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Preparation, Characterization and Evaluation of a Poly Methyl Methacrylate Bone Cement Modified by Montmorillonite Loaded with Silver Nano Particles

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ABSTRACT

Purpose: The aim of this study was to prepare and characterize poly methyl methacrylate (PMMA) composite bone cement filled with montmorillonite (MMT) and loaded with silver nanoparticles (AgNPs). **Materials and methods:** AgNPs were synthesized by the chemical reducing method. Characterization was done using Field emission scanning electron microscope (FE-SEM), X-ray diffraction analysis (XRD) and Thermogravimetric analysis (TGA). Two and four weight percentages of MMT powder and the prepared MMT/AgNPs powder were added to the bone cement. A total of 75 PMMA bone cement specimens were prepared and divided into 5 groups (control group, PMMT/MMT 2%, PMMT/MMT 4%, PMMT/MMTAgNps 2% and PMMT/MMTAgNps 4%). Then they were tested regarding the compressive strength, flexural strength and antibacterial activity. **Results:** SEM images showed the layered MMT with light spots indicating presence of AgNPs that was confirmed by XRD; while TGA showed its thermal stability. Compressive strength results revealed statistically insignificant difference between the control and the tested groups while the 4% PMMA/MMT showed statistically significant higher values than the 2%. Statistically

KEYWORDS

Polymethyl methacrylate, silver nanoparticles, montmorillonite.

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insignificant difference in flexural strength results were found between the 2% and 4% concentrations within the PMMA/MMT/AgNPs group, and within the PMMA/MMT group. Antibacterial results revealed statistical significant differences in all the tested groups ($p<0.001$). **Conclusion:** MMT could be used as a controlled delivery system to hold AgNPs that proved to have antibacterial effect when added to PMMA cement. Addition of 4% MMT to PMMA bone cement improved its compressive strength.

INTRODUCTION

Filling gaps between metallic joint prostheses and the host bone requires efficient grouting agent like acrylic bone cement. Acrylic bone cements proved to be successful in restoring facial malformations, defects in mandibular and chin bone and in repairing orbital defects [1]. In 1943, PMMA bone cement was used for the first time to close cranial defects as well as for dental fixatives and fixtures. In the United States (1970), the Food and Drug Administration; permitted the use of bone cement technology [2].

One of the major causes for implant failure is the lack of its primary stability, together with loss of surrounding bone, infection, and overloading. Thus, PMMA bone cement is used to provide the needed initial stabilization and fixation of the artificial implant to hard tissues [3].

Despite its admirable record of performance; bone cement weakness was found to be the result of fatigue failure by cracking or impact failure. This type of failure can lead to implant loosening and revision surgery [4]. Being a brittle material its tensile strength (24–50 MPa) is significantly lower than its compressive strength (73–120 MPa) [5].

Attempts to bridge early fatigue cracks and stop their propagation was tried through loading bone cements with different filler types (carbon fibers, glass fibers, polyethylene fibers, long macroscopic and stainless steel fibers). Clays also were found to be successful fillers in polymer matrix due to their characteristic lamellar morphology and high aspect ratio [6]. Montmorillonite (MMT) with its nanoscale layered silicates characteristics when incorporated into polymers; improved their mechanical, thermal, and the marginal properties of the tested polymers [7].

Despite stringent antiseptic operating measures, the patient is at danger of developing a deep wound infection. Infections of this type are rarely cured without the removal or revision of the affected implant. Gram-positive staphylococcus aureus causes the majority of implant-associated infections [8].

Antibiotics administered systemically are ineffective in obtaining high local tissue drug concentrations, and large systemic doses of antibiotics can result, posing organ toxicity risks such as hearing and kidney damage. As a result, impregnating the bone cement with powdered antibiotics such as gentamicin or vancomycin is another option [9,10]. The development of non-antibiotic antimicrobial treatments is critical since the widespread use of bone cement loaded with antibiotic may contribute to the emergence of resistant bacterium strains, and antibiotic action is also short-lived [11,12].

When silver ions are introduced into bone cement, they have antibacterial properties without affecting the biomaterial's cytotoxicity. Silver nanoparticles was incorporated in removable denture base acrylic resins, in direct resin composite restorative materials, and was tried in root canal irrigating solutions, obturation materials, adhesives used in orthodontic, periodontal membrane, and with coatings of dental titanium implant [13].

