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Impact of adding 3% titanium oxide nanoparticles in different denture base materials on some of the biological, physical, and mechanical properties properties: *In-vitro* study

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Background/aim

Acrylic resin denture base material with all its varieties and curing techniques enormously influence their physical, biological, and mechanical properties. This study designed to evaluate and compare the impact of acrylic resin reinforcement with titanium oxide nanoparticles (TiO₂NP) on physical, biological, and mechanical criteria of two dissimilar denture base materials.

Materials and methods

This study was constructed on 120 specimens of 2 different types of acrylic resin denture base materials. The specimens were divided equally into 2 groups (60 each), group I (heat cured acrylic resin) and group II (microwave cured acrylic resin). Each group was additionally divided into two subgroups (each 30). Subgroup A as control, while subgroup B was modified with 3% TiO₂NP. Biological evaluation was performed on *Enterococcus faecalis by using antimicrobial assay by agar diffusion test* color stability via employing spectrophotometer, and the Modulus of elasticity through universal testing machine.

Results

Regarding the results of biological assessment, groups IA and IIA (control groups) revealed no inhibitory zone, while groups modified with 3% $\rm TiO_2NP$ group IIB demonstrated significantly higher (P < 0.05) inhibitory zone when compared with group IB. Concerning color stability, Regarding color stability, Group IA and IIA (control groups) demonstrated the highest color changes, while Group IB and IIB (modified groups) demonstrated the lowest color changes. In modulus of elasticity, groups IB and IIB were significantly higher (P < 0.05) than groups IIA and IIB.

Conclusion

Adding 3% TiO₂NP to heat and microwave cured resins induced antibacterial characteristics against E. faecalis. Besides improvement of the evaluated mechanical (modulus of elasticity) and color stability properties.

Keywords:

biological, denture base material, nanoparticles, physical, titanium oxide, mechanical properties

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Previous publication/presentations mentioned: (a) A comparative clinical study of the effect of denture cleansing on the surface roughness and hardness of two denture base materials. (b) Mechanical properties, color stability and biological characteristics of acrylic resin denture base materials containing titanium oxide

nanoparticles: *in-vitro* study. (c) Dimensional accuracy of implant impression obtained from poly-siloxane condensation silicone: an *in-vitro* study. (e) Flexural and tensile strength of acrylic resin denture base materials processed by three different methods. (f) Comparative study clarifying the most suitable material to be used as partial denture clasps.

Introduction

Denture base is determined as the denture's foundation which substitutes the whole dentition and related structures in both maxilla and mandible through its

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wide-ranging values [1]. Resting on the fundamental areas, prosthetic teeth attachment, transferring the intense occlusal forces to the basal structures besides replacing both bulk and shape of the underlying alveolar tissues are examples of its importance [2]. Denture base material has two main forms; metallic (cobaltchromium alloy) and nonmetallic (acrylic resin) [3,4]. Biocompatibility and advancement of the nonmetallic denture base's properties justify its remarkable employment in dentistry than the metallic one [5].

Acrylic resin as the outmost utilized denture base form is supplied as both liquid and powder. The liquid (monomer) embraces of chiefly unpolymerized methyl meth acrylate (MMA), petty percent of hydroquinone as an inhibitor and glycol dimethacrylate as a cross linking agent enhancing its distortion resistance [6,7]. The acrylic resin's extra response that necessitates initiator stimulation through energy for processing to take place is known as polymerization reaction [8].

Heat cure acrylic resin is the outmost regularly employed nonmetallic denture base polymerization reaction relies on thermal energy to take place represented as either a short or long water bath cycle [9]. Although heat cure polymethyl meth acrylate (PMMA) possesses several notable advantages, including its nontoxic nature, insolubility, pleasing aesthetics, affordability, extended shelf life, and ease of processing and repair, it is also important to acknowledge its associated drawbacks. These drawbacks such tissue hypersensitivity, as instability, dimensional discoloration, susceptibility to bacterial colonization. As a result, it necessitates the development of innovative resins and employs advanced processing techniques in order to enhance its overall properties and functions [10–12].

