100	300			
G4:7% S	5	100	120	44.72135955	100	200			

HS: Highly significant P< 0.01

Further analysis using Mann- Whitney U test showed that a highly significant difference was achieved among the experimental groups in comparison to the control group concerning viable bacterial counting, table (3).

Table 3. Mann-Whitney U test show the difference between each 2 groups

Groups		Mann-Whitney U test	p-value
G1:0% S	G2:3% S	0	0.008 (HS)
	G3:5% S	0	0.008 (HS)
	G4:7% S	0	0.006 (HS)
G2:3% S	G3:5% S	0	0.008 (HS)
	G4:7% S	0	0.007 (HS)
G3:5% S	G4:7% S	9.5	0.439

HS: Highly significant P< 0.01

IV. Discussion

Initial adherence and colonization of oral micro organisms on several prosthodontic materials may lead to biofilm formation and may contribute to dental caries and gingival inflammation. The use of prosthodontic appliances containing antimicrobial agents is advocated to combat this problem.

The acrylic resin in pure state do not exhibit any bacterial activity while the antibacterial activity of such material (denture base material) can be of great advantage for patient wearing removable restorations .Several attempts have been made to incorporate antibacterial agents to acrylic resin and they found a clear [1,10,15].

The present study could not conclude whether the antimicrobial effect was resulted from release of s contents from the modified acrylic samples or direct contact between the modified acrylic samples and microbial cells.

Our results showed that Siwak powder in 5% and 7% concentrations exhibit the lowest microbial adherence in comparison to 3% which demonstrate the highest microbial adherence, this may be contributed to the presence of:

Fluoride: the results of recent studies have shown that fluoride can affect bacterial metabolism via various actions with different mechanisms." It can act directly as an enzyme inhibitor , for example for the glycolytic enzyme enolase, which is prevented in a quasi-irreversible manner" [12] . Direct action seems also to occur in inhibition of heme-based peroxidases with binding of fluoride to heme. The flavin-based peroxidases of different oral bacteria are insensitive to fluoride. The antibacterial actions of fluoride was regarded to be complex but to be controlled by weak-acid effect[12].

The nitrate ($No3^-$) content has been reported to prevent active transfer of oxidative phosphorylation and oxygen uptake by staphylo coccus aureus [12]," in addition to thiocyanate (SCN^-) content s of siwak which acts as a substrate for lactopeoxidase to produce hypothiocyanite $OSCN^-$ in presence of hydrogen peroxide , $OSCN^-$ react with sulfhydryl groups in bacterial enzymes which lead to bacterial death" [17] .

The potential antibacterial action of siwak could be related to Ca and P anionic components [18]. this could be explained that the difference in osmotic concentrations of these ions within the bacteria and around them , these ions are considered as simple nutritional requirements of certain bacteria therefore when the external concentration of (Ca,P) increased the intracellular content o f (Ca, P) may drop rapidly and this affect the active transport system of most bacteria [14].

V. Conclusion

Within the limitations of the present study, the modified acrylic resin combined with siwak displayed the highest anti microbial properties against oral flora in ratios 5% and 7% of siwak powder addition.

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