When employed alone, agglomeration between pure nanoparticles is a prevalent issue. Maintaining the nanoparticles on the external surfaces and/or within the interlamellar gaps of clay during preparation of nanoparticles is one of the most successful strategies for overcoming agglomeration [11].

Therefore, the aim of this study work was to investigate the effect of the addition of the silicate layered structure, montmorillonite, loaded with silver nanoparticles on the mechanical performance and antibacterial activity of poly methyl methacrylate bone cement.

MATERIALS AND METHODS

A commercially available PMMA surgical bone cement (Laboratorios SL S.A San Fernando-Argentina), sodium montmorillonite nanoclay powder NaMMT was purchased from Sigma-Aldrich (USA), silver nitrate $AgNO_3$ used as silver precursor was obtained from Merck (Germany), sodium borohydride $NaBH_4$ used as reducing agent (Sigma-Aldrich, USA). All the aqueous solutions were prepared in double distilled water.

Ethical Committee:

This research work got the approval of the ethical committee of Faculty of Dental Medicine, Girls Branch, Al-Azhar University. The Ethics Code: REC-MA-21-01.

Specimens' Grouping:

A total of 75 PMMA bone cement specimens were prepared for this study. Specimens were divided as shown in table (1).

Specimens' preparation:

A- Preparation of nano-silver loaded MMT particles

Silver nanoparticles (Ag NPs) were prepared using the chemical reducing method. The $AgNO_3$ was used as a silver precursor together with sodium boron hydride (NaBH4) as a reducing agent. All the aqueous solutions were prepared in double distilled water [8]. The MMT (75g) was suspended into (500ml) solution containing 7.8g silver nitrate $AgNO_3$ and the suspension was kept under stirring for 24 h at room temperature. Then a freshly prepared 0.18 mole (6.97 g) of $NaBH_4$ was poured into the suspension and stirring was continued for about 1 hour to complete the cation exchange according to the following equation [8]:

$$Ag+MMT + BH4^{-}+ 3H2O \rightarrow Ag°/MMT + B(OH)_3+ 3.5\ H_2$$

Afterwards, centrifugation of the suspension was done at 15,000 rpm for 20 minutes, the precipitate was washed twice with distilled water to eliminate the silver ion residue then dried overnight in an oven at 50ºC.

B- Characterization of the prepared nano-silver loaded MMT powder:

The prepared powder was characterized as follows:

1. Field emission scanning electron microscope (FE-SEM):

Investigation was performed using the Quanta FEG 250 (FEI, UK) to study the morphology of MMT/Ag nanocomposites.

2. Powder X-ray diffraction analysis(XRD):

XRD analysis was employed using diffractometer (PANalytical EMPYREAN, UK) with a curved position sensitive detector PSD (reflection mode, using Cu-$K_{\alpha 1}$ radiation), allowing continuous data collection over 120° to 0°with 0.026° steps in 2-theta. With constant conditions (45 KV, 30 mA) at 25°C diffraction patterns.

Table (1)*: Specimens grouping*

Material / Testing method	PMMA (control)	PMMA + MMT		PMMA +MMT + Ag NPs		Total
		2wt%	4 wt%	2wt%	4 wt%	
Compressive Strength	5	5	5	5	5	25
Flexural Strength	5	5	5	5	5	25
Antibacterial Activity	5	5	5	5	5	25
Total	15	15	15	15	15	75

3. Thermogravimetric analysis (TGA):

The thermal reduction properties were explored by using SDT Q600 V20.9 Build 20 thermal gravimetric and differential thermal analyzer (USA) with a constant heating rate of from 28°C to 1000°C under air atmosphere

C-Preparation of PMMA bone cements composites:

Two different weight percentages (2 wt% and 4 wt %) of both the MMT powder and the previously prepared MMT loaded with AgNPs were added to the bone cement powder used in this study.

All the cement specimens were prepared according to manufacturer's instructions. The powder was manually mixed with the liquid monomer at a 2:1 P/M ratio for 30 sec at 25ºC ±1. The homogenous dough obtained was then kept for about 2-4 min (depended on the sample) to reach the sticky state. In this step each mix was poured into the specially prepared molds for further testing.

