Introduction to experimental studies
on antibiotic-containing bone cement
Antibiotic-containing bone cement

In Part II of this thesis, pre-mixed tobramycin-containing bone cement was evaluated in experimental studies. In numerous hospitals, especially in the United States, antibiotic-containing bone cement is prepared by adding tobramycin powder intraoperatively to bone cement and subsequent hand-mixing [Heck, 1995] Pre-mixed antibiotic-containing bone cements are marketed in Europe, but regulations of the Federal Drug Agency (FDA) do not allow the use of these cements in the United States (U.S.). Although registration regulation for new bone cements have become less strict recently, both in Europe and in the U.S., strict regulations for manufacturing of bone cements should guarantee constantly reproducible manufacturing conditions and the safe use of these products clinically [Khn, 2000] Therefore, it is somewhat inconsistent that U.S. regulations allow less constant reproducible manufacturing conditions, i.e. the hand mixing of antibiotics and bone cement in the operating room by the surgical team. This hand mixing of antibiotics with bone cement does not allow for a consistent and controllable quality of the cement. A standardized preparation of antibiotic-containing bone cement benefits not only future infection-based efficacy studies, but also the evaluation of its biomechanical properties. Although adding low quantities (approximately one gram per unit) of tobramycin has minimal effects on the fatigue life of cement, amounts of more than two gram of antibiotics per unit of cement have shown to decrease the strength of cement [Davies, 1991, Lautenschlager, 1976].

Tobramycin

Since 1969, various combinations of antibiotics and bone cement have been used in the prevention and treatment of arthroplasty infections [Buchholz, 1970, Elson, 1977a, Murray, 1984]. Tobramycin, developed in 1968, is one of the aminoglycoside antimicrobial agents. Other members of this group are gentamicin, streptomycin, kanamycin, amikacin and netilmicin. Like other aminoglycosides, tobramycin is heat-stable, which makes it suitable for incorporation in polymethylmethacrylate (PMMA). Aminoglycosides are effective
primarily against most staphylococci, certain mycobacteria and aerobic Gram-negative bacilli such as enterobacteriaceae or Pseudomonas aeruginosa [Edson, 1999, Wingard, 1991]. The antimicrobial spectrum of tobramycin includes pathogens like staphylococci and aerobic Gram-negative bacilli, which are most frequently cultured from orthopaedic implant infections [Scott, 1999]. Aminoglycosides are bactericidal by binding to bacterial ribosomes and interfering with protein synthesis, and by disrupting the cell membrane. Differences in structure of the compounds determine the spectrum of activity against bacteria, the risk of toxicity in patients and occurrence of resistance. Aminoglycosides enter the inner cell membrane of the bacteria through an energy-dependent, aerobic process. Binding to the 30S-ribosomal subunit inhibits protein synthesis and induces misreading of the mRNA. These effects, together with cell membrane-related effects cause bacterial cell death [Edson, 1999, Greenwood, 1998, Lorian, 1996]. Antibacterial activity of aminoglycosides is concentration dependent, meaning that an increased drug concentration will enhance the bactericidal activity [Lode, 1998]. The respiratory processes needed for aminoglycoside uptake are absent in anaerobes, streptococci and enterococci, resulting in relative resistance [Greenwood, 1998]. Due to the high polarity of aminoglycosides, these compounds do not enter phagocytic cells and thus they are not active against intracellular bacteria [Greenwood, 1998, Wingard, 1991]. Resistance mechanisms involve most commonly aminoglycoside-modifying enzymes. These enzymes are plasmid-mediated, which means that this genetically information can spread easily to different bacterial species [Wingard, 1991]. The structure of a particular aminoglycoside determines its inactivation by the enzymes. Aminoglycosides can share some structural groupings vulnerable to modifications, which explains the emergence of cross-resistance between gentamicin and tobramycin. In a recent study however, more than half of gentamicin-resistant isolates of Enterobacteriaceae showed susceptibility for tobramycin [Adwan, 1998]. Susceptibility studies have shown that tobramycin is generally more active in vitro against most strains of P. aeruginosa, and gentamicin is more active against Serratia species [Edson, 1999]. Resistance rates of P. aeruginosa strains have been reported to be 7-14% for gentamicin versus 4% for tobramycin [Edson, 1999, Lorian, 1996]. but other authors found no difference between the two (18%) [Schmitz, 1999b]. However, data reported in literature on aminoglycoside susceptibility are of limited value for the surgeon with respect to the choice of a specific type of antibiotic-containing bone cement in the individual patient. Resistance to aminoglycosides, just as to other antibiotics, varies by location and local
usage patterns that can change over time [Schmitz, 1999a]. This is illustrated by the fact that - despite close association of aminoglycoside resistance with methicillin resistance [Schmitz, 1999b] - restricted use of gentamicin has been shown to induce reemergence of gentamicin-susceptible methicillin-resistant S. aureus [Aubry-Damon, 1997]. Therefore, it is more prudent to rely on data, if available, specific to the hospital where antibiotic-containing bone cement is to be used for prevention of prosthesis infection. When an infected arthroplasty is being revised and culture results are known, the selection of the antibiotic-containing bone cement should be tailored to the causative pathogens.

