JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Antibacterial effect of acrylic bone cements loaded with drugs of different action's mechanism

Lizette Morejón Alonso[1], Iran Fernández Torres[2], Ángela M. Zayas Tamayo[2], Oscar Ernesto Ledea Lozano[2], Ivette Durán Ramos[1], José Ángel Delgado García-Menocal[1], Nely Rios-Donato[3], Eduardo Mendizábal[3]

[1] *Center of Biomaterials, University of Havana, Havana, Cuba*
[2] *National Center for Scientific Research, Havana, Cuba*
[3] *CUCEI, University of Guadalajara, Jalisco, Mexico*

Abstract

Introduction: Antibiotic-loaded bone cements of poly(methyl methacrylate) are considered as very useful biomaterials for the management of corporal deep osseous infections. However, the high prevalence of resistant germs and polymicrobial infections makes it necessary to search for new formulations of bone cements containing antibiotics for local antibacterial therapy. In this work, bone cements loaded with drugs with different mechanism of action were evaluated to determine its antibacterial effectiveness on *Pseudomonas aeruginosa*.

Methodology: Poly(methyl methacrylate) cements loaded with 10 wt.% of Oleozon®, mixtures of Ciprofloxacin/Meropenem and Ciprofloxacin/Meropenem/Oleozon® were prepared. The *in vitro* drugs release in water was followed by UV-Vis spectroscopy, and their antibacterial activity against *Pseudomonas aeruginosa* was evaluated for 11 days using the microdilution method.

Results: All the extracts demonstrated an inhibitory effect on the growth of the strain during the whole trial period. Extracts from cement with Oleozon® only presented a total antibacterial inhibitory effect during 20 hours for the extracts taken at day 1 while the extracts from the cements loaded with mixtures of Ciprofloxacin/Meropenem and Ciprofloxacin/Meropenem/Oleozon® showed complete inhibition of the growth of the microorganism, even at 11 days. At the end of the trial period, some of the drugs remained inside the matrices, indicating that they can be released for a longer time in treatments.

Conclusions: The results indicated a positive antibacterial effect by the combined used of the two or the three drugs tested against the Gram-negative bacilli *Pseudomonas aeruginosa*, so these proposal may be a valid alternative to be considered by surgeons.

Key words: Antibiotic loaded bone cement; local antibiotic therapy; osteomyelitis; periprosthetic joint infections.

J Infect Dev Ctries 2019; 13(6):487-495. doi:10.3855/jidc.10716

(Received 17 July 2018 – Accepted 23 April 2019)

Introduction

Septic processes of joint prostheses, open bone fractures, non-unions of fractures and osteomyelitis illness are deep infections of the musculoskeletal system that are considered severe disorders due to the psychological and physical consequences for patients, as well as the complexity of their treatment [1–3].

In recent years, periprosthetic joint infections (PJIs) have been reduced in many countries to 1-2 % [4,5] or less [6]. However, the high incidence of pathologies or traumatic orthopedic lesions that conduces to total joint replacements is increasing around the world and therefore, also the number of patients with the possibility to develop PJIs [7]. Osteomyelitis (an inflammatory process of the entire bone with the bone destruction caused by an infecting organism [8]) remains likewise as a severe worldwide problem, causing plenty of hospital admissions and requiring considerable expenses [9]. To treat it, the efforts are made by multidisciplinary specialists to diminish costs, co-morbidities and mortality in patients.

The management of bone infections is often characterized by the use of systemic antibiotic therapy, local drugs administration and surgical treatments. Surgical options of PJIs include [3]:

- irrigation and cleaning with retention of the prosthesis (success rate 0%-89%) [10];
- single-stage revision surgery (success rate > 80 %) [11]
- two-stage revision surgery (success rate 87 %) [12]
- arthrodesis (success rate varying from 60 %-100 %) [3]
- amputation

Bone cements of poly(methyl methacrylate) (PMMA) loaded with antibiotics represent the current gold standard for local antibiotic delivery in many of these surgical treatments. They can be used in the fixation of the primary joint prosthesis, fixation of the prosthesis in single-state or in the two-stage revision surgeries as well as temporary devices for local drug release in the form of PMMA-chain beads or PMMA-joint spacers.

As local drug carrier, the acrylic cements in some cases perform a prophylactic function because they incorporate low doses of the drug, but in others, they are designed to release high antibiotics concentrations directly to the affected tissues or to joint spaces [13] without severe risks of systemic toxicity [14]. They facilitate the formulation of custom-made drug carriers directly in the surgical room and allow surgeons choose the antibiotic and its dose according to the virulence, antibiotic sensitivity profile of the causative germs and the medical condition of patients [15,16].

Among the most common pathogens reported as responsible for Osteomyelitis in humans are recurrently the *Staphylococcus* species, followed by *Enterobacteriaceae* and *Pseudomonas* species [17], while in the Osteomyelitis associated with implants the *Staphylococcus epidermidis* is present more than 90 % of the time [18]. For PJIs, the spectrum of causative bacteria is broad, including: Coagulase-negative staphylococci (30-43 %); *Staphylococcus aureus* (12-23 %); Streptococci (9-10 %); Enterococci (3-7 %); Gram-negative bacilli (3-6 %); anaerobes (2-4 %); multiple pathogens (polymicrobial) (10-12 %) or unknown microbes (10-11 %) [19].

The great diversity of germs together with the recent growth of antibiotic-resistant strains, the formation of bacterial biofilm on the surface of the materials, or the presence of polymicrobial infections complicate in great grade the management of the deep musculoskeletal infections, and for these reasons, they still represent a major challenge [20,21].

