The Effect of Newly Prepared Cleansing Agent on The Surface Roughness and Tensile Strength of Highly Impact Acrylic Denture Base Material

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ABSTRACT

Aims: To evaluate the effect of two prepared and commercial solution on surface roughness and tensile strength of highly impact acrylic denture base material. Materials and Methods: The total number of specimens were one hundred and fifty. They were prepared from highly impact acrylic and subdivided into five groups for each solution (EDTA, Soda+H₂O₂, Lacalut, Corega and distilled water). Two laboratory tests were used for this research. Surface Roughness and tensile strength test. The surface roughness test specimens were constructed with dimensions (10×10×2±0.03mm) (length, width, and thickness respectively). According to ADA specification no.12 the tensile test specimens were constructed with dimensions 90×10×3±0.03mm (length, width, and thickness respectively). The immersion periods in this research are (2day, 7 day and one month). ANOVA and Duncan multiple range test were used. The statistical results were considered significant at p ≤ 0.05. Results: the results showed that (soda+H₂O₂) has no significant change on the surface roughness and tensile strength of highly impact acrylic denture base material in (2day, 7 day, and 1month). Conclusion: (soda+H₂O₂) has the lowest effect on the surface roughness and tensile strength of highly impact acrylic denture base material in (2day, 7 day, and 1month)

Keywords: Denture cleanser, Highly impact, lacalut


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INTRODUCTION

Polymethyl methacrylate (PMMA) has been used in dental prosthetic devices for almost 70 years. Three fundamental features have contributed for its success: excellent appearance, simple processing technique and easiness of the repair. However, the resistance to impact and fracture of PMMA during function are low fracture. (1-3) The denture base resin is subject-
ed to various stresses during function. During fabrication of a denture, the physical and mechanical properties influence by cure condition and choice of materials. Each cure cycle or fabrication technique is a compromise that attempts to optimize the properties thought important for a given application. Dentist and manufacturers of denture base materials have long been searching for ideal materials and designs for dentures. So far, the results have been noteworthy, although there are still some physical and mechanical problems with these materials. Many attempts have been made to enhance the strength properties of acrylic denture bases including the addition of metal wire. The primary problem of using metal wire reinforcement is poor adhesion between wire and acrylic resin. Although several methods have been used to improve the adhesion between these components, enhancement in mechanical properties, such as transverse strength and fatigue resistance, was not significant. Modifications of chemical structure, by the addition of cross-linking agents such as polyethylene glycol dimethacrylate or by copolymerization with rubber, have been attempted. Various types of fiber including carbon fiber whisker fiber, aramid fiber, polyethylene fiber, and glass fiber have been used as a reinforcement. Reinforcement with fibers enhances the mechanical strength characteristics of denture bases, such as the transverse strength, ultimate tensile strength and impact strength. In addition, fiber reinforcement has advantages compared with other reinforcement methods, including improved esthetics, enhanced bonding to the resin matrix, and ease of repair. Cleansers and cleaning methods used may have harmful effect on the plastic or metal component of the denture. Knowledge of constituents of denture cleansers, their efficiency, adverse effect and safety would aid in dispensing appropriate information to the patient, so the dentist must be able to recommend a denture cleanser that is effective, non deleterious to denture material and safe for patient use.

During this research of the effect of denture cleanser on the properties of denture base materials, the chemicals disinfectants (Chlorhexidine gluconate, sodium hypochlorite and gluteraldehyde) reduced the tensile strength of denture base material, but this reduction is not significant. The aims of this study are to evaluate the effect of two prepared and two commercial solutions on surface roughness and tensile strength of highly impact acrylic denture base material after (2day, 7 day and 1 month).

