Gerjon Hannink

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UNDER
IMPACTION
RECONSTRUCTION
GRAFTING

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CONTRIBUTING PUBLICATIONS

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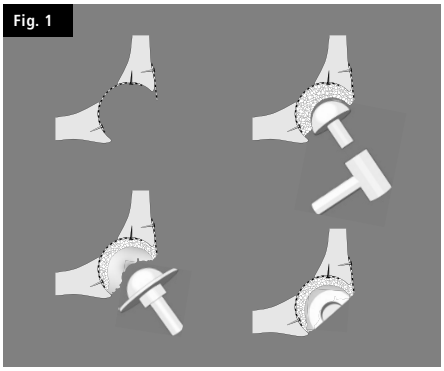
GENERAL INTRODUCTION

INTRODUCTION

Total hip arthroplasty (THA) is one of the most successful medical procedures. The clinical results are very satisfying, with restoration of hip function and relieve of pain symptoms that are associated with osteoarthritis of the hip. The success of THA in combination with the increasing possibilities in hip surgery have led to an extension of the indications for THA. Nowadays, also young patients with severe bone defects due to congenital hip disease or rheumatoid arthritis are indicated for THA. Unfortunately, the survival of hip prostheses is not unlimited. The main reason for loosening of hip implants is aseptic loosening.[1] Aseptic loosening is a complex and multi-factorial event, however the underlying mechanism is still unknown. Although fluid flow and implant motion are associated with bone resorption, the importance of particles in aseptic loosening seems certain.[2, 3] Wear particles that originate from the implant surfaces can induce an inflammatory reaction, resulting in peri-prosthetic bone loss and loosening of the implant. Other factors that may induce prosthetic loosening include infection, fractures or implant related problems.[4-6] Loosening of hip implants necessitates revision surgery. Particularly more active younger patients may require two or more revisions during their lifetime. One of the major problems encountered in hip revision surgery is the loss of bone stock due to the loosening process itself and the removal of the failed implants. Several techniques are available for dealing with bone loss and bone defects in hip revision surgery.[7-14] The type of technique used for reconstruction depends on the type of bone defect present. The American Academy of Orthopaedic Surgeons (AAOS) classification for the acetabulum and proximal part of the femur is used most frequently and describes the type of defect adequately.[15, 16] It distinguishes between segmental, cavitary, and combined segmental-cavitary defects as well as defects with a pelvic discontinuity and/or arthrodesis. Of the many methods of reconstruction available to the surgeon performing a revision, only two techniques have the aim to reconstitute the bone stock, i.e. bone impaction grafting and the use of structural allograft. The objective of bone impaction grafting is to achieve stability of an implant with the use of impacted, morselized bone graft and subsequently allow the restoration of living bone stock by bone ingrowth (Fig. 1 and 2).

Bone impaction grafting has been used successfully as a reconstruction technique on both the acetabular and femoral side in revision total hip replacement. This technique is based on the pioneering work of the groups in Nijmegen,[17] the Netherlands and Exeter,[18] England, and its long term results have been favorable.[19-22] However, there are numerous important issues related to the bone impaction grafting technique. A clear understanding of the mechanical and biological behavior of the morselized grafts in combination with an adequate surgical technique will optimize the technique and improve the clinical outcome. The purpose of this chapter is to discuss the current scientific knowledge of biomechanical and biological characteristics of the bone impaction grafting technique and its future development.



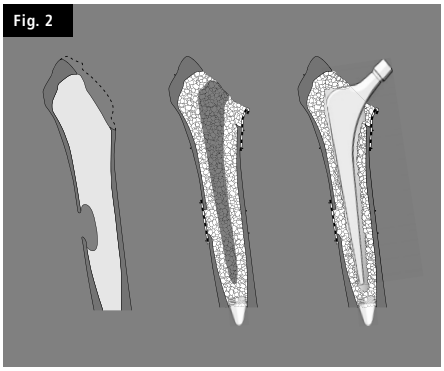


Fig. 1
Bone impaction grafting of the acetabulum. Segmental defects are reconstructed with a metal wire mesh to contain the defect. Trabecular bone grafts are inserted into the defect and firmly impacted using metal impactors and a hammer. A cemented cup is placed after reconstruction of the defect.

Fig. 2
Bone impaction grafting of the femur. A bone plug is introduced into the femoral canal with a central rod attached to the plug. Trabecular bone grafts are introduced into the femoral canal and firmly impacted with metal impactors sliding of the central rod. Proximal impaction is done using a stem phantom. The canal is retrogradely filled with bone cement, followed by placement of the femoral stem.