The strategies to face up this new and complex context include the use of novel antimicrobial agents with high bone penetration for oral and parenteral antibiotic treatment such as: linezolid, daptomycin and tigecycline [22]; the exploration of the use of non-classical antibiotics to load acrylic bone cement such as: amphotericin B [23], grepafloxacin [24], teicoplanin [25] or meropenem [26]; the use of combined drugs in the cements in order to increase the antimicrobial spectrum and the potential antibiotics concentrations in tissues as gentamicin/linezolid [27], vancomycin/linezolid [27], gentamicin/teicoplanin [28], vancomycin/daptomycin [29], vancomycin/cefazolin [30], ciprofloxacin/ceftazidime [31], ciprofloxacin/meropenem [31] or the development of biodegradable implants for local antibiotics release in Osteomyelitis therapy [32].

Nowadays, the best evidence for an adequate antibiotic selection is available mainly for staphylococci species while for other bacteria such as streptococci, enterococci or Gram-negatives, the evidence for antibiotic selection is less clear [33]. Given the great problem that represent the emergence of resistant Gram-negative organisms as in the case of *Pseudomonas aeruginosa*, nowadays, it is vital to test new drugs with novel mechanisms of antibacterial action or the proposal of new combination of drugs eluting from acrylic bone cements in special with different action's mechanism on bacteria to broad the antibacterial spectrum and counteracting further resistance mechanisms.

Ozonated sunflower oils have been effective in the treatment of viral, bacterial, fungal and protozoal infections [34–36]. *In vitro* tests of ozonized vegetable oils have shown their activity against a large number of microorganism species. The values of Minimum Inhibitory Concentration (MIC) vary depending on the type of microorganisms and the physical-chemical characteristics of the oils, obtaining values between 0.1 mg mL^{-1} to 17.5 mg mL^{-1}. Oleozon® a Cuban drug obtained from the reaction of ozone with sunflower seed oil has antimicrobial activity demonstrated in different studies, its biological activity is attributed mainly to peroxidic species, including α-hydroxy-hydroperoxides, peracids, α-acyloxy-hydroperoxides and ozonides [37]. The hypothesis established is that damage to the cell membrane occurs due to the oxidation produced by peroxides and alterations in enzymatic complexes essential for the microbial cell [38].

In this work, was evaluated the effect of acrylic cements loaded with combined drugs of different action's mechanism such as ozonized sunflower oil and ozonized oil/Ciprofloxacin/Meropenem on *Pseudomonas aeruginosa* cultures as an example of common Gram-negative germ in PJIs, in Osteomyelitis or in open bone fractures. The possibility to include Oleozon® in the acrylic cement formulations is likely to increase the option of treatments to be considered by surgeons and represents the first report of this alternative. Moreover bearing in mind that in developing countries medical products that are marketed internationally are not always available in their hospitals, and facing the urgent clinical necessity

to provide an efficient treatment in severe septic processes associated with bone injuries, National Health Systems have to develop domestic formulations for such cases.

Methodology

Acrylic bone cements formulations

Acrylic cements formulations were prepared using conventional components of bone cements and high antibiotic dose (an equivalent of 4g of antibiotic per 40 g of cement) as is recommended for acrylic cements intended for antibacterial therapy. Table 1 shows the composition of the acrylic bone cements tested. Cement samples (unloaded and loaded with antibiotics) were hand mixed at room temperature and cured in spherical molds of silicon (0.6 Ø cm) for about 24 hours prior to testing.

In vitro tests

For the *in vitro* antimicrobial tests, the spherical cement probes were immersed in 2.5 mL of double-distilled water at 37 ± 0.5 ºC, and at 1, 3, 5, 7, 9 and 11 days after the initial immersion, the water with the drug released (extract) was replaced with 2.5 mL of fresh double-distilled water. Half of the extract was used for the quantification of the antibiotic released, and the other half was used for the *in vitro* antimicrobial tests. The tests were carried out in triplicate for each cement formulation.

Antibiotic release assay

Drug release was followed by UV-Vis spectroscopy. The solution absorbance's measurements were made using a spectrophotometer Rayleigh UV-2601 (Beijing Rayleigh Analytical Instrument Corp., Beijing, China), at wavelengths of 228, 272 and 297 nm in correspondence with the UV-Vis maximums of absorption for Oleozon®, Ciprofloxacin and Meropenem respectively. The antibiotic concentration was calculated according to the Beer–Lambert's law using a calibration curve for the Cement-Oleozon®(CO) extracts. For the extracts from the cements with a combination of drugs: Ciprofloxacin/Meropenem (CM) or Ciprofloxacin/Meropenem/Oleozon® (CMO), first was determined the extinction coefficient (ε) of the antibiotics at their respectively absorption maximums using standard solutions. Then, to take into account the contribution of each species to the global absorbance at each wavelength, an appropriate set of equations was used to calculate the concentration of each species in the extract. The percentages of antibiotic released were calculated with respect to the total amount incorporated in each spherical probe.

Antimicrobial *in vitro* assay

The broth microdilution method according to CLSI-M100-S24 (2014) was followed to evaluate the antimicrobial effectiveness of each cement formulation [39].