Anatase is the most favorite crystalline form of titanium oxide's (TiO₂) assembly, owing to its nontoxicity, great surface area, enormous photo catalytic activity, reasonably affordable besides both its photo chemical and minimal temperature Thus, anatase-TiO₂ is employed for stability. disinfection and purification of different environmental elements as water and air [13,14]. Evolution in PMMA characteristics via addition of formulated titanium oxide nanoparticle (TiO₂NP) has been stated recently and apparent in acrylic resin's physical, biological, mechanical, and viscoelastic criteria advancement. Hence, abundant utilization of TiO₂NP in versatile dental specialties is currently apparent [10,15].

One of the recent acrylic resin form's innovations took place via microwave curing which is remarkably di-methacrylate distinguished via its liquid (monomer) instead of PMMA, together with attraction and rehabilitation of microwave energy polymerization for disseminated dielectric heat which speedily elevates the temperature using the nonmetallic microwave translucent oven flask [16,17].Sequentially, microwave cured acrylic resin denture base is advantageous with its dimensional stability, trivial residual monomer content, enhanced adaptation, nominal time, and properties advancement [18,19].

Because acrylic resin is porous, prosthetic patients are more susceptible to the formation of oral biofilm and intraoral bacterial infections. This susceptibility is exacerbated by oral hygiene practices and the presence of infectious disorders that promote bacterial adhesion and growth [20]. Enterococcus faecalis (E. faecalis), a strong and highly prevalent intra-oral bacterial species, is particularly problematic in immunocompromised and prosthetic patients, potentially causing harm to these individuals. As a result, when selecting the base material for dentures, considering the biological factor becomes priority [21,22].

Since the acrylic resin denture base material with all its diversities is frequently exposed to liquid sorption, food debris, ecological conditions, and absorbing various contaminants ending up with discoloration and inferior esthetics. Hence, color stability is a crucial physical parameter in denture base material [23]. Methods employed for assessing color stability are either subjective or objective and the spectrophotometer with all its natures is rated as the most preferable objective technique for its easiness and accuracy [24].

Moreover, while denture base resins are often exposed to repeated mechanical stresses within the oral cavity, which can negatively affect their durability and functionality, only a limited number of studies have explored their mechanical characteristics under dynamic loads during activities like chewing and swallowing, as opposed to static loads. Permanent deformation caused by mastication can be tolerated by denture base materials possessing a higher modulus of elasticity. Because of flexure, upper denture fractures always happen through the midline of the denture. As a result, the denture foundation needs to have enough flexural modulus to withstand fracture [25].

Prosthetic appliances' durability and success greatly rely on their denture base resins' features whether

biological, physical, or mechanical. These properties can be simply modified by incorporating any additive to PMMA [7]. As the existing literatures lacked comprehensive discussions regarding the influence of incorporating TiO₂NP into various types of acrylic resin denture base materials, this research was designed with the dual objective of assessing and contrasting the effects of reinforcing acrylic resin with TiO2NP on the biological, physical, and mechanical properties of two distinct denture base materials.

Materials and methods

Materials

This study was conducted using two distinct forms of accessible acrylic resin denture base materials, including the conventional heat cured acrylic resin (Acrostone; Acrostone Dental Factory—Industrial Zone, Salam City A.R. E-WHW Plastic, England) and Microwave cured acrylic resin (Protechno; Internacional, Poligono Emporda Garrotaxa, Vilamalla Girona, Spain). Moreover, TiO₂NP (Sigma Aldrich company, USA) were added in modified groups.

Study design

A total number of 120 specimens were constructed utilizing metal patterns with different dimensions and shapes vary according to evaluated property and fabricated according to ADA specification no. 12. A metal disc of 10 mm in diameter ×2 mm in thickness was fabricated for biological property assessment, whereas a metal disc of 50 mm×0.05 mm in diameter and thickness for color stability evaluation, while a rectangular metal plate of 60 mm lengths × 10 mm width $\times 2 \, \text{mm}$ thicknesses mechanical properties evaluation. These 120 specimens were divided equally into two main groups, group I of conventional heat cured acrylic resin and group II of microwave cured acrylic resin. Each group was additionally divided into two subgroups (30 each) as follows:

- (1) Group IA: Conventional heat cured PMMA control specimens.
- (2) Group IB: Specimens as group IA modified with 3% TiO₂NP.
- (3) Group IIA: Microwave cured PMMA control specimens.
- (4) Group IIB: Specimens as group IIA modified with 3% TiO₂NP.