D-Mechanical testing:

1. Compressive strength:

A total of 25 cylindrical specimens were used for this test. The mix was poured into a specially designed metallic mold 6 mm internal diameter and 12 mm in height. The polymerized material was then removed from the mold, and the test was performed using a universal testing machine (UTM) (SHIMADZU 5 KN, AUTOGRAPH AG_XPLUS, Japan) at cross head speed of 2 mm/min [4]. The compressive strength was calculated by the UTM software (Trapezium X) [14].

2. Flexural strength:

A total of 25 bar shaped specimens 60 x 10 x 3 mm in dimension were prepared using a specially designed metallic mold. The Specimens were subjected to three-point bend test using a universal testing machine (LLOYD INSTRUMENTS LR5K, UK) at cross head speed of 2 mm/ min on span length 46 mm; test proceeded until fracture.[15] The maximum loads were obtained and the flexural strength was calculated (MPa) using the following equation [15]:

Flexural strength: $\sigma = 3F_{max}.l / 2bh^2$

Where F_{max} is the maximum load recorded before fracture in N,

l is the span between the supports

b is the width of the specimen in mm

h is the height of the specimen in mm

E- Antimicrobial Assessment:

Circular discs (10 mm diameter and 2 mm thickness) of the control and experimental bone cement specimens were tested against staphylococcus aureus a gram positive bacterium which is the main causative bacteria of osteomyelitis. The antibacterial activity of the tested specimens was evaluated according to the disc diffusion method with the determination of the diameter of inhibition zones in millimeters [11].

Statistical analysis

The mean and standard deviation values were calculated for each group in each test. Normality of data was explored using Shapiro-Wilk tests and Kolmogorov-Smirnov. In non-related samples; independent sample t-test was used to compare between two groups. In more than two groups in non-related samples, one-way ANOVA followed by post hoc Tukey test was used to compare between them. The interactions between different variables were tested using two-way ANOVA test. The significance level was set at $P \leq 0.05$. IBM® SPSS® Statistics Version 20 for Windows was used for statistical analysis.

RESULTS

1. Characterization of the prepared MMT-AgNPs powder:

1.a. SEM examination :

Results of the SEM images, showed a layered flake morphology which is typical of the MMT structure while the light spots indicate silver particles in the nanometric range, well dispersed over the surface and in between the individual layers of MMT with diameter from 13 up to 95 nm (Fig. 1).

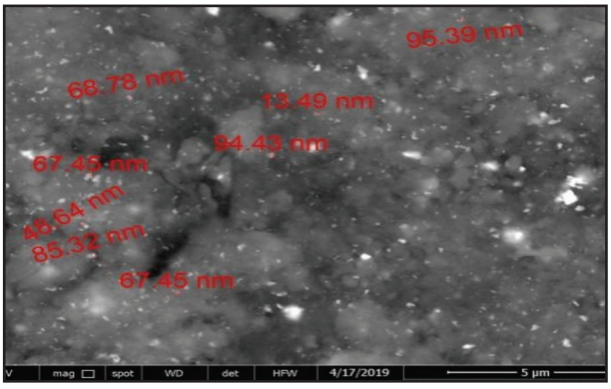

Figure (1): Scanning electron micrograph for the prepared MMT-AgNPs powder

1.b. XRD analysis :

XRD analysis is shown in Figure (2). The peak found at 6.9° is assigned to an interlayer distance (d_{001}-spacing) of 1,27 nm, distinctive of MMT crystalline structure [16].

Furthermore, all possible diffractions corresponding to presence of AgNPs, namely, at 38.13° revealing 113 plane, in addition to another one at 44.63° indicating 222 plane, are also found, which supports the formation of a hybrid composite material comprising the MMT as a base loaded by AgNPs, in particular that the d_{001}-spacing is significantly expanded with respect to its original state [17].

1.c. TGA results:

The thermodegradative behavior of the prepared composites under air atmosphere is shown in figure (3). Initial weight loss appeared at 90°C with no significant weight loss detected up to 600°C.

