Ibraheem *et al.*
Bulletin of the National Research Centre (2023) 47:91
https://doi.org/10.1186/s42269-023-01065-2

Bulletin of the National Research Centre

RESEARCH

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Effect of titanium oxide nanoparticles on polymerization reaction of heat and microwave cured polymethylmethacrylate: in vitro study

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Abstract

Background Titanium Oxide Nanoparticles (TiO_2NPs) have been introduced to polymethyl methacrylate (PMMA) denture base to enhance its mechanical, physical, and biological performance.

Objectives This research aimed to assess the effect of adding TiO_2NPs on the degree of polymerization and morphological characterization of heat and microwave cured PMMA.

Materials and methods Fabrication of PMMA specimens which were categorized according to the curing technique into heat cured (group I) and microwave cured (group II). Each group was subdivided into two subgroups A and B according to the addition of TiO_2NPs. A total number of 24 specimens were fabricated. Each subgroup contains 6 specimens. The specimens were characterized by Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray analysis (EDX). The polymerization reaction of specimens was assessed by Attenuated Total Reflection Fourier-Transform Infra-Red Spectroscopy (ATR-FTIR). Results were statistically analyzed using an independent T-test and one-way ANOVA. The significance level was set at $p < 0.05$.

Results SEM/EDX revealed that TiO_2 NPs appeared as bright areas in the matrix of both groups. The matrix showed organic impurities and minute internal cracks, but group IIB (microwave-cured PMMA with TiO_2NPs) showed higher organic impurities than heat-cured PMMA. The FTIR revealed that the addition of 3% by weight TiO_2NPs decreased the degree of polymerization of both heat and microwave-cured PMMA but the decrease was insignificant ($p > 0.05$).

Conclusions Incorporation of 3% by weight TiO_2NPs does not significantly affect the degree of polymerization of both heat and microwave-cured PMMA. Hence, denture materials can benefit from the advantages of the TiO_2NPs without any alterations to their structures.

Keywords Heat-cured denture PMMA, Microwave cured PMMA, Titanium oxide nanoparticles, SEM/EDX, Polymerization reaction, ATR-FTIR

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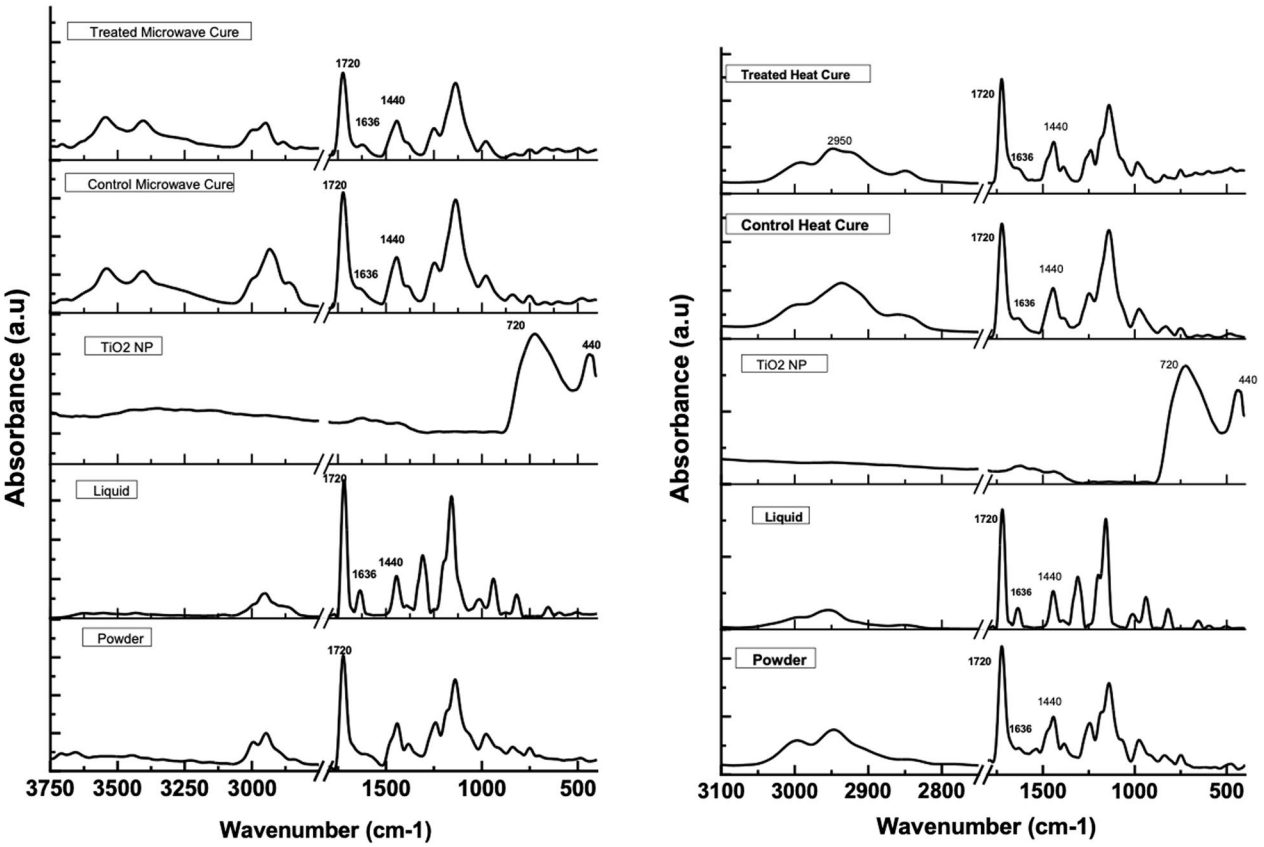
Graphical abstract