MATERIALS AND METHODS

The total number of specimens was one hundred and fifty. Seventy five for each test were prepared from highly impact acrylic and subdivided into five groups for each solution. The immersion periods in this study are (2day, 7 day and one month)

Highly impact acrylic (vertex-dental) used in this research mixed according to the manufacture instruction. The liquid powder ratio is 1 ml liquid and 1.2 mg powder, adding powder to the liquid and then mixing the powder to liquid for 30 min, leave the mixing for 8 min in room temperature 22 °C until reach to the dough stage adding the highly impact acrylic to the flask through in room temperature 22 °C and then press the flask by press, and putting immediately inside hot water approximately 70°C for 90 min and then rising the degree of temperature to the 100 °C for 30 min and the remove the flask and leave it to cool. Two laboratory tests were used for this research, tensile strength and Surface roughness test. According to ADA specification no.12 the tensile test specimens were constructed with dimensions 90×10×3±0.03mm (length, width, and thickness respectively) Then universal testing machine (Gunt, Germany) was used to measure the tensile strength of specimens The force at failure was recorded in Newton (N) and the true tensile strength value was calculated by the following formula: Tensile strength = F(N)/A (mm²). The surface roughness test specimens were constructed with dimensions (10×10×2±0.03mm) (length, width, and thickness respectively) The surface roughness (Ra) values were measured using a profilometer (Stylus 10 UK) which can measure small surface variations by moving a diamond stylus in
contact with the surface. The specimens were fabricated by using Type III model dental stone (Zhermack SPA Rovigo, Italy) as a mold. This study deals with five solutions (table 1).two experimental prepared solutions, solution one (Ethylene Diamin Tetra acetic Acid) EDTA and solution two (soda Na₂CO₃ and Hydrogen peroxide H₂O₂) two commercial denture cleanser tablets (Corega, lacalut) for comparison and distilled water as a control solution. Every solution was diluted in 100 ml of distilled water.

The following equations illustrate the preparation of the above solutions
1-EDTA

\[\text{CH}_3\text{COOH} \rightarrow \text{CH}_3-\text{CH}_2-\text{N}-(\text{CH}_3\text{COOH}) \rightarrow \text{Ca}\]

2-Soda +H₂O₂

Artificial Saliva was developed in order to bring the trials closer to real in-mouth conditions. Indeed, its mineral composition is close to that of resting mixed saliva. By mixing the following compounds in distilled water, the artificial saliva solution were prepared. (18)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentrations (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.4</td>
</tr>
<tr>
<td>KCl</td>
<td>0.4</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.79</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.78</td>
</tr>
<tr>
<td>UREA</td>
<td>1</td>
</tr>
<tr>
<td>DISTELD WATER</td>
<td>1 L</td>
</tr>
</tbody>
</table>

The fresh solutions were prepared daily at the beginning of soaking trial (1/2h). The specimens were removed from the solution washed with distilled water, and dried in air by shaking the specimen for about 30 seconds. The solutions were removed, the beakers were cleaned and the specimens were immersed in distilled water for 8 hs at (21±2°C) then immersed in artificial saliva for about 15.5 h at (37±1°C) in the incubator. According to method described previously The immersion periods in this study are (2day, 7 day and one month). (19)

Lacalut denture cleanser, release an active oxygen, and Corega denture cleanser, release an active CO₂, used in this study and prepared as manufacture instruction

The following statistical methods were used to analyse and assess the results via SPSS V. 11.5 for Windows:
1. Descriptive statistics include mean ± standard deviation values.
2. ANOVA and Duncan's multiple range test were used. The statistical results were considered significant at p ≤ 0.05.

RESULTS

Tensile Strength

The One Way Analysis of variance (ANOVA) as shown in Tables (1,2) demonstrated that there was significant difference at P≤ 0.05 in the tensile strength of highly impact acrylic resin in 2day and 7 day and no significant difference in 1 month. Figures (1-3) demonstrated the mean ± SD values and Duncan's multiple range test of tensile strength. In 2 day showed the highest value in (EDTA) and lowest value in (Distilled water) .in 7 day showed the highest value in (Corega) and the lowest value in (Lacalut).
Table (1): Solutions Preparation

<table>
<thead>
<tr>
<th>Solution no.</th>
<th>Material 1</th>
<th>Weight or volume</th>
<th>Material 2</th>
<th>Weight or volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EDTA</td>
<td>4 g</td>
<td>H2O2</td>
<td>25%</td>
</tr>
<tr>
<td>2</td>
<td>Soda</td>
<td>100 ml</td>
<td>Distilled Water</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Distilled Water</td>
<td>1 tab = 3.25 g</td>
<td>Corega</td>
<td>1 tab = 2.85</td>
</tr>
</tbody>
</table>