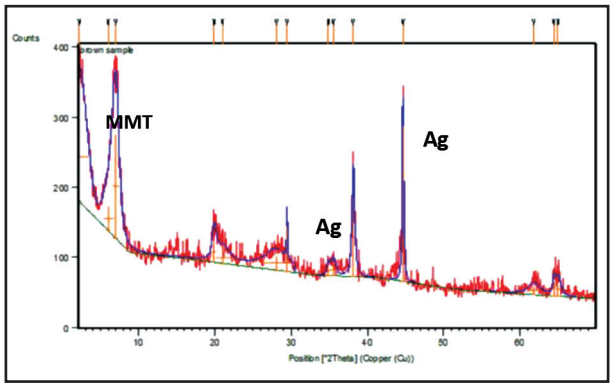

Figure (2): XRD pattern of MMT loaded by AgNPs

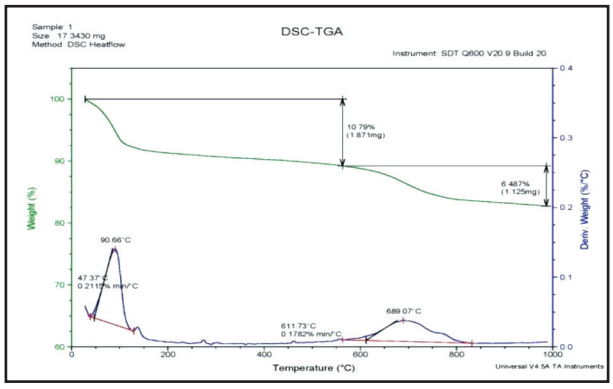

Figure (3): Thermogravimetric traces of MMT-AgNPS hybrid

2. Mechanical testing

2.a. Compressive strength:

Table (2 and 3), and figure (4) show the mean, and standard deviation (SD) values of compressive strength of different groups with different concentrations. Results revealed statistically insignificant difference between the control and experimental groups. However, upon comparing two concentrations in the PMMA/MMT group, the 4% concentration showed statistically significant higher compressive strength values compared to the 2% concentration.

On the other hand, the PMMA/MMT/AgNPs group showed statistical insignificant difference between the two concentrations. Upon general comparison between the 2% and 4% concentrations regardless of the presence or absence of AgNPs, insignificant difference was found in compressive strength values.

Table (2): *The mean, and standard deviation (SD) values of compressive (MPa) and flexural strength (MPa) of the tested groups.*

Variables	Compressive strength		Flexural strength	
	Mean	SD	Mean	SD
Control	78.06	0.70	98.24	8.18
PMMA/MMT/2%	71.05	8.29	101.46	10.54
PMMA/MMT/4%	81.74	2.09	94.87	15.74
PMMA/MMT/AgNps/2%	76.50	5.08	88.95	14.19
PMMA/MMT/AgNps/4%	74.84	1.98	89.62	7.66
p-value	0.019*		0.399ns	

**; significant (p<0.05) ns; non-significant (p>0.05)*

Table (3): *The mean and standard deviation (SD) values of compressive and Flexural strength (MPa) of different groups with different concentrations.*

Variables	Compressive strength					Flexural strength				
	(PMMA/MMT)		(PMMA/MMT/ AgNPs)		p-value	(PMMA/MMT)		(PMMA/MMT /AgNPs)		p-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Conc 2%	71.05	8.29	76.50	5.08	0.245ns	101.46	10.54	88.95	14.19	0.152ns
Conc 4%	81.74	2.09	74.84	1.98	0.001*	94.87	15.74	89.62	7.66	0.522ns
p-value	0.023*		0.515ns			0.459ns		0.928ns		

**; significant (p<0.05) ns; non-significant (p>0.05)*

2.b. Flexural strength *:*

Table (2 and 3), and figures (5) show the mean, standard deviation (SD) values of flexural strength of different groups with different concentrations. Results showed statistically insignificant difference between the control group and each of the experimental groups. There was also statistical insignificant difference between the 2% and 4% concentrations within the PMMA/MMT/AgNPs group, nor within the PMMA/MMT group. A statistical insignificant difference was also found between (PMMA/MMT) and (PMMA/MMT/AgNPs) between both 2% concentration and the 4% concentration.

