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Influence of Antimicrobial Nanoparticles on Flexural Strength and Hardness of Polymethylmethacrylate

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Abstract

BACKGROUND: Polymethylmethacrylate (PMMA) is commonly used for dental appliances but has several shortcomings that could benefit from improvement with the use of nanoparticles (NPs).

AIM: The purpose of this study was to modify PMMA with three different antimicrobial NPs; Graphene oxide nanosheets (nGO), Titanium dioxide NPs (TiO2 NPs) and curcumin (CUR)-loaded graphene oxide nanosheets alone, and in combination and assess the flexural strength and hardness of the different groups.

MATERIALS AND METHODS: The material used in this study was chemically cured PMMA that was modified with nGO, TiO_2 NPs and GOCUR alone and in combination to give 6 groups; Group A: PMMA, Group B: PMMA with nGO, Group C: PMMA with TiO_2 NPs, Group D: PMMA with TiO_2 and GO NPs, Group E: PMMA with GOCUR, and Group F: PMMA with TiO_2 NP, and GOCUR. The Six groups were tested for flexural strength and hardness. Statistical analysis was and data were expressed as means and standard deviation. Data was explored for normality using the Kolmogorov-Smirnov test of normality. The ANOVA test was used to compare between groups, followed by Bonferroni's *post hoc* test for pairwise comparison. The significance level was set at $p \leq 0.05$.

RESULTS: The highest flexural strength was recorded in Group C (52.26 ± 5.48 MPa) and the lowest value was in Group A (24.94 ± 5.37 MPa). The highest hardness was recorded in Group F (23.29 ± 0.8 HV) and the lowest value was in Group A (15.88 ± 1.02 HV).

CONCLUSION: The modification of PMMA with NPs with proven antimicrobial activity can increase the flexural strength and hardness of the material. GO, TiO_2 and, GOCUR NPs were each used alone and in different combinations, and all the groups displayed higher flexural strength and hardness than the unmodified PMMA.

Introduction

Polymethylmethacrylate (PMMA) is a popular and versatile dental material used mainly for removable dental appliances [1]. Its wide use and acceptance in dentistry are due to its excellent esthetics, low cost, availability, ease of processing, and repair [2]. Although, PMMA displays desirable properties several shortcomings have created high demand for the development of a modified version of the material.

One shortcomings of PMMA is that its contamination and accumulation of microbes from the oral environment adversely affects the health of patients [2], [3]. Several attempts have been made at improving the properties of PMMA and giving it antimicrobial characteristics through different additions, including the use of nanomaterials [2], [3], [4].

Using nanoparticles (NPs) is helpful in creating an antimicrobial PMMA and combating the potential problem of microbes adhering to dental appliances [1]. NPs combined with polymers such as PMMA were found to exhibit superior antimicrobial properties in the oral cavity [5].

Despite the importance of developing a PMMA with antimicrobial resistance, it is crucial not to compromise the mechanical properties of the appliance. Mechanical properties are important for the evaluation and determination of the longevity, and quality of service provided by an appliance. Higher mechanical properties will allow the resistance of; uneven masticatory forces present in the oral cavity, scratches, wear, and sudden fractures [4], [6], [7].

The purpose of this study was to modify PMMA with three different antimicrobial NPs; Graphene oxide nanosheets (nGO), Titanium dioxide NPs (TiO_2 NPs), and Curcumin (CUR) loaded graphene oxide nanosheets (nGOCUR) alone, and in combination, and assessing the flexural strength and hardness of the different groups. The modified groups were compared to the unmodified PMMA and each other.

Material

Chemically cured acrylic resin material composed of PMMA powder and methylmethacrylate liquid (Acrostone, Egypt) was used as the polymeric base of the denture base material. Titanium (IV) isopropoxide, 97% (Alfa Aesar), Graphite powder (extra pure, TM Media, India), and CUR (crystalline 99%, Loba Chemie) were prepared into nanomaterials and used as inorganic fillers to modify the denture base material. The inorganic fillers were prepared in Nano Gate lab, Cairo, Egypt.

