Antibiotic release from bone cement under simulated physiological conditions
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Chapter 3

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Introduction

Gentamicin has been added to bone cement utilized in joint arthroplasties for over three decades. The purpose of this admixture is to reduce the risk of implant failure due to infection. At first it was claimed that the initial release would eliminate intra-operative contamination and the release observed up to months postoperatively would be effective in preventing haematogenous infection.\(^1\) Despite the lack of detailed knowledge about the mechanism of release and therefore long-term risks, such as the development of resistance among silently residing biofilm bacteria or haematogenously infecting bacteria,\(^2\) it is widely being used as a routine prophylactic measure.\(^3\)

One of the main concerns involves recognition of the biofilm mode of growth, as the preferred mode of bacterial growth in nature.\(^4\) Bacteria in a biofilm possess increased antibiotic resistance as compared to organisms in suspension. This has often been ascribed to protection offered by bacterial slime,\(^5\) but may also be due to physiological changes of bacteria in an adhered state.\(^6-8\) This phenomenon has spurred caution against routine prophylactic use of antibiotic-loaded bone cement, particularly as to the long-term low release that has been associated with the development of gentamicin resistance.\(^9,10\)

Several \textit{in vitro} studies have indeed shown bacterial growth on antibiotic-loaded bone cements,\(^11-16\) although one may wonder whether the geometry of the surfaces evaluated in the experimental set-ups used has an influence on these results. Most geometries used differ greatly from the situation in the close vicinity of an implanted gentamicin-loaded bone cement mantle \textit{in vivo}, which impedes the build-up of high antibiotic concentrations in close proximity of the bone cement. In a cadaveric pig femur it has been shown that the boundary layer between bone cement and bone had widths of 50 to 500 \(\mu\)m along 15% of the interfacial circumference.\(^17\) Inside a simulation of such a prosthesis-related gap, the attained antibiotic concentrations have been shown to be much higher than those reached in geometries in which release was studied so far.\(^18\)

The aim of the current study was to investigate bacterial survival in a simulated prosthesis-related interfacial gap, with a geometry closely similar to the clinical situation using gentamicin-loaded and plain versions of commercially available bone cements. This
was performed with bone cement blocks before (“pre-elution”) and after (“post-elution”) initial elution, in analogy with high initial burst release intra-operatively and the long-term, low release postoperatively.

**Materials and methods**

**Bone cements and preparation method**

The following bone cements were used in the present study as donated by their respective distributors: CMW 1 Radiopaque G, containing 1.7 w/w% gentamicin base, plain CMW 1 Radiopaque (DePuy CMW, United Kingdom), Palacos R-G containing 0.84 w/w% gentamicin base, Palacos R (Schering-Plough, The Netherlands), Palamed G, containing 0.86 w/w% gentamicin base and Palamed (Ortomed, The Netherlands) (see also Table 2-1). Bone cements were prepared by mixing the powder with the liquid, strictly according to the manufacturer’s instructions and maintaining sterile conditions. This was performed manually with a spatula in a ceramic bowl, under atmospheric pressure and at ambient temperature. At the time specified for start of application, as stated in the respective manuals, the doughy bone cement was spread over a polytetrafluoroethylene (PTFE) mould, fitted with stainless steel strips with a thickness of 200 µm.

After application of the bone cement, the mould was covered with copier overhead film (MC 110, Océ, The Netherlands) and compressed between two glass plates. The film facilitated removal of the glass plates after hardening of the bone cement. The glass plates were compressed up to the time specified for final hardening, after which they were left in place for 24 h. The stainless steel strips were subsequently removed and the bone cement blocks were gently punched out of the mould. This yielded blocks with a central gap, as detailed in Figure 3-1. The gap had a surface area of 0.61 cm$^2$ and a volume of 6 µl. The blocks were macroscopically examined and those with visibly entrapped gas bubbles or other defects in proximity of the surface were discarded.

The blocks were divided in two groups. One part was kept in dry storage, designated “pre-elution” bone cement. The other part was submersed in a surplus volume of phosphate buffered saline (PBS = NaCl 8.76 g/l, K$_2$HPO$_4$ 0.87 g/l, KH$_2$PO$_4$ 0.68 g/l; pH set at 7.0) for 3 weeks at 37°C using a Gyrotory® Water Bath Shaker (Model G 76, New Brunswick
Scientific Co. Inc., U.S.A.). Finally, these blocks (called “post-elution” bone cement), were separated from the fluid and left to dry for 3 days, while observing sterility precautions.

