Research Paper

# Diagnosis of Persistent Infection in Prosthetic Two-Stage Exchange: Evaluation of the Effect of Sonication on Antibiotic Release from Bone Cement Spacers

Sandrine Mariaux, Ulrika Furustrand Tafin, Olivier Borens✉

Service of Orthopaedics and Traumatology, Lausanne University Hospital, Avenue Pierre-Decker 4, 1011 Lausanne Switzerland

✉ Corresponding author: Prof. Olivier Borens, Lausanne University Hospital (CHUV), Rue du Bugnon 46, 1011 Lausanne, Switzerland. Mobile: +41 79 556 47 76; Phone: +41 21 314 27 89 or +41 21 314 27 52; Fax: +41 21 314 27 55; Email: Olivier.Borens@chuv.ch

## Abstract

***Introduction***: When treating periprosthetic joint infection with a two-stage procedure, antibiotic-impregnated spacers can be used in the interval between prosthetic removal and reimplantation. In our experience, cultures of sonicated spacers are most often negative. The objective of the study was to assess whether that sonication causes an elution of antibiotics, leading to elevated antibiotic concentrations in the sonication fluid inhibiting bacterial growth and thus causing false-negative cultures.

***Methods***: A prospective monocentric study was performed from September 2014 to March 2016. Inclusion criteria were a two-stage procedure for prosthetic infection and agreement of the patient to participate in the study. Spacers were made of gentamicin-containing cement to which tobramycin and vancomycin were added. Antibiotic concentrations in the sonication fluid were determined by mass-spectometry (LC-MS).

***Results***: 30 patients were identified (15 hip and 14 knee and 1 ankle arthroplasties). No cases of culture positive sonicated spacer fluid were observed in our serie. In the sonication fluid median concentrations of 13.2µg/ml, 392 µg/ml and 16.6 µg/ml were detected for vancomycin, tobramycin and gentamicin, respectively. According to the European Committee on antimicrobial susceptibility testing (EUCAST), these concentrations released from cement spacer during sonication are higher than the minimal inhibitory concentrations (MICs) for most bacteria relevant in prosthetic joint infections.

***Conclusion:*** Spacer sonication cultures remained sterile in all of our cases. Elevated concentrations of antibiotics released during sonication could explain partly negative-cultured sonicated spacers. Indeed, the absence of antibiotic free interval during the two-stages can also contribute to false-negative spacers sonicated cultures.

Key words: Infection, Two-Stage Exchange, Sonication, Spacer

## Introduction

Periprosthetic joint infection is a common complication following joint arthroplasty, estimated at 1 and 2% for total hip arthroplasty and total knee arthroplasty, respectively. As the incidence of prosthetic joint replacements increases, the infection problem is going to remain an important issue in the future.

One of the possible options for the treatment of periprosthetic joint infections is a two-stage exchange procedure. During the interval between removal of the prosthesis and reimplantation, antibiotic-impregnated cement spacers can be used. They have

the advantages of local antibiotic release, dead space management and prevention of soft tissue retraction. However, the spacer can also act as a foreign body and thus be colonized by biofilm forming microorganisms. In the literature, most studies report cases of spacer infection at the second-stage procedure [1-4].

Sonication is a method to take off the bacterial biofilm containing adherent microorganisms on a prosthetic implant by ultrasound. By this process, the bacteria return to a planktonic state, can be incubated and analyzed. It increases significantly the sensitivity of bacterial cultures from 61% for standard cultures to 79% after sonication [5]. This is thus a useful method for diagnosis of periprosthetic infection. However, its use for diagnosis of persistent infection at the spacer stage is unclear so far.

According to Nelson et al [1], 50% of their removed and sonicated spacers were infected at the time of the second stage procedure. In the group with positive sonication results, 50% of patients had a re-infection at 2 years follow-up. In our experience, cultures of sonicated spacers were always negative. However, in their series, the interval during the two-stages was longer and antibiotics were suspended 6 weeks before reimplantation. Those two reasons could increase the probability to identify persistent infection.