GENERAL OVERVIEW OF GRAFT INCORPORATION

There is a complex and delicate balance between the mechanical demands of achieving initial stability and the biology of long term incorporation. Incorporation is clearly a biological process, although it depends on a certain degree of mechanical stability. It is not known how much incorporation is needed for long-term success or how long the process of incorporation takes. Histological retrieval analysis has confirmed that remodeling does occur with gradual but variable ingrowth. A goat model has been used to analyze the incorporation and remodeling process of impacted bone grafts histologically at different time points after acetabular impaction bone grafting.[23] At three weeks, the first signs of revascularization and active bone remodeling were seen, progressing to the incorporation of two thirds of the graft at six weeks. The twelve week specimens showed almost total graft incorporation, with the graft mostly being replaced with normal viable bone in the revascularized areas. Complete consolidation and revascularization of the graft were observed in all specimens at 24 and 48 weeks. On the acetabular side, based on human biopsies taken between 1 and 72 months, histological data showed that impacted morselized graft completely transfers into a normal trabecular bony structure with time.[24] A more extensive study of human samples taken after twenty-one revision arthroplasties with impaction bone grafting revealed in some cases a process of endochondral ossification in the graft bed.[25] In another study, twenty-four acetabular bone biopsy specimens were obtained 3 months to 15 years after acetabular reconstruction in primary and revision total hip arthroplasties in combination with a cemented cup.[26] Histological examination showed rapid revascularization of the graft, directly followed by osteoclastic resorption and woven bone formation directly on the graft remnants.

Studies using positron emission tomography have shown that the initial increase in blood flow and bone remodeling following surgery reduced to baseline levels by one year, indicating that the incorporation is complete by this stage.[27] After 6 years, bone metabolism in most areas of the proximal femur was significantly reduced compared to the elevated activity during the first year after surgery, and also normalized compared to the contralateral healthy femur.[28] The rate of incorporation may be related to the degree of impaction. A study comparing bone ingrowth into impacted and non-impacted graft in a bone chamber showed that increasing the degree of impaction reduces ingrowth.[29] However, it is questionable if the used degree of impaction will be ever reached in clinical reconstructions. On the other hand, fibrous tissue ingrowth increases the stability, suggesting that complete osseous remodeling might not even be necessary for a good clinical result.[30] This hypothesis has been supported by retrieval studies indicating that complete graft incorporation was not a prerequisite for clinical success if the resulting construct provided mechanical stability.[31] An observational study of stable implants showed incomplete remodeling and graft incorporation, and the authors concluded that a stable construct could be provided by fibrous tissue ingrowth in areas of unincorporated graft.[32] Clearly, there is still controversy about both the nature and the need for graft incorporation. It may be that fibrous tissue ingrowth can provide an acceptable degree of stability in the long term if full osseointegration does not occur. In the following part relevant items will be discussed in detail.

MATERIALS

Autogenous bone graft is considered the gold standard material for bone grafting because it is osteoconductive, osteoinductive, and it provides osteogenic cells. In addition, there is no risk of infectious disease transmission and it is not accompanied by an immune response that often is present when allografts are used. However, donor site morbidity and lack of volume usually prevents harvesting autograft from an individual at the same time as performing their revision hip surgery. The advantages of allograft bone include unlimited availability and the avoidance of the morbidity associated with harvesting autograft. The disadvantages of allograft bone grafts are immune response, lack of osteogenic cells, decreased activity of osteoinductive factors, and the risk of infectious disease transmission. Marrow elements within the cancellous bone are thought to be responsible for the majority of this immunogenic response. In the past, this has been recognized only as a minor problem. However, there has been recent interest in the detrimental effect of this immunogenic response, with a number of authors reporting improved incorporation and biomechanical performance with histocompatibility-matched grafts.[33, 34]

The osteoarthritic femoral head harvested during hip replacement is the most common source of fresh frozen allografts for impaction bone grafting. It contains three types of tissues: cancellous bone in its bulk, cortical bone (mainly from the neck) and remnants of articular cartilage or synovial lining. During the processing of these femoral heads into bone chips it is important that no cartilage is included, as this may influence mechanical stability and incorporation.[26, 35] It was shown that the presence of cartilage prevented efficient impaction of the morselized grafts, probably due to its elastic nature.[35] In addition, cortico-cancellous grafts without articular cartilage had stiffness values as good as in their control group. It seems unlikely that a few cortical bone fragments will significantly change the biological response of the graft. However, cancellous bone usually is revascularized rapidly and replaced by new host bone. In contrast, cortical bone is revascularized slowly, requiring osteoclasts to resorb bone to provide channels for the invasion of vessel from the host. This osteoclastic activity weakens the graft compared with normal bone for a significant period depending on the size of the graft.[36]

