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Effect of Photodynamic Disinfection on milled acrylic flexural strength (an-in vitro study)

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Abstract

Background: Photo-dynamic disinfection is a new efficient and easy specific method for disinfection to get red-off colonized micro-organisms over the denture surface. Denture surface should be highly polished and with high mechanical properties to prevent further colonization of bacteria. Contaminated denture may cause many problems starting from oral stomatitis up to pneumonia. The aim of the current study is to investigate the effect of Photodynamic disinfection on flexural strength of milled versus heat cured acrylics after a period of time simulating 3 years of usage.
Materials & methods: Preparation of 50 rectangular bar samples (2mm × 2mm × 25mm) of milled acrylic , applying of a continuous 3 days,19 hours and 15 minutes of disinfection = 12 month of usage (15 minutes a day) ,7 days, 14 hours and 30 minutes = 24 months and finally at the end of the 11 days, 9 hours and 45 minutes of disinfection which simulate 3 years of daily disinfection with photodynamic method and compare it with the positive control (1% NaOCl disinfection) and negative control (distilled water) groups and preparing another 50 samples of heat cured acrylic with the same parameters and propose them to the same conditions and comparing them to milled samples then flexural strength is tested after each interval and compared to baseline specimens with no disinfection at all .
Results: The mean flexural strength values of all disinfectant groups at different intervals showed non-significant difference in flexural strength. But there was a significant difference between the time intervals for each disinfectant group.
Conclusion: within the limitation of this study, it was concluded that Photodynamic disinfection can be used safely to disinfect complete dentures. Regarding this size of samples heat cured acrylic resin showed higher flexural strength than the milled samples. Milled samples weren't affected neither by disinfectants nor by aging periods.

1. Introduction

Oral stomatitis is a very common oral manifestation in complete denture patients particularly in the developing world [1]. Photodynamic therapy is a new, easy and cheap specific promising method for denture disinfection [2]. A photo synthesizer solution is applied in a way to release oxygen radicals which can specifically kill microorganisms upon exposure to specific light wavelength; each photo synthesizer is stimulated by specific range of light wavelengths. [3], [4], [5]. Mechanical removal of colonized micro-organisms is still the most efficient way to remove this microfilm but in another easy way, light application may increase the frequency of denture disinfection [6]. It is of clinical importance to determine whether denture disinfection by photodynamic method alters the mechanical properties of acrylic resins as fracture resistance of an acrylic denture base. This common problem occurs during masticatory function because of base deformation and consequent resin fatigue **[7]**. Many studies showed the sensitivity of denture flexural strength to the method of denture disinfection [8]**.** Because hygiene procedures have been shown to alter the physical and mechanical properties of acrylic resins, so **the aim of this study** was to compare between the flexural strength of the pre-polymerized CAD/CAM milled acrylic resin and the conventional heat polymerized acrylic resin after being disinfected with photodynamic therapy (PDT) and Na-hypochlorite 1%.
Measure the flexural strength of each PMMA after each disinfection, during a period of time simulating 3 years of usage.

2. Material and method

This study is designed to assess the hypothesis that photodynamic disinfection will reduce flexural strength of both milled and heat cured acrylic.

2.1. Specimens preparation:

A. Construction of specimens of pre-polymerized acrylic resin (wiessen CE 0197-iso13485-Germany)

50 specimens were milled by CAD/CAM fabricated into (25 x 2 x 2 mms.) rectangular bars in accordance to the American National Standards Institute/American Dental Association specification # 27.
After milling of the specimens; they were de-sprued, finished and polished.

B. Construction of specimens of conventional heat cured acrylic resin (Acrostone-Egypt)

50 Wax blocks were milled by CAD/CAM with the same dimensions of milled acrylic bars. The wax models were de-sprued, flasked and then wax was eliminated. The mold cavity was packed with heat cured acrylic resin. The acrylic resin was packed in the upper half of the flask and pressed well. Enough material was used to insure over packing on the first closure and two pieces of wet cellophane papers were placed over the acrylic resin. The two compartments were assembled, and positioned in a hydraulic press where it was subjected to gradual pressure. The flask was removed from the press and opened carefully and the excess acrylic was trimmed of with a sharp knife. Final closure was done after painting the mold with separating medium, and then the flasks were closed under the press until metal to metal contact is reached. The pressure was maintained for one hour to allow for bench setting.
The flasks were then removed and flask frames were tightened to maintain pressure and the assemblies were introduced into an automatic curing unit.

