Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v26e-79814E

e-ISSN 1809-6891
Veterinary Medicine | Research article

Influence of silver nitrate on the mechanical properties of orthopedic bone cement and *in vitro* antibacterial activity

Influência do nitrato de prata nas propriedades mecânicas do cimento ósseo ortopédico e atividade antibacteriana *in vitro*

Alini Osowski[1], Sheila Canevese Rahal[1], Celso Roberto Ribeiro[2], Luiz Donizete Campeiro Junior[1], José Ivaldo de Siqueira Silva Júnior[1], Guilherme Rech Cassanego*[1], Paulo Henrique Gilio Gasparotto[3]

1 Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Botucatu, São Paulo, Brazil.
2 Laboratório de Ensaios Mecânicos e Metalográficos (LEMM), Jaú, São Paulo, Brazil.
3 Laboratório de Sanidade Animal - MICROVET, Ji-Paraná, Rondônia, Brazil.
*corresponding author: guilherme.rech@unesp.br

Received: June 01, 2024. Accepted: October 31, 2024. Published: February 13, 2025. Editor: Luiz Augusto B. Brito

Abstract: This study aimed to evaluate the influence of silver nitrate (AgNO3) on the mechanical properties of polymethyl methacrylate (PMMA) bone cement by static testing and *in vitro* antibacterial activity. Two groups were formed: Group 1, control (n=10); and Group 2, cement with silver nitrate (n=10). In Group 1, the cement was prepared manually by mixing 20 g of PMMA powder with 10 ml of methyl methacrylate liquid. In Group 2, the cement was prepared as in Group 1; however, 0.25 g of silver nitrate was added to 20 g of PMMA powder. No statistical differences were observed for bending strength with (61.80 ± 4.96 MPa) or without silver nitrate (60.20 ± 5.88 MPa). Statistical differences were verified in the higher compressive strength for the control (78.60 ± 3.20 MPa) compared to cement with silver nitrate specimens (74.20 ± 1.61 MPa). Antimicrobial sensitivity testing showed no effect of cement with silver nitrate on *Staphylococcus aureus*, *Streptococcus sp*., *Pseudomonas aeruginosa*, and *Escherichia coli*. In conclusion, the concentration of silver nitrate did not change the mechanical properties of the PMMA bone cement in the bending test, but it had a negative effect in the compression testing. In addition, the product did not reduce the bacterial load in the *in vitro* test.

Key-words: polymethylmethacrylate; mechanical testing; sample; static testing

Resumo: O objetivo do estudo foi avaliar a influência do nitrato de prata (AgNO3) nas propriedades mecânicas do cimento ósseo de polimetilmetacrilato (PMMA), por meio de testes estáticos, bem como a atividade antibacteriana *in vitro*. Foram constituídos dois grupos: Grupo 1 – controle (n=10), Grupo 2 – cimento acrescido de nitrato de prata (n=10). No Grupo 1 o cimento foi preparado manualmente usando 20 g de pó de PMMA. No Grupo 2 o cimento foi preparado como no Grupo 1, a diferença foi que 0,25 g de nitrato de prata foi adicionado a 20 g de pó de PMMA. Dez corpos de prova de cada grupo foram designados para o ensaio de flexão em quatro pontos e 10 para o ensaio de compressão. Não foram observadas diferenças estatísticas para a resistência à flexão com (61,80 ± 4,96 MPa) ou sem nitrato de prata (60,20 ± 5,88 MPa).

Diferenças estatísticas foram verificadas na resistência à compressão, sendo maior para os corpos de prova controle (78,60 ± 3,20 MPa) comparado ao nitrato de prata (74,20 ± 1,61 MPa). Amostras dos produtos foram analisadas pelo teste de sensibilidade antimicrobiana, porém não foi verificado efeito com relação ao *Staphylococcus aureus*, *Streptococcus sp*., *Pseudomonas aeruginosa* e *Escherichia coli*. Conclui-se que na concentração empregada, o nitrato de prata não alterou as propriedades mecânicas do cimento ósseo de PMMA no teste de flexão, porém influenciou negativamente no teste de compressão. Além disso, o produto não permitiu a redução da carga bacteriana no teste *in vitro*.

