Original Article

Rifampin-loaded Mesoporous Silica Nanoparticles Improved Physical and Mechanical Properties and Biological Response of Acrylic Bone Cement

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Abstract

Background: Acrylic bone cement, which is used to fix implants in the knee and hip, is prone to contamination with various types of infections. Adding small amounts of different antibiotics to the cement can help prevent and treat infections. Rifampin antibiotic has been added to bone cement to create an appropriate antimicrobial response in the treatment of resistant coagulase-negative staphylococci (CoNS) biofilms, but there are some challenges such as reducing mechanical properties and prolonging the setting time of the cement. Loading the antibiotic in the nanoparticle could eliminate these challenges. **Methods:** In this study, rifampin-loaded mesoporous silica nanoparticles (MSNs) were added to bone cement, and the polymerization components, mechanical properties, drug release, antibacterial activity, and cellular response were investigated and compared with commercial pure cement and the cement containing free rifampin. **Results:** Loading rifampin into MSN improved compressive strength by 57.52%. Cement containing rifampin loaded into MSN showed remarkable success in antibacterial activity. The growth inhibition zone created by it in the culture medium of *Staphylococcus aureus* and CoNS was 15.44% and 11.8% greater, respectively, than in the cement containing free rifampin. In other words, according to the results of spectrophotometric analysis of cement samples over 5 weeks, MSNs caused a 33.2 ± 0.21-fold increase in rifampin washout from the cement. Cellular examination of the cement containing rifampin loaded into MSN compared to commercial pure cement showed an acceptable level of cell viability. **Conclusion:** Rifampin loading in MSN limited the reduction of cement strength. It also improved the drug release pattern and prevented antibiotic resistance.

Keywords: *Bone cement, infection, joint replacement, mesoporous silica nanoparticle, rifampin*

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Introduction

Infections occur in 7% to 9.1% of joint replacement surgeries. Coagulase-negative staphylococci (CoNS) are the second most common cause of infections within 3 months after joint replacement surgery and the primary cause of infections from 3- to 12-month postsurgery.[1] Acrylic bone cement used for attaching metallic implants to bones is susceptible to contamination with CoNS.[2] The concept of using antibiotic-loaded cement for prosthesis fixation to prevent infection was proposed by Buchholz and Engelbrecht. This method is now common and has proven efficacy in preventing and treating deep bone infections.[3]

Staphylococcal infections of bones and joints respond to different combinations of recommended antibiotics. Among many antibiotics studied, aminoglycosides such as gentamicin and glycopeptides such as vancomycin have good activity against peri-implant infections but carry the risk of developing resistant bacteria. Therefore, it is necessary to investigate other antibiotics such as rifampin, which has strong activity against biofilms created by CoNS, particularly drug-resistant strains. Rifampin is known as the cornerstone of CoNS treatment.[4] However, adding antibiotic powder to cement has minimal effectiveness, with only 10% to 20% of the added antibiotic being bioavailable.[5] In addition, the addition of various antibiotics to bone cement poses various challenges. Some antibiotics, like gentamicin, face microbial resistance, whereas others, like vancomycin with higher molecular weight, leave larger cavities after washing out from the cement. Furthermore, antibiotics like

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ciprofloxacin can lead to skeletal–muscular side effects in patients.[6-8]

Adding rifampin to bone cement also comes with associated issues despite its desirable antimicrobial performance. Bone cement containing the antibiotic rifampin shows a significant reduction in mechanical properties to the extent that its compressive strength falls below the minimum acceptable strength of orthopedic cement (70 GPa). Studies in this regard point to the hydroquinone group in the chemical structure of rifampin as the main factor contributing to these issues. Hydroquinone interferes with the initiation of the polymerization reaction of bone cement, resulting in delayed cement setting time and preventing the involvement of a portion of methyl methacrylate monomers during incomplete polymerization, thus creating voids in the cement structure that can explain the reduction in mechanical strength.[9]

