

Research Paper

Effect of Sonication on the Elution of Antibiotics from Polymethyl Methacrylate (PMMA)

Anne Kummer, Ulrika Furustrand Tabin, Olivier Borens 

University Hospital Lausanne (CHUV), Service of Orthopedics and Traumatology, University of Lausanne, Lausanne, Switzerland

✉ Corresponding author: Prof. Olivier Borens; Unit of Traumatology, Unit of Septic Surgery, Service of Orthopedics and Traumatology, University Hospital Lausanne (CHUV), Rue du Bugnon 46, CH-1011 Lausanne. Phone number: +41 21 314 27 52; Fax number: +41 21 314 2379; olivier.borens@chuv.ch

© Ivyspring International Publisher. This is an open access article distributed under the terms of the Creative Commons Attribution (CC BY-NC) license (<https://creativecommons.org/licenses/by-nc/4.0/>). See <http://ivyspring.com/terms> for full terms and conditions.

Received: 2017.08.19; Accepted: 2017.10.11; Published: 2017.11.13

Abstract

Background: In the setting of prosthetic joint infections treated with a two-stage procedure, spacers can be sonicated after removal. We hypothesize that the sonication process may cause an increased elution of antibiotics from the spacer, leading to elevated concentrations of antibiotics in the sonication fluid inhibiting bacterial growth. We aimed to evaluate in vitro the influence of sonication on the elution of antibiotics from polymethyl methacrylate (PMMA) over time and to determine whether these concentrations are above the minimum inhibitory concentrations (MIC) for microorganisms relevant in prosthetic joint infections.

Methods: PMMA blocks impregnated with vancomycin, fosfomycin, gentamicin or daptomycin were incubated in phosphate-buffered saline (PBS) at 37°C for up to 6 weeks. PBS was changed once a week. Concentrations were determined from samples of each antibiotic every week, and after 5 minutes of sonication at 2, 4 and 6 weeks.

Results: With sonication there was a trend toward an increase of the elution of antibiotics. This increase was significant for vancomycin at 2 and 4 weeks ($p=0.008$ and 0.002 respectively) and for fosfomycin at 2 weeks ($p=0.01$).

Conclusion: The effect of sonication could play a role in clinical results, especially for daptomycin and gentamicin for which the MIC is close to the concentration of antibiotics at 4 and 6 weeks.

We conclude that elution of antibiotics from PMMA along with the effect of sonication could inhibit bacterial growth from spacers, resulting in false negative results in the setting of two-stage exchange procedures for prosthetic joint infections.

Key words: sonication, antibiotic elution, bone cement, polymethyl methacrylate, prosthetic joint infection

Introduction

Prosthetic joint infections represent one of the most serious complication after prosthetic joint implantation with a cumulative incidence of 1-2% over the lifetime of the prosthetic joint[1]. In Europe, surgical site infections occur with a cumulative incidence of 1.2 % for hip arthroplasties and 0.8% for knee arthroplasties[2]. Sonication of explanted prosthetic implants is known to increase detection of prosthetic joint infection[3-5].

According to the current guidelines[1], patients

with prosthetic joint infections with symptoms lasting more than 3 weeks are candidates for an exchange procedure. Depending on the germ, the resistance profile, the soft tissue situation and other factors, the treating team has to choose between a one-stage or a two-stage procedure[6]. The strategy of a two-stage procedure consists of prosthetic component and complete cement removal as well as debridement of infected tissue, followed by implantation of an antibiotic-impregnated bone cement spacer[1,7,8].

After an interval of 2 to 8 weeks[6,9], the second stage procedure is performed with reimplantation of a definitive prosthetic joint. Given that the spacer can act as a foreign-body on which microorganisms can adhere and form biofilms [10,11], it can be sonicated after removal in order to detect possible microorganisms.

Acrylic bone cement consists of copolymer of acrylic acid and acrylic esters leading to a higher hydrophilicity compared to pure PMMA[12]. In addition to this, Bettencourt et al.[13] showed that under contact with fluids, the cement surface is changing to a more hydrophilic state. Antibiotic release from bone cement depends on the surface and the water-absorbing properties of the cement[12]. Antibiotic loaded bone cement are used in clinical practice for the treatment of osteomyelitis and prosthesis infection and have proven to be able to maintain therapeutically relevant levels of antibiotics[14].