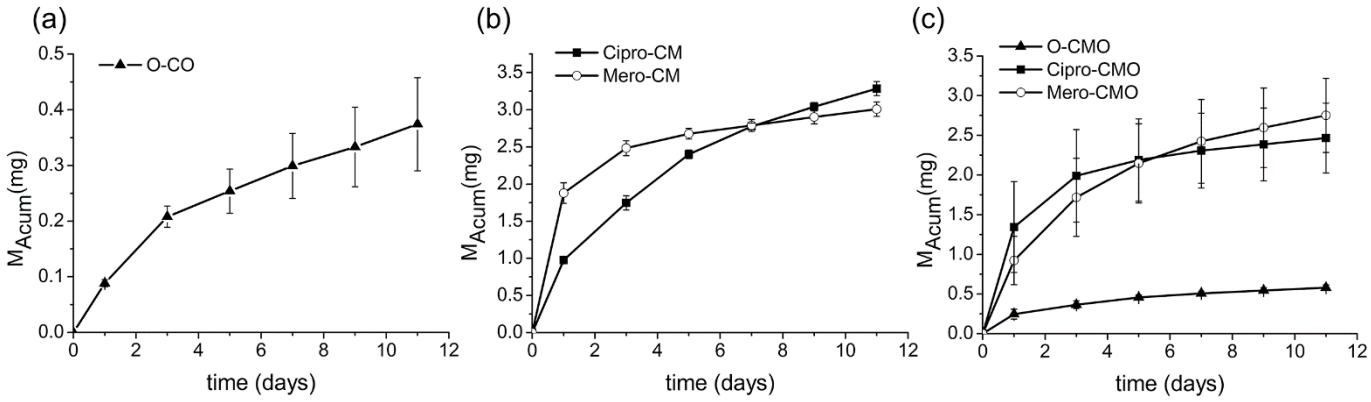
Isolates of the control strain *Pseudomonas aeruginosa* (ATCC 27853) were cultured during 18-20 hours at 37 °C in tryptone soy agar (Merck, Darmstadt, Germany) as the first stage. Afterward, the inoculum preparation was carried out by re-suspension of 3-5 bacterial colonies in Mueller Hinton broth (Merck, Germany), followed by incubation with shaking for 3-5 hours at 35 ºC until reaching a turbidity equal to 0.5 McFarland determined with the assistance of the DIRAMIC-10 equipment (CNIC, Havana, Cuba). Serial dilutions were made until obtaining a concentration of microorganisms of 5×10^4 CFU mL^{-1}. Later, in 96-well plates (BRAND GMBH Plates, Wertheim, Germany), 100 µL of the microorganism was inoculated aseptically and 100 µL of the extract of each cement formulation was added. Readings at a wavelength of 405 nm were performed on micro-ELISA equipment (TECNOSUMA®, Havana, Cuba) at different intervals during the first 24 hours to visualize the kinetics of death of the microorganisms in contact

Table 1. Composition of the acrylic bone cement samples tested*.

	Liquid Part				Solid Part				
CEMENT SAMPLE	**Methyl methacrylate (MMA)[a] (vol.%)**	**N,N-dimethyl-p-toluidine (DMPT)[a] (vol.%)**	**Hydroquinone (HQ)[a] (ppm)**	**Oleozon®[b] (vol.%)**	**Poly (methyl methacrylate) beads (PMMA)[c] (wt.%)**	**Benzoyl peroxide (BP)[a] (wt.%)**	**Barium Sulfate (BS)[d]**	**Ciprofloxacin[e] (wt.%)**	**Meropenem[e] (wt.%)**
C	97.3	2.3	80	-	87.4	2.6	10	-	-
CO	87.3	2.3	80	10	87.4	2.6	10	-	-
CM	97.3	2.3	80	-	77.4	2.6	10	5	5
CMO	87.3	2.3	80	10	77.4	2.6	10	5	5

*A 2/1 Solid/Liquid ratio was used for all formulations and the curing process was carried out at room temperature; [a] Sigma-Aldrich (USA); [b] Supplied by National Center for Scientific Research (Cuba); [c] Bonar Polymer Ltd. (England); [d] J.T Baker (USA), [e] Supplied as active powder principles by BioCubaFarma (Cuba).

Figure 1. Average experimental cumulative amount released from antibiotic-ALBCs and S.D. versus time. a) Cement-CO, b) Cement-CM, c) Cement-CMO. -▲- M_{Acum} of Oleozon®, -■- M_{Acum} of Ciprofloxacin, -○-M_{Acum} of Meropenem.



with cement extracts in comparison with curves of spontaneous growth of the *Pseudomonas aeruginosa* strain. During the experiments, the plates were maintained at 37 °C for an adequate incubation of the strain. All the tests were performed in triplicate.

Results

In vitro antibiotics release

In this work, the kinetics of drug release from acrylic bone cements was determined *in vitro*. The drug (Oleozon®) was used alone and in combination with Ciprofloxacin/Meropenem. Figure 1 shows the cumulative release of Oleozon® from Cement-O, of Ciprofloxacin or Meropenem from Cement-CM and the three antibacterial drugs from Cement-CMO.

All cements showed an initial rapid release of the antibiotic, and then the elution rate decreased to maintain a pattern of sustained drug release over time. This biphasic profile is attributed to the rapid dissolution of the antibiotic on the surface of the cement and then to the drug dissolution/diffusion through cracks, gaps or elution paths that allow the water to penetrate the PMMA matrix and reach and dissolve the antibiotic [40].

Because the oily nature of the Oleozon® (hydrophobic drug), this drug was released at a lower rate and in smaller amount compared to Ciprofloxacin or Meropenem drugs (Figure 1a and Table 2).