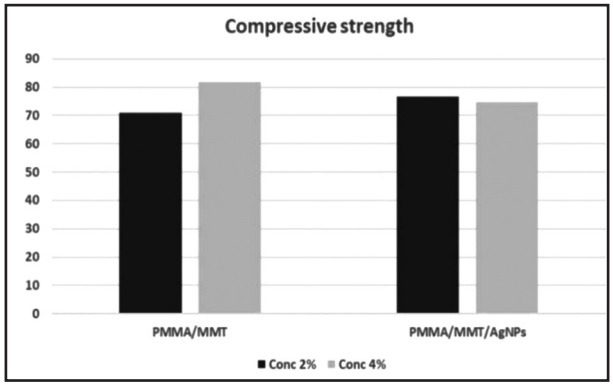

Figure (4): Compressive strength values of different groups with different concentrations

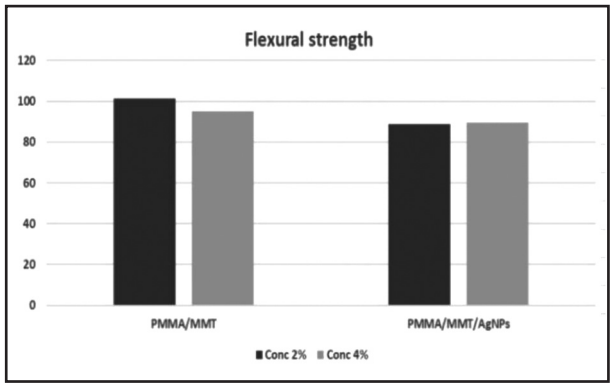

Figure (5): Flexural strength values of different groups with different concentrations

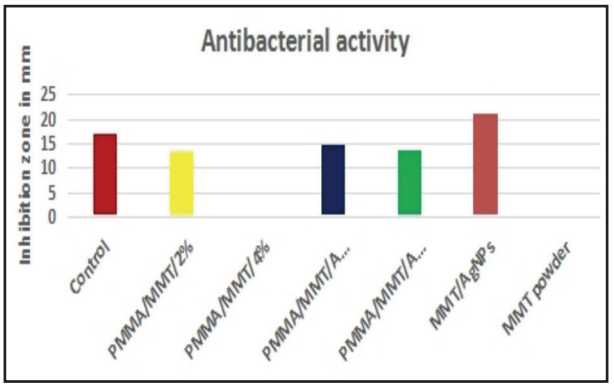

Figure (6): Antibacterial activity of the tested groups

3. Antimicrobial assessment:

Table (4) and figure (6) show the antibacterial activity of the tested groups. Results revealed a statistically significant difference between all the tested groups ($p<0.001$). The MMT powder as well as the 4% MMT / PMMA cement, showed no antimicrobial activity with a statistically significant difference with all the other tested groups ($p<0.001$). While the MMT-AgNPs groups showed a statistically significant difference with higher antimicrobial effect than each of the other tested groups.

Table (4) *The mean, standard deviation (SD) values of Antibacterial activity (Inhibition zone in mm).*

Variables	Antibacterial activity	
	Mean	SD
Control	17.00 b	2.65
PMMA/MMT 2%	13.33 b	2.08
PMMA/MMT 4%	0.00 c	0.00
PMMA/MMT/AgNps 2%	14.67 b	1.15
PMMA/MMT/AgNps 4%	13.67 b	0.58
MMT/AgNPs powder	21.33 a	0.58
MMT powder	0.00 c	0.00
p-value	<0.001*	

*Means with different letters indicates significant difference *; significant ($p<0.05$)*

DISCUSSION

Nanoclays are constructed of layered silicates, their low cost, swelling qualities, and high cation exchange capacities, make them a favourable choice as fillers for the preparation of polymer composites. Montmorillonite, saponite, and laponite are examples of these clays. Due to its availability, exfoliation / intercalation chemistry, reactivity and high surface area, montmorillonite is considered the most extensively utilized clay in polymer nanocomposites [18, 19].

Among various nanoparticles with antibacterial effect, silver nanoparticles are considered popular as it contains 15,000 silver atoms, with smaller diameters less than 100 nm and due to the large surface to volume ratio; AgNPs possess antimicrobial activity. Additionally, they're of low cost and have shown low cytotoxicity and immunological response. Silver nanoparticles are also characterized by continuous release of silver ions that adhere to the cell wall and cytoplasmic membrane, responsible for microbial death [20,21].