Some in vitro studies have shown that low-frequency ultrasound could increase the release of gentamicin[15] or vancomycin[16] from acrylic bone cement. We hypothesize that the sonication process may cause an increased elution of antibiotics from PMMA, leading to elevated concentrations in the sonication fluid inhibiting bacterial growth (false negative cultures).

We aimed to evaluate in vitro the influence of sonication on the elution of antibiotics from PMMA over time (up to 6 weeks in accordance with our clinical practice for the two-stage exchange procedure) and to determine whether these concentrations are above the minimum inhibitory concentrations (MIC) for microorganisms relevant in prosthetic joint infections.

Methods

Test specimens were made of 5.0 g of cement powder, containing undermentioned amount of antibiotics, and 2.5 ml of monomer liquid. First, a spatula was used to mix the two components in a bowl at room temperature and finally round specimens (height = 15.0 mm and diameter = 6.0 mm) were formed by using predefined forms (stainless steel with 20 drill holes). PMMA blocks impregnated with vancomycin (2 g/40 g PMMA), gentamicin (0.5 g/40 g PMMA), daptomycin (1.5 g/40 g PMMA) or fosfomycin (1.5 g/40 g PMMA) were placed in 15 ml Falcon tubes containing 5 ml phosphate-buffered saline (PBS). Vancomycin, daptomycin and fosfomycin cements contains in addition 0.5 g/40 g PMMA of gentamicin. These loads of antibiotics correspond to the formulation of bone cement used in clinical practice for spacers (i.e. Copal® G+V, Heraeus

Medical GmbH, Germany). Tubes were incubated statically at 37°C for up to 6 weeks in order to mimic human conditions. The PBS was changed once a week in order to imitate the physiological turn-over and to prevent to build up high concentrations that could limit further release. Samples, in triplicate, were taken every week, before changing the PBS, in order to determine the antibiotic release over time. In addition, at 2, 4 and 6 weeks, tubes containing each antibiotic and PBS in triplicate were sonicated for 5 minutes at a frequency of 40 Hz, then samples were taken in order to determine the effect of sonication on the release. Sonicator BactoSonic (Bandelin GmbH, Berlin, Germany) was used.

Samples were stored in -80°C until all samples have been collected. The antibiotic concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The lower limits of quantification were 0.1 µg/mL for vancomycin and daptomycin, 0.5 µg/mL for gentamicin and 1.0 µg/mL for fosfomycin.

In order to determine whether these concentrations are above the minimum inhibitory concentrations (MIC) for microorganisms relevant in prosthetic joint infections, we used breakpoint tables from the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[17], which are summarized in *table 1*.

Table 1. Clinical minimum inhibitory concentrations (MIC) in µg/ml (from EUCAST breakpoint tables [17])

	Vancomycin	Fosfomycin	Daptomycin	Gentamicin
<i>S. aureus</i>	2	32	1	1
Coagulase-negative staphylococci	4	32	1	1
<i>Streptococcus</i> spp	2	-	1	-
<i>Enterobacteriaceae</i>	-	32	-	2-4
<i>Pseudomonas</i> spp.	-	-	-	4
<i>Enterococcus</i> spp.	4	-	-	-
<i>Corynebacterium</i> spp.	2	-	-	1
<i>Acinetobacter</i> spp.	-	-	-	4
Gram positive anaerobes	2	-	-	-

For statistical analysis, unpaired *t* test was performed using Prism 5.0 (GraphPad Software, La Jolla, CA). A *P* value <0.05 was considered to be significant. Results are presented as mean and standard deviation of triplicate experiments.

Results

The release of antibiotics during 6 weeks of incubation in PBS is shown in *Figure 1*. A burst release was observed during the first week of incubation for fosfomycin, daptomycin and gentamicin with mean

concentrations of 414.3 µg/ml, 31.4 µg/ml and 83.2 µg/ml respectively. These concentrations are 13-30 times higher the MIC for most bacteria relevant in the context of prosthetic joint infections. Release of vancomycin was stable over the 6 weeks with a mean concentration between 6.6 and 8.8 µg/ml (7.9 µg/ml during the first week) and are above the MIC for most bacteria.

