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The Influence of Natural Products as Denture Cleansers on Candida albicans Colonization to Cobalt–Chromium Alloy Denture Base Material.

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الخلاصة

الهدف: يهدف البحث الحالي إلى تقييم تأثير مختلف منظفات الأطقم على درجة التصاق مستعمرات المبيضات البيض (Candida albicans) على مادة قاعدة الطقم المصنوعة من الكروم– كوبالت وقياس الخشونة الناتجة عن استعمال هذه المنظفات. المواد وطرق العمل: تم تحضير ست وثلاثين عينة من مادة الكروم– كوبالت واستخدمت خمس منظفات، أربع منها محضرة وهي (الشب، الملح، الصودا والخل الصودا وزيت الزعتر) وواحدة تجارية (Protefix) واستخدم الماء المقطر كمجموعة المقارنة (control). تم غمس العينات في منظفات الأطقم لمدة شهر واحد، إذ احتوى كل منظف على ست عينات باستثناء ستة عينات في مجموعة الماء باعتبارها المجموعة المقارن بها. غمست نصف العينات لكل منظف لمدة نصف ساعة في اليوم والنصف الأخر لمدة ثمان ساعات في اليوم خلال الشهر، وقبل الفحص المايكروبايولوجي اجري فحص خشونة العينات باستخدام جهاز(Profilometer). تم خزن معلقات المبيضات البيض مع العينات بدرجة حرارة37 سيليزية ومن ثم غمست العينات في منظفاتها لمدة ساعة واحدة. استخدم المجهر الضوئي في فحص وتعداد خلايا (Candida albicans) الملتصقة وفحص درجة مقاومة المنظفات لالتصاق هذه الخلايا. النتائج: دللت النتائج على وجود تغيير غير معنوي في خشونة سطح عينات الكروم– كوبالت في منظفات الأطقم عند غمسها لمدة نصف ساعة، بينما ظهر تغيير معنوي عند الغمس لمدة ثمان ساعات، كما بينت النتائج تغييرا معنويا في عدد مستعمرات مبيضات (Candida albicans) على سطح عينات الكروم– كوبالت بحيث أظهرت كل المنظفات التصاقا أقل من مجموعة الماء. وأظهر منظف (الصودا + زيت الزعتر) تأثيراً أقل على خشونة سطح الكروم– كوبالت ومن ناحية التصاق مبيضات (Candida albicans) بالمقارنة مع بقية المنظفات. الاستنتاجات: توصلت الدراسة إلى أن جميع المنظفات المستخدمة كانت فعالة في درجة مقاومتها لالتصاق مبيضات (Candida albicans) على سطح عينات الكروم كوبالت وأظهرت كذلك خشونة اقل من مجموعة الماء.

الكلمات المفتاحية: منظفات الأطقم، قاعدة الطقم من مادة الكروم كوبالت، المبيضات البيض

ABSTRACT

Aims: The study aims to evaluate the influence of various denture cleansers on colonization of *Candida albicans* to Cobalt–Chromium alloy denture base and the subsequent roughness assosiated with these cleansers. **Materials and methods:** Thirty–six samples of Co–Cr denture base and five cleansers, four prepared(alum, salt, soda+vinegar, soda+thymol), one commercial (protefix) and distilled water(D.W) as a control were used. Samples were immersed in denture cleansers for one month in which each cleanser had 6 samples excluding 6 samples in D.W as a control. Half of samples for each cleanser were immersed ½ hr per day and the other half immersed 8hrs per day through one month, before microbiological examination, samples were tested for surface roughness using profilometer. *Candida albicans* cell suspension was incubated with the test samples for 1hr at 37°C after which the test samples were immersed in their cleansers for 1hr. Visualization, inspection and enumeration of adherent C.albicans cells and detection of the anti–adherent effect of the cleansers was achieved by using light microscopy. **Results**: The results demonstrated insignificant difference in surface roughness of Co–Cr alloy denture base in the cleansers at 1/2 hr and a significant difference at 8 hrs immersion. There was a significant difference in C.albicans colonization to Co–Cr denture base in which all the cleansers showed less adhesion than control. The results also revealed that (soda+Thymol oil) cleanser expressed the least values of colonization and roughness among other cleansers. **Conclusions:** The cleansers were effective as anti–adherent yeast cells to Co–Cr denture base and showed roughness degrees less than control.