Results of XRD analysis of the prepared powder; revealed that the loading of AgNPs took place not only on the surface but extended also to the interlamellar space between the platelets of the MMT. This was in full matching with the finding of SEM assessment results. Regarding the TGA, the

initial weight loss appeared at 90°C is attributed to the liberation of physio-chemical adsorbed water molecules on the surface. On the other hand, absence of significant weight loss (change in the nature of the formed hybrid material as a result of, for example, dehydroxylation of MMT or oxidation of AgNPs) up to 600°C, suggesting that this hybrid is highly stable under the working conditions.

Although statistically insignificant differences in compressive strength was found between the control group and the experimental groups; initial drop in compressive strength from 78 MPa for the control group to 71 MPa for the PMMA loaded with 2% MMT. On the other hand, an increase in compressive strength of PMMA loaded with 4% MMT was noticed reaching 81 MPa. This could be attributed to that the layered structure of MMT nanocomposite became more compacted at higher loading percentage. These findings indicate that the MMT has no deteriorating effect on PMMA compressive strength.

On the other hand; loading of MMT with AgNps and their presence in between MMT layers, resulted in an increase in the inter-layer spacing, increasing the chance of sliding of the clay platelets over each other. Accordingly; the compressive strength declined slightly at MMT/AgNPs /2% with respect to the control group of PMMA. This explanation was confirmed by the further drop in the compressive strength for MMT/AgNPs with 4% loading. Such findings were supported by SEM results that showed the dispersion of silver particles over the surface and in between the individual layers of MMT.

It is well known that the organophilization of hydrophilic montmorillonite before insertion into a hydrophobic matrix such as PMMA causes good dispersion, helps the dispersion into the inter-layer distance by the polymer molecules and hence improves the mechanical strength [22].As this is not the case, because the MMT used in the current study was kept unmodified to preserve its natural interlayer spacing that allow it to be loaded with AgNPs which may lead to some agglomeration and poor compatibility with the polymer matrix.

The agglomerated particles may act as stress concentration areas inside the polymer matrix, decreasing the mechanical strength of the polymerized material. This was observed and expressed in the results where the flexural strength shows slight increase after addition of 2% MMT to reach average of 101.5 MPa then with increasing the loading percentage of MMT alone or with presence of another heterogenic filler (AgNPs) causing slight drop of the flexural strength with statistically insignificant differences in mean flexural strength values between the tested groups [23].

This was in agreement with other experimental studies regarding the mechanical properties of nanoclay-reinforced PMMA. They have concluded that there is a direct correlation between the concentrations of MMT particles incorporated within PMMA resin matrix and its flexural strength which can be adversely affected by increasing the added percentage above 2% [16,22]. It is well known that; poor interfacial bonding between filler and surrounding matrix prevents effective stress transfer between them, hence; affecting the mechanical properties of composite material.

On the other hand; despite of the simplicity and widely used blending method in the current study; proper dispersion of the nanofiller within the polymer matrix is more difficult and probably may affect the strength properties of the tested composites [24,25].

Regarding the antibacterial activity, it was stated that the PMMA itself has a slight bacteriostatic effect on the growth of staphylococci and other pathogenic organisms due to the liberated heat of polymerization, along with the elaboration of the residual monomer that may also play a significant role which explains the antibacterial effect that appeared when PMMA bone cement specimens (control group) were tested in the current study [26].

As the nanometer scale range from (1nm to 100nm), the small-sized silver nanoparticles presented a higher antibacterial property. The size of the nanoparticles prepared in current study was in the range of (13 nm up to 95 nm) as previously shown on SEM images with heterogeneous size distribution. This might have led to high agglomeration tendency resulting in a decrease in the total surface area compared to well dispersed nanoparticles affecting the amount of released silver ions.

Possible entrapment of AgNPs within the silicate layered structure of the MMT and the polymerized PMMA matrix may have a significant role in delaying the antibacterial effect of silver ions exchanged in the clay. Subsequently, this may lead to a decrease in the onset of their inhibitory effect but keep more sustained release due to the ability of silver nanoparticles to continually release silver ions over time [27,28]. This comes in agreement with other studies who stated that MMT can hold and support the AgNPs from being easily aggregated assuring slow but long lasting release of silver ions which may be due to the interactional force between AgNPs and MMT [29].

