Gentamicin-loaded bone cement with clindamycin or fusidic acid added: Biofilm formation and antibiotic release

Daniëlle Neut,[1,2] Erik P. de Groot,[1] Rick S. Z. Kowalski,[3] Jim R. van Horn,[2] Henny C. van der Mei,[1] Henk J. Busscher[1]

[1]*Department of Biomedical Engineering, University Medical Center Groningen, and University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands*

[2]*Department of Orthopedic Surgery, University Medical Center Groningen, and University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*

[3]*DePuy CMW, Cornford Road, Blackpool, FY4 4QQ, United Kingdom*

Received 20 September 2004; accepted 15 October 2004
Published online 10 March 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30253

Abstract: The formation of staphylococcal biofilms on experimental bone cements, loaded with 0.5 or 1.0 g of active gentamicin and an additional equivalent amount of gentamicin, clindamycin, or fusidic acid was investigated. The biofilms were formed in a modified Robbins device over a 3-day time span and the influence of the additional antibiotics was quantified by expressing the number of colony forming units relative to the corresponding bone cement containing only gentamicin. Combinations of gentamicin with either fusidic acid or clindamycin reduced growth of clinical isolates of both gentamicin-sensitive *Staphylococcus aureus* and gentamicin-resistant coagulase-negative staphylococci to approximately 28%. To determine whether adding a second antibiotic has influence on the gentamicin release, cement blocks were placed in phosphate buffer and aliquots were taken at designated sampling intervals. The influence of the additional antibiotics was quantified by expressing the percentage released of the total amount of antibiotic incorporated in the different bone cements. After 3 days, all bone cements had released similar percentages of gentamicin, whereas more clindamycin and fusidic acid were released after doubling their concentration in the bone cements. In conclusion, bone cements loaded with combinations of gentamicin and clindamycin or fusidic acid are more effective in preventing biofilm formation than bone cements with gentamicin as a single drug. In addition, the presence of clindamycin or fusidic acid in gentamicin-loaded bone cement has no influence on the total gentamicin release. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 73A: 165–170, 2005

Key words: gentamicin-loaded bone cement; fusidic acid; clindamycin; elution; biofilm

INTRODUCTION

In 1970, Buchholz and Engelbrecht[1] introduced the use of acrylic bone cement as a release system for antibiotics in joint arthroplasties to achieve high local concentrations of antibiotics around an implant. The clinical efficacy of antibiotic-loaded bone cements has initially been considered quite good, and studies have reported lower rates of infection in total hip replacements when using antibiotic-loaded bone cement in contrast to when plain cement was used.[2] Almost 90% of all orthopedic surgeons in the United States use prophylactic antibiotic-loaded bone cement for the fixation of implants.[3] This is despite the fact that the release mechanisms of antibiotic from bone cements is poorly understood and controlled, with some reports claiming the release of antibiotic from bone cements for up to 5 years after implantation.[4] This long-term release of low concentrations of antibiotics around an implant has been associated with the introduction of antibiotic-resistant bacteria.[5]

Gentamicin is in Europe the most commonly used antibiotic in bone cement because of its wide antibacterial spectrum and its ability to remain stable at high temperatures reached during polymerization of the polymethylmethacrylate. In a study of 33 infected hip joints, Weber and Lautenbach[6] noted that 29% of bacteria isolated preoperatively were resistant to gentamicin. Unfortunately, after the use of gentamicin-loaded bone cement, resistance increased to 41% of bacteria isolated postoperatively. The increase in gentamicin resistance among bacterial strains responsible

Correspondence to: D. Neut; e-mail: d.neut@med.umcg.nl

for orthopedic infections has stimulated the development of bone cements containing a combination of gentamicin and a second antibiotic.

Recently, Copal® bone cement, containing a combination of gentamicin and clindamycin, has been introduced on the European commercial market. The combination of gentamicin and clindamycin has a synergistic bactericidal effect on >90% of the bacteria common to infected arthroplasty cases.[7] Gonzalez Della Valle et al.[8] found that the presence of tobramycin has a synergistic-like effect on the bactericidal activity of vancomycin. In contrast, the combination of gentamicin and clindamycin in bone cement inhibited growth of *Staphylococcus epidermidis*, but in an adhesion assay the combination was not significantly more effective than a monotherapy of gentamicin.[9] There are also conflicting studies concerning the amount of antibiotics released when using a combination of drugs. Some reports show a synergistic effect,[10] whereas others show an inhibitory effect.[11]

Fusidic acid is an antibiotic that has been available since the 1960s and a small number of studies have examined the use of fusidic acid for the treatment of bone infections, yielding evidence of good efficacy.[12] Moreover, fusidic acid remains stable when exposed to the high temperatures generated during the curing of the bone cement.[12] For this reason, the properties of fusidic acid incorporated into bone cement have been examined. Beeching et al.[13] showed large inhibitory effects of fusidic acid on the growth of *Staphylococcus aureus* for up to 7 weeks, with the largest inhibitory effect in the first week.

