The Influence of Chlorhexidine Diacetate Salt Incorporation Into Soft Denture Lining Material on Its Antifungal And Some Mechanical Properties

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ABSTRACT

Background: One of the most common problem associated with the used of soft denture lining material is microorganisms and fungal growth especially Candida albicans, which can result in chronic mucosal inflammation. The aim of this study was to evaluate the influence of chlorhexidine diacetate (CDA) salt Incorporation into soft denture lining material on antifungal activity; against Candida albicans, and the amount of chlorhexidine di-acetate salt leached out of soft liner/CDA composite. Furthermore, evaluate shear bond strength and hardness after CDA addition to soft liner

Materials and methods: chlorhexidine diacetate salt was added to soft denture lining material at four different concentrations (0.05%, 0.1% and 0.2% by weight). Four hundred and fifty specimens were made and divided into four groups according to the test to be performed. Disk diffusion test was used to evaluate the antifungal activity of the soft liner/CDA composite after four different periods of incubation in artificial saliva. UV spectroscopy was used to evaluate the amount of accumulative and periodic CDA released in artificial saliva after 2 days, 2 weeks and 4 weeks incubation in artificial saliva. The shear bond strength and shore A hardness was measured after 2 and 4 weeks incubation in artificial saliva and the results were statistically analyzed.

Results: All experimental groups showed a highly significant increase in diameter of inhibition zone around the test specimen in compare with control group. The release of Chlorhexidine showed to be dose dependent. The shore A hardness a highly significant increase with the addition of CDA and as for shear bonding strength, the addition of CDA at 0.5% and 1.5% percentage resulted in a highly significant decrease in bond strength, while 2.5% and 3.5% percentage showed non-significant differences in compare with control.

Conclusion: soft denture lining material with antifungal properties was the result of CDA salt incorporation which indicate that chlorhexidine was released in affected concentration from soft liner/CDA composite. This incorporation resulted in Hardness increase and did not affect the shear bond strength for 2.5% and 3.5% percentage. Keywords: Soft denture liners, antifungal activity, chlorhexidine diacetate salt.

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INTRODUCTION

Lack of antimicrobial properties is one of the most common problem associated with the used of soft denture lining material. Denture stomatitis is most associated by poor oral hygiene along with fungal and microorganism growth. (1, 2)

The routine treatment denture stomatitis includes prescription of antifungal drugs, denture repair or replacement and application of prophylactic measures (3, 4), and this treatment can be further complicated for patients with special needs or elderly because this type of patients have difficulty in keeping clean denture, and following antifungal drug routine. (5)

Chlorhexidine di-acetate salt and the related compounds are used as antiseptics and disinfectants, and exhibit a broad spectrum of antimicrobial activity including Candida albicans (6.7). In the present study heat cure acrylic-based soft denture lining material impregnated with CDA salt to produce resin material with antifungal properties that has the ability to reduce Candida albicans growth and evaluate whether soft liner properties will be affected by this addition or not.

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MATERIALS AND METHODS:

Heat cured acrylic-based soft denture liner material (Vertex™Soft, Vertex-Dental, Netherlands) was impregnated with chlorhexidine diacetate salt (Xi'an Lyphar Biotech Co. ,China) in different percentages (0. 5%, 1.5% ,2.5%cand 3.5 by weight). A total of Four hundred fifty specimens were prepared and divided into four groups according to the test to be performed.

Evaluating the antifungal activity of soft liner/CDA samples by using disk diffusion test:

Sample fabrication

Samples were made by using plastic model with the dimension of (10 mm diameter and 3.0 mm thickness) (8) which were invested in hard but flexible silicon material then after that both silicone and the plastic pattern were invested in stone to form silicone-stone mould. The soft lining material was mixed, packed and cured as instructed by manufacturer, and as for experimental samples, chlorhexidine diacetate salt was added to the liner powder and mixed manually (9, 10).

The samples were finished, polished, sterilized by autoclave after the curing process was complete and storage in artificial saliva.