Palavras-chave: polimetilmetacrilato; ensaio mecânico; corpo de prova; ensaio estático

1. Introduction

Orthopedic bone cement based on polymethyl methacrylate (PMMA) has been used for years in various surgical procedures, such as fixation of prostheses, oncological surgeries, and percutaneous vertebroplasty [1-4]. However, owing to the occurrence of infections, antibiotic-loaded bone cement has been used as a prophylactic agent in prosthetic surgeries, for the treatment of infections associated with these procedures, and in chronic osteomyelitis treatment, especially as pearls or spacers[2,4]. Concerns associated with the local administration of antibiotics include side effects such as cytotoxicity and systemic toxicity, the occurrence of resistant organisms due to exposure to subtherapeutic concentrations of antibiotics, antibiofilm effects dependent on the ability of bacteria to survive in high local antibiotic concentrations, only heat-stable antibiotics suitable for incorporation in PMMA, and the use of antibiotic whose pharmacokinetic data are unknown, among others[4-7].

Owing to the challenges related to the treatment of prosthetic joint infection and the potential to induce antibiotic resistance, other approaches have been proposed to reduce or eliminate the antibiotic load in PMMA cement[8]. One option is silver, which has been employed in industrial, domestic and healthcare fields, because of its antibacterial, antiviral and antifungal properties[9,10]. In the medical field, silver has been utilized in both mineral and compound forms, including silver zeolite, silver nitrate, and nanosilver (related to the advancement of nanotechnology)[9]. The use of PMMA bone cement with nanoparticles could be a mechanism to diminish the incidence of resistance because of its multifaceted mechanism of action[10,11].

However, any material incorporated into PMMA cement must preserve or improve its mechanical properties to maintain functionality, especially if used for implant fixation[3,12]. This study aimed to evaluate the influence of silver nitrate on the mechanical properties of PMMA bone cement using static testing and *in vitro* antibacterial activity.

2. Material and methods

2.1 Preparation of samples

Commercial bone cement was used to prepare samples containing PMMA (81.0%), barium sulfate (10%), and benzoyl peroxide (2.25%) in the powder component, and monomethyl methacrylate (99.24%), N,N-dimethyl p-toluidine (0.75%), and hydroquinone (0.04%) in the liquid component.[3] Bone cement was sterilized using ethylene oxide. Two groups were formed: Group 1, bone cement (control) (n=20), and Group 2, bone cement with silver nitrate (n=20). Ten samples from each group were assigned to the four-point bending test, and 10 to the compression test. In Group 1, the cement was manually prepared by mixing 20 g of powder with 10 ml of liquid in a PET (polyethylene terephthalate) bowl using a plastic spatula. The cement in the dough phase was placed in an aluminum metallic mold according to the ISO 5833 standard and pressed manually until polymerization. After 24 hours at room temperature (23° ± 2°C), hardened bone cement was removed from the mold.

For the four-point bending tests, the samples were 75-mm length, 10-mm width, and 3-mm thickness. For the compression tests, the samples were 6-mm in diameter, and 12-mm in length. In Group 2, cement was prepared as in Group 1. The difference was that 0.25 g of silver nitrate ($AgNO_3$) P.A. grade was added to 20 g of polymethylmethacrylate powder. The mixture was manually mixed for two minutes before adding the liquid component. In both groups, sample preparation procedures were carried out under aseptic conditions in a climate-controlled room (23ºC) by the same investigator, based on ISO 5833:2002 (implants for surgery – acrylic resin cements). Before the mechanical testing, the samples were maintained for 24 h in a climate-controlled room (23ºC).

2.2 Mechanical testing

A four-point bending test (Figure 1a) was conducted following ISO-5833(2002) Annex F (determination of the bending strength of polymerized cement). A universal testing machine (EMIC DL-10.000) with a maximum capacity of 10,000 kgf and a 1000 N load cell was utilized for the test. The distance between the support rollers was 60 mm. In addition, the distance between the loading rollers and the support and loading rollers was 20 mm. The rollers have an outer diameter of 3 mm. The samples were tested at a rate of 5 mm/min. The bending strength was measured until complete displacement (break of the samples) was achieved using the TESC software. The testing was conducted in a controlled environment with a temperature of 23° ± 2°C and a relative humidity of 60%. Each sample was identified from 1 to 10 in the software according to the group.