**Bacterial strains, gentamicin susceptibility and growth conditions**

Six bacterial strains (Table 3-1) isolated from patients with orthopaedic prosthesis infections treated in the University Hospital of Groningen, were used in this experiment. E-tests (AB Biodisk, Sweden) were used to establish the gentamicin susceptibility of the strains. The strains were chosen to reflect the spectrum of causative bacteria in deep infections, but also to cover a range of gentamicin susceptibilities.

The bacteria were cultured from cryopreservative beads (Protect, Technical Service Consultants Ltd., United Kingdom) onto blood agar plates at 37°C in ambient air for 3 days. From these plates, one colony was sub-cultured on blood agar plates for another 24 h to insure purity of the culture. One colony from this plate was used to create a pre-culture in 10 ml tryptone soy broth (TSB, Oxoid, United Kingdom) under the same incubating conditions, yielding a mean growth density after 24 h for all bacteria of $2.1 \times 10^8$ cfu / ml, as determined by counting the number of colony forming units after growth of serial dilutions on TSB agar plates. This pre-culture was subsequently diluted in TSB at 1:10, to provide new nutrients, prior to filling the gaps in the bone cement with 6 µl of this dilution. These inoculated bone cement blocks were incubated for 24 h in a water vapour saturated environment at 37°C before microbiological evaluation. This procedure was performed both for the pre-elution and
for the post-elution bone cement samples.

**Quantification of biofilms**

After biofilm growth in the gaps, the bone cement blocks were broken to expose the gap surface and both sides were scraped with a stainless steel surgical blade to harvest the bacteria adhering to the biomaterial surface. The blade was wiped with a soaked cotton swab which was then put in 4.5 ml of 9 g/l sodium chloride, vortexed and sonicated for 60 s in a 35 kHz ultrasonic bath (Transsonic TP 690-A, Elma® Germany). Serial dilutions were made and poured on TSB agar plates for overnight incubation at 37°C and enumeration on the next day. The agar plates that had not shown any growth on the first day were left in the incubator for up to one week, after which they were checked again. This was done in order to detect possible slowly growing sub-populations that can be seen after exposure to a hostile environment such as a high antibiotic concentration.20

All results were expressed in \(10^{10}\) log cfu/cm² and experiments were carried out in triplicate with separately cultured strains, unless bacterial growth was fully absent on the gentamicin-loaded variant in the first experiment.

**Statistical analysis**

To determine effects of the antibiotic on bacterial survival and growth, \(10^{10}\) log cfu/cm² values for gaps in gentamicin-loaded bone cements were compared with those for the plain bone cement, employing a one-tailed Student’s t-test for paired samples.

**Results**

**Gentamicin susceptibility**

All strains used are shown in Table 3-1, together with their gentamicin susceptibility. Two strains, a coagulase-negative staphylococcus (CNS) 7334 and *Staphylococcus aureus* 7323, were sensitive to gentamicin. Two strains, *Pseudomonas aeruginosa* 5148 and CNS 7353, had intermediate sensitivities to gentamicin (1-4 µg/ml), of which the latter was able to show resistant sub-populations with a gentamicin susceptibility up to 32 µg/ml, while two other strains, CNS 5234 and CNS 5115, were gentamicin-resistant with a sensitivity above 4 µg/ml.21
Quantification of biofilms on bone cement samples

A summary of the number of colony forming units harvested from the bone cement surfaces is provided in Table 3-1 as well. The procedure applied to create so-called post-elution samples did not affect biofilm formation. On the plain bone cements, results were similar for pre-elution and post-elution samples. Hence, both data sets were averaged and displayed in one column in Table 3-1. Sizeable numbers of bacteria were found on all plain bone cements (10 log cfu / cm² ranged from 5.2 for CNS 7334 on Palacos R to 7.2 for *P. aeruginosa* 5148 on Palamed).

However, neither the two gentamicin-sensitive strains nor the two strains with intermediate gentamicin susceptibility were able to survive in gaps in pre-elution gentamicin-loaded bone cements. Also the resistant sub-population of CNS 7353 and the resistant CNS 5234 did not survive after 24 h of incubation. The two gentamicin-sensitive strains and the intermediately sensitive *P. aeruginosa* 5148 were also unable to survive in gaps made in post-elution gentamicin-loaded cements, despite the absence of the high initial burst release of antibiotics. The other intermediately sensitive strain, CNS 7353, showed significantly reduced survival on post-elution CMW 1 Radiopaque G (p=0.008), Palacos R-G (p=0.004) and Palamed G (p<0.001) as compared with the plain counterparts.

