Summary
Antibiotic-loaded bone cement has been in use for over thirty years for the fixation of total joint arthroplasties, although its mechanism of action is still poorly understood. Chapter 1 reviews the backgrounds of acrylic bone cements, prosthesis-related infection and antibiotic-loaded bone cements. The rate of deep infection around implants of around 1% to 2% may be underestimated with classical microbiological techniques. Direct contamination is likely to cause the majority of these infections, which are difficult to treat due to the biofilm mode of growth of bacteria. It is shown that antibiotic-loaded bone cement has a significant effect on bacteria, decreasing the likelihood of infection and improving the efficacy of infection treatment. However, recently antimicrobial resistance among bacteria has been ascribed to the antibiotic-loaded bone cement. The nature of the release of gentamicin from bone cement is bi-phasic with a peak release in the first hours, followed by a steadily decreasing but ongoing release that can be measured for months. The fundamental background of this release remains a matter of conjecture to the biomedical field. The unresolved issues both regarding the action of antibiotic-loaded bone cement and the nature of the antimicrobial resistance necessitate further research into the interaction of antibiotic-loaded bone cement and bacteria. Many lines of research try to counteract the decreased effectiveness of antibiotics against bacteria in biofilms. The use of ultrasound is an example of such a line of work. With the use of low-frequency ultrasound an enhanced antimicrobial effect of antibiotics was found, called the bio-acoustic effect. Similarly, ultrasound has been implied in drug delivery systems. Considering that the majority of antibiotics remain locked in the bone cement, ultrasound could play a role in accessing this reservoir.

The aim of the work described in this thesis therefore is to gain insight in the mechanism of action of antibiotic-loaded bone cement. Specifically, antibiotic release in simulated physiological conditions and the effect this has on bacteria has been researched. A secondary aim was to study the possibility of accessing the antibiotic reservoir in antibiotic-loaded bone cement by use of ultrasound.

Numerous studies have been published on gentamicin release from bone cements, but none have been able to estimate the local concentrations in the prosthesis-related interfacial gap, present after implantation. In Chapter 2 the aim was to develop a method allowing determination of antibiotic release in such a gap. 200 µm wide gaps with a volume of 6 µl and a surface area of 0.6 cm² were created by inserting stainless steel strips in gentamicin-loaded bone cement plugs prior to polymerization. After hardening, the gap surface was exposed to 6
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µl or 10 ml of phosphate buffered saline (PBS). Within 2 h, gentamicin concentrations in the gaps reached around 4000 µg/ml for four different gentamicin-loaded CMW and Palamed bone cements and 2500 µg/ml for Palacos R-G. Concentrations measured in the larger volume were several hundred-times lower than in the gaps. This simulated prosthesis-related interfacial gap model offers new insights in the clinical efficacy of antibiotic-loaded bone cements. It is demonstrated that concentrations up to 1000-fold the antibiotic resistance levels for most bacterial strains causing implant infection can be achieved in a realistic in vitro model.

Clinical experience and in vivo studies indicate a beneficial effect of antibiotic-loaded bone cement, although in vitro studies have shown biofilm formation on these bone cements. The latter studies, however, have neglected the possibility of a build-up of a high local antibiotic concentration in the gap between bone cement and bone. In Chapter 3 bacterial survival in a simulated prosthesis-related gap was investigated. Similar samples with a gap as in Chapter 2 were made of three different commercially available bone cements (CMW 1 Radiopaque, Palacos R and Palamed) both with and without gentamicin (plain bone cement). These were stored dry (‘pre-elution’) or submersed in PBS to simulate initial gentamicin release (‘post-elution’). Gaps were subsequently inoculated with bacteria isolated from infected orthopaedic prostheses after estimation of their gentamicin sensitivity. Bacterial survival was measured 24 h after inoculation. All strains survived in plain bone cements. In pre-elution gentamicin-loaded bone cements only the most gentamicin-resistant strain survived, but in post-elution samples more strains survived. The high gentamicin concentrations demonstrated inside the gaps in Chapter 2, sufficed to kill only gentamicin-sensitive strains. Clinically, this could explain the increased prevalence of prosthesis-related infections with gentamicin-resistant bacteria.