PREPARATION AND PROCESSING

MORSELIZATION

The size and grade of the bone particles are important for the initial mechanical stability of the impacted morselized graft. Particles should be as large as practical to ensure stability, i.e. they should be the largest size that can be fitted between the host cortical bone and the phantom prosthesis. That size is thought to be between 3 and 5 mm in diameter for femoral revisions.[37, 38] On the acetabular side, the ideal size is larger; research suggests 8 to 10 mm diameter chips provide the best initial stability.[39-41] More proximal in the femur, these larger grafts are now also used to improve the initial stability of the stem. Another advantage of larger particles is that they result in a more porous and more permeable impacted graft. This is important because a higher degree of impaction may make it more difficult for new bone to grow into the impacted mass. A study comparing bone ingrowth into non-impacted and impacted bone showed that a higher degree of impaction reduces bone ingrowth.[29] Also larger voids facilitate cement penetration, which may contribute to an improved initial stability. Implants supported by impacted morselized bone graft can migrate as a result of shear within the layers of the impacted graft. Shear strength of the graft layer is improved by using morselized grafts with a range of particle sizes.[42] However, using a range of particle sizes reduces graft permeability, since the pores between the larger particles will be filled with smaller particles. A reduction in permeability may reduce bone ingrowth, but this can be offset by the improved mechanical resistance to shear. More work is needed to clarify the interaction between these mechanical and biological factors.

RINSING

Rinsing grafts has been shown to improve the shear strength of impacted allografts.[42] The mechanisms for this are two fold. First, the removal of fat and marrow fluid reduces the lubrication of particles and thereby increasing frictional resistance. Secondly, removal of fat before impaction increases the compactability of the graft, allowing greater interdigitation between particles. These advantages have been borne out in a whole construct model of femoral impaction grafting in bovine femora and during in vivo human studies.[43] The authors reported a statistically significant reduction in subsidence using rinsed graft compared to using non-rinsed graft. Similar improvements in cup stability have also been demonstrated in an acetabular model.[41] In this study, the force required to tilt the acetabular component by 16 degrees increased from 3450 N to 7000 N when the graft was rinsed with saline before impaction. Rinsing therefore certainly increases mechanical stability. However, the biological effects of rinsing are not as clear. On the one hand, freshly milled graft may contain growth factors and cytokines that stimulate the incorporation of the morselized graft bed by encouraging ingrowth and new bone formation. On the other hand, immunogenic factors present in the freshly frozen allograft bone may reduce bone ingrowth. Recently, a goat model was used to study the effect of rinsing on bone ingrowth into morselized impacted autograft and allograft bone.[44] Without rinsing, the autograft bone showed more bone ingrowth than did the allograft bone. Rinsing prior to impaction reduced ingrowth into the autograft bone but increased ingrowth into the allograft bone.

Similar results have been found with solvent defatted allograft in non-impacted bone chamber models in rabbits.[45] It is not clear whether this increased ingrowth is secondary to the more porous nature of rinsed bone or whether there is some form of biological inhibition from the marrow. Allograft produces an immunogenic response to the graft and most antigenic cells are found in the marrow. Rinsing the graft removes most of the marrow, resulting in a reduction in the immunological load to the patient. Furthermore, the risk of disease transmission is reduced with removal of blood, marrow, cells and bacteria. Rinsing the bone bed also reduces thromboembolic extravasation of bone marrow during cement application. Sherman et al.[46] compared hemodynamic and blood gas changes during THA in a dog model and suggested that thorough bone lavage before cementing had a highly effective prophylactic effect on cardiovascular complications. Similarly, Byrick et al.[47] found a significant reduction of fat emboli using high-volume pulsed lavage in comparison to low-volume lavage and no lavage during cemented arthroplasty in a dog model. The most effective method of rinsing grafts has not yet been determined.

IRRADIATION

Gamma radiation has been widely used for sterilization of bone allografts due to its efficacy against both bacterial and viral disease transmission. During gamma irradiation sterilization highly reactive hydroxyl radicals are formed due to ionization of water molecules. It has been speculated that these free radicals impair the integrity of collagen molecules.[48] In addition, radiation may also denature bone morphogenetic proteins, thereby reducing the osteoinductive potential of the allograft. Due to changes in mechanical properties of bone by gamma irradiation it has been used with some cautiousness in clinical practice, especially in load bearing applications. Gamma irradiation at clinically relevant levels (at least 25 kGy) has no effect on the mechanical properties of frozen unprocessed human cancellous bone but irradiation at a higher dose of 60 kGy was found to significantly reduce the compressive failure stress and the elastic modulus.[49] The effect of irradiation on freeze dried bone is different. A dose of 25 kGy in combination with freeze drying has been shown to reduce the ultimate strength by 42.5%.[50] However, it was also shown that irradiated freeze-dried impacted grafts provide a more stable femoral reconstruction than fresh frozen grafts when tested in a hip simulator.[51] On the other hand, the higher compactness caused by impaction of irradiated bone may reduce the speed of the incorporation process and reduce the cement penetration.