I Carriers of antimicrobials for local drug delivery

In Chapter 7, antimicrobial-loaded biomaterials are discussed that can be implanted locally for the prophylaxis or treatment of musculoskeletal infection. Each of these carriers, of which tobramycin-containing bone cement is just one example, has its specific indications for use in the management of these infections, because properties of these materials, such as form and biodegradability, vary. The multitude of antibiotics and other antimicrobial substances that are incorporated in these carriers, together with the material properties, determine the drug-release profiles that can be found either in vitro or in vivo.

I Release of tobramycin from tobramycin-containing bone cement

The efficacy of antibiotic-containing bone cement in preventing or treating arthroplasty infection depends on the antibiotic release characteristics. In Chapter 8, the release of tobramycin from pre-mixed tobramycin-containing bone cement in the rabbit's femur was investigated. In order to be effective in preventing or treating arthroplasty infection, antibiotic-containing bone cement should release the antibiotic in concentrations above the susceptibili-
ty level of the microorganism involved. Secondly, this concentration should be reached at the site of the implant, especially at the bone-cement interface and in the surrounding bone. In addition, the antibiotic concentration in serum should not exceed toxic levels, to limit risk of side effects. Therefore, both serum and bone concentrations of tobramycin were investigated in the rabbit model described in this chapter.

Susceptibility of bacteria to tobramycin

Still, it is difficult to translate results from susceptibility tests and release studies to clinical efficacy of antibiotic-containing bone cement. Several factors may influence the effect of antibiotics released from bone cement on the bacteria. This complicates the prediction whether the use of local antibiotic-containing bone cement will prevent or inhibit bacterial growth in the setting of implant infection. Firstly, concentrations of antibiotics that are higher than the minimum inhibitory concentration (MIC), will theoretically eliminate bacteria, defined as susceptible to these antibiotics [Johansson, 1991]. However, when initial release of antibiotic is high, even bacteria that are defined as resistant, based on their MIC, may be killed [Scott, 1999]. Secondly, in device-related infection, standard susceptibility tests do not correlate with treatment success [Blaser, 1995]. The microenvironment in arthroplasty infection might favor bacteria, as it includes the presence of foreign bodies and eventually devitalized bony due to previous surgical procedures or to the infectious process itself. In such a setting, bacteria can alter their phenotype, rendering them less susceptible to local defense mechanisms and immune responses of the body and to antibiotics. This phenomenon has been described for different biomaterials especially in relation with the capability of some bacteria to produce a polysaccharide biofilm on the surface of the implant. These phenotypic alterations in vivo may not be detected by standard susceptibility tests, since these tests measure in vitro phenotypic susceptibility of bacterial isolates [Johansson, 1991]. Gristina showed that despite normal MIC levels of tobramycin in broth suspension, exposure to higher levels of tobramycin of Staphylococcus epidermidis, enclosed in a biofilm on a stainless steel implant, did not result in a total reduction of viable bacteria [Gristina, 1987]. Similar results were obtained by Nickel et al. after exposure of P. aeruginosa, growing as a biofilm on urinary catheters, to
tobramycin [Nickel, 1985]. Other in vitro studies showed that gentamicin or tobramycin, impregnated in PMMA bone cement, could not completely prevent biofilm formation after incubation with *S. epidermidis* [Chang, 1991, Chang, 1992, Oga, 1992]. However, the addition of aminoglycosides to the PMMA reduced adherence and viability of these microorganisms [Chang, 1991, Chang, 1992, Oga, 1992]. In fact, in contrast to plain bone cement, the addition of gentamicin prevented infection after implantation of PMMA, preincubated with *S. epidermidis* [Chang, 1994, von Eiff, 1998]. Darouiche *et al.* showed that *S. epidermidis*, grown on stainless steel nuts in presence of vancomycin, could not be eradicated completely, even though high levels of vancomycin were reached in the biofilm [Darouiche, 1994]. Binding of antibiotics to glycocalyx material and consequent inhibition of diffusion is not a major mechanism of antibiotic resistance in biofilms, as has been shown for tobramycin and vancomycin [Darouiche, 1994, Nichols, 1988]. More likely, an altered physiology and growth rate of the bacteria diminish the antimicrobial effect in the biofilm environment [Darouiche, 1994, Nichols, 1988]. Another aspect of the problem of extrapolating laboratory results to the clinical situation, which has been described as the minefield of difficulties, is related to aminoglycoside-containing polymers: [Greenwood, 1981]. Von Eiff has shown that in the presence of gentamicin-containing beads, *S. aureus* can transform into so-called small colony variants (SCVs). These SCVs show a decreased growth rate, are therefore more resistant to antibiotics, especially to aminoglycosides, and may be easily missed in the clinical laboratory [Proctor, 1998, Proctor, 1995, von Eiff, 1997, von Eiff, 1998]. Subsequently, testing of susceptibility of only the parent strain may give misleading results [Proctor, 1998, von Eiff, 1997].