Figure 1b (Cement-CM) shows that initially, the release of Meropenem is faster than that of Ciprofloxacin; from day 7 to 9, the amounts of drug released were statistically similar and by day 11, the quantity of Ciprofloxacin liberated was statistically higher than that of Meropenem. However, for the Cement-CMO sample, the differences between the amounts of drug released (Ciprofloxacin or Meropenem) were not statistically different during the entire *in vitro* assay (11 days), Figure 1c. The presence of Oleozon® led to a modification of the release pattern of the Ciprofloxacin and Meropenem, reducing the elution rate of both drugs. The elution rate of Ciprofloxacin was the one that showed the greatest decrease. The cumulative release of the Ciprofloxacin and Meropenem from Cement-CMO samples decreased in comparison with the Cement-CM samples, 13 % for Ciprofloxacin and 5.6 % for Meropenem (Table 2). Conversely, the amount of Oleozon® released increased slightly by the presence of the two other hydrophilic drugs in Cement-CMO almost 3 %. The dispersion of the cumulative release data of Ciprofloxacin and Meropenem indicates that the release process was made more complex by the presence of

Table 2. Amount and cumulative percent of drugs released after 11 days of *in vitro* antibiotic release assay.

ALBC	Amount released (mg)	Cumulative released (%)
CO	0.37 ± 0.08	8.6 ± 0.6
CM		
Ciprofloxacin	3.28 + 0.09	44.7 ± 3.2
Meropenem	3.01 ± 0.10	40.8 ± 2.0
CMO		
Oleozon	0.58 ± 0.03	11.5 ± 0.2
Ciprofloxacin	2.5 ± 0.4	31.6 ± 5.4
Meropenem	2.8 ± 0.5	35.2 ± 5.8

Oleozon® in the system (a hydrophobic drug) and therefore, higher standard deviations were obtained in the accumulated concentrations of these drugs.

In vitro antibacterial effectiveness

In this work, the antibacterial activity of the new formulations of antibiotic-loaded bone cements (ALBC) was determined against one of the bacilli that causes the highest number of intrahospitalary infections and the most complicated of the nosocomial infections to treat: *Pseudomonas aeruginosa* [41].

Figure 2 summarizes the antibacterial activity of the extracts from the Cement-CO, Cement-CM and Cement-CMO taken at 1, 5 and 11 days as representative of the antibacterial test. It is observed that in Figures 2a-c the extracts of the acrylic bone cement without any drug present immediately after inoculation of the plates a short period where D/Do (optical density ratio, $D_t/D_{initial}$) is almost constant. This effect is a consequence that the bacteria first synthesize the enzymes that allow them to multiply later, so the cells may grow in size but not in numbers. Then, the number of bacteria increases with time even at 24 hours of the assay, indicating that bacterial growth is not limited by the depletion of essential nutrients and/or the formation of an inhibitory product.

It is possible to appreciate from Figure 2a, that the extracts for all ALBCs have significant antibacterial effect during the first day. However, the extracts from the Cement-CO, after the first 20 hrs started to lose effectiveness. The extracts from Cement-CO of day 1 released to the surrounding medium 88.4 ± 6.3 µg of Oleozon® and this amount inhibited around 77 % the growth of the strain. Extracts from days 5 and 11 caused less inhibition, Figures 2b and 2c. The statistical analyses indicated no differences in the D/Do values of the Cement-CO extracts taken at the day 3, 5, 7, 9 and 11 day of the assay after 24 hours of being in contact with the strain. In all cases, the inhibitory effect on the bacterial growth was about 50 %.

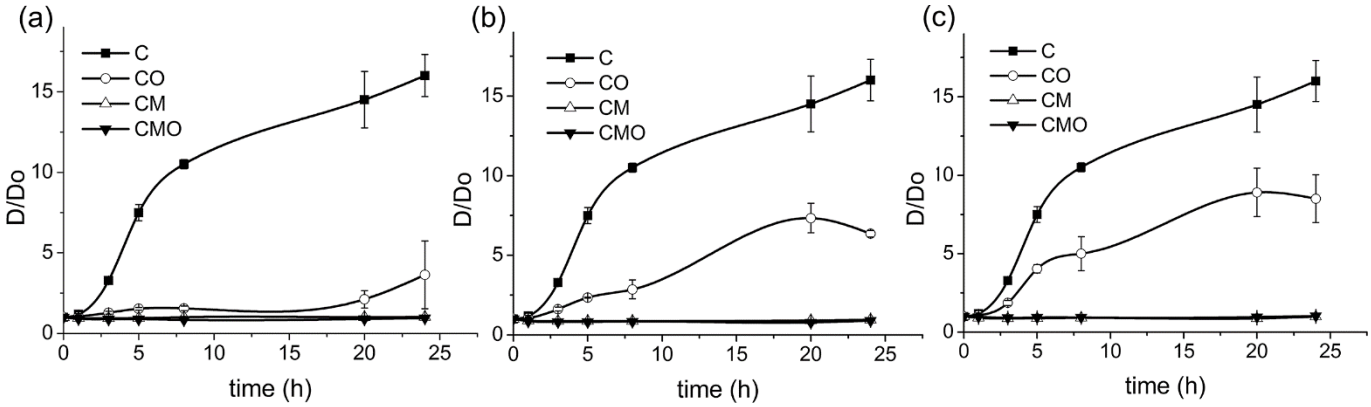
The extracts of the Cement-CM and Cement-CMO at 1, 5 and 11 days completely inhibited the growth of the bacilli which indicate that probably the antibacterial effectiveness will be maintained for more time, Figure 2a-c.