C. Curing of the acrylic resin model

Long curing cycles were used for polymerization at 75 °C for 9 hours. After complete curing, the flasks were left to cool slowly into the water bath then left outside for bench cooling to room temperature. Then models were de-flasked, inspected, finished and polished**.**

2.2. Randomized controlled trial formation

The 100 specimens were divided into 50 CAD/CAM milled and 50 heat cured then randomizer mobile application was used for random selection of each group (n=15) specimens where the control groups aged in distilled water, 1 % NaOCl disinfection groups and PDT disinfection groups. And (n= 5) samples were selected by the same application. For baseline groups of both heat cured and milled acrylic, despite aging protocol require a base line group for each disinfectant but no need for repeated measuring as the same material is in the same conditions for sure will give the same results so we repeated the same results of baseline groups in all disinfectant. So ultimately (n=20) for each disinfectant.

A. Negative Control group:

In which each 15 specimens milled & 15 heat cured PMMA were immersed in distilled water.

B. Positive control group

Each 15 Specimens were immersed in 1% Na-hypochlorite. Which prepared by diluting commercial Clorox containing 5 % Na-hypochlorite.
In this protocol of disinfection; the specimens were immersed in 1% NaOCl for 15 days continuously, which is considered a simulation of 3 years with daily immersion for 20 minutes.
Flexural strength tests were performed at 12 months, 24months and 36months simulations during this regime of disinfection.
1 hour = 3 immersions of 20 minutes
No. of immersions /day = 3 × 24 = 72
Therefore, 1day of full immersion = 72 days of usage.
3 years (1095 days) of disinfection = 1095÷ 72 =15 days of full immersion based on recommended duration of disinfection (20 minutes daily).
As flexural strength test was supposed to be done before the use of any disinfectant so it was the baseline, immersed and tested after 5 days of immersion = (12 month of use) ,10 days = 24 months and finally at the end of the 15th day of immersion which will simulate 3 years of daily disinfection .

C. Test groups of Photodynamic disinfection

I. **Preparation of photosynthetizer:** A stock solution (1.5 g/L) of curcumin was prepared in dimethylsulfoxide (DMSO) (0.1 %) and then diluted in autoclaved distilled water (980 mL) to obtain the concentration used (30 mg/L). The curcumin salt used had 1 g of salt containing 0.654 g of the curcumin plus curcuminoid, but in our study, natural curcumin (curcumin 53.4 % and curcuminoid 46.16 %) was used. In which specimens were immersed for 5 minutes before each light application.

II. **Instrumentation to perform PDD:** Each specimen received~ 200 J/cm^2 to be disinfected. A device based on blue light-emitting diode (LED) with wavelength of (455±30 nm) was custom made using 300-mWatt average optical power. The light was applied for 10 min, which led to an energy density (radiation dose/ fluency) delivered of ~200 J/cm^2. The time was calculated from following equation: fluency= PD×T, where P is peak density of light beam which calculated by dividing power of light measured with m Watt by the distance from the UV emitter. The LED UV light beam is radiating divergently unlike the laser parallel beam so we considered a total energy per unit of area reaching the surface as the delivered dose, but this was not necessarily uniformly absorbed. The dose delivery was approximated, because different areas and several distances were irradiated. Although we applied the blue LED for 10 min for each application, now each single disinfection took 5 minutes immersion in curcumin solution and 10, min light application, 15 min total time so for accumulative duration to simulate 12 months, 24months and 36months intervals at which flexural strength tests will be performed: 1hour = 4 disinfections of (20 minutes immersion in curcumin solution and 40 minutes light application) .No. of disinfections /day = 4 × 24 = 96

Therefore, 1day of full immersion = 96 days of usage.
3 years (1095 days) of disinfection = 1095÷ 96 =11 days, 9 hours and 45 minutes of full immersion based on recommended duration of disinfection (15 minutes daily).
Tested after 3 days,19 hours and 15 minutes of disinfection = (12 month of use) ,7 days, 14 hours and 30 minutes = 24 months and finally at the end of the 11 days, 9 hours and 45 minutes of disinfection which will simulate 3 years of daily disinfection.

2.3. Flexural strength test

The flexural strength was measured using a three -points bending test in a Universal Testing Machine with 50 kgf load cell at crosshead speed of 1mm/min. The flexural strength (S) of each rectangular specimen was calculated using the Equation:

$$\mathbf{S} = \frac{\mathbf{3PL}}{\mathbf{2bd^2}}$$

Where P is the maximum load, L is the distance between the supports, b is the specimen width, and d is the specimen thickness. Mean flexural strengths were calculated in MPa.