Preparation of the inorganic fillers.

Graphene oxide nanosheets (nGO)

The improved hummer method was used to prepare the GO nanosheets. $KMnO_4$ was added slowly in 6 equal portions to a 9:1 mixture of concentrated H_2SO_4/H_3PO_4 and graphite flakes, producing a slight exothermic reaction (35−40°C). The reaction mixture was then heated to 50°C and stirred for 12 h and then it was cooled to room temperature and poured onto ice containing 30% H_2O_2. It was then neutralized by multiple washings with distilled water and then centrifugated at 4000 rpm for 4 h resulting in supernatant material that was decanted. The residual material left behind was washed in sequence with 200 ml distilled H_2O 3 times, 200 ml of 30% HCl and 200 ml of ethanol 2 times. The solution was then filtered using the U.S. Standard testing sieve and then filtered through polyester fiber membrane and the obtained product was dried in vacuum for 48 h at 45°C [8].

Titanium Dioxide (TiO2) Nanoparticles

TiO_2 NPs were prepared by hydrolysis of titanium (IV) isopropoxide in propan-2-ol at 80°C for 4 h. The colloidal suspensions were adjusted to low pH using nitric acid (HNO_3) and then additionally heated at 80°C for 24 h. The final form was a powder of spherical particles with an average size of 15 ± 3 nm constituting 95–97% anatase and 3–5% brookite [9], [10].

Curcumin Loaded Graphene Oxide Nanosheets (nGOCUR)

The loading of CUR on nGOs was carried out by mixing GO in ddH_2O at a concentration of 2 mg/ml with 2 ml CUR dissolved in ultrapure ethanol at a concentration of 300 μg. The samples were incubated at 70°C for 12 h for complete ethanol evaporation [11], [12].

The inorganic fillers were mixed with the acrylic resin powder of PMMA using a mixing mill for 15 min to get a homogenous distribution of the phases [13].

Table 1: PMMA and its modifications

Group	Composition
A	PMMA
B	PMMA 50 g/GO 0.001 g
C	PMMA 50 g/TiO_2 0.5 g
D	PMMA 50 g/TiO_2 0.5 g/GO 0.001 g
E	PMMA 50 g/GOCUR 0.001 g
F	PMMA 50 g/TiO_2 0.5 g/GOCUR 0.001 g

(Table 1) shows the different groups prepared in the study with the amount in grams of PMMA and inorganic fillers in each group.

Methods

Sample size calculation

Sample size calculation was performed using the G-power test and ANOVA test was used for comparison according to a previous study by Zidan *et al*. in 2019 [14].

In the flexural strength test, a total of 42 samples (7 in each group) were sufficient to detect a large effect size of 0.65 with a power of 0.8 and a $p \leq 0.05$. In the hardness test, a total of 36 samples (6 in each group) were sufficient to detect a large effect size of 0.7 with a power of 0.8 and a $p \leq 0.05$.

Specimen preparation

A total of 78 specimens were prepared for the tests conducted. The molds were made from an aluminum alloy with cavities with the dimensions of 65 mm (length) × 10 mm (width) × 2.5 mm (depth). The powder in each group was mixed with the liquid monomer according to manufacturers' instructions, mixing was continued until a consistent mixture was obtained. When the mixture reached the dough stage, it was packed inside the molds.

Before pouring the mixture into the mold, a separating medium of sodium alginate was applied to the mold for easy separation. The mix was left to set completely and then removed from the mold [14].

Flexural strength test

The flexural strength (MPa) of the specimens was evaluated using the 3-point bending test in a universal testing machine (Model STM-50, Santam, Tehran, Iran) with a cross-head speed of 5 mm/min. The 42 specimens (7 in each group) were stored in distilled water at room temperature for 10 days and then retrieved and placed on supporting jigs 40 mm apart. A loading force was applied using a centrally located plunger with a diameter of 20 mm and the maximum load exerted on the specimens until fracture was recorded. The flexural strength was calculated as $F=3PL/2bd^2$, where F is the

flexural strength, P is the applied load, L is the support span length, b is the sample with and d is the sample thickness [6], [14].