Discussion

Local antibiotic therapy using acrylic bone cements as drug carrier is a common procedure for treatments of PJIs, Osteomyelitis or septic open bone fractures, therapy that have demonstrated excellent clinical results over the years. For a suitable antimicrobial performance, ALBCs must supply high drugs concentrations to tissues during the early postoperative period, levels above the Minimal Inhibitory Concentrations (MICs) of the causative organism(s). The simultaneous addition of two antibiotics or more into bone cement, in addition to increasing the antimicrobial spectrum, it usually results in a greater elution compared to bone cement loaded with one antibiotic [16], these effect is named *Passive Opportunism* [42]. The release of the more soluble drug open cavities and cracks in the cement matrix facilitating the elution of the remaining antibiotic from layers' nearest to surface. This effect has been reported when using specific antibiotics pairs such as Ciprofloxacin/Meropenem [31].

In this sudy, an effect of *Passive Opportunism* due to the presence of Oleozon® was not observed in the water-soluble drugs (Table 2). However, this *Passive Opportunism* effect was found for the release of Oleozon® due to the presence of Ciprofloxacin and

Figure 2. D/Do ratio during 24 hours to be in contact the microbial strain *Pseudomonas aeruginosa* culture with extracts of the bone cement formulations recollected at: a) 1 day, b) 5 days and c) 11 days. -■- extract of cement without any drugs, -○-extract of Cement-CO, -△- extract of Cement-CM, -▼- extract of Cement-CMO.



Meropenem, since these drugs upon dissolution increase the elution pathways of the drug occluded in the innermost layers of the matrix (Figure 1a and Table 2).

Reports of experimental cements with only Meropenem in a concentration of 10% by weight show a good release behavior. For example, Samuel *et al*. [26] showed that Meropenem elutes in pharmacologically measurable concentrations from ALBC during a period of 3 to 27 days depending on the concentration of antibiotic used; on day 27 only the 2.18 % of the initial amount of the drug was released. However, the extracts retained their bioactivity against several types of microorganisms typical of infected arthroplasties for at least three weeks [26].

The concentration of Meropenem extracts reported by Samuel *et al*. [26] at 24 hours using ALBC cylinders (1.6 cm length and 1.2 cm in diameter) loaded with 10 wt.% of Meropenem, and 30 mL of a saline solution reached 27.94 mg L^{-1}; while in our work the concentration of Meropenem detected at 24 hours was around 751.6 mg L^{-1} for the Cement-CM and 369.0 mg L^{-1} in the Cement-CMO. Gálvez-López *et al*. [43] used ALBC beads (0.5 cm in diameter) loaded with 10 wt.% of Meropenem immersed in 1 mL of PBS at 37 °C, obtaining a concentration around 67 mg L^{-1} in the extract on the first 48 h. By comparing the amount of Meropenem liberated per unit of probe area, it was found that the ALBC tested by Samuel released 0.1 mg cm^{-2} at 24 h, the ALBC used by Gálvez-López liberated 0.085 mg cm^{-2} in the first 48 hrs. In this work, in the first 24 hours were released 1.66 mg cm^{-2} when using Cement-CM, and 0.82 mg cm^{-2} when using Cement-CMO. At day 6 Samuel *et al*. reported that the extract had 0.018 mg cm^{-2} of Meropenem, whereas at day 7, Gálvez-López *et al*. [43] reported 0.012 mg cm^{-2} in the extract. At day 7 in our test the Cement-CM released 0.098 mg cm^{-2} and the Cement-CMO 0.244 mg cm^{-2}. Therefore, in our study, the amount of drug released initially was higher, and higher amounts of the drug were released for a longer time than the values reported by Samuel *et al*. and Gálvez-López *et al*.

According to the first national prospective surveillance study assessing antimicrobial activity of Meropenem against clinical isolates from French hospitals, the MIC50 and MIC90 determined by the agar dilution reference method were 0.5 mg L^{-1} and 4 mg L^{-1} respectively [44], values that were overcomed several times for the Meropenem eluting concentrations from Cement-CM and Cement-CMO obtained in this work. The greater concentrations of Meropenem detected in this work were associated with the *Passive Opportunism* reported previously for the Ciprofloxacin/Meropenem couple drugs [31].

The acrylic beads used in clinical are usually loaded with gentamicin are most often fabricated in chains of 30 beads that locally produce concentrations around 300 mg L^{-1} far above the MIC values for most micro-organisms [45].

Reports on the use of acrylic bone cements loaded with 10% by weight of Ciprofloxacin or with Oleozon® were not found in the literature. So, this work is an original proposal.

Antibacterial effectiveness on Pseudomonas aeruginosa of the cement formulations

The evaluation of the delivery capacity to the biological medium of a drug from medical devices for local antibiotic therapy (as cement beads or acrylic joint spacers) together with the evaluation of its antimicrobial effectiveness remains of great interest in current medicine.

Pseudomonas aeruginosa is strain that can propagate on medical devices, hospital environment and even in disinfectants [46]. It is considered as an opportunism Gram-negative microorganism that commonly appears in concomitancy with other germs in the PJIs or in Osteomyelitis disease associated with injection drug use, catheter-related infections or after urinary tract instrumentation [32]. At present time, *Pseudomonas aeruginosa* has grown in importance as a causative agent of deep infections on the rising number of high-energy traumas associated with open fractures, as a consequence of traffic accidents and war injuries [22,32,47]. It is argued that *Pseudomonas aeruginosa* is one of the most difficult germ to eradicate, once established within the joint [48,49] because of its resistance to a variety of antimicrobial agents [50] and to its ability to acquired further resistance, even in anti-pseudomonal chemotherapy [41], extreme treatments are required [46]. The presence of this virulent pathogen in PJIs may require multiple revisions if its eradication is not achieved [51].

