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Research Article

Antibiotic Elution from Cement: An *In Vitro* Study

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Abstract

Prosthetic joint infection is a serious complication of total hip replacement. Cement removal during two stage revisions can be associated with significant morbidity. Exposing new surface area of cement may achieve therapeutic antibiotic elution.

Five blocks containing polymethylmethacrylate (PMMA) and vancomycin were stored in normal saline at 37°C and allowed to reach steady state. Different interventions to expose new surface area were performed at six weeks.

Post intervention, blocks with the greatest increase in surface area showed the greatest increase in vancomycin levels, the highest increase being 641.77% compared with the control (5.88%).

This study confirms antibiotics remain trapped within PMMA after elution ceases. Further release of vancomycin from cement occurs after exposing new surface area. This seems proportional to the exposed surface area size.

Keywords: Cement; Antibiotic; Elution; Total Hip Replacement; Infection

Introduction

The rate of deep infection of primary total hip or knee replacement (THR or TKR) has been reported to be 1-2% [1-3]. Several authors have shown a decrease in infection rate when using antibiotic impregnated cement when compared to plain cement in the primary THR or TKR [4-7]. The surgical management for deep infection often involves removal of the infected prosthesis and implantation of a new prosthesis, either as a one or two stage procedure. In a two stage procedure, all the infected components and all of the cement are removed, and after a minimum of six weeks with tailored antibiotic administration, implantation of a new prosthesis is performed if there is evidence that the infection has resolved. Two stage revisions have reported success rates of 90-97% [8-10]. During the interval, a cement spacer (often impregnated with antibiotics) is sometimes used. Removal of cement has associated morbidity including prolonged surgical time, increased blood loss and fracture [11,12].

In 1960, Charnley [13] first reported the use of polymethylmethacrylate (PMMA) cement for fixation of prosthetic joint implants, such as total hip or knee replacement. Buchholz., *et al.* [14] later added antibiotics to bone cement with a potential role in the prevention and treatment of infection. Antibiotic-loaded cements are now widely used as prophylaxis or as a therapeutic modality for prosthetic joint infection in THR or TKR [15-17]. Using antibiotic impregnated cement has now become a recommended practice [18,19].

Antibiotic impregnated cement elutes antibiotic over time *in vitro*, peaking at day 1 [20] and then has a slow sustained release over time [21]. Powles., *et al.* [22] showed that gentamicin could be released from cement by fracturing the cement mantle years after the original procedure. It has been estimated that only 5-18% of antibiotic is released from the cement [23].

The exact elution mechanics of antibiotics is complex and is not fully understood. It is dependent on the type and concentration of antibiotic, the type of cement and the conditions under which the cement is mixed [24]. It was initially thought that antibiotics diffused through the solid PMMA matrix [25-27].

Other authors have subsequently shown that elution of cement occurs through the surface of cement and through an intercon-

nected series of cracks and voids in the polymer matrix [24,28-31]. Van de Belt., *et al.* [32] showed that the kinetics of antibiotic release was to some extent through the surface initially, but sustained release depended on the penetration depth as determined by the bulk porosity of the cement.

The aim of this study was to investigate whether further antibiotic is eluted from antibiotic impregnated cement *in vitro* after increasing the surface area of the cement at 6 weeks. This may have clinical relevance in deep infection after primary joint replacement. It may be more therapeutic to expose a new surface area of antibiotic impregnated than removing it all during a two-stage revision. Our theory is that this will increase the local concentration of antibiotic in the infected joint and prevent the morbidity of removing all the cement during the first stage of the revision.

Materials and Methods

2g of powdered vancomycin was mixed with 80g of PMMA cement (Stryker SimplexP). The liquid monomer was then added and this was hand mixed with a plastic spatula until a consistent liquid was achieved. Five 15mL cement blocks were created with stainless steel screws inserted into two of these blocks. The cement was allowed to set before each block was placed into individual 1L plastic bottles of sterile 0.9% normal saline solution. The bottles were then stored in an incubator at 37^0C.

At each time point the bottles were removed from the incubator. Samples of 2mL from each bottle were taken. The bottles were then returned to the incubator until the next time point. A total of ten sets of samples were tested prior to intervention with another ten sets taken post intervention (Table 1).

Pre intervention	Post intervention
1 Hr	1 Hr
2 Hrs	2 Hrs
4 Hrs	4 Hrs
8 Hrs	16 Hrs
1 Day	1 Day
2 Days	2 Days
5 Days	5 Days
2 Weeks	2 Weeks
3 Weeks	3 Weeks
6 Weeks	6 Weeks

Table 1: Time points for sampling.