Fosfomycin concentration decreased to 13.3 µg/ml after 2 weeks of incubation and became undetectable after 6 weeks. Daptomycin concentrations decreased gradually from 5.9 µg/ml after 2 weeks to 2.6 µg/ml after 6 weeks. These concentrations remain above the MIC. Concentrations of gentamicin decreased from 17.8 µg/ml after 2 weeks to 6.0 µg/ml after 6 weeks and these values remain above the MIC.

Figure 2 shows the effect of sonication on the antibiotic release from PMMA at 2, 4 and 6 weeks of incubation. With sonication process, there was a trend toward an increase in the elution of antibiotics. A significant increase in concentrations after sonication was observed for vancomycin at 2 weeks (10.6 µg/ml vs 8.6 µg/ml, p=0.008) and 4 weeks (12.3 µg/ml vs 8.8 µg/ml, p=0.002) and for fosfomycin at 2 weeks (19.6 µg/ml vs 13.3 µg/ml, p=0.01).

Considering the MIC for most relevant bacteria in the setting of prosthetic joint infection, concentrations of vancomycin always remained above the MIC. Daptomycin and gentamicin concentrations decreased but remained above MIC at 4 and 6 weeks. On the other hand, fosfomycin concentrations were below the MIC, except for the elution at the first week.

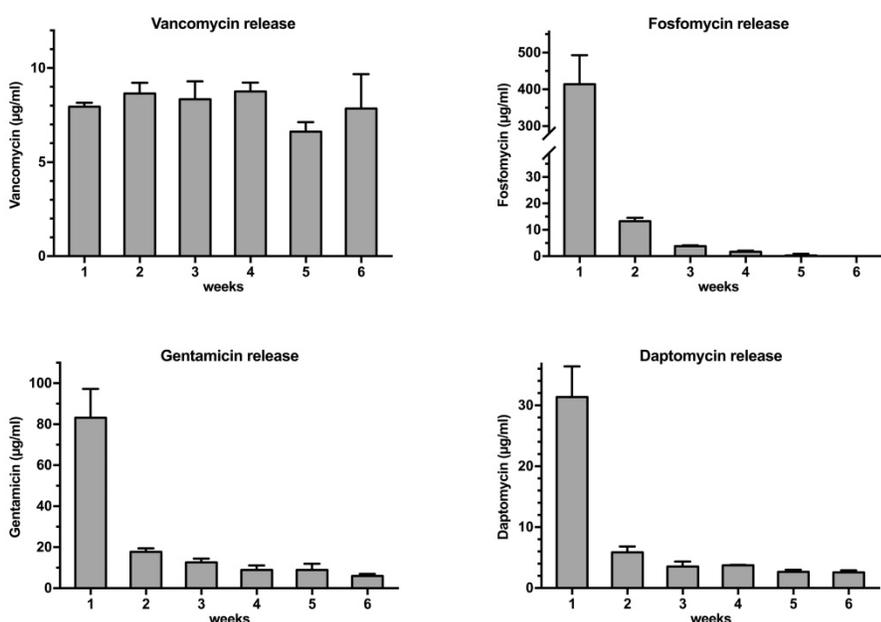


Figure 1. Antibiotic release in PBS at 37°C during 6 weeks. Mean and standard deviation of triplicate experiments.

Discussion

The major finding of this study was a trend toward an increase in antibiotics elution from PMMA after sonication process. Although significant difference was only found for vancomycin and fosfomycin, this trend was observed for all studied antibiotics and remained over time, up to 6 weeks. This observed effect could play a role in clinical results, especially for daptomycin and gentamicin for which the concentration of antibiotics at 4 and 6 weeks were close to the MIC.

Our results are in agreement with the work of Wendling et al.[16] who found that low-frequency ultrasound (25.5 kHz) increase elution of vancomycin from PMMA, and with the study of Hendriks et al.[15] that showed an increased release of gentamicin from bone cement using ultrasound at 46.5 kHz frequency. The present study used sonication at 40 kHz. The selected intervals of 2,4 and 6 weeks for sonication process correspond to different potential time points in clinical practice for reimplantation[1,6,9]. Our results support the hypothesis that sonication could lead to false negative results in clinical practice, in the setting of two-staged exchange procedure.