In previous efforts to preserve the mechanical properties of rifampin-loaded bone cement, microcapsules of alginate and polyhydroxy butyrate–valerate (PHBV) were assessed for their beneficial value. The isolation of rifampin during the polymerization process through microencapsulation techniques enables the addition of rifampin to bone cement, facilitating better release of rifampin from the cement. However, this study does not provide a report on the extent of the release of free monomers or the cellular response to the cement containing encapsulated rifampin.[10] On the other hand, adding biodegradable biomaterials can lead to a decrease in the long-term mechanical properties of cement. In short term, infection is the most important factor leading to failure in joint replacement surgeries, whereas noninfectious loosening becomes more significant over longer periods of time. Currently, it has been proven that there is a connection between the cavities created in the cement structure and the reduction in mechanical properties, which increases the noninfectious loosening.[7,11] The study by Tunney *et al*. in 2008 indicates that adding chitosan as a biodegradable polymer to the cement containing gentamicin results in a reduction of the long-term mechanical properties of the cement. Scanning electron microscopy (SEM) images taken from the fracture surface of the cement immersed in phosphate-buffered saline (PBS) indicate an increase in the volume of voids compared to the freshly cured cement.[12] The possibility of loading drugs into nanoceramics and their high biocompatibility have led to extensive use of nanoceramic in drug delivery systems. Mesoporous silica nanoparticles (MSNs) as a biocompatible nanoceramic have been used in gentamicin-loaded bone cement. Gentamicin in MSN-containing cement has a release efficiency of over 70%, whereas the addition of free gentamicin powder to bone cement has a release efficiency of approximately 10%. This increase in release can be attributed to the increased water affinity of MSN-containing cement. The contact angle of MSN is 0, whereas the contact angle of cement is 90°. The cement containing MSN should have a lower contact angle, but a specific value has not been reported. Despite further cement washing and higher release of antibiotics, MSNs remain undissolved and do not create voids in the cement structure. It has been reported that approximately 96% of MSNs remain in the structure of bone cement after 6 months.[13]

In this study, to prevent adverse reaction of antibiotic and the bone cement, the rifampin was loaded in MSNs, then was added to bone cement. Then, the loading rate of rifampin in MSNs and its effect on bone cement mechanical properties, drug release, and antibacterial activity of the bone cement against bacteria responsible for bone infections were evaluated.

Materials and Methods

Materials

Bone cement arthroplasty from the company Synimed (France), rifampin from Hakim Pharmaceutical Company (Iran), and mesoporous MSNs with pore sizes of 2–6 nM from US Nano (USA) were obtained.

Rifampin loading rate and optimization of mesoporous silica nanoparticle amount

Concentrations of 0, 0.04, 0.08, 0.12, 0.16, and 0.2 gr/mL of methanol solution containing rifampin were prepared, and a standard curve was obtained using ultraviolet (UV)-Vis at a wavelength of 254 nm. MSN powder and rifampin were dissolved in methanol with 2:2 weight ratio of MSN to rifampin and 2:1 weight ratio of MSN to rifampin. The solutions were stirred at a speed of 500 rpm for 24 h. Then centrifuged at 1400 rpm for 20 min, and the supernatant was collected. The remaining amount of rifampin in the solution was measured using UV-Vis spectrophotometry. The amount of rifampin loaded in MSN was obtained by calculating the difference between the initial amount and the remaining amount in the supernatant.[14,15]

Using the UV-Vis examination of the supernatant of the solutions after centrifugation; the loading efficiency of rifampin in MSN was determined in two weight ratio of MSN to rifampin (2:2 and 2:1) approaches.

According to the provided Equation 1, the required amount of MSN for loading 1 g of rifampin in each approach was estimated, and initial bone cement samples were prepared to measure the compressive strength resulting from the use of both approaches [Table 1].

$$X = (100 - \Sigma) / \Sigma \quad (1)$$

Where, the required amount of MSN for loading 1 gram of rifampin (x) at different loading efficiencies (∑).

Optimized sample preparation

The amount of antibiotic powder added is considered at the minimum common level (1 gr) per 40 gr package of commercial cement to focus more on reducing the

Table 1: Compressive strength samples to find the optimal loading approach

Samples	Additives to 40 g cement
B	-
B2:2	The required amount of MSN for loading 1 g of rifampin in 2:2 approach
B2:1	The required amount of MSN for loading 1 g of rifampin in 2:1 approach

MSN – Mesoporous silica nanoparticles

compressive strength caused by the undesirable chemical reaction between rifampin and cement. Adding higher amounts than 1 gr of antibiotics will decrease the mechanical strength of the cement due to the formation of empty cavities after washing the antibiotic mass. However, adding 1 gr of them does not have a significant effect on the mechanical strength unless they create cavities due to a chemical reaction like rifampin.[9] The test samples are as follows: B1 (pure bone cement), B2 (cement containing 1 g of rifampin), and B3, which indicates cement containing 1 g of rifampin loaded into an optimal amount of MSN.