The compression test (Figure 1b) was conducted following ISO-5833(2002) Annex E (determination of the compressive strength of polymerized cement). A universal testing machine (EMIC DL-10.000) with a maximum capacity of 10,000 kgf and a 2000 N load cell was used for the test. The sample was positioned between the two plates and then force was applied at a test speed of 22 mm/min. The compressive strength was calculated using the peak load. The testing was performed in a controlled environment with a temperature of 23° ± 2°C and a relative humidity of 60%.

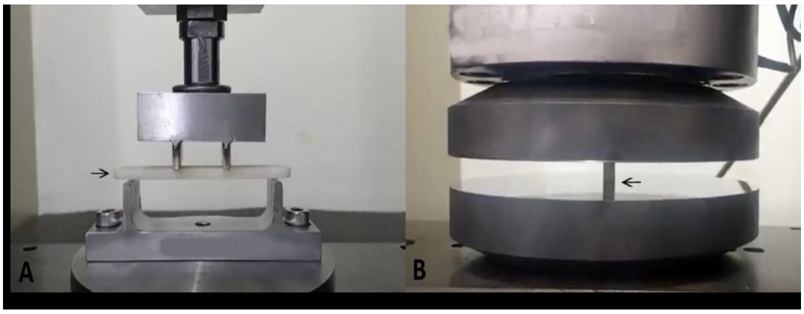

Figure 1. (A) Four-point bending test setup along with one sample (arrow). (B) Compression test setup along with one sample (arrow).

2.3 Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution. Student's t test was used to compare the control and cement with silver nitrate according to the mechanical test. The significance level was set at $p < 0.05$. Statistical evaluation was performed using SigmaStat 3.5 software.

2.4 Microbiological analysis

Microbiological analyses (bacterial and fungal tests) were performed on samples from both groups that were not subjected to mechanical testing. All the samples were manufactured and manipulated under aseptic conditions. After external cleaning with 70% alcohol, the samples were broken, crushed, and immersed in brain heart infusion (BHI) broth. The incubation was at 35-37ºC for 24 h. Then, the material was streaked to blood agar, Tryptic Soy Agar (TSA), and MacConkey agar with incubation between 35ºC and 37ºC for bacterial culture. For fungal testing, the material was transferred to Sabouraud-dextrose agar with incubation at 20-25°C.

2.5 Antimicrobial sensitivity testing

Specific bacteria, including *Staphylococcus aureus*, *Streptococcus sp*., *Pseudomonas aeruginosa*, and *Escherichia coli*, were used to test the antimicrobial sensitivity of the samples from groups 1 and 2. Each colony was placed in BHI broth and then incubated at 35º-37ºC for 24 h. A cotton swab was used to create a lawn culture of the bacterial suspension on Mueller-Hinton or MacConkey agar (for Gram-negative bacteria). Samples from groups 1 and 2 were then placed on the plates, and the plates were incubated at 35º-37ºC for 48 hours.

3. Results

No statistical differences were observed in the bending strength between the control and the cement with silver nitrate *(*$p = 0.519$). Statistically significant differences were observed in the compressive strength ($p = 0.001$), which was higher in the control samples. The values are listed in Table 1. Graphs of the bending and compressive strengths of the control and cement with silver nitrate samples are shown in Figures 2 and 3, respectively.

Table 1. Comparison between the control (Group 1) and bone cement with silver nitrate (Group 2), according to mechanical tests.

