Microencapsulation of Rifampicin: A Technique to Preserve the Mechanical Properties of Bone Cement

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Received 19 January 2017; accepted 19 May 2017
Published online 23 May 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.23614

ABSTRACT: Two-stage exchange with antibiotic-loaded bone cement spacers remains the gold standard for chronic periprosthetic joint infection (PJI). Rifampicin is highly efficient on stationary-phase staphylococci in biofilm; however, its addition to PMMA to manufacture spacers prevents polymerization and reduces mechanical properties. Isolation of rifampicin during polymerization by microencapsulation could allow manufacturing rifampicin-loaded bone cement maintaining elution and mechanical properties. Microcapsules of rifampicin with alginate, polyhydroxybutyratehydroxyvalerate (PHBV), ethylcellulose and stearic acid (SA) were synthesized. Alginate and PHBV microcapsules were added to bone cement and elution, compression, bending, hardness, setting time and microbiological tests were performed. Repeated measures ANOVA and Bonferroni post-hoc test were performed, considering a $p < 0.05$ as statistical significance. Bone cement specimens containing alginate microcapsules eluted more rifampicin than PHBV microcapsules or non-encapsulated rifampicin over time ($p < 0.012$). Microencapsulation of rifampicin allowed PMMA to preserve mechanical properties in compression and bending tests. Cement with alginate microcapsules showed similar behavior in hardness tests to control cement over the study period ($73 \pm 1.68 H_D$). PMMA with alginate microcapsules exhibited the largest zones of inhibition in microbiological tests. Statistically significant differences in mean diameters of zones of inhibition between PMMA loaded with alginate-rifampicin ($p = 0.0001$) and alginate-PHBV microcapsules ($p = 0.0001$) were detected. Rifampicin microencapsulation with alginate is the best choice to introduce rifampicin in PMMA preserving mechanical properties, setting time, elution, and antimicrobial properties. The main applicability of this study is the opportunity for obtaining rifampicin-loaded PMMA by microencapsulation of rifampicin in alginate microparticles, achieving high doses of rifampicin in infected tissues, increasing the successful of PJI treatment.
 J Orthop Res 36:459–466, 2018.

Keywords: rifampicin-loaded bone cement; rifampicin microencapsulation; bone cement spacers; periprosthetic infection

Periprosthetic infection (PJI) is a major complication, with a raised prevalence in the last decades because of the increased rate of primary and revision arthroplasties.[1] The high susceptibility of implanted devices to infection is due to a locally acquired host defense defect. Rapid formation of a biofilm resistant to host defense mechanisms and antimicrobial agents causes the persistence of infection.[2,3]

Two-stage exchange remains the gold standard for treatment in chronic PJI. It includes the removal of the prosthesis and the implantation of a temporary antimicrobial-impregnated bone cement spacer that maintains joint space and allows for the direct delivery of antibiotics to the infected tissues. There exists a great deal of data of the use of certain antimicrobial agents in PMMA, being aminoglycosides and vancomycin the most common agents used. The increasing emergence of multiresistant bacterial strains threatens the effectiveness of local antibiotic treatment.[4,5]

The role of rifampicin against device-associated staphylococcal infection has been demonstrated. Rifampicin is highly efficient on adherent and stationary-phase staphylococci and is now a standard combination antibiotic in the systemic treatment of (PJI).[6,7] However, its addition to bone cement to manufacture spacers is not possible yet, because of the deterioration of the mechanical properties and the prevention of complete polymerization. The exact mechanism of this interaction has not been elucidated.[4,8–10]

Isolation of rifampicin during the polymerization process by microencapsulation techniques could allow adding rifampicin to bone cement, preserving good elution, and mechanical properties.

The aims were:

(1) Determine the feasibility of loading rifampicin into microcapsules and the capability of microencapsulated rifampicin to elute to a physiologic liquid medium.
(2) Determine the setting time of PMMA loaded with microencapsulated rifampicin and the consequences on mechanical properties.
(3) Quantify the in vitro release profile of microencapsulated rifampicin-loaded PMMA.
(4) Determine the effectiveness of microencapsulated rifampicin-loaded PMMA against a common strain of *Staphylococcus aureus*.