Table (2): ANOVA for Comparison of tensile strength among time intervals

<table>
<thead>
<tr>
<th>Time</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 day</td>
<td>Between Groups</td>
<td>0.001</td>
<td>7</td>
<td>0.000</td>
<td>8.013</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.001</td>
<td>32</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.002</td>
<td>39</td>
<td></td>
<td>3.434</td>
</tr>
<tr>
<td>7 day</td>
<td>Between Groups</td>
<td>0.001</td>
<td>7</td>
<td>0.000</td>
<td>3.434</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.002</td>
<td>32</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.003</td>
<td>39</td>
<td></td>
<td>1.187</td>
</tr>
<tr>
<td>1 month</td>
<td>Between Groups</td>
<td>0.001</td>
<td>7</td>
<td>0.000</td>
<td>1.187</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.003</td>
<td>32</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.004</td>
<td>39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means with the different letter are statistically significant different at p < 0.05

Figure (1): Mean ± SD and Duncan's multiple rang test of tensile strength for Comparison among time intervals. (2 Days)

Means with the different letter are statistically significant different at p < 0.05

Figure (2): Mean ± SD and Duncan's multiple rang test of tensile strength for Comparison among time intervals. (7 Days)
Means with the same letter are statistically no significant different at p < 0.05

Figure (3): Mean ± SD and Duncan's multiple range test of tensile strength for Comparison among time intervals. (1 Month)

The One Way Analysis of variance (ANOVA) as shown in Table (3) demonstrated the differences between the solution and showed that there was significant difference at P≤ 0.05 in the tensile strength of highly impact acrylic resin in (EDTA, Distilled water, Corega, Lacalut) and no significant difference in solution (Soda+H2O2). Tensile strength of highly impact acrylic resin, in comparison between solution, Figures (4-8) demonstrated the mean ± SD values and Duncan's multiple range test of tensile strength. In EDTA showed the highest value in 2 day and lowest value in 1 month. In (Soda+H2O2) showed no differences. In (Distilled water) the highest value in 7 day and lowest value in 1 month. In (Corega) showed the highest value in 7 day and lowest value in 1 month. In (Lacalut) showed the highest value in 2 day and lowest value in 1 month.

Table (3): ANOVA for Comparison of tensile strength among five solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Between Groups</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F–value</th>
<th>p–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>Between Groups</td>
<td>0.001</td>
<td>2</td>
<td>0.001</td>
<td>12.793</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.001</td>
<td>12</td>
<td>0.000</td>
<td>12.793</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.002</td>
<td>14</td>
<td>0.000</td>
<td>12.793</td>
<td>0.001*</td>
</tr>
<tr>
<td>Soda + H2O2</td>
<td>Between Groups</td>
<td>0.000</td>
<td>2</td>
<td>0.000</td>
<td>2.358</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.001</td>
<td>12</td>
<td>0.000</td>
<td>2.358</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.002</td>
<td>14</td>
<td>0.000</td>
<td>2.358</td>
<td>0.137</td>
</tr>
<tr>
<td>Corega</td>
<td>Between Groups</td>
<td>0.001</td>
<td>2</td>
<td>0.000</td>
<td>14.713</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.000</td>
<td>12</td>
<td>0.000</td>
<td>14.713</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.001</td>
<td>14</td>
<td>0.000</td>
<td>14.713</td>
<td>0.001*</td>
</tr>
<tr>
<td>Lacalute</td>
<td>Between Groups</td>
<td>0.001</td>
<td>2</td>
<td>0.000</td>
<td>8.397</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.000</td>
<td>12</td>
<td>0.000</td>
<td>8.397</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.001</td>
<td>14</td>
<td>0.000</td>
<td>8.397</td>
<td>0.005*</td>
</tr>
<tr>
<td>Water</td>
<td>Between Groups</td>
<td>0.001</td>
<td>2</td>
<td>0.000</td>
<td>8.895</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.000</td>
<td>12</td>
<td>0.000</td>
<td>8.895</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.001</td>
<td>14</td>
<td>0.000</td>
<td>8.895</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

