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Effect of adding natural coconut oil on the antifungal, flexural strength, and hardness properties of heat cure acrylic resin (an *in vitro* study)

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ABSTRACT

Purpose. This study's objective was to estimate the effect of various quantities of the addition of natural coconut oil according to concentrations (1%, 2%, 3%) on the heat cured denture base material's anti-fungal, flexural strength, and hardness properties.

Material and method. A total of 120 samples were created and (divided into three groups each groups contain forty samples) built regarding the attitude test: *Candida albicans* adherence test, flexural strength test, and hardness test. Next, each group was subdivided into three subgroups (control 0%, 1%, 2% and 3%) based on the various quantities of the added natural coconut oil, with (n=10 samples for each subgroup). Each group was assessed at 48 hours. One-way ANOVA and other statistical methods were used to analyze the data, Duncan and Dunnett's tests for comparison means among all groups with control at $p \leq 0.05$.

Results. A One-way ANOVA showed a significant difference in the mean values of *Candida albicans* adherence, with the additive of natural coconut oil groups (1%, 2%) showing the highest antifungal effect compare with (3% additive groups). Adding 1% coconut oil had no significant difference on hardness and flexural strength, while adding 2% and 3% additive of coconut oil groups showed a significantly decrease than control groups at $p \leq (0.05)$.

Conclusion. The antifungal properties of heat-cured acrylic resin are enhanced by the addition of coconut oil. However, when used in high concentrations (greater than 1%), it can result in a decrease in hardness and flexural strength.

Keywords: heat cured acrylic resin, natural oil, fungal, physical properties

INTRODUCTION

Heat cured denture-base resins widely used for fabrication of denture bases, have a characteristic of satisfactory working properties, inexpensive equipment, simple processing technique, and ability to be repaired, but there are some disadvantages such as the accumulation of plaque [1].

Bad oral hygiene of complete denture wearers may be due to factors such as advanced age patients, handicapped patients, difficulties in drug application, and inability to preserve treatment. These factors allow the microbial cells to attach themselves on a surface and enhance denture plaque development, which conversely leads to denture stomatitis in the oral tissue close to the surface [2].

The fitting surface of the heat cured denture base is the major reservoir of *Candida albicans.* To prevent the accumulation of fungi on the inner surfaces of dentures, several methods have been used, including incorporating of antifungal substances inside the denture resin material [3,4] .

Natural coconut oil, an essential cooked oil, has been used in food production for several years and has other medical uses; in dentistry, coconut oil has been used for therapeutic purposes as a mouthwash and topical application to reduce the growth of fungi [5,6].

The extracted oils have efficient antifungal properties. In this research, natural coconut oil was incorporated with polymethylmethacrylate to im-

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prove the antifungal efficiency contra *Candida albicans* [7] .

Flexural strength and hardness are essential because denture bases are subjected to fracture after clinical use [8,9] .

This study's objective was to estimate the influence of various quantities of the addition of natural coconut oil according to concentrations (1%, 2%, 3%) on the heat cured denture base material's anti-fungal, flexural strength, and hardness properties.

The addition of various quantities of natural coconut oil (1%, 2%, and 3%), according to the null hypothesis, would have no influence on the antifungal, flexural strength, and hardness properties of heat-cured denture base material.

MATERIALS AND METHODS

The study conducted by the University of Mosul, College of Dentistry (REC reference No. UoM. Dent/ DM.L. (42/21 in 20/4/2021)) has been approved by the research Ethics Committee board.