Background

One of the most essential components of intraoral prosthetic appliances is the denture base since it provides profound attachment of the artificial teeth, which accordingly aids in the replacement of the entire dentition besides both the maxillary and mandibular accompanying foundation. Furthermore, the denture base has an additional task as improvement of the masticatory performance, preservation of tissue health, as well as adequate reform of the occlusal forces across the entire denture base (The Glossary of Prosthodontic Terms 2017; Lai et al. 2004).

Denture bases can be categorized based on their material into metallic and non-metallic forms. Each has its advantages and deficiency concerning biocompatibility and the polymerized acrylic resin material verified is much preferable to the metallic one (Ahmed et al. 2019; Marcauteanu et al. 2008; Carr and Brown 2015).

The non-metallic acrylic resin denture base is provided as powder and liquid. The powder (polymer) comprises pre-polymerized spheres of PMMA and a minor expanse of benzoyl peroxide as an initiator. The liquid (monomer) is predominantly unpolymerized methyl methacrylate (MMA) with slight sums of hydroquinone as an inhibitor (Rueggeberg 2002; Memon et al. 2001; O'Brien 2002).

The polymerization reaction of the heat-cured acrylic resin is usually initiated by employing thermal energy, which takes place via a short or long round of water bath. Heat-cured acrylic resin denture base showed numerous advantages such as satisfying shelf lifetime, convenient processing, non-toxicity, the potentiality to repair, maximum esthetics, and tolerability. However, it also reveals a few drawbacks as tissue irritation which is initiated by residual monomer content and ends up with hypersensitivity. As well as water sorption, solubility, and polymerization shrinkage that compromise both the material's durability and dimensional instability (Ahmed et al. 2019; Marcauteanu et al. 2008; Carr and Brown 2015; Virendra 2004; McCabe and Walls 2013).

Accordingly, polymerization shrinkage impacts the mechanical features and aesthetics; also, it initiates surface roughness and microorganisms' sheltering

promoting microbial settlement into dentures' pores which greatly badly affects the patient's general health. Meanwhile, no single denture detergent is worthy of diminishing microorganisms from denture surfaces, even though it is highly attainable in a wide diversity (Ahmed et al. 2019; Kumar et al. 2010).