Key words: Denture cleansers, Co–Cr denture base, *Candida albicans*

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INTRODUCTION

Metal denture bases may be made from different materials like gold, Co–Cr, Ni–Cr, and stainles steal. In dentistry, chrome containing alloys are the principle materials from which removable partial

denture frameworks, major connectors and denture bases are constructed, in repairing broken frameworks, tooth born splint, implant, fixed prosthesis and in medical device industry [1–4]. The popularity of Cr–Co alloy materials is increased as they are rigid, strong in thin section, having good thermal conductivity and low density, so the denture is fairly light.

Denture base materials collect oral deposits in the same manner as natural teeth which is a significant factor for denture stamatitis [5]. The fungal organisms that are most commonly associated with denture plaque are of genus candida. These yeasts are present in the saliva of a majority of denture wearers and display an affinity for adherence to denture materials, [6] so the presence of a prosthetic device is one of the several reasons for infection by candida [7,8]. Since the microporous surface of the denture provides a wide range of enviroment to support microorganisms that can threaten the health of a physically vulnerable patient, therefore; unclean dentures represent both an esthetic a health concern for the person who use them [6].

It is well established that the use of denture cleansers helps to control or reduse the amount of plaque residing on denture surface [9]. Denture cleanliness is essential to prevent malodor, poor esthetic and accumulation of plaque and important for long–term success of prosthodontic treatment. The most common commercial denture cleansers use immersion technique which is the suitable method for many elderly patients in long–term care hospital because of disease and poor dextrity.[5]

There are large number of solutions, tablets and powders available for cleaning dentures. An ideal denture cleanser should be non toxic, bactericidal, fungicidal and compatible with denture base, [9] i.e it must clean effectively without adversly affecting denture base material properties especially roughness because rough surface is unfavorable and may affect plaque formation or inhibit its removal.[10,11] The purpose of the current study is to determine the effect of five immersion–type denture cleansers on colonization of *Candida albicans* to a Co–Cr alloy denture base material and the changes produced in surface roughness.

MATERIALS AND METHODS

Wax patterns (30*20*1)mm in dimension, [12] with a hole at one side of the sample were prepared to manufacture thirty–six samples of Co–Cr alloy denture base material (Biosil, Germany). The patterns were invested with phosphate–bonded investment material (Biosint–Supra, Degussa, Germany) in accordance with the manufacturers instruction. Investment molds were placed in a casting furnace (KaVo,Germany) and heated at a constant rate to 1050 °C with the total heating time about 150 min. according to manufacturers instruction. The investment mold and refractory crucible containing the metal were placed in the casting machine (Motor–cast, Degussa,Germany). After heating the mold , it was placed in the cradle of the machine with the sprue holes facing the crucible. When the metal was completely molten, the heat source was removed and the casting arm of the machine was rotated to thrust the molten metal into the mold [13]. After bench cooling, the samples were removed from the mold, sandblasted to remove exccess of the investment and high speed micromotor (Strong 204, Korea) was used for sprue removal with separating disks. Finishing and polishing were performed with carborandum wheels, special stone burs, brushes and rubber wheels.

Denture cleansers that are used in this study are five solutions and distilled water as a control ,[14] the composition of the prepared solutions is shown in Table(1).

Table (1): Denture cleansers used in the study

Material	Composition and Manufacturer
Sol. 1	Protefix tablet/Germany in 100 ml of distilled water
Sol. 2	Alum powder/Sweden (5gm) in 100 ml of distilled water
Sol. 3	Sodium chloride salt/Iraq (40gm) in 100 ml of distilled water
Sol. 4	Sodium bicarbonate/China (9.52gm)+clear commercial vinegar /Jordan (16 ml) in 100 ml of distilled water
Sol. 5	Sodium bicarbonate/China (2.38gm)+Thymol oil/Iraq (1.24gm) in 100 ml of distilled water

Before microbiology, the 36 samples of Co–Cr were immersed in denture cleansers for one month, six samples in every cleanser, and six samples in distilled water as a control. Half of samples for each cleanser were immersed for 1/2 hr and the other half were immersed for 8 hrs per day throughout one month [(14)], excluding the control group where 6 samples were immersed in distilled water for one month. The samples of each solution were held in glass beakers by dental floss in which the sample was completely covered with the cleanser solution.

