Summary and discussion
Approximately one million total hip replacement operations are performed worldwide annually, mostly for osteoarthritis and rheumatoid arthritis. 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. The number of revision surgeries in elderly patients is likely to increase considerably in the next decades, due to the tendency to insert orthopaedic implants at younger ages and the longer life expectancy of patients. Revision surgery has a high complication rate in elderly patients and is associated with less improvement in social outcome, compared to primary hip arthroplasty in patients of all ages. Many studies have focused on the relatively good technical outcome of revision hip arthroplasty in patients of all ages. However, the impact on the patient’s life, especially in elderly patients has been underemphasised.

This thesis was set up to develop and analyse an alternative treatment for hip prosthesis loosening. We used a gene-directed enzyme-prodrug therapy to kill and remove interface tissue, after which we injected bone cement around the prosthesis percutaneously under CT- and fluoroscopy guidance. The first chapter describes the social and medical impact of revision hip arthroplasty in patients 80 years and older, and thereby the reason we searched for an alternative treatment. The next three chapters describe preclinical studies, in chapter 3 and 4 cell studies are described that show that interface cells can be killed by the gene therapy. Chapter 5 shows the importance of a pre-treatment arthrogram. In chapters six to eight the clinical protocol is outlined, and results are shown. Finally, chapter nine describes a short case series of patients who had percutaneous cement injections without previous interface removal.

Revision surgery in octogenarians

In Chapter 2 we retrospectively reviewed all patients 80 years and older undergoing revision total hip arthroplasty in two hospitals in the Netherlands between 1994 and 2007. Primary objective was social outcome after hospital admittance for revision hip surgery. Secondary objectives were occurrence of complications during hospital stay, patient survival, and use of walking aids before and after revision surgery. After hospital admittance for revision surgery eventually 75% of patients could return to their previous social situation, in 12% the social situation worsened, and in 4% the situation improved. For the patients living independently before the revision surgery, we used a logistic regression analysis to predict which patients could return home, and which patients had to go to a nursing facility for a longer period of time. The regression analysis revealed that the sole predictor for returning home was the presence of a spouse. This
Chapter 10

A feature was also mentioned in the study by Strehle et al., who found that 95% of patients living with a spouse could return to home, compared to 70% of patients not living with a spouse. Although revision surgery of the hip prosthesis was intended to improve the ADL (Activities in Daily Living) of the patients, our study shows that the social situation worsened more than improved. There can be several reasons for this. One explanation can be that although function of the hip is improved by revision surgery, the patient’s condition is deteriorated by the hospital stay. Although there was no correlation between hospital stay and the number of complications occurred, the duration of hospital stay was long (mean of 34 days), and the amount of complications was high, with a mean of 1.3 complications per patient, and a statistically significant correlation between ASA - category (American Society of Anesthesiologists) and number and seriousness of complications. 26% of the patients had a delirium, implicating that the revision surgery had a high impact on physical and mental status. Beside deterioration of the physical and mental status of the patient during hospital stay, another explanation for worsening in social situation is that the situation at home was already unacceptable, and the hospital admittance was the trigger to change it. Another feature that involves elderly patients is that the risk for peri-prosthetic fractures is higher than in younger patients. This is explained by the fact that elderly patients usually have a poorer bone stock. Although we only studied octogenarians, we found that 13% of patients in ASA-3 had a periprosthetic fracture, compared to 5.4% of patients in ASA-2 ($p = 0.10$). In conclusion, chapter 2 shows that revision hip surgery has a high impact on the physical, mental and social status of elderly patients. Indications for revision surgery should be extensively considered, and consequently there remains a group of elderly patients with serious comorbidity and/or a low bone stock, who are not eligible for revision surgery. For these patients the alternative treatment described in this thesis is developed.