Advancement of acrylic resin was carried out with a particularly formulated liquid (monomer) and non-metallic flask to be cured by microwave energy using dielectric heating. Employment of the microwave non-metallic flask promotes waves' reflection on the flask's surface, hinders their impact on the resin and both evenly distribute the heat and speedily leverages the temperature (Elboraey et al. 2016; Muñoz-Bonilla and Fernández-García 2012; Negreiros et al. 2009; Santos et al. 2012).

Microwave curing of acrylic resin provides a superior adaptation of the processed denture bases due to homogenous heating, dimensional stability, negligible residual monomer content, and short curing time (Schneider et al. 2002; Keenan et al. 2003).

Consequently, pioneering resins and processing approaches have been expected adjoining nanoparticles (NPs), metal, and metal oxides NPs that achieve more novel traits, especially than those of the premier metal to augment acrylic resin's versatile criteria and performance (Gurbuz et al. 2010; Souza Júnior et al. 2006).

Titanium oxide has three famous forms according to its crystalline structures (anatase, rutile, and brookite). The anatase form is the most common form utilized in the dental field due to its distinguished photocatalytic activity which promotes its superiority over others, non-toxicity, pleasing color, and relatively inexpensive. Being biologically and chemically inert, photo catalytically stable, easy to construct and utilize, and non-hazardous to the environment and humans. (Gurbuz et al. 2010; Koutis and Freeman 2001; Macwan et al. 2011).

The addition of specifically formulated TiO_2NPs to PMMA promotes acrylic resin's innovative criteria such as biocompatibility, antifungal effect, superior biocidal activity, dimension stability, and minimal polymerization shrinkage (Koutis and Freeman 2001; Macwan et al. 2011).

One of the most versatile microscopy approaches providing magnifications up to 50,000×is Electron Microscopy (EM) which is recognizable for its amazing quality of 3-D images. Coupling with energy-dispersive X-ray analysis (EDX) provides additional knowledge about the elemental configuration map of the sample (Lin et al. 2014, 2016).

Scanning electron microscopy (SEM) is a non-destructive device that analyzes surface details down to nanoscale by utilizing the electron beam probe and creates magnified images with high resolution. Elemental analysis of the acrylic resin is performed by energy dispersive X-ray fluorescence (EDX) spectrometer; which quantitatively defines the sample's elements by eradicating them with X-rays and then analyzing the reemitted fluorescent X-rays. SEM/EDX provides information about the surface elemental composition of areas as small as nanometers in diameter (Asaka et al. 2004; Nam et al. 2012).

Attenuated Total Reflection Fourier-Transform Infra-Red Spectroscopy (ATR-FTIR) is one of the most accepted tools for analyzing the degree of conversion and polymerization of PMMA acrylic resin. Furthermore, it is a powerful analytical technique that has been employed as a quantitative measure for identifying and monitoring both the setting reactions and polymerization of diverse forms of dental materials (Bartoloni et al. 2000).

The Null hypothesis assumes that addition of TiO_2NPs to both heat and microwave-cured acrylic resin denture base materials does not affect polymerization reaction and microstructure.

Hence, the current research utilized the ATR-FTIR and SEM/EDX to assess and relate the impact of adding TiO_2NPs on the polymerization reaction and microstructure of the PMMA in both heat and microwave-cured acrylic resin denture base materials.

Materials and methods

Study design

- Group 1: Twelve samples of heat-cured PMMA were fabricated and divided into two equal subgroups Group IA: Conventional heat-cured PMMA and Group IB: Heat-cured PMMA incorporated with 3% by weight TiO_2NPs (6 each subgroup).
- Group II: Twelve samples of microwave-cured PMMA were fabricated and divided into two equal subgroups Group IIA: Microwave-cured PMMA and Group IIB: Microwave-cured PMMA incorporated with 3% by weight TiO_2NPs (6 each subgroup).
- The decision to use 3% of TiO_2NPs by weight was based on previous researches that utilized the same percentage to assess different characteristics of adding TiO_2NPs to PMMA denture base material (Azeez et al. 2021).

Characterization of PMMA

Groups I and II were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The degree of polymerization reaction of PMMA was assessed by Attenuated Total Reflection Fourier-Transform Infrared spectroscopy (ATR-FTIR).