At the end of the immersion period and before undergoing microbiological experiment, the surface of the samples had to be examined for roughness as there is an association between roughness and microbial attachment. Surface roughness of the tested samples was measured by using a stylus profilometer (Tylor–Hobson, England). Three readings of each sample were recorded and the avarage roughness(Ra) , the arithmatic mean of all deviations of the roughness profile within the total measuring lenghth was taken.

Microbiology:

A culture of *Candida albicans* was obtained from several patients wearing upper complete dentures with candida infection. To ensure the purity, the *Candida albicans* was cultivated on Sabouraud Dextrose agar and germ tube test. The culture was then inoculated in 100 ml of brain–heart infusion broth (BHI) and incubated for 18 hrs at 37°C without agitation.[(15)] Cells were harvested by refrigerated centrifuge (6000 rpm/4°C/15 min) and washed twice in phosphate–buffer saline PBS. Microorganism cells were resuspended in the same buffer to an optical density of 0.5(540 nm) spectrophotometrically which represents ($8.62\pm2.87\times10^6$). Harvested cells were kept in PBS at 4°C in refrigerator. (2 ml) of *C.albicans* suspension in PBS was added to petri dish that contained the test samples and incubated for 1hr at 37°C. Samples with adherent microorganisims were removed from incubator, washed by dipping gently 10 times in 100 ml of PBS in order to remove the loosely adherent cells and were dried by lying horizontally inside the hood. Samples of Co–Cr were distributed according to their cleansers in which every cleanser had 6 samples, and 6 samples were immersed in distilled water as a control, time of immersion in denture cleansers and distilled water was 1hr, after that the samples were removed from cleansers and distilled water, washed by dipping in 100 ml of PBS to remove the adherent cells that were affected by the action of cleansers. Retained *Candida albicans* cells to Co–Cr samples were fixed by immersing in 80% methanol for 30 sec., allowed to dry by lying horizontally inside the hood and stained with crystal violet for 1 min., then the samples were washed with PBS for 30 sec.and air dried. Adherent yeast cells in each sample were enumerated under light microscope at (X100) magnification with a light source directed from above in three randomly selected fields of view for each sample and the final results were expressed as the number of the yeast cells/mm^2.[(16)]

RESULTS

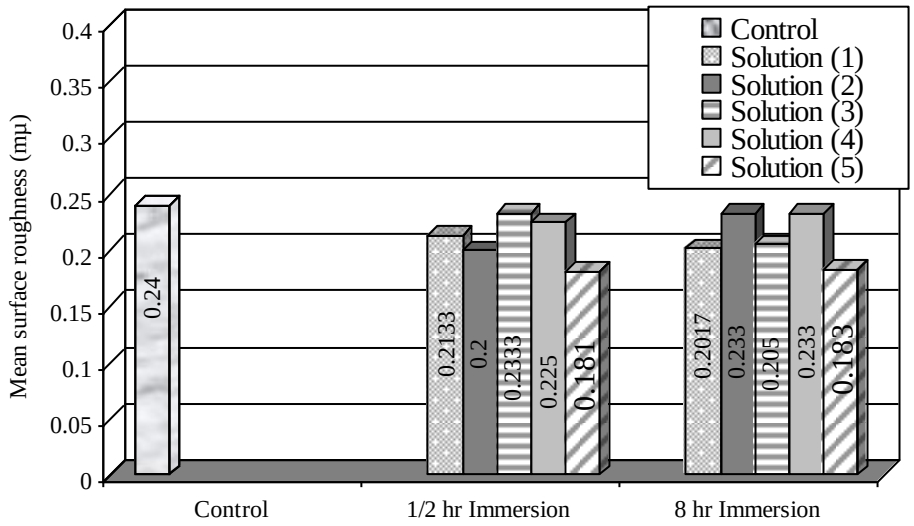
Table (2) demonstrated insignificant difference in roughness of Co–Cr alloy denture base material in different cleansers at ½ hr immersion and a significant difference at 8 hrs immersion.

