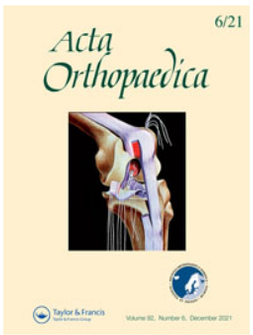

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The effect of mixing on gentamicin release from polymethylmethacrylate bone cements

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ABSTRACT We compared the release of gentamicin from 6 different commercially available, antibiotic-loaded PMMA bone cements used for vacuum- and hand-mixed cement using a Cemvac vacuum mixing system. We also measured the release of gentamicin after manual addition of the antibiotic to different commercial, unloaded bone cements after hand-mixing. The porosity of cements was reduced in all vacuum-mixed cements, as compared with hand-mixed cements, concurrent with a statistically significant reduction (3 of 6) or increase (1 of 6) in the total amounts of gentamicin released. The total gentamicin release was studied in 3 of the brands after manual addition and mixing of the antibiotics. We found that the release of antibiotics was lower than in samples made from industrial mixing. In conclusion, the manual addition and mixing of gentamicin in PMMA bone cements leads to a lower release of antibiotics than that in corresponding commercially available antibiotic-loaded cements, while vacuum-mixing only leads to a minor reduction in antibiotic release, as compared to hand-mixing.

The use of polymethylmethacrylate (PMMA) bone cement loaded with antibiotics has become increasingly common in the treatment of infected knee and hip arthroplasties and also as prophylaxis in primary joint replacement. Nowadays about 90% of the orthopedic surgeons in the USA use this method of local antibiotic delivery (Heck et al. 1995). In the USA, no antibiotic-loaded bone cements are commercially available and surgeons manually add the antibiotics to the polymer powder during surgery. This is unlike the situation in Europe, where a variety of different antibiotic-loaded bone cements are commercially available. In vitro and in vivo studies have shown that only small amounts of the antibiotics incorporated in bone cement are actually released (Schurman et al. 1978, Hoff et al. 1981, Törholm et al. 1983, Bunetel et al. 1989, Chohfi et al. 1998), because the release is largely a surface phenomenon. Antibiotics elute rapidly from the outer 50–100 μm of bone cement. Thereafter only small amounts slowly diffuse through the polymer matrix (Penner et al. 1996, Van de Belt et al. 2000).

The sustained release of antibiotics from bone cements is largely affected by the penetration of fluids into the polymer matrix, which requires a certain porosity of the cement. The porosity can be increased, for instance, by inclusion of soluble dextran (Kuechle et al. 1991) or of a second antibiotic (Penner et al. 1996), but adverse effects on the mechanical properties of the cement limit the extent to which this can be done. In fact, to avoid the inclusion of air in bone cements, that may act as foci for initiation of a crack, polymer powder and monomer liquid are often mixed under lower than atmospheric pressure (Lidgren et al. 1984, Wixson et al. 1987, Bishop et al. 1996, Fritsch 1996). Vacuum-mixing reduces the porosity of bone cement with a concurrent increase in mechanical properties, but it is not known to what extent this procedure affecs the release of antibiotics from the cement. Moreover, it is not known whether

the peroperative manual addition of antibiotics to the polymer powder will yield a bone cement with antibiotic release properties comparable to those of industrially manufactured, commercially available antibiotic-loaded bone cements.

Therefore, we compared the release of antibiotics from 6 commercially available gentamicin-loaded bone cements after vacuum- and hand-mixing. The release of gentamicin after the manual addition of the antibiotic to different commercial, unloaded bone cements was also measured after hand-mixing.

Material and methods

Cement preparatŠn: Hand- versus vacuum-mixing

We used 6 gentamicin-loaded bone cements: Palacos R (Schering-Plough, Maarssen, The Netherlands) and Palamed (Merck Biomaterial GmbH, D-64271 Darmstadt, Germany) both with 2.1 w/w% gentamicin sulfate and CMW1, CMW3, CMWendurance, and CMW2000 (DePuy CMW, Conford Road, Blackpool Lancashire FY4 4QQ, UK) all with 4.2 w/w% gentamicin sulfate. The cements were prepared by adding the polymer powder to the monomer liquid in the mixing cartridge of a vacuum-mixing system (Cemvac, Cemvac System AB, Linköping, Sweden). It was then mixed by moving and turning a mixing paddle up and down in the mixing cartridge in a vacuum of 0.2 bar. Hand-mixing was done in the same way, but at atmospheric pressure. After mixing, the cement was driven to the top of the cartridge with the cement gun for curing. The hand- and vacuum-mixed cements were radiographed to visualize effects of the mixing-mode on porosity. Samples were then sawed from the cured cements into discs of 1 cm thick. Each sample weighed 800 mg and had a total surface area of 4 cm^2. 3 samples of each were used to analyze release of gentamicin.