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Table 3-1. Clinically isolated bacterial strains used in this study with their gentamicin susceptibility. Also the number of colony forming units (10 log cfu / cm²) harvested from gaps prepared in both pre-elution (+ pre) and post-elution (+ post) gentamicin-loaded and plain (-) bone cements is shown. Results are averages from three separate experiments, with an average standard deviation of 0.6 10 log cfu / cm², unless no growth was found in the gentamicin-loaded bone cement. The value for the - groups represents the average of pre-elution and post-elution plain bone cements.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (µg / ml)</th>
<th>CMW 1</th>
<th>Palacos R</th>
<th>Palamed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ pre</td>
<td>+ post</td>
<td>+ pre</td>
<td>+ post</td>
</tr>
<tr>
<td>CNS 7334</td>
<td>0.25</td>
<td>-</td>
<td>5.8</td>
<td>5.2</td>
</tr>
<tr>
<td>S. aureus 7323</td>
<td>1.0</td>
<td>-</td>
<td>7.1</td>
<td>6.5</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> 5148</td>
<td>2</td>
<td>-</td>
<td>7.0</td>
<td>7.1</td>
</tr>
<tr>
<td>CNS 7353</td>
<td>3 – 4 (32)</td>
<td>-</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>CNS 5234</td>
<td>24</td>
<td>-</td>
<td>2.2</td>
<td>5.9</td>
</tr>
<tr>
<td>CNS 5115</td>
<td>&gt; 256</td>
<td>6.0</td>
<td>6.8</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*a*: below detection.

*b*: (32) indicates a resistant sub-population
The highly gentamicin-resistant strain CNS 5115 showed reduced survival in gaps made in pre-elution gentamicin-loaded bone cements compared with plain bone cements. In post-elution samples however, this difference was not present. CNS 5234, being less resistant than CNS 5115, did not survive in pre-elution gentamicin-loaded bone cements, but showed reduced growth in gaps made in post-elution blocks, with respective p-values of 0.098, 0.006 and 0.062 for CMW 1 Radiopaque G, Palacos R-G and Palamed G.

**Discussion**

In the present study, differences in bacterial survival have been investigated on gentamicin-loaded and plain bone cements in an *in vitro* simulation of a prosthesis-related interfacial gap. Earlier *in vitro* studies have shown statistically significant reductions in bacterial growth on antibiotic-loaded bone cements as compared with plain bone cements, but bacterial survival and growth was generally not below detection as in the present study.\(^{11-16}\) Moreover, since antibiotic-loaded bone cements remain *in situ* for many years post-surgery while showing long-term release of minor amounts of antibiotic, experiments were also done with bone cements after the initial burst release of antibiotics. Interestingly, in gaps in pre-elution bone cements only the highly resistant CNS 5115 strain could survive, but in these post-elution bone cements survival was also possible for less resistant strains.

The difference between the earlier *in vitro* studies and the simulated prosthesis-related gap model centres around a different ratio of exposed area of bone cement (A) over the volume (V) involved (see Table 3-2 for a summary of area over volume ratios in the literature). For studies with bone cement samples in a test tube, the area over volume ratio (A/V) is between 0.30 and 3.0 cm\(^{-1}\),\(^{11,12}\) while in a modified Robbins device the area to volume ratio ranges from 0.030 to 0.00041 cm\(^{-1}\).\(^{13-16}\) In the gap model as applied here the area to volume ratio is much higher and equals 100 cm\(^{-1}\). Previously,\(^{18}\) we have shown that such a high area over volume ratio yields gentamicin concentrations up to 4000 µg/ml in gaps in pre-elution bone cements. This exceeds the MIC’s of the 5 non-surviving strains in this study by at least two orders of magnitude, but is only about 15 times higher than the MIC of the surviving CNS 5115.

Bacterial killing in gaps in post-elution bone cement occurred only in sensitive strains and to a lesser extent in progressively more resistant strains. Using identical methods as
Bacterial survival in a simulated interfacial gap

Table 3-2. Summary of in vitro studies on the survival of bacteria on antibiotic-containing bone cements, together with an estimate of the area to volume ratios (A/V) in the experimental set-ups employed.