It is known that the mechanism of action of the antibiotic on bacteria are quite diverse. Most current bactericidal antimicrobials inhibit DNA synthesis, RNA synthesis, cell wall synthesis, or protein synthesis [52]. Antibiotic-mediated cell death is a complex process that begins with the physical interaction between a drug molecule and its bacterial-specific target and involves alterations to the affected bacterium at the biochemical, molecular and ultrastructural levels [53].

Combinatorial antibiotic treatments can have diverse effects on bacterial survival. Antibiotics can be more effective when combined, displaying either an additive effect (effect equal to the sum of treatments) or a synergistic effect (effect greater than the sum of treatments). The combination of antibiotics can also be antagonistic [53].

In this work, three types of drugs were used alone or in combination as part of an ALBC formulation. Ciprofloxacin belonging to the family of the fluoroquinolones contributes to the inhibition of nucleic acid synthesis because it affects DNA replication [53], Meropenem a β-lactam antibiotic interferes with specific steps in homeostatic cell wall biosynthesis [53] whereas Oleozon® affects the cell membrane perhaps as a consequence of the peroxidation and modifications in enzymatic complexes essential for bacteria.

Results of this work indicate a better antibacterial effect by the combined used of two or the three drugs in ALBC against Gram-negative bacilli. However, the period of the antibacterial effectiveness tested was too short to prove differences between Cement-CM and Cement-CMO formulations.

Since the groups of bacteria most sensitive to Oleozon® are mycobacteria and gram-positive cocci, it is expected that the incorporation of this drug to bone cement can contribute to eradicating them in the PJI or in Osteomyelitis. In addition, taking into account the wide availability of sunflower oils, Oleozon® becomes a competitive antimicrobial agent that, together with the proven synergic effect that is induced when the Ciprofloxacin/Meropenem combination is used [31], makes these new formulations of cement be a valid ALBCs alternative to be considered by surgeons.

Conclusion

Bone cements loaded with antibiotics were prepared with Oleozon®, mixtures of Ciprofloxacin/Meropenem and of Ciprofloxacin/Meropenem/Oleozon®. All the drugs elute from the cement probes as active ingredients against *Pseudomonas aeruginosa* cultures, and at the end of the trial period, some of the drugs remained inside the matrices, indicating that they can be used for a longer time in treatments. The cement extracts with Oleozon® demonstrated an inhibitory effect on the growth of the strain during the whole trial period, however, at the end of the test (11 days), only a decrease of about 50 % of the bacterial growth was obtained. The extracts from cements with mixtures of Ciprofloxacin/Meropenem and Ciprofloxacin/Meropenem/Oleozon® showed complete inhibition of the growth of the microorganisms, even at 11 days, time that the test lasted. The incorporation of Oleozon® to the acrylic cement alone or in combination with other drugs (as Ciprofloxacin/Meropenem) may contribute to eliminating mycobacteria and Gram-positive cocci, the most germs in the PJIs or in Osteomyelitis, in addition to Gram-negative germs as *Pseudomonas aeruginosa*.

In future work, *in vitro* studies will be carried out with other types of strains present in septic musculoskeletal processes to confirm the antibacterial effectiveness of the proposed acrylic cement formulations. *In vivo* studies will also be carried out to verify if cement formulations with Oleozon® are effective for the control of sepsis

Acknowledgements

L. Morejón thanks to the company BioCubaFarma (Cuba) and to the National Center for Scientific Research (Cuba) for supplying the drugs used in this work as well as the support received by the National Program of Basic Sciences (PNCB, Cuba) through the project P223LH001-060.

References

1. Senn D, Gehmert S, Ochsner PE, Kühn K-D, Nowaskowski AM (2017) Case Report Therapy for Chronic Recurrent Osteomyelitis with Multi-Resistant Pseudomonas aeruginosa using Local Antibiotic Release by a Polymethylmethacrylate. Surg Infect case Reports 2: 26–30.
2. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA (2016) Periprosthetic joint infection. Lancet 387: 386–394.
3. Vilchez-Cavazos F, Villarreal-Villarreal G, Peña-Martinez V, Acosta-Olivo C (2017) Management of perisprosthetic infections. World J Clin Infect Dis 7: 11–20.
4. George DA, Gant V, Haddad FS (2015) The management of periprosthetic infections in the future: A review of new forms of treatment. Bone Jt J 97–B: 1162–1169.
5. Bistolfi A, Massazza G, Verné E, Massè A, Deledda D, Ferraris S, Miola M, Galetto F, Crova M (2011) Antibiotic-Loaded Cement in Orthopedic Surgery: A Review. ISRN Orthop 2011: 1–8.
6. Malizos KN, Poultsides LA (2010) The Socio-economic Burden of Musculoskeletal Infections. In Meani, E., Romanò, C., Crosby, L., Hofmann G, editor. Infection and Local Treatment in Orthopedic Surgery. Springer. Available: https://www.springer.com/fr/book/9783540479994.
7. Kurtz S, Ong K, Lau E, Mowat F, Halpern M (2007) Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Jt Surg - Am 89: 780–785.
8. Lew DP, Waldvogel FA (2004) Osteomyelitis. Lancet 364: 369–379.
9. Frank D, Montskó G, Juricskay I, Borsiczky B, Cseh G, Kocsis B, Nagy T, Nagy ÁK, Kovács GL, Miseta A (2011) Clindamycin Release Determined by High Performance Liquid Chromatography from a Novel low-Cost Local Drug Delivery