Preclinical studies
For the removal of the interface tissue between prosthesis and bone a gene-directed enzyme prodrug therapy (the vector CTL102 in combination with the prodrug CB1954) is used. The primary effect of CTL102 infection of a cell is the delivery of the Ntr gene expression cassette. After cell entry and unpackaging from the viral capsid, the CTL102 genome exists as an extra chromosomal DNA element from which the machinery of the infected cell transcribes Ntr mRNA. This in turn will be translated into active nitroreductase, which will accumulate in the cytosol. Intracellular Ntr activity is responsible for the activation of prodrug CB1954 to a toxic bifunctional alkylating agent inside the cell. Cells able to bioactivate CB1954 are cytotoxically affected by crosslink formation.
at very high frequency. As a consequence, cells activating CB1954 are destroyed.\textsuperscript{18,34} The intended outcome is that infected cells expressing Ntr, which take up CB1954, will be killed by the activated prodrug. Synovial tissue has the histological and histochemical characteristics of interface tissue\textsuperscript{43} and in a previous study in our lab by Goossens \textit{et al.}\textsuperscript{46} it was demonstrated that genes can be transferred to synovial tissue \textit{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 \textbf{Chapter 3} the efficient killing of interface cells by gene-directed enzyme prodrug therapy is shown. To test the susceptibility of interface cells to HAdV-5 vectors, primary cultures of interface cells were exposed to the HAdV-5 vector Ad.CMV.LacZ. The transduction efficiency increased with vector concentration and at 400 pfu/ cell 88\% of cells expressed the reporter gene. Separately, the vector CTL102 and the prodrug CB1954 were not toxic to the interface cells at low to intermediate doses. However, CTL102-transduction of interface cells resulted in a 60-fold sensitisation to the prodrug CB1954. Before intra-articular injection of a therapeutic ingredient the position of the needle in the joint space is usually confirmed by injection of a small amount of contrast medium into the cavity, while making fluoroscopic images. For the use in a clinical study, a possible effect of contrast medium on the efficiency of transduction and killing of interface cells should be tested. Results in chapter 3 show that the contrast medium has no effect on the viability of interface cells but, in the presence of iodide-containing contrast medium, transduction of the cells by an adenoviral vector is almost negligible. The mechanism for this remains unclear, however we showed that the effect is not caused by a change in the cells themselves as transient exposure does not lead to inactivation of the vector. Furthermore, the effect is independent on the receptor, and is not caused by the iodide itself. In conclusion, chapter 3 shows that interface cells can be killed by the Ntr/CB1954 enzyme prodrug approach. However, the currently employed contrast medium cannot be used to verify the position of the needle during intra-articular injection of the vector and prodrug, given the effect on the transduction of cells.

\textbf{Chapter 4} describes two methods we tested to optimise transgene expression. When the gene expression can be made more predictable and efficient, the vector dose can be decreased. This has several advantages, including less evocation of an immune response and a smaller demand for the production of clinical grade adenovirus.\textsuperscript{66} One of the methods to increase gene expression is to facilitate interaction of the promoter with the DNA sequences. Normally, the chromatin of DNA is wrapped around a complex of histone proteins. Sodium butyrate (NaB) causes hyperacetylation of histones, which alters the chromatin structure and increases gene expression. Furthermore, NaB probably upregulates transcription factors which also increases reporter-gene expres-
We showed that NaB at a concentration of 6mM increases reporter gene expression in vitro with a factor 7 to 16 compared to a control condition without NaB. NaB has two main disadvantages that could withhold use in a clinical study. The first disadvantage is that NaB is rapidly metabolised in vivo (half-life of 6.1 min), making it difficult to maintain at an effective therapeutic level. To overcome this problem esterified butyrate derivatives with longer half-lives have been developed. Probably, when a high concentration of NaB can be achieved locally (when injected in a closed compartment, like a joint), NaB can be advantageous to increase transgene expression in vivo. The second disadvantage is that the effect of NaB will not only be on the transgene intended, but also on other genes that are being transcribed. Additional studies are needed to examine the long-term effects of NaB before it can be used in a clinical gene therapy study.

In an attempt to increase local effect on transgene expression while keeping systemic effects at a minimum, a vector was developed with a ubiquitous chromatin opening element (UCOE) inserted in the vector DNA. The mode of action of this UCOE is that the promoter is packed in a DNA sequence containing methylation-free CpG-islands, making the promoter resistant to heterochromatin-mediated silencing of these genes. This will prevent that transgene expression decreases over time. In our study we found no difference in expression between the vector with and without UCOE. A possible explanation can be that the promoter is not silenced in the first days after infection, rendering no effect of an anti-silencer insert. In a clinical study where transgene expression is needed for a longer period of time, insertion of a UCOE in the vector DNA could probably be a good option.