Commercial Synicem® arthroplasty acrylic bone cement is purchased. The mixing of the additive antibiotic powder and bone cement powder is done by manual stirring in a mortar, and then, it is added to the liquid part of the cement.[16]

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra were obtained using an FTIR spectrometer (model: Varian FTS 1000). Each sample was subjected to 20 scans in the spectral range of 500–4000 cm^{-1}, with a resolution of 4 cm^{-1} to ensure accurate and reliable spectral data acquisition. The mechanism involved in FT-IR spectroscopy is based on the absorption of infrared radiation by the sample, which causes molecular vibrations that are characteristic of the various functional groups present in the material. These vibrations lead to specific peaks in the FT-IR spectrum, which allow the identification of chemical bonds and functional groups in the sample.

Surface morphology of the bone cement

Cross-sectional fracture and intact surfaces were prepared for morphology investigation using SEM from samples that had been stored in PBS for 5 weeks. Sample preparation was done by coating with a gold layer of 40-µm thickness using the sputter coater method, and SEM imaging was performed.[7]

Mechanical tests

After mixing the liquid and cement powder, the cement is molded in Teflon molds with dimensions of 6 mm × 12 mm. According to ISO 5883-2002 standard, the compressive strength test was conducted after 24 h of cement incubation in PBS solution at a temperature of 37°C ± 1°C with a pressure application rate of 25 mm per minute.[17]

To evaluate the cement–bone adhesion, holes with a diameter of 6 mm are created in cortical bone samples. After mixing the liquid and cement powder, the cement is injected into the holes and allowed to set. The bone is sliced to a thickness of 5 mm, and after placing it in grips, a 5-mm diameter rod is brought close to the hole at a speed of 0.5 mm per minute. The force required to push out the hardened cement from the bone holes is measured as the shear strength along the common surface.[18]

Contact angle

For each case, samples with a smooth surface were prepared, and distilled water drops with volume of 5 µL were placed on it. The obtained images were analyzed by Image J software to measure the angles.[19]

In vitro elution of antibiotic

Samples with cylindrical shapes in sizes of 6 mm × 12 mm were prepared and placed in 5 mL of PBS solution. After drawing the standard rifampin curve in PBS solution, the release rate was examined at 48, 24, 6 h, and the end of the first and third weeks by taking 1 mm of the solution each time and replacing it with 1 mL of fresh PBS. The withdrawn liquid was measured for rifampin release using the UV-Vis method at a wavelength of 254 nm.[10,13]

Biological evaluation

The antimicrobial activity of cement samples is being evaluated by diffusion in an agar environment. After culturing bacteria in test tubes until reaching a turbidity of 0/5 McFarland, the solution was used to cover the entire surface of the agar with a sterile swab. Then, bone cement discs (2 mm × 6 mm) were placed on a plate. After 18 h of incubation, the zone of inhibition was measured using antibiotics released from the cement pieces and compared.[20]

Cell culture tests and evaluation of cells were done by the MTT method and using MG63 cell grouping. 10000 cells were placed on the each test samples. The MTT test is based on the reduction of tetrazolium salts by the metabolic activities of the living cell (by dehydrogenase enzyme), and as a result of the reduction process, violet-colored formazan crystals are formed. Then, these crystals are dissolved in a suitable solvent, and the amount is measured by spectrophotometric methods. The amount of color created (due to the dissolution of formazan crystals) can indicate the percentage of living cells.

Investigation of cytotoxicity was done on the 1st and 7th days after cell culture on the samples by MTT test. For this purpose, 3 samples from each group were placed in a culture container of 24 cells per day, and about 10^5 cells were cultured on them. MTT powder was also dissolved in PBS with a concentration of 5 mg/mL and sterilized by

filter. At the end of each day, the culture medium in the well was drained and replaced with a mixture of DMEM and MTT in a ratio of 10:1 and added to each plate and incubated for 4 h at 37°C and 5% CO2. After 4 h of incubation, 200 µL of dimethyl sulfoxide was added to each well and successive pipetting was done to dissolve the blue-colored formazan inside the cell and a uniform solution obtained. Then the absorbance of the extract was read with a spectrophotometerin the wavelength 590 nm. Higher absorption values mean more formation of formazan, which is directly proportional to the number of living cells.[21]

Statistical analysis

The data were processed using Microsoft Excel 2003 software. Graphs were plotted in OriginPro software, and statistical analysis was performed using GraphPad Prism software ver.10.3.1.509. Each sample was tested three times in the experiment, and the mean ± standard deviation was reported. A one-way ANOVA analysis was used to compare the means of two samples, and statistical significance (when the $P < 0.05$) was determined.