System: A New Potential Treatment Option for Chronic Osteomyelitis. J Chemother 23: 282–284.
10. Romanò CL, Manzi G, Logoluso N, Romanò D (2012) Value of debridement and irrigation for the treatment of peri-prosthetic infections. A systematic review. HIP Int 22 Suppl 8: 19–24.
11. Wolf CF, Gu NY, Doctor JN, Manner PA, Leopold SS (2011) Comparison of One and Two-Stage Revision of Total Hip Arthroplasty Complicated by Infection. J Bone Jt Surgery-American Vol 93: 631–639.
12. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR (2013) Diagnosis and management of prosthetic joint infection: Clinical practice guidelines by the infectious diseases Society of America. Clin Infect Dis 56: 1–25.
13. Anagnostakos K (2017) Therapeutic Use of Antibiotic-loaded Bone Cement in the Treatment of Hip and Knee Joint Infections. J Bone Jt Infect 2: 29–37.
14. Springer B, Lee G, Osmon D, Haidukewych G, Hanssen A, Jacofsky D (2004) Systemic safety of high-dose antibiotic-loaded cement spacers after resection of an infected total knee arthroplasty. Clin Orthop Relat Res 427: 47–51.
15. Ohtsuru T, Morita Y, Murata Y, Munakata Y, Itoh M, Kato Y, Okazaki K (2018) Custom-made, antibiotic-loaded, acrylic cement spacers using a dental silicone template for treatment of infected hip prostheses. Eur J Orthop Surg Traumatol 28: 615–620.
16. Martínez-Moreno J, Merino V, Nácher A, Rodrigo JL, Climente M, Merino-Sanjuán M (2017) Antibiotic-loaded Bone Cement as Prophylaxis in Total Joint Replacement. Orthop Surg 9: 331–341.
17. Gogia JS, Meehan JP, Di Cesare PE, Jamali AA (2009) Local Antibiotic Therapy in Osteomyelitis. Semin Plast Surg 23: 100–107.
18. Ciampolini J, Harding KG (2000) Pathophysiology of chronic bacterial osteomyelitis . Why do antibiotics fail so often ? Pathophysiology of chronic bacterial osteomyelitis . Why do antibiotics fail so often? Postgr Med J 76: 479–483.
19. Nieto MI (2015) Clinic, epidemiology and prognosis of joint prosthesis infection. Multicentric study Doctorate thesis in Medicine. Sevilla University. 204 p.
20. Bozic KJ, Kurtz SM, Lau E, Ong K, Chiu V, Vail TP, Rubash HE, Berry DJ (2010) The epidemiology of revision total knee arthroplasty in the united states. Clin Orthop Relat Res 468: 45–51.
21. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J (2012) Economic burden of periprosthetic joint infection in the united states. J Arthroplasty 27: 61–5.e1.
22. Gomes D, Pereira M, Bettencourt AF (2013) Osteomyelitis: An overview of antimicrobial therapy. Brazilian J Pharm Sci 49: 13–27.
23. Marra F, Robbins GM, Masri BA, Duncan C, Wasan KM, Kwong EH, Jewesson PJ (2001) Amphotericin B-loaded bone cement to treat osteomyelitis caused by Candida albicans. Can J Surg 44: 383–386.
24. Efstathopoulos N, Giamarellos-Bourboulis E, Kanellakopoulou K, Lazarettos I, Giannoudis P, Frangia K, Magnissalis E, Papadaki M, Nikolaou VS (2008) Treatment of experimental osteomyelitis by Methicillin Resistant Staphylococcus Aureus with bone cement system releasing grepafloxacin. Injury 39: 1384–1390.
25. Chang Y, Chen WC, Hsieh PH, Chen DW, Lee MS, Shih HN, Ueng SWN (2011) In vitro activities of daptomycin-, vancomycin-, and teicoplanin-loaded polymethylmethacrylate against methicillin-susceptible, methicillin-resistant, and vancomycin-intermediate strains of Staphylococcus aureus. Antimicrob Agents Chemother 55: 5480–5484.
26. Samuel S, Mathew BS, Veeraraghavan B, Fleming DH, Chittaranjan SB, Prakash JAJ (2012) In vitro study of elution kinetics and bio-activity of meropenem-loaded acrylic bone cement. J Orthop Traumatol 13: 131–136.
27. Snir N, Meron-Sudai S, Deshmukh AJ, Dekel S, Ofek I (2013) Antimicrobial Properties and Elution Kinetics of Linezolid From Polymethylmethacrylate. Orthopedics 36: e1412–e1417.
28. Anagnostakos K, Kelm J, Regitz T, Schmitt E, Jung W (2005) In vitro evaluation of antibiotic release from and bacteria growth inhibition by antibiotic-loaded acrylic bone cement spacers. J Biomed Mater Res B Appl Biomater 72: 373–378.
29. Parra-Ruíz FJ, González-Gómez A, Fernández-Gutiérrez M, Parra J, García-García J, Azuara G, De la Torre B, Buján J, Ibarra B, Duocastella-Codina L, Molina-Crisol M, Vázquez-Lasa B, San Román J (2017) Development of advanced biantibiotic loaded bone cement spacers for arthroplasty associated infections. Int J Pharm 522: 11–20.
30. Paz E, Sanz-Ruiz P, Abenojar J, Vaquero-Martín J, Forriol F, Del Real JC (2015) Evaluation of elution and mechanical properties of high -dose antibiotic-loaded bone cement: comparative “in vitro” study of the influence of vancomycin and cephazolin. J Arthroplasty 30: 1423–1429.
31. Morejón Alonso L, Mendizábal Ruiz AP, Muñiz Flores JA, Rodríguez Herrera DL, Rodríguez García O, Ledea Lozano OE, Delgado García-Menocal JA, Fuentes Estévez G (2018) Evaluation of acrylic bone cements with single and combined antibiotics: Release behavior and in vitro antibacterial effectiveness. Int J Polym Mater Polym Biomater 67: 830–838.
32. Chihara S, Segreti J (2010) Osteomyelitis. Disease-a-Month 56: 6–31.
33. Moriarty TF, Kuehl R, Coenye T, Metsemakers W-J, Morgenstern M, Schwarz EM, Riool M, Zaat SAJ, Khana N, Kates SL, Richards RG (2016) Orthopaedic device-related infection: current and future interventions for improved prevention and treatment. EFORT Open Rev 1: 89–99.
34. Almeida NR, Beatriz A, Micheletti AC, Arruda EJ (2012) Ozonized vegetable oils and therapeutic properties: A review. Orbital - Electron J Chem 4: 313–326.
35. Rajabi O, Sazgarnia A, Abbasi F, Layegh P (2015) The activity of ozonated olive oil against leishmania major promastigotes. Iran J Basic Med Sci 18: 915–919.
36. Curtiellas V (2014) Los aceites ozonizados en el tratamiento de enfermedades infecciosas. 34: 64–68.
37. Martinez Tellez G, Ledea Lozano O, Díaz Gómez M (2006) Measurement of peroxidic species in ozonized sunflower oil. Ozone Sci Eng 28: 181–185.
38. Curtiellas V, Ledea O, Rodríguez S, Ancheta O, Echevarria M, Sanchez E, Fernández I (2008) OLEOZON® effects on viability, cellular permeability and ultrastructure of Staphylococcus aureus. Rev CENIC Ciencias Biol 39: 128–131. [Article in Spanish].
39. Clinical and Laboratory standard institute (CLSI). (2014) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Doc M100-S24 (ISBN 1-56238-897-5).
40. Anagnostakos K, Kelm J (2009) Enhancement of antibiotic elution from acrylic bone cement. J Biomed Mater Res - Part B Appl Biomater 90 B: 467–475.