Figure.1 plastic model and silicone silicone-stone mould used in disk

Isolation of C. albicans:

A sterile cotton swab was used to isolate Candida albicans from the oral cavity of patients that visit the college of dentistry with denture stomatitis symptoms by rubbing the lesion with the swab [(11)]. Than after that Sabouraud dextrose agar (which was prepared as instructed by the manufacturer) was used to incubate the swab aerobically for 24-48 at 37CA, and then kept at 4°C for further investigation. [(12)]

Identification of C.:

To identify Candida albicans germ tube formation procedure was used [(13)] and API API-Candida (bioMérieux) system was used also which is a biochemical method for identification.

Preparation of culture media for disk diffusion test:

Sabouraud dextrose agar was used as a culture media for disk diffusion test which was prepared as instructed by the manufacturer. [(14)]

Evaluation disk diffusion test:

To produce a yeast stock suspension with turbidity equal to 0.5 McFarland, 5 mL of 0.85 % sterile normal saline was used to suspend isolated colonies of C. albicans and McFarland densitometer was used to ensure that the suspension had turbidity equal to0.5 McFarland. The agar plate was swabbed carefully in 3 directions by sterile swab after being dipped into suspension and excess fluid was pressed out to achieve even growth on the surface of the agar plate. After that the agar surface has been left for about 5 minute, then the experimental and control disks were placed on the surface of the culture media and incubated at 37º C for 48 h. aerobically. The inhibition zone that may appear around the disks was measured by using electronic digital caliper. This procedure was repeated for the samples that incubated in artificial saliva for 2 days, 2 and 4 weeks. [(15)]

Chlorhexidine diacetate salt release test:

A round plastic model with the dimension of 10mm in diameter and 3mm in thickness was used to prepare the specimens that used to evaluate CDA release [(16)]. All specimens were placed in a plastic plane tubes and immersed in 1mL of artificial saliva and kept at 37◦C for 4 weeks and during this period the incubation solution was replace every 2 days and with each replacement the artificial saliva of each tube was collected, and the change in optical density was measured by UV spectroscopy (UV-160AShimadzu, Japan) at a wave length of 257.5 nm. [(17, 18)]

Shore A hardness test

To evaluate hardness of soft denture lining material, soft liner disk with dimension (3mm, 30 mm) thickness and diameter on top of acrylic disk with the same dimension was used [(19,20)].Two plastic patterns on top each other with the previously mentioned dimension was used to made silicone-stone mould. Rapid heat cured acrylic resin (Vertex™ Rapid Simplified, Vertex-Dental, Netherlands) was mixed, packed into the space created by removing the upper pattern while the lower one still present to act as a spacer for lining material and cured as instructed by manufacturer then after completing the polymerization process, the acrylic resin disk was removed from the mould, trimmed and polished. The acrylic disk was then returned to the mould after removing the plastic spacer and soft liner mixed, packed against acrylic disk and cured as instructed by manufacturer. Shore A durometer (TH 200, Germany) was used to measure soft liner hardness .The testing value was taken as an average of five different reading that were taken directly from the scale reading of durometer.

Shear bond strength test

Shear bonding strength between acrylic denture base and soft lining material was evaluate by using two acrylic block with dimensions of (75 mm*25 mm*5 mm, length, width, depth respectively) with stopper of depth about 3 mm [(21)] Rapid heat cured acrylic resin (Vertex™ Rapid Simplified, Vertex-Dental, Netherlands) was used. Mixing packing and curing was done as instructed by the manufacturer. One block of acrylic put over the other block leaving a space between them with the dimensions (25 mm*25 mm*3 mm length, width, depth respectively) for reline material application, that filled with wax. Then the whole specimen (the 2 blocks with wax) was invested into silicon material to fabricate a mould for final specimen curing. Wax elimination procedure was done and the formed space (25mm × 25mm × 3mm) was filled with soft lining material and curing was carried out (Fig.2 A&B). The specimens were tested using Instron testing machine (Instron 1195, England) at load cell capacity of (100Kg) and cross head speed equal to (0.5mm/min). The shear bond strength was calculated by dividing the maximum load

required for the sample to fail on cross section area of the testing sample according to (ASTM specification D-638m, 1986). Formula

A

B

Figure 2: (A) Acrylic block used in test, (B) Custom-fabricated flask

RESULTS:

FTIR analysis showed that there was no chemical interaction between the soft lining material and CDA. The antifungal activity and the release of Chlorhexidine showed to be dose and time dependent (increase with concentration and decrease as incubation period increase).