Mechanical tests	Control (MPa)	Bone cement with silver nitrate (MPa)	*P*-value
Bending strength	60.20 ± 5.88	61.80 ± 4.96	0.519
Compressive strength	78.60 ± 3.20	74.20 ± 1.61	0.001

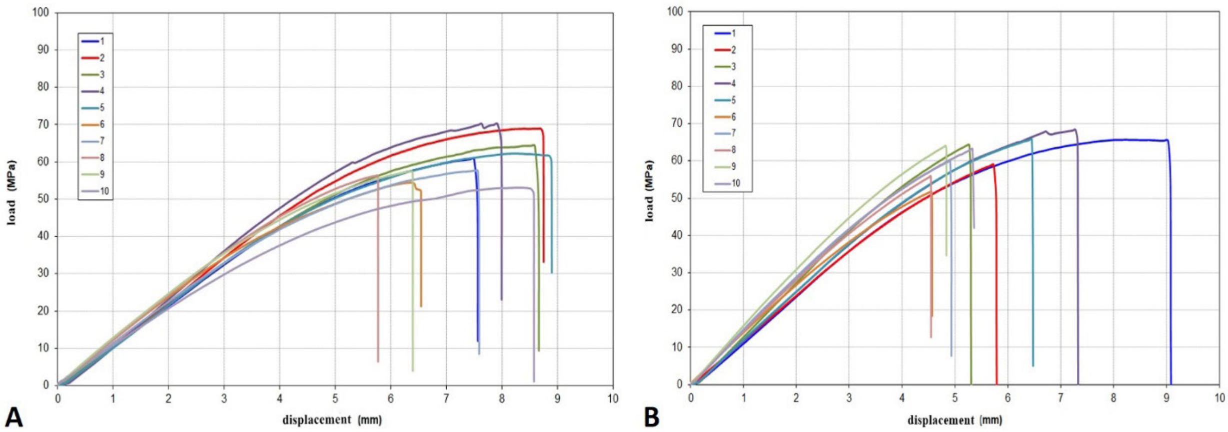

Figure 2. Load-displacement curves obtained during four-point bending testing of 10 samples from the control cement (A) and 10 samples from cement with silver nitrate (B).

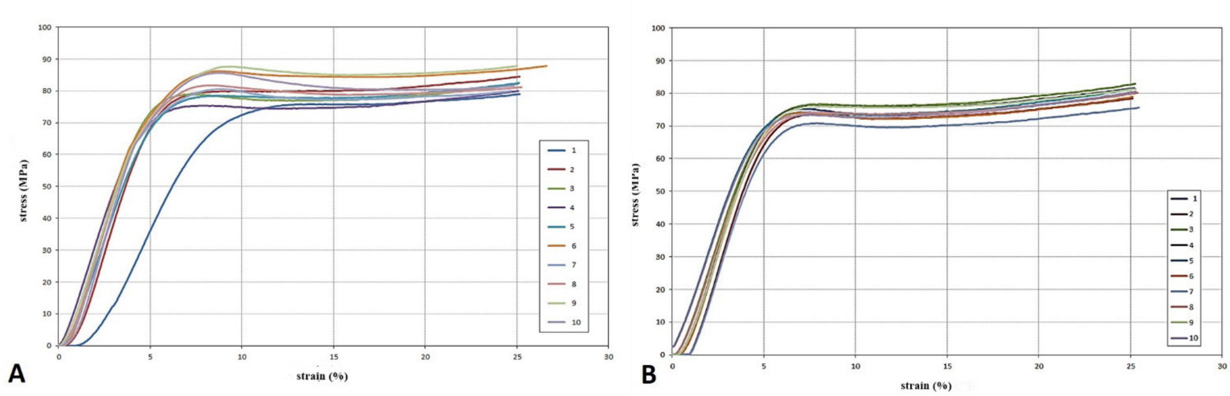

Figure 3. Stress-strain curves obtained during compressive testing of 10 samples from the control cement (A) and 10 samples from cement with silver nitrate (B).

Microbiological analysis did not detect bacterial or fungal growth in samples from Group 1 or Group 2. Antimicrobial sensitivity testing showed no effect of cement with silver nitrate on *Staphylococcus aureus*, *Streptococcus sp.*, *Pseudomonas aeruginosa,* and *Escherichia coli*.

4. Discussion

The present study verified that the mechanical properties of bone cement were influenced by the addition of silver nitrate. Destructive tests with a single load to failure, as used in the current study, and dynamic tests involving cyclic loading are frequently used to evaluate the mechanical properties of bone cement[3,13,14]. In addition, the mechanical properties of bone cement can be classified into into short- and long-term, in which the tensile strength, compressive strength, bending strength, and modulus of elasticity are considered important short-term properties[14]. Two short-term properties were analyzed in the present study, and both bending and compression tests were carried out as determined by ISO 5833:2002.

