Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release

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Abstract

In this study, the release of gentamicin as a function of time was measured for six different gentamicin-loaded bone cements and related with the surface roughness, porosity and wettability of the cements. Initial release rates varied little between the six bone cements (CMW1, CMW3, CMW Endurance, CMW 2000, Palacos, and Palamed) and ranged from 8.6 to 14.1 \( \mu \)g/cm\(^2\)/h. The total amounts of gentamicin released after 1 week varied between 4.0 and 5.3% of the total amount of antibiotic incorporated for the CMW cements and was 8.4% for Palacos. Palamed released after 1 week significantly more of the gentamicin incorporated (17.0%). The wettability of all cements was similar (water contact angles between 70 and 80°), but the surface roughness and the porosity of the cements varied markedly. Initial release rates increased with surface roughness, although the correlation coefficient was low (0.64), while total amounts released increased linearly (correlation coefficient 0.97) with the bulk porosity of the cements. Consequently, it can be concluded that the release kinetics of gentamicin from bone cements is controlled by a combination of surface roughness and porosity.

Keywords: Polymethylmethacrylate; Gentamicin; Bone cement; Surface roughness; Porosity; Wettability

1. Introduction

The incorporation of antibiotics in polymethylmethacrylate (PMMA) bone cements for the treatment and prevention of infection in orthopedics has become common clinical practice during the last two decades and almost 90% of all orthopedic surgeons in the USA use antibiotic-loaded bone cement for the fixation of implants \cite{1}. Apart from implant fixation, antibiotic-loaded bone cements are used in orthopedics for temporary beads and spacers. Incorporation of antibiotics in bone cements and their release over time is thought to yield higher antibiotic concentrations to the (infected) bone or tissue site than can be achieved by systemic routes. The aminoglycoside gentamicin has developed as the most widely used antibiotic in bone cements due to its wide-spectrum antimicrobial activity, excellent solubility and resistance against elevated temperatures, as during polymerization \cite{2}. There are conflicting reports in the literature concerning the gentamicin elution properties of different acrylic bone cements, which are partly due to the lack of a standardized, in vitro test method \cite{3–5}.

Release of antibiotics from PMMA bone cements is largely influenced by the penetration of dissolution fluids into the polymer matrix, which requires a certain porosity of the cement \cite{6,7}. The porosity of the polymer matrix depends on air entrapment during the wetting and stirring of the cement powder during transfer to the cement gun and on effects of monomer boiling \cite{8}. Penetration of dissolution fluids into pores of the polymer matrices also depends, however, on the wettability of the bone cement surface, which makes antibiotic release essentially a surface phenomenon. By consequence, surface roughness is an important characteristic of antibiotic-loaded bone cements.

Despite the widespread clinical use of antibiotic-loaded bone cements, there are growing doubts about the...
clinical efficacy of incorporating antibiotics into bone cements. Since the release mechanisms are poorly understood and difficult to control, while maintaining an adequate mechanical strength of the cement, release rates are generally low and sub-inhibitory antibiotic concentrations result over extended periods of time that may stimulate antibiotic resistance amongst infectious microorganisms [9]. Recently, the isolation of a gentamicin-resistant staphylococcal strain from an infected total hip arthroplasty, fixed with gentamicin-loaded bone cement, has been described [10]. Hence, it can be concluded that the efficacy of antibiotic-release by currently available antibiotic-loaded bone cements for primary implant fixation can be debated.

The aim of this study is to compare the release of gentamicin as a function of time for six different bone cements and to relate the antibiotic release measured with the surface roughness, bulk porosity and wettability of the cements.

2. Materials and methods

2.1. Cement disc preparation

Six gentamicin-impregnated bone cements were used: CMW1, CMW3, CMW Endurance, and CMW2000 all with ±2.5 w/w% gentamicin, corresponding 4.22 w/w% gentamicin sulphate (DePuy International Ltd., Corford Road, Blackpool Lancashire FY4 4QQ, UK), Palacos (Schering-Plough, Maarssen, The Netherlands), and Palamed both with ±1.25 w/w% gentamicin, corresponding with 2.04 w/w% gentamicin sulphate (Merck Biomaterial GmbH, D-64271 Darmstadt, Germany). The compositions of the cements are shown in Table 1, as taken from the manufacturers information leaflets.

