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Antibiotic-loaded acrylic bone cements: An in vitro study on the release mechanism and its efficacy

Marta Miola [a,*], Alessandro Bistolfi [c,d], Maria Carmen Valsania [c], Carlotta Bianco [c], Giacomo Fucale [b], Enrica Verné [a]

[a] Applied Science and Technology Department, Politecnico di Torino, Italy
[b] Chemical, Clinical and Microbiological Analyses Dept., CTO, Turin, Italy
[c] Department of Orthopaedics, Traumatology and HM, University of Turin, Italy
[d] AO CTO, M Adelaide Hospital, Turin, Italy

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ABSTRACT

An in vitro study was carried out in order to investigate the antibiotic release mechanism and the antibacterial properties of commercially (Palacos® R + G and Palacos® LV + G) and manually (Palacos® R + GM and Palacos® LV + GM) blended gentamicin-loaded bone cements.
Samples were characterized by means of scanning electron microscopy (SEM) and compression strength was evaluated. The antibiotic release was investigated by dipping sample in simulated body fluid (SBF) and periodically analyzing the solution by means of high pressure liquid chromatography (HPLC). Different antibacterial tests were performed to investigate the possible influence of blending technique on antibacterial properties.
Only some differences were observed between gentamicin manually added and commercial ones, in the release curves, while the antibacterial effect and the mechanical properties seem to not feel the blending technique.

1. Introduction

Bacterial contamination during operative procedures for total joint replacement and the subsequent deep wound infection is one of the worst adverse events that can still occur in the modern surgery [1,2]. Open procedures always have a risk of contamination, but the presence of biomaterials increases the risk of infection due to their susceptibility to bacterial colonization [3,4]. This event depends on several aspects, such as the physicochemical surface properties of biomaterials as well as the cell surface structure and receptors of bacteria [5,6]. Host tissues can develop an increased susceptibility to infections after implantation of a prosthetic device and if bacteria reach the biomaterial surface they can easily adhere and proliferate on it, causing septic mobilization of the prosthesis [4].

Aiming to prevent this event, several research studies have been carried out, mainly focused on the local delivery of antibiotics. For this purpose, the use of antibiotic-loaded bone cements, based on PMMA and co-polymers, was first introduced in 1970 by Buchholz and Engelbrecht [7], and today this is a well established strategy in order to prevent periprosthetic infections, osteomyelitis and generally in the treatment of musculoskeletal infections [8,9].

As reported in literature [5], the release of antibiotic from bone cement is a complex process that depends on several variables, like the chemical formulation of the cement, its viscosity, the mixing conditions and the type of antibiotic itself. Some studies [10,11] have underlined that antibiotic is mainly released from the surface, even if physiological fluids seem to enter the polymeric structure of acrylic matrix leading to antibiotic elution across cracks and pores. However, the elution of the antibiotic incorporated in the bulk is not complete, and until now very few studies [10,12] reported a complete characterization in terms of release mechanism. Generally, the release kinetic has been correlated to the degree of porosity rather than to the amount of antibiotic, in some cases to different blends of various antibiotics in the same cement.

Another topic that is not fully discussed in literature is the effect of the mixing pattern on antibiotic release. Antibiotic powders could be industrially blended to the solid fraction of bone cement during its production, or can be added to polymeric matrix by the surgeon at the surgical site. The use of commercially available antibiotic-loaded bone cements is most common in Europe, while manual addition of antibiotic powder to traditional bone cement during surgery is preferred in the United States. Some studies have been performed in order to individuate the best blending technique [13,14], but till now the best mixing pattern is still an open problem.

Until now, commercially available products are mainly low-dose antibiotic-loaded bone cements approved and diffused for the second stage of revisions, after eradication of the infection which caused

* Corresponding author at: Politecnico di Torino, Dipartimento di Scienza Applicata e Tecnologia-DISAT, Corso Duca degli Abruzzi 24, 10129 Torino, Italy. Tel.: +39 0110904717; fax: +39 0110904699.
E-mail address: marta.miola@polito.it (M. Miola).

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septic mobilization of the primary total joint arthroplasties and for first implants in selected cases or critical patients such as immunosuppressed, diabetics, and elderly patients.

The clinical experience, together with new data from the earliest experimental studies, opened the question of the eventual extension of high-dose antibiotic-loaded bone cements to the prophylaxis in primary total joint replacement, so any approach to the understanding of the release mechanism and efficacy of these local drug delivery biomaterials should be encouraged.

In this work, a deep investigation of the antibiotic release mechanism from commercially and manually blended antibiotic-loaded bone cements together with a complete evaluation of antibacterial properties have been proposed, aiming to give further support to the literature concerning this very controversial topic.

2. Materials and methods

The bone cements used in this research work are reported in Table 1. They belong to the Palacos® commercial stock and were purchased at Heraeus Kulzer s.r.l. Both high (Palacos R®) and low viscosity (Palacos LV®) commercial cements were selected for this study. The plain cements were manually blended with gentamicin and compared to the commercially available gentamicin-loaded ones (Palacos R + G® and Palacos LV + G®). The gentamicin was added as sulfate (Farmalabor), since also commercial Palacos® contains this kind of antibiotic, and in the same amount of commercial references. Before mixing, the acrylic powders (solid phase of the cement precursors) and gentamicin sulfate were analyzed by scanning electron microscopy (SEM — FEI, Quanta Inspect 200) and energy dispersion spectrometry (EDS — EDAX PV 9900) to observe their morphologies and compositions.