The bending strength was 60.20 MPa for control and 61.80 MPa for bone cement with silver nitrate, while the compressive strength was 78.60 MPa for control and 74.2 MPa for bone cement with silver nitrate. These values differ from those cited by ISO 5833 and ASTM F 451, in which commercial cements must vary within a limited range, including a bending strength of 67-72 MPa and a compressive strength of 80-94 MPa[3]. In general, PMMA bone cement has a compressive strength higher than its bending strength, which is superior to the tensile strength[14] observed in the control cement and bone cement with silver nitrate. Surgical PMMA cement is a brittle material analogous to concrete, characterized by weak tensile and shear strengths but strong compression[13].

The ratio of 0.25 g of silver nitrate per 20 g of PMM powder adversely affected the compressive strength but did not interfere with the bending strength. Another study that used 0.25 g of silver microparticles per 20 g of PMMA noted that the silver group was weaker than the control in both uniaxial compression and four-point bending tests[15]. In contrast, a study that incorporated silver nanoparticles in three ratios (0.25, 0.5, and 1.0%) into bone cement found that the mechanical properties by static testing (four-point bending, compression, and fracture toughness testing) and dynamic testing were not different from those of standard cement[16]. The addition of 0.5% silver nanoparticles to 2% dental acrylic resin (PMMA) promoted a higher increased the bending strength (three-point bending), fracture toughness, impact strength, and hardness; however, the compressive strength was found to be not influenced by the differences in percentage[17]. The differences in the products and protocol for silver nitrate incorporation may have interfered with the results of these studies.

The silver nitrate used in the present study was not present as nanoparticles, which are most frequently incorporated in PMMA-based cements used in medicine and dentistry[15,16,18-23]. Silver nanoparticles (1-100 nm in diameter) are composed of clusters of silver atoms[11,17] and can be synthesized using several techniques including physical, chemical, and biological procedures[10]. Despite their beneficial effects, previous studies have shown that silver nanoparticles may induce damage to human lung fibroblasts, immune system suppression, abnormalities in zebrafish embryos, and disruption of cell membranes[9]. Additionally, silver complexed with certain ligands can be more cytotoxic than silver nitrate; however, it can be less toxic to related ligands[24].

Microbiological analysis confirmed sterilization of the products by both bacteria and fungi. However, antimicrobial sensitivity testing showed that the bone cement with silver nitrate did not reduce bacterial growth. A study found that silver nanoparticles were effective in reducing the biofilm formation of *Aeromonas spp*., but were unable to inactivate the growth of the isolates in bactericidal concentration tests, whereas silver nitrate showed efficiency in different concentrations. Additionally, the authors noted that in the presence of an efflux pump inhibitor, one isolate that was initially resistant to nanoparticles became sensitive[25]. In contrast, *in vitro* studies using different methodologies have verified that silver particles exhibited antibacterial and antibiofilm activities[18,21,22,26]. The percentage of silver nitrate added to the bone cement, but not in the nanoparticle form may have influenced the results. Future studies in which these variables are changed are necessary for comparison purposes.

5. Conclusion

The concentration of silver nitrate used did not alter the mechanical properties of the of polymethyl methacrylate (PMMA) bone cement in the bending test; however, it did negatively affect the compression test results. Additionally, the product did not effectively reduce the bacterial load in the in vitro test.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: A. Osowski, S. C. Rahal and C. R. Ribeiro. Data curation: C. R. Ribeiro and H. G. Gasparotto. Formal analysis: A. Osowski, S. C. Rahal, C. R. Ribeiro, L. D. Campeiro Junior and P. H. G. Gasparotto. Funding acquisition: S. C. Rahal, C. R. Ribeiro and L. D. Campeiro Junior. Project management: A. Osowski, S. C. Rahal and C. R. Ribeiro. Methodology: A. Osowski, S. C. Rahal, C. R. Ribeiro, L. D. Campeiro Junior and P. H. G. Gasparotto. Supervision: S. C. Rahal and C. R. Ribeiro. Investigation: A. Osowski, S. C. Rahal and C. R. Ribeiro. Data curation: C. R. Ribeiro and H. G. Gasparotto. Visualization: J. I. S. Silva Júnior and G. R. Cassanego. Writing (original draft): A. Osowski, S. C. Rahal and C. R. Ribeiro. Writing (proofreading and editing): A. Osowski, S. C. Rahal, C. R. Ribeiro, L. D. Campeiro Junior and G. R. Cassanego.