Cements were prepared by mixing the powdered poly(methylmethacrylate) with the liquid monomer methylmethacrylate in a bowl with a spatula. Manual mixing was done according to the manufacturers instructions. Mixing resulted in a liquid cement. The liquid cement was poured into a polytetrafluoroethylene mould (200 × 40 × 3.2 mm), containing holes of 6 mm diameter. The filled mould was pressed between two glass plates for 25 min. After hardening of the cement, cement discs were pulled out of the mould, and stored under dark, sterile conditions at room temperature. The total surface area of one disc was 1.17 cm² and a disc weighed 100 mg.

2.2. Gentamicin release rates

A gentamicin-loaded cement disc was immersed in 10 ml phosphate buffer saline, PBS, (NaCl 8.76 g/l, K₂HPO₄ 0.87 g/l, KH₂PO₄ 0.68 g/l, pH 7.4) and stirred at 37°C. At designated sampling intervals (6, 24, 30, 48, 72 and 168 h), the disc was removed, placed in 10 ml of fresh PBS and a 1 ml sample of the stored PBS solution was taken.

These samples were analyzed for gentamicin using fluorescence polarization immunoassay (Abbott AxSym; Abbott Laboratories, Abbott Park, IL). The immunoassay technique is based on a competitive-binding assay methodology. The reagents contain gentamicin-specific antibodies and gentamicin labeled with fluorescein, a fluorescent tracer. The tracer, when excited by plane polarized light, emits fluorescence with a degree of polarization inversely related to its rate of rotation. Unbound tracer becomes randomly oriented and the polarization of fluorescence is low. Tracer binding to its specific antibody results in the tracer rotating at a slower rate and an increase in the polarization of emitted light. Unlabeled gentamicin in the sample competes with the tracer for

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>CMW1 (w/w%)</th>
<th>CMW3 (w/w%)</th>
<th>CMW Endurance (w/w%)</th>
<th>CMW2000 (w/w%)</th>
<th>Palacos (w/w%)</th>
<th>Palamed (w/w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Powder</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Poly(methylmethacrylate)</td>
<td>84.73</td>
<td>83.88</td>
<td>64.53</td>
<td>73.50</td>
<td>82.66</td>
<td>85.22</td>
</tr>
<tr>
<td>Poly(MMA/styrene)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Poly(MMA/styrene)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Barium sulfate</td>
<td>9.10</td>
<td>10.00</td>
<td>9.75</td>
<td>7.80</td>
<td>14.81</td>
<td>11.75</td>
</tr>
<tr>
<td>Zirconium dioxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Benzoil peroxide</td>
<td>1.95</td>
<td>1.90</td>
<td>1.80</td>
<td>2.24</td>
<td>0.49</td>
<td>0.99</td>
</tr>
<tr>
<td>Gentamicin sulphate</td>
<td>4.22</td>
<td>4.22</td>
<td>4.22</td>
<td>4.22</td>
<td>2.04</td>
<td>2.04</td>
</tr>
<tr>
<td><strong>Liquid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMA</td>
<td>98.23</td>
<td>96.54</td>
<td>98.01</td>
<td>97.99</td>
<td>97.84</td>
<td>97.98</td>
</tr>
<tr>
<td>N,N-Dimethyl-p-toluidine</td>
<td>0.81</td>
<td>2.49</td>
<td>1.98</td>
<td>1.99</td>
<td>2.12</td>
<td>2.02</td>
</tr>
</tbody>
</table>

*% by weight (w/w) of powder component and liquid component, respectively. Other constituents such as ascorbic acid, ethanol, chlorophyll and hydroquinone are not mentioned in this table.*
Fig. 1. Gentamicin release rates of different antibiotic-loaded bone cements as a function of time during exposure to phosphate-buffered saline, together with a hypothetical ideal release kinetics. Results are averages of three experimental runs, with separately prepared discs used and data were examined for statistical significance using the Student $t$-test.

2.3. Surface roughness

As all cement samples were prepared by pressing the filled Teflon moulds against the same glass plate, a comparison of the roughness of the various cement samples yields information relevant to the different brands. The surface roughness ($R_A$) was determined with a stylus instrument, the Perthometer C5D (Perthen, Germany) equipped with a 5 μm stylus (opening angle of 90°). Tracings were made over a length of 1.5 mm per cement sample. Surface roughness values presented were obtained by averaging $R_A$ values from five different tracings over one cement disc, while three separately prepared discs of each cement were examined. Data were examined for statistical significance using the Student $t$-test.