The aim of this study was to determine the effect of adding clindamycin or fusidic acid to a gentamicin-loaded bone cement on the formation of a staphylococcal biofilm on acrylic bone cement and on the release of the antibiotics. For completeness, commercially available Copal® bone cement, containing a combination of gentamicin and clindamycin, was also evaluated. Because gentamicin resistance is the main rationale for loading bone cements with combinations of different antibiotics, both a gentamicin-sensitive and a resistant strain were used.

MATERIALS AND METHODS

Bone cement disc preparation

Six of the polymethylmethacrylate bone cements evaluated were manufactured by DePuy International Ltd. (Blackpool, Lancashire FY4 4QQ, UK). SmartSet® HV bone cements were loaded with 0.5 or 1.0 g of active gentamicin (sulfate) per package of 40.0 g of cement powder and an additional, equivalent amount of clindamycin (hydrochloride), fusidic acid (sodium fusidate), or gentamicin (sulfate). For completeness, Copal® (Merck Biomaterial GmbH, D-64271 Darmstadt, Germany) was used as well, which contains 1.0 g of active gentamicin (sulfate) and 1.0 g of active clindamycin (hydrochloride) per package of 42.6 g of cement powder.

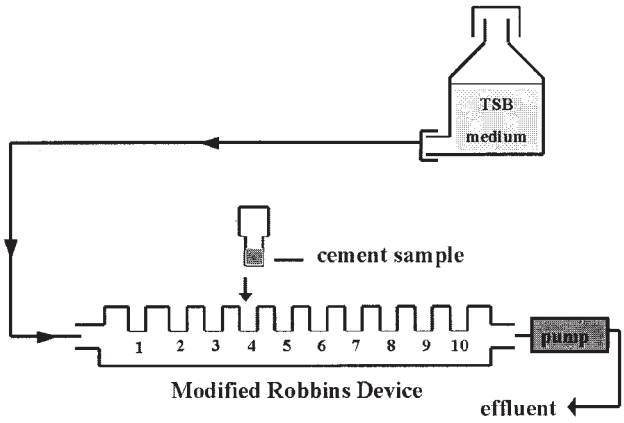

Figure 1. Schematic representation of the modified Robbins device.

The cements were prepared by adding the liquid monomer to the methacrylate powder. After mixing for 30 s with a spatula, the liquid cements were poured into 3.2-mm-thick Teflon molds containing holes with a diameter of 6 mm. This filled mold was manually pressed between two glass plates covered with copier overhead film (Océ, MC 110, 's Hertogenbosch, The Netherlands) to facilitate removal after hardening, up to the time specified for final hardening. After 24 h, the cement discs were pulled out of the mold and stored in the dark at room temperature until further use. The total surface area of each disc was 1.2 cm^2 and one disc weighed about 100 mg. All procedures were performed under sterile conditions.

Bacterial strains and biofilm formation

The bacterial strains were obtained from hip replacement infections in patients from the Department of Orthopedic Surgery at the University Medical Center Groningen, The Netherlands. A gentamicin-sensitive *S. aureus* 2580 [minimal inhibitory concentration (MIC) value of 0.75 μg/mL] and a gentamicin-resistant coagulase-negative *Staphylococcus* 2577 (MIC value >256 μg/mL) were used as representatives of the most common pathogens in orthopedic infections.[14]

The bacteria were aerobically cultured from cryopreservative beads (Protect Technical Service Consultants Ltd., UK) onto blood agar plates at 37° C for 24 h. One colony from this plate was used to create a preculture in 10 mL of Tryptone Soya Broth (TSB) (Oxoid, Basingstoke, Great Britain) under the same incubating conditions. This preculture was used to inoculate a second culture (400 mL), which was grown overnight and used to inoculate a modified Robbins device (MRD), essentially a hollow rectangular cubicle (620 × 20 × 20 mm) with 10 holes in which cement discs can be plugged (see Fig. 1). The cement discs were glued onto

