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

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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 associated 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 (Profifix) 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 cleaners 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 cleaners 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

INTRODUCTION

Metal denture bases may be made from different materials like gold, Co–Cr, Ni–Cr, and stainless 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, im-
plant, fixed prosthesis and in medical de-
vice 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 de-
nature 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 mate-
rials, (6) so the presence of a prosthetic device is
one of the several reasons for infection by
candida (7,8). Since the microporous sur-
face of the denture provides a wide range
of enviroment to support microorganisms
that can threaten the health of a physically
vulnerable patient, therefore; unclean de-
natures 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 re-
duce 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 elde-
ry patients in long–term care hospital
because of disease and poor dexterity. (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, (6) i.e it must
clean effectively without adversely affect-
ing denture base material properties espe-
cially roughness because rough surface is
unfavorable and may affect plaque forma-
tion or inhibit its removal. (10,11) The pur-
pose of the current study is to determine
the effect of five immersion–type denture
cleansers on colonization of _Candida albici-
cans_ to a Co–Cr alloy denture base ma-
terial and the changes produced in surface
roughness.

**MATERIALS AND METHODS**

Wax patterns (30*20*1)mm in di-
msion, (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 pat-
terns were invested with phosphate–
boned investment material (Biosint–
Supra, Degussa, Germany) in accordance
with the manufacturers instruction. In-
vestment 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 ma-
chine (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 cool-
ing, the samples were removed from the
mold, sandblasted to remove exces of
the investment and high speed micromotor
(Strong 204, Korea) was used for sprue
removal with separating disks. Finishing
and polishing were performed with carbo-
randum wheels, special stone burs, brush-
es and rubber wheels.

Denture cleansers that are used in
this study are five solutions and distill-
ed water as a control (14) the composi-
tion of the prepared solutions is shown in
Table(1).
Table (1): Denture cleansers used in the study

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

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 average roughness (Ra), the arithmetic mean of all deviations of the roughness profile within the total measuring length 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±2.87×10^6). 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 microorganisms 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. Immersion of Co–Cr 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 1/2 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

<table>
<thead>
<tr>
<th>Immersion period</th>
<th>Sum of Square</th>
<th>df</th>
<th>Mean Square</th>
<th>F– value</th>
<th>P– value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2 hr</td>
<td>Between groups</td>
<td>0.014</td>
<td>5</td>
<td>0.003</td>
<td>1.873</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>0.046</td>
<td>30</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.016</td>
<td>35</td>
<td>0.003</td>
<td>1.873</td>
</tr>
<tr>
<td>8 hrs</td>
<td>Between groups</td>
<td>0.015</td>
<td>5</td>
<td>0.003</td>
<td>3.041</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>0.046</td>
<td>30</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.060</td>
<td>35</td>
<td>0.003</td>
<td>3.041</td>
</tr>
</tbody>
</table>

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.

Table (3) revealed a significant difference in Candida albicans colonization to Co–Cr alloy denture base between different cleansers (p ≤ 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/mm²) in relation to the other four solutions, and the control group recorded the highest value in fungal cell adhesion (3.66 cell/mm²). 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.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F–value</th>
<th>P–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>33.356</td>
<td>5</td>
<td>6.671</td>
<td>2.489</td>
<td>0.044</td>
</tr>
<tr>
<td>Within groups</td>
<td>128.667</td>
<td>48</td>
<td>2.681</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>162.023</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 multiple range test for the effect of denture cleansers on Candida albicans colonization to Co–Cr alloy denture base.

<table>
<thead>
<tr>
<th>Denture cleansers</th>
<th>N</th>
<th>Mean (cell / mm²)</th>
<th>Duncan’s group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution (1)</td>
<td>6</td>
<td>2.1667</td>
<td>AB</td>
</tr>
<tr>
<td>Solution (2)</td>
<td>6</td>
<td>2.000</td>
<td>A</td>
</tr>
<tr>
<td>Solution (3)</td>
<td>6</td>
<td>1.8889</td>
<td>A</td>
</tr>
<tr>
<td>Solution (4)</td>
<td>6</td>
<td>1.4444</td>
<td>A</td>
</tr>
<tr>
<td>Solution (5)</td>
<td>6</td>
<td>1.2222</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>3.6667</td>
<td>B</td>
</tr>
</tbody>
</table>

N: number.

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

<table>
<thead>
<tr>
<th>Variables / Colonization</th>
<th>Person Correlation</th>
<th>P–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness</td>
<td>0.110</td>
<td>0.430</td>
</tr>
</tbody>
</table>

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 incidence of fungal infection (17).

Denture cleansers are performed to thwart plaque formation and improve esthetic of the device. When considering practical plaque control 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\textsuperscript{(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.\textsuperscript{(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 \textit{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\textsuperscript{(15)}. Immersion in denture cleansers for detecting their effect on fungal attachment was one hour\textsuperscript{(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 \textit{Candida albicans} colonies, this result agreed with other researchers about thymol as antimicrobial product especially against \textit{Candida albicans}\textsuperscript{(19–21)}. Solution(4), Soda+vinegar came in the second level in \textit{C. albicans} attachment scale, attributing this finding to the effectiveness of vinegar in killing microorganisms.\textsuperscript{(22)} and the antifungal activity of sodium bicarbonate. Solution(3), saturated salt occupied the third level, its very fast, broad spectrum microbicidal active product\textsuperscript{(23)}. Alum, solution(2) was also effective as a cleanser against \textit{C. albicans} attachment, this confirm the findings of (Ibrahim et al)\textsuperscript{(24)}, who mixed the alum with a vaccine in mice as an adjuvant against multiple strains of \textit{C. albicans}. The protefix tablet, solution(1) showed antifungal activity but less than natural cleansers, its action resulted from the oxidizing ability of the peroxide decomposition and the effervescing action of evolved oxygen\textsuperscript{(14)}. The control (water group) showed the highest number of \textit{Candida albicans} colonies and came in the last level of the scale.

Adhesion of \textit{C. albicans} to Co–Cr was supported by roughness measurements in which denture cleaners 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\textsuperscript{(8,15)}. The smoothing effect of the cleanser solutions may be due to the deposition of the organic components of the cleaners 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\textsubscript{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\textsuperscript{(12)}.

**CONCLUSIONS**

Relying on the results of the study, Co–Cr denture base observed roughness values and number of \textit{Candida albicans} colonies in the denture cleaners 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**

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Denture cleansers & C. albicans colonization to Co–Cr alloy