The antibacterial activity against E. faecalis was assessed by measuring the diameter of the inhibition zone in millimeters representing the biological properties. The color changes were assessed utilizing spectrophotometer to evaluate physical properties, and the modulus of elasticity was evaluated by universal testing machine to estimate the mechanical criteria.

Sample size calculation

Sample size was considered based on a preceding study as a reference [26]. Hence, the apparently passable sample size was 10/subgroup, once each group's response was ordinarily spread through standard deviation 0.11, difference 0.15, 80% power and 0.05 type I error probability. Independent t-test was performed by utilizing P.S. Power3.1.6.

Ethical approval

The current study adhered to the ethical guidelines outlined by the World Medical Association's Code of Ethics, following the principles set forth in the 1975 Declaration of Helsinki. Approval for this research was obtained from the Medical Research Ethical Committee (MREC) of the National Research Centre (NRC) in Cairo, Egypt, under approval number 54312012023.

Methods

Conventional metal flask was employed to attain molds of conventional heat cured acrylic resin while a special microwave plastic flask was purchased from Tecno-Flask (Protechno, Can Viloca, Spain) and utilized for the microwave one. The inferior section of the dental flask was occupied with dental plaster that was bought from Elite (Rock Stone, Zhermack Clinical, Italy), which was blended rendering to the manufacturer's guidelines (i.e., 50 ml/100 g). Each of the three metal patterns utilized in the current research was coated with the separating medium; that was purchased from Acrostone, Egypt, followed by another layer of plaster mix. Sequential to plaster setting (30 min), coating both the plaster and metal patterns with a separating medium then another layer of plaster was infused into the superior part of the flask with vibration by aid of mold vibrator. Plaster was left to harden for (60 min), then finally the flask was deflasked, metal patterns were detached, and the mold was gained which later on helped in construction of acrylic resin specimens.

Specimen preparation

The conventional heat cured PMMA was prepared and packed using a stainless-steel spatula following the manufacturer's guidelines. As approached the dough stage, it was carefully placed into the plaster mold. The metal flask was then compressed using a hydraulic press and positioned in the water bath curing unit (water bath curing unit; type 5518, KaVo EWL, Biberach, Germany) for a duration of 30 min at 70°C, accompanied by an extra 30 min at 100°C for the heat curing process. Afterward, the flask was grabbed out of the water bath and left till reaching room temperature before deflasking. Finally, finishing and polishing of the specimens were carried out.

The microwave-cured denture base material was prepared according to the manufacturer's instructions, which specified a powder-to-liquid ratio of 2:1 by weight (a powder ECO-CRYL M). Once mixture reached an appropriate doughy consistency, it was carefully packed into the mold. A special nonmetallic flask was then subjected to manual pressure and placed inside the microwave oven for 3 min at 500 W for curing. Post curing, the flask was removed from the microwave and till achieving room temperature for 30 min. Subsequently, it was dipped in cold water for 20 min to facilitate deflasking. Deflasking was carried out by gently tapping the flask to release the specimens, and finally, all the specimens were finished and polished.

Acrylic resin specimens of both group IB and IIB containing TiO₂NP (modified group). TiO₂NP powder was added (3% by weight) to the polymer of heat cured and microwave cured resins, respectively, by employing the weighing balance which was bought from Adam Equipment 124 precision weighing balance, UK in the Central Service Unit at National Research Centre, National Research Centre (NRC), Cairo, Egypt. Then specimens' curing of each subgroup was performed as that of group IA and IIA, respectively.

Biological, physical, and mechanical properties assessments

Biological assessment

The isolated colonies of organisms were cultured in 5 mL of broth medium at 37°C and left to grow overnight (16–18 hours). Then, the cultures were diluted, and the organism inoculum size was standardized by measuring the absorbance at 600 nm "absorbance of 0.5 at 600 nm follows the McFarland standard, which indicates the number of bacteria within a given range to standardize microbial testing". The provided resins were inserted in 1 mL of PBS pH7.4 and incubated at 37°C for 30 minutes to enhance the release of active substance, followed by vigorous vortex stock solution. The released solution was centrifuged at 8000xg for 10 minutes and the pellet

was resuspended in 200 μ L of PBS and used for antimicrobial assessment. In petri dishes, the *E. faecalis* pathogen was cultured in Luria-Bertani (LB) broth. A150 μ l of the organism was gently spread throughout the plate, then dry any extra liquid solution. Each agar plate was divided into 5 sections and labelled with the corresponding used resin. 100 μ l of each tested sample was added to disc in the tested dish, and 100 μ l of the PBS was added as negative control. The plates were incubated inverted overnight or until cells have grown out completely. After 7 days, the area of inhibited bacterial growth was measured and scaled with a ruler. as displayed in Figure 1.

