Al-Azhar Journal of Dentistry

Volume 6 | Issue 3 Article 15

Restorative Dentistry Issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

7-1-2019

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How to Cite This Article

ebida, marwa; Kholief, Dina; and Kabeel, Shereen (2019) "Effect of Reinforcement of Denture Base Resin with Zirconia on Bacterial Colonization and Some Mechanical Properties," *Al-Azhar Journal of Dentistry*: Vol. 6: Iss. 3, Article 15.
DOI: https://doi.org/10.21608/adjg.2019.5668.1017

AL-AZHAR
Dental Journal
For Girls

The Official Publication of The Faculty of Dental Medicine For Girls, Al-Azhar University Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 6, No. 3, July (2019) — PP. 377:384

Effect of Reinforcement of Denture Base Resin with Zirconia on Bacterial Colonization and Some Mechanical Properties

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http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2019.5668.1017

ABSTRACT

Purpose: The aim of the study was to compare between zirconia reinforced acrylic resins with that conventional acrylic resins (PMMA) regarding bacterial colonization and some mechanical properties (flexural strength, surface roughness, and surface hardness). **Material and methods:** Clinical study: sixteen completely edentulous patients were randomly selected which their ages ranged from 50-60 years. Patients were divided into two groups. Patients in the first group received maxillary and mandibular complete denture made of PMMA (Group I) and patients in the second group received maxillary and mandibular complete denture made of ZrO_2 reinforced acrylic resins (Group II). Microbial biofilm was evaluated after three, six and nine months for each patient. Laboratory study: sixty specimens were constructed. The specimens were divided into two groups. Thirty specimens of PMMA (Group I) and thirty specimens of ZrO_2 reinforced PMMA (Group II) for testing FS, surface hardness and surface roughness **Results:** The results of microbial biofilm evaluation in group II was significantly higher than that found in group I. The laboratory study results showed that FS and surface roughness in ZrO_2 reinforced PMMA were significantly higher than conventional PMMA. Further, the study showed that an insignificant increase of surface hardness in ZrO_2 reinforced PMMA when compared with conventional PMMA. **Conclusion:** Reinforcement of acrylic resin with ZrO_2 in PMMA results in an increase of the microbial colonization and surface roughness of denture and improvement of some mechanical properties as flexural strength and surface hardness.

KEYWORDS

Zirconia, Microbial evaluation,
Flexural strength,
Surface hardness,
Surface roughness

INTRODUCTION

Complete dentures are dental prosthesis that replaces the entire complete loss of natural teeth and its associated structures of the mandible and the maxilla. The conventional complete denture is still

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the treatment of choice for medical and financial reasons, although the increase usage of dental implants in the treatment of edentulous patients [1].

The most widely used material for construction of denture base is heat cured polymethyl methacrylate (PMMA). It has several advantages such as it is non-toxic, non-irritant, not soluble in oral fluids, good aesthetical aspect, easy manipulation, and easy repair but its drawback are the weakness in its resistance, its high permeability, and presence of residual monomer [2].

ZrO_2 is a hard white amorphous powder that is obtained from zirconium which is found naturally. It is produced by thermal processes.ZrO_2 is a material that very resistance to crack propagation and It has premium mechanical properties with high strength , fracture toughness and high thermal expansion, so it is often the best material for joining ceramic and steel [3].

There are several approaches to improve the properties of acrylic resins. One of that methods is the addition of zirconia as a filler to PMMA as it is biocompatible, has high mechanical properties and has additional advantage of zirconia when is used as filler is superior esthetics. So, the ZrO_2 powder has been chosen for improvement of properties of PMMA [4-6].

MATERIAL AND METHODS

The clinical study made on sixteen completely edentulous patients whose maxillary ridges covered with firm and healthy mucosa free from any signs of inflammation, ulceration, and flappy tissues. Patients with xerostomia or excessive salivation and Heavy smokers were excluded. All patients accepted this dental treatment and informed about the steps of this study and signed a written consent with the Research Ethics Committee (REC) approval. Dividing of sixteen patients into two groups eight patient for each one:

Group I: Each patient received maxillary and mandibular complete denture made of conventional heat cured acrylic resin. **Group II:** Each patient received maxillary and mandibular complete denture made of ZrO_2 reinforced acrylic resin.

For each patient, Primary alginate impression for maxillary and mandibular arch were made using stock trays of suitable size then, a secondary impression was taken using zinc oxide and eugenol impression material, occlusion blocks were fabricated and centric relation in patient's mouth was recorded at the correct vertical dimension. Mounting of The maxillary and mandibular casts on a semi-adjustable articulator (Hanau articulator) and setting up of artificial teeth using cross-linked acrylic artificial teeth and waxing up was carried. The denture was tried in the patient mouth to check denture extension, retention, stability, tongue space, vertical dimension, and centric occluding relation. Flasking of the waxed up denture was done.