2.4. Porosity

For each cement, three samples were fractured and the broken surfaces sputter-coated with a 3 nm thick gold and palladium layer for examination at 5.0 kV in a JEOL field emission scanning electron microscope type 6301F. The total surface area of the pores was measured and divided by the total surface area of the cross section of the disc, which was 19.2 mm$^2$ for all samples, to obtain a percentage porosity.

2.5. Wettability

Advancing-type contact angles were measured with water on all bone cement surfaces by putting 1 μl droplets on each sample. Water droplets were observed with a video-camera, connected to a counter-monitor for observer-independent readings. Three separately prepared samples were analyzed per cement, putting three droplets on each sample.

3. Results

3.1. Gentamicin release rates

Fig. 1 summarizes the gentamicin release as a function of time for the six different acrylic cements, expressed in μg/cm$^2$/h. After a high initial release, a rapid decay in release rates can be observed for all cements within 30 h. Notably, the initial release rates are slightly different for each cement and highest for Palamed. Release rates after 168 h essentially become below the detection limit, and the total amount released after 1 week can be taken as an estimate of the total amounts released, which are summarized in Table 2, expressed as a percentage of the total amount of gentamicin incorporated. The CMW cements all release between 4.0 and 5.3% of the gentamicin incorporated, while Palacos released 8.4% in total, significantly more than CMW cements ($p < 0.05$). Palamed released significantly more gentamicin (17.0%) than the other cements ($p < 0.05$).

3.2. Surface roughness

Differences in surface roughness between the six bone cements existed (Table 2), despite the fact that all were prepared by the same procedure, likely because the surface roughness measured is a partial result of size differences in polymer beads used protruding out of the polymer matrix. The surface roughness of the CMW cements varied between 0.16 and 0.33 μm, Palamed (0.49 μm) was significantly ($p < 0.05$) rougher than the other cements.

3.3. Porosity

Fig. 2 shows an electron micrograph of sectioned CMW 3 and Palamed. Clearly, Palamed contains more pores than CMW 3, while also the size of the pores in Palamed is slightly larger. As a consequence, the percentage porosity of Palamed is higher than that of CMW 3 (Table 2). As can be seen in Fig. 3 a high-porosity
Table 2
The initial gentamicin release rate as calculated by linear regression analysis over the first 6 h of release, total gentamicin release after 1 week as a percentage of the total amount incorporated, surface roughness, bulk porosity and water contact angles of the different gentamicin-loaded bone cements employed in this study. The values are expressed as mean ± SD

<table>
<thead>
<tr>
<th>Bone cement</th>
<th>Initial release rate (µg/cm²/h) ± SD</th>
<th>Total release after 1 week (%) ± SD</th>
<th>Surface roughness (µm) ± SD</th>
<th>Porosity (%) ± SD</th>
<th>Contact angle (degrees) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMW 1</td>
<td>13.1 ± 0.6</td>
<td>5.3 ± 0.2</td>
<td>0.33 ± 0.08</td>
<td>4.9 ± 0.5</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>CMW 3</td>
<td>11.8 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>0.30 ± 0.06</td>
<td>3.4 ± 0.8</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>CMW endurance</td>
<td>12.4 ± 1.1</td>
<td>5.1 ± 0.4</td>
<td>0.20 ± 0.04</td>
<td>2.8 ± 0.8</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>CMW 2000</td>
<td>9.2 ± 0.3</td>
<td>4.0 ± 0.2</td>
<td>0.16 ± 0.03</td>
<td>3.1 ± 1.1</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Palacos</td>
<td>8.6 ± 0.6</td>
<td>8.4 ± 0.4</td>
<td>0.29 ± 0.04</td>
<td>5.3 ± 0.5</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>Palamed</td>
<td>14.1 ± 0.3</td>
<td>17.0 ± 0.8</td>
<td>0.49 ± 0.16</td>
<td>10.4 ± 3.5</td>
<td>80 ± 5</td>
</tr>
</tbody>
</table>

Fig. 2. Scanning electron micrographs comparing sectioned CMW 3 (a) and Palamed (b) cement discs. The bars represent 1 mm.

