1

General introduction

In a short version awarded the SIROT-prize, Istanbul 2005
Hip prosthesis loosening and the problems in elderly patients

Approximately one million total hip replacement operations are performed worldwide annually, and this number is likely to increase considerably in the next decades. A major complication in total hip arthroplasties is loosening of the prosthesis leading to pain and walking difficulties and a higher risk for dislocations and pathological fractures. Within ten years of primary hip replacement 7-13 percent of patients need revision surgery due to loosening of the implant. Revision surgery has a high morbidity and mortality rate, especially in elderly patients with comorbidity. In the United States Medicare Population 5.3% of 3,165 patients undergoing revision surgery at age 80 and older died within 90 days of surgery. This was 1.9 times higher than a comparable Medicare cohort that had not undergone revision total hip replacement. Strehle et al. registered complications and social outcome in a cohort of 53 patients undergoing revision total hip arthroplasty older than age 80 years. They reported a total mean blood loss of 4,730mL and a mean duration of the procedure of 200 minutes. Eleven patients (21%) were admitted postoperatively to the intensive care unit, and mean hospital stay was 30 days. Complication rate was higher in patients with comorbidity. Of the 53 patients followed, three patients died during hospital stay, ten patients formerly living alone in a house or an apartment went to nursing care institutions and five patients became dependent on outside help from family members, neighbours or health care institutions. These figures indicate that revision surgery can be a heavy burden for elderly patients and the indication needs to be reconsidered thoroughly before these patients can be operated. Consequently, there remains a group of elderly patients with comorbidity who are not eligible for surgery and experience incapacitating pain and dependency in activities of daily living. Currently there are no alternative treatments for revision surgery.

Aseptic loosening

Aseptic loosening by particulate-induced osteolysis is the most common cause of implant failure. Wear particles, such as particles of polyethylene and metal, are phagocytosed by macrophages, leading to secretion of inflammatory cytokines. The resulting chronic inflammation eventually produces a pseudomembrane of synovium-like interface tissue with activated macrophages, fibroblasts, giant cells and osteoclasts.

At present, experimental approaches to the aseptic loosening problem are preventative rather than therapeutic. Preclinical studies have shown that bisphosphonates
might be useful to prevent aseptic loosening, but up till now clinical evidence is missing that bisphosphonates will prevent aseptic loosening at longer term. An alternative preventative approach for aseptic loosening involves gene therapy, e.g. using an osteoclast inhibitory protein, osteoprotegerin, delivered by a vector that delivers the gene inside the cell, such as an adeno-associated vector. Osteoprotegerin serves as a competitive inhibitor for the differentiation of osteoclasts, thereby preventing osteoclast activation. The vector to express the active ingredient was delivered by intra-muscular injection into the quadriceps muscles of mice. The effect on osteoclasts was therefore systemic and this appeared to be successful in inhibiting the osteolysis that was seen in untreated controls. Although the results of these experimental animal data are interesting, it is unclear what the long-term systemic effects of prolonged elevations in serum osteoprotegerin might be. Before clinical application a deleterious effect on normal osteoclast function needs to be excluded.

In summary, experimental studies on alternatives for revision surgery are primary preventative and not yet in clinical trials.

Removal of interface tissue

This thesis describes an approach to stabilise loosened hip prostheses as an alternative to regular revision surgery. The technique involves, among other things, injection of bone cement around the loosened prosthesis percutaneously, while the prosthesis remains in place. Before bone cement can be injected to stabilise the prosthesis, interface tissue preferably needs to be removed to leave space for the cement. As the periprosthetic space is a more or less closed compartment local application of a toxic component could be a good option. Non-surgical removal of interface tissue has not been described in the literature. However, from the early 1950s several chemical agents have been used for intra-articular chemical synovectomy in patients with rheumatoid arthritis. Synovial tissue has the histological and histochemical characteristics of interface tissue, and results of studies with synovial tissue could therefore be an indicator for outcome of studies with interface tissue. Chemical agents as osmic acid, nitrogen mustard, and thiopeta have been shown successful in non-controlled studies, but not in controlled studies and are currently not in use because of potential hazards. Cyto-statics that are used in cancer therapy act by inhibition of cell division. As the cells in interface tissue are only slowly dividing cells, the use of cytostatics to remove interface tissue is not a good option. Another approach to killing pathological cells is to introduce a gene into the target cells that encodes an enzyme capable of converting a pro-drug of relatively low toxicity into a potent cytotoxic drug. As the prodrug is converted...
to the toxic derivative locally, occurrence of systemic adverse effects will be low. This approach is known as gene-directed enzyme prodrug therapy (GDEPT).\textsuperscript{23}

**Gene therapy**

The definition of gene therapy (according to a medical dictionary) is the insertion of normal DNA directly into cells to correct a genetic defect. It involves the treatment of disease by replacing, altering, or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for a disease.