Results

Rifampin loading rate and optimization of mesoporous silica nanoparticle amount

Figure 1 shows the UV-Vis examination of the supernatant of the solutions after centrifugation; the loading efficiency of rifampin in MSN was determined in two approaches.

According to the provided Equation 1, the required amount of MSN for loading 1 g of rifampin in each approach was estimated, and initial bone cement samples were prepared to measure the compressive strength resulting from the use of both approaches. Table 2 indicates the required amount of MSN for loading 1 g of rifampin at different loading efficiencies, and Figure 2 shows the effect of these values on the compressive strength of cement.

Figure 2 shows the results of the compressive strength test of cementitious parts containing MSN. The amount of MSN added to the cement in each part is specified in Table 2. The values obtained from the compressive strength test indicated that adding MSN to the cement up to about 4 g does not create significant changes in the compressive strength of the samples (B2:2) while increasing the MSN to 6.49 g leads to a significant drop in the strength of the cement samples (B2:1). Therefore, for the loading of rifampin in MSN, the 2:2 approach was used. In this loading method, 1 g of rifampin is loaded into approximately 4 g of MSN and then added to the cement. Table 3 shows the prepared samples. These values are expressed per 40 g of cement (the common weight of a commercial bone cement packet). These samples were used for subsequent tests.

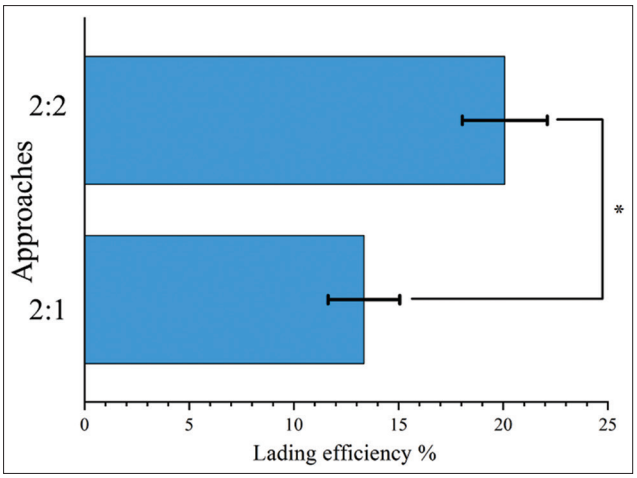

Figure 1: The loading efficiency in two approaches: 2:2 and 2:1 (mesoporous silica nanoparticles: rifampin) ****P* **< 0.05

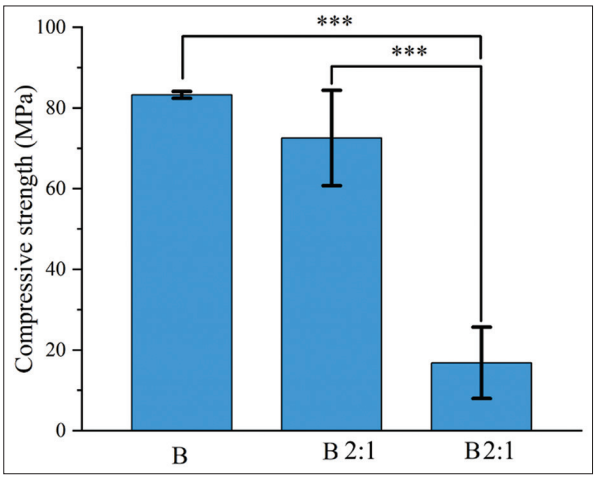

Figure 2: Compressive strength in the commercial sample (B), containing 4 gr of mesoporous silica nanoparticles (MSN) (B2:2) and containing 6.49 gr of MSN (B2:1) ******P* **< 0.01

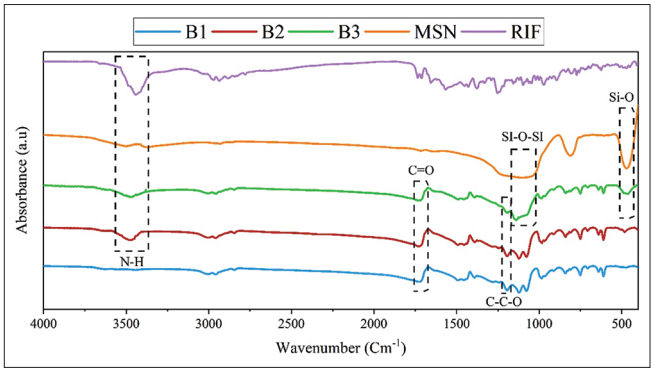

Figure 3: Fourier transform infrared results of the bone cements. B1: pure bone cement, B2: bone cement with 1 gr rifampin, B3: bone cement with 1 gr rifampin loaded in mesoporous silica nanoparticles (MSN), MSN: pure mesoporous silica nanoparticles and rifampicin: pure rifampin. MSN: Mesoporous silica nanoparticles, RIF: Rifampicin