The objective of the study was to assess whether that spacer sonication causes an elution of antibiotics, leading to elevated antibiotic concentrations in the sonication fluid inhibiting bacterial growth and causing false-negative cultures.

## Method

A prospective monocentric study was performed from September 2014 to March 2016 at the Lausanne University Hospital (CHUV). Inclusion criteria were patients who were operated for a periprosthetic joint infection treated with two-stage exchange and who gave their informed consent to the study. The study was approved by the local ethical committee.

The diagnosis of infection was confirmed either by multiple positive periprosthetic cultures and/or, sonication of the prosthesis at the first stage of the procedure. The threshold of ≥50 CFU was defined as positive cultures, being a sign of infection [5]. Moreover, patients with fistula were considered infected even if all cultures samples were negative.

30 consecutive patients were included: 15 total hip arthroplasty (THA), 14 total knee arthroplasty (TKA), 1 total ankle arthroplasty (TAA). 8 patients were female and 22 were male. 8 patients were diabetic (26.6%). Mean age was 66 years old (range 28-85). The bacteria identified were *Staphylococcus*

*epidermidis (8)*, *S. aureus (7)*, *S. capitis (3)*, *Streptococcus dysgalactiae (4)*, *S. milleri (2)*, *S. pneumoniae (1)*, *S. salivarius (1)*, *Enterococcus faecalis (1)*, *Cutibacterium acnes (1)*, *Clostridium celecrescens (1)* and *Campylobacter fetus (1)*.

At the first stage of the procedure, the prosthesis was removed and was sent for sonication to the laboratory of microbiology [5-6], Wide debridement was performed collecting at least 2-3 periprosthetic tissues samples which were sent for culture. Then a handmade spacer was formed. For the production of the spacer 40g of the shelf cement containing 0,5gr of gentamycin (Palacos R+G, Hereaus Medical, Berlin, GER) were handmixed with supplemental 1.2g tobramycin and 2g vancomycin. Empiric intravenous antibiotics were administrated postoperatively followed by specific intravenous antibiotics, once the susceptibility tests were available. Rifampin was not introduced before the second stage was completed, in order to avoid development of rifampin-resistant bacteria. Indeed, Achermann et al proved that PJI with high initial bacterial load, inappropriate initial debridement and length of intravenous antibiotics shorter than 2 weeks were risk factors to develop resistance to rifampin. Moreover, in presence of wound discharge or sinus tract, the use of rifampin could select rifampin-resistant skin micro-organisms and could cause surinfection. They also attested that even if rifampin is postponed for several days, it does not alter survival rate of the prosthetic implant. For those reasons, we chose to introduce rifampin only after the second stage, when wound was calm [7].

A short interval from 2 to 4 weeks was chosen for each case; the best time of reimplantation being decided depending on local status (acceptable quality of bone or soft tissue at the time of implant removal), pathogen involved (absence of difficult-to-treat microorganisms such as rifampicin-resistant staphylococci, ciprofloxacin-resistant gram-negative bacteria, fungi) and decreasing of C-reactive protein (CRP) and white cell count, without any strict cut-off value. No antibiotic free period was performed between the 2 stages.

At the second stage, the spacer was removed, a wide debridement was performed and the new prosthesis was implanted. At this stage, cultures of 2-3 samples were done and the spacer was sonicated. For the purpose of the study, concentration of each antibiotic in the sonication fluid was measured.

Our protocol of sonication consists in two minutes at 40kHz using sonication device Bactosonic (Bandelin GmbH, Berlin, Germany). It was based on the protocol published by Trampuz et al, and adapted according to the Microlabs standard operating procedures [5]. A minimum of phosphate buffered

saline (PBS) fluid was poured in the sterile container containing the spacer. The quantity of fluid was depending on the size of the spacer. Unfortunately, the quantity could not be standardized for every case and was not measured systematically. Therefore, the concentration of antibiotics is only indicative for the purpose of the study. It does not imply that the antibiotic concentrations are efficient enough to treat locally the infection. Indeed, these are *ex vivo* antibiotic concentrations and do not represent the local concentration of antibiotics around the spacer in the patient.