Cement preparatŠn: Manual additŠn of antibŠtics

Gentamicin sulfate (DePuy CMW, Conford Road, Blackpool, Lancashire FY4 4QQ, UK) was manually added to the polymer powder of unloaded cements CMW3, Palacos R, and Palamed and mixed for 3 minutes in a bowl with a spatula. 1.69 grams of gentamicin sulfate was added to 40 gram CMW3 polymer powder, 0.84 grams of gentamicin sulfate to 40 grams of Palacos R polymer powder and 0.92 grams of gentamicin sulfate to 44 grams of Palamed polymer powder. The powdered polymer PMMA with antibiotic added was hand-mixed with the liquid monomer methylmethacrylate in a bowl with a spatula, according to the manufacturer's instructions. The still liquid cement was poured into a polytetrafluoroethylene mould (200 × 40 × 3.2 mm), containing holes 6 mm in 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 and weight of one disc was 1.2 cm^2 and 100 mg.

Gentamicin release rates

Gentamicin-loaded cement discs were 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 specific sampling intervals (6, 24, 168 h) the discs were removed, placed in fresh 10 ml PBS and a 1 mL sample of the gentamicin-PBS solution was taken. The gentamicin content of the solution was determined by fluorescence polarization immunoassay (Abbott AxSym; Abbott Laboratories, Abbott Park, Illinois). The immunoassay technique is based on a competitive-binding assay method. 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. The binding to a specific antibody makes the tracer rotate at a slower rate and increases the polarization of emitted light. Unlabeled gentamicin in the sample competes with the tracer for a limited number of antibody sites. If the sample contains a high concentration of gentamicin, the degree of polarization is low. If the sample contains a low concentration of free gentamicin, more tracer is bound and the degree of polarization is high. The AxSym calculates the

concentration of gentamicin present in the specimen on te basis of a calibration curve using the proportional relationship between the gentamicin concentration and the degree of polarization (Price and Newman 1991).

Statistics

We used one-tailed Student's t-test for two samples with equal variance and the 95% confidence limits were computed. The experiments were done in triplicate.

Results

After a high initial release, all cements showed a rapid decline in their release rates within 24 h (Figures 1 and 2). The initial rates were slightly different in each brand of cement and highest for Palamed (Figure 1). After 168 h, the release rates fell below the detection limit.

The total release of gentamicin for up to 168 h was 0.1–0.5% lower in vacuum-mixed CMW cements than in the hand-mixed ones. On the contrary, vacuum-mixed Palacos R released 0.4% more gentamicin after 168 h than did hand-mixed Palacos R (Table 1). These variations in total release were minor, however, compared to the effects of vacuum-mixing on the total release of gentamicin from Palamed, decreasing from 8.0% of the total amount incorporated after hand-mixing to 6.5% after vacuum-mixing.

The manual addition and distribution of the antibiotics in the three types of cements showed that the total release was significantly less than from their commercially available antibiotic-loaded counterparts (Table 2). The reductions in antibiotic release are relatively minor (0.8% less) for CMW3, but much grater (5.2% less) for Palacos R and Palamed (9.6% less).

The radiographs of hand- and vacuum-mixed cements showed a reduction in the number and size of air inclusions in all vacuum-mixed versions (Figure 3).

Discussion

Vacuum-mixing is recommended nowadays, because it improves the mechanical properties of cements (Linden and Gillquist 1989, Askew et al. 1990, Lewis et al. 1997). In this study, vacuum-mixing of nearly all gentamicin-loaded bone cements provided significant but small reductions in antibiotic release. The largest reduction in antibiotic release after vacuum-mixing was seen with Palamed, which also had fewer inclusions of air in the vacuum-mixed cement than in hand-mixed cement. Vacuum-mixing was effective in reducing the number and size of large voids in high-viscosity (e.g., Palamed) and low-viscosity cements (e.g., CMW3).