There are few clinical reports of impaction grafting with irradiated bone grafts in hip revision surgery on both the femoral and acetabular side, using radiological and clinical scores as outcome measures. A study evaluating femoral impaction grafting with the Exeter hip using non-rinsed irradiated (25 kGy) bone grafts showed a lacking radiologic incorporation and trabecular remodeling.[52] In contrast, in a 2 to 7 years follow up study using rinsed fresh and irradiated (25-30 kGy) allograft bone in 41 revision femoral athroplasties no significant difference was found in clinical or radiological outcome.[53] Comparing fresh-frozen and irradiated allograft bone in impaction grafting of the acetabulum, no obvious difference in clinical performance with evidence of incorporation and remodeling after 6 months and 13 months for the irradiated graft, using two different radiologic criteria were found.[54] Buckley et al.,[55] using non washed irradiated (25 kGy)

bone for acetabular revision surgery, showed a 88% survival at a mean follow up of five years, which is comparable with that of studies using non irradiated bone. In addition, Hamer et al.[56] histologically examined five cases of rerevisions where irradiated cortical allograft had been used. All cases showed favorable histologic features, with soft tissue attachment by fibrous adhesion, union of graft to host and osseous remodeling. In addition, irradiation of unprocessed femoral heads has also been shown to produce altered lipids from the marrow that are cytotoxic to osteoblast cultures, which may have an impact on bony ingrowth into a graft and long-term survival.[57]

ETHYLENE OXIDE

One other common method of terminal sterilization, often used as alternative for gamma irradiation, is ethylene oxide treatment. There is controversy regarding the suitability of ethylene oxide sterilization for bone grafts,[58] with some authors arguing that it damages the osteoinductive potential of the graft,[59, 60] while others state that it does not.[61, 62]

Ethylene oxide is also potentially carcinogenic and residual gas can cause inflammatory reaction in the recipient. A medium term follow up of ethylene oxide sterilized freeze-dried allograft in acetabular revision only reported one revision for aseptic loosening in 21 cases at a mean 5.8 years, although 62% did show significant acetabular component migration.[63]

FREEZE-DRYING

Freeze-dried cancellous allograft is known to have inferior mechanical characteristics compared with fresh-frozen bone and is rarely used in bone impaction grafting. In vitro impaction studies comparing freeze-dried irradiated allograft with fresh-frozen, have shown that both types of graft reach the same maximal stiffness of 55 MPa, but the freeze-dried bone required three to four times fewer impactions to reach this level.[51] In a separate study, the same authors showed less subsidence in a full hip simulator model with freeze-dried graft.[64] Benefits may include a reduced risk of femoral fracture because of the lower impaction energy required and a reduced risk of disease transmission. However, caution is required when interpreting these studies, as the fresh frozen graft used as a control was not rinsed, a factor that can greatly influence the compactability and stability of the graft. Only few clinical studies have reported the results of this type of graft. A very short-term study, documented no failures at a mean follow-up of 14 months in 40 femoral revisions.[65] The authors used bone scintigraphy in nine patients and found high levels of new bone formation around the prosthesis. In addition, a medium term outcome study of both femoral and acetabular revisions in 32 patient showed a 91% survivorship with all failures on the acetabular side.[66] Thien et al.[67] reported an overall survival rate of 86% at an average follow-up of 7 years for 7 acetabular revisions using impacted freeze-dried cancellous bone chips.

IMPACTION

Probably the most critical aspect of the bone impaction grafting technique is the graft impaction. During the process of graft impaction, plastic deformation of and intergranular motion occur, leading to denser packing of the graft and a permanent deformation with a reduced volume. Only after impaction the morselized graft is strong enough to carry the load imposed by the patient. In vitro studies have confirmed the importance of impaction: the migration of hip stems within a reconstruction with impacted grafts can be largely predicted from the density of the impacted graft. The degree of impaction is mainly influenced by the impaction force (the energy applied per impact) and the number of cycles. Gie et al.,[18] in their original paper on impaction grafting in the femur, recommend "vigorous impaction" and commented on the impressive stability that can be achieved with such a technique. Although using more force creates a more impacted graft bed with improved resistance to mechanical loading, it also has a downside. Studies have shown intra-operative fracture of the femur to be the most common complication of the technique.[68] As argued before, the higher compactness caused by impaction of bone may also reduce the speed of the incorporation process and reduce the cement penetration. An alternative technique to impact morselized bone grafts on the acetabular side with instruments available in every orthopaedic theatre is the so-called "reversed reaming technique". In this technique, the acetabular reamer is used in a reversed direction in combination with manual compression on the reamer. However, it was found that reversed reaming (with small particles) led to an increase of migration of approximately 60% compared to the traditional technique using metal impactors, a hammer and large bone chips.[40]

The impaction energy required for the graft bed to achieve sufficient weight bearing capacity is determined not only by the impaction force and the number of cycles, but also by the material properties of the graft, the particle size and grading and the fat content. Using stronger and stiffer material, larger and better graded particles, and the removal of fat are all strategies that surgeons could employ to reduce the impaction effort and the probability of intraoperative fracture.