<table>
<thead>
<tr>
<th>Bone cement</th>
<th>Antibiotic</th>
<th>Bacterial strains</th>
<th>A/V (cm⁻¹)</th>
<th>Survival</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMW</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.00041</td>
<td>Yes</td>
<td>14</td>
</tr>
<tr>
<td>Palacos R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 1</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.00091</td>
<td>Yes</td>
<td>15</td>
</tr>
<tr>
<td>CMW 3</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.0012</td>
<td>Yes</td>
<td>16</td>
</tr>
<tr>
<td>CMW 2000</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.003</td>
<td>Yes</td>
<td>17</td>
</tr>
<tr>
<td>CMW Endurance</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.0091</td>
<td>Yes</td>
<td>18</td>
</tr>
<tr>
<td>Palacos R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palamed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMW 3</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.0012</td>
<td>Yes</td>
<td>16</td>
</tr>
<tr>
<td>Palacos R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMMA bone cement</td>
<td>Tobramycin</td>
<td>CNS</td>
<td>~ 0.03</td>
<td>Yes</td>
<td>13</td>
</tr>
<tr>
<td>Simplex P</td>
<td>Tobramycin</td>
<td>S. aureus</td>
<td>~ 0.3</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>Palacos R</td>
<td>Vancomycin</td>
<td>CNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMMA and orthodontic resin liquid</td>
<td>Gentamicin</td>
<td>CNS</td>
<td>~ 3</td>
<td>Yes, except for Proteus mirabilis</td>
<td>CNS</td>
</tr>
<tr>
<td>CMW 1</td>
<td>Gentamicin</td>
<td>S. aureus</td>
<td>0.00091</td>
<td>Yes, except for resistant strain</td>
<td>This study</td>
</tr>
<tr>
<td>Palacos R</td>
<td>CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palamed</td>
<td>P. aeruginosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

~ signifies approximately

employed previously for the pre-elution bone cements, gentamicin concentrations in post-elution gaps were measured. After 2 h of release, gentamicin concentrations for CMW 1 Radiopaque G, Palacos R-G and Palamed G of 75, 180 and 650 µg/ml could be measured, respectively. This approaches or even exceeds the MIC values of the strains involved in this study. Consequently, this study shows that gentamicin-sensitive strains, able to survive in earlier models with a lower area to volume ratio, were eliminated in a simulated prosthesis-related interfacial gap, both in pre-elution as in post-elution bone cements.
From a clinical point of view it is important to discuss on the basis of the present results, whether gentamicin-loaded bone cement is useful in primary arthroplasty and in revision arthroplasty. Examinations in animals after primary simulated arthroplasty have indicated that prophylactic use of antibiotic-loaded bone cements protects against infection after intra-operative challenge with gentamicin-sensitive bacteria.\textsuperscript{22-25} Randomized controlled prospective clinical trials have also shown that antibiotic-loaded bone cements provide a protective effect against deep infection.\textsuperscript{26-28} This is further supported by recommendations to use gentamicin-loaded bone cement in combination with systemic antibiotics in primary arthroplasty, based on a cost-effectiveness analysis and an empirical analysis.\textsuperscript{29,30} The current study also points at an efficacy of gentamicin-loaded bone cement. However, it also indicates that gentamicin-loaded bone cement may select for gentamicin-resistant strains. Similar observations have been made in clinical practice.\textsuperscript{9,31} In summary, gentamicin-loaded bone cement can be considered to be effective in primary arthroplasty. The current trend of adding ‘last resort’ antibiotics, such as vancomycin, to bone cement must be considered as a dangerous one, as it may lead to a similar selection of vancomycin-resistant strains in subsequent infections.\textsuperscript{32}

Revision arthroplasty is known to have a higher infection risk than primary arthroplasty.\textsuperscript{33} If gentamicin-loaded bone cement has been used in primary surgery, bacteria involved in these infections may already have survived a high gentamicin concentration inside a prosthesis-related gap and are probably gentamicin-resistant. Subsequent use of gentamicin-loaded bone cement would therefore be less efficacious, whilst still carrying the risk of further selecting resistance. Obviously, this does not apply to revision arthroplasty in the absence of previous use of gentamicin-loaded bone cement.

In conclusion, the current study offers a fundamental background for evaluating the efficacy of use of gentamicin-loaded bone cements in clinical orthopaedics. Inside a simulated prosthesis-related interfacial gap, high gentamicin concentrations can be attained. Both in pre-elution and in post-elution bone cements these concentrations appear to be successful in killing commonly infecting bacterial strains with MIC’s that are over 100-fold lower. However, concurrently a selection of resistant strains can be seen.
References


Bacterial survival in a simulated interfacial gap