MATERIALS AND METHODS

Synthesis and Characterization of Microcapsules

Microcapsules containing rifampicin Rifaldin® (Sanofi®, Barcelona, Spain) as core material were prepared employing alginate, polyhydroxybutyratevalerate (PHBV), ethylcellulose

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and stearic acid (SA) as shell materials. The ionic gelation process was used to prepare the alginate microcapsules and solvent evaporation method to prepare the PHBV, ethylcellulose and SA ones.[11]

The rifampicin content in the microparticles was measured by dissolving 5 mg of microcapsules in 5 ml of dichlorometane/methanol solution (10%/90%). In alginate microcapsules, rifampicin content was quantified by dissolving the microcapsules in ethylenediaminetetraacetic acid (EDTA) 0.25 M because alginate cannot dissolve in dichloromethane and methanol. Solution was centrifuged at 4000 rpm for 5 min. The concentration of rifampicin in the supernatant was assayed by ultraviolet-visible spectrophotometry (UV-Vis, Cary 4000) (Agilent Technologies®, Santa Clara, CA) at 334 nm. Each sample was analyzed in triplicate.

To characterize the process of microencapsulation three parameters were described: Rifampicin content (% Rifampicin), microcapsules production yield and rifampicin encapsulation efficiency. Rifampicin content was defined as the ratio of the weight of rifampicin obtained over the weight of microcapsules. Microcapsules production yield was determined by the ratio of the total amount of raw material used in the process to the weight of the microparticles obtained. Rifampicin encapsulation efficiency was defined as the ratio of the actual amount of encapsulated rifampicin over the total amount of rifampicin used.[12] Five specimens of each sample of microcapsules were analyzed in triplicate.

The morphology of the microparticles was observed under scanning electron microscopy (SEM) (Philips X-30®, Philips Electronic Instruments, NJ). It was spherical in samples of PHBV and ethylcellulose and irregular in SA and alginate (Fig. 1).

In Vitro Elution Analysis of Encapsulated Rifampicin

Five milligram of microcapsules were weighed into test tubes with 5 ml of phosphate buffer (PBS) at pH 7.4, which were placed at 37°C. After 6, 24, and 48 h and 1, 2, and 5 weeks, 1 ml was removed and replaced with 1 ml of new PBS. Each sample was analyzed in triplicate. Concentrations of rifampicin were determined by UV-Vis spectrophotometry. Prior to the measurements, the corresponding calibration curve was prepared.

PHBV and alginate microcapsules showed better elution properties and were selected to the following tests. Microencapsulation and elution tests were performed again. Each sample was analyzed in triplicate.

Bone Cement Specimens

Bone cement was prepared manually in an appropriate sterile container with a spatula, following the ASTM F451:99 "Standard Specification for acrylic bone cement"[13] and the International Standard ISO 5833:2002 "Implant for surgery-acrylic resin cements."[14] Four groups were defined, group one (control): Bone cement specimens without rifampicin, group two: Bone cement specimens with non-encapsulated rifampicin, group three: Bone cement specimens with PHBV microcapsules containing rifampicin and group four: Bone cement specimens with alginate microcapsules containing rifampicin. Rifampicin powder or rifampicin microcapsules (1.25 or 5 wt%, respectively) were incorporated to the powder of acrylic bone cement following Frommelt's recommendations to homogeneously disperse the antibiotic.[15] The polymer powder and the monomer liquid were mixed and introduced, in doughy consistency, in different molds to prepare the bone cement samples (Fig. 2). Table 1 shows the quantitative composition of acrylic bone cement.

Specimens for elution and compression testing were cylinder-shaped (12 mm high and 6 mm diameter). Specimen dimensions for bending and hardness tests were $80 \times 10 \times 4$ mm. ISO 5833:2002 and ISO 7619:2011[14,16] were followed. In microbiologic assays, cylinder specimens of 2 mm high and 12 mm diameter were used.

Mechanical Tests

Compression tests were performed using the universal testing machine ELIB 20W (Ibertest®, Madrid, Spain) and four-point bending tests with the universal testing machine IBTH/500 (Ibertest®, Madrid, Spain), according to ISO 5833:2002 Standard.[14] Mechanical tests were done 1 week