For Group I: processing of waxed-up upper and lower dentures into conventional heat cure acrylic resin by mixing powder of PMMA with monomer in a ratio of 2.7:1 then packing into mold space in the dough stage and for Group II: Waxed-up upper and lower dentures were processed into ZrO_2 reinforced acrylic resin which is formed by adding 10 wt% ZrO_2 powder with an average particle size of 5-10 µm to PMMA. Mixing and blending were done to obtain a consistent and uniform mix by treating filler particles with 1 wt% of saline coupling agent before the mix and then mixing powder with the monomer in a ratio of 2.7:1 and packed in the dough stage into mold space. For both groups, trying the closes of flasks was done and flasks were clamped followed by Bench curing that done for 20 min and resin were processed in a 74°C water bath for eight hours and then the temperature was increased to 100°C for one hour. Curing, deflasking, finishing, and polishing was performed by a routine method. Finally, each patient delivered his denture and instruction were given to the patients for proper hygiene of the oral cavity, cleaning of the denture and follow up.

Microbial evaluation

For clinical study sixteen samples were collected by swabbing the palatal mucosa and fitting surface of maxillary dentures after patients rinsed their mouth with tap water for both patients groups in sterile tubes containing 2ml normal saline after 3, 6 and 9 months from denture insertion. Samples were stored in a cold place (icebox) and transported to the laboratory within one hour.

The microbial evaluation was made through total colony forming unit and total counts of three standard strain organisms (C. albicans, S. mutants, lactobacilli) obtained from National Research Centre) NRC(culture collection.

Colony forming unit (CFU) was done through using conventional plate count agar. the sample was suspended in it which was incubated at 35 ±1˚C for 48 h, then CFU was enumerated and calculated per ml of sample.

CFU/ml was calculated using the formula:

$$\frac{\text{(no. of colonies} \times \text{dilution factor)}}{\text{volume of a culture plate}}$$

Mechanical properties evaluation

For laboratory study, a total of sixty specimens were constructed. According to the type of denture base resin the specimens were divided into two groups. According to sample dimensions and sort of test, each group was divided into three subgroups. Group I: Thirty specimens of conventional acrylic resin samples and Group II: Thirty specimens of zirconia reinforced acrylic resin samples for the flexural strength, surface hardness and surface roughness tests for both group. Sample preparation: the dimensions of rectangular metal dies for measuring flexural strength were 65 mm length × 10 mm width × 2.5 mm thickness. The dimensions of rectangular metal dies for measuring surface hardness and surface roughness were 30 mm length × 10 mm width × 2.5 mm thickness . Standardized specimens were prepared according to International Standards Organization (ISO) Specification No. 1567 for heat cured acrylic resins and ZrO_2 reinforced acrylic resins.

Flasking the metal dies using type III dental stone. The two halves of the flask were separated after setting of dental stone and the dies were removed from molds without distorting the mold space. Acrylic resins samples and ZrO_2 reinforced acrylic resins samples were prepared as mentioned before and placed in the mold space to fabricate the test samples.

The flexural strength test of specimens were tested by using three-point bending test in Universal Testing Machine**.** A specimen was centrally loaded with a load cell of 5 KN at a crosshead speed of 5 mm/min over a two-point support span set at a distance of 20 mm. Specimens were subjected to compression loading until fracture occurred. Flexural strength is the maximum load required for rupture that was recorded.

FS represents the limiting stress at which failure or instability is imminent. The value of the calculation of FS was guided by the formula:

$$\sigma_f \quad \frac{3FL}{2bd^2}$$

Where; **F** is the maximum load, **L** is span, **b** is the specimen width and **d** is the specimen thickness.

Digital Display Vickers Micro-hardness Tester was used to determine surface Micro-hardness of the specimens with a Vickers diamond indenter and a 20X objective lens. The surface of the specimens was applied to a load of 200g for 20 seconds. Three indentations, which were equally placed over a circle and separated from the adjacent indentations by 0.5mm, were made on the surface of each specimen.

USB Digital microscope with a built-in camera was used for photographed the surface roughness of Specimens that was connected with an IBM compatible personal computer using a fixed magnification of 120X. The resolution of images were 1280×1024 pixels per image. Microsoft office picture manager was cropped images to 350 x 400 pixels to specify/ standardize area of roughness measurement.

Statistical analysis

All measurements were recorded and tabulated. Statistical analysis was then performed using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA). Data related to microorganisms count showed a parametric distribution. Therefore, both groups was compared by using independent t-test, parametric data of Flexure strength at Maximum Flexure load, surface hardness and surface roughness were compared using independent t-test. The significant level was set at $P < 0.05$.