Observed elution rate of antibiotics were close to in vitro experiment of Galvez-Lopez[18] for gentamicin, vancomycin and daptomycin, although antibiotic concentrations in the cement and the method used was different than the ones used in our study. Observed elution kinetics were similar to elution reported in a study from Gasparini et al[19] although in this study a different methodology was used.

In an in vivo study, Hsieh et al.[20], using 4 g vancomycin and 4 g aztreonam per 40 g PMMA hand-made spacers, found high levels of vancomycin with more than 1000 µg/ml on post-operative day 1, and still more than 500 µg/ml on day 7. However, in another in vivo study, using 2 g vancomycin per 40 g PMMA, different concentrations of vancomycin and gentamicin from spacers were measured, with peak mean concentrations of 37 µg/ml, and 21 µg/ml respectively[21]. This study found higher concentrations of antibiotics in the drainage fluid from beads than from spacer (2-6 time higher), due to their larger surface area, according to the

authors.

Most studies[18,19,22] showed a burst release of antibiotics during the first hours or days, followed by a more stable rate of elution from PMMA. Nevertheless, Galvez-Lopez et al.[18] found three different patterns of elution depending on the antibiotic tested. The majority of antibiotics showed a triphasic pattern with a progressive increase in the first 24 hours, following by a decrease and a final phase with a low and steady decline, but some antibiotics like vancomycin and gentamicin showed constant elution kinetics during 30-days. We found similar results, although some differences were observed during the first week as we found no burst release for vancomycin.

In a prospective study, Nelson et al.[23] followed 36 patients undergoing a two-stage procedure. They found that positive sonication of spacers was predictive of failure (defined by reinfection at 2-year follow-up). Fifty percent (9 of 18) patients with positive sonication were reinfected, compared to 11% (2 of 18 patients) with negative sonication. In a recent prospective analysis of 50 spacers, Esteban et al.[24] showed that high colony counts from sonicate culture of antibiotic-load cement spacer was associated with worse clinical outcomes.

However, sonication reliability of bone cement remains unclear. Some discrepancies between sonication results and clinical outcomes following two-staged procedures were reported. Sorli et al.[25] found clinical failure in 18 of 55 patients following a

two-stage procedure. Among these failures, only 7 had been diagnosed with a subclinical infection (positive culture from sonicated spacer fluid or two or more tissue specimen positive). These results are in agreement with our personal experience, according to which sonicated spacers often are culture negative. This fact could be explained by the elution of antibiotics from spacer by sonication according to the results of the present in vitro study.

There are however some limitations in this study. First, this is an in vitro experiment and, although samples were kept at 37° and the PBS was changed once a week to mimic physiologic fluid turnover, only assumptions can be made for application in clinical practice. In most in vitro studies on antibiotic elution PBS is changed once a day the first week, even more often the first 24 hours[18,22,26,27] in order to accurately analyze antibiotic elution. However, the main objective of the present study was to examine the effect of sonication, whereas antibiotic elution was a secondary objective. We used blocks of PMMA impregnated with 1.25-5 % w/w concentrations of antibiotics. Giving the variety of PMMA shapes and antibiotic concentrations in PMMA used in clinical practice, elution rate could be very different and could lead to concentrations apart from MIC. In such cases, sonication process could have no effect on the final result of spacer sonication. Despite these limitations, we think that results from the present study could be useful in clinical practice in the setting of two-stage exchange procedures and suggest to be careful in case of negative sonicated culture.

We conclude that the sonication process increases antibiotic elution from PMMA, leading to higher antibiotic concentrations in the sonicated fluid, and could therefore lead to false negative culture results of sonicated spacers.

Abbreviations

PMMA: polymethyl methacrylate; MIC: minimum inhibitory concentration; PBS: phosphate-buffered saline; EUCAST: European Committee on Antimicrobial Susceptibility Testing; PCR: polymerase chain reaction.