Three groups of one hundred twenty samples were prepared, each of which groups contained 40 samples built on the attitude test: *Candida albicans* adherences test, flexural strength test, and hardness test. Next, each group was subdivided into three subgroups (control 0%, 1%, 2% and 3%) according to the amount of natural coconut oil that has been added, with ten samples each for every subgroup. As shown in Table 1, each group was evaluated after (48 h) in distilled water. The control group was mixed according to the manufacturer's instructions. Natural coconut oil was added to the liquid part of the heat cured resin at different concentrations (1%, 2%, 3%). The volume of natural coconut oil was measured via a micropipette and subtracted from the liquid volume of resin to obtain an accurate P/L ratio, 3:1 by volume, the monomer was blended with additives using a spatula until an identical mixture was produced. The powder was then added to the mixture and blended carefully. Following the manufacturer's instructions, the samples were packed directly into the designer mold and cured in a water bath for (30 min at 70°C and then for 30 min at 100°C). The dimensions of each sample were determined using a digital caliper (LEZACO, ART. 2771, China). All samples were detected by Fourier transform infrared spectroscopy before any test was performed. Each group was assessed at (48 hours in distilled water) for *Candida albicans* adherence test, flexural strength, and hardness test groups. The sampling distribution was randomly assigned by a blinded laboratory assistant.

Preparation of the additive material (natural coconut oil)

According to a chilling and thawing method, crushed coconut meat (500 g) was mingled with warm water (1:1) for a whole day, then mixed in an electric mixer for 5 min and filtered to extract coconut milk. The filtered milk was placed in the refrigerator for the whole day until the coconut cream was produced. The coconut cream was filtered for impurities by slow heating coconut cream for 30 min and released the oil was separated from obstinate residue by filtering through muslin cloth and static residue was more heated to separate extra oil, the extracted oil was kept in a dark room at (5°C) [10,11].

Fourier transform infrared spectroscopey

The chemical structure of the heat cured denture base was checked after the addition of coconut oil (1%, 2%, and 3%) by Alfa Burke instrument. The measurements were taken at the (University of Mosul, College of Dentistry).

Candida albicans adherence test

Dimensions of the samples (10mm × 10mm × 2mm) [12] of the tested material was fabricated. The samples were stored at 37ºC in distilled water. All samples were disinfected by autoclave (15 pounds/ inch2 /121°C) for fifteen minutes. Then, the samples were placed in tube contain suspension of *Candida*

TABLE 1. The sample groups

Test groups	Number of sample	Subgroups	Number of samples (n)
Candida albicans adherence test	N= 40	1. Control (0%) 2. Additive of coconut oil with (1%) 3. Additive of coconut oil with (2%) 4. Additive of coconut oil with (3%)	n= 10 n= 10 n= 10 n= 10
Flexural strength test	N= 40	1. Control (0%) 2. Additive of coconut oil with (1%) 3. Additive of coconut oil with (2%) 4. Additive of coconut oil with (3%)	n= 10 n= 10 n= 10 n= 10
Hardness test	N= 40	1. Control (0%) 2. Additive of coconut oil with (1%) 3. Additive of coconut oil with (2%) 4. Additive of coconut oil with (3%)	n= 10 n= 10 n= 10 n= 10