After sonication, a sample of sonication fluid was collected under laminar flow for measurement of antibiotic concentration. Antibiotic concentrations in the sonication fluid were determined by liquid chromatography associated with mass-spectometry (LC-MS).

The analysis was performed during the first 3-4 days after sonication and samples were kept at -80 Celsius degrees between the different stages of the procedure.

## Results

At reimplantation, cultures of tissue samples and spacer sonication fluid were all negative.

At a mean follow-up of 12.8 months (range from 1 to 24 months), we had two persistent infections: one patient infected with *S. epidermidis* and one patient infected with *methicilin-resistant S. aureus (MRSA)*. Four patients had a re-infection (13.3%): one hematogenous THA infection by *S. aureus* caused by diabetic foot ulcer 9 months later, one hematogenous THA infection by *S. aureus* 5 months later and two cases of persistent serous discharge of wound 1 month after reimplantation (1 THA infection by *E. faecalis* and 1 TKA infection by *E. cloacae)*. As the bacteria identified were different from the first stage procedure, they were treated by debridement, changing of the mobile part and implant retention. Re-infection appeared between 1 and 8 months after reimplantation (mean: 3.5 months).

In the sonication fluid, median concentrations of 13.2µg/ml (min. 1.4 µg/ml, max 49.2 µg/ml), 39.2 µg/ml (min. 0 µg/ml, max 1068,8 µg/ml), and 16.6 µg/ml (min 0 µg/ml, max 169.7 µg/ml) were detected for vancomycin, tobramycin and gentamicin, respectively (Table 1). The detailed antibiotic concentrations are listed in Table 2. According to the European Committee on antimicrobial susceptibility testing (EUCAST), these antibiotic concentrations released from cement spacer during sonication are higher than the minimal inhibitory concentrations (MICs) for most bacteria relevant in PJI (Table 3). Only one case (Case 7) showed antibiotic concentration for

*S. aureus* lower than MIC. Moreover, despite standardized protocol and operative report, tobramycin and vancomycin were not mixed to the Palacos R+G cement.

**Table 1.** Mean antibiotic concentrations in spacer sonicated fluid

|  | Gentamicin mg/l | Tobramycin mg/l | Vancomycin mg/l |
|---|---|---|---|
| **Median** | 16.6 | 392 | 13.2 |
| **Min; Max** | 2.2; 169.7 | 4.7; 1068 | 1.4; 49.2 |

## Discussion

In our serie, the survival rate free-of infection was 80 % at a mean follow-up of 12.8 months. Those results are similar to other studies where survival rates were between 67% and 94% [1],[9],[10],[8],[11]. Four on six infections in our serie were newly acquired infections with different germs compared to the initial infection in 3 cases. Two cases were persistent infections. We can conclude that 28 on 30 cases were true-negative spacer sonication cultures.

From the literature, we already know that bacteria can adhere on cement spacer despite a high load of antibiotics [12]. *In vitro* and *in vivo* studies have shown that antibiotics are released from cement spacers in high concentrations during the first few days after implantation [13-14]. After a peak of antibiotic levels during the few days of spacer implantation, a lower residual antibiotic concentration persists during the following weeks [15-16].