Acknowledgments

The authors thanks Coordination for the Improvement of Higher Level Personnel (Capes - Finance *Code 001*), LEMM (Mechanical and Metallography Testing Laboratory), and National Council for Scientific and Technological Development (CNPq – PQ 305813/2023-4).

References

1. Webb JC, Spencer RF. The role of polymethylmethacrylate bone cement in modern orthopaedic surgery. The Journal of Bone and Joint Surgery. British volume. 2007; 89(7):851–857. https://doi.org/10.1302/0301-620X.89B7.19148

2. Magnan B, Bondi M, Maluta T, Samaila E, Schirru L, Dall'Oca C. Acrylic bone cement: current concept review. Musculoskeletal Surgery. 2013; 97(2):93–100. https://doi.org/10.1007/s12306-013-0293-9

3. Cavalu S. Acrylic bone cements: new insight and future perspective. Key Engineering Materials. 2017; 745:39-49. https://doi.org/10.4028/www.scientific.net/KEM.745.39

4. van Vugt TAG, Arts JJ, Geurts JAP. Antibiotic-loaded polymethylmethacrylate beads and spacers in treatment of orthopedic infections and the role of biofilm formation. Frontiers in Microbiology. 2019; 10:1626. https://doi.org/10.3389/fmicb.2019.01626

5. Hayes G, Moens N, Gibson T. A review of local antibiotic implants and applications to veterinary orthopaedic surgery. Veterinary and Comparative Orthopaedics and Traumatology. 2013; 26(4):251–259. https://doi.org/10.3415/VCOT-12-05-0065

6. Walker LC, Baker P, Holleyman R, Deehan D. Microbial resistance related to antibiotic-loaded bone cement: a historical review. Knee Surgery, Sports Traumatology, Arthroscopy. 2017; 25(12):3808–3817. https://doi.org/10.1007/s00167-016-4309-5

7. Schwarz EM, McLaren AC, Sculco TP, Brause B, Bostrom M, Kates SL, Parvizi J, Alt V, Arnold WV, Carli A, Chen AF, Choe H, Coraça-Huber DC, Cross M, Ghert M, Hickok N, Jennings JA, Joshi M, Metsemakers WJ, Ninomiya M, Nishitani K, Oh I, Padgett D, Ricciardi B, Saeed K, Sendi P, Springer B, Stoodley P, Wenke JC; Hospital for Special Surgery. Biofilm Symposium Workgroup. Adjuvant antibiotic-loaded bone cement: Concerns with current use and research to make it work. Journal of Orthopaedic Research. 2021; 39(2):227–239. https://doi.org/10.1002/jor.24616

8. Lewis G. Antibiotic-free antimicrobial poly (methyl methacrylate) bone cements: A state-of-the-art review. World Journal of Orthopedics. 2022; 13(4):339–353. https://doi.org/10.5312/wjo.v13.i4.339

9. Brennan SA, Ní Fhoghlú C, Devitt BM, O'Mahony FJ, Brabazon D, Walsh A.Silver nanoparticles and their orthopaedic applications. The Bone & Joint Journal. 2015; 97-B(5):582–589. https://doi.org/10.1302/0301-620X.97B5.33336

10. Naganthran A, Verasoundarapandian G, Khalid FE, Masarudin MJ, Zulkharnain A, Nawawi NM, Karim M, Che Abdullah CA, Ahmad SA. Synthesis, characterization and biomedical application of silver nanoparticles. Materials (Basel). 2022; 15(2):427. https://doi.org/10.3390/ma15020427

11. Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology. 2010; 28(11):580–588. https://doi.org/10.1016/j.tibtech.2010.07.006

12. Lewis G. Properties of nanofiller-loaded poly (methyl methacrylate) bone cement composites for orthopedic applications: a review. Journal of Biomedical Materials Research. 2017; 105(5):1260–1284. https://doi.org/10.1002/jbm.b.33643

13. Saha S, Pal S. Mechanical properties of bone cement: a review. Journal of Biomedical Materials Research. 1984; 18(4):435–462. https://doi.org/10.1002/jbm.820180411

14. Wang J-S, Dunne N. Bone cement fixation: acrylic cements. In: Revell PA, ed. Joint Replacement Technology. United Kingdom: Woodhead Publishing; 2008. p. 212-51.