2.4. Statistical analysis

Data Explored for normality using Kolmogorov–Smirnov test. Three -way ANOVA was used to compare between tested groups, Disinfection and aging period for flexural strength followed by multiple comparison with Tukey HSD. Significant level was set at 0.05 (α=0.05). Statistical analysis was done using Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

3. Results

It was found that there was a significant difference between the heat cured acrylic samples flexural strength mean values compared to milled acrylic samples flexural strength in each time variable and in each type of disinfection. For negative control group (distilled water) mean value of base line heat cured samples 275.3 ± 46.7 MPa, at T_1 220.8 ± 44.5 MPa, at T_2 200.5 ± 34.2 MPa and at T_3 178.7 ± 31.3 MPa. For negative control group mean value of base line milled samples 166.4 ± 32.2 MPa, at $T_1$163.6 ± 8.3 MPa, at T_2 153.9 ± 18.0 MPa and at T_3 149.3 ± 23.7 MPa . The difference between the groups was statistically significance as revealed by three way ANOVA test ($P < 0.05$) except for T_3 of negative control group of heat cured compared to milled acrylic. All heat cured samples showed higher flexural strength compared to milled ones but for T_3 this was insignificant.
For positive control group (1% NaOCl) mean value of base line heat cured samples 275.3 ± 46.7 MPa at T_1 236.8 ± 34.2 MPa, at T_2 279.8 ± 26.4 MPa and at T_3 181.9 ± 21.4 MPa. For positive control group mean value of base line milled samples 166.4 ± 32.2 MPa, at T_1 162.2 ± 16.5 MPa, at T_2 165.3± 18.5MPa and at T_3 157.4± 19.3MPa. The difference between these groups was statistically significant except for T_3 of positive control group of heat cured compared to milled acrylic.
For the test group of photo dynamic disinfection mean value of base line heat cured samples 275.3 ± 46.7 MPa, at T_1 213.5± 32.3MPa, at T_2 221.3± 25.9 MPa and at T_3 220.5± 48.8MPa. For the test group of photo dynamic disinfection mean value of base line milled samples 166.4 ± 32.2 MPa, at T_1 165.9± 15.3MPa, at T_2 161.3± 29.2MPa and at T_3 172.2± 9.2MPa. The difference between these groups was statistically significant. All heat cured samples here showed higher flexural strength than milled samples **(Table 1)**.
So all heat cure acrylic samples in all disinfectants showed higher flexural strength than milled acrylic but T_3 groups in both negative and positive control groups the difference had insignificant values

4. Discussion

The flexural strength of a material is defined as the maximum bending stress that can be applied to that material before it fractures. Meanwhile the denture is always subjected to this bending force during biting and mastication. It is of a clinical importance to investigate this force. Conventionally heat cured acrylic resin have been used as the main material of denture fabrication. But the digital revolution of dental industry using CAD/CAM proposed the pre polymerized acrylic resin blocks as a new promising material. Pre-polymerized blocks of acrylic resin that cure under extremely heavy pressure over and over again to get red off all insulting residual monomer particles and its sequelae. So it was anticipated that the milled samples would show higher flexural strength than the heat cured ones, but unlikely the heat cured samples were much stronger than the milled. This reduction of mean flexural strength values of milled acrylic than the heat cured samples as shown in **(Table 1)**. where P-value <0.001 for all disinfectant groups in milled compared to heat cured PMMA may be contributed to surface damage caused by CAD/CAM.CAD/CAM machining is crushing and crumbling together with plastic deformation in a microscopic scale of material surface causing formation of micro-cracks. These data agreed with the published literature by Sindel J et al., 1998 [9], this in turn will reduce flexural strength. In general, non-significant difference between the mean values of flexural strength of each disinfection groups in comparison to each other in both types of PMMA. This result is in agreement with the study of Pisani M et al., 2010 [10] who found no differences in the flexural strength of conventional hot water polymerized acrylic resin and microwave-polymerized acrylic resin after immersion in distilled water and 1% Sodium hypochlorite solution for 15 days. But aging the heat cured samples in 1% NaOCl and distilled water increased flexural strength of PMMA where P-value <0.001. This result is in agreement with the study of Galav A et al., 2010 [11] who found that PMMA specimens showed an increase in the fracture

Table 1: Mean and standard deviation (SD) for flexural strength.