The release kinetics of gentamicin from bone cements is determined by a combination of surface roughness and porosity (Van de Belt et al. 2000). The former is thought to control the initial rates of release of antibiotics from bone cements, while the latter has been related to sustained release over longer periods of time. In our study, the surface finish was determined by sawing and was probably similar for all cements. Therefore, the differences in gentamicin release must be due to different porosities of the cements and the effects of vacuum-mixing can be expected to be largest in bone cements with a high bulk porosity. In all cements, vacuum-mixing, indeed, led to a reduction in porosity and in 5 of 6 cases lower amounts of gentamicin were released. Palacos R, however, showed a greater release of antibiotic after vacuum-mixing, despite a reduction in the porosity (Figure 3). We suggest that this was caused by an increase in the number and distribution of micropores (< 1 mm diameter), which develop during polymerization by evaporation of the volatile monomer. Prechilling can reduce the maximum temperature reached during polymerization of bone cement and consequently the evaporation of the volatile monomer. In this respect it is important to note that all bone cements in this study were mixed at room temperature without prechilling. Prechilling before vacuum-mixing is recomended only by the manufacturer of Palacos R, probably to reduce the evaporation of the volatile monomer.

In the USA, antibiotic-loaded bone cements are not FDA-approved and therefore not commercially available. Yet, many surgeons add the antibiotics to the polymer powder manually during surgery to obtain optimal, assumed protection of their

