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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL PROPERTY OF ZINC ACETATE AND ITS EFFECT ON FLEXURAL STRENGTH OF DENTURE BASE RESIN-AN IN VITRO STUDY

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ABSTRACT

Purpose: The intaglio surface of complete denture is not polished so due to rough surface adhesion of microorganisms to denture surface. This study aimed to measure the antimicrobial activity and flexural strength of heat cure acrylic resin after incorporating zinc acetate in different percentage.

Materials and Method: A total number of thirty-two disk shaped (6mm x 1mm) and rectangular shaped samples (65mm x 10mm x 2.5mm) were prepared from heat-polymerized acrylic resin incorporated with Zinc acetate. The zone of inhibition diameters were measured with zone measuring scale (in mm) and this measurement indicated the microbial susceptibility to the material. And, the flexural strength of the specimens was determined using a 3-point bend testing device in a Lloyd's Universal Testing Machine. The measured values were submitted to statistical analysis by descriptive statistics, independent sample't' test and one way ANOVA followed by Scheffe's Post Hoc test.

Results: One way ANOVA descriptive analysis detected the different percentage of zinc acetate in heat polymerized acrylic resin showed significant difference. The increasing order of antimicrobial activity is: Group A (control) < Group B (2.25% zinc acetate) < Group C (5% zinc acetate) < Group D (7.25% zinc acetate). However, the increasing order of flexural strength is: Group D (7.25% zinc acetate) < Group C (5% zinc acetate) < Group B (2.25% zinc acetate) < Group A (control).

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INTRODUCTION

A complete denture serves various functions like aesthetics, mastication and speech for edentulous patients. If geriatric patients lack in adequate denture hygiene this will lead to biofilm accumulation and oral infections like denture stomatitis. Polymethylmethacrylate is a main component of denture base polymer. This usually have low resistance to impact, flexure or fatigue. And if denture breaks it is usually repaired with repair acrylic resin. Which has further low impact strength. Many studies have been done by incorporating various components in heat cure acrylic resin to improve its strength. Recent trends involves with the studies with incorporating metal strengtheners in on various mechanical and antimicrobial properties of heat polymerized acrylic resin. It was claimed that high concentration of zinc ion have some antimicrobial properties.

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Zinc oxide is popular to use as components of cements and periodontal dressing and as filler in endodontic gutta percha cones. Hence, the purpose of this study is to evaluate the effect of zinc acetate when added at different concentrations, its antimicrobial efficiency in PMMA denture base resin and its flexural strength with PMMA denture base resin.

MATERIALS AND METHODS

A total number of 64 specimens were used in study. 32 disc shaped specimens were fabricated for testing antimicrobial activity and 32 rectangular specimens were fabricated for testing flexural strength. Test specimens were divided into 4 groups based on concentration of zinc acetate in heat cure acrylic resin.

- **Group A:** heat cure acrylic resin with 0% zinc acetate (control)
- **Group B:** heat cure acrylic resin with 2.5% zinc acetate (by weight)

- **Group C:** heat cure acrylic resin with 5% zinc acetate (by weight)
- **Group D:** heat cure acrylic resin with 7.5% zinc acetate (by weight)

Each group will comprise of 16 specimens out of which 8 (disc shaped specimens) were assessed for the antimicrobial activity while the other 8 (rectangular shaped specimens) were evaluated for flexural strength.

Assessment of antimicrobial activity

The saliva was discarded and the specimens were subjected to the antimicrobial activity assays using Agar disc diffusion method. For obtaining the inoculum,

Candida albicans

The reference strains (Candida albicans ATCC 28366) were inoculated in Mueller Hinton broth. After incubation at 37°C for 24 hours, the turbidity of Candida albicans inoculum was adjusted to tubes 1 McFarland standard. Mueller Hinton Agar plates were swabbed using sterile cotton swab dipped in 1 McFarland matched Candida albicans culture in Mueller Hinton broth. Specimens measuring 6mm in diameter and 1mm in thickness were placed in 6mm diameter wells prepared on surface of Mueller Hinton Agar plates. The plates were kept at room temperature for 120 min for diffusion of the antimicrobial agent and then incubated in aerobiosis at 37° C for 24 hours. The zone of inhibition diameters were measured with zone measuring scale (in mm) and this measurement indicated the microbial susceptibility to the material12.

Staphylococcus aureus

The reference strains (Staphylococcus aureus) were inoculated in Mueller Hinton broth. After incubation at 37°C for 24 hours, the turbidity of Staphylococcus aureus inoculum was adjusted to tubes 0.5 McFarland standard. Mueller Hinton Agar plates were swabbed using sterile cotton swab dipped in 0.5McFarland matched Staphylococcus aureus culture in Mueller Hinton broth. Specimens measuring 6mm in diameter and 1mm in thickness were placed in 6mm diameter wells prepared on surface of Mueller Hinton Agar plates. The plates were kept at room temperature for 120 min for diffusion of the antimicrobial agent and then incubated in aerobiosis at 37° C for 24 hours. The zone of inhibition diameters were measured with zone measuring scale (in mm) and this measurement indicated the microbial susceptibility to the material12.