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki in 1975. This study has been approved by the Medical Research Ethical Committee of the National Research Center, Cairo, Egypt with approval number 44311122022.

Materials

- Conventional heat-cured acrylic resin *(Acrostone; Acrostone dental factory—industrial zone Salam city A.R.E-WHW Plastic England).*
- Microwave cured acrylic resin *(Protechno; PoligonoEmpordaInternacional, Garrotaxa, Vilamalla Girona -Spain).*
- Dental plaster *(Elite Rock Stone- Zhermack Clinical- Italy).*
- Titanium Oxide Nanoparticles *(TiO2 NPs; Sigma Aldrich Company, St. USA).*

Methods

a) Fabrication of Heat-Cured PMMA Specimens (Group I):

Twelve specimens were fabricated 6 in each subgroup. A round metal ring measuring (50 mm in diameter and 0.05 mm in thickness) according to ADA specification NO.12) was invested into a dental plaster to have a standardized mold.

For Group IA, polymer and monomer (2:1 ratio) were mixed according to the manufacturer's instructions, when it reached the dough stage it was packed in the conventional dental flask. Then it was cured following a long heating cycle in the water bath curing unit for 30 min at 70 °C *(Water bath curing unit: Type 5518, KaVo EWL, Biberach, Germany).* After curing of PMMA, each flask was opened and the PMMA sample was finished and polished.

For Group IB, the Titanium Oxide Nanoparticles (TiO_2NPs) 3% by weight were added to the polymer powder of the heat-cured PMMA and mixed manually. Then the polymer was mixed with monomer and when it reached the dough stage it was packed in a dental flask, cured, finished, and polished as previously.

a) b. Fabrication of Microwave Cured PMMA Specimens (Group II)

Twelve samples were fabricated for group II and then divided into 2 equal subgroups (6 specimens per subgroup). For group IIA, Microwave cured acrylic resin *(Protechno; PoligonoEmpordaInternacional, Garrotaxa, Vilamalla. Girona -Spain).* The polymer and monomer (ratio 2:1) were mixed following the manufacturer's instructions. When it reached the dough stage, it was packed in a special flask *(Tecno-Flask, Protechno, Can Viloca-Spain).*The flask was inserted into the microwave oven for 3 min at 500 watts. After curing, each flask was opened and the PMMA samples were finished and polished.

For group IIB, TiO_2NPs 3% by weight was added to the polymer powder of the microwave PMMA and mixed manually. Then the polymer was mixed with monomer and when reached the dough stage it was packed in the specific flask, cured, finished, and polished as previously.

Characterization of PMMA

The microstructure of the heat and microwave-cured PMMA with and without TiO_2 NPs was examined by scanning electron microscopy and energy dispersive X-ray analysis *(SEM/EDX: a JSM-6060LV scanning microscope, JEOL, Peabody, MA)* in the Central Service Unit at National Research Centre. Changes in crystalline microstructure, together with the particle size and morphology of the prepared samples were identified.

Polymerization reaction and cross-linking of PMMA (ATR-FTIR measurements)

Three small pieces of every complete hard-set specimen from each subgroup (n = 6) were examined using the Spectrometer *(Bruker Optics Vertex 70 FT-IR spectrometer, Germany)* within 24 h from preparation. ATR-IR spectra were collected utilizing the attenuated total reflection unit (ATR-diamond crystal) in absorbance form, 64 scans, and 4 cm^{-1} resolution in the wave number range 4000–400 cm^{-1}. The ATR-IR spectra were recorded for the individual components (powder and liquid) of each material and the TiO_2NPs powder as well. The recorded spectrum for any specimen in this work was an average of three replicates.

Sample size calculation

The sample size was calculated by Software is P.S power version 3.1.6 depending on a previous study as a

reference (Ayaz et al. 2014). According to this study, the minimally accepted sample size was 6 per subgroup, when the response within each subject group was normally distributed with a standard deviation of 0.01, the estimated mean difference was 0.018 when the power was 80% and type I error probability was 0.05.