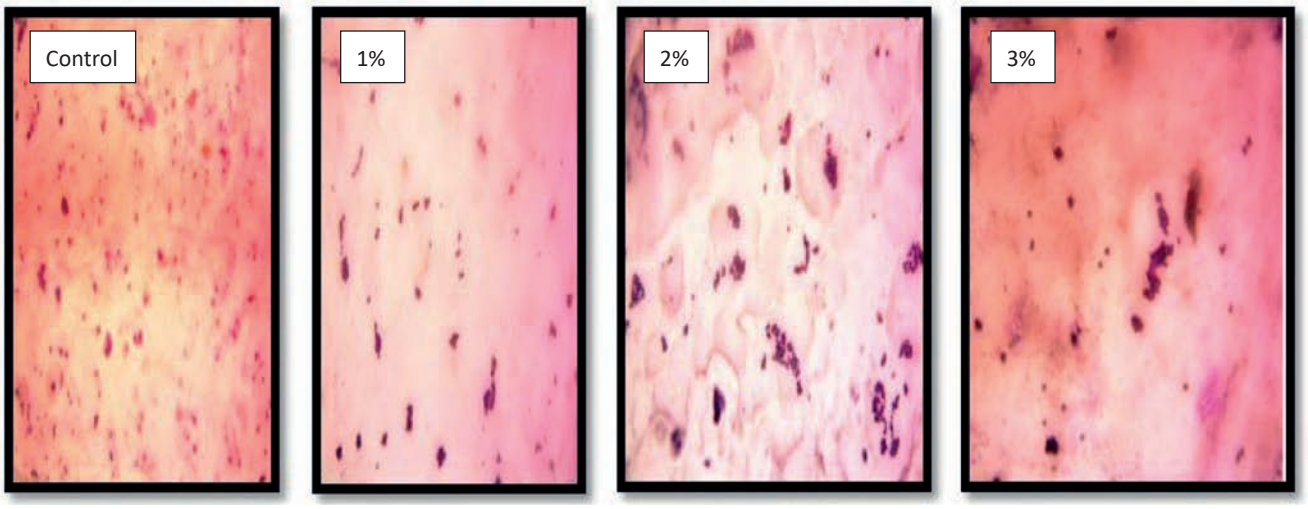

FIGURE 1. Show adherence cell of candida albican under a light microscope (control and 3%, 1% additive groups)

albicans (0.5) McFarland standards and incubated for 1 hr at 37°C. After that, the samples were cleaned for one minute using phosphate-buffered saline, and then, dehydration of the samples was done. These adherent cells were fixed using 80% methanol for 30 s and, then stained with violet crystal for (1 minute). Next, the number of adherence cells of *Candida albicans* attached to the tested material was calculated using a computerized microscope (Karl Olb, Germany) used for examination with a magnification of 40, Candida albicans looked like round or egg-shaped violet cells, as shown in Figure 1.

Flexural Strength Test

Samples were prepared at 64 × 10 × 3 in length, width, and thickness ±0.03 mm, respectively (ADA No.12, 2002 specification) [13]. Three-points bending test was carried out utilizing an Instron universal testing apparatus (Model; AEL.1000–400, China). The device had two components: a loading plunger in the center and a support roof that was divided into sections to mimic a denture, moderate intermolar space. The load plunger was fixed halfway between two corresponding supports, the supports were firmly corresponding to one another and upright to the central line, and the sample was held at each end of the two supports. The transverse test was carried out at a steady 5 mm/min crosshead speed, and the samples were redirected until they fractured. The formulation for calculating the flexural strength was (S = 3PL/2bd2, where *P* is the peak load applied, *L* is the span length, *b* is the width, and *d* is the thickness).

Hardness Test

Hardness was tested by a type D Durometer (Shore D, Shaw, China) hardness tester, equipped with a round steel ball with a 1.25mm in diameter indenter. The samples were retained on the solid apparatus and subjected to a load of 44.5N by the needle that was kept at a distance of 12 mm from the specimen. The hardness value was determined visually by reading the analog after (1) second of load application. The dimensions of the samples were 30 × 15 × 3 in length, width, and thickness ±0.03 mm, and five measurements were recorded for each sample.

Statistical analysis

Used to analyze the data (Statistical Package for Social Sciences version 22), using ANOVA, and Duncan and Dunnett's tests for comparison means among all groups with control, at $p \leq 0.05$.

RESULTS

All samples were detected by Fourier transform infrared spectroscopy before any test was established. The graphs show that there was no chemical change in the chemical structure, but there were physical changes only, as shown in Figure 2, which means that the bond between resin and natural coconut oil was physical rather than chemical [14-16].

Evaluating *Candida albicans* adherence: The counts of adherence cells of the three additive groups (1%, 2%, 3%) showed a lowering in mean values when compared with the control groups. Additive groups (1%) had the lowest mean value of the count of adherence cells of *Candida albicans* (652.2 cells), while the mean value of 2% and 3% additive groups coconut oil, 680.9, 750.6 cells mean value respectively, when compared with control (999.9 cells) as shown in (Figure 3 A). *Candida albicans* adherence cells were decreased significantly after the addition of coconut oil (1%, 2%, 3%) as compared with the control at $p \leq 0.05$, as shown in Table 2.