Fabrication of specimens for assessment of flexural strength

A metal mold with three plates was fabricated, of which middle plate had windows measuring 65x10x2.5 mm as per ADA specification no. 12. This was done to get uniform size of wax pattern to make acrylic resin test specimens. The flexural strength of the specimens was determined using a 3-point bend testing device in a Lloyd's Universal Testing Machine. The device consisted of a loading wedge and a pair of adjustable supporting wedges placed at 50 mm apart.

The peak load was converted to flexural strength by the formula:

S = 3PL/2bd2
Where S = flexural strength (N/mm2)
P = load at fracture
L = distance between jig supports
b = specimen width
d = specimen thickness

Table 1. Antimicrobial activity of control (GROUP A) Zinc acetate 2.25% (GROUP B), 5%(GROUP B) and 7.25% (GROUP C) by incorporating in heat cure acrylic resin, respectively on candida and staphylococcus aureus

ONEWAY Descriptives						
		N	Mean	Std. Deviation	Minimum	Maximum
Candida	Group A	4	.0000	.00000	.00	.00
	Group B	4	1.3750	.25000	1.00	1.50
	Group C	4	3.2500	.28868	3.00	3.50
	Group D	4	4.6250	.25000	4.50	5.00
	Total	16	2.3125	1.83371	.00	5.00
Staphylococcus aureus	Group A	4	.0000	.00000	.00	.00
	Group B	4	1.7500	.28868	1.50	2.00
	Group C	4	2.7500	.28868	2.50	3.00
	Group D	4	4.2500	.28868	4.00	4.50
	Total	16	2.1875	1.61116	.00	4.50

N Mean Std. Deviation Minimum Maximum

CANDIDA
GROUP A 4 .0000 .00000 .00 .00
GROUP B 4 1.3750 .25000 1.00 1.50
GROUP C 4 3.2500 .28868 3.00 3.50
GROUP D 4 4.6250 .25000 4.50 5.00
Total 16 2.3125 1.83371 .00 5.00

STAPHYLOCOCCUS

AUREUS
GROUP A 4 .0000 .00000 .00 .00
GROUP B 4 1.7500 .28868 1.50 2.00
GROUP C 4 2.7500 .28868 2.50 3.00
GROUP D 4 4.2500 .28868 4.00 4.50
Total 16 2.1875 1.61116 .00 4.50

- This table shows descriptive statistics of antimicrobial activity of group A, group B, group C and group D on candida and staphylococcus aureus.
- It shows means of imbibition zone produced by different percentage of zinc acetate in heat cure acrylic resin for candida and staphylococcus aureus.

Table 2. Evaluation of flexural strength of heat cure acrylic resin after incorporating different percentage of zinc acetate Dependent variable**: Flexural strength**

ONEWAY Descriptives FLEX_STR				
	N	Mean	Std. Deviation	Std. Error
Group A	8	105.1176	1.52783	.54017
Group B	8	97.5417	.94347	.33357
Group C	8	87.7508	1.28865	.45561
Group D	8	78.2979	.78299	.27683
Total	32	92.1770	10.32720	1.82561

This table shows descriptive statistics of control group with all the other test groups.

Dependent variable**: Flexural strength**
ONEWAY Descriptives
FLEX_STR
N Mean Std. Deviation Std. Error
Group A 8 105.1176 1.52783 .54017
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Total 32 92.1770 10.32720 1.82561

This table shows descriptive statistics of control group with all the other test groups.

RESULTS

The mean change was compared between different percentage of zinc acetate in denture base resin for antimicrobial activity and flexural strength. To facilitate comparison of relative values, the accuracy was expressed in percent of mean change rather than the absolute values as shown in Table l, and ll. There was seen increase in antimicrobial property with increase in percentage of zinc acetate. When Group C (5% zinc acetate) and Group B (2.25% zinc acetate) were compared, Group C shows 57% and 36.36% increase in antimicrobial property for candida and staphylococcus aureus, respectively. However, when Group D (7.25% zinc acetate) and Group C (5% zinc acetate) were compared, Group D shows 29% and 35.29% increase in antimicrobial property for candida and staphylococcus aureus, respectively. However, effect of incorporation of zinc acetate in denture base resin was negative. With, increase in concentration of zinc acetate there was seen decrease in flexural strength. There was subsequent decrease in flexural strength by 7.2% in Group B, 16.52% in Group C and 29.51% in Group D respectively, as compared to Group A.