Hardness test

A total of 36 specimens (6 in each group) were evaluated using a fully automatic Vicker hardness ($HV_{0.05}$) testing machine (Tukon 1102, Wilson, Buehler, Germany). The indenter had a square-based diamond pyramid and the value of the load was fixed at 50 g for 10 s. The load was applied smoothly, without impact, and after removal, the indentation was observed with a magnifying lens and the two impression diagonals were measured (to the nearest 0.1-μm) and averaged. The Vickers hardness (HV) was calculated as $HV = 1854.4L/d^2$, where L is the load and d is the averaged diagonals [6], [14].

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows. The data were expressed as means and standard deviation and it was explored for normality using the Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of the data were normally distributed (parametric data), so one-way analysis of variance ANOVA test was used to compare between groups, followed by Bonferroni's post hoc test for pairwise comparison. The significance level was set at $p \leq 0.05$.

Results

Flexural strength

The highest mean value for flexural strength (MPa) was recorded in Group C (52.26 ± 5.48 MPa) and the lowest value was in group A (24.94 ± 5.37 MPa). There was a statistically significant difference between all tested groups (p = 0.00) (Table 2).

Microhardness

The highest mean value for microhardness (HV) was recorded in Group F (23.29 ± 0.8 HV) and the lowest value was in group A (15.88 ± 1.02 HV). There was a statistically significant difference between all tested groups (p = 0.00) (Table 3).

Table 2: Mean flexural strength (MPa) of each group and comparison between them

Group	Mean	Standard deviation	p
A	24.94^{d}	5.37	0.000*
B	$35.54^{b,c}$	4.26	
C	52.26^{a}	5.48	
D	43.29^{b}	6.46	
E	42.58^{b}	3.12	
F	$31.59^{c,d}$	4.80	

Significance level p < 0.05, *Significant. Bonferroni post hoc test for pair-wise comparison, the means sharing the same superscript letter are not significantly different.

Table 3: Mean microhardness (HV) of each group and comparison between them

Group	Mean	Standard deviation	p
A	15.88^{d}	1.02	0.000*
B	$19.07^{b,c}$	0.89	
C	18.45^{c}	1.04	
D	18.13^{c}	0.63	
E	20.32^{b}	0.34	
F	23.29^{a}	0.80	

Significance level p < 0.05, *Significant. Bonferroni post hoc test for pair-wise comparison, the means sharing the same superscript letter are not significantly different.

Discussion

NPs range in size between 1 and 100 nm and have very promising antimicrobial activity. This activity is attributed to its small size, large surface area to volume ratio, and increased chemical reactivity. NPs are incorporated in dental materials because their large surface area and high charge density enable them to interact with bacterial cells that are negatively charged causing antibacterial activity [1]. The addition of NP as fillers also influences other properties of the modified material including its mechanical properties.

PMMA is an acrylic resin that is commonly used when fabricating an appliance's denture base. These resins could benefit from the application of NPs to improve their properties especially mechanically. A study by Gad *et al.* in 2016 found that the addition of NPs improved the mechanical and physical properties of acrylic resin [7].

In this study, different metal oxide NPs with proven antimicrobial effects were added to chemically cured PMMA to avoid thermal damage to the fillers during heat-induced polymerization. The flexural strength and hardness of the modified PMMA (Group B, C, D, E, and F) were tested and compared to unmodified PMMA (Group A) and each other. These two mechanical properties were tested because low flexural strength is one of the major causes of denture base failure while low scratch resistance can lead to scratches which can accumulate plaque and weaken the base [3].

nGOs were used as fillers in the PMMA because of their biocompatibility, high mechanical properties, and proven antimicrobial activity against oral bacteria and fungus [15], [16], [17]. A study by Lee *et al.* in 2018 incorporated nGO into PMMA and found that it roughened its surface and increased its hydrophilicity without compromising its flexural strength and surface hardness. The nGO incorporated PMMA was tested against 4 microorganisms commonly found in the oral environment and was found to have an antimicrobial effect against all four species [3].