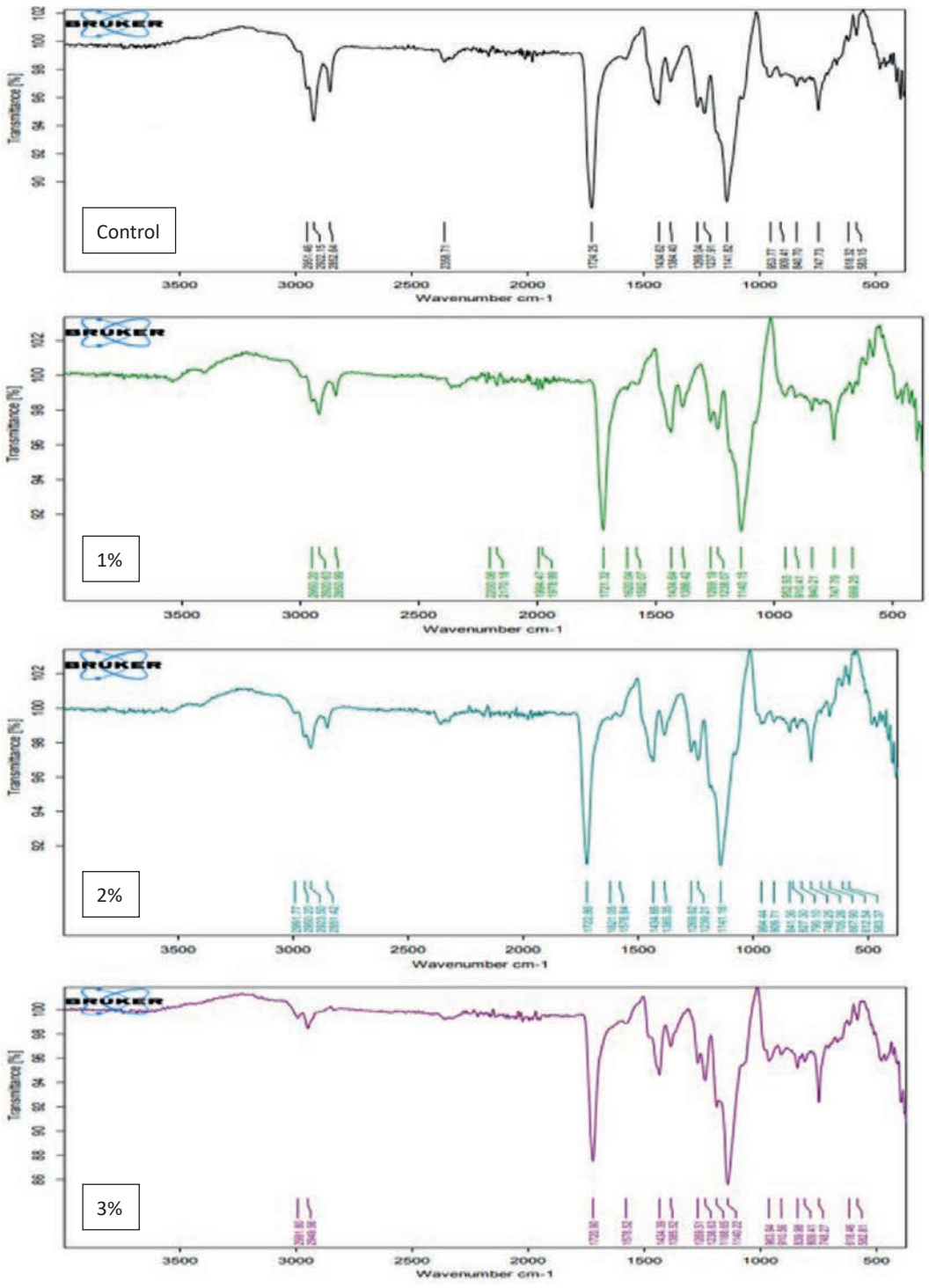

FIGURE 2. FTAR shows there is no chemical change in the properties of the substance between nature coconut oil and heat cure resin in different concentrations of oil

TABLE 2. Mean, and standard deviation of the *Candida albicans* adherence of control and three additive groups (1%, 2%, 3%)

Subgroups	Mean	±SD	P-value
Control without additive	999.86[a]	±.051	
1% additive natural coconut oil	652.20[b]	±.00	0.00*
2% additive natural coconut oil	680.90[c]	±.00	0.00*
3% additive natural coconut oil	750.60[d]	±.00	0.00*

Different in letters indicate statistical differences within colum, and the same letters lowercase indicate non statistical differences.
±SD: standard deviation.*: significant differences at a level of 0.05

Flexural strength and hardness test

The mean flexural strength and hardness of control and three additive groups (1%, 2%, 3%) are shown in (figure 3 B, C).