In all studies a good exposure of the target tissue is essential, and for clinical studies in which the active ingredient is injected intra-articularly, the active ingredient must remain in the joint for a sufficiently long period. Beside size of the therapeutic particle, the integrity of the surrounding joint capsule (containment) is important in retaining the active particles within the joint space. Containment of the joint space can be visualised by arthrography in which the joint space is filled with radio-opaque contrast medium and this procedure is visualised with fluoroscopy. Efficacy of intra-articular treatment is also dependent on the joint volume. In large joint spaces the active ingredient will be more diluted than in small joint spaces or a higher dose will be required with the potential for increased toxicity. In a clinical study where intra-articular injection is used as the method of delivery of the therapeutic ingredient, it is important to perform an arthrogram in the inclusion period. This ensures that patients who have a non-contained joint can be excluded from the study. Furthermore, the volume of the joint space is known and this is important for the preparation of the therapeutic solution. Chapter 5 shows a retrospective study of 221 hip arthograms performed...
for diagnosis of prosthesis loosening. In 74% of the arthrograms the joint space was contained, and these patients would have been suitable for intra-articular therapy. The mean volume of the contained joints was 31 mL. This chapter shows that, as 26% of the patients have a non-contained joint and the volume differs considerably among patients, it is important to perform an arthrogram before intra-articular therapy to know which patients should be excluded from treatment and what volume can be injected.

Clinical studies
After completion of the preclinical studies a study protocol for a phase-1 clinical trial was developed. In this study 12 patients were included. Treatment involved intra-articular injection of the human adenoviral-5 vector CTL102 and, 2 days later, intra-articular injection of the prodrug CB1954. 7 Days later the loosened hip prosthesis was refixed by percutaneous peri-prosthetic bone cement injection. The study protocol was approved by the Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands); The Ministry of Housing, Spatial Planning, and the Environment (VROM, The Hague, The Netherlands), and the local ethics committee and was conducted according to the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, Somerset West, and Edingburgh), and in accordance with the Guidelines for Good Clinical Practice (CPMP/ICH/135/95-July 17, 1996). Chapter 6 describes the clinical protocol in detail.

As the clinical study is a phase-1 trial, safety is the primary objective. Recommendations for grading of acute and subacute toxic side effects (World Health Organisation, 1979) were used to report adverse events. Adverse events were recorded up to 6 weeks after vector injection. Chapter 7 describes all adverse events that occurred during the first 6 weeks after gene therapy. No dose limiting toxicity occurred. Vector administration could be continued until the proposed highest dose of 1 x 10^{11} particles. Nine of 12 patients experienced nausea and vomiting starting 6 h after prodrug injection, with one patient needing parenteral fluid administration because of dehydration. These side effects are likely to be caused by the prodrug (and not the vector), as their occurrence decreased after lowering the prodrug dose and did not increase with the 30-fold increase in HAdV-5 vector. Besides nausea and vomiting eight patients had a rise in aspartate aminotransferase (AST), with a maximum 4 days after prodrug injection, and four of these patients also had a rise in alanine aminotransferase (ALT). These hepatic side effects were asymptomatic and completely reversible. However, as 2 of the patients had grade 2 rises in AST and ALT, and one patient needed parenteral feeding due to dehydration, prodrug dose was lowered to 16 mg/m² after 4 patients were treated. Pathophysiology of the gastrointestinal adverse events of the prodrug is
unknown; two possible explanations are considered. First, the most likely explanation is indirect gastrointestinal epithelial toxicity. Another explanation might be conversion of CB1954 to the activated form by Ntr from *E. coli* present in colonic flora. CB1954 metabolism is predominantly hepatic. This underscores that the rise in transaminase levels can be explained by liver cell damage by CB1954 metabolites during drug clearance. In conclusion, no dose-limiting toxicity occurred with Ntr-CB1954 gene therapy. However, prodrug dose was lowered to 16 mg/m² due to inconvenient gastrointestinal and hepatic side effects.