		Heat		Milled		p-value
Baseline		**Mean**	**SD**	**Mean**	**SD**	
		275.3	46.7	166.4	32.2	<0.001*
Water	12 Months	220.8	44.5	163.6	8.3	0.005*
	24 Months	200.5	34.2	153.9	18.0	0.02*
	3 Years	178.7	31.3	149.3	23.7	0.140 NS
NaOCl	12 Months	236.8	34.2	162.2	16.5	<0.001*
	24 Months	279.8	26.4	165.3	18.5	<0.001*
	3 Years	181.9	21.4	157.4	19.3	0.218 NS
PDT	12 Months	213.5	32.3	165.9	15.3	0.018*
	24 Months	221.3	25.9	161.3	29.2	0.003*
	3 Years	220.5	48.8	172.2	9.2	0.016*

Different lowercase letter within each column indicates significant difference at p<0.05
Different uppercase letter within each row indicates significant difference at p<0.05
*=significant, NS=Non-significant

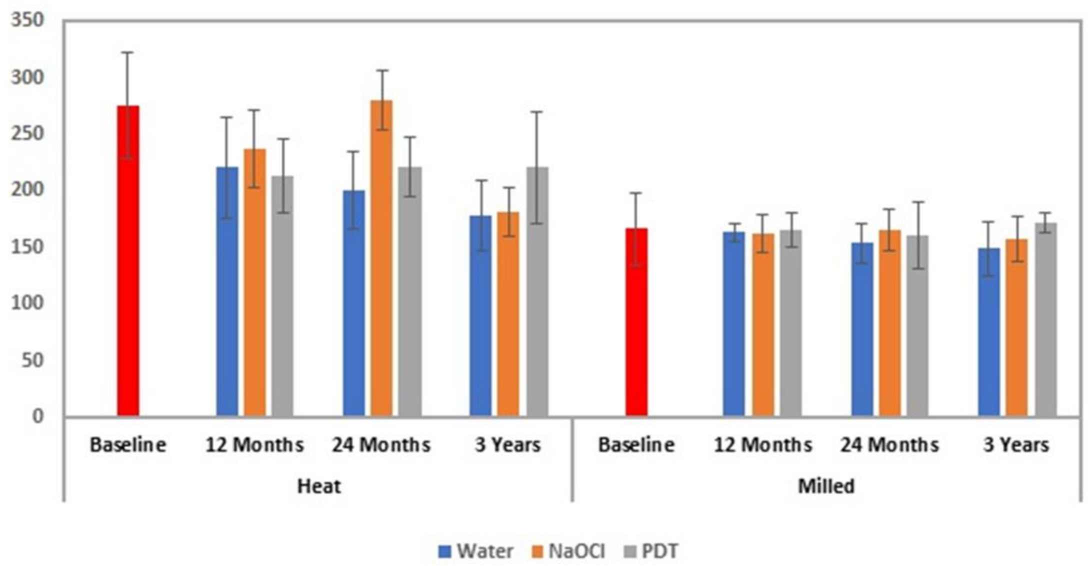

Figure 1: Bar chart for mean flexural strength.

strength after 21 days of water storage interval when compared with 7 and 14 days of interval which may be contributed to more water sorption and more increase in sample size which directly affect the flexural strength. The water uptake was probably restricted to more superficial layers, as the water storage time 7 days was comparatively short. At the initial stages, the effect of water in monomer hydrolysis is low or nonexistent as proved by Thompson G et al., 2014 [12]. A longer water storage time might have resulted in a greater difference because of the influence of water in more central parts of the material that weakens PMMA due to hydrolysis of the monomers, giving rise to a constant decrease in its mechanical properties to reducing the flexural strength by aging time till it reaches 3 years of usage. This results are compatible with the study of Abdulmohsen B et al., 2016 [13].That not present neither in PD disinfection nor in milled samples due to dry samples in case of PD disinfection as UV waves act to dry the specimens so no chance for monomer hydrolysis which agree with the results of the study of Örtengren U et al., 2000 [14]. Less monomer content and less water sorption of high-pressure polymerization of CAD/CAM milled acrylic reduces the monomer to least amounts so minimal hydrolysis occurs. These results agree with the study of. Al-Qarni et al., 2019 [15]. Who found that milled acrylic had much less water sorption in comparison to the heat cured acrylic. This can explain the results of the milled acrylic specimens, as the Flexural strength nearly sustained without change throughout the whole disinfection periods and techniques.

5. Conclusions

Within the limitation of this study, it was concluded that:

1. Photodynamic disinfection can be used safely to disinfect complete dentures and the hypothesis was rejected.

2. Regarding this size of samples heat cured acrylic resin showed higher flexural strength than the milled samples

3. Milled samples weren’t affected neither by disinfectants nor by aging periods.

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