Statistical analysis

Statistical analysis was performed with SPSS 20, Graph Pad Prism, and Microsoft Excel 2016. All data were explored for normality by using the Shapiro–Wilk Normality test and presented as means and standard deviation (SD) values. An Independent t-test was used to compare the two groups. While the Way ANOVA test was employed to compare all groups.

Results

(A) SEM/EDX

SEM/EDX images (Fig. 1) revealed that TiO_2NPs were in the nanoscale range from 80 to 100 nm on the manufacturer's authority but it was agglomerated within the PMMA matrix of both groups. The agglomerated TiO_2NPs in the PMMA matrix appeared as bright areas (Hua et al. 2013).

Group IA had a non-homogenous mix of Heat cured PMMA and lofty entrapment of air, revealing a highly porous mix, while group IIA structure was more compact than group IA.

(B) Polymerization Reaction (ATR-FTIR Measurements).

An Independent t-test was used to compare the degree of polymerization between the subgroups (IA and IB) of heat-cured PMMA (group I) with and without TiO_2NP. Data presented that adding TiO_2NPs decreased the degree of polymerization which was insignificant as $P > 0.05$ as presented in Table 1 and Fig. 2.

Table 1 Degree of polymerization for all groups and subgroups

	N	Group I (heat cured PMMA)		Group II (microwave cured PMMA)	
		M	SD	M	SD
Subgroup A (control)	6	44.65	4.03	41.99	9.53
Subgroup B (with TiO_2 NPs)	6	43.79	2.95	41.52	0.95
P value		0.68		0.91	

N—Count M—mean SD—standard deviation

The microwave-cured PMMA (group II) displayed also an insignificant difference between the two subgroups (IIA and IIB) with and without TiO_2NPs as $P > 0.05$ as presented in Table 1 and Fig. 2.

One Way ANOVA test was utilized to compare all groups and subgroups. Data revealed that there was a decline in the degree of polymerization, but it was of insignificant difference as $P > 0.05$ as presented in Table 2.

Discussion

Polymethylmethacrylate (PMMA) is the most used material in the field of prosthodontics. But it shows weak physical and mechanical properties. Many experiments have been undertaken to improve the strength of PMMA to prevent fracture and clinical failure. Among these approaches is adding TiO_2NPs to the denture base material since it increases the surface hydrophobicity, reduces the adherence of biomolecules, aids in coloring, has antimicrobial properties, and improves the mechanical properties of PMMA resins (Azeez et al. 2021).