After the final set of pre intervention samples were taken at 6 weeks, the cement blocks were removed from their bottles and the following interventions were performed on separate blocks: removal of screw (bottle 2), removal of screw and shattering of block (bottle 3), single burr hole (bottle 4) and multiple burr holes (bottle 5). The remaining block was kept as the control (bottle 1) (Table 2).

	Additional component	Intervention
Bottle 1	Nil	Nil
Bottle 2	Stainless steel screw	Screw to be removed at 6 weeks
Bottle 3	Stainless steel screw	Screw to be removed, and block shattered at 6 weeks
Bottle 4	Nil	Single burr hole
Bottle 5	Nil	Multiple burr holes

Table 2: Interventions.

When all the interventions had been completed, the cement blocks were returned to their original bottles of normal saline. While handling the blocks, care was taken to prevent cross contamination.

Post intervention samples were taken for vancomycin levels over a further six weeks.

The samples were all analyzed using a homogeneous enzyme immunoassay technique which was calibrated prior to analysis. Any samples, which were not immediately analyzed, were stored at 4^0C for a maximum of 36 hours until analysis took place.

Results

Over the initial six weeks prior to intervention, all of the cement blocks demonstrated similar vancomycin elution profiles (Figure 1). An initial high rate of antibiotic release was observed in all blocks over the first 24hours. Following this there was a decline in elution rates (Figure 2), and the vancomycin concentrations peaked between 5 days to 3 weeks. A degree of variability in peak concentrations was noted between cement blocks (peak concentration: mean 11.78mcg/mL, min 9.2, max 17.9, SD 3.65). All bottles subsequently demonstrated a slow regression in levels. The raw data is summarized in table 3.

After the interventions were performed, the greatest rise in vancomycin levels was observed in the shattered cement block

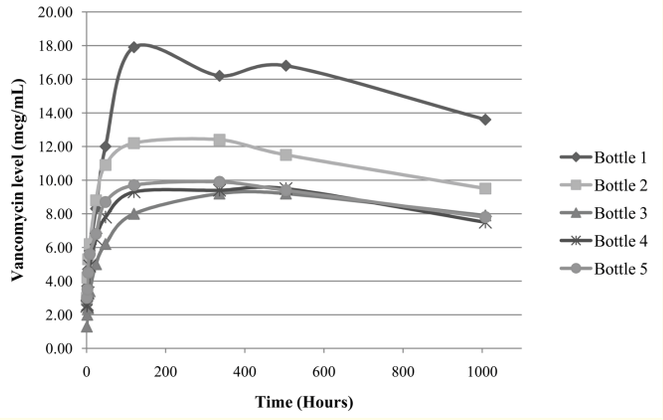

Figure 1: Vancomycin levels pre intervention.

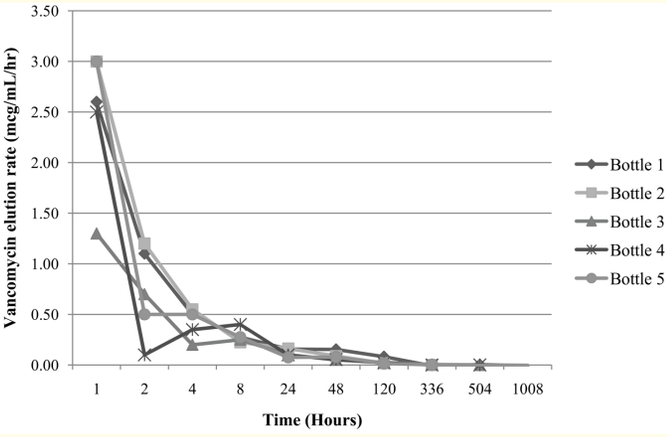

Figure 2: Vancomycin elution rates.

Time	Vancomycin Levels (mcg/mL)				
	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Bottle 5
1 Hr	2.60	3.00	1.30	2.50	3.00
2 Hrs	3.70	4.20	2.00	2.60	3.50
4 Hrs	4.70	5.30	2.40	3.30	4.50
8 Hrs	5.80	6.20	3.40	4.90	5.60
1 Day	8.30	8.80	5.00	6.50	6.80
2 Days	12.00	10.90	6.20	7.80	8.70
5 Days	17.90	12.20	8.00	9.30	9.70
2 Weeks	16.20	12.40	9.20	9.40	9.90
3 Weeks	16.80	11.50	9.20	9.50	9.40
6 Weeks	13.60	9.50	7.90	7.50	7.80

Table 3: Pre intervention vancomycin levels.