CEMENTING

Modern cementing techniques have contributed to improved long-term results in total hip arthroplasty. Thorough cleaning of the bone bed, the use of a distal intramedullary plug, and a proximal seal (representing cement pressurizing techniques) reduce the risk for revision by approximately 20% for each.[69] In addition, new implant designs improve cement penetration, increase the stability of the bone-cement interface and consequently lengthen the lifetime of femoral and, less evidently, acetabular components. Most basic research studies regarding cementing technique are focused on the femur and the femoral component of THA. However, there have been few basic research studies addressing the effect of cementing technique on cement pressurization and penetration in the acetabulum.[70, 71]

For primary total hip replacements, adequate bone cement intrusion into the cancellous bone bed has been shown to be important for success. With increased depth of cement penetration, the strength of the bone cement interface is enhanced.[72, 73] Pressurization and lavage of cancellous bone have been identified to be significant factors with regard to improved cement penetration and consequently improved shear strength. Blood flow may obstruct cleaning and cement intrusion into trabecular bone, which could have a significant effect on results. The optimal amount of cement interdigitation in the revision of failed hip replacements with impaction allografting is not known. Excessive cement intrusion may prevent the remodeling of the bone graft or the cortex if the cement reaches the endosteal surface.[74] On the other hand, insufficient cement interdigitation may lead to stem subsidence.[75] The parameters that determine the cement intrusion into the cancellous bone bed are not well understood.

ALTERNATIVE MATERIALS USED IN IMPACTION GRAFTING

The concept of adding an alternative material to bone allograft is attractive because it reduces dependency on donor supply and raises the possibility of improving the biomechanics of the graft. The shear strength of impacted bone graft has been shown to increase with the addition of small bioglass particles.[76] Other investigators have confirmed that adding stronger or stiffer particles (such as ceramic particles) to the morselized bone improves the mechanical stability. Using an in vitro acetabular model, a 27% reduction in migration of the acetabular component was found by adding a porous tricalciumphosphate (TCP) and hydroxyapatite (HA) mixture to human allografts, whereas a 55% reduction was found when a solid TCP/HA mixture was used.[77] In vitro models using human cadaver femora have also shown increased mechanical stability with a TCP/HA graft extender, but worryingly, also reported a higher risk of (iatrogenic) femoral fractures.[78] An animal model of femoral revision using supplementary TCP/HA mixtures showed no statistically significant differences in loosening or subsidence rates at 18 months.[79] Graft incorporation of TCP/HA particles has been observed in a goat model of contained acetabular deficiencies reconstructed with a 50/50 mixtures of cancellous bone and TCP/HA granules.[80] Although the mechanical stability may be improved with these extenders, the biological aspects of graft incorporation and remodeling have yet to be fully studied. A denser graft may reduce cement penetration and also reduce ingrowth and lead to greater failure. A further concern is the possibility of third body wear of the articulating surface by the TCP/HA particles. Clinically, Oonishi et al. [81] applied HA granules for acetabular reconstruction utilizing a double cementation technique for over a decade. They first used a cement layer in order to completely seal the ceramic materials in a reconstructed acetabulum. Then, they applied a second cement layer on top of the first one to fixate the acetabular cup. After performing an analysis of retrieved specimens, they reported that the HA granules evoked little foreign body reaction after 4-10 years follow-up. The main questions belonging to alternative materials are therefore not mechanical but biological. Clearly, before general clinical implementation of these alternative materials, more data are needed.

GROWTH FACTORS AND ADDITIVES

There has been considerable interest in graft additives to improve allograft incorporation. Growth factor enhanced allograft incorporation could improve clinical outcome after revision surgery by accelerating new bone formation and thereby improving implant stability. Particularly bone morphogenetic protein-7 (BMP-7 or osteogenic protein-1 (OP-1)) has been studied extensively.

OP-1 has been shown to increase bone ingrowth in bone chamber models.[82] However, the density of the new bone may be reduced because of an increased resorption. An animal study of femoral impaction grafting with additional OP-1 showed enhanced initial graft resorption and hastened graft incorporation and remodeling.[83] However, there was one case of excessive stem subsidence in the OP-1 group, suggesting a relation between increased early graft resorption and reduced mechanical stability. No effects of OP-1 on the early and late incorporation of impacted bone grafts were found using a realistic acetabular model in goats.[84] A weight bearing animal model of impaction grafting showed that weight bearing increased graft remodeling but this was not further enhanced by additional OP-1.[85]

Accelerated resorption may have negative consequences for the outcome of bone impaction grafting, since resorption before the formation of bone may compromise implant fixation. A solution can be an osteoconductive material providing initial stability after reconstruction.[86, 87] The role of BMPs in hip revision surgery may then be to serve as a promotor of bone formation in combination with a slow resorbing or unresorbable graft materials.[88, 89]

The use of BMPs in hip reconstructive surgery is not without limitations and potential complications.[90] A BMP cannot overcome a poor biologic or biomechanical environment. In order to induce bone formation and remodeling implant stability must be present if as well as a viable cell source, vascularity, and absence of infection. In addition, maintenance of the BMP at the implantation site and delivery of an appropriate dose by an appropriate carrier material are essential for successful osteoinduction.