CONCLUSIONS

Within the limitations of the current study, it could be concluded that:

1. Montmorillonite nanoparticles with their unique structure are able to hold silver nanoparticles in their interlamellar space and the external surface layer.
2. Addition of 2% and 4% montmorillonite to poly methyl methacrylate bone cement has no deteriorating effect on the compressive and flexural strength.
3. The prepared 2% and 4% MMT-AgNPs hybrid composites proved to have antibacterial effect.

RECOMMENDATIONS

Further studies are recommended by using different blending methods and/or organic modification of the MMT filler particles that may improve the mechanical and biological properties of the investigated PMMA bone cement. Also, it's recommended to test the antibacterial effect of the added AgNPs over extended time intervals to assure their prolonged sustained effect.

Conflict and Interest:

The authors have no conflicts of interests to declare

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REFERENCES

1. Sugita Y, Okubo T, Saita M, Ishijima M, Torii Y, Tanaka M et al. Novel Osteogenic Behaviors around Hydrophilic and Radical-Free 4-META/MMA-TBB: Implications of an Osseointegrating Bone Cement. Int. J. Mol.Sci. 2020; 21:2405
2. Daniel G, Kasa B, Regis L, Simon C, Mark S, Lowry C. Comparative Study of Antibiotic Elution Profiles From Alternative Formulations of Polymethylmethacrylate Bone Cement. J Arthroplasty. 2019; 34: 1458-61.
3. Al Thaher Y, Alotaibi HF, Yang L, Prokopovich P. PMMA bone cement containing long releasing silica-based chlorhexidine nanocarriers. PLOS ONE. 2021; 16: e0257947.
4. Nikolaidis A, Koulaouzidou E, Gogos Ch and Achilias D. Synthesis and Characterization of Dental Nanocomposite Resins Filled with Different Clay Nanoparticles. Polymers. 2019; 11:730.
5. Shuaia C, Penga B, Liua M, Pengb S, Feng P. A self-assembled montmorillonite-carbon nanotube hybrid nanoreinforcement for poly-L-lactic acid bone scaffold. Mater. Today Adv. 2021; 11: 100158.
6. Prokopovich P, Leech R , Carmalt C, Parkin S ,Perni S.A novel bone cement impregnated with silver–tiopronin nanoparticles: its antimicrobial, cytotoxic, and mechanical properties. Int. J. Nanomedicine. 2013; 8: 2227-37.
7. Sultan A, Samuel T, Umpierrez E, Swiergosz A, Rabin J, Mahmood B, et al. Routine use of commercial antibiotic-loaded bone cement in primary total joint arthroplasty:

a critical analysis of the current evidence. Ann Transl Med. 2019 ; 7: 73.