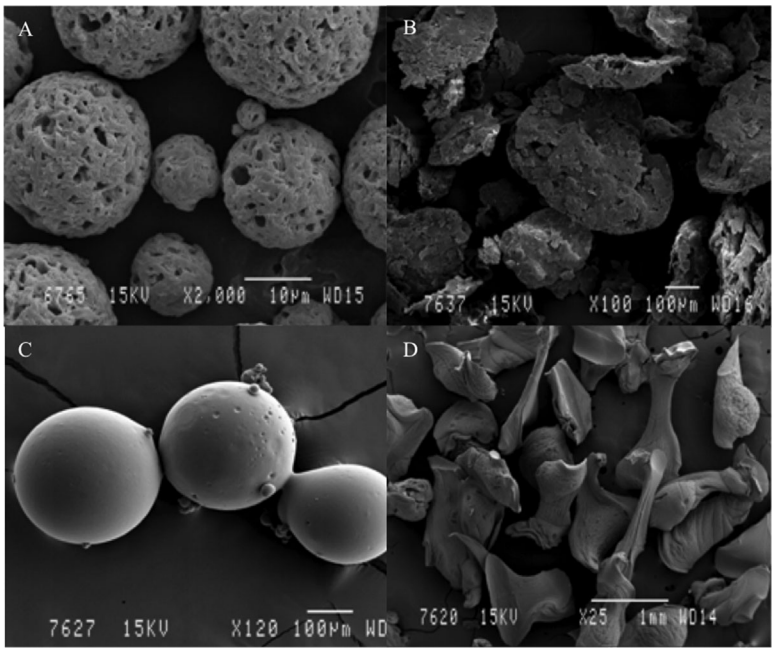

Figure 1. SEM images of the microcapsules. (A) PHBV. (B) Stearic acid. (C) Ethylcellulose. (D) Alginate.

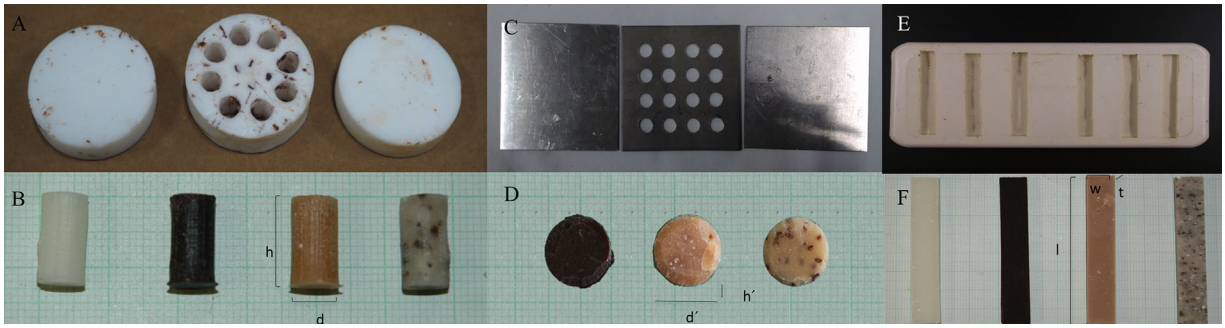

Figure 2. Molds and bone cement samples. (A and B) Elution and compression tests (h: height, d: diameter). (C and D) Microbiological tests (h': height, d': diameter). (E and F) Flexion tests (l: length, w: width, t: thickness).

after sample preparation. Shore D hardness measurements were performed using a Bareiss® Durometer (Bareiss®, Neurtek, Eibar, Spain) at 15, 30, 45, 60 min, 2, 3, 4, and 24 h after bone cement mixture. Bending tests were repeated at 45 min with these samples. Five specimens of each sample were analyzed in triplicate.

Rifampicin Elution Fom Bone Cement Samples

Twenty-four hour after preparation, five specimens of each group (non-encapsulated Rifampicin, PHBV and alginate microcapsules containing rifampicin) were immersed into individual test tubes with 5 ml of PBS and were placed in an incubator at 37°C. At 6, 24, and 48 h and 1 week, cylinders were removed from the tubes and immerse into new PBS, and rifampicin concentration was measured by UV-Vis spectrophotometry. The corresponding calibration curve was previously prepared.

Microbiological Tests

The antimicrobial activity of bone cement specimens with non-encapsulated rifampicin, PHBV and alginate microcapsules was assessed by disk diffusion test. Eight agar plates were seeded with *S. aureus* ATCC®29213™ by inoculation with an overnight broth culture adjusted to MacFarland 0.5 turbidity. Diameters of zones of inhibition were measured at 24 h and photographs were taken. Each plate was measured in triplicate.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 22.0 for Mac (SPSS Inc. Chicago) using repeated measures ANOVA and Bonferroni post hoc analysis. Differences were considered to be statistically significant at a level of $p < 0.05$.

RESULTS

Microencapsulation parameters of PHBV, SA, ethylcellulose and alginate are summarized in Table 2. Ethylcellulose showed the highest rifampicin content, microcapsules production yield and rifampicin encapsulation efficiency. Differences in rifampicin content between samples of PHBV, SA, ethylcellulose and alginate reached statistical significance ($p < 0.003$).