(bottle 3), which reached a peak concentration of 58.6 mcg/mL at 5 days. Bottle 5 contained the block with multiple burr holes and demonstrated a moderate increase in vancomycin levels, reaching a peak concentration of 11.10mcg/mL also at 5 days. Post intervention data is shown in table 4 and figure 3.

Time	Vancomycin levels (mcg/mL)				
	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Bottle 5
0 Hrs	13.60	9.50	7.90	7.50	7.80
1 Hr	13.90	9.20	35.20	7.50	9.10
2 Hrs	14.30	9.10	38.30	7.80	9.10
4 Hrs	14.00	9.40	44.60	7.70	9.80
16 Hrs	13.60	9.30	50.50	7.50	10.10
1 Day	14.00	9.40	53.40	7.70	10.40
2 Days	14.40	9.20	55.00	7.40	11.00
5 Days	13.60	9.20	58.60	6.80	11.10
2 Weeks	12.40	8.30	57.20	6.90	10.30
3 Weeks	10.80	7.40	49.80	6.40	9.50
6 Weeks	9.20	6.70	43.20	5.90	8.50

Table 4: Post intervention vancomycin levels.

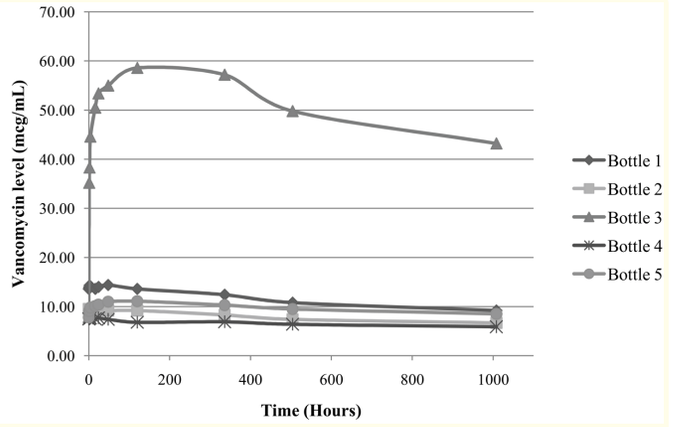

Figure 3: Vancomycin levels post intervention.

These results correspond to a 641.77% rise in vancomycin level in bottle 3 and a 42.31% increase in bottle 5. The cement blocks in bottles 1 (control) and 4 (single burr hole) failed to show a rise in elution greater than 6% (bottle 1 increased 5.9% and bottle 4 increased 3.8%). No increase was observed in bottle 2 which had the block with the screw removed. Table 5 contains a summary of these findings. A graphical representation of combined pre and post intervention levels is displayed in figure 4.

	6 weeks vancomycin level (Pre intervention) *	Peak vancomycin level (Post intervention)	Time of peak level	% Rise post intervention
Bottle 1	13.6	14.4	2 Days	5.88
Bottle 2	9.5	Decline only	N/A	N/A
Bottle 3	7.9	58.6	5 Days	641.77
Bottle 4	7.5	7.8	2 Hours	3.84
Bottle 5	7.8	11.1	5 Days	42.31

Table 5: Summary of findings post intervention.

* In mcg/mL.

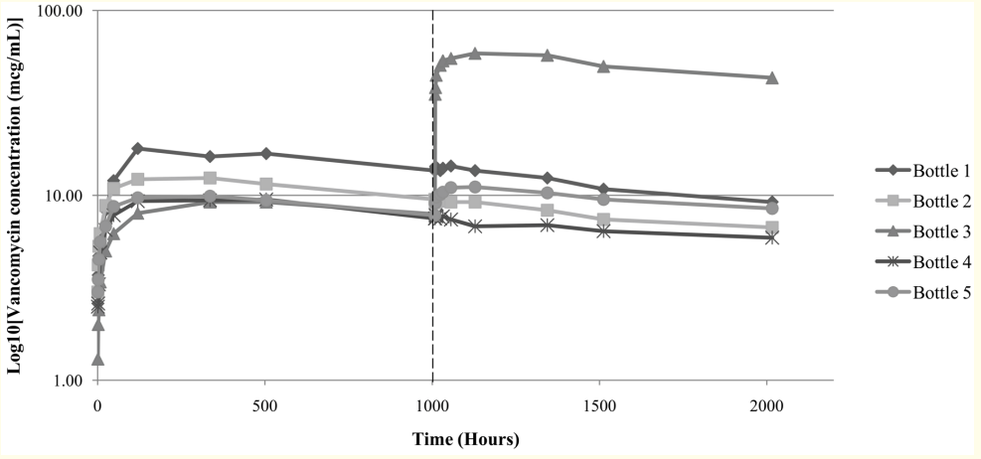

Figure 4: Combined pre and post intervention vancomycin levels.