Table (2) Analysis of Variance of the mean surface roughness of Co–Cr alloy denture base in different denture cleansers and different durations of immersion

Immersion period		Sum of Square	df	Mean Square	F–value	P–value
1/2 hr	Between groups	0.014	5	0.003	1.873	0.129
	Within groups	0.046	30	0.002		
	Total	0.060	35			
8 hrs	Between groups	0.015 0. 30	5	0.003	3.041	0.025
	Within groups	0.046	30	0.001		
	Total		35			

df: degree of freedom

Figure (1) showed that in both immersion periods, denture cleansers observed a reduction in roughness values in comparison to the control (distilled water), and solution 5 (Soda+Thymol oil) observed the least values in both immersions (0.181µm and 0.183µm) respectively.



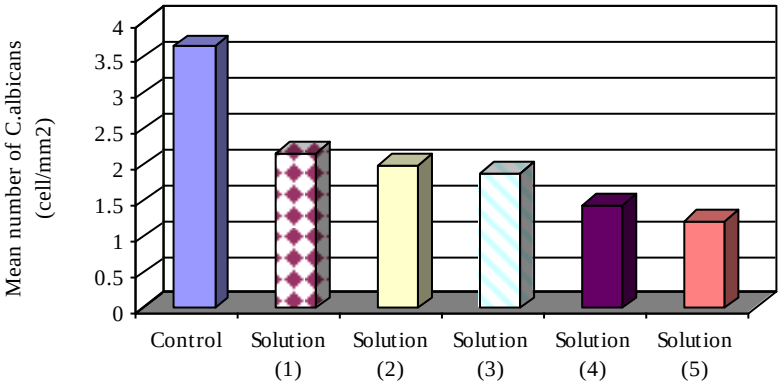
Figure(1) Mean surface roughness of Co–Cr alloy denture base in denture cleansers with 1/2hr and 8 hrs durations of immersion

Table (3) revealed a significant difference in Candida albicans colonization to Co–Cr alloy denture base between different cleasers($p \leq 0.05$). Duncan's multiple range test, Figure (2) and Table (4) showed that *Candida albicans* adhesion in denture cleansers was significantly less than control in which solution(5) recorded the least adhesion value (1.22 cell/mm^2) in relation to the other four solutions, and the control group recorded the highest value in fungal cell adhesion (3.66 cell/mm^2). Table(5) showed a positive but insignificant relation between roughness and *Candida albicans* colonization.

Table (3) Analysis of Variance of the effect of denture cleansers on the *Candida albicans* colonization to Co–Cr alloy denture base.

	Sum of Squares	df	Mean Square	F–value	P–value
Between groups	33.356	5	6.671	2.489	0.044
Within groups	128.667	48	2.681		
Total	162.023	53			

df: degree of freedom



Figure(2) The effect of denture cleansers on the mean number of *Candida albicans* colonies on Co–Cr alloy denture base

Table (4) Duncan's mutiple range test for the effect of denture cleansers on *Candida albicans* colonization to Co–Cr alloy denture base.

Denture cleansers	N	N	Mean (cell / mm^2)	Duncan's group
Solution (1)	6		2.1667	AB
Solution (2)	6		2.000	A
Solution (3)	6		1.8889	A
Solution (4)	6		1.4444	A
Solution (5)	6		1.2222	A
Control	6		3.6667	B

N: number.

Table (5) The power of correlation between the roughness and *Candida albicans* colonization.

Variables	Person Correlation	P–value
Roughness / Colonization	0.110	0.430

P> 0.05 (Not significant).

DISCUSSION

Metal denture bases proved to be effective in decreasing the fungal growth typically present in complete denture than acrylic and provide an alternative dental service for patient who are particularly prone to higher incidance of fungal infection [17].