Fourier transform infrared spectroscopy

Figure 3 shows the result of the FTIR test that evaluates

chemical functional groups of the samples. Specific peaks of C = O (1728 cm^{-1}) and C-C-O (cm^{-1})[22] which indicate the presence of polymethyl methacrylate as the main constituent of acrylate bone cement were observed. The peaks created by bands Si-O (468 cm^{-1}) and Si-O-Si (1105 cm^{-1})[23] indicate the presence of MSN. Furthermore, the peak created by N-H (3476 cm^{-1})[24] was observed in samples containing rifampin.

Surface morphology of the bone cement

Figure 4 shows the SEM analysis of fractured surface from the samples with 5 weeks immersed in PBS; this indicates a 11.98% reduction in the area of the surface holes of sample B3 compared to sample B2. In addition, each of the samples B2 and B3 show an increase in the area of the surface holes by 66.89% and 46.89%, respectively, compared to sample B1.

Table 2: The required amount of mesoporous silica nanoparticles for loading 1 g of rifampin (x) at different loading efficiencies (∑) *P*<0.05

Weight ratio of MSN:rifampin (g:g)	Rifampin in supernatant (%)	∑ (%)	x (g)
2:2	79.92±2.03	20.07±2.03	4.01±0.51
2:1	86.65±1.71	13.34±1.71	6.49±0.94

MSN – Mesoporous silica nanoparticles

Table 3: Samples coding for physiochemical and biological tests

Samples	Additives to 40 g cement
B1	-
B2	1 g rifampin
B3	1 g rifampin loaded in 4 g MSN

MSN – Mesoporous silica nanoparticles

Mechanical tests

The measured compressive strength in samples B1, B2, and B3 is 83.27 ± 0.85 MPa, 12.86 ± 0.21 MPa, and 60.76 ± 8.29 Mpa, respectively; hence, the compressive test results [Figure 5a] indicate a significant reduction (84.55%) in the compressive strength of cement due to the addition of rifampin (B2). This value was measured at 27.03% in B3. The examination of the elastic modulus in this test shows that the addition of rifampin, whether added freely or loaded into MSN, reduces the elastic modulus of cement. Changing the strength values in sample B3 compared to sample B2 demonstrates the relative success of the rifampin isolation by loading method in MSN.

The force required to extrude cement from the bone hole in the push-out test was measured as 799.55 ± 9.61 N, 104.36 ± 17.2 N, and 624.30.29 N in samples B1, B2, and B3, respectively. The study of the push-out test [Figure 5b] conducted with cement-filled bone samples showed 86.94% reduction in bone and B2 cement joint season strength. The push-out test showed a decrease in the adhesion of the cement containing rifampin to bone. Furthermore, the comparison of B2 and B3 samples showed that rifampin loading in MSN prevented the reduction of push-out force by 65.09%.