SOV: Source of variance; SS: Sum of Squares; df: Degree of freedom; MS: Mean Square
* indicated significant difference at p < 0.05.
Means with the different letter are statistically significant different at p < 0.05

Figure (4): Mean ± SD and Duncan's multiple range test of tensile strength for EDTA

Means with the same letter are statistically no significant different at p < 0.05

Figure (5): Mean ± SD and Duncan's multiple range test of tensile strength for (Soda + H2O2)

Means with the different letter are statistically significant different at p < 0.05

Figure (6): Mean ± SD and Duncan's multiple range test of tensile strength for (Corega)
Means with the different letter are statistically significant different at p < 0.05

**Figure (7):** Mean ± SD and Duncan's multiple rang test of tensile strength for (Lacalute)

![Graph showing tensile strength for Lacalute](image)

Means with the different letter are statistically significant different at p < 0.05

**Figure (8):** Mean ± SD and Duncan's multiple rang test of tensile strength for (Water)

![Graph showing tensile strength for Water](image)

**Surface Roughness**

The One Way Analysis of variance (ANOVA) as shown in Table (4) demonstrated that there was significant difference at P≤ 0.05 in the surface roughness of highly impact acrylic resin among time intervals. Surface roughness of highly impact acrylic resin, in comparison between time intervals, (Figures 9-11) demonstrated the mean ± SD values and Duncan's multiple range test of surface roughness.

In 2 day showed the highest value in (Distilled water) and lowest value in (Lacalut). In 7 day showed the highest value in (Lacalut) and the lowest value in (Corega). In 1 month showed highest value in (Lacalut) and the lowest value in (Soad+H2O2).

**Table (4):** ANOVA for Comparison of surface roughness among time intervals

<table>
<thead>
<tr>
<th>Time</th>
<th>Source of Variance</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 day</td>
<td>Between Groups</td>
<td>5.057</td>
<td>7</td>
<td>0.722</td>
<td>7.444</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>3.105</td>
<td>32</td>
<td>0.097</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8.162</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 day</td>
<td>Between Groups</td>
<td>7.098</td>
<td>7</td>
<td>1.014</td>
<td>21.590</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>1.503</td>
<td>32</td>
<td>0.047</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8.600</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>Between Groups</td>
<td>14.257</td>
<td>7</td>
<td>2.037</td>
<td>27.542</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2.366</td>
<td>32</td>
<td>0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16.623</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SOV: Source of variance; SS: Sum of Squares; df: Degree of freedom; MS: Mean Square
* indicated significant difference at p < 0.05.
Means with the different letter are statistically significant different at p < 0.05

**Figure (9):** Mean ± SD and Duncan's multiple range test of surface roughness for Comparison among time intervals. (2 Days)

Means with the different letter are statistically significant different at p < 0.05

**Figure (10):** Mean ± SD and Duncan's multiple range test of surface roughness for Comparison among time intervals. (7 Days)

Means with the different letter are statistically significant different at p < 0.05

**Figure (11):** Mean ± SD and Duncan's multiple range test of surface roughness for Comparison among time intervals. (1 Month)
The One Way Analysis of variance (ANOVA) as shown in Table (5) demonstrated that there was a significant difference at P≤ 0.05 in the surface roughness of high impact acrylic in solutions (EDTA, distilled water, corega, lacalut) and no significant difference in (Soda+H2O2). Surface roughness of highly impact acrylic resin, in comparison between five solutions, figures (12-16) demonstrated the mean ± SD values and Duncan’s multiple range test of surface roughness. In (EDTA) showed the highest value in 7 day and lowest value in 2 day. In (Soda+H2O2) showed no differences. In (distilled water) the highest value in 1 month and lowest value in 2 day. In (Corega) showed the highest value in 2 day and lowest value in 1 month in (Lacalut) showed the highest value in 1 month and lowest value in 2 day.