Of unknown risk is the role of antibodies that develop in some patients after implantation. Little is known of the effects of these antibodies on bone induction and future healing particularly after repeat application of a BMP, which is not advised.[91] However, BMPs likely play an important role in future hip reconstructive surgery where bone defects and deficits are present.

In addition, the action of bisphosphonates on impacted allograft has also been studied. Aspenberg and Astrand[89] showed in a bone chamber model that the addition of alendronate prevented graft resorption but allowed bone ingrowth. Furthermore, the combination of OP-1 and bisphosphonate (clodronate) increased final graft density and bone ingrowth.[92]

CONCLUSION

Bone impaction grafting in combination with cemented implants for revision surgery of the hip has appeared to be a successful technique for dealing with bone loss. The success of this method depends on achieving adequate initial stability followed by a biological response of graft incorporation and remodeling. Manipulation of the graft to improve one of these factors (biomechanical vs biological) may be detrimental to the other. The ideal graft has not yet been defined. Fresh frozen femoral heads are the most frequently used, but concern remains over the potential for transmission of disease and the future ability to maintain an adequate supply of graft. The ideal size of bone chips for the acetabulum has been well defined but evidence on the femoral side is less certain. Rinsing grafts has been shown to be beneficial but the most efficient method has not been established. One of the greatest problems in assessing impaction bone grafting as a technique and interpreting the literature are the large number of variables and the diverse methods of outcome assessment. Radiologic follow-up needs better definition, specifically with regard to the early signs of failure and the correlation between radiologic and histological changes that may be relevant in clinical practice. Many authors have reported high rates of incorporation, but there are no accepted criteria for such incorporation, and radiographic appearances may well lag behind the clinical situation. Graft incorporation and remodeling has been demonstrated histologically but in vivo methods of monitoring these are limited to bone scintigraphy and positron emission tomography scanning. Less invasive and more sensitive methods of monitoring remodeling would be useful. Impaction grafting is likely to remain one of the preferred options for revision hip surgery. The clinical results are good and support recommendations for the continued use and development of the technique. However, recently, a high risk of complications in revisions using bone impaction grafting in large acetabular bone defects and pelvic discontinuities was reported.[93] Further work is needed on the most appropriate production and processing techniques of the graft in order to generate mechanically stable constructs advantageous to biological incorporation and remodeling.

STRUCTURE OF THIS THESIS

The work in this thesis has evolved around the ongoing development of the bone impaction grafting technique. In this thesis is focused on how the incorporation of impacted morselized bone grafts and bone substitute materials can be influenced and optimized. Several factors play a role in the incorporation of bone grafts and bone substitutes into a new bony structure. To gain more insight in some of these factors, series of animal experiments were performed.

Titanium chambers have been used for almost three decades for studies of bone regeneration in a separate environment without the direct effects of surrounding tissues. By isolating the chambers from surrounding soft tissues to prevent interference with the bone ingrowth process, a standardization of the conditions for a regenerative response can be achieved. The effect of growth factors on bone conductive materials are usually seen at an early stage during bone ingrowth. It is then difficult to find the right time to measure these effects, if new ingrown bone rapidly fills the chamber. Therefore, we used the bone conduction chamber (BCC), a chamber which is never completely filled with bone. The final amount of ingrown bone in the chambers can be used as a marker for incorporation.

The BCC has several applications. Osteoconductive materials can be tested inside the chamber, and the local conditions for bone ingrowth may be manipulated by adding growth factors and drugs.

Using the BCC various materials and factors have been tested for their effect on bone graft incorporation and bone healing. However, biomaterials often have to be crushed to fit in this small chamber. Since cellular responses to biomaterials are influenced by the size and shape of particles, research concerning the evaluation of biomaterials is limited by the dimensions of this bone chamber. In chapter 2 we developed and tested three new large titanium bone chambers, based on the bone conduction chamber, in order to be able to investigate the in vivo influences of bone processing, growth factors and biomaterials on tissue and bone ingrowth, biomaterial resorption and incorporation under reproducible, non-load bearing conditions.

A major point of discussion is the benefit of rinsing of bone grafts. Both rinsing before and before and after impaction are used clinically. However, rinsing before and before and after impaction might have quite different effects on the incorporation of grafts. It is not known how rinsing of morselized bone grafts again after impaction affects the incorporation process. By the impaction procedure many microfractures are created, and biologically active factors in the bone matrix consequently are released. Rinsing the grafts again after impaction might have a negative effect on bone induction if growth factors released by impaction are washed away. In chapter 3 we studied if growth factors are released in physiologically relevant concentrations from the mineralized matrix by firm impaction, and if these growth factors stimulate bone formation in vitro.