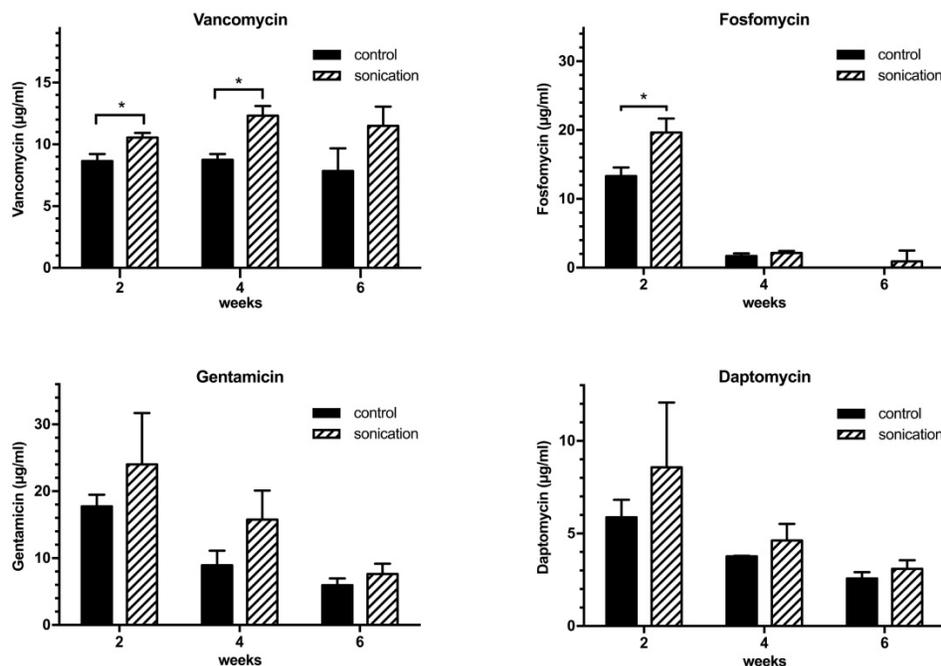


Figure 2. Effect of sonication on antibiotic release. Mean and standard deviation of triplicate experiments. *indicates statistical significance (p<0.05).

Author Contributions Statement

Anne Kummer: substantial contributions to the acquisition, analysis and interpretation of data; drafting the paper.

Ulrika Furustrand: substantial contributions to research design, and the acquisition, analysis and interpretation of data; revising the paper critically.

Olivier Borens: substantial contributions to research design, and the acquisition, analysis and interpretation of data; revising the paper critically.

Competing Interests

The authors have declared that no competing interest exists.