The shore A hardness showed a highly significant increase with the addition of CDA and decrease as incubation period increase for control and 3.5% group .As for shear bonding strength, the addition of CDA at 0.5% and 1.5% percentage resulted in a highly significant decrease in bond strength, while 2.5% and 3.5% percentage shown non-significant differences in in compare with control, while all group showed increased with time

Table1: Descriptive statistics and one-way ANOVA of disk diffusion test

duration	groups	mean	S.D.	f- test
dry	Cl	0		2884.773
	0.5	13.18	0.402	
	1.5	15.38	0.41	
	2.5	18.06	0.578	
	3.5	22.52	0.763	
2 day	C	0		3692.685
	0.5	12.6	0.337	
	1.5	14.38	0.371	
	2.5	16.47	0.469	
	3.5	18.55	0.497	
2 weeks	C	0		1872.346
	0.5	11.09	0.716	
	1.5	13.21	0.247	
	2.5	14.96	0.538	
	3.5	17.3	0.587	
4 week	C	0	0	2863.686
	0.5	10	10	
	1.5	11.84	0.422	
	2.5	13.59	0.495	
	3.5	15.66	0.472	

Table2: LSD test between disk diffusion test means.

duration	Conce.		MD	p-value
Dry	C	0.5%	-13.18	0
		1.5%	-15.38	0
		2.5%	-18.06	0
		3.5%	-22.52	0
	0.5%	1.5%	-2.2	0
		2.5%	-4.88	0
		3.5%	-9.34	0
	1.5%	2.5%	-2.68	0
		3.5%	-7.14	0
	2.5%	3.5%	-4.46	0
2 days	C	0.5%	-12.60	0
		1.5%	-14.38	0
		2.5%	-16.47	0
		3.5%	-18.55	0
	0.5%	1.5%	-1.78	0
		2.5%	-3.87	0
		3.5%	-5.95	0
	1.5%	2.5%	-2.09	0
		3.5%	-4.17	0
	2.5%	3.5%	-2.08	0
2 weeks	C	0.5%	-11.09	0
		1.5%	-13.21	0
		2.5%	-14.96	0
		3.5%	-17.3	0
	0.5%	1.5%	-2.12	0
		2.5%	-3.87	0
		3.5%	-6.21	0
	1.5%	2.5%	-1.75	0
		3.5%	-4.09	0
	2.5%	3.5%	-2.34	0
4 weeks	C	0.5%	-10	-
		1.5%	-11.84	0
		2.5%	-13.59	0
		3.5%	-15.66	0
	0.5%	1.5%	-1.84	0
		2.5%	-3.59	0
		3.5%	-5.66	0
	1.5%	2.5%	-1.75	0
		3.5%	-3.82	0
	2.5%	3.5%	-2.07	0

Table 3: Descriptive statistics and one-way ANOVA of periodic CDA release

Duration	Conce.	Mean	S.D.	F-test
2 days	C	0	0	301.038
	0.5	0.025	0.005	
	1.5	0.051	0.005	
	2.5	0.155	0.03	
	3.5	0.209	0.02	
2 weeks	C	0	0	797.508
	0.5	0.004	0.003	
	1.5	0.013	0.003	
	2.5	0.043	0.006	
	3.5	0.101	0.008	
4 weeks	C	0	0	591.077
	0.5	0	0	
	1.5	0.001	0.001	
	2.5	0.003	0.003	
	3.5	0.059	0.007	