Tobramycin-containing bone cement and prophylaxis of infection

Thus, the efficacy of antibiotic-containing bone cement to prevent or treat arthroplasty infection cannot be extrapolated just from susceptibility studies. Therefore, we performed various animal studies to evaluate the efficacy of the new tobramycin-containing bone cement. Firstly, in *Chapter 9*, the efficacy of this bone cement was evaluated for the prevention of local *S. aureus* and *S. epidermidis* infection. These bacterial species were used because they
are most frequently isolated in arthroplasty infection. Incidence rates after total joint arthroplasty of *S. epidermidis* (26-38% of infections) are somewhat higher than those for *S. aureus* (16-24% of infections) and have increased over the last decade [Fitzgerald, 1994, Garvin, 1993, Ostendorf, 2001, Sanzen, 1988, Tsukayama, 1996].

In literature, the optimal mode of administration of antibiotics - prior to or during surgery - is still subject of discussion. Dutch guidelines state that there is no indication for the use of antibiotic-containing bone cement in primary arthroplasty, if operated under prophylaxis of systemic antibiotics and an ultra-clean air system [Dutch Institute for healthcare improvement, 1994].

Data from the Swedish hip arthroplasty registry show however an increased use of antibiotic-containing bone cement in primary hip arthroplasty from approximately 10% of all primary hip arthroplasties performed in 1978 to 80% in 1996 [Swedish-National-Hip-Arthroplasty-Registry, 1998]. The different modes of administration of antibiotics have been compared for efficacy by only a few experimental and clinical studies. Petty *et al.* showed in a study in dogs that systemic antibiotic treatment as well as local treatment with antibiotic-containing bone cement reduced infections of the implant bed, but only the latter was found to be significantly different from controls [Petty, 1988]. Josefsson *et al.* compared prophylaxis with systemic antibiotics versus gentamicin bone cement in total hip arthroplasty in a prospective randomized clinical trial [Josefsson, 1990, Josefsson, 1993]. At 5 years follow-up, significantly more infections occurred in the group receiving systemic antibiotics. However, at 10-years follow-up of 1688 hips, infection rates in the systemic antibiotics group and in the antibiotic-containing bone cement group were no longer significantly different. In a similar study, McQueen *et al.* found no difference between these two modes of infection prophylaxis in 401 patients, at two years follow-up [McQueen, 1990].

It is obvious from the few available studies that there is still a lack of scientific proof regarding the efficacy of systemic (intravenous) and local (bone cement) administration of antibiotics to prevent implant bed infection. In **Chapter 10**, we evaluated the efficacy of either intravenous cefazolin or tobramycin-containing bone cement in preventing experimental implant infection. Cefazolin was used for systemic administration, since it is used widely by orthopaedic surgeons for treatment and prevention of staphylococcal infections.
Tobramycin-containing bone cement and treatment of infection