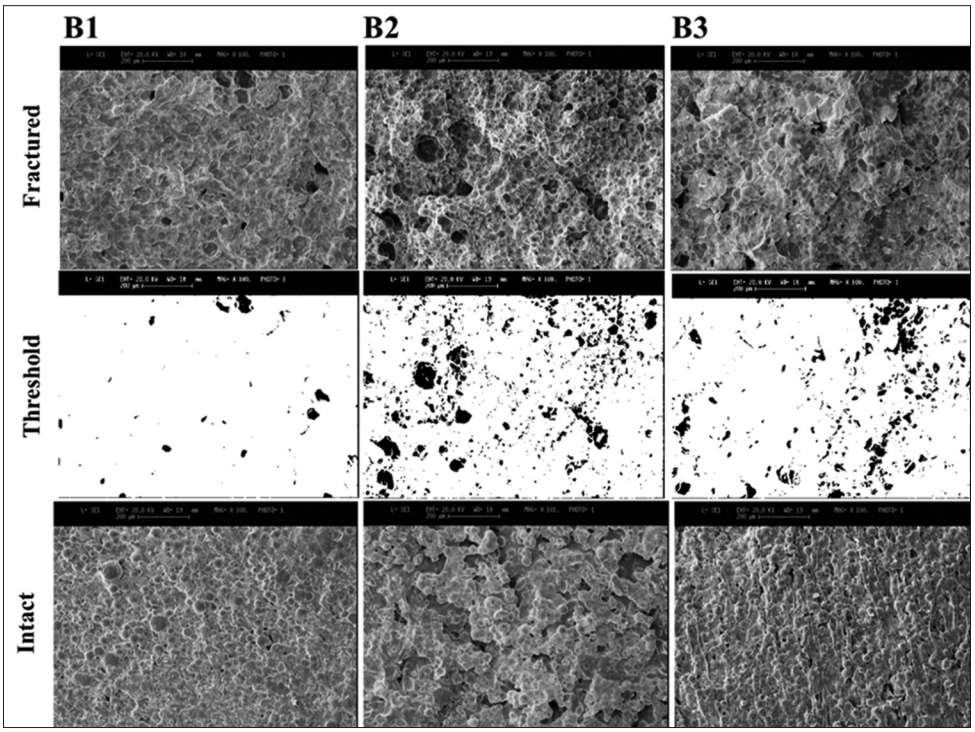

Figure 4: Scanning electron microscopy images of bone cement samples after 5 weeks of immersion in phosphate-buffered saline. The intact and fracture surfaces of samples B1, B2, and B3 are shown. Holes are shown in the fractured surface images

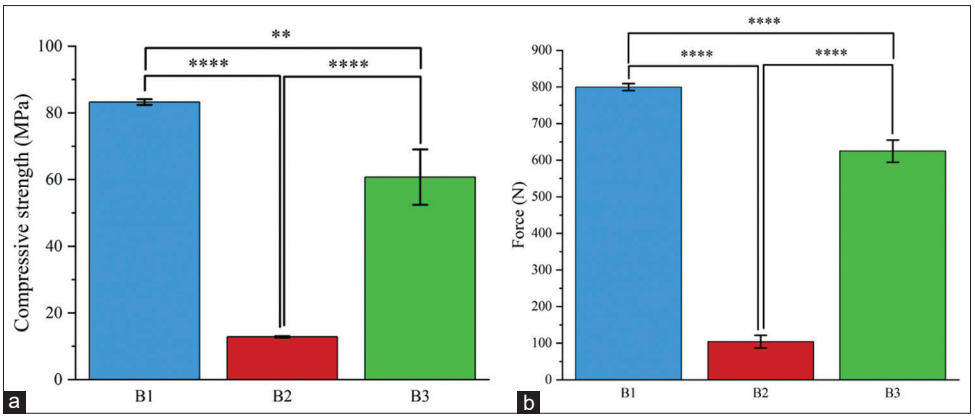

Figure 5: Mechanical tests: The compressive strength (a). The amount of force required to push out the cement from the bone cavity (b) **$P < 0.01$, ****$P < 0.0001$

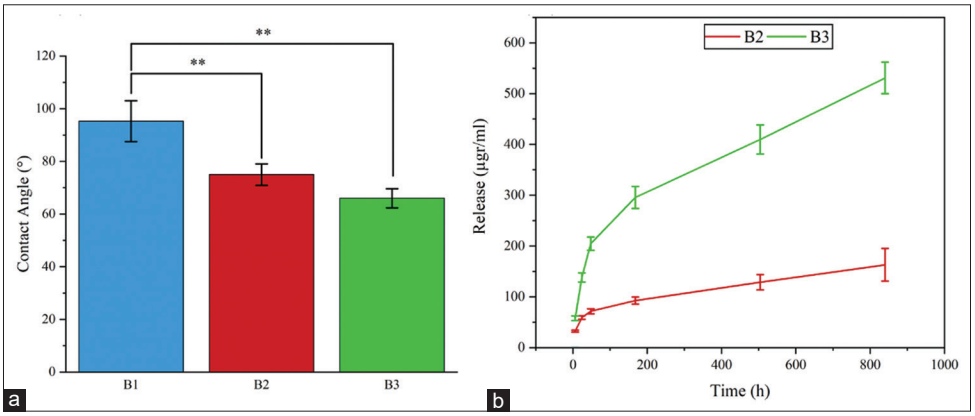

Figure 6: The contact angle of a water droplet with the surfaces of cement samples: B1 (95.26 ± 7.76), B2 (75 ± 4.08), and B3 (65.99 ± 3.63) (a). The cumulative release curve during 5-week immersion in phosphate-buffered saline at 37.5°C (b) **$P < 0.01$

Contact angle

The measurement of contact angle on the sample surfaces [Figure 6a] indicates a decrease in contact angle when antibiotics are added to the cement. The contact angle of commercial bone cement (B1) was measured as 95.26° ± 7.76°. This angle is reduced by 22% with rifampin in B2 and 31% with MSN-loaded rifampin in B3. The angles measured were B1 (95.26° ±7.76°), B2 (75° ±4.08°), and B3 (65.99° ±3.63°).