### Table (5): ANOVA for Comparison of surface roughness among five solutions.

<table>
<thead>
<tr>
<th>Solution</th>
<th>SS (Between Groups)</th>
<th>df</th>
<th>MS</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>3.467</td>
<td>2</td>
<td>1.734</td>
<td>20.260</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>4.494</td>
<td>12</td>
<td>0.086</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.494</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soda + H2O2</td>
<td>1.445</td>
<td>2</td>
<td>0.722</td>
<td>3.186</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>2.720</td>
<td>12</td>
<td>0.227</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.165</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corega</td>
<td>0.571</td>
<td>2</td>
<td>0.285</td>
<td>14.189</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>0.241</td>
<td>12</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.812</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacalut</td>
<td>8.352</td>
<td>2</td>
<td>4.176</td>
<td>66.639</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>0.752</td>
<td>12</td>
<td>0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.104</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.392</td>
<td>2</td>
<td>0.196</td>
<td>5.751</td>
<td>0.018*</td>
</tr>
<tr>
<td></td>
<td>0.409</td>
<td>12</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.801</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SOV: Source of variance; SS: Sum of Squares; df: Degree of freedom; MS: Mean Square
* indicated significant difference at p < 0.05.
Means with the different letter are statistically significant different at p < 0.05.

Figure (13): Mean ± SD and Duncan's multiple range test of surface roughness for (Soda + H2O2)

Means with the different letter are statistically significant different at p < 0.05.

Figure (14): Mean ± SD and Duncan's multiple range test of surface roughness for (Corega)

Means with the different letter are statistically significant different at p < 0.05.

Figure (15): Mean ± SD and Duncan's multiple range test of surface roughness for (Lacalute)
**DISCUSSION**

*(Ethylene Diamen Tetra acetic Acid)*

*EDTA* show significant change in surface roughness and tensile strength in 2 days, 7 days and one month because it release CO$_2$ and form weak sodium bicarbonate that effect on the surface roughness of highly impact acrylic denture base material$^{[20]}$. *Soda+H$_2$O$_2$* show there was no significant change in surface roughness and tensile strength in 2 days, 7 days and one month because it release O$_2$ which the different reaction from other solution and it was not effect on the surface roughness of highly impact acrylic denture base material$^{[21]}$.

*Distilled water* show significant change in surface roughness and tensile strength in 2 days, 7 days and one month that agreement with Pavarina et al$^{[22]}$ stated that prolonged immersion of denture teeth in water caused softening of the acrylic resin. Absorbed water has been shown to affect the surface properties of all forms of acrylic$^{[23]}$ *(Lacalut denture cleanser)* show significant change in surface roughness and tensile strength in 2 days, 7 days and one month because it release CO$_2$ and O$_2$ and form weak sodium bicarbonate that effect on the surface roughness of highly impact acrylic denture base material this result disagreement with other studies Salman and Saleem$^{[24]}$ show that there was no significant difference between pre and post soaking for heat cured acrylic group. *(Corega)* show significant change in surface roughness and tensile strength in 2 days, 7 days and one month because it release CO$_2$ and form weak sodium bicarbonate that affect on the surface roughness of highly impact acrylic denture base material that disagreement with Ural$^{[25]}$ show the test specimens that immersed in water with Corega Tabs did not show a significant increase in surface roughness of the specimens. FDA is asking manufacturers of denture cleansers to include a warning in the label about persulfates, which are known to cause allergic reactions in some people. Persulfates are used in most denture cleansers as part of the cleaning and bleaching process. The agency is also recommending that manufacturers consider appropriate alternatives to persulfates. The use (EDTA and soda+H$_2$O$_2$) not cause allergic reactions because this solution not have Persulfates and it were more safe than other solution$^{[26]}$.

**CONCLUSION**

*Soda+H$_2$O$_2$* had no significant difference in surface roughness and tensile strength in (2day, 7day, and 1month). In regarding the type of solutions there was no significant difference in tensile strength after (1 month) immersion period. There was a significant difference among the solution surface roughness after difference immersion period (2day, 7 day and 1month).

**REFERENCES**


26. www.fda.gov/consumer