RESULTS

Clinical study:

1- Comparison of colony forming unit, Streptococcus_mutants (S. mutants) and lactobacilli between both groups

The mean value and standard deviation of CFU ($x10^3$) in both groups are presented in table (1). At 3 months, a higher mean value was recorded in group II (ZrO_2 reinforced acrylic resin PMMA) with no significance difference (P=0.22). While at 6 and 9 months, a higher mean value was recorded in group II (ZrO_2 reinforced acrylic resin PMMA) with an extremely significant difference (P=0.000) and (P=0.000) respectively.

The mean value and standard deviation of S. mutants count in both groups presented in table (1). At 3,6 and 9 months, a higher mean value was recorded in group II (ZrO_2 reinforced acrylic resin PMMA)than group I , with a significant difference between groups (P=0.011), (P=0.012) and(P=0.000) respectively.

The mean value and standard deviation of Lactobacilli count in both groups presented in table (1).At 3, 6 and 9 months, a higher mean value was recorded in group II (ZrO_2 reinforced acrylic resins PMMA), with a significance difference (P=0.028), a highly significant difference (P=0.0003) and an extremely significant difference (P=0.000) respectively.

Table (1): *Comparison of mean values and standard deviation of CFU (x10³), S. mutants and lactobacilli between groups (independent t-test)*

C.F.U	Times	Groups	Mean	Std. Dev.	T	P
	3 months	Group I	147.39	18.42	0.5	0.22ns
		Group II	154.44	15.45		
	6 months	Group I	154.78	20.70	5.56	0.000*
		Group II	212.00	38.41		
	9 months	Group I	161.7	30.67	7.02	0.000*
		Group II	231.89	29.29		
S.mutans						
	3 months	Group I	40.56	12.08	2.69	0.011*
		Group II	53.33	16.12		
	6 months	Group I	56.11	16.92	2.67	0.012*
		Group II	70.56	15.52		
	9 months	Group I	82.78	20.62	6.05	0.000*
		Group II	130.6	26.46		
Lactobacilli						
	3 months	Group I	368.89	114.24	2.3	0.028*
		Group II	440.00	63.99		
	6 months	Group I	621.94	210.59	4.04	0.0003*
		Group II	960.56	286.28		
	9 months	Group I	999.44	340.75	6.99	0.000*
		Group II	1625.78	168.52		

*Significance level p<0.05, * significant, ns= non-significant*

2- Comparison of Candida albicans between both groups

The mean value and standard deviation of C. Albicans count in both groups are presented in table (2) At 3 months, a higher mean value was recorded in group II , with no significant variation between both groups (P=0.14). While, at 6 months, a higher mean value was recorded in group II, with a considerable variation between both groups (P=0.043).At 9 months, a higher mean value was recorded in group II, with an extremely significant variation between both groups (P=0.000).

Table (2): *Comparison of mean values and standard deviation of C. albicans count between group I and group II (independent t-test)*

Times	Groups	Mean	Std. Dev.	T	P
3 months	Group I	12.78	3.08	1.51	0.14^{ns}
	Group II	14.56	3.94		
6 months	Group I	16.94	4.06	2.12	0.043*
	Group II	20.00	4.64		
9 months	Group I	22.22	6.74	6.44	0.000*
	Group II	38.89	8.67		

*Significance level p<0.05, * significant, ns=non-significant*

Laboratory study:

A- Flexure strength at Maximum Flexure load (MPa)

Comparison of Flexure strength at Maximum Flexure load (MPa) in both subgroup IA (conventional heat-cured acrylic resin samples) and subgroup IIA (ZrO_2 reinforced acrylic resin samples) was presented in Table (3). A higher mean value of Flexure strength was recorded in subgroup IIA. Independent t-test detected that this variation was statistically significant (p=0.033).

Table (3): *Comparison of Flexure strength at Maximum Flexure load (MPa) in subgroups IA and IIA (independent t-test).*

Subgroups	Mean	Std. Dev.	Std. Error Mean	T	P
Subgroup IA	60.368	5.91	2.642	2.32	0.033*
Subgroup IIA	66.376	5.73	4.801		

*Significance level P<0.05, *significant*

B- Surface hardness and surface roughness

Comparison of surface hardness in both subgroups IB (conventional heat-cured acrylic resin samples) and IIB (ZrO_2 reinforced acrylic resin samples) was presented in Table (4). A higher mean value and standard deviation of surface hardness were recorded in subgroup IIB. Independent t-test detected that this variation was not statistically significant (p=0.69).

Comparison of surface roughness in both subgroups IC (conventional heat-cured acrylic resin samples) and IIC (ZrO_2 reinforced acrylic resin samples) that was measured in micron was presented in Table (4). A higher mean value of surface roughness was recorded in subgroup IIC. Independent t-test detected that this variation was statistically significant (p=0.008).