Discussion

Our results demonstrate that increasing the surface area of antibiotic impregnated cement after reaching a steady state lead to an increased elution of antibiotics *in vitro*. The increase in antibiotic elution seems proportional to the amount of surface area exposed. The clinical correlation may be that exposing a new surface area of cement in a two-stage revision for an infected cemented hip prosthesis would lead to elution of further antibiotics. This may be therapeutic and would also decrease the need for removing all the cement at the time of revision.

Removal of cement from a femur for an infected THR can involve a prolonged operative time, increased blood loss and lead to femoral fracture or perforation [11,12]. Thorough debridement and exposure of a new surface by taking away a portion of the cement could avoid these complications. The second stage of the revision could then involve a cement-in-cement revision of the femoral stem in THR, which has been shown to be effective 12 years post operatively [33].

Powles., *et al.* [22] looked at a similar phenomenon *in vitro* and found comparable results. Five patients with primary THR with gentamicin impregnated cement underwent revision 6.8 years post operatively.

Gentamicin concentration in joint capsule, fluid and membrane were measured before and after disruption of the cement mantle, and considerably higher concentrations of gentamicin were measured after disruption of the cement. They concluded that samples taken from tissue for microbiology should be performed before the cement mantle is disrupted, as they may be contaminated by gentamicin.

Our results support other research, which has established that the elution of antibiotic from cement is biphasic, with an initial peak and then a decline [20,21,34-36]. There was an inhomogeneity of antibiotic elution, which is similar to other *in vitro* [37] and *in vivo* [20] studies. Anagnostakos., *et al.* [20] attributed this variability to the manual incorporation of the vancomycin into the cement powder. This inhomogeneity did not affect our results, as each cement block acted as its own control.

Before intervention, the concentrations in the samples started declining at two to three weeks. This may be due to the instabil-

ity of vancomycin in saline at body temperature. Vancomycin is known to undergo conversion to an inactive crystalline degradation product over time [38], and this process is accelerated at higher temperatures [39]. Kowk., *et al.* [40] demonstrated a 5.4% decrease in vancomycin concentration after 7 days in normal saline at 37C. However, Wood., *et al*. [39] found that vancomycin was stable in normal saline with less than 10% degradation for up to 62 days at 25C.

The different interventions were performed at six weeks as acute deep infections can present at this time.

Different levels of surface area exposure were chosen to simulate clinical practice. Removal of the screw only (bottle 2) simulated removing the prosthesis only. Bottles 3-5 were increasing levels of surface exposure. Bottle 3 especially showed a dramatic rise in vancomycin elution post intervention (shattering), in keeping with its much higher increase in surface area. This, as well as the modest increase in the elution of bottle 5 (multiple holes) compared with bottle 4 (single hole) suggests that the increase in elution of antibiotic is proportional to the increase of surface area.

Investigators have demonstrated that a larger initial surface area can increase the amount of antibiotic eluted. Holtom., *et al.* [41] compared the elution of vancomycin from solid spacers and fenestrated spacers *in vitro*, and found that the fenestrated spacers, which had 40% greater surface area than solid spacers, had an average of 20% more antibiotic eluted on any given day. Anagnostakos., *et al.* [20] compared the elution of antibiotics from spacers with beads *in vivo* and found that although the dose of antibiotic was lower in the spacers, the beads had greater elution due to their larger surface area.

Vancomycin was chosen as it is a popular antibiotic for use in bone cement [34-36,41-43]. Studies have demonstrated that low concentration vancomycin impregnated bone cement maintains sustained release and has minimal impact on the mechanical properties of the cement [34,36,44,45]. It also possesses stable bactericidal activity throughout cement polymerisation and has synergistic effects with other antibiotics [37,42]. As the incidence of methicillin resistant staphylococcus aureus (MRSA) increases in the community [10,43], more vancomycin impregnated cement in primary joint replacements may be used in the future.