DISCUSSION

Denture hygiene is indispensable for general health, especially in elderly patients who cannot adequately brush their dentures because of disease, dementia and poor dexterity. Beyond the concern for the aesthetics, the lack of adequate denture hygiene can cause biofilm accumulation and oral infection such as denture stomatitis. It is a common infection characterized by oral tissue and colonization of the intaglio surface of prostheses by microorganisms1. By incorporating antimicrobial agent in denture base resin, change in physical properties should also be seen. In the present study, zinc acetate was added to heat polymerized acrylic resin provide inherent antimicrobial property against Candida albicans and staphylococcus aureus as compare to surface treatment. The addition of zinc acetate to heat polymerized acrylic resins is consistent with the current trend of incorporating antimicrobials into dental materials. However, its feasibility is related to improvement of the material's physical, mechanical and biological properties. This study was done to evaluate the antimicrobial activity of heat cure acrylic resin after incorporating different percentages of zinc acetate, and to assess whether the addition of zinc acetate alters the flexural strength of the denture bases.

Test specimen were divided into 4 groups based on concentration of zinc acetate in heat cure acrylic resin.

- **Group A:** heat cure acrylic resin with 0% zinc acetate (control)
- **Group B:** heat cure acrylic resin with 2.5% zinc acetate (by weight)
- **Group C:** heat cure acrylic resin with 5% zinc acetate (by weight)
- **Group D:** heat cure acrylic resin with 7.5% zinc acetate (by weight)

It was observed that the control specimens (fabricated without incorporation of zinc acetate), did not have antimicrobial activity, which agrees with the results of previous studies that report little or no antimicrobial activity of the tested materials. The addition of 2.25% of zinc acetate to heat polymerized acrylic resin was enough to provide antimicrobial activity against the test strains. A significant increase in antimicrobial activity was observed with the increase in the concentration of zinc acetate. The higher the percentage of zinc acetate, the greater the antimicrobial activity. The smallest inhibition halos were observed for the heat polymerized acrylic resin specimens with 2.25% of zinc acetate against Candida albicans and staphylococcus aureus isolate, while the largest inhibition halos were observed for heat polymerized acrylic resin specimens with 7.25% zinc acetate against Candida albicans and staphylococcus aureus isolate. These results may be attributed to zinc, which leached out from the resins.

Flexural strength

Addition of zinc acetate is critical to enhance the antimicrobial property. But at the same time zinc acetate might interference with polymeric chain. Thus, by measuring the flexural strength, the quality of polymerization might be evaluated to some extent in addition to determination of denture base resistance to force and trauma. Polymethylmethacrylate (PMMA) has been widely used as a main component of denture base polymers for many years. It has been found that the prosthesis made of this material gets fractured or cracked in clinical use. One of the factor that causes fracture is considered to be low resistance to impact, flexural or fatigue 5. A metal mold with three plates was fabricated, of which middle plate had windows measuring 65x10x2.5 mm as per ADA specification no 12. This was done to get uniform size of wax pattern, to make acrylic resin test specimens. The flexural strength of the specimens was determined using a 3-point bend testing device in a Lloyd's Universal Testing Machine. The device consisted of a loading wedge and a pair of adjustable supporting wedges placed 50mm apart.

The Peak load was converted to flexural strength by the formula:

S = 3PL/2bd2

Where S= Flexural strength (N/mm2)
P = load at fracture
L = distance between jig supports
b = specimen width
d = specimen thickness

A statistically significant decrease of flexural strength, in comparison to the control groups, was observed with the addition of 2.25%, 5% and 7.25% to heat polymerized acrylic resin, which is consistent with the findings of previous investigations done using other antimicrobial agents (C.P. Bardon 2006, Gaurav Puri 2008)13. Lowest flexural strength was observed with specimens containing 7.25% of zinc acetate and highest flexural strength was observed with specimens containing 0% of zinc acetate. It was observed that as the concentration zinc acetate in heat polymerized acrylic resin increases, the flexural strength decreases. the decrease of flexural strength values in this study (as depicted in Table 2), is in agreement with the results of the study done by Addy and Handlery, who reported that the addition of a similar agent to methacrylates negatively affected their mechanical properties3. Zinc reduces the number of double bond in PMMA. Therefore, cross linkage reduces and strength of PMMA decreases. So, addition of zinc acetate in a denture base resin increases the antimicrobial property but it has negative effect on flexural strength. It is important to realize that the least mean flexural strength value (78.2979 MPa), obtained for the samples in which the percentage of zinc acetate was as high as 7.25%, is more than the minimum flexural strength value of 65 MPa set forth by the ADA specification no.12. Hence this slight reduction may not have any clinical relevance1.

Conclusion

Within the limitations of this study, it can be concluded that the addition of zinc acetate decreases the flexural strength of heat cure denture base resin. However, addition of small concentration of zinc acetate to polymethylmethacrylate may be effective against microorganism and therefore its effect on flexural strength may be less significant than the potential benefits, especially for the patients who do not follow an adequate denture cleaning protocol.

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