TABLE I
Positions of Different Bone Cement Samples in the Modified Robbins Device

Position	Type of Bone Cement
1 and 2	0.5 g gentamicin, combined with 0.5 g gentamicin
3 and 4	1.0 g gentamicin, combined with 1.0 g gentamicin
5 and 6	0.5 g gentamicin, combined with 0.5 g clindamycin or 0.5 g fusidic acid
7 and 8	1.0 g gentamicin, combined with 1.0 g clindamycin or 1.0 g fusidic acid
9 and 10	Copal® (1.0 g gentamicin combined with 1.0 g clindamycin)

Note that flow is directed from position 1 to 10.

the sample holders with silicone paste under aseptic conditions. The silicone paste did not have any antibacterial properties. The bone cement discs were distributed over the 10 sample positions as indicated in Table I. Care was taken to avoid cross influences between samples, by placing cements with only gentamicin upstream (positions 1–4), whereas placing samples with higher antibiotics downstream of the low concentrations. The positions 5–8 were always filled with a bone cement containing gentamicin in combination with either fusidic acid or clindamycin. Positions 9 and 10 were reserved for Copal®.

The MRD was inoculated with the second culture and left for 5 h, allowing adherence of the bacteria to the different bone cement samples. Hereafter, the device was perfused for 72 h with TSB growth medium at a flow rate of 63 mL/h. Three separate runs were performed with each antibiotic combination. For each run, new cement discs were made and placed in the device. The temperature of the MRD was maintained around 37°C during the experiment.

Biofilm evaluation

The cement discs were removed from the MRD, put in 2 mL 0.9% NaCl, vortexed for 10 s, and finally sonicated for 60 s for microbiological evaluation. Serial dilutions were poured onto TSB agar plates. After incubation overnight, the numbers of colony forming units (CFU) were counted, and expressed relative to the surface area of the cement discs (CFU/cm^2).

Antibiotic release

For the measurement of antibiotic release, sterile cement blocks were placed in 10 mL of phosphate buffer saline at pH 7.0 and stored in a dark environment at 37°C. At designated time intervals (1, 6, 24, and 72 h), 500-μL samples were taken. The aliquots were stored in a dark environment at −20°C.

Gentamicin concentrations were measured using a procedure proposed by Sampath and Robinson[15] and modified by Zhang et al.[16] Briefly, an *o*-phthaldialdehyde reagent was made and stored for 24 h in a dark environment. The gentamicin sample, *o*-phthaldialdehyde, and isopropanol (to avoid precipitation of the products formed) were mixed in equal proportions and stored for 30 min at room temperature. The *o*-phthaldialdehyde reacted with the gentamicin amino groups and chromophoric products were obtained, whose absorbances were measured at 332 nm[17] using a Spectronic® 20 Genesys™ spectrophotometer (Spectronic Instruments Inc., Rochester, NY). The gentamicin percentages released were calculated with respect to the total amount incorporated for all cements used.

Clindamycin and fusidic acid concentrations were measured using high-performance liquid chromatography. The clindamycin and fusidic acid percentages released of the total amount incorporated were calculated for the concerned cements.

Data for total antibiotic release (%) were evaluated for statistical significance using an independent sample *t* test. The number of study units was three for all experiments and a 95% ($p < 0.05$) confidence level was adapted for statistical significance.

RESULTS

Biofilm formation

Table II summarizes the effects of adding clindamycin or fusidic acid to gentamicin-loaded bone cement on bacterial growth on the cement blocks. For all runs, the number of colony forming units per square centimeter on the bone cement discs (CFU/cm^2) was calculated. Combinations of 0.5 g of gentamicin with either fusidic acid or clindamycin reduced growth of the gentamicin-sensitive *S. aureus* 2580 to approximately 28%. The gentamicin-resistant coagulase-negative Staphylococci 2577 showed similar growth reductions.

Doubling the content of gentamicin from 0.5 to 1.0 g was less beneficial in reducing growth and colony

TABLE II
Number of Colony Forming Units per Square Centimeter on the Bone Cement Discs (CFU/cm^2) for Biofilms of *S. aureus* 2580 and CNS 2577 in a Modified Robbins Device

	CFU/cm^2 ($\times 10^7$)	
Bone Cement	*S. aureus*	CNS
0.5 g gentamicin		
and 0.5 g gentamicin	27.1	10.8
and 0.5 g fusidic acid	5.9	3.6
and 0.5 g clindamycin	9.9	1.2
1.0 g gentamicin		
and 1.0 g gentamicin	12.6	6.6
and 1.0 g fusidic acid	4.2	1.5
and 1.0 g clindamycin	2.6	1.3
Copal	4.2	0.7

All results are from triplicate experiments with separately cultured bacteria and different cement blocks, yielding an average standard deviation of 50%.