41. Rossolini GM, Mantengoli E (2005) Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. Clin Microbiol Infect 11: 17–32.
42. Penner MJ, Masri BA, Duncan CP (1996) Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. J Arthroplasty 11: 939–944.
43. Gálvez-López R, Peña-Monje A, Antelo-Lorenzo R, Guardia-Olmedo J, Moliz J, Hernández-Quero J, Parra-Ruiz J (2014) Elution kinetics, antimicrobial activity, and mechanical properties of 11 different antibiotic loaded acrylic bone cement. Diagn Microbiol Infect Dis 78: 70–74.
44. Joly-Guillou ML, Kempf M, Cavallo JD, Chomarat M, Dubreuil L, Maugein J, Muller-Serieys C, Roussel-Delvallez M (2010) Comparative in vitro activity of Meropenem, Imipenem and Piperacillin/tazobactam against 1071 clinical isolates using 2 different methods: A French multicentre study. BMC Infect Dis 10: 1–9.
45. Kuiper JW (2014) Treatment of acute periprosthetic infections with prosthesis retention: Review of current concepts. World J Orthop 5: 667.
46. Xu J, Duan X, Wu H, Zhou Q (2013) Surveillance and correlation of antimicrobial usage and resistance of Pseudomonas aeruginosa: A hospital population-based study. PLoS One 8: 1–7.
47. De Carvalho VC, De Oliveira PRD, Dal-Paz K, De Paula AP, Félix C da S, Munhoz Lima ALL (2012) Gram-negative osteomyelitis: Clinical and microbiological profile. Brazilian J Infect Dis 16: 63–67.
48. Buchholz H, Elson R, Engelbrecht E, Lodenkämper H, Röttger J, Siegel A (1981) Management of deep infection of total hip replacement. J Bone Jt Surg Br 63: 342–353.
49. Cherney DL, Amstutz HC (1983) Total hip replacement in the previously septic hip. J Bone Jt Surg - Ser A 65: 1256–1265.
50. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH (1999) Emergence of Antibiotic-Resistant Pseudomonas aeruginosa : Comparison of Risks Associated with Different Antipseudomonal Agents. 43: 1379–1382.
51. Scott CP, Higham PA (2003) Antibiotic Bone Cement for the Treatment of Pseudomonas Aeruginosa in Joint Arthroplasty: Comparison of Tobramycin and Gentamicin-Loaded Cements. J Biomed Mater Res - Part B Appl Biomater 64: 94–98.
52. Walsh CT (2003) Antibiotics: Actions, origins, resistance. Washington, D.C: ASM Press. 3060 p.
53. Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: From targets to networks. Nat Rev Microbiol 8: 423–435.

Corresponding author

Lizette Morejón Alonso, PhD
Polymeric Biomaterials Department, Center of Biomaterials,
University of Havana, Cuba,
Ave. Universidad, e/ Ronda y G, Vedado, La Habana 10400, Cuba
Tel. 53 (7) 8700594
Fax: 53 (7) 8735863
Email: lizette@biomat.uh.cu; lizettemorejon@gmail.com

Eduardo Mendizábal Mijares, PhD
CUCEI, University of Guadalajara, Jalisco, Mexico
Blvd. Marcelino García Barragán #1421, esq Calzada Olímpica,
C.P. 44430, Guadalajara, Jalisco, México.
Tel: 52 (33) 1378 5900, Ext: 27541
Fax: 52 (33) 3619 7316
Email: lalomendizabal@gmail.com

Conflict of interests: No conflict of interests is declared.