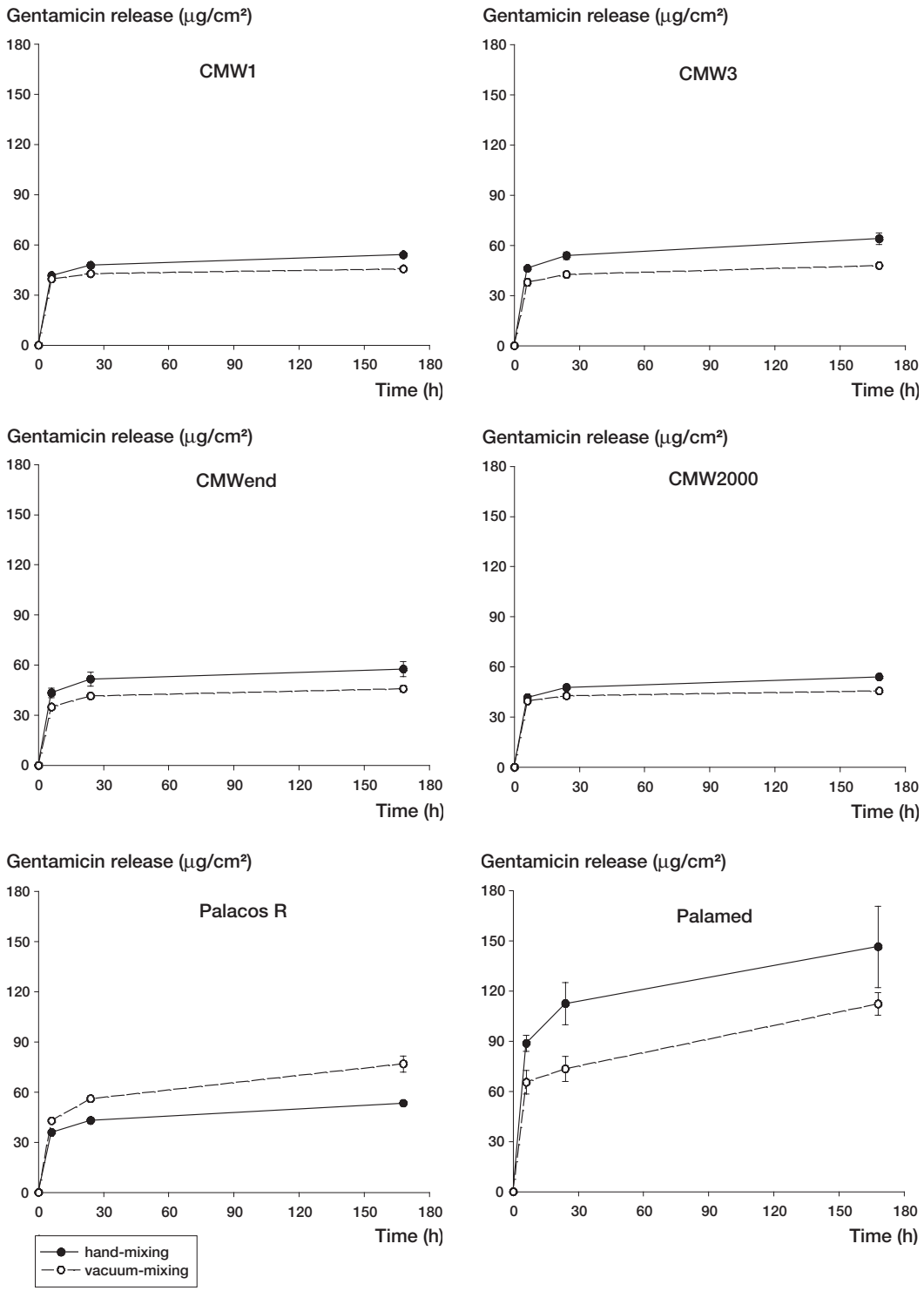

Figure 1. Cumulative gentamicin release (µg/cm^2) of various antibiotic-loaded bone cements as a function of time during exposure to phosphate-buffered saline. The findings are given as averages of three experimental runs, with bars indicating the SD. The dotted lines represent vacuum-mixed cements, while the continuous lines represent hand-mixed ones.

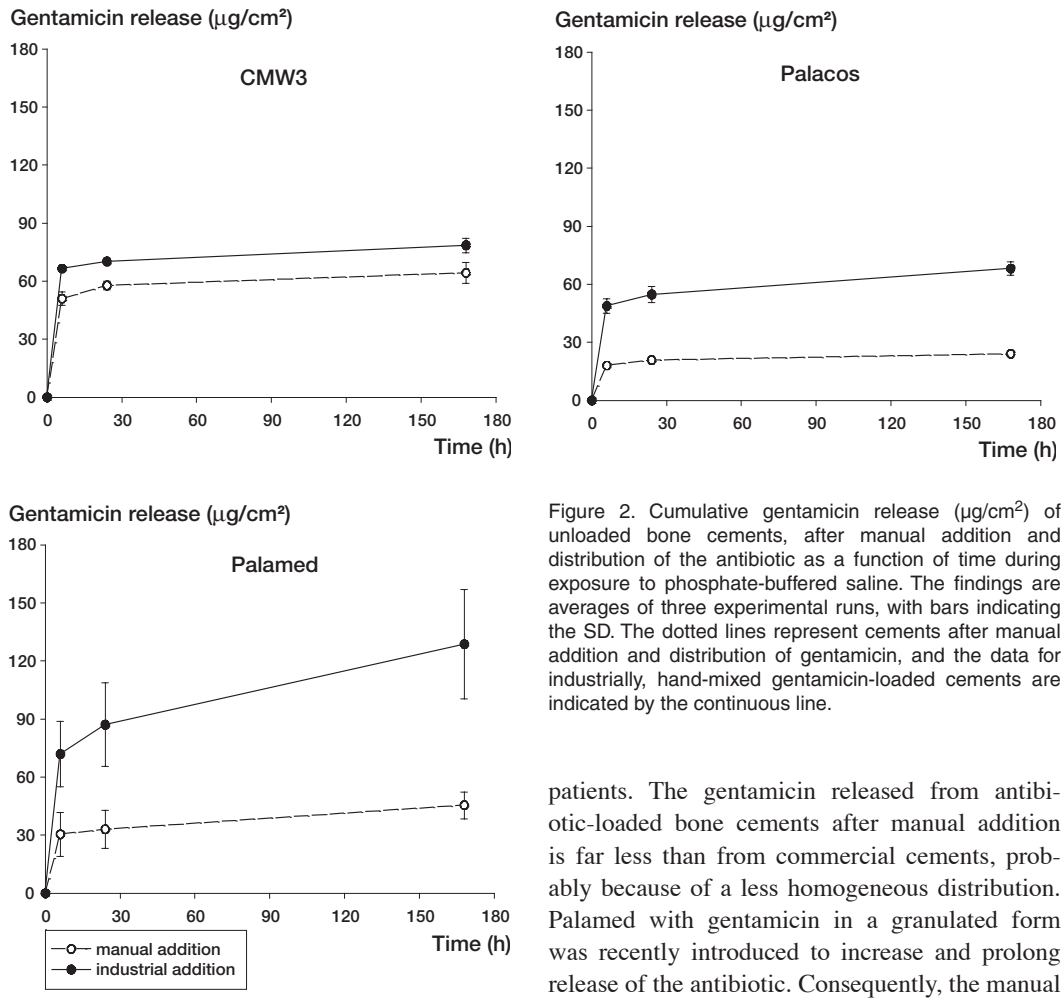

Figure 2. Cumulative gentamicin release (μg/cm^2) of unloaded bone cements, after manual addition and distribution of the antibiotic as a function of time during exposure to phosphate-buffered saline. The findings are averages of three experimental runs, with bars indicating the SD. The dotted lines represent cements after manual addition and distribution of gentamicin, and the data for industrially, hand-mixed gentamicin-loaded cements are indicated by the continuous line.