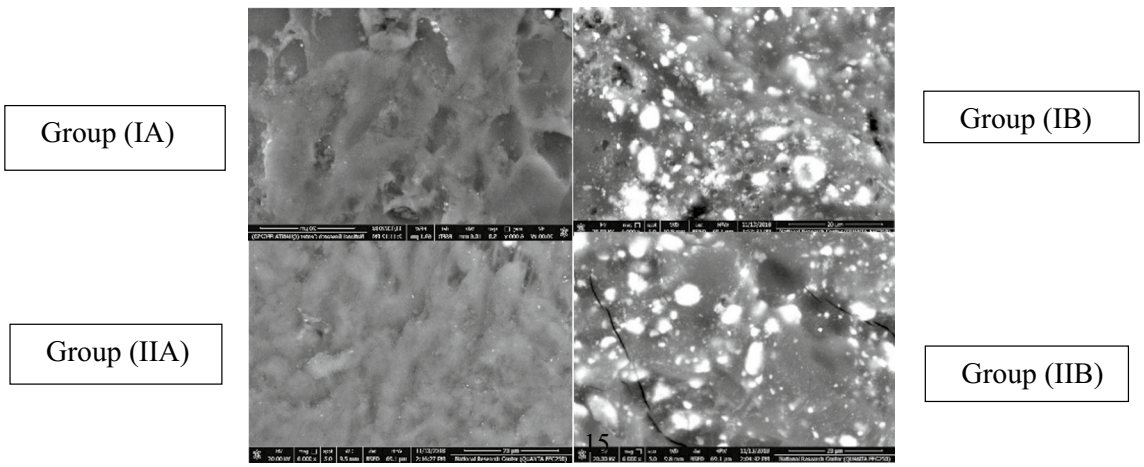


Fig. 1 SEM/EDX images for all studied groups

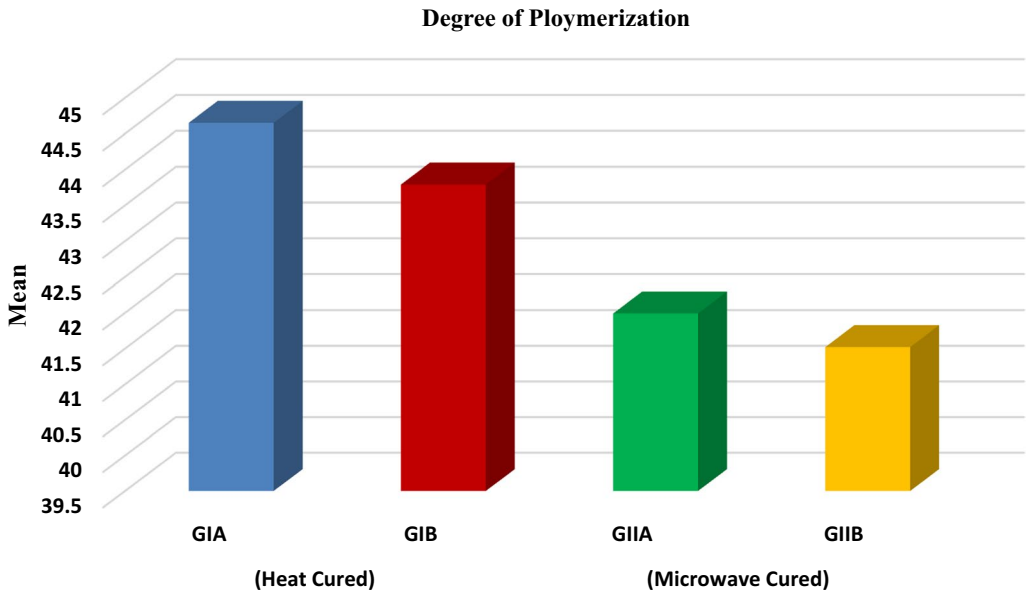


Fig. 2 Bar chart representing the mean degree of polymerization in all groups

Table 2 Comparing the degree of polymerization between all groups and subgroups

	Subgroups	Min	Max	M	SD	P value
Group I Heat cured PMMA	IA (Control)	41.22	51.57	44.65	4.03	0.72
	IB (WithTiO_2 NPs)	42	47.21	43.79	2.95	
Group II Microwave cured PMMA	IIA (Control)	29.45	52.62	41.99	9.53	
	IIB (WithTiO_2 NPs)	40.57	42.47	41.52	0.95	

Min—minimum Max—maximum. M—mean SD—standard deviation

The SEM/EDX and ATR-FTIR were used to assess and relate the impact of adding 3% by weight TiO_2NPs on the polymerization reaction and microstructure of the PMMA in both heat and microwave-cured acrylic resin denture base materials.

SEM/EDX was employed in this study as SEM reveals the surface morphology, irregularities, and supplementary imperfections of TiO_2NPs mixed with PMMA powder, while EDX evaluated the distribution of TiO_2NPs in specimens and fillers integration within the polymer matrix (Alrahlah et al. 2018; Trapalis et al. 2003; Chowdhury et al. 2021).

For group IB and group IIB; the TiO_2NPs appeared as bright areas in the matrix of both groups. Both groups showed organic impurities and minute internal cracks, but group IIB showed higher organic impurities due to expanding curing temperature of the microwave-cured PMMA.

ATR-FTIR spectrometer was employed in this research. Due to its sensitivity, extreme wavenumber accuracy, and its speed high-resolution scan (0.1 ~ 0.005 cm 1). (Chowdhury et al. 2021).

The ATR-FTIR spectra of the hard-set materials of group I and group II and their subgroups with and without the addition of 3% TiO_2NPs together with their components; powder and liquid were recorded within 24 h from setting presented in Figs. 3 and 4, respectively.