Alginate microcapsules showed higher rifampicin elution in PBS than PHBV, ethylcellulose and SA microcapsules ($p = 0.0001$). The cumulative rifampicin release over study period is shown in Figure 3A. SA and ethylcellulose microcapsules showed low elution rate at 6 h, so they were ruled out. PHBV and alginate microcapsules were selected to continue the study.

The mean microcapsules production yield was 70% and 135% in PHBV and alginate samples, respectively. Rifampicin-Alginate microcapsules production yield was greater than 100%. This fact was attributed to the use of a $CaCl_2$ (1.5 wt%)/chitosan (0.5 wt%) solution to induce ionic gelation. Calcium ions and chitosan, used as counterions, were anchored to alginate structure during microcapsule formation, contributing to final microcapsule mass. Alginate microcapsules showed higher encapsulation efficiency than PHBV. Rifampicin content was also higher in alginate samples than PHBV.

Alginate and PHBV microcapsules elution values as a function of time are shown in Table 3. Statistically significant differences between rifampicin concentrations eluted by alginate and PHBV microcapsules were reached at 6, 24, 48 h and 1 week ($p < 0.004$). Alginate microcapsules showed higher cumulative rifampicin elution over time than PHBV microcapsules (Fig. 3B).

Table 1. Quantitative Composition of Acrylic Bone Cement

Powder Phase		
Pre-polymerised polymer	Colacryl 866—polymethyl methacrylate (PMMA)	36.36 g*
Initiator	Colacryl 866—benzoyl peroxide (BPO)	
Radiopaque agent	Barium sulphate ($BaSO_4$)	3.54 g
Liquid Phase		
Monomer	Methyl methacrylate (MMA)	19.4 ml
Activator	N,N-Dimethyl-p-toluidine (DmpT)	160 µl

*2.5% of BPO is included in PMMA.

Table 2. Microencapsulation Characteristics and Rifampicin Elution at the Beginning and the End of the Study, of PHBV, Stearic Acid, Ethylcellulose, and Alginate Microcapsules

Sample	Polymer	Microcapsules Obtained (g)	Microcapsules Production yield (%)	Rifampicin Content (%)	Encapsulation Efficiency (%)	Elution 6 h (μg/ml)	Elution 5 Weeks (μg/ml)
M1	PHBV	0.70	70.63	1.86	11.67	15.58	31.43
M2	Stearic acid	0.71	69.55	0.31	1.84	3.27	5.13
M3	Ethylcellulose	0.93	89.42	6.99	54.17	3.70	43.97
M4	Alginate	2.32	135	5.43	60.02	28.27	122.97

Regarding mechanical properties, bending modulus showed the greatest differences 45 min after bone cement manufacturing (Table 4). Cement with alginate, PHBV microcapsules and non-encapsulated rifampicin showed a 7.5%, 21%, and 59% reduction in the bending modulus respectively, compared to the control cement. Bone cement with alginate or PHBV microcapsules and control bone cement exceeded the minimum values for bending modulus (1800 MPa) according to Standard ISO 5833.[14] There were significant differences between control-rifampicin ($p = 0.0001$), control-PHBV ($p = 0.036$), rifampicin-PHBV ($p = 0.0001$), and rifampicin-alginate ($p = 0.0001$).

Bending modulus results at 1 week are shown in Figure 4A. Bone cement containing non-encapsulated rifampicin caused an 18% reduction in the bending modulus compared to control cement. The addition of PHBV and alginate microcapsules to bone cement did not significantly alter the bending modulus compared to control cement ($p = 1$). All results showed higher bending modulus than the minimum established by ISO 5833.[14]

Figure 4B shows the compressive strength of the bone cement samples at 1 week. Specimens with PHBV and alginate microcapsules showed 14% lower compressive strength (91.26 and 91.35 MPa, respectively) than the control cement (106.2 MPa). However, they were considerably higher than ISO 5833 requirements (70 MPa).[14] Bone cement containing non-encapsulated rifampicin showed a 30% reduction (74.04 MPa) compared to control cement. Statistically significant differences were found when comparing the mean values of the compressive strength between control-rifampicin, control-alginate, PHBV-control, PHBV-rifampicin, and alginate-rifampicin (all $p = 0.0001$).

Cement with alginate microcapsules showed similar behavior in hardness tests to control cement over the study period ($73H_D$), reaching the maximum hardness within 15 min (Fig. 5). Cement with non-encapsulated rifampicin could not be tested at 15 min because it was too liquid for the assay, it started to acquire hardness at 30 min ($33H_D$) and achieved similar hardness to control cement within 45 min ($67H_D$).