Table 4: LSD test between periodic CDA release test mean

	Conce		MD	p-value
2 days	C	0.5%	-0.025	0
		1.5%	-0.051	0
		2.5%	-0.155	0
		3.5%	-0.209	0
	0.5%	1.5%	-0.026	0
		2.5%	-0.13	0
		3.5%	-0.184	0
	1.5%	2.5%	-0.104	0
		3.5%	-0.158	0
	2.5%	3.5%	-0.054	0.002
2 weeks	C	0.5%	-0.004	0.008
		1.5%	-0.013	0
		2.5%	-0.043	0
		3.5%	-0.101	0
	0.5%	1.5%	-0.009	0
		2.5%	-0.039	0
		3.5%	-0.097	0
	1.5%	2.5%	-0.03	0
		3.5%	-0.088	0
	2.5%	3.5%	-0.058	0
4 weeks	C	0.5%	0	.
		1.5%	-0.001	0.155
		2.5%	-0.003	0.062
		3.5%	-0.059	0
	0.5%	1.5%	-0.001	0.155
		2.5%	-0.003	0.062
		3.5%	-0.059	0
	1.5%	2.5%	-0.002	0.261
		3.5%	-0.058	0
	2.5%	3.5%	-0.056	0

Table 5: Descriptive statistics and one-way ANOVA of shore A hardness test

Duration	Conce	Mean	S.D.	F-test
2 weeks	C.	78.423	1.728	24.486
	0.5%	81.535	0.402	
	1.5%	82.768	1.317	
	2.5%	83.475	1.401	
	3.5%	84.225	1.928	
4 weeks	C.	75.708	1.761	61.969
	0.5%	82.106	1.78	
	1.5%	83.007	0.745	
	2.5%	84.044	0.976	
	3.5%	81.838	0.909	

Table 6: LSD test between shore A hardness test mean

duration	Conce		M.D	p-value
2 weeks	C	0.5%	-3.113	0.002
		1.50%	-4.346	0
		2.5%	-5.052	0
		3.5%	-5.802	0
	0.5%	1.5%	-1.233	0.098
		2.5%	-1.94	0.011
		3.5%	-2.69	0.011
	1.5%	2.5%	-0.707	0.772
		3.5%	-1.457	0.322
	2.5%	3.5%	-0.75	0.854
4 weeks	C	0.5%	-6.398	0
		1.5%	-7.299	0
		2.5%	-8.336	0
		3.5%	-6.13	0
	0.5%	1.5%	-0.901	0.132
		2.5%	-1.938	0.002
		3.5%	0.268	0.65
	1.5%	2.5%	-1.037	0.084
		3.5%	1.169	0.052
	2.5%	3.5%	2.206	0

Table 7: Descriptive statistics and one-way ANOVA of shear bonding strength test.

Duration	Conce.	mean	SE	F-test
2 weeks	C	0.415	0.059	10.183
	0.5%	0.303	0.019	
	1.5%	0.332	0.03	
	2.5%	0.352	0.02	
	3.5%	0.391	0.068	
4 weeks	C	0.574	0.051	6.261
	0.5%	0.449	0.098	
	1.5%	0.481	0.099	
	2.5%	0.514	0.082	
	3.5%	0.599	0.054	

Table 8: LSD test between shear bonding strength test mean

duration	Conce.		MD	p-value
2 week	C	0.5%	0.111	0.001
		1.5%	0.082	0.012
		2.5%	0.063	0.057
		3.5%	0.023	0.922
	0.5%	1.5%	-0.029	0.114
		2.5%	-0.049	0
		3.5%	-0.088	0.017
	1.5%	2.5%	-0.02	0.447
		3.5%	-0.059	0.151
	2.5%	3.5%	-0.039	0.447
4 week	C	0.5%	0.126	0.022
		1.5%	0.093	0.115
		2.5%	0.06	0.317
		3.5%	-0.025	0.825
	0.5%	1.5%	-0.032	0.945
		2.5%	-0.065	0.509
		3.5%	-0.15	0.006
	1.5%	2.5%	-0.033	0.924
		3.5%	-0.118	0.034
	2.5%	3.5%	-0.085	0.089

DISCUSSION

The most common problem associate with the use of soft denture lining material is fungal colonization especially Candida albicans and along with plaque accumulation inflammation and infection of denture bearing area will develop.[23]

In this study an attempt was made to produce soft liner with antifungal activity by incorporating chlorhexidine diacetate salt.