Denture cleansers are performed to thwart plaque formation and improve esthetic of the device. When considering practical plaque cotrol on Co–Cr denture base, the choice of a denture cleanser depends on its composition and the compatibility between materials should be considered to avoid or minimize alteration of

properties [12].

Prior to commencement of the study, Co–Cr samples were soaked daily for 1/2 hr and 8 hrs as a part of patient daily regimen for a month. In most microbiological studies, it would be better to diagnose the material for roughness because it has been emphasized that surface roughness controls the initial microbial adherence and determine its colonization by different microorganisms.[8] In this study 8 hrs immersion showed a significant difference in roughness, while 1/2 hr immersion expressed roughness insignificantly, this could be attributed to the long contact of the denture cleanser material with the surface of Co–Cr alloy denture base.

The study observed the initial attachment of *C. albicans* cells after one hour incubation period. Initial retention and/or attachment are best monitored over a short period, enabling cell–substratum interaction to be visualized and recorded [15]. Immersion in denture cleansres for detecting their effect on fungal attachment was one hour[18].

In regard to anti–yeast colonizing order, denture cleansers revealed their activity in descending order as: soda+thymol, solution(5) was the best cleanser and observed the least number of *Candida albicans* colonies, this result agreed with other researchers about thymol as antimicrobial product especially against *Candida albicans* [19–21]. Solution(4), Soda+vinegar came in the second level in *C. albicans* attachment scale, attributing this finding to the effectiveness of vinegar in killing microorganisms, [22] and the antifungal activity of sodium bicarbonate. Solution(3), saturated salt occupied the third level, its very fast, broad spectrum microbicidal active product [23]. Alum, solution(2) was also effective as a cleanser against C. albicans attachment, this confirm the findings of (Ibrahim et al)[24], who mixed the alum with a vaccine in mice as an adjuvant against multiple strains of C. albicans. The protefix tablet, solution(1) showed anti–fungal activity but less than natural cleansers, its action resulted from the oxidizing ability of the peroxide decomposition and the effervescing action of evolved oxygen [14]. The control (water group) showed the highest number of *Candida albicans* colonies and came in the last level of the scale.

Adhesion of *C. albicans* to Co–Cr was supported by roughness measurements in which denture cleansers observed roughness values less than control and this association proved the hypothesis that the retention of yeast is favored on the rougher surface because of increased surface area available for colonization [8,15].

The smoothing effect of the cleanser solutions may be due to the deposition of the organic components of the cleansers on the alloy surface or it may be due to the uniform dissolution of the alloy surface in such solutions, while the increased surface roughness behavior with distilled water may be attributed to its normal content of O_2 on one hand and the absence of oxidizing agent in its composition on the other hand, inaddition distilled water lacks organic materials and detergents that are present in cleanser formulation which may be deposited on the metal surface and give a false indication about surface topography [12].

CONCLUSIONS

Relying on the results of the study, Co–Cr denture base observed roughness values and number of *Candida albicans* colonies in the denture cleansers less than the group of distilled water, and this action was most marked with soda+thymol oil cleanser group. There was a positive relation between Candida albicans colonization and roughness measurement.

REFERENCES

1. Wataha JC. Alloys for prosthodontic restorations. *J Prosthet Dent***.** 2002; 87: 353–363.
2. Gapido CG, Kobayashi H, Miyakawa O, Kohno S. Fatique resistance of cast occlusal rests using Co–Cr and Ag–Pd–Cu–Au alloys *J Prosthet Dent*. 2003; 90: 261–9.
3. Cruz AR, Velez EJ, Soto RV. Mechanics of biomaterials: human face reconstruction prostheses. *Application of Engineering Mechanics in Medicine, GED–University of Puerto Rico, Mayagüez.* 2005, C1–C17.
4. Claudio R, Hernandez E, Alex Hernandez A, Mercado F. Mechanics of biomate-