Additive groups with 1% coconut oil specified a non-significant difference compared with control groups at p ≤0.05, while additive groups with (2% and 3% of coconut-oil) showed significant difference than control groups, as shown in Table 3 and Table 4.

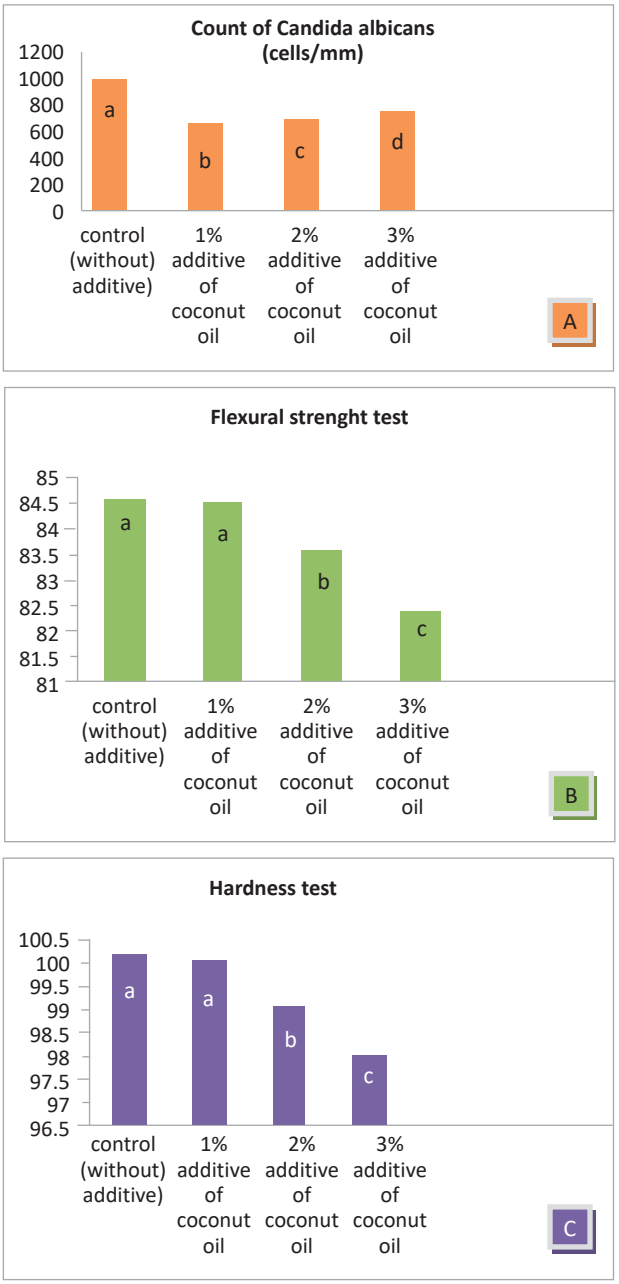

FIGURE 3. Mean value of three additive groups (1%, 2%, and 3%) and control group for: A. adherence cell of *Candida albicans* test; B. flexural strength test; C. hardness test

DISCUSSION

The ongoing research hypothesis was not accepted, because natural coconut oil when, added to heat acrylic resin in different concentrations (1%, 2%, 3%), displayed a significant decrease in the *Candida albican* adherences. A statistically non-significant difference, especially in small amount addition, between the flexural strength and hardness with the (1%) addition group and the control.