The results of this study showed that the antifungal activity of CDA is a concentration and time dependent (the antifungal activity increase with concentrations of added drug and decrease when the period of incubation in artificial saliva increase) in which 3.5% group showed that highest mean value during all incubation period (22.52, 18.55, 17.3, 15.66 mm.) (Table 1)

The explanation for this could be due to increase in the amount of chlorhexidine leaching out of test specimens with the increase in the concentration of the added drug and decrease when the period of incubation in artificial saliva increases. [24]

And this was confirmed by the result of chlorhexidine diacetate release test that was conducted in this study which showed that the release is also concentrations and time dependent (increase with concentrations and decrease when the period of incubation in artificial saliva increase) which 3.5% group showed that highest mean value during all incubation period (0.209, 0.101, 0.059) (Table 3).

With the higher concentration appeared during the first 2 days and the explanation for that is the result of surface release of CDA first and followed by slow release which could be the cause of processes more complex, which involving the formation of fluid clusters around the drug molecules and the interaction of these clusters with the mechanism of acrylic resin for fluid absorption. [25,26]

The result of this study showed significant increase in the mean value of hardness for experimental group in compare with control group and from both periods of incubation in artificial saliva(Table 6), in which control group showed the lowest mean value for both incubation period (78.423, 75.708) (Table 5) .

This finding can be explained by the fact that added antifungal agent like chlorhexidine dictate in to soft liner material may affect the plasticizers ability for softening gel formation and its ability for polymeric chains penetration, and CDA salt may act like fillers that increase soft liner hardness and resistance when dispersed inside it [27]. This study also showed that the mean value of hardness significant decrease for both control group and 3.5% group, and a non-significant different for 0.5%, 1.5%, 2.5% group with time. The decrease in the mean value of hardness for control group and 3.5% group could be the result water absorption that may act as additional plasticizers that improve the resiliency of the material and cause decrease in hardness.And as for the non-significant different in mean value of hardness for 0.5%, 1.5%, 2.5% group with time could be the result of insufficient water absorption by these groups during the second period of evaluation. [28, 29]

As for shear bonding strength this study showed that the mean value of shear bonding strength for 0.5% and 1.5% groups was a significant decrease in the in compare with control, this can be attributed to leakage of residual monomer and other soluble impurities into artificial saliva which will leave empty spaces for water to get in [30], and in addition to that CDA is another factor which increases the micro porous as it is water soluble, so it dissolves and create more spaces that filled with water which result in swelling and stress build up at bonding interface. And as for2.5%, 3.5% group, it showed a non-significant difference in the means value of shear bonding strength in

compare with control group. And this could be the result of leaching out the plasticizer, which in turn leads to the increased stiffness.[31] This study also showed significant increase in the mean value of shear bond strength for all group (control and experimental) [32,33].

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الخلاصة

إن عملية استيطان بطانة طقم الاسنان اللينة من قبل المبيضات البيض والكائنات الدقيقة الاخرى لايزال يمثل مشكلة جدية كان الغرض من هذه الدراسة هو تقييم تأثير دمج نسب مختلفة من احد املاح الكلورهكسدين في مادة التبطين الاكريليكية المعالجة حراريا اللينة لطقم الاسنان على النشاط المضاد للفطريات (ضد المبيضات البيض) ، وعلى قوة الربط القصية وقوة الصلابه . علاوة على ذلك، تقييم كمية الكلورهكسدين المتحررة من مركب البطانة اللينة مع ملح الكلورهكسدين.