In vitro elution of antibiotic

The liquid spectrofluorometric analysis from the immersion sample [Figure 6b] revealed that the most effective release of antibiotics from the cement containing antibiotics occurs during the initial hours of soaking in the PBS. The release of rifampin from samples B2 and B3 within the first 24 h was measured to be 36.73% and 26.4% of the total release, respectively.

Continuing the spectrofluorometric analysis for up to 5 weeks showed that the overall release of rifampin from B3 was 2.23 ± 0.2 times more than B2.

In the analysis of the results obtained by measuring the contact angle of the surface of the cement samples, the B3 sample was only 12% more hydrophilic than the B2 sample, but the results of measuring the release of rifampin from both samples show that during 5 weeks of release in PBS, the B3 sample had more than twice the release of rifampin than the B2 sample.

Biological evaluation

Figure 7a-c shows the antimicrobial activity of cement discs indicated by the inhibition zone measurement against *Staphylococcus aureus* and *Staphylococcus saprophyticus* (CoNE) bacteria around the cement discs. The cement containing rifampin creates a larger inhibition zone (14.5%) in the CoNE environment compared to Aureus. In addition, the inhibition zone around the B3 was measured to be 15.44% and 11.8% larger than B2 in the CoNE and Aureus environments.

The results of the MTT assay indicate a decrease in cell viability in the presence of bone cement compared to the control group without the sample. According to Figure 7d at the end of the 1st day, sample B1 showed 8.48% less cell viability compared to the control sample. At the end of the 1st day, samples B2 and B3 showed 13.31% and 14.12% less cell viability, respectively, compared to the

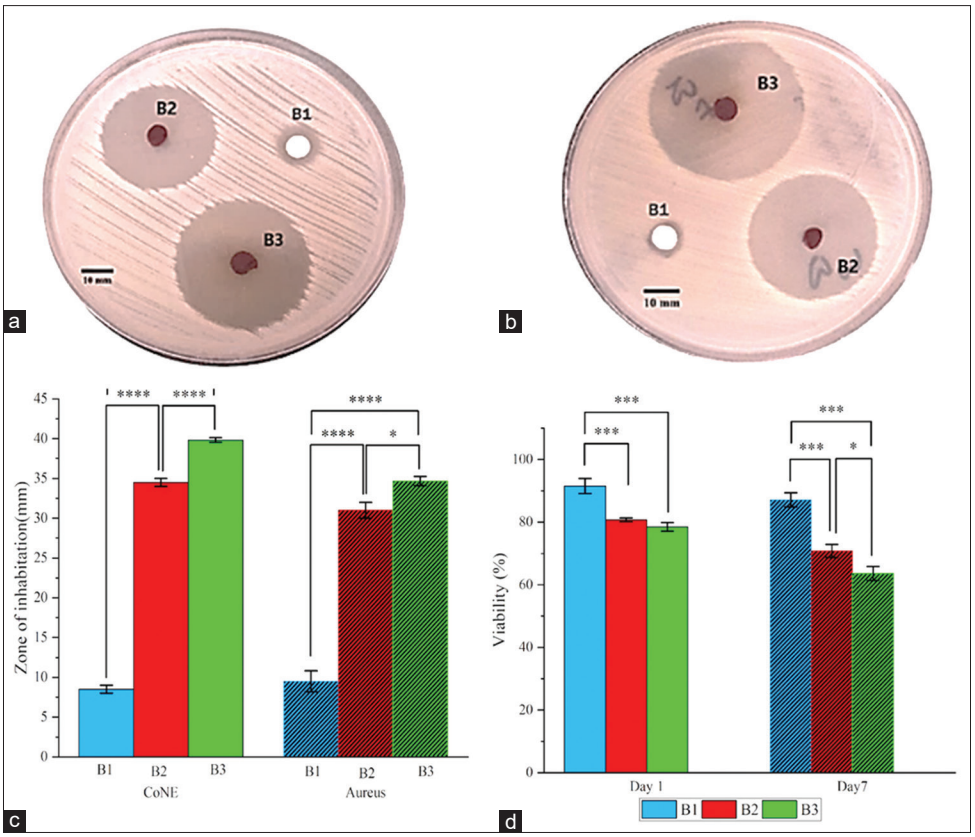

Figure 7: Inhibition zone of bacterial growth in the culture medium of *Staphylococcus aureus* (a) and *Staphylococcus saprophyticus* (b). The diagram of the antimicrobial test (c). The results of examining the cellular response of cement pieces **P < 0.05, **P < 0.01,*P<0.001, ****P < 0.0001* (d)**

pure cement sample (B1). At the end of the 7th day of the experiment, sample B1 showed 12.86% less cell viability compared to the control sample, and samples B2 and B3 showed 18.7% and 26.97% less cell viability, respectively, compared to the pure cement sample (B1).