Secondary objectives in the study were (1) to establish that this procedure does not cause shedding of the virus into the environment (2) histological investigation of biopsies of interface tissue (3) to investigate the possibility to stabilise the loosened prosthesis by means of bone cement and (4) to investigate clinical results, i.e., pain relief and improvement in ADL. Secondary objectives are discussed in Chapter 8. Shedding of the virus in blood plasma, urine, and stool samples, and in nose and throat swabs, was measured by quantitative polymerase chain reaction (PCR) analysis. Two patients in the highest vector dose group (1 x 10¹¹ particles) showed virus load in the blood plasma after 24 h. Therefore, these patients were kept in isolation and new samples were taken the next day, which showed negative. In previous studies with CTL102 as a vector no shedding was shown after 24 h. An explanation for the presence of vector in our study can be that the vector is injected in the joint, which is a more or less closed space from which particles will slowly release. Although adenoviral vector DNA could not be detected outside the joint after 48 h, puncture of joint fluid showed positive for adenoviral DNA even after 11 months in one of the patients. In seven patients needle biopsies provided a sufficient amount of tissue for histological analysis. These samples mostly showed tissue necrosis without apoptosis. This is in concordance with the study by Lipinski *et al.*, who concluded that in CTL102-CB1954 suicide gene therapy, cells appeared to die by pathways that suggest necrosis more than classical apoptosis. Percutaneous cement injection was used to stabilise the prosthesis after killing of the interface tissue. Chapter 8 shows that part of the radiolucent zone can be filled with cement, but not all. The question remains whether stabilisation of the prosthesis with cement injection will lead to increase of bone stock over time, due to reduction of stress shielding of the bone, and consequent bone loading after fixation of the prosthesis. Finally, clinical outcome was assessed by Harris Hip Score and by Visual Analogue Scales (VASs) for pain, walking distance, and activities of daily living. All these features of clinical outcome improved after cement injection, especially in the two highest dose groups. Whether this difference in dose groups is caused by a higher vector dose or by an increasing learning curve for the percutaneous cement injection cannot be differentiated. However, this is inevitable when implementing a new tech-
nique. In conclusion, gene therapy to remove interface tissue, followed by percutaneous cement injection to stabilise the prosthesis is a safe and feasible approach. Clinical function improves according to the patient’s opinion and radiographs show partial filling of the radiolucent zone with cement. However, it remains to be seen whether stabilisation of the prosthesis leads to increase in bone stock over the longer term.

A drawback of gene therapy to remove interface tissue is that patients need to be admitted to the hospital for at least one week. Furthermore, most of the patients suffered from systemic side effects by the prodrug. To investigate whether percutaneous periprosthetic cement injection was possible without previous removal of interface tissue, we performed a small case series on 7 patients. This case series and the results are discussed in Chapter 10. Seven elderly patients not eligible for regular revision hip arthroplasty due to serious comorbidity or poor bone stock were entered in the case series. These patients received CT- and fluoroscopy guided periprosthetic cement injection, like the patients in the gene therapy trial, but without the gene therapy. A mean volume of 16 ml of bone cement could be injected around the stem, and 5.5 ml around the cup of the prosthesis. All patients reported clinical improvement. Due to the low numbers of patients in both studies no conclusions can be drawn on the best method to refix the prosthesis. Furthermore, no predictions can be made for results at the longer term follow up. Although the percutaneous cement injection is a relatively small procedure, we recommend only to use it in patients who are ineligible for revision surgery, as regular revision surgery has proven to be effective although complications may occur. Furthermore, long term results of the percutaneous injections are yet unknown. On the other hand, after percutaneous cement injection, the option for revision surgery is still open. A drawback of percutaneous cement injection is that it can only be performed when the position of the prosthesis is good and the patient’s complaints are not caused by excessive polyethylene wear or recurrent dislocations. Especially in patients with high risks, it is very important to verify that the pain is caused by loosening of the prosthesis, e.g., by marcainisation, and not by other causes (e.g. lower back pathology), before considering revision surgery. In conclusion, chapter 10 shows that percutaneous cement injection around an aseptically loosened prosthesis without previously removing the interface tissue is possible, but since the interface tissue is not removed, it will probably be limited to rare indications. Furthermore, based on the current data no conclusions can be made on the preference to remove the interface tissue before injecting cement. However, it seems logical that removal of the interface tissue will give a better stabilisation compared to a situation where the interface tissue is left in place.