2.1. Cement preparation and characterization

Antibiotic-loaded commercial cements were prepared following the procedure recommended by the supplier company. Cements with manually added gentamicin were prepared by mechanically mixing the antibiotic with the poly(methyl acrylate, methyl methacrylate) powders for 10 min, maintaining the same monomer/polymer ratio and introducing the same antibiotic amount of the respective commercial ones (0.024%wt and 0.012%wt respectively for low and high viscosity cements). The gentamicin powder was blended with the polymer powder before the addition of the liquid monomer.

The polymer–monomer mixing was manually performed under a laminar flow cabinet and the mixture was transferred into a polished aluminum mould (100 × 100 × 5 mm with 25 holes of 10 mm in diameter), which was closed with two polished aluminum plates in order to allow the polymerization process and obtain identical samples [13].

After setting the cylindrical samples were removed from the mould and characterized.

Sample surfaces and sections were characterized by means of scanning electron microscopy in order to observe their morphologies and porosity. Besides, pore-size distribution was further investigated on sample cross-sections through image analysis (software Leica QWin). The analyses were performed in triplicate.

Table 1
Description of the bone cements used.

Name	Description
Palacos® R + G	Commercial Palacos high viscosity cement containing gentamicin
Palacos® LV + G	Commercial Palacos low viscosity cement containing gentamicin
Palacos® R + GM	Commercial Palacos high viscosity cement with manually added gentamicin
Palacos® LV + GM	Commercial Palacos low viscosity cement with manually added gentamicin

2.2. Antibiotic release

In order to estimate the amount and the kinetics of antibiotic release, gentamicin-containing samples were dipped into 30 ml of a simulated body fluid (SBF — Kokubo [15]) up to 28 days at 37 °C. The SBF volume was selected on the basis of Kokubo protocol, using a volume (V)/surface (S) ratio of at least:

$$V(\mathrm{ml}) = S\left(\mathrm{mm}^2\right)/10$$

2 ml of SBF solution was periodically spiked and analyzed by means of high pressure liquid chromatography (HPLC) technique. All the chemicals employed were of analytical grade. Acetonitrile, tris(hydroxymethyl)aminimethane (Tris), and 1-fluoro-2,4-dinitrobenzene (FDNB) were from Sigma-Aldrich (Milan, Italy). Gentamicin sulfate was a gift of Farmalabor (Milan, Italy). The analytical method was adapted from Arcelloni et al. (2001) [16]. A Hewlett Packard (Agilent, USA) apparatus and a column (LiChroCART® 250 × 4.4 mm–LiCrosphere® 100 RP-18 (5 μm)) equipped with diode array (DAD) were employed. The flow-rate was 1.3 ml/min and the separation of the gentamicin derivatives was monitored with UV detection at 365 nm. Previously, a calibration curve was performed using different gentamicin solution concentrations (2.5, 5, 10, 30, 50, 100 mg/l), taking into account the three main chemical species: C1, C1a, and C2. The limit of detection (LOD), and the limit of quantification (LOQ) were 1 mg/l and 2.5 mg/l, respectively; the CV (coefficient of variation) was <8%.

The release test was performed in triplicate.

At the end of test, samples were gently washed in distilled water, analyzed by SEM observation, to investigate the effect of dipping in SBF on their surface morphology, and used for some of the antibacterial test, afterwards described.

2.3. Mechanical properties

The bone cements were also prepared using an aluminum mould of 90 × 90 × 24 mm with 25 holes of 12 mm in diameter, in order to prepare samples for mechanical compressive test, in accordance with ASTM D 695-96 standard [17]. Then, samples were polished with SiC abrasive papers to remove all superficial roughness.

Compressive test was performed on five specimens, using an Instron machine at 2 mm/min crosshead speed; the average and standard deviation were calculated. Statistical analyses were performed by using the ANOVA test.

The mechanical properties were also investigated for samples aged for 14 days in SBF solution in order to estimate a possible variation of compressive strength.

2.4. Antibacterial test

Antibacterial tests were performed in order to verify any difference, in terms of microbial adhesion and proliferation, between Palacos® + G and Palacos® + GM (both R and LV). Moreover, some analyses were performed to well investigate the antibiotic release mechanism and in particular to verify if the release is only a surface phenomenon or if bulk diffusion occurs [11].

The inhibition halo evaluation (a semi-quantitative test) and the colony forming unit counts (CFU — a quantitative one), were carried out in accordance to the National Committee for Clinical Laboratory Standards (NCCLS) for antimicrobial susceptibility [18,19]. For both tests, a bacterial broth was prepared by dissolving a lyophilized disk of *Staphylococcus aureus* strain (ATCC 29213) in 5 ml of brain–heart