TABLE III
Total Amounts of Gentamicin, Clindamycin, and Fusidic Acid Released After 72 h, Expressed as a Percentage of the Total Amount Included, of Seven Different Gentamicin-Loaded Bone Cements

Bone Cement	Total Release After 72 h (%) ± SD		
	Gentamicin	Clindamycin	Fusidic Acid
0.5 g gentamicin			
and 0.5 g gentamicin	6.8 ± 1.6	—	—
and 0.5 g fusidic acid	8.2 ± 1.4	—	0.8 ± 0.3
and 0.5 g clindamycin	8.1 ± 2.3	18.6 ± 3.6	—
1.0 g gentamicin			
and 1.0 g gentamicin	9.3 ± 0.7	—	—
and 1.0 g fusidic acid	7.7 ± 2.0	—	1.8 ± 0.3
and 1.0 g clindamycin	8.2 ± 0.9	32.0 ± 6.0	—
Copal®	16.8 ± 5.6	17.2 ± 1.5	—

The values are expressed as mean of three separate experiments ± SD.

growth was reduced to 46 and 61% for the gentamicin-sensitive and resistant strain, respectively. Adding fusidic acid or clindamycin to bone cement with a double gentamicin content resulted in further growth reductions, both for the gentamicin-sensitive and the gentamicin-resistant strain. Copal® showed similar growth reductions.

Antibiotic release

Table III summarizes the total release (%) of gentamicin, clindamycin, and fusidic acid after 3 days for all bone cements involved in this study. Release was most rapid during the first 6 h and continued at a much lower rate thereafter for all three antibiotics. Gentamicin release from the cements containing only gentamicin amounted to 123 μg after 3 days, respectively, for the cement containing 0.5 g of gentamicin, whereas for the cement containing 1.0 g of gentamicin the number was 356 μg. Evidently, disproportionally more gentamicin is released by doubling its concentration in the bone cement. This increase in gentamicin release was statistically significant ($p < 0.05$).

Addition of a second antibiotic increased the total release (%) of gentamicin after 3 days (see Table III) for the 0.5-g gentamicin-loaded bone cement. In contrast, adding a second antibiotic to the 1.0-g gentamicin-loaded cement decreased the total release of gentamicin. However, this did not apply for Copal® (see Table III). Copal® showed a statistically significantly higher ($p < 0.05$) gentamicin release (17% of the total amount incorporated instead of 8%) than the other 1.0-g gentamicin bone cements. In addition, Copal® showed a statistically significant ($p < 0.05$) decrease in the clindamycin release (17% of the total amount incorporated instead of 32%) when compared with the other 1.0-g clindamycin-loaded bone cement.

Similar to gentamicin, more clindamycin and fusidic acid are released by doubling their concentration in the bone cement. This increase in antibiotic release was statistically significant ($p < 0.05$) both for clindamycin and fusidic acid.

DISCUSSION

Combining two antibiotics in bone cement is a common clinical practice in the United States although, until recently, only mono-antibiotic-loaded bone cement has been commercially available in Europe. The increase in gentamicin resistance among bacterial strains responsible for orthopedic infections has stimulated the industrial development of bone cements containing a combination of gentamicin and a second antibiotic. In this study, we found that bone cements loaded with combinations of gentamicin and clindamycin or fusidic acid are more effective in preventing bacterial growth than bone cements with gentamicin as a monotherapy. The choice of these additional antibiotics can be rationalized. Susceptibility to clindamycin of bacteria adhering to polymethylmethacrylate has not been shown to change because of growth in the adhered state.[18] The combination of gentamicin and clindamycin in bone cement has recently been shown to be able to produce larger zones of inhibition against *S. epidermidis* than bone cements with only gentamicin.[9] Fusidic acid, with its steroid-like structure, is a lipophilic antibiotic that could be applied as a "slime buster."[9] It has good efficacy against staphylococci and bone penetration and has therefore been suggested as a promising agent against bone infections.[12]