The rate of antibiotic elution may have been affected by the concentration of vancomycin in the solution.

We were unable to confirm if the plateau represents complete elution of antibiotics or simply an equilibrium state between concentration in solution and surface concentration. For this reason, we chose to maintain the same bottle of saline throughout the entire experiment. Ideally, a model that simulates constant flow of saline past the blocks would more closely simulate the body metabolizing antibiotic. We recognize one of limitations of this study was its *in vitro* nature. Further research is needed to investigate whether the rate of antibiotic elution will exceed the MIC required to kill common pathogens.

Conclusion

In summary, our results indicate that increasing the surface area of antibiotic impregnated cement after elution reaches a steady state lead to further elution of antibiotic. This is proportional to the amount of new surface area exposed. This may have clinical implication for patients who have deep infections of prosthetic joint replacements, with antibiotic impregnated cement in their primary procedure. Further *in vivo* studies need to be performed prior to use in clinical practice.

Acknowledgements

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Bibliography

1. Haaker R., *et al.* "Osteomyelitis after endoprostheses". *Orthopade* 33 (2004): 431.

2. Leone JM and Hanssen AD. "Management of infection at the site of a total knee arthroplasty". *Journal of Bone and Joint Surgery American* 87 (2005): 2335.

3. Zimmerli W., *et al.* "Prosthetic-joint infections". *New England Journal of Medicine* 351 (2004): 1645.

4. Buchholz HW., *et al.* "Antibiotic-loaded acrylic cement: current concepts". *Clinical Orthopaedics and Related Research* 190 (1984): 96.

5. Josefsson G., *et al.* "Prophylaxis with systemic antibiotics versus gentamicin bone cement in total hip arthroplasty. A five-year survey of 1688 hips". *Clinical Orthopaedics and Related Research* 253 (1990): 173.

6. Persson U., *et al.* "The economics of preventing revisions in total hip replacement". *Acta Orthopaedica Scandinavica* 70 (1999): 163.

7. Chiu FY., *et al.* "Cefuroxime-impregnated cement in primary total knee arthroplasty: a prospective, randomized study of three hundred and forty knees". *Journal of Bone and Joint Surgery American* 84 (2002): 759.

8. Volin SJ., *et al.* "Two-stage reimplantation of total joint infections: a comparison of resistant and non-resistant organisms". *Clinical Orthopaedics and Related Research* 427 (2004): 94.

9. Haleem AA., *et al.* "Mid-term to long-term follow-up of two-stage reimplantation for infected total knee arthroplasty". *Clinical Orthopaedics and Related Research* 428 (2004): 35.

10. West rich GH., *et al.* "Modern treatment of infected total knee arthroplasty with a 2-stage reimplantation protocol". *Journal of Arthroplasty* 25 (2010): 1015.

11. Akiyama H., *et al.* "Computer-assisted fluoroscopic navigation system for removal of distal femoral bone cement in revision total hip arthroplasty". *Journal of Arthroplasty* 22 (2007): 445.

12. Nelson CL. "Cemented femoral revision: technique and outcome". *American Journal of Orthopedics* 31 (2002): 187.

13. Charnley J. "Anchorage of the femoral head prosthesis to the shaft of the femur". *Journal of Bone and Joint Surgery British* 42-B (1960): 28.

14. Buchholz HW., *et al.* "Management of deep infection of total hip replacement". *Journal of Bone and Joint Surgery British* 63-B (1981): 342.

15. Diefenbeck M., *et al.* "Prophylaxis and treatment of implant-related infections by local application of antibiotics". *Injury* 37.2 (2006): 95.

16. Jiranek WA., *et al.* "Antibiotic-loaded bone cement for infection prophylaxis in total joint replacement". *Journal of Bone and Joint Surgery American* 88 (2006): 2487.

17. Youngman JR., *et al.* "Antibiotic-loaded cement in revision joint replacement". *Hospital Medicine* 64 (2003): 613.

18. Pitto RP and Spika IA. "Antibiotic-loaded bone cement spacers in two-stage management of infected total knee arthroplasty". *International Orthopaedics* 28 (2004): 129.

19. Cerretani D., *et al.* "The *in vitro* elution characteristics of vancomycin combined with imipenem-cilastatin in acrylic bonecements: A pharmacokinetic study". *Journal of Arthroplasty* 17 (2002): 619.

20. Anagnostakos K., *et al.* "Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers *in vivo*". *Acta Orthopaedica* 80 (2009): 193.