Physical assessment

In this study, color stability was utilized to assess the physical characteristics of acrylic resin. Acrylic resin specimens (*N*=10 in each group) were immersed in tea. Color stability evaluation for each specimen was recorded pre and 7 days post of their immersion in prepared tested solution via utilizing spectrophotometer (Neu-Isenburg, Germany), as displayed in Fig. 2.

The color changes of each group were calculated via employing the C.I.E. L, a, b, uniform color scale. The magnitude of total color alteration was formulated by: $\Delta E \left[\Delta E = (2\Delta L + 2 \Delta a + 2 \Delta b)^{1/2}\right] [27]$, where ΔE is degree of color change, ΔL is L specimen – L standard, Δa is a specimen – a standard and Δb is b specimen – b standard.

Figure 1



Shows agar diffusion assay illustrated the antimicrobial potential of all groups on *Enterococcus faecalis*. (a) negative control group, (b) Group IA, (c) Group IB, (d) Group IIA, (e) Group IIB.



Shows spectrophotometer used in evaluation of color changes.

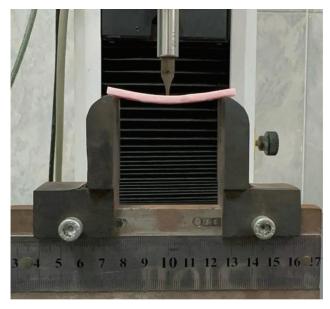
Mechanical properties assessment

The modulus of elasticity was measured using a Universal testing machine(INSTRON, 3345 England.). The specimens were centered on the machine's supports and gradually applied perpendicular to the midpoint of the strips. The load was recorded until the maximum capacity was reached. Load-deflection curves were recorded using computer software(Bluehill v1.5; Instron Ltd).. The elastic modulus was calculated using the formula E = FL3 /4Ybd3, where E represents the flexural modulus, F represents the maximum load, L represents span length, Y represents recorded deflection, b represents specimen width, and d represents specimen thickness, as displayed in Figure 3 [28].

Statistical analysis

All data were collected, revised, coded, and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean and standard deviations. To evaluate the data's normality, both the Shapiro-Wilk and Kolmogorov test were employed, and it was observed that all data from the various groups exhibited a normal distribution. Subsequently, the comparison between two groups was done using Student t-test, while the comparison among the different groups was driven employing the one-way ANOVA test, accompanied by the Tukey Post Hoc test to facilitate several comparisons. A significance threshold of P less than 0.05 was adopted.

Figure 3



Shows evaluation of acrylic resin specimen modulus of elasticity through universal testing machine.

Results

Biological assessment

Regarding the bacterial evaluation of denture base materials specimens using the inhabitation zone of E. faecalis, the comparison between all groups displayed highly significant difference at P value less than 0.01. In addition, the multiple comparisons showed that negative control and control groups of conventional heat cured PMMA (group IA) and microwave cured PMMA (group IIA) were displayed no inhibition zone, while group of heat cured specimens modified with 3% TiO₂NP (group IB) exhibited 2.5 ± 0.12 mm, which was significantly lower (P<0.05) than Microwave cured specimens modified with 3% TiO₂NP (group IIB), which revealed 2.5 ± 0.22 mm, as presented in Table 1.

Physical assessment

Regarding the color changes ΔE of denture base specimens using spectrophotometer, comparison between different groups revealed highly significant difference as < 0.0001. In addition, the multiple comparisons revealed that the control groups of conventional heat cured PMMA (group IA) which exhibited 18.07 ± 6.27 and microwave PMMA (group IIA) which exhibited 28.2 ± 2.99 demonstrated the highest color changes. Followed by heat cured specimens modified with 3% TiO_2NP (group IB) which exhibited 13.18 ± 1.29. On the other hand, Microwave cured specimens modified with $3\% \text{ TiO}_2\text{NP}$ (group IIB) 6.78 ± 0.51 revealed the least amount of color changes, as presented in Table 2.