15. Ficklin MG, Kunkel KA, Suber JT, Gerard PD, Kowaleski MP. Biomechanical evaluation of polymethyl methacrylate with the addition of various doses of cefazolin, vancomycin, gentamicin, and silver microparticles. Veterinary and Comparative Orthopaedics and Traumatology. 2016; 29(5):394–401. https://doi.org/10.3415/VCOT-16-01-0005

16. Slane J, Vivanco J, Rose W, Ploeg HL, Squire M. Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles. Materials Science & Engineering. 2015; 48:188–196. https://doi.org/10.1016/j.msec.2014.11.068

17. Alsukhayri AA, Alwagdani AA, Ibrahim MI, Fahmi MK. (2019). Effect of silver nanoparticles fillers addition on flexural strength, fracture toughness, impact strength, compressive strength and hardness of heat-polymerized acrylic resin. *International Journal of Advanced Research. 2019;* 7(9):1419-1422. http://dx.doi.org/10.21474/IJAR01/9798

18. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E, Domann E, Schnettler R. (2004). An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomaterials. 2004; 25(18):4383–4391. https://doi.org/10.1016/j.biomaterials.2003.10.078

19. Oei JD, Zhao WW, Chu L, DeSilva MN, Ghimire A, Rawls HR, Whang K. Antimicrobial acrylic materials with in situ generated silver nanoparticles. Journal of Biomedical Materials Research. 2012; 100(2):409–415. https://doi.org/10.1002/jbm.b.31963

20. Sodagar A, Kassaee MZ, Akhavan A, Javadi N, Arab S, Kharazifard MJ. Effect of silver nano particles on flexural strength of acrylic resins. Journal of Prosthodontic Research. 2012: 56(2):120–124. https://doi.org/10.1016/j.jpor.2011.06.002

21. Prokopovich P, Leech R, Carmalt CJ, Parkin IP, Perni S. A novel bone cement impregnated with silver-tiopronin nanoparticles: its antimicrobial, cytotoxic, and mechanical properties. International Journal of Nanomedicine. 2013; 8:2227–2237. https://doi.org/10.2147/IJN.S42822

22. Prokopovich P, Köbrick M, Brousseau E, Perni S. (2015). Potent antimicrobial activity of bone cement encapsulating silver nanoparticles capped with oleic acid. Journal of Biomedical Materials Research. 2015; 103(2):273–281. https://doi.org/10.1002/jbm.b.33196

23. Ghaffari T, Hamedirad F, Ezzati B. In vitro comparison of compressive and tensile strengths of acrylic resins reinforced by silver nanoparticles at 2% and 0.2% concentrations. Journal of Dental Research, Dental Clinics, Dental Prospects. 2014; 8(4):204–209. https://doi.org/10.5681/joddd.2014.037

24. Clement JL, Jarrett PS. Antibacterial silver. Metal-based Drugs. 1994; 1(5-6):467–482. https://doi.org/10.1155/MBD.1994.467

25. Braga NB, Pires LCSR, Oliveira HP, Costa MM. 2018. Antimicrobial and antibiofilm activity of silver nanoparticles against *Aeromonas* spp. isolated from aquatic organisms. Pesquisa Veterinária Brasileira. 2018; 38(2):244-249. https://doi.org/10.1590/1678-5150-PVB-4805

26. Jackson J, Lo J, Hsu E, Burt HM, Shademani A, Lange D. The combined use of gentamicin and silver nitrate in bone cement for a synergistic and extended antibiotic action against gram-positive and gram-negative bacteria. Materials (Basel). 2021; 14(12):3413. https://doi.org/10.3390/ma14123413