Beeching et al.,[13] who also studied a combination of gentamicin with fusidic acid, demonstrated an enhanced inhibitory effect for the first 3 days that disappeared after 5 days. The disappearance of an enhanced effect was attributed to a possible physical incompatibility of gentamicin and fusidic acid in solution. An advantage of combining gentamicin with fusidic acid in orthopedic infections might be the lipophilic character of fusidic acid that enables it to be more homogenously spread throughout the polymer matrix.[19] High homogeneity is required for a sustained and prolonged antibiotic release and effective inhibition of bacterial colonization.[19] Combined with the ability to act as a "slime buster," fusidic acid seems an ideal antibiotic to incorporate in bone cement. The present study showed that despite a low total release of fusidic acid after 3 days, bone cements loaded with a combination of gentamicin and clindamycin or fusidic acid yield additional antimicrobial efficacy. For fusidic acid, this may signify that the lipophilic character of

fusidic acid enables it to be more effective against bacteria colonizing bone cement.

The type of the antibiotic influences its release from bone cement. For example, within the first few hours, gentamicin elutes in much higher concentrations than does clindamycin.[4] The elution rate of both antibiotics decays with time but the elution rate of gentamicin decays much faster than that of clindamycin.[4] Although other authors have reported clindamycin release characteristics from bone cement that are superior to those of gentamicin,[20] the opposite has also been found.[21] Literature is equally divided as to the release of fusidic acid from bone cement.[13,20,21] Also, the concentration and possible combinations that are used influence the release of antibiotics.[10,22] This might be attributable to the existence of more and larger pores and cracks in the bone cement[10] as a result of greater disturbances of the polymethylmethacrylate matrix. Our release experiments showed that the total gentamicin release after 3 days was significantly higher when more gentamicin was incorporated in the experimental cements. The existence of more pores and cracks in the bone cement might be beneficial to the antibiotic release, but the strength of the bone cement is affected by adding antibiotics to it.[23,24] High quantities of antibiotics or combined antibiotics may lead to incomplete polymerization of the cement and significantly prejudices its mechanical properties.

The elution kinetics of different combinations of antibiotics depends not only on the type of antibiotic used and the mixing ratio, but also on the type of bone cement used. Our results indicated that Copal® released much more gentamicin than the experimental bone cement with 1.0 g of gentamicin and clindamycin, despite a similar antibiotic content. This could be explained by the difference in the porosity of bone cement. Copal® is made of Palacos cement, whereas the experimental cements were made of CMW cements. Palacos bone cement has a higher porosity than CMW cements, yielding higher gentamicin release rates.[25] Striking, Copal® released far less clindamycin than the experimental bone cement with a similar clindamycin content. The difference in hydrophobicity of Palacos and CMW cements may explain this.[25]

Doubling the gentamicin content in experimental bone cement yielded a reduction in bacterial growth of the gentamicin-resistant bacterial strain because of the very high local concentrations achieved. The gentamicin-resistant strain used has a gentamicin MIC value >256 μg/mL, whereas local antibiotic concentrations achieved with antibiotic-loaded bone cement can increase above 450 μg/mL.[26] However, the higher gentamicin release from Copal® after 3 days, as compared with the experimental bone cement with the same antibiotic content, is not accompanied by an additional antimicrobial effect found in the Robbins device, probably because of the lower clindamycin release. Additional comparison between the Robbins device experiment and the gentamicin release showed no relation in either the 0.5-g gentamicin group or the 1.0-g gentamicin group. The reason for the additional antimicrobial effect for the bone cements containing combinations of antibiotics must therefore be found in the presence of the second antibiotic (so called multidrug targeting), and not in the increase of gentamicin release.

In conclusion, the addition of a second antibiotic to gentamicin-loaded bone cements shows an additional antimicrobial effect against pathogens occurring in orthopedic joint surgery, both against gentamicin-resistant and gentamicin-sensitive strains. Furthermore, gentamicin-loaded bone cement with clindamycin or fusidic acid added releases after 3 days similar percentages of gentamicin.