rials: urology and gastroenterology prosthetic device. *Application of Engineering Mechanics in Medicine, GED–University of Puerto Rico, Mayagüez.* 2005, H1–H22.
5. Gornitsky M, Paradise I, Landaverde G, Malo AM, Velly AM. A clinical and microbiological evaluation of denture cleansers for geriatric patients in long term care institutions. *J Can Dent Assoc*. 2002; 68(1): 39–45.
6. Shay K. Denture Hygiene: a review and update. *J Contemp Dent Pract.*2000;1(2): 1–8.
7. Compagnoni MA, Souza RF, Marra J, Pero AC, Barbosa DB. Relationship between candida and nocturnal denture wear: quantitative study. *J Oral Rehabil*. 2007; 34: 600–605.
8. Pereira–Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JMC. Development of candida–associated denture stomatitis: new insights. *J Appl Oral Sci*. 2008; 16(2): 86–94.
9. Sheen S, Harrison A. Assessment of plaque prevention on denture using an experimental cleanser. *J Prosthet Dent.* 2000; 84(6): 594–601.
10. Harrison Z, Johnson A, Douglas CW. An in vitro study into the effect of a limited range of denture cleansers on surface roughness and removal of candida albicans from conventional heat cured acrylic denture base material. *J Oral Rehabil.* 2004; 31: 460–467.
11. Barbosa SH, Zanta RL, Navarro MF, Nunes OB. Effect of different finishing and polishing techniques on the surface roughness of microfilled, hybrid and packable composite resin. *Braz Dent J*. 2005; 16(1): 39–44.
12. Al–Nu'aymi LAM. The effect of denture cleansers on the surface roughness,weight and dissolution of cobalt–chromium denture base casting alloy.Thesis;College of Dentistry, University of Baghdad; 2002.
13. Al–Ni'aimi AJM. Effect of laser welding on the tensil strength and radiographic analysis of Co–Cr repaired joints.Thesis; College of Dentistry, University of Mosul; 2008.
14. Khalil SM. New denture cleansers. Thesis; College of Dentistry, University of Mosul; 2007.
15. Taylor R, Maryan C, Verran J. Retention of oral microorganisms on cobalt– chromium alloy and dental acrylic resin with different surface finishes. *J Prosthet Dent*. 1998; 80(5): 592–7.
16. Al–Irheyim RNA. Evaluation of surface roughness and adherence of candida albicans on some denture base and denture lining materials. Thesis; College of Dentistry; University of Mosul; 2009.
17. Perezous LF, Stevenson GC, Flaitz CM, Goldschmidt ME, Engelmeier RL, Nichols CM. The effect of complete dentures with a metal palate on Candida species growth in HIV–infected patients. *J Prosthodont.* 2006; 15: 306–31.
18. Kassab NHN. Antifungal effect of some agents on Candida albicans growth on acrylic resin denture base surface.Thesis; College of Dentistry, University of Mosul; 2002.
19. Nazer AI, Kobilinsky A, Tholozan JL, Dubois–Brissonnet F. Combination of food antimicrobials at low levels to inhibit the growth of Salmonella species, Typhimuriumi: a synergistic effect. *Food Microbiol.*2005; 22: 391–398.
20. Sekino S, Ramberg P. The effect of a mouth rinse containing phenolic compounds on plaque formation and developing gingivitis. *J Clin Periodontal.*2005; 32: 1083–1088.
21. Hazzit M, Baaliouamer A, Verissimo AR, Faleiro ML, Miguel MG. Chemical composition and biological activities of Algerian Thymus oils. *Food Chemistry*. 2009; 116(3): 714–721.
22. Basson NJ, Quick AN, Thomas CJ. Household products as sanitizing agents in denture cleansing. *J Dent Assoc S Africa*. 1992; 47: 437.
23. Baltch AL, Smith RP, Franke MA, Ritz WJ, Michelsen P, Bopp LH, Singh JK. Microbicidal activity of MDI–P against Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Legionella pneumophilla. *Am J Infect Control.* 2000; 28(3): 251–257.
24. Ibrahim AS, Spellberg BJ, Avanesian V, Fu Y, Edward Jr JE. The anti–candida vaccine based on the recombinant N–terminal domain of ALs1p is broadly active against disseminated candidiasis. *Infect Immun.* 2006; 74(5): 3039–3041.