Gamma irradiation has been widely used for sterilization of bone allografts. However, gamma irradiation alters proteins. This is favorable when it reduces immunogenicity, but is undesirable when osteoinductive proteins are damaged. Although the effect of gamma irradiation on BMPs has been studied, effect of irradiation on the process of incorporation of morselized bone chips

remains unclear. In chapter 4 we studied the effects of sterilization by gamma irradiation on the incorporation of impacted morselized allografts.

The clinical application of osteogenic proteins offers solutions to many challenging problems in orthopaedics. Bone morphogenetic proteins have the potential to improve clinical outcome after revision surgery by improving graft incorporation and implant fixation. A major concern in using growth factor is the potential stimulation of the osteoclast lineage. A solution would be using an osteoconductive material resistant to resorption and providing initial stability after reconstruction. Growth factors may promote bone formation in combination with such graft materials. In chapters 5 and 6 we studied whether OP-1 promotes the incorporation of impacted morselized allografts and TCP/HA into host bone, if bone formation is preceded by an initial process of accelerated resorption, and if a dose related bone graft remodeling response to OP-1 exists.

In addition, maintenance of the BMP at the implantation site and delivery of an appropriate dose by an appropriate carrier material are essential for successful osteoinduction. At locations with less favorable conditions the BMP clearance might be faster than the bone induction response of the host bone. In chapter 7 we developed a new carrier based delivery system with a localized sustained release. Heparin was covalenty attached to a collagen coated TCP/HA bone substitute and loaded with BMP-7. The location of the BMP on and its release from the coated granules was analyzed.

One of the more promising groups of synthetic graft materials for augmenting acute fracture healing are the calcium phosphate based cements. There has been a great interest in calcium phosphate cements as bone substitute materials because they can be molded to fill a void or defect and are osteoconductive. The suggestion has been made that they might also compete with the PMMA bone cements and apatite coatings for fixation of metal endoprostheses in orthopaedics and oral implantology. In chapter 8 we evaluated a new calcium phosphate cement and compared its crystallographic properties, biological activity, osteoconductive properties and resorption rate with two other commonly used calcium phosphate cements.

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CHAPTER TWO
DEVELOPMENT OF A LARGE BONE CHAMBER

INTRODUCTION

Calcium phosphate-based materials, such as tricalcium phosphate and hydroxyapatite are popular bone graft substitutes in a wide range of applications.[1-3] These materials have a proven osteocompatibility and can act as osteoconductive material. Promising is the combination of both biomaterials and bone inductive substances. There are in vivo models, e.g. critical size defects and titanium chambers,[4-7] in which various materials and factors have been tested for their capacity in graft incorporation and bone healing. In the evaluation of osteoconductive materials, the defect created should be large enough to challenge the adjacent bone with a space that can hardly fill spontaneously. The effects of growth factors on bone conductive materials are usually seen at an early stage during bone ingrowth. It is then difficult to find the right time to measure these effects, if new ingrown bone rapidly fills the defect or chamber.

Particularly, the 'bone conduction chamber' (BCC) of Aspenberg has appeared to be a useful tool for quantifying bone regeneration under the most variable conditions in both rats and goats.[8, 9] This bone chamber is unlikely to be completely filled with bone, so that the effects of growth factors, processing of bone and biomaterials within these chambers can be evaluated as differing final amounts of formed bone. However, biomaterials often have to be crushed to fit in this small bone chamber. Although these materials are identical in composition, this process creates irregularly shaped, sharp or jagged edged particles. When granules of these biomaterials are implanted into bone, bone growth behavior and cellular reactions are different due to the size and shape of the granules.[10-14] Biological responses such as inflammation, bone bonding and resorption of bioactive ceramics are very important in clinical applications.[15] Thus, the dimensions of this bone chamber limits research concerning the evaluation of biomaterials.

Therefore, we developed and tested three new large titanium bone chambers, based on the BCC, in order to be able to investigate the in vivo influences of biomaterials, growth factors and bone processing on tissue and bone ingrowth, biomaterial resorption and incorporation under reproducible, non-load bearing conditions.

MATERIALS AND METHODS

BONE CONDUCTION CHAMBER

Our basic model is the BCC which is a model for membranous ossification (Fig. 1a).[6] The BCC consists of a titanium screw with a cylindrical interior space. It is made up of two threaded half cylinders held together by a hexagonal closed screw cap. One end of the implant is screwed into the bone. The interior of the chamber has a diameter of 2 mm, and a length of 7.5 mm. There are two ingrowth openings for bone ingrowth located at the bone end of the chamber. Thus, the ingrowing tissues enter the cylindrical space from the bone compartment. The chamber extends far out into the subcutaneous region and the ingrown bone-derived tissue can fill the chamber without competing with other tissues. Thus, the tissue ingrowth distance from the holes towards the other end of the chamber can be used to estimate tissue regeneration. The geometry of the chamber makes it easy to distinguish areas for histomorphometry.[6] Since the tibial cortex in rats is thinner than that in goats, we adjusted the BCC for use in goats. A 1 mm disk was placed in the cap of the BCC to provide for location of the ingrowth holes of the chamber to be deeper down, just at the level of the endosteum after the implant is screwed in.[7]