المواد وطرق البحث: تم دمج ملح الكلورهكسدين مع مادة التبطين الاكريليكية اللينة لطقم الاسنان بنسب وزنية مختلفة (0.5%,1.5%,2.5%,3.5%). تم نشر مسحوق ملح الكلورهكسدين في بوليمر مادة التبطين اللينة يدوياً باستخدام السباجيوله وبعد ذلك اضيف سائل البطانة اللينة ومزجت معا.تم اعداد اربع مائة وخمسين (450) عينة وتم تقسيمها الى اربع مجموعات وفقا لنوع الاختبار المراد اجرائه. اجري اختبار مسح الموجات تحت الحمراء لتحديد ما اذا هنالك اي تفاعل كيميائي بين ملح الكلورهكسدين ومادة التبطين اللينة. تم تقييم نشاط مزيج مادة التبطين اللينة مع ملح الكلورهكسدين ضد الفطريات على اربع فترات زمنيه مختلفة وباستخدام طريقة اختبار انتشار القرص. وقد تم قياس كمية الكلورهكسدين المتحررة في اللعاب الاصطناعي كل يومين لمده شهر بواسطة التحليل الطيفي للامتصاص وتم قياس قوة الصلابه باستخدام جهاز شور أي ديروميتير و تم قياس قوة الربط القصية بين البطانة اللينة ومادة قاعدة طقم الاسنان باستخدام جهاز انسترون. تم تحليل النتائج احصائيا باستخدام اختبار ANOVAواختبار LSDو اختبار.

النتائج: اظهرت نتائج مطياف الاشعة تحت الحمراء عدم وجود اي تفاعل كيميائي بين مادة التبطين اللينة وبين ملح الكلورهكسدين،وقد اظهرت نتائج اختبار انتشار القرص ازدياد قطرمنطقة التثبيط مقارنة بالمجموعة الضابطة، مع نقصان قطرمنطقة التثبيط بازدياد طول فترة حضانة العينات في اللعاب الاصطناعي. وقد تم الكشف عن اثر ملح الكلورهكسدين المتحرر في محلول اللعاب الاصطناعي خلال فترة الحضانة المستعملة مع نقصان في كمية الكلورهكسدين المتحرره بازدياد طول فترة حضانه العينات في العاب الاصطناعي . أظهرت النتائج زيادة كبيرة جدا في قوة الصلابه بعد إضافة ملح الكلورهكسدين لجميع المجاميع مقارنة بالمجموعه الضابطه ، و ان ازدياد فترة الحضن في العاب الاصطناعي أدت الى انخفاض قوة الصلابه للمجموعه 3.5% و المجموعه الضابطه فقط مع تغيرات غير ملحوضه على بقية المجموعات ، وقد لوحظ ان اضافة ملح الكلورهكسدين ادت الى انخفاض ملحوظ و بدرجة كبيرة في قوة الربط القصية لمجموعة 0.5% و 1.5% و تم العثور على اختلافات غير ملحوظة في قوة الربط القصية للمجموعات 2.5% و3.5% مع ازدياد القوة الرابطه القصية بازدياد فترة حضانة العينات في العاب الاصطناعي لجميع العينات،

الاستنتاج: ان اضافة ملح الكلورهكسدين الى مادة التبطين الاكريليكية اللينة لطقم الاسنان يساعد على انتاج مادة تبطين لينة مع خصائص مضادة للفطريات،و ذللك لتحرر الكلورهكسدين بتراكيز فعاله وبالتالي تقليل قابلية حصول التهاب الفم الناتج عن طقم الاسنان. ان المادة المطورة لاتزال تحافظ على توافق حيوي. وقد اسفرت هذه الاضافة عن انخفاض في قوة الربط القصي لمادة التبطين للمجموعتان 0.5% و1.5% و لم توثر على قوة الربط القصيه للمجموعتان 2.5% و 3.5 % ، في حين ادت إضافة ملح الكلورهكسدين الى ازدياد قوة الصلابه لجميع المجموعات