In our study and after explantation of the spacer, the concentrations of antibiotics in the spacer sonication fluid were sufficiently high for microbial growth inhibition of most bacteria responsible for prosthetic joint infection, even if important variability between patients was observed. However, the volume of fluid used in the sonication process was not standardized; those results do not represent the in vivo concentrations. Hendricks et al also demonstrated that sonication tends to increase antibiotic release *in vitro* [17]. The same results were found by Kummer et al. *In vitro* polymethyl methacrylate (PMMA) scaffolds containing antibiotics were stored in 37°C for up to 6 weeks. Sonication increased antibiotics elution, especially during the first 2 weeks. The release was more stable for vancomycin, in comparison with gentamycin that decreased over time. However, all concentrations were above MICs of microorganisms responsible for most frequent PJI infection [18]. Ensing et al added that increased antibiotic release from cement blocks by ultrasounds is active on bacteria in planktonic state as bacteria in biofilms. However, the efficiency of

antibiotics differs depending on the germs. Indeed, S. aureus and *Coagulase-negative staphylococcus* are more susceptible than *Pseudomonas* [19]. However, Clauss et al disagree with the results cited above. In their study *in vitro*, in which PMMA samples were exposed to bacteria for 1-2 days, bacterial growth was not altered by release of antibiotics. They found >500CFU/ml in

sonication fluid when *S. aureus* and *E. faecalis* were tested. However, *C. acnes* was influenced by antibiotic release. That phenomenon increased by a longer interval between sonication and time of analysis [20]. This shows that the reaction is different depending on the bacteria involved.

**Table 2.** Detailed antibiotic concentrations

| Patients | Implants | Primary infection | Antibiotics concentrations | | | Re-infection |
|---|---|---|---|---|---|---|
| | | | Gentamicin mg/l | Tobramycin mg/l | Vancomycin mg/l | |
| 1 | THR | *Streptococcus dysgalactiae* | 8.12 | 64.98 | 19.216 | None |
| 2 | TKR | *S. epidermidis* | 39.49 | 185.064 | 89.733 | Persistent infection with cutaneous fistula |
| 3 | THR | *methicilin-resistant S. epidermidis* | 5.062 | 7.841 | 5.652 | None |
| 4 | THR | *Cutibacterium acnes* | 4.916 | 4.675 | 3.491 | None |
| 5 | THR | *methicilin-resistant S. epidermidis* | 9.893 | 16.879 | 7.148 | None |
| 6 | THR | *S. aureus* | 25.53 | 37.708 | 17.398 | Re-infection by *S. aureus* |
| 7 | TKR | *S. aureus* | 1.439 | 0 | 0 | None |
| 8 | THR | *S. epidermidis* | 16.134 | 76.711 | 81.183 | None |
| 9 | THR | *methicilin-resistant S. epidermidis* | 6.665 | 7.166 | 2.489 | Re-infection by *Enterococcus faecalis* |
| 10 | TAR | *Staphylococcus capitis* | 13.152 | 12.404 | 5.554 | None |
| 11 | TKR | *Streptococcus pneumoniae* | 8.2 | 13.9 | 3.3 | None |
| 12 | THR | *Enterococcus faecalis* | 33 | 66.6 | 17.7 | None |
| 13 | THR | *S. aureus* | 49.2 | 153.3 | 77.7 | None |
| 14 | THR | *S. epidermidis* | 17 | 15.6 | 2.5 | None |
| 15 | TKR | *Streptococcus dysgalactiae* | 22.92 | 99.721 | 169.686 | Re-infection by *Staph aureus* |
| 16 | TKR | *methicillin-resistant S. aureus* | 10.716 | 39.208 | 16.624 | Re-infection by *Enterobacter cloacae* |
| 17 | THR | *Streptococcus milleri* | 4.65 | 5.00 | 2.56 | None |
| 18 | TKR | *Streptococcus salivarus* | 10.36 | 5.89 | 2.30 | None |
| 19 | TKR | *Streptococcus dysgalactiae* | 43.42 | 884.94 | 285.92 | None |
| 20 | THR | *Campylobacter fetus* | 23.86 | 25.43 | 200.94 | None |
| 21 | TKR | *Streptococcus milleri* | 23.63 | 425.23 | 2.76 | None |
| 22 | TKR | *methicillin-resistant S. aureus* | 25.85 | 1068.61 | 371.35 | None |
| 23 | TKR | *methicillin-resistant S. aureus* | 24.36 | 751.26 | 8.99 | Persistent infection with cutaneous fistula |
| 24 | TKR | *Streptococcus dysgalactiae* | 9.79 | 6.20 | 213.85 | None |
| 25 | THR | *S. epidermidis* | 42.38 | 84.92 | 24.84 | None |
| 26 | THR | *Staphylococcus capitis* | 1.50 | 17.62 | 15.62 | None |
| 27 | TKR | *Clostridium celerecrescens* | 8.08 | 6.18 | 2.19 | None |
| 28 | TKR | *methicilin-resistant S. epidermidis* | 24.03 | 460.7 | 107.29 | None |
| 29 | THR | *S. aureus* | 9.12 | 16.08 | 6.53 | None |
| 30 | TKR | *Staphylococcus capitis* | 64.28 | 1068.8 | 225.93 | None |