Fig. 3. Scanning electron micrograph showing the matrix of a high-porosity cement with interconnecting pores. The bar represents 100 µm.

3.4. Wettability

Water contact angles on the different bone cements ranged between 70 and 80° (Table 2).

4. Discussion

This study compares the release of gentamicin from antibiotic-loaded bone cements in relation with the surface roughness, porosity and wettability of different cements. The mechanism by which bone cements release incorporated antibiotics is under debate. Some authors favor a diffusion theory and explain the sustained release by diffusion of antibiotics either through the entire polymer matrix or through capillaries within the matrix [12,13]. Most authors, however, support the idea that release is essentially a surface phenomenon [14–20].

Fig. 4 shows the relationship between initial gentamicin release rates with bulk porosity and surface roughness. As can be seen, porosity does not relate with the initial release rates, but release rates increase with surface roughness. This can be understood simply by the fact that a rougher surface constitutes a larger area for release and supports the view that antibiotic release is a surface phenomenon. In Fig. 5 the total amounts of gentamicin released are plotted as a function of porosity and surface roughness. The total amounts released increase linearly with bulk porosity, pointing to a diffusion mechanism. These observations indicate that the kinetics of antibiotic release are initially controlled to some extent by surface phenomena, while the sustained release over
a time span of several days depends on the penetration depth as determined by the bulk porosity of the cement. It has been demonstrated that all antibiotics incorporated into a bone cement can be released, provided the geometry applied does not exceed 100 μm thickness [16]. Consequently, it can be concluded that by adjusting surface roughness and bulk porosity of antibiotic-loaded cements, one can control both the initial as well as the sustained release of the antibiotic. Fig. 6 shows a schematic presentation of the different mechanisms that control antibiotic release over time of a low- (Fig. 6a–c) and high-porosity cement (Fig. 6d–f), including the surface-controlled phase (Fig. 6b and e) and the sustained release by diffusion (Fig. 6c and f). Sustained release requires the penetration of dissolution fluids into the interconnecting pores and cracks, which is dictated by the wettability of the polymer matrices and by the number and sizes of the pores in the polymer matrix. Water contact angles were between 70 and 80°, i.e., all cements were fairly hydrophobic, indicating that penetration will be equally slow for all cements. On the basis of the model presented in Fig. 6, the differences in release kinetics observed can therefore only be explained by the number and size of the pores in the cements. Ideally, the release kinetics should be such that sufficient antibiotics elute over a defined period of time and should stop, because subinhibitory concentrations might introduce resistant strains. As obvious from Fig. 1 none of the cement brands demonstrates the idealized release kinetics, which should start horizontal and end vertical. The total release is a combination of initial release and sustained release. As can be calculated from Fig. 1 the CMW brands release almost 80% of the gentamicin within 6 h, whereas Palacos and Palamed release 65 and 53%, respectively. The sustained release of the latter two cements possibly leads to subinhibitory antibiotic concentrations in vivo with the risk of inducing resistant strains.

A previous study demonstrated that the antibiotic release rate of an antibiotic-loaded bone cement almost
Fig. 6. Proposed sequential steps in the release of gentamicin from a low-porosity (a–c) and a high-porosity (d–f) bone cement. After a high initial release (b and e), there is a slow sustained release in time (c and f), which depends on the penetration depth of the fluid through connection pores.

tripled on increasing the porosity from 38 to 69% [21], but this involved calcium phosphate bone cement with other likely penetration properties than acrylic bone cements. In many other studies, a clear distinction between initial release rate and total amounts released is not made, contributing to the confusion about the actual mechanism of antibiotic release. In order to prolong or limit the release of antibiotics, it may appear tempting to change the porosity of bone cements as can be done by manipulating the polymer composition or change the way of mixing. However, these positive effects will, at a certain point, be negated by insufficiency of the mechanical cement properties that constitute its primary function [22,23].

In conclusion, this study demonstrates that the initial release of gentamicin from an acrylic polymer matrix is partially a surface phenomenon, whereas the total amount released depends on bulk porosity. This conclusion may open new pathways for the design of antibiotic-loaded bone cements with better controlled release kinetics. As long as we are not able to reach the goal of the ideal controlled release, the use of antibiotic-loaded bone cement for fixation of implants should be critically considered.

References