Bone cement specimens containing alginate microcapsules eluted more rifampicin than specimens with PHBV microcapsules or non-encapsulated rifampicin at 6, 24, 48 h and 1 week ($p < 0.012$) (Fig. 6). A total of 48.89% of rifampicin included in PMMA with alginate microcapsules was eluted in the first 24 h.

Microencapsulated rifampicin in bone cement preserved its antimicrobial properties. Mean values of diameters of zones of inhibition were 20.78 ± 2.23, 4.89 ± 2.93, and 9.33 ± 4.41 mm for cement with alginate microcapsules, cement with PHBV microcapsules and cement with non-encapsulated rifampicin, respectively. Statistically significant differences were detected between PMMA with alginate microcapsules and PMMA with non-encapsulated rifampicin ($p = 0.0001$) and PMMA with alginate microcapsules

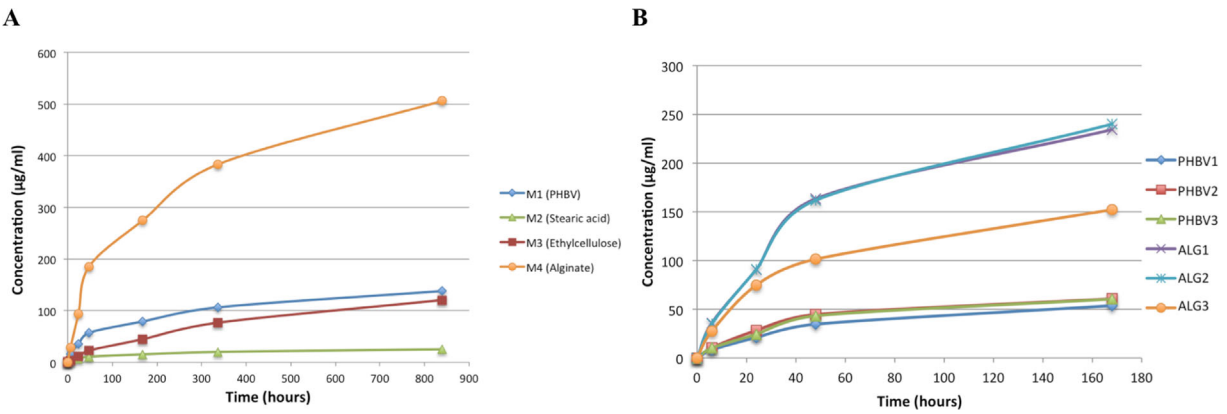

Figure 3. (A) Cumulative rifampicin elution from PHBV, stearic acid, ethylcellulose and alginate microcapsules over 5 weeks. (B) Cumulative rifampicin elution from PHBV and alginate microcapsules over 1 week.

Table 3. Elution Results of PHBV and Alginate Microcapsules Over the Study Period (Mean ± Standard Deviation)

	6 h	24 h	48 h	1 Week
Sample	CC (μg/ml)	CC (μg/ml)	CC (μg/ml)	CC (μg/ml)
PHBV 1	8.23 ± 0.95	12.73 ± 1.68	13.63 ± 3.25	19.10 ± 1.21
PHBV 2	10.53 ± 0.81	17.67 ± 2.65	16.40 ± 1.60	16.17 ± 2.25
PHBV 3	10.23 ± 1.36	14.10 ± 0.2	18.83 ± 2.25	17.23 ± 1.02
ALGINATE 1	35.97 ± 5.96	54.80 ± 2.10	72.70 ± 3.83	70.87 ± 3.75
ALGINATE 2	35.10 ± 6.23	55.70 ± 6.82	71.07 ± 11.32	78.17 ± 7.34
ALGINATE 3	27.50 ± 1.23	47.20 ± 2.90	56.63 ± 2.53	51.03 ± 3.51

and PMMA with PHBV microcapsules ($p = 0.0001$). Photographs of the zones of inhibition are shown in Figure 7.