The first attempt for human gene therapy was reported in 1975 when Rogers and Terheggen combined their knowledge on the Shope rabbit papilloma virus that induces arginase,\textsuperscript{104} and on the disease argininemia, a genetic disease involving a low arginase-level, causing spastic diplegia, epileptic seizures, and mental retardation.\textsuperscript{119} They inoculated fibroblasts from humans with arginase deficiency with the Shope virus, resulting in an induction in arginase activity.\textsuperscript{105} However, in a clinical trial in three patients, intravenous injection of the Slope virus was unsuccessful.\textsuperscript{118}

In recent years the potential role of gene therapy has been expanded to gene therapy as a tool for delivering individual proteins to specific tissues and cells. This also encompasses delivering proteins to kill cells (e.g. in cancer) and introducing therapeutic proteins locally for a longer period of time (e.g. in rheumatoid arthritis). The most common method to introduce the gene into the cell is by using a virus as a vector, as viruses are known to deliver their DNA into the host cell for replication. The adenoviral (Ad) vectors are popular for gene therapy since they are very efficient at infecting dividing as well as non-dividing cells, are easy to produce in large titers,\textsuperscript{38} and provide ample space for transgenes. Adenoviral vectors deliver their gene outside the host cell genome, thereby minimising the risk for disturbing normal cellular gene expression. The expression of their inserted gene is transient due to cellular and humoral immune responses\textsuperscript{130} which could actually be an advantage when only transient expression is required for adequate therapy. The presence of anti-Ad neutralising antibodies tends to be ubiquitous in human adults and greatly reduces virus dissemination while peak transgen expression in the targeted tissue is only minimally reduced.\textsuperscript{15} Moreover, the virus has a high particle size which, when introduced in a more or less closed compartment, prevents most of it from diffusing into other tissues. Thus, an adenoviral vector can ideally be used to deliver a gene to the interface tissue in the periprosthetic space. When using adenovirus 5 as a vector to express the gene Escherichia coli nitroreductase (Ntr), infected cells become extremely sensitive to the prodrug CB1954. This prodrug causes death of the infected cells.\textsuperscript{34} In a study by Goossens et al.,\textsuperscript{46} it was demon-
strated that genes can be transferred to synovial tissue in vivo in rhesus monkeys by direct injection into the joint, and that the synoviocytes can be killed with injection of a specific prodrug. In our laboratory, previous experiments have shown the efficacy of the infection and destroying of synoviocytes and fibroblasts from interface tissue by HAdV-5-Ntr and CB1954.

Cement injection

One of the major difficulties in revision surgery is the removal of cement from the femoral shaft without fracturing the femur which may be eggshell thin. Therefore debate exists whether this cement should be removed completely before a new stem can be cemented. Chapchal et al. advocated in 1973 to remove all old bone cement despite it being time-consuming and hazardous. Later, this advise was more differentiated by Charnley et al. who stated that the difficulties of complete removal, the risk of fracture, of interrupting blood supply and of reduction in the amount of cancellous bone may together represent a greater risk than that of bond failure at an old-new cement interface. They recommended, in the replacement of a non-infected femoral prosthesis, to ream out sufficient cement to permit a loose fit of the new prosthesis. Biomechanical studies showed that recementing over old cement is a practical alternative when all the blood is removed from the old cement, the old cement is rasped and the newly inserted cement is as fresh as possible to assure the presence of non-activated monomer that can be activated by the benzoyl peroxide activator still present in the old cement giving a greater interface strength. Lieberman et al. showed this in clinical practice in 19 patients where a new prosthesis was cemented in an old cement mantle. The technique involved rasping and drying of the surface before applying fresh cement, to increase interface strength between the old and the new cement. Since the mid 1980s percutaneous cement injection is used for vertebroplasty in patients with painful vertebral lesions to relieve pain and provide strength. For this purpose low viscosity PMMA-cement with additional radio-opacity has been developed together with cement-guns and needles for injecting the cement. As aseptic loosening results in a radiolucent line around the prosthesis and the pain from loosening is caused by movement of the prosthesis within the bone, it would be worthwhile trying to inject cement in the periprosthetic zone percutaneously. Ideally three or more injection sites should be used to allow stabilisation in a 3D-space. In this way the prosthesis could again be stabilised in the bone, leading to decrease in pain and improvement in walking. Furthermore, the mechanical stress on the bone by the loose prosthesis is reduced, thus allowing for reconstituting of the resorbed bone. Percutaneous cement
injection in the periprosthetic space has not been described in the literature. To our knowledge, this procedure has not been studied as an alternative to revision surgery. To assess if the periprosthetic space is accessible to bone cement, an arthrography of the hip can be useful. With arthrography, the periprosthetic space can be visualised when the contrast medium is easily distributed around the prosthesis. However, arthrography of the hip in a loosened prosthesis often shows just a small line (i.e. <1 mm) of contrast medium between bone and cement. It should be questioned if this area is large enough to inject a sufficient amount of cement to stabilise the prosthesis, particularly since the cement has a higher viscosity than the contrast medium. Therefore, before cement can be injected, the interface tissue preferably has to be removed.