Table 1 Inhibition zone (mm) regarding Enterococcus faecalis in all groups and subgroups

Inhibition Zone (mm)	М	SD	P-value
Negative control	0.0 ^a	0.0	_
Group IA			
Conventional heat cured PMMA	0.0 ^a	0.0	
Group IB			
Heat cured PMMA incorporated with 3 % by weight TiO₂NP	2.5 ^b	0.12	<0.0001*
Group IIA			
Microwave cured PMMA	0.0 ^a	0.0	
Group IIB			
Microwave cured PMMA incorporated with 3 % by weight TiO ₂ NP	4.5 ^c	0.22	

All data with different superscript letters (a, b, c) were significantly different at *P-value* less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation, TiO₂NP: titanium oxide nanoparticles. *Significant difference as *P* less than 0.05.

Table 2 Color changes in control and modified groups with 3% TiO₂NP using spectrophotometer

Color changes (Δ E)	Mean	SD	Р
Group IA			
Conventional heat cured PMMA	18.07 ^a	6.27	
Group IB			
Heat cured PMMA incorporated with 3% by weight TiO ₂ NP	13.18 ^b	1.29	< 0.0001*
Group IIA			
Microwave cured PMMA	282 ^c	2.99	
Group IIB			
Microwave cured PMMA incorporated with 3% by weight TiO ₂ NP	6.78 ^d	0.51	

All data with different superscript letters (a, b, c) were significantly different at P value less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation, TiO_2NP : titanium oxide nanoparticles. *Significant difference as P less than 0.05.

Mechanical properties assessment

Regarding modulus of elasticity of denture base materials specimens using universal testing machine, comparison between different groups revealed highly significant difference as P=0.0001. In addition, the multiple comparisons revealed that the controls groups of conventional heat cured PMMA (group IA) which exhibited 217.59 ± 32.88 and microwave cured PMMA (group IIA) which exhibited 218.14 ± 50.31 demonstrated the the lowest modulus of elasticity. Followed by heat cured specimens modified with 3% TiO_2NP (group IB) which exhibited 311.8 ± 51.18. On the other hand, microwave cured specimens modified with 3% TiO_2NP (group IIB) 740.5 ± 43.26 revealed the highest modulus of elasticity, as presented in Table 3.

Discussion

In this study, we meticulously considered various parameters in the experimental setup. We prepared metal specimens with precise shapes and dimensions to serve as molds for creating acrylic resin specimens, aligning with ADA specification no.12 [29]. For heat cured acrylic resin specimens, the conventional method was followed using a metal flask, while for microwave cured ones, a special microwave plastic flask was employed to avoid potential issues from microwave energy reflection [16,30].

All acrylic resin denture base materials in each group were manufactured following the recommended

Table 3 Modulus of elasticity in control and modified groups with 3% TiO₂NP using Universal testing machine

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Storage modulus	Mean	SD	Р	
Group IA				
Conventional heat cured PMMA	217.59 ^a	32.88		
Group IB				
Heat cured PMMA incorporated with 3% by weight TiO ₂ NP	311.8 ^b	52.18	0.0001	
Group IIA				
Microwave cured PMMA	218.14 ^a	50.31		
Group IIB				
Microwave cured PMMA incorporated with 3% by weight TiO ₂ NP	740.5 ^c	43.26		

All data with different superscript letters (a, b, c) were significantly different at *P* value less than 0.05, using ANOVA. M: mean, PMMA: polymethyl meth acrylate, SD: standard deviation, TiO₂NP: titanium oxide nanoparticles. *Significant difference as *P* less than 0.05.

guidelines to ensure top-quality results. To enhance the biocompatibility, stability, antifungal PMMA's properties, and mechanical strength TiO2NP was introduced, specifically using the anatase form for its superior photocatalytic activity and stability [31,32]. To minimize any potential negative effects of TiO₂NP, especially with increased concentrations, we added only 3% by weight to the two different PMMA resin types used for specimen fabrication [33].