DISCUSSION

The concern about increasing bacterial resistance is leading to consider different antibiotics, with high anti-staphylococcal activity (linezolid, quinolones, and rifampicin), to be added to bone cement. Anagnostakos et al.[17] determined the in vitro elution characteristics of linezolid alone and in combination with gentamicin when used in PMMA hip spacers. Matos et al.[18] studied a new delivery system of levofloxacin by calcium phosphate particles, which were added to PMMA. However, the addition of rifampicin to PMMA prevented complete polymerization becoming unsuitable for use in clinical practice, despite the adequate in vitro release and good activity against *S. aureus*.[4,10] The proposed mechanism is that rifampicin reacts with dibenzoyl peroxide (initiator) and/or dimethyl-p-toluidine (activator) being unable to react with the methylmethacrylate, and therefore the radical polymerization is inhibited.[19]

Rifampicin has been already encapsulated in lipids, PHBV, alginate, polylactic-co-glycolic acid (PLGA) and other polymers[20–23] to improve bioavailability and reduce dose for the treatment of pulmonary tuberculosis. There is only one report about the addition of microcapsules containing antibiotics to PMMA. Shi et al. fabricated PLGA microspheres (10 or 15 wt%) with colistin, which were loaded to PMMA to control drug release.[24] No studies were found about the microencapsulation of rifampicin for addition to bone cement in order to preserve mechanical properties and setting characteristics. Our results showed the possibility of adding microencapsulated rifampicin in PMMA for use in cement spacers in PJI. Elution and mechanical tests revealed that microencapsulation could be a suitable method to carry rifampicin in bone cement.

Elution of rifampicin microcapsules in PBS has been already described. Duran et al.[20] studied the elution kinetics of PHBV microparticles containing rifampicin by adding 5 mg of microcapsules to 5 ml of PBS with 10% ethanol. Concentration was determined spectrophotometrically at 1, 3, 6, 11, and 23 h. They observed an initial burst (almost 90% within 24 h) followed by sustained release. Large size microcapsules (high PHBV concentration) showed lower rate of elution in 24 h. We cannot make direct comparisons with our study because the composition of the microcapsules was different and the elution assay was performed in a different way. Despite this, the elution curve followed the same trend. Wu et al.[25] created PLGA and alginate microspheres with rifampicin and observed that smaller microcapsules had a higher release rate and a shorter lag phase. Sarfaraz et al.[26] prepared rifampicin biodegradable microcapsules with sodium alginate and Carbopol 974P® as coating materials. The elution kinetics was influenced by the coating ratio, as proportion of Carbopol 974P® increased, rifampicin release rate decreased. We obtained similar elution curves but the influence of the amount of polymer in the coat was not analyzed. Our results showed that elution of the microcapsules in PBS correlated with elution in cement. The elution tests confirmed the ability of rifampicin to elute from microcapsules and PMMA. Preservation of antimicrobial activity of rifampicin was confirmed by microbiological tests.

Han et al.[10] reported that rifampicin-loaded bone cement could not be used to manufacture spacers

Table 4. Mean Bending Modulus, Standard Deviation and Reduction Compared to Control of Specimens, 45 min After Bone Cement Manufacturing

	Bending Modulus (MPa)	Standard Deviation	Reduction Compared to Control
Control	2333	41	
RIF	954	279	−59.11%
PHBV	1844	216	−20.96%
Alginate	2158	195	−7.5%

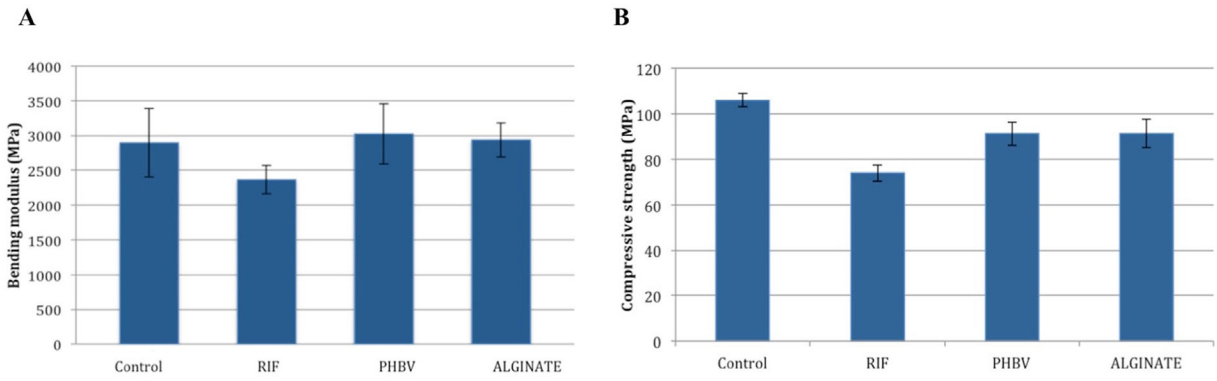

Figure 4. (A) Bending modulus of the bone cement samples. (B) Compressive strength of the bone cement samples.