More generally, such biomaterial-related infections with antibiotic-resistant strains threaten the use of biomaterials. In orthopaedics, this led to use of bone cement loaded with multiple antibiotics. In Chapter 3, a highly gentamicin-resistant strain showed survival in a gap environment in gentamicin-loaded bone cement. Chapter 4 therefore aims to evaluate survival of such strains in bone cements with two antibiotics, in which half of the gentamicin is replaced by clindamycin or fusidic acid. Three highly gentamicin-resistant, but clindamycin and fusidic acid sensitive, coagulase-negative staphylococcal strains from orthopaedic prosthesis-related infections were used. There was a trend toward less survival
for the combination of gentamicin and clindamycin, but not for that of gentamicin and fusidic acid. In conclusion, a combination of antibiotics in bone cement does not uniformly lead to better results than use of gentamicin as a single antibiotic.

The release of antibiotics is incomplete and mainly confined to the surface. Walking results in cyclic loading, which may lead to cracks in bone cement. These cracks would present new surface for further antibiotic release. The goal of Chapter 5 was to compare antibiotic release from cyclically loaded bone cement with release from unloaded bone cement. Two models of the frontal aspect of a femoral stem were cemented with CMW 1 Radiopaque G, Palacos R-G and Palamed G. Both were immersed in water, and the gentamicin concentration in the water was monitored. One model was cyclically loaded at 5 Hz during immersion achieving relevant stresses in the bone cement mantle. After $10.8 \times 10^6$ cycles, equivalent to five to ten years walking, the bone cement mantles were analysed. Cyclic loading resulted in increased initial release of gentamicin from Palamed G, but not from CMW 1 Radiopaque G and Palacos R-G. The release characteristics could not be related to cracks, but Palamed G has been demonstrated before to have an aberrant antibiotic release profile as compared with the other two bone cements. There was no progressive increased release with further cyclic loading in any of the bone cements, as expected on the basis of a non-linear damage accumulation scenario. This may be explained by the absence of communication between internal cracks in the bulk with the elution fluid.

The release profile of antibiotics from antibiotic-loaded bone cement, used to prevent infections in total joint arthroplasty, is neither complete nor ideal. Ultrasound has been used to allow drugs to cross otherwise impermeable barriers. The aim of Chapter 6 was to establish a possible effect of ultrasound on antibiotic release from bone cements. Samples were made of the same commercially available gentamicin-loaded bone cements as in Chapters 3 and 5. Part of the samples was allowed to release gentamicin for 3 weeks before insonation. An insonation device was specifically developed and produced an ultrasound field with a time average acoustic intensity of 167 mW/cm$^2$ at a frequency of 46.5 kHz. The samples were exposed to the ultrasound field or not exposed to it as a control. The amount of gentamicin released was measured by fluorescence polarization immuno-assay. There was a limited increase of gentamicin release with application of ultrasound in fresh samples, but not in the samples that had been allowed to release gentamicin. For fresh samples, a linear regression model showed that this ultrasound effect was statistically significant. The
mechanism behind these observations is not clear, but it is suggested that micro-streaming or localized temperature rise may be involved.

In the general discussion, Chapter 7, a more fundamental approach is taken to the mechanism of release of antibiotics from acrylic bone cements. The results from a study performed in association with the Department of Physics indicate that the initial burst release is caused by the dissolution of antibiotic particles that are readily available on the surface of the bone cement either directly or through surface irregularities. The long-term release appears to be the result of the water very slowly entering the polymer matrix and carrying the antibiotic to the surface. These findings are corroborated by the findings of Chapters 5 and 6. Next, the methodological aspects of the experiments performed for Chapters 2 till 6 are critically reviewed. From this analysis further experiments are suggested. Finally, the conclusions from this thesis are extrapolated to the clinical application of bone cement.

This thesis suggests that use of antibiotic-loaded bone cement is effective. Nevertheless, the value of antibiotic-loaded bone cement to the clinician is not likely to extend beyond the first days after implantation. The ongoing low-level release of antibiotics, that may have limited side-effects, calls for the development of a more optimal antibiotic carrier for orthopaedic surgery.