THR= Total Hip Replacement; TKR= Total Knee Replacement; TAR= Total Ankle Replacement

**Table 3.** Minimal Inhibitory Concentration (MIC) breakpoint.

| | Gentamycin (mg/l) | Tobramycin (mg/l) | Vancomycin (mg/l) |
|---|---|---|---|
| *Staphylococcus aureus* | 1 | 1 | 2 |
| *Coagulase-Negative Staphylococcus* | 1 | 1 | 4 |
| *Streptococcus spp* | - | - | 2 |
| *Enterobacteriae* | 2-4 | 2 | - |
| *Pseudomonas spp* | 4 | 4 | - |
| *Enterococcus spp* | - | - | 4 |
| *Corynebacterium spp* | 1 | - | 2 |
| *Acinebacter spp* | 4 | 4 | - |
| *Clostridium difficile* | - | - | 2 |
| *Gram positive anaerobes* | - | - | 2 |
| *Cutibacterium acnes* | - | - | - |
| *Campylobacter spp* | - | - | - |

Spp= species; - = not available; Values from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. 2017. http://www.eucast.org.

Concerning *in vivo* studies, there is no consensus on the quantity and type of antibiotics needed in spacers. Corona et al. have tested spacers containing different loads of antibiotics, either gentamicin alone, or gentamicin and vancomycin [21]. However, there was no statistically significant difference between different groups in terms of re-infection and complication rate. Those results are similar to the study of Nettrour which showed 88% of survival free of infection, with no difference between groups (tobramycin, vancomycin or both of them; with dose of antibiotics contained in the cement either below or above 4g) [11]. Nevertheless, some authors published that association of antibiotics would be more efficient than one antibiotic alone due to synergic effect [13],[15]. The quantity of antibiotics is then at the discretion of the surgeon.

In our study, the combination of 3 antibiotics was chosen; vancomycin to cover Gram positive infection, tobramycin to cover Gram negative and gentamicin already present in the cement; to be active against all bacteria mostly responsible for prosthetic joint infection, even the more virulent ones. Based on our experience a short interval exchange, once the micro-organism is identified at the first stage procedure, is an acceptable option for eradication of infection. IV- antibiotics were continued during the whole interval between the two stages. From our point of view, this limits the risk of colonization of the cement spacer.

However, even with standardized protocols, local antibiotics concentrations have shown some discrepancy between individuals. Indeed, elution depends on surface area, that is different for each joint and each patient due to the centromedullary width and size of the bones; characteristics and quantity of antibiotics, and characteristics of bone cement (porosity and roughness) [3],[22]. The type of spacer used does not seem to interfere significantly with antibiotic release. In their review, Pivec et al. showed no difference in re-infection rate in articulating versus static spacers in TKA [23]. Moreover, handmade versus prefabricated spacers had similar re-infection rates. The only significant difference was a tendency of increased dislocation, and fracture rate for spacers made intraoperatively by the surgeon [24].

In conclusion, in our study of explanted spacers, antibiotic concentrations released from cement spacer during sonication are high enough to cause culture-negative spacer sonicated fluid. Therefore, sonication of cement spacers does not seem relevant for diagnostic purposes. Indeed, a negative spacer sonication does not confirm that periprosthetic infection is completely cured. However, the absence of an antibiotic free interval in our serie between the two-stages can also contribute to false-negative cultures.