21. Anagnostakos K and Kelm J. "Enhancement of antibiotic elution from acrylic bone cement". *Journal of Biomedical Materials Research - Part B: Applied Biomaterial* 90 (2009): 467.

22. Powles JW., *et al.* "Gentamicin release from old cement during revision hip arthroplasty". *Journal of Bone and Joint Surgery British* 80-B (1998): 607.

23. Törholm C., *et al.* "Total hip joint arthroplasty with gentamicin-impregnated cement. A clinical study of gentamicin excretion kinetics". *Clinical Orthopaedics and Related Research* 181 (1983): 99.

24. Jiranek W. "Antibiotic-loaded cement in total hip replacement: current indications, efficacy, and complications". *Orthopedics* 28.8 (2005): 873.

25. Bayston R and Milner RD. "The sustained release of antimicrobial drugs from bone cement: An appraisal of laboratory investigations and their significance". *Journal of Bone and Joint Surgery British* 64 (1982): 460.

26. Law HT., *et al.* "*In vitro* measurement and computer modelling of the diffusion of antibiotic in bone cement". *Journal of Biomedical Engineering* 8 (1986): 149.

27. Wahlig H and Dingeldein E. "Antibiotics and bone cements. Experimental and clinical long-term observations". *Acta Orthopaedica Scandinavica* 51 (1980): 49.

28. Kuechle DK., *et al.* "Elution of vancomycin, daptomycin, and amikacin from acrylic bone cement". *Clinical Orthopaedics and Related Research* 264 (1991): 302.

29. Schurman DJ., *et al.* "Antibiotic-acrylic bone cement composites. Studies of gentamicin and Palacos". *Journal of Bone and Joint Surgery American* 60 (1978): 978.

30. Wroblewski BM. "Leaching out from acrylic bone cement. Experimental evaluation". *Clinical Orthopaedics and Related Research* 124 (1977): 311.

31. Baker AS and Greenham LW. "Release of gentamicin from acrylic bone cement. Elution and diffusion studies". *Journal of Bone and Joint Surgery American* 70 (1988): 1551-1557.

32. Van de Belt H., *et al.* "Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release". *Biomaterials* 21 (2000): 1981.

33. Mandziak DG., *et al.* "Cement-within-cement stem exchange using the collarless polished double-taper stem". *Journal of Arthroplasty* 22 (2007): 1000.

34. Anagnostakos K., *et al.* "Antibiotic-impregnated PMMA hip spacers: current status". *Acta Orthopaedica* 77 (2006): 628.

35. Hsieh P., *et al.* "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". *Journal of Orthopaedic Research* 24 (2006): 1615.

36. Bertazzoni Minelli E., *et al.* "Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty". *Journal of Antimicrobial Chemotherapy* 53 (2004): 329.

37. Anagnostakos K., *et al.* "*In vitro* evaluation of antibiotic release from and bacterial growth inhibition by antibiotic-loaded acrylic bone cement spacers". *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 72B (2005): 373.

38. Cheng C., *et al.* "94 Degradation of vancomycin to CDP-1 as measured by the COBAS (R) INTEGRA". *Therapeutic Drug Monitoring* 19 (1997): 570.

39. Wood MJ., *et al.* "Stability of vancomycin in plastic syringes measured by high-performance liquid chromatography". *Journal of Clinical Pharmacy and Therapeutics* 20 (1995): 319.

40. Kwok AK., *et al.* "An *in vitro* study of ceftazidime and vancomycin concentrations in various fluid media: implications for use in treating endophthalmitis". *Investigative Ophthalmology and Visual Science* 43 (2002): 1182.

41. Holtom PD., *et al.* "Relation of surface area to *in vitro* elution characteristics of vancomycin-impregnated polymethylmethacrylate spacers". *American Journal of Orthopedics* 27 (1998): 207.

42. Hsieh P., *et al.* "Liquid gentamicin and vancomycin in bone cement: A potentially more cost-effective regimen". *Journal of Arthroplasty* 24 (2009): 125.

43. Tunney MM., *et al.* "Antimicrobial susceptibility of bacteria isolated from orthopedic implants following revision hip surgery". *Antimicrobial Agents and Chemotherapy* 42 (1998): 3002.

44. Breusch SJ and Kühn KD. "Bone cements based on polymethylmethacrylate". *Orthopade* 32 (2003): 41.

45. Adams K., *et al.* "*In vitro* and *in vivo* evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads". *Clinical Orthopaedics and Related Research* 278 (1992): 244.