patients. The gentamicin released from antibiotic-loaded bone cements after manual addition is far less than from commercial cements, probably because of a less homogeneous distribution. Palamed with gentamicin in a granulated form was recently introduced to increase and prolong release of the antibiotic. Consequently, the manual

Table 1. Total amounts of gentamicin released after 168 h, expressed as a percentage of the total amount of 6 commercially available gentamicin-loaded bone cements, prepared by hand- or vacuum-mixing. The values are the mean of 3 separate experiments. N = 3 for each statistical analysis

Cement	Total amounts released (%)		95% CI	P-value
	Hand-mixed	Vacuum-mixed		
CMW1	1.56	1.32	0.07	0.001
CMW3	1.84	1.37	0.13	0.001
CMWend	2.13	2.07	0.15	0.2
CMW2000	1.32	1.02	0.06	<0.001
Palacos R	3.46	3.84	0.26	0.02
Palamed	8.02	6.48	2.60	0.13

Table 2. Total amounts of gentamicin released after 168 h, expressed as a percentage of the total amount of 3 gentamicin-loaded bone cements, prepared by hand-mixing after manual or industrial addition of the antibiotic. The values are the mean of 3 separate experiments. N = 3 for each statistical analysis

Cement	Total amounts released (%)		95% CI	P-value
	Industrial addition	Manual addition		
CMW3	4.53	3.71	0.46	0.01
Palacos R	8.08	2.86	0.54	<0.001
Palamed	14.81	5.24	4.12	0.004

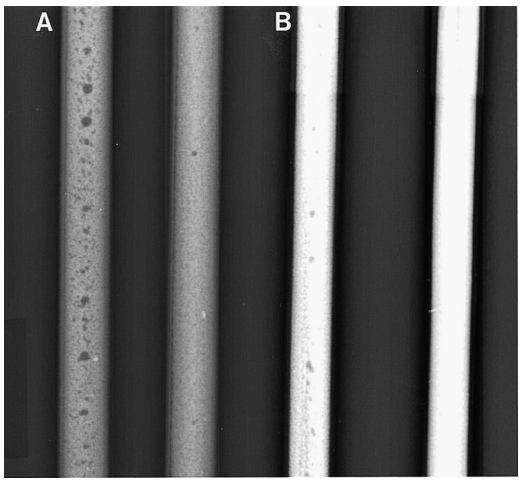

Figure 3. Radiographs of CMW2000 (A) and Palacos R (B) bone cements, prepared by (left) hand- and (right) vacuum-mixing. The width of each cement column is 10 mm.

addition of nongranulated gentamicin sulfate to unloaded Palamed had the greatest effect on the antibiotic release rate. It must be emphasized that although the reduction in gentamicin release rates was highest for Palamed, its release rates remain much higher than CMW3 or Palacos R after the manual addition of antibiotics.

Hand-mixing with a spatula of commercially available antibiotic-loaded bone cements in a bowl resulted in a much larger total antibiotic release, than hand-mixing in a Cemvac system. The difference in the method of hand-mixing could be one factor of importance, but the difference in the sample size used was probably still more important. Cement samples made with the Cemvac system had a 4-times larger surface area (4 cm^2 instead of 1.2 cm^2), while the weight of the samples was 8 times greater (800 mg versus 100 mg). Therefore, the ratio of the cement surface area to the weight of the Cemvac cement samples was half the ratio of the samples made with a bowl and spatula. Since antibiotic release from gentamicin-loaded bone cement is mainly a surface phenomenon, the total gentamicin release (expressed as a percentage of the total amount included) was largely affected by the difference in the ratio of the surface area to the weight.

In conclusion, vacuum-mixing reduces the porosity of bone cements, and gave more or less marked changes in gentamicin release. Manual addition of gentamicin followed by hand-mixing leads to a lower release of antibiotics than from their corresponding commercially available antibiotic-loaded counterparts, especially in Palamed and Palacos R cements.

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No competing interests declared.

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