In revision surgery, antibiotic-containing bone cement is often used, since revised implants become more frequently infected than primaries [Fitzgerald, 1977, Nasser, 1992, Wilson, 1990b]. The higher infection rate is not only the result of another operation in compromised tissue, but the previously mentioned difficulties in diagnosing implant infection may play their role as well. When an implant is inserted in an implant bed that has not been totally cleared from bacteria, the second implant is likely to become infected too. In case a prosthesis is infected, rigorous treatment modalities are necessary to eliminate the infection, but the best choice for treatment of an infected total joint prosthesis still remains to be clarified. Consensus exists among most orthopaedic surgeons to remove the infected prosthesis if possible, because the infection is difficult to treat in presence of foreign material covered with bacteria [Brandt, 1997]. Such a revision operation can be performed either as a one-stage procedure or as a two-stage procedure. In the one-stage revision, the infected implant is removed and, after debridement and lavage of the implant bed, replaced by a new prosthesis during the same session. In a two-stage revision, the insertion of the new implant is postponed until several weeks after removal of the infected implant. During this period, the infection is treated with systemic antibiotics and/or local antibiotic-containing beads. The new prosthesis is inserted not until the infection parameters have regained normal levels. The use of antibiotic-containing bone cement for fixation of the revision prosthesis is preferred, given the higher incidence of infection after revision in comparison with primary joint prostheses. Where most surgeons choose the two-stage procedure for exchange of an infected prosthesis, also large series have been reported using the one-stage procedure, especially in Europe [Buchholz, 1981, Raut, 1995]. So far, reviews of literature on arthroplasty infection reported success rates of 82-83% for one-stage revisions, and 91-93% for two-stage revisions [Garvin, 1995, Pagnano, 1997]. Clinically, direct comparison between a one-stage or two-stage revision of a total joint prosthesis is difficult because of the lack of prospective, randomized studies evaluating the timing of this procedure [Garvin, 1995]. Clearly, the initial costs of a one-stage procedure as opposed to the two-stage procedure are less. Fewer operations means also less inconvenience for the patient. These advantages must be weighed against the risk of a re-infection of the prosthesis since frequently a second operation is needed anyhow. Together
with the increase of the number of revision procedures a patient will undergo, there is an increased risk of complications due to bone loss and of potentially fatal outcome. The risk of a re-infection after the first revision procedure, rather than the costs associated with the initial procedure itself, is probably the major factor in comparing the cost-effectiveness between the one- or two-stage procedure. Comparative trials between both options have been advocated for to clarify this matter [Gillespie, 1997]. The need for long-term follow-up in the evaluation of infection rates has also been emphasized [Raut, 1995]. Some consensus exists on the indications for a one-stage revision. Infections caused by less virulent bacteria, fully susceptible to the antibiotics used for treatment, in otherwise healthy patients who lack additional risk factors for infection (rheumatoid arthritis, diabetes, decubitus, need for bone grafting) can probably be treated safely and effectively by a one-stage procedure. Using these criteria, two small series (respectively, 15 patients with a mean follow-up of 53 months and 20 patients with a mean follow-up of 10 years) reported no recurrence of infections [Mulcahy, 1996, Ure, 1998]. Altered circumstances (e.g. the emergence of resistant bacterial strains as the causative organisms in infected arthroplasties) can pose the surgeon to choose for a two-stage procedure, even when he favors the one-stage revision [Elson, 1994]. Still, the criteria for one- or two-stage revisions are subject to debate. For instance, Raut argued that a discharging sinus is not always a contraindication for a one-stage revision of an infected hip prosthesis [Raut, 1994]. The outcome of one-stage revisions of infected total knee prostheses has been reported to be inferior to those of hip prostheses, probably due to lower vascularity and minimal soft-tissue coverage of the knee [von Foerster, 1991]. To prevent a recurrent infection after implant revision, antibiotic-containing bone cement should be able to treat an already established osteomyelitis. This can be investigated in a one-stage revision model, since it lacks the temporarily treatment with antibiotic-containing beads or a spacer that could reduce the infection. Therefore, in Chapter 11, a one-stage revision model was used to test the efficacy in treating an infection with either tobramycin-containing bone cement or systemic cefazolin.
Aims of Part II of this thesis:

- To review the literature on antimicrobial-loaded carriers that are clinically available or are being developed for use in the management of musculoskeletal infection (Chapter 7)
- To investigate the in vivo release of tobramycin in blood and bone as a function of time, after insertion of premixed tobramycin-containing cement. (Chapter 8)
- To investigate the efficacy of premixed tobramycin-containing bone cement in the prevention of *S. aureus* and *S. epidermidis* infections. (Chapter 9)
- To investigate the efficacy of both premixed tobramycin-containing bone cement and systemic cefazolin in the prevention of *S. aureus* infection. (Chapter 10)
- To investigate the efficacy of tobramycin-containing bone cement and systemic cefazolin for treatment of *S. aureus* infection in a one-stage revision model. (Chapter 11)