Discussion

The cement containing rifampin can be used in the prevention and treatment of deep bone infections, but the undesired reaction between cement and rifampin poses a challenge to its use. Preventing direct contact between rifampin and cement during polymerization was one of the goals of this study. Rifampin was loaded onto an inactive carrier in the cement and then added. Measurement of various components, including mechanical properties, indicated the relative success of this method in addressing the challenge associated with the cement containing rifampin. A previous study by Sanz-Ruiz *et al*. in 2018[10] used the encapsulation method by putting rifampin in alginate microcapsules and PHBV microcapsules to isolate it from the cement monomers. Although they reported improving in mechanical properties, it is not possible to say whether rifampin isolating and preventing its adverse reaction with bone cement improved mechanical properties or not because the effect of alginate and PHBV on cement properties was not measured in this study.

The significant reduction in compressive strength in the cement containing rifampin may be due to the formation of deep cavities due to undesirable interaction between cement and rifampin, causing the lack of progress in the polymerization reaction. In our study, the loading of rifampicin (RIF) in MSN reduces the direct contact between hydroquinone and cement components and leads to the maintenance of the relative mechanical properties of the cement. The results showed that by loading rifampin in MSNs, the voids decreased by 12%, whereas the compressive strength increased by 57.52%. Moreover, in sample B3, the drug is loaded in MSN. Therefore, drug release from these nanoparticles does not cause a lot of voids in the cement substrate, while in B2, the drug is mixed with the cement and its release creates a lot of pores. On the other hand, the presence of MSN particles in the B3 sample is effective in the mechanical strengthening of the structure.

According to a study by Funk *et al*. in 2019,[9] drug release from the bone cement is a function of contact angle and the possibility of water penetration into the internal structure of bone cement due to the presence of pores and cavities. Our research revealed that although the pores present in the cement B3 are approximately 12% less than in the cement B2, the antibiotic release from B3 cements is about 2.34 times more than B2 ones. This phenomenon

is due to the lower contact angle and better wetting properties of B3 samples caused by the presence of MSN superhydrophilic particles. The obtained results are also in agreement with Krainer and Hirn[25] who reported an exponential relationship between the increase in wettability and the increase in the amount of drug release. The release of antibiotics from cement samples containing free antibiotics (B2) reached its maximum level in the initial 24 h and then decreased, while the loaded antibiotics in MSN showed more stability in release.

Considering the cellular response of cement samples and comparing them with the control group (cells without cement sample) showed that the cells are less compatible with the cement containing the antibiotic rifampin. The results are in agreement with the study of Duewelhenke *et al*. in 2007.[26] Local concentration of antibiotics can disrupt the metabolism of mitochondria and reduce the speed of proliferation of osteoblast cells. This disruption in the process of proliferation and metabolism of cells by antibiotics such as fluoroquinolones, clindamycin, and rifampin develops more than glycoside antibiotics such as gentamicin and glycopeptides such as vancomycin. The results of cell response also supported the results of drug release; however, in all groups, cell viability was more than 80% on the 1st day and more than 50% on the 7th day, which indicated the absence of cytotoxicity according to the standards.

Conclusion

Bone cement with added antibiotics is usually effective in preventing and treating bone infections. RIF is one of the most effective antibiotics against common bacteria in bone infections. The challenges associated with the use of the cement containing RIF include a significant reduction in strength, an increase in setting time, and an increase in the level of unreacted monomers. In this study, loading of RIF in MSN was used to overcome the strong decrease in cement strength. Reduction of direct contact between cement and RIF led to relative preservation of mechanical properties. As highly hydrophilic compounds, MSNs increase the wettability in the cement matrix, thereby improving drug release and preventing antibiotic resistance. Based on the obtained results, it seems that the leakage of RIF from MSNs into the cement prevents the complete separation of RIF during mixing. Therefore, it is suggested to use more reliable isolation methods such as nanoencapsulation in MSN in the future studies.

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Conflicts of interest

There are no conflicts of interest.

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