because of its delayed polymerization. They studied the rifampicin elution from CMW®3 (Depuy, Warsaw, IN) discs cements in PBS on days 1, 3, 7, 14, and 30, using 1, 2, or 4 g rifampin per 40 g PMMA. Rifampicin was only detected on day 1 in the samples that contained 2 g rifampicin (0.7 ± 0.4 ug/ml/day) and up to 2 weeks in the samples that contained 4 g (0.1 ± 0.0 ug/ml/day). In our study, the amount of rifampicin added to bone cement was lower. However, elution from non-encapsulated rifampicin and alginate microcapsules showed statistically significant differences at 24 and 48 h ($p = 0.001$ and $p = 0.05$, respectively). Alginate microencapsulation resulted in an earlier elution than non-encapsulated rifampicin. The reduction in the mechanical properties of bone cement when high dose of antibiotics is added has been reported. He et al.[27] studied the amount of gentamicin that adversely affected the mechanical properties of Palacos®. A maximum of 6.5 wt% of antibiotic was recommended to preserve compression properties. Lautenschlager et al.[28] concluded that the addition of 10.1 wt% of gentamicin decreased compression strength below 70 MPa.

No answers were found about mechanisms to improve rifampicin-loaded bone cement properties. To our knowledge, this is the first experimental study that describes the microencapsulation of rifampicin as a technique to preserve the mechanical properties and the setting time of PMMA. Bone cement containing alginate microcapsules showed improved compression, flexion and hardness properties than bone cement with non-encapsulated rifampicin ($p < 0.05$). Rifampicin microencapsulation with alginate allowed obtaining similar setting time and hardness than control cement, taking less than 15 min to complete polymerization. In our mechanical tests, the polymerization of non-encapsulated rifampicin-loaded bone cement was delayed by 45 min, which is unsuitable for manufacturing bone cement spacers in the operating room. Longer times of set, 122.5 ± 31.1 min, were reported by Han et al.[10]

Microencapsulation of rifampicin does not alter the microbiological properties. Duran et al.[20] placed 100 ml of PHBV microcapsules containing rifampicin (4 mg/ml) in agar plates seeded with *S. aureus* ATCC®6538™. Diameters of zones of inhibition at 20 and 24 h of incubation were similar to those obtained after placing non-encapsulated rifampicin in the plates. Rifampicin added to bone cement preserves microbiological activity. Beeching et al.[9] manufactured CMW®1 discs loaded with rifampicin, and observed annular inhibition zones of 9 mm in agar plates seeded with *S. aureus* ATCC®25923™. They documented that rifampicin produced tacky black PMMA

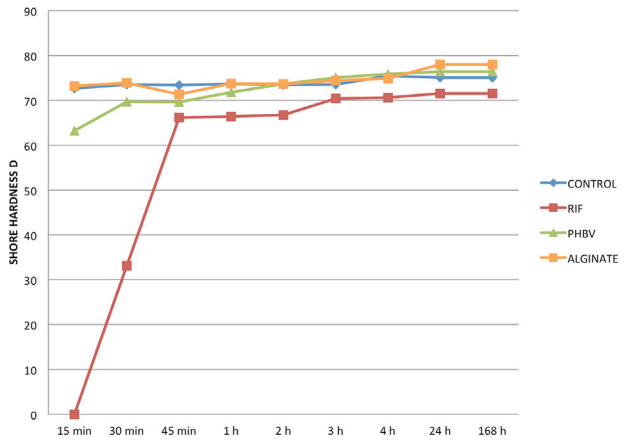

Figure 5. Hardness behavior over time (Shore D scale).

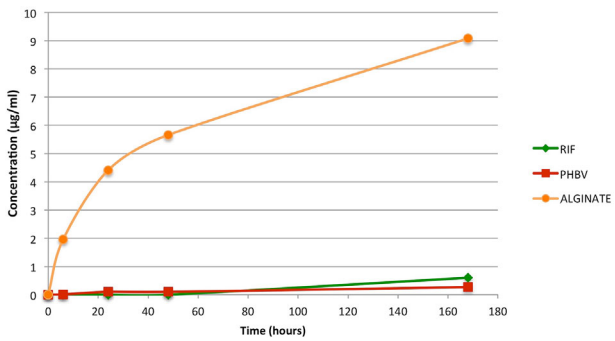

Figure 6. Cumulative rifampicin elution from bone cement specimens over 1 week.