**Aims and outline of this Thesis**

The aim of this thesis was to evaluate risks and benefits of revision hip arthroplasty in a retrospective cohort of patients 80 years and older and to develop and assess an alternative treatment for revision hip arthroplasty for elderly patients with a high risk for complications due to serious comorbidity or a low bone stock (i.e. high likelihood of femoral fracture). In Chapter 2, we analysed the risks of revision hip arthroplasty in elderly patients. We studied the burden of hospital stay and occurrence of complications and the benefits of improvement in social outcome. We assessed social outcome of 145 patients 80 years and older undergoing 183 hospital admittances for revision surgery of their hip prostheses. Primary objective was to investigate whether hip revision surgery in elderly patients could improve social outcome (housing situation and independency in ADL (activities in daily living)). Secondary objectives were occurrence of complications during hospital stay, patient survival, and use of walking aids before and after revision surgery.

In Chapter 3, we studied whether cells from the interface tissue between prosthesis and bone could be killed by Gene-directed Enzyme Prodrug Therapy (GDEPT). We investigated whether these cells could be transduced by a human adenoviral-5 vector carrying the *E.coli*-derived nitroreductase gene (CTL102) and sensitised to the prodrug CB1954. First, we exposed the cells to various concentrations of Ad.CMV.LacZ to determine the infectivity of interface cells. In the next experiment, interface cells were exposed to various concentrations of CTL102 and subsequently to various concentrations of CB1954 to study cell-killing potential of the Ntr/CB1954 GDEPT. In this chapter, we also discuss the influence of iodide-containing contrast medium on adenovirus-mediated gene transfer.

Chapter 4 describes two alternative methods to optimise short-term transgene
Chapter 1

expression. In clinical studies it is essential to have a predictable and adequate expression. When the gene expression can be made more efficient and predictable, the vector dose can be decreased. This has several advantages, including less evocation of an immune response, a smaller demand for the production of clinical grade adenovirus, and less adverse events. A Ubiquitous Chromatin Opening Element (UCOE) was inserted in an Ad.CMV.Luc vector, and sodium butyrate (NaB) was added to the culture medium of interface cells in various concentrations to study the effect on transgene expression. Both these methods have a theoretical potential to enhance expression without increasing the amount of viral particles. The two methods were tested individually and in combination to evaluate their effect on transgene expression.

For the clinical use of intra-articular treatments a good exposure of the target tissue is essential and the injected active ingredient must remain in the joint for a sufficient amount of time to ensure adequate therapy. Beside size of the therapeutic particle, the integrity of the surrounding joint tissue (containment) is important in retaining active particles within the joint space. Efficacy of intra-articular therapy is also dependent on the joint volume, because this determines the concentration of the therapeutic ingredient. Chapter 5 shows a retrospective analysis of 221 hip arthrograms performed for diagnosis of prosthesis loosening. All arthrograms were studied for leakage of contrast medium and injected volume. This analysis was performed to determine the percentage of hip prostheses that would be eligible for therapy using intra-articular delivery of genes and proteins.

After these pre-clinical studies, a phase-1 clinical gene therapy approach was designed to destroy the periprosthetic loosening membrane, and enable refixing of the hip prosthesis with percutaneous bone cement injections under radiological guidance. In this phase-1/2 dose escalating gene therapy trial twelve patients were treated. Chapter 6 shows the protocol of the phase-1 clinical study on gene therapy in aseptic prosthetic replacement loosening, carried out in the Leiden University Medical Center between June 2004 and February 2006.

The results of the clinical study are described in two chapters. As the study is a phase-1 clinical study, safety is the primary objective. Chapter 7 describes all adverse events of the clinical study and their possible relations to gene therapy, cement injection or other features of the study. Chapter 8 describes the secondary objectives of the clinical study. Virus shedding was quantitatively measured by analysis of urine, stool, blood, and nose and throat swabs. Biopsies from periprosthetic interface tissue, taken during the cementing procedure, were investigated for apoptotic and necrotic tissue. X-rays of the hip before and after the cementing procedure were analysed for increase in cement thickness. Finally, for clinical evaluation, Harris Hip Score and Visual Analogue Scales for pain, walking distance, and independency for activities in daily liv-
In Chapter 9 a small case series is described in which percutaneous peri-prosthetic cement injection is performed in elderly patients with hip prosthesis loosening, without previous gene therapy to remove the interface tissue. This series was performed to study whether cement injection is feasible without previous interface tissue removal.

Finally, in Chapter 10, a general discussion is given on percutaneous peri-prosthetic cement injection with or without gene therapy as an alternative to revision surgery, based on the work presented in this thesis.