References

- [1] Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2013; 56: 1–25.
- [2] European Centre for Disease Prevention and Control. Surveillance of Surgical Site Infections in Europe, 2008–2009. 2012.
- [3] Corvec S, Portillo ME, Pasticci BM, Borens O, Trampuz A. Epidemiology and new developments in the diagnosis of prosthetic joint infection. *Int J Artif Organs*. 2012; 35: 923–34.
- [4] Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*. 2007; 357: 654–63.
- [5] Renz N, Cabric S, Janz V, Trampuz A. Sonication in the diagnosis of periprosthetic infections: Significance and practical implementation. *Orthopade*. 2015; 44: 942–5.
- [6] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med*. 2004; 351: 1645–54.
- [7] Cui Q, Mihalko WM, Shields JS, Ries M, Saleh KJ. Antibiotic-impregnated cement spacers for the treatment of infection associated with total hip or knee arthroplasty. *J Bone Joint Surg Am*. 2007; 89: 871–82.
- [8] Senthil S, Munro JT, Pitto RP. Infection in total hip replacement: meta-analysis. *Int Orthop*. 2011; 35: 253–60.
- [9] Baker RP, Furustrand T, U, Borens O. Patient-adapted treatment for prosthetic hip joint infection. *Hip Int*. 2015; 25: 316–22.
- [10] Bertazzoni Minelli E, Della Bora T, Benini A. Different microbial biofilm formation on polymethylmethacrylate (PMMA) bone cement loaded with gentamicin and vancomycin. *Anaerobe*. 2011; 17: 380–3.
- [11] Anagnostakos K, Hitzler P, Pape D, Kohn D, Kelm J. Persistence of bacterial growth on antibiotic-loaded beads: is it actually a problem? *Acta Orthop*. 2008; 79: 302–7.
- [12] Kühn K-D. PMMA Cements. Berlin, Germany: Springer Berlin Heidelberg; 2014.
- [13] Bettencourt A, Calado A, Amaral J, Alfaia A, Vale FM, Monteiro J, et al. Surface studies on acrylic bone cement. *Int J Pharm*. 2004; 278: 181–6.
- [14] Moojen DJ, Hentenaar B, Charles Vogely H, Verbout AJ, Castelein RM, Dhert WJ. In vitro release of antibiotics from commercial PMMA beads and articulating hip spacers. *J Arthroplasty*. 2008; 23: 1152–6.
- [15] Hendriks JG, Ensing GT, van Horn JR, Lubbers J, van der Mei HC, Busscher HJ. Increased release of gentamicin from acrylic bone cements under influence of low-frequency ultrasound. *J Control Release*. 2003; 92: 369–74.
- [16] Wendling A, Mar D, Wischmeier N, Anderson D, McIlff T. Combination of modified mixing technique and low frequency ultrasound to control the elution profile of vancomycin-loaded acrylic bone cement. *Bone Joint Res*. 2016; 5: 26–32.
- [17] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. <http://www.eucast.org>.
- [18] Gálvez-López R, Peña-Monje A, Antelo-Lorenzo R, Guardia-Olmedo J, Moliz J, Hernández-Quero J, et al. Elution kinetics, antimicrobial activity, and mechanical properties of 11 different antibiotic loaded acrylic bone cement. *Diagn Microbiol Infect Dis*. 2014; 78: 70–4.
- [19] Gasparini G, De Gori M, Calonego G, Della Bora T, Caroleo B, Galasso O. Drug elution from high-dose antibiotic-loaded acrylic cement: a comparative, in vitro study. *Orthopedics*. 2014; 37: e999-1005.
- [20] Hsieh PH, Chang YH, Chen SH, Ueng SW, Shih CH. High concentration and bioactivity of vancomycin and aztreonam eluted from Simplex™ cement spacers in two-stage revision of infected hip implants: A study of 46 patients at an average follow-up of 107 days. *J Orthop Res*. 2006; 24: 1615–21.
- [21] Anagnostakos K, Wilmes P, Schmitt E, Kelm J. Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers in vivo. *Acta Orthop*. 2009; 80: 193–7.
- [22] Baleani M, Persson C, Zolezzi C, Andollina A, Borrelli AM, Tigani D. Biological and biomechanical effects of vancomycin and meropenem in acrylic bone cement. *J Arthroplasty*. 2008; 23: 1232–8.
- [23] Nelson CL, Jones RB, Wingert NC, Foltzer M, Bowen TR. Sonication of antibiotic spacers predicts failure during two-stage revision for prosthetic knee and hip infections. *Clin Orthop*. 2014; 472: 2208–14.
- [24] Esteban J, Gadea I, Pérez-Jorge C, Sandoval E, García-Cañete J, Fernandez-Roblas R, et al. Diagnosis of spacer-associated infection using quantitative cultures from sonicated antibiotics-loaded spacers: implications for the clinical outcome. *Eur J Clin Microbiol Infect Dis*. 2016; 35: 207–13.
- [25] Sorli L, Puig L, Torres-Claramunt R, González A, Alier A, Knobel H, et al. The relationship between microbiology results in the second of a two-stage exchange procedure using cement spacers and the outcome after revision total joint replacement for infection: the use of sonication to aid bacteriological analysis. *J Bone Joint Surg Br*. 2012; 94: 249–53.
- [26] Neut D, de Groot EP, Kowalski RS, van Horn JR, van der Mei HC, Busscher HJ. Gentamicin-loaded bone cement with clindamycin or fusidic acid added: biofilm formation and antibiotic release. *J Biomed Mater Res A*. 2005; 73: 165–70.
- [27] Hendriks JG, Neut D, van Horn JR, van der Mei HC, Busscher HJ. The release of gentamicin from acrylic bone cements in a simulated prosthesis-related interfacial gap. *J Biomed Mater Res B Appl Biomater*. 2003; 64: 1–5.