In this study, the addition of nGO to the PMMA (Group B) increased its flexural strength and hardness

significantly compared to the unmodified PMMA. This is due to the higher elastic modulus of the nGO which reinforced the PMMA [18]. Despite the low percentage of nGO dispersed in the PMMA, it could increase the mechanical properties significantly by reducing stress concentration in the matrix [19].

TiO_2 has been incorporated in various biomaterials including composites because of their antimicrobial effectiveness and the ability to produce self-cleaning dental materials. A study by Sodagar *et al.* in 2016 assessed the effectiveness of nanotitanium dioxide and nanosilicone dioxide against cariogenic bacteria and they were found to show strong antimicrobial activity [20].

In this study, the group with the TiO_2 NPs added to PMMA (Group C) showed a significant increase in flexural strength and hardness. The results could also be due to an increase in the number of bonds between the matrix and the fillers which require more energy to break increasing the mechanical properties over all of the modified PMMA [21].

A study by Hashem *et al.* in 2017 found that the modification of PMMA with 1%, 2%, and 3% TiO_2 NPs resulted in increased hardness values of the tested material by 20%, 30% while the flexural strength increased by 95%. This increase could be due to the NPs acting as rigid bodies in the PMMA matrix, therefore increasing the stiffness and reducing the mobility of the matrix [22].

In this study both nGO and TiO_2 NPs were added each on their own (Group B and C) and together (Group D) to PMMA. All 3 groups gave a modified material with higher flexural strength properties and surface hardness compared to the unmodified materials.

CUR is a low molecular weight polyphenol with antimicrobial effects and antifungal potential. The problem with the clinical use of CUR is its insolubility in water, limiting its bioavailability and stability. To improve its properties it should be encapsulated in a drug delivery system [23].

A study by Sodagar *et al.* in 2016, 1% CUR NP was added to orthodontic composite and the antimicrobial effect and shear bond strength were assessed. It was found that the composite had significant antimicrobial activity against cariogenic bacteria and there was no adverse effect on the shear bond strength. The major drawback to the use of the CUR NPs was their insolubility [24].

In this study nGO, is used as the drug delivery system for CUR and added to PMMA (Group E). As far as we know this is the first study to modify PMMA by incorporating nGOCUR. This modification could be very beneficial because denture base materials could positively benefit from having a drug with antimicrobial potential. After testing the mechanical properties of this group it was found that it was still significantly higher than PMMA and also higher than the nGO without the CUR. The addition of the drug to the nGO positively affected the strength properties.

Another modification was also tested in this study both TiO_2 NP and nGOCUR were added to PMMA (Group F). This combination of NPs reduced the flexural strength of the PMMA where it was the lowest of all the modified groups and although it was higher than the unmodified PMMA (Group A) the increase was not significant. On the other hand, the hardness in this group was found to be significantly the highest among all the groups. This could mean that the combination of NPs increased the surface bond strength of the particles but negatively affected the bulk of the specimen. It could also be due to excess air bubbles in the body of the specimen during the mixing of the polymer powder and liquid.

Conclusion

Within the limitations of this study, it can be concluded that the modification of PMMA with NPs with proven antimicrobial activity can increase the flexural strength and hardness of the material. GO, TiO_2, and GOCUR NPs were each used alone and in different combinations, and all the groups displayed higher flexural strength and hardness than the unmodified PMMA. This increase in mechanical properties could be because of the higher modulus of elasticity of the NPs or an increase in the number of bonds between the matrix and the fillers. No direct relationship was found between increasing the number and types of NPs and an increase in both flexural strength and hardness.

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