References

1. Buchholz HW, Engelbrecht H. Depot effects of various antibiotics mixed with Palacos resins. Chirurg 1970;41:511–515.
2. Thierse L. Experiences with Refobacin-Palacos with regard to deep late infections following hip-joint endoprosthesis surgery. Z Orthop Ihre Grenzgeb 1978;116:847–852.
3. Heck D, Rosenberg A, Schink-Ascani M, Garbus S, Kiewitt T. Use of antibiotic-impregnated cement during hip and knee arthroplasty in the United States. J Arthroplasty 1995;10:470–475.
4. Wahlig H, Dingeldein E. Antibiotics and bone cements. Experimental and clinical long-term observations. Acta Orthop Scand 1980;51:49–56.
5. van de Belt H, Neut D, van Horn JR, van der Mei HC, Schenk W, Busscher HJ. Antibiotic resistance: to treat or not to treat? Nat Med 1999;5:358–359.
6. Weber FA, Lautenbach EE. Revision of infected total hip arthroplasty. Clin Orthop 1986;211:108–115.
7. Kühn KD. Bone cements up-to-date comparison of physical and chemical properties of commercial materials. Berlin: Springer-Verlag; 2000. p 257–258.
8. Gonzalez Della Valle A, Bostrom M, Brause B, Harney C, Salvati EA. Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement. Acta Orthop Scand 2001;72:237–240.
9. Konig DP, Schierholz JM, Hilgers RD, Bertram C, Perdreau-Remington F, Rutt J. *In vitro* adherence and accumulation of *Staphylococcus epidermidis* RP 62 A and *Staphylococcus epidermidis* M7 on four different bone cements. Langenbecks Arch Surg 2001;386:328–332.
10. Penner MJ, Masri BA, Duncan CP. Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. J Arthroplasty 1996;11:939–944.
11. Klekamp J, Dawson JM, Haas DW, DeBoer D, Christie M. The use of vancomycin and tobramycin in acrylic bone cement: biomechanical effects and elution kinetics for use in joint arthroplasty. J Arthroplasty 1999;14:339–346.
12. Atkins B, Gottlieb T. Fusidic acid in bone and joint infections. Int J Antimicrob Agents 1999;12(Suppl 2):S79–S93.
13. Beeching NJ, Thomas MG, Roberts S, Lang SD. Comparative *in vitro* activity of antibiotics incorporated in acrylic bone cement. J Antimicrob Chemother 1986;17:173–184.

14. Garvin KL, Hinrichs SH, Urban JA. Emerging antibiotic-resistant bacteria. Their treatment in total joint arthroplasty. Clin Orthop 1999;369:110–123.
15. Sampath SS, Robinson DH. Comparison of new and existing spectrophotometric methods for the analysis of tobramycin and other aminoglycosides. J Pharm Sci 1990;79:428–431.
16. Zhang X, Wyss UP, Pichora D, Goosen MF. Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties. J Pharm Pharmacol 1994;46:718–724.
17. Frutos CP, Diez PE, Barrales-Rienda JM, Frutos G. Validation and *in vitro* characterization of antibiotic-loaded bone cement release. Int J Pharm 2000;209:15–26.
18. Arciola CR, Campoccia D, Montanaro L. Effects on antibiotic resistance of *Staphylococcus epidermidis* following adhesion to polymethylmethacrylate and to silicone surfaces. Biomaterials 2002;23:1495–1502.
19. Schierholz JM, Steinhauser H, Rump AF, Berkels R, Pulverer G. Controlled release of antibiotics from biomedical polyurethanes: morphological and structural features. Biomaterials 1997;18:839–844.
20. Hill J, Klenerman L, Trustey S, Blowers R. Diffusion of antibiotics from acrylic bone cement *in vitro*. J Bone Joint Surg Br 1977;59:197–199.
21. Picknell B, Mizen L, Sutherland R. Antibacterial activity of antibiotics in acrylic bone cement. J Bone Joint Surg Br 1977; 59:302–307.
22. Baker AS, Greenham LW. Release of gentamicin from acrylic bone cement. Elution and diffusion studies. J Bone Joint Surg Am 1988;70:1551–1557.
23. Moran JM, Greenwald AS, Matejczyk MB. Effect of gentamicin on shear and interface strengths of bone cement. Clin Orthop 1979;141:96–101.
24. Lautenschlager EP, Jacobs JJ, Marshall GW, Meyer PR Jr. Mechanical properties of bone cements containing large doses of antibiotic powders. J Biomed Mater Res 1976;10:929–938.
25. van de Belt H, Neut D, Uges DR, Schenk W, van Horn JR, van der Mei HC, Busscher HJ. Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release. Biomaterials 2000;21:1981–1987.
26. Kendall RW, Duncan CP, Smith JA, Ngui-Yen JH. Persistence of bacteria on antibiotic loaded acrylic depots. A reason for caution. Clin Orthop 1996;329:273–280.