Our study has some limitations. The number of patients is relatively small, although relatively high compared to most studies on spacers sonication. The difference of spacer size between patients and the absence of standardization of the quantity of liquid in sonication are also issues to measure precisely antibiotic concentrations. This explains the variation of antibiotic concentration between patients in our series and this is the reason why our results should not be compared with *in vivo* studies. Indeed, the antibiotic concentrations in our study are not representative of the local antibiotic concentration around the spacer in the patient. The length of follow-up of our study can also be considered too short to highlight low-grade bacteria such as

*Cutibacterium acnes* and *Enterococci*. However, the aim of this study was to measure the elution of antibiotics out of the explanted spacer through sonication.

## Conclusions

Spacer sonication cultures remained sterile in all of our cases. Elevated concentrations of antibiotics released during sonication could explain at least partly negative-cultured sonicated spacers. Therefore, sonication of cement spacers does not seem relevant for diagnostic purposes. Indeed, a negative spacer sonication does not confirm the absence of periprosthetic infection and does not help to predict which patients will suffer from a persistent infection.

## Acknowledgements

### IRB/Ethical Committee Approval

This study was approved by the independent local ethics committee (Commission cantonale (VD) d'éthique de la recherche sur l'être humain). Protocol 136/15 on 18th July 2014.

## Competing Interests

Sandrine Mariaux and Ulrika Furustrand Tafin have no disclosures.

Here are disclosures of Prof. Olivier Borens: paid presentations for Zimmer-Biomet, Medacta, Lima and Heraeus. Unpaid consultant for Medacta, Lima and Zimmer-Biomet. Research support from Matthys, Bonesupport and Lima. Part of orthopaedic publications board of Journal of Bone and Joint Infection. Board member of Swiss orthopaedics, former Board member of European Bone and Joint infection society, President of AOTrauma Switzerland, Trustee AOFoundation.

## References

[1]  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 Relat Res 2014;472:2208–14. doi:10.1007/s11999-014-3571-4.

[2]  Sorlí 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. J Bone Jt Surg Br 2012;94B:249–53. doi:10.1302/0301-620X.94B2.27779.

[3]  Mariconda M, Ascione T, Balato G, Rotondo R, Smeraglia F, Costa GG, et al. Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol for infected joint arthroplasty. BMC Musculoskelet Disord 2013;14. doi:10.1186/1471-2474-14-193.

[4]  Schmolders J, Hischebeth GT, Friedrich MJ, Randau TM, Wimmer MD, Kohlhof H, et al. Evidence of MRSE on a gentamicin and vancomycin

impregnated polymethyl-methacrylate (PMMA) bone cement spacer after two-stage exchange arthroplasty due to periprosthetic joint infection of the knee. BMC Infect Dis 2014;14:1–5. doi:10.1186/1471-2334-14-144.

[5] 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. doi:10.1056/NEJMoa061588.

[6] Borens O, Yusuf E, Steinrücken J, Trampuz A. Accurate and early diagnosis of orthopedic device-related infection by microbial heat production and sonication. J Orthop Res Off Publ Orthop Res Soc 2013;31:1700–3. doi:10.1002/jor.22419.

[7] Achermann Y, Eigenmann K, Ledergerber B, Derksen L, Rafeiner P, Clauss M, et al. Factors associated with rifampin resistance in staphylococcal periprosthetic joint infections (PJI): a matched case-control study. Infection 2013;41:431–7. doi:10.1007/s15010-012-0325-7.

[8] Mortazavi SMJ, Vegari D, Ho A, Zmistowski B, Parvizi J. Two-stage Exchange Arthroplasty for Infected Total Knee Arthroplasty: Predictors of Failure. Clin Orthop Relat Res 2011;469:3049–54. doi:10.1007/s11999-011-2030-8.