E. faecalis was chosen for the biological assessment of PMMA resins in this study due to its versatile nature. It is considered one of the most strong and prevalent intraoral species, capable of existing as both single cocci and in chains [20,34]. In addition, it exhibits broadspectrum colonization within the oral cavity and is associated with oral mucosal lesions, particularly in immunocompromised patients. In terms of the physical properties of acrylic resin, we focused on color stability, as it plays a significant role in esthetics and patient satisfaction [35,36]. To ensure precision, we utilized a spectrophotometer, and consistency was evaluated employing Commission International de L'Eclairage uniform color scale (CIE scale) [37]. Furthermore, this research assessed the viscoelastic properties using a constant frequency of 1 Hz and a temperature range of 5-50°C to simulate masticatory rhythms and the intraoral temperature conditions, ensuring that the resins perform well in clinical settings [38].

Outcomes of this research displayed that both group IA and G IIA (without TiO2NP) showed no inhibition zone, while in modified groups (with TiO₂NP) revealed inhibition zone. This might be related to the TiO2NP which is an effective antimicrobial additive due to its chemical stability, non-toxicity, and ability to inhibit pathogenic bacteria adherence. PMMA resin reinforcement with 3% TiO₂NP significantly reduces microbial count and attachment, particularly E. faecalis, and enhances their biological properties.

On the other hand, group IIB showed higher inhibitory zone than group IIA, this might be attributed to the different nature of both acrylic resin and their variance in the curing method as proved by other studies [9,10]. Moreover, microwave curing offers distinct advantages over water bath curing due to its rapid temperature increase and heating capabilities. When using water baths, the formation of bubbles occurs, which can become trapped within the polymer matrix, leading to excessive porosity and surface irregularities [39,40]. Conversely, heat cured PMMA specimens tend to harbor more bacteria and facilitate their colonization, thereby reducing their ability to create a sufficient inhibitory zone [41].

The current research findings revealed that both the control and modified heat cured PMMA groups had the uppermost span of color variations group IA (18.07 ± 6.27) and group IIA (28.2 ± 2.99) , succeeded by the control microwave cured acrylic resin group IB (13.18 ± 1.29) , then the modified microwave one group IIB (6.78 ± 0.51) displayed the least amount of color changes individually.

This can be attributed to the fact that color stability is influenced not only by the type of PMMA resin but also by the processing method, chemical composition, and specific standards applied. Certain studies have explored how the processing method can impact the degree of color stability during the PMMA's polymerization process [9,10]. It has been noted that shorter processing times result in better color stability. As a result, since microwave polymerization only takes around 3 min. compared to the 9 hours required for the hot water bath technique, the color stability of the PMMA specimens was significantly better in the microwave cured ones compared to those cured through heat [42,43]. The study found that adding 3% TiO₂NP to PMMA resin types reinforces the microwave polymer matrix, limiting color changes and absorbing more amount of light than the control specimens, resulting in more opaque and glossier specimens [8,44].

Findings of this research, concerning the modulus of elasticity in mechanical properties evaluation, revealed insignificant difference between both control groups of heat cure PMMA group IA which exhibited the lowest modulus of elasticity and microwave one group IIA. followed by modified heat cured PMMA group IB, while modified microwave cured PMMA group IIB displayed the highest storage modulus. The flexural strength and modulus of acrylic resin were greatly enhanced by the incorporation of TiO₂ NPs. TiO₂ NP addition results in a concentration-dependent process which increases flexural strength modulus. Physically rigid particles, TiO2 NPs can fill voids in the material's matrix. They may absorb energy and stresses before failure if they are distributed properly. Because they are physically inflexible, TiO2 nanoparticles can be very effective at absorbing energy from the material. These nanoparticles can assist in more uniformly distributing stresses throughout the matrix when external pressures or stresses are applied to the material. They serve as stress absorbers, lowering 88

the concentration of tension at particular locations. Additionally, the greatest flexural strength and modulus were produced by lower concentrations of TiO₂ NPs, which is consistent [45,46].

Conclusion

Within the limitation of the present study, it can be concluded that both heat and microwave cured acrylic resins have a comparable mechanical feature regarding the modulus of elasticity; as well as absence of antibacterial properties. Microwave cured resin has a lower color stability property compared to that of heat cured resin. Adding 3% TiO2NP to heat and microwave cured resins induced antibacterial characteristics against *E. faecalis*. Besides improvement of the evaluated mechanical (modulus of elasticity) and color stability properties.

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Conflicts of interest

There are no conflicts of interest.

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