The final presented spectrum of each inspected sample was the average of three replicate spectra recorded from three dissimilar measurements. The spectra display the typical absorption bands of the PMMA molecular composition; two medium-intensity broad peaks around 2950 cm^{-1} of the C–H, and a strong peak around 1440 cm^{-1} are assigned to C–C stretching vibrations. Two bands that appear strongly intense around 1720 cm^{-1} and 1148 cm^{-1} are specific to C=O and C–O stretching vibrations, respectively. The key absorption band in the polymerization process of the vinyl C=C group appears at 1638 cm^{-1} with medium peak height (Jawad et al. 2016). The spectral features before and after curing reveal no remarkable change except the decrease in peak height at 1636 cm^{-1}. The addition of the TiO_2NPs

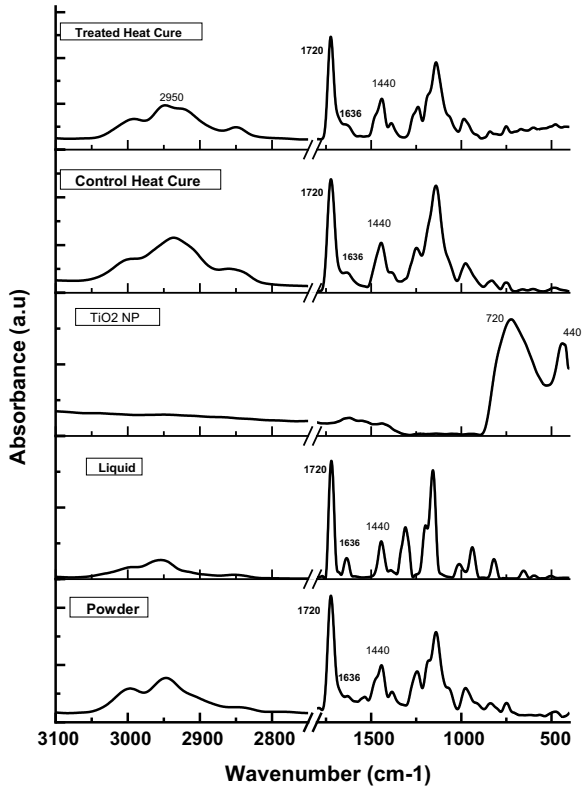


Fig. 3 ATR-FTIR spectra of heat-cured denture individual components, control, and TiO_2 NPs treated complete set specimens

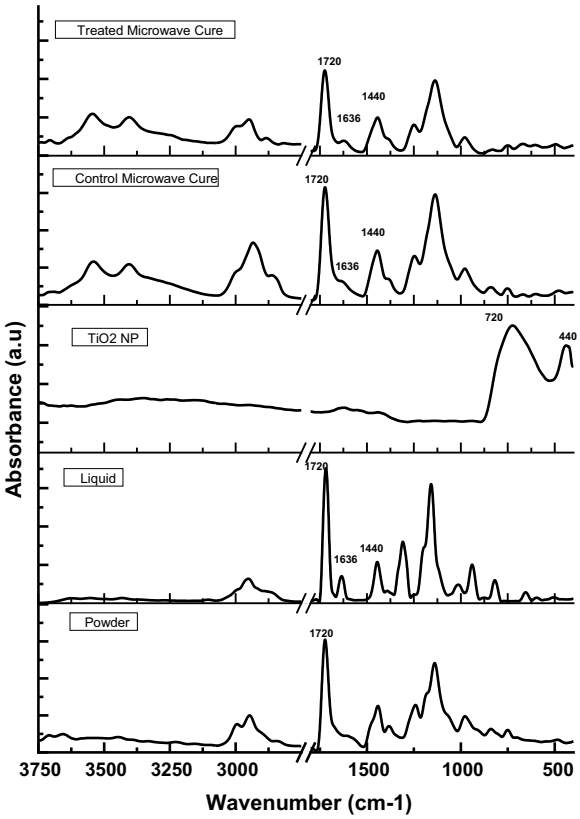


Fig. 4 ATR-FTIR spectra of microwave-cured denture individual components, control, and TiO_2 NPs treated complete set specimens

produced no observable effect on the characteristic absorption peaks of the polymerized resins. Relying on the quantitative analysis of the FTIR spectra, the polymerization rate of the hard-set resin was evaluated by using the absorbance ratio of the peak height (H) of the reacting aliphatic C=C group situated at 1636 cm^{-1} to that of non-reacting C–C group at 1440 cm^{-1} as an internal reference. The percentage degree of polymerization reaction (%DP) was calculated via the following equation (Khalil et al. 2007).