[9] Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, et al. Culture-negative prosthetic joint infection. Clin Infect Dis Off Publ Infect Dis Soc Am 2007;45:1113–9. doi:10.1086/522184.

[10] Kurd MF, Ghanem E, Steinbrecher J, Parvizi J. Two-stage Exchange Knee Arthroplasty: Does Resistance of the Infecting Organism Influence the Outcome? Clin Orthop Relat Res 2010;468:2060–6. doi:10.1007/s11999-010-1296-6.

[11] Nettrour JF, Polikandriotis JA, Bernasek TL, Gustke KA, Lyons ST. Articulating Spacers for the Treatment of Infected Total Knee Arthroplasty: Effect of Antibiotic Combinations and Concentrations. Orthopedics 2013;36:e19–24. doi:10.3928/01477447-20121217-13.

[12] Anagnostakos K, Fürst O, Kelm J. Antibiotic-impregnated PMMA hip spacers: current status. Acta Orthop 2006;77. doi:10.1080/17453670610012719.

[13] 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. doi:10.3109/17453670902884700.

[14] Bertazzoni Minelli E, Benini A, Samaila E, Bondi M, Magnan B. Antimicrobial activity of gentamicin and vancomycin combination in joint fluids after antibiotic-loaded cement spacer implantation in two-stage revision surgery. J Chemother 2015;27:17–24. doi:10.1179/1973947813Y.0000000157.

[15] Bertazzonni Minelli E, Benini A, Magnan B, Bartolozzi P. Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty. J Antimicrob Chemother 2004;53. doi:10.1093/jac/dkh032.

[16] Fink B, Vogt S, Reinsch M, Büchner H. Sufficient release of antibiotic by a spacer 6 weeks after implantation in two-stage revision of infected hip prostheses. Clin Orthop Relat Res 2011;469. doi:10.1007/s11999-011-1937-4.

[17] Hendriks JGE, 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 Controlled Release 2003;92:369–74. doi:10.1016/S0168-3659(03)00361-4.

[18] Kummer A, Tafin UF, Borens O. Effect of Sonication on the Elution of Antibiotics from Polymethyl Methacrylate (PMMA). J Bone Jt Infect 2017;2:208–12. doi:10.7150/jbji.22443.

[19] Ensing GT, Neut D, van Horn JR, van der Mei HC, Busscher HJ. The combination of ultrasound with antibiotics released from bone cement decreases the viability of planktonic and biofilm bacteria: an in vitro study with clinical strains. J Antimicrob Chemother 2006;58:1287–90. doi:10.1093/jac/dkl402.

[20] Clauss M, Laschkolnig E, Graf S, Kühn K-D. Influence of Sonication on Bacterial Regrowth from Antibiotic Loaded PMMA Scaffolds - An In-vitro Study. J Bone Jt Infect 2017;2:213–7. doi:10.7150/jbji.22382.

[21] Corona PS, Barro V, Mendez M, Cáceres E, Flores X. Industrially Prefabricated Cement Spacers: Do Vancomycin- and Gentamicin-impregnated Spacers Offer Any Advantage? Clin Orthop Relat Res 2014;472:923–32. doi:10.1007/s11999-013-3342-7.

[22] Jaeblon T. Polymethylmethacrylate: properties and contemporary uses in orthopaedics. J Am Acad Orthop Surg 2010;18:297–305.

[23] Pivec R, Naziri Q, Issa K, Banerjee S, Mont MA. Systematic Review Comparing Static and Articulating Spacers Used for Revision of Infected Total Knee Arthroplasty. J Arthroplasty. 2014 Mar;29(3):553-7.e1. doi: 10.1016/j.arth.2013.07.041. .

[24] Citak M, Masri BA, Springer B, Argenson J-N, Kendoff DO. Are Preformed Articulating Spacers Superior To Surgeon-Made Articulating Spacers in the Treatment Of PJI in THA? A Literature Review. Open Orthop J 2015;9:255–61. doi:10.2174/1874325001509010255.