This study revealed that the incorporation of natural coconut oil (1%, 2%,3%) into the heat resin, resulted in lowering in the number of adhered cells of *Candida albicans* in three additional groups compared with the control groups. Coconut oil as a bioactive component. Lauric acid, present in a great percentage of natural coconut oil, has strong antioxidant and antimicrobial properties, which are important for the alteration of the immune cell of *Candida albicans*, it has a fungicidal effect, particularly against *Candida albicans*. This result agreed with Pragati et al. who said: "specimens containing coconut oil interfered with *Candida albicans* growth" [17].

TABLE 3. Mean, and standard deviation of the flexural strength of control and three additive groups (1%, 2%, 3%)

Subgroups	Mean	±SD	P-value
Control without additive	999.86[a]	±.051	
1% additive natural coconut oil	652.20[b]	±.00	0.00*
2% additive natural coconut oil	680.90[c]	±.00	0.00*
3% additive natural coconut oil	750.60[d]	±.00	0.00*

Different in letters indicate statistical differences within column, and the same letters lowercase indicate non statistical differences.
±SD: standard deviation,*: significant differences at a level of 0.05

TABLE 4. Mean, and standard deviation of the hardness of control and three additive groups (1%, 2%, 3%)

Subgroups	Mean	±SD	P-value
Control without additive	100.18[a]	±0.006	
1% additive natural coconut oil	100.16[a]	±.0.006	0.30
2% additive natural coconut oil	99.10[b]	±.00	0.00*
3% additive natural coconut oil	97.74[c]	±.00	0.00*

Different in letters indicate statistical differences within column, and the same letters lowercase indicate non statistical differences.
±SD: standard deviation.* : significant differences at a level of 0.05

The mean values of the counts of adherent cells in group 1% show the lowest value when compared with the control group, this result can be due to the antimicrobial activity of coconut natural oil, which has good inhibition for *Candida albicans* adhesion cells, especially in a little amount of addition [18-21]. Zinah M and Ghassan N, 2020 [22] said: "Incorporation of a small amount of natural coconut oil (1.5%) has improved the antifungal competence compared to control and experimental groups". This result was in agreement with this study.

This research was installed to contrast the flexural strength and hardness of (additive groups and control) in three different concentrations (1%, 2%, and 3%). The results showed that the addition of oil with a small amount (1%) showed a non-significant difference from the addition with a high amount (2%,3%) when compared with the control, lowering in mean values that was gained by the (2% and 3%) addition groups, could be explained to reason that coconut (oil) migrated to the superficial strata that differentiate an oily layer that has an effected the bonding of material or another reason that coconut oil had a role in elastomeric properties to heat cure acrylic [23,24] Bushra and Ghassan, 2020 [25] said:

"The incorporation (1% coconut oil) was the extreme salutary effects versus candida, with small effect on the shear strength and hardness" this outcome was in agreement with our study.

The outcomes of Al-Nema et al. and Hatim et al. [26,27], and Ban [28], disagreed with this research. These scientists concluded that there was a significant increase in transverse strength and decrease in hardness following the addition of natural oil to the acrylic resin. However, these results disagreed with this study, due to different natural antifungal oils used.

The limitations of this study are that, the data from this in vitro study are different from those obtained from intraoral dental prostheses, which are influenced by factors such as saliva, artificial teeth, and oral mucosa's resilience. Therefore, more studies are required to improve the clinical significance of this research in an *in vivo* study that can be applied under simulated oral conditions.

CONCLUSION

The present study helps to choose the best concentration of the addition of natural coconut oil with heat cure resin that has improved antifungal activity against *Candida albicans*, and it appeared that addition (1% of organic coconut oil) produced the most beneficial results versus *Candida albicans*, with no effect on the (flexural strength and hardness) properties of heat cured resin material.

The antifungal properties of heat-cured acrylic resin are enhanced by the adddition of coconut oil. However, when used in high concentrations (greater than 1%), it can result in a decrease in hardness and flexural strength.

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Conflicts of interest: none

Financial support: none

Ethical policy and institutional review board statement:
The study was approved by the Research Ethics Committee board (the University of Mosul, College of Dentistry, REC reference No UoM. Dent/ DM.L. 42/21 in 20/4/2021), and there are no biohazardous and toxic materials/substances.
This study was in accordance with institution guidelines

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