8. Chen I-Ch, Su Chen-Y, Nien Wei-H, Huang Tzu-T, Huang Chang-H, Lu Yung-Ch, Chen Yu-J, Huang Gwo-Ch, and Fang Hsu-W. Influence of Antibiotic-Loaded Acrylic Bone Cement Composition on Drug Release Behavior and Mechanism. Polymers 2021; 13: 2240.
9. Shameli K, Bin Ahmad M, Zargar M, Zin W, Yunus W. Synthesis of silver nanoparticles in montmorillonite and their antibacterial behavior. Int. J. Nanomedicine. 2011; 6: 581-90.
10. Xu G, Qiao X, Qiu X, Chen J. Preparation and Characterization of Nano-silver Loaded Montmorillonite with Strong Antibacterial Activity and Slow Release Property. J. Mater. Sci. Technol., 2011;27: 685-90
11. Bakhtiari S, Bakhsheshi-Rad H, Karbasi S, Tavakoli M, Razzaghi M, Ismail A et al. Polymethyl Methacrylate-Based Bone Cements Containing Carbon Nanotubes and Graphene Oxide: An Overview of Physical, Mechanical, and Biological Properties. Polymers. 2020; 12: 1469.
12. Nurazzi, N.M, Sabaruddin, F.A, Harussani, M.M, Kamarudin S.H, Rayung M, Asyraf, M.R. et al. Mechanical Performance and Applications of CNTs Reinforced Polymer Composites—A Review. Nanomaterials 2021; 11: 2186.
13. Salaie R, Besinis A, Le H, Tredwin C, Handy RD. The biocompatibility of silver and nanohydroxyapatite coatings on titanium dental implants with human primary osteoblast cells. Mater. Sci. Eng. C. 2020;107:110-210.
14. Bistolfi A., Ferracini R., Albanese C. PMMA-Based Bone Cements and the Problem of Joint Arthroplasty Infections: Status and New Perspectives. Materials 2019;12: 4002.
15. Bacali C, Baldea I, Moldovan M, Carpa R, Olteanu D. Flexural strength, biocompatibility, and antimicrobial activity of a polymethyl methacrylate denture resin enhanced with graphene and silver nanoparticles. Clin. Oral Investig. 2020; 24:2713-25.
16. Fernandez C, Sokolonski A.R, Fonseca M.S, Stanisic D, Araújo D.B, Azevedo V et al. Applications of Silver Nanoparticles in Dentistry:Advances and Technological Innovation. Int. J. Mol. Sci. 2021; 22: 2485.
17. Dantas de Oliveira A, Gonçalves C. Polymer Nanocomposites with Different Types of Nanofiller. Nanocomposites - Recent Evolutions. Subbarayan Sivasankaran, 2019. DOI: 10.5772/intechopen.81329.
18. Erezuma I, Eufrasio-da-Silva T, Golafshan N, Deo K, Mishra Y, Castilho M,et al. Nanoclay Reinforced Biomaterials for Mending Musculoskeletal Tissue Disorders. Adv. Healthcare Mater.2021;10; 210-17.
19. Shanmuganathan R, Karuppusamy I, Muthupandian S. and Harshiny M. Synthesis of Silver nanoparticles and their biomedical applications-A comprehensive review. Curr Pharm Des. 2019; 25: 2650-60.
20. Liao C, Li Y, Tjong S. Bactericidal and cytotoxic properties of silver nanoparticles. Int J Mol Sci. 2019;20:449.
21. Barzegar A, Ghaffari T. Nanoclay-reinforced polymethylmethacrylate and its mechanical properties. Dent Res J (Isfahan). 2018; 15: 295-301.
22. Castro D, Nascimento C, Alves O ,Santos E, Agnelli J, Reis A, et al. Analysis of the oral microbiome on the surface of modified dental polymers. Arch Oral Biol. 2018;93:107-14.
23. Zhou W, Jia Z, Xiong P. Bioinspired and biomimetic AgNPs/gentamicin-embedded silk fibroin coatings for robust antibacterial and osteogenetic applications. ACS Appl Mater Interfaces. 2017;9:25830-46.
24. Soleymani S, Bakhsheshi-Rad H, Karbasi S, Tavakoli M, Razzaghi M, Ismail A.F, RamaKrishna S. and Berto F. Polymethyl Methacrylate-Based Bone Cements Containing Carbon Nanotubes and Graphene Oxide: An Overview of Physical, Mechanical, and Biological Properties. Polymers. 2020; 12: 1469.
25. Raji M, Mekhzoum M, Rodrigue D, El Kacem Q, Bouhfid R. Effect of silane functionalization on properties of polypropylene/clay nanocomposites. Compos. B. Eng. 2018;146:106-15.
26. Passador F, Ruvolo-Filho A, Pessan L. Nanocomposites of polymer matrices and lamellar clays. In: Nanostructures. 1st ed. Elsevier. 2017:187-207.
27. Shanmuganathan R, MubarakAli D, Prabakar D. An enhancement of antimicrobial effificacy of biogenic and ceftriaxone-conjugated silver nanoparticles: green approach. Environ Sci Pollut Res Int. 2018;25 :10362–70.
28. Iconaru S, Groza A, Stan G, Predoi D, Gaiaschi S, Trusca R et al. Preparations of Silver/Montmorillonite Biocomposite Multilayers and Their Antifungal Activity. Coatings. 2019; 9:817.
29. Yin IX, Yu OY, Zhao IS. Developing biocompatible silver nanoparticles using epigallocatechin gallate for dental use. Arch Oral Biol. 2019;102:106-12.