$$\%DP = \left(1 - R_p/R_m\right) \times 100$$

where R_p and R_m are the infrared absorbance ratio (H1636/H1440) of the hard-set polymer and the monomer, respectively.

All calculated values of the %DP for group I and group II, control and treated subgroups revealed that the addition of 3% TiO_2NPs to the powder component of the resin either heat or microwave cured slightly reduced the polymerization degree by 1.93% for group I and 1.12% for group II. This may be owed to that the amount of titanium powder added slightly decreased the chance of direct contact between the liquid and the powder molecules.

TiO_2NPs neither affected the chemical structure nor the polymerization mechanism of the used denture materials apart from a slight decrease in the %DP. This can be attributed to its nature of being a photocatalytic agent and not a thermo-catalytic one (Macwan et al. 2011). Since the incorporated TiO_2NPs appeared to act just as filler without any chemical bonding with the PMMA, it may play a role in decreasing the curing shrinkage of the thermoset (Fernàndez-Francos et al. 2013).

Nazirkar et al. (Nazirkar et al. 2014) reported that adding TiO_2NPs could interfere with the polymerization process, but they did not explain the possible mechanism. While Tandra et al. (Tandra et al. 2018) spotted that the formation of greater quantities of TiO_2NPs agglomerates affects the polymerization reaction. And the increased concentration of TiO_2NPs increases the amount of residual monomer which acts as a plasticizer (Sodagar et al. 2013). This plasticizing effect reduces the monomer conversion rate, which affects the polymerization rate (Shibata et al. 2007).

Limitations of the study

There were a few limitations faced during accomplishing the current study as; the manual integration of TiO_2NPs into the polymer powder of the PMMA. Another difficulty was the acquisition of a specialized device that is microwave safe.

Conclusions

Within the limitation of this laboratory study, it could be concluded that:

The addition of 3% by weight TiO_2NPs did not affect the degree of polymerization of heat and microwaved cured TiO_2NPs/PMMA composite. Therefore, the denture can obtain all the privileges of the TiO_2NPs as strengthen nanofiller with no impact on their chemical microstructures.

To reach the clinical application stage of TiO_2NPs/PMMA composite, future studies that utilize different concentrations of TiO_2NPs and more PMMA samples are recommended. Also, the antimicrobial properties and thermal coefficient of both types of TiO_2NPs/PMMA composite could be studied.

Abbreviations

TiO_2NPs	Titanium Oxide Nanoparticles
PMMA	Polymethyl methacrylate
MMA	Methyl Methacrylate
NPs	Nanoparticles
EM	Electron Microscopy
SEM	Scanning Electron Microscopy
EDX	Energy Dispersive X-ray analysis.
ATR-FTIR	Attenuated Total Reflection Fourier-Transform Infra-Red Spectroscopy

Acknowledgments

Not applicable.

Author contributions

All authors made substantial contributions to conception and design of the research. I.E, E.A, M.A, and D.W participated in the study design, dental practical work, scientific writing, and revising of the manuscript. K.S assessed the degree of polymerization and morphological characterization of PMMA. All authors have read and approved the manuscript.

Funding

This research received no external funding.

Availability of data and materials

Data are available upon request.

Declarations

Ethics approval and consent to participate

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki in 1975. This study has been approved by the Medical Research Ethical Committee of the National Research Center, Cairo, Egypt with approval number 44311122022.

Consent for publication

Was not applicable.

Competing interests

The authors declare no competing interest.

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Received: 22 April 2023 Accepted: 8 June 2023
Published online: 26 June 2023

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