Table (4): *Comparison of surface hardness and surface roughness in both subgroup IB and IIB and subgroup IC and subgroup IIC (independent t-test).*

Surface hardness								
Subgroups	Mean	Std. Dev.	Std. Error Mean	Mean difference	95% Confidence Interval of the Difference		T	P
					Lower bound	Upper bound		
Subgroup IB	41.291	3.020	0.780	-0.623	-3.85	2.60	0.40	0.69^{ns}
Subgroup IIB	41.914	5.217	1.347					
Surface roughness								
Subgroup IC	0.253	0.001	0.000	0.0015	-.006	0.0019	3	0.008*
Subgroup IIC	0.256	0.003	0.001					

*Significance level P<0.05, *significant*

DISCUSSION

This study was designed as a prospective comparative clinical and laboratory trials. The success or failure of an oral prosthesis depends upon several factors including the technical and planning skills of the prosthodontics, properties of the material used and the health and tolerance of oral tissues[7] .

In the present study, the edentulous maxillary ridge were selected rather than the mandibular ridges, since, the amount of mucosa in contact with denture is generally greater than in mandible, and due to excessive saliva in the floor of the mouth which implies that the maxillary denture usually possess great clinical importance for detection of bacterial colonization[8] .

The edentulous ridge had normal morphology and cover with firm healthy mucosa to ensure favorable conditions for prosthetic procedures and patient had no oral lesions when examined [9].

There was a critical increase in the total count of microorganisms in group II (upper ZrO_2 reinforced PMMA denture) during the follow-up periods when compared to group I (upper conventional PMMA denture) in this study.

This study had proved that both the nature of the denture base and its roughness, have a high effect on the degree of microbial colonization of the material. The count of adherent organisms after 3 months was significantly higher on group II than on the group I and also, increasing surface roughness leaded to increase microbial colonization for both types of acrylic [10].

High microbial colonization was found in group II may be due to the high surface energy of the particle of ZrO_2, this finding was in agreement with another study who demonstrated that Surface free energy (SFE) is a contributing factor which affects microbial adhesion and plaque maturation on surfaces [11].

This study showed that surface roughness in group II (ZrO_2 reinforced PMMA) was significantly higher than group I (conventional PMMA). The surface roughness of denture material is critical factor as it affects the oral health of tissues that was in direct contact with the fitting surface of dentures[12].

This result was in agreement with another study who showed that surface roughness is a very important factor that affecting microbial colonization on denture as rougher surfaces had more microorganism count than on the smoother surfaces [13].

The results of this study showed that flexural strength of subgroup IIA (ZrO_2 reinforced acrylic resin) was significantly higher when compared to subgroup IA (Conventional heat cured acrylic resin). This result was in agreement with another study who showed that significant improvements in the mechanical properties with reinforcement of oxide particles [5].

The results are also in agreement with previous studies that revealed that the fractures of the denture can be reduced by increasing the strength of PMMA through addition of ZrO_2. The addition of zirconia in various dental materials was found to have significant improvement on the mechanical properties [4, 13].

The finding of the present study may be explained on the basis that ZrO_2 powder was added to PMMA in concentrations of 10% after treating with the saline coupling agent that provide excellent chemical bond between ZrO_2 and acrylic resin. Using of zirconia as filler particles of size 5–10 μm helps in filling the interstitial matrix space of PMMA. The flexural strength increasing may result from filling of interstitial space of an acrylic resin matrix with ZrO_2 that made interruption of crack propagation [5, 6].

On the other hand, another study showed that a slight decrease in flexural strength which may be explained on the basis of clustering the particles within the resin that leading to weakness of the material [14].

The results of this study revealed that insignificant increase in surface hardness in subgroup IIB (ZrO_2 reinforced PMMA samples) when compared with subgroup IB (conventional PMMA samples). This is finding is in agreement with the study who showed an insignificant increase in surface hardness of ZrO_2 reinforced PMMA when compared with conventional PMMA [4]. Further, another studies showed that the addition of ZrO_2 to PMMA leaded to significant increase of fracture toughness, impact strength and surface hardness of PMMA [15, 16].

This study showed that surface roughness in subgroup IIC (ZrO_2 reinforced PMMA) was significantly higher when compared to group IC (conventional PMMA). This may result from void formation from entrapped air and moisture, incomplete wetting of the fillers by the resin due to increasing fillers content. Furthermore, ZrO_2 affects the integrity of the polymer matrix as it acts as an interfering factor [17-19].

CONCLUSION

It was concluded that:

I- Reinforcement of ZrO_2 particles in PMMA results in an increase the microbial colonization and surface roughness of denture.

II- Reinforcement of ZrO_2 particles in PMMA has critical role in the improvement of some mechanical properties as flexural strength and surface hardness.

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