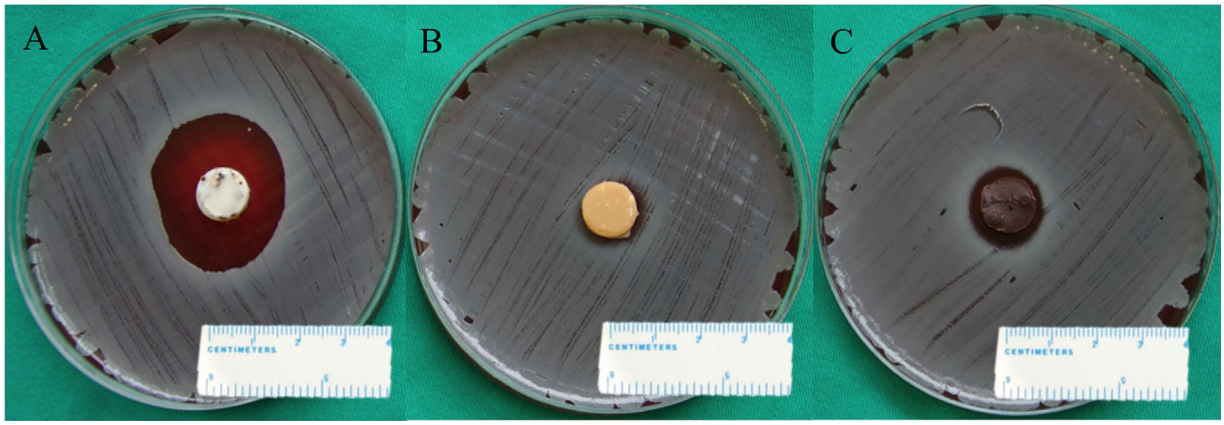

Figure 7. Zones of inhibition produced by bone cement with alginate microcapsules (A), PHBV microcapsules (B) and rifampicin (C) Powder Phase.

that did not harden for several days. Aiken et al.[29] studied the antimicrobial efficacy of rifampicin combined with calcium sulfate beads by a disc diffusion assay (ratio 600 mg: 20 g) against *S. aureus* ATCC®6538™. Zones of inhibition of bacterial growth around beads revealed that antimicrobial efficacy was unaltered after 42 days. We observed that zones of inhibition produced around bone cement containing alginate microcapsules were larger than bone cement with non-encapsulated rifampicin ($p = 0.0001$), because alginate increased bone cement porosity and enhanced the elution of rifampicin.

The main applicability of this study is the opportunity for obtaining rifampicin-loaded bone cement by microencapsulation of rifampicin in alginate microparticles. The adequate elution and mechanical properties could allow intraoperative manufacturing of bone cement spacers for the treatment of PJI. Achieving high local doses of rifampicin in infected tissues potentially increases the treatment success.

We acknowledge several limitations. In vitro results may not accurately reflect in vivo conditions; therefore, animal studies are needed to better understand the effects of rifampicin-loaded bone cement in PJI. In elution tests, imprecision in the manual extraction of the aliquots or inhomogeneous distribution of the microcapsules in PBS could result in the variability observed between the samples. The number of samples was also limited but several previous biomaterial studies used similar number of samples.[30] Synergism of rifampicin with other antibiotics was not studied, as it was not a purpose of the study. Our purpose was to release rifampicin from a PMMA with good mechanical properties and conventional setting time. We recognize that, in clinical practice, rifampicin should be used in combination with other antibiotics for manufacturing bone cement spacers.

In conclusion, microencapsulation of rifampicin with alginate is a good technique to introduce rifampicin in PMMA, without being detrimental to mechanical properties and setting time. Preservation of elution and antimicrobial properties allows create bone cement spacers for local delivery of rifampicin in the treatment of PJI.

AUTHORS' CONTRIBUTIONS

PS-R: original idea, designed and performed the experiments, paper construction. EC-L: designed and performed the experiments, paper construction. JCDR-R: performed sample measurement and paper review. FA-A: performed sample measurement and helped with editing of manuscript. YB-I: designed the experiments, paper review. EP-J: performed sample measurement and data analysis. MS-N: performed sample measurement and data analysis. MAL-P: performed sample measurement and data analysis. JV-M: data analysis, paper review. All authors have read and approved the final submitted manuscript.

ACKNOWLEDGMENTS

Carmen de Pablos, who was a student of the Mechanical Engineering Master of ICAI, performed some mechanical tests. Microbiology Department of Gregorio Marañón General Hospital helped us with the microbiological tests. The authors thank them for their contributions.

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