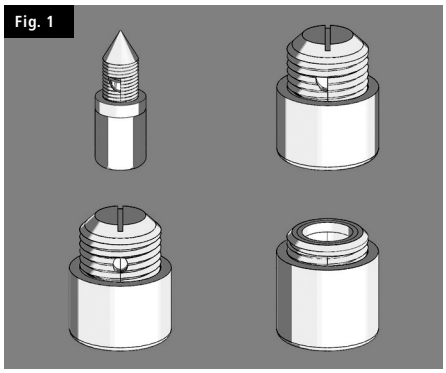
NEW CHAMBERS

All three new chambers are modifications of the BCC. Similar to the BCC, all new models were made of two threaded half-cylinders held together by a cylindrical closed screw cap and fabricated of commercially pure titanium. The interior of the new chambers has a diameter of 6 mm, and a length of 8.5 mm, so all chambers have the same large volume. The outer diameter is 8 mm, the overall length 13 mm. All ingrowth openings were located at the same height at endosteal level, only shape and size differed. The first model (BCC+) had two ingrowth openings, similar in shape and size to that of the BCC, which are located at the bottom of the chamber and are positioned subcortically when implanted into the bone (Fig. 1b). The second model (ROU) had two round ingrowth openings, each 1.5 mm in diameter (Fig. 1c). The third model (BOT) had no ingrowth openings present at the side of the cylinder but had an open bottom to allow bone and tissue ingrowth (Fig. 1d). All new chambers were designed for implantation in the proximal medial metaphysis of the goat tibia.

SURGICAL TECHNIQUE/ ANIMALS AND OPERATIONS

Seven mature Dutch milk goats (Capra Hircus Sana) (48–61 kg) were obtained from the Central Animal Laboratory, University of Nijmegen, the Netherlands. The goats received two empty bone chambers at each side in the cortical bone of the proximal medial tibia (Fig. 2). The position of implantation among the chambers and the side for each type of chamber were randomized. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen, the Netherlands.

Anaesthesia was accomplished by intravenous administration of pentobarbital (CEVA Santé Animale, Maassluis, the Netherlands) (0.5 ml/kg) and maintained after intubation with nitrous oxide, oxygen and isoflurane (1.5-2%). Under aseptic conditions, a longitudinal incision was made in



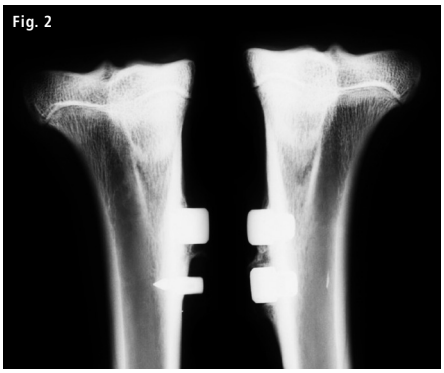


Fig. 1
The bone conduction chamber and the three modified models. All models are made of two threaded half-cylinders held together by a closed screw cap and fabricated of commercially pure titanium. A disk is placed in the cap of the BCC to lower the ingrowth holes to the level of the endosteum. All ingrowth openings are located at the same level in the bone. From left to right: BCC, BCC+, ROU and BOT.

Fig. 2
Radiograph of implanted bone chambers in the proximal medial tibia of the goat.

the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, a hole was drilled through the medial cortex at approximately 4 cm from the joint cleft using a 3.1 mm drill for the BCC and a 7.3 mm watercooled hollow diamond tipped drill (Surgical Diamond Instruments, Scientific Developments GmbH, Munich, Germany) for the modified chambers. The hole was tapped and bone debris from drilling was removed. The bone chamber was screwed in manually. The second bone chamber was placed at a distance of 10 mm from the first one. This was repeated for the other side. The skin was sutured in two layers. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure the animals received subcutaneous ampicillin (Albipen LA, Intervet International BV, Boxmeer, the Netherlands) (15 mg/kg/48 h) three times. The goats received intravital fluochromes (calcein green) (20 mg/kg) at 8 days and 1 day before killing.
After 12 weeks all goats were killed with an overdose of pentobarbital. Tibiae were removed, and the bone chambers with surrounding cortex were fixed in 4% buffered formalin. After 1 day the content was removed from the chambers and fixed additionally.

µCT

All specimens were scanned in a µCT (µCT20, Scanco Medical AG, Bassersdorf, Switzerland) with an isotropic spatial resolution of 9 µm to evaluate the distribution of ingrown bone within the chamber. Bone could be present around the ingrowth holes only and could therefore be missed during histological and histomorphometrical evaluation. In addition, the morphology of mineralized trabecular microstructure was evaluated.
The scans were segmented into binary images. A local segmentation algorithm consisting of a standard edge-detection algorithm, extended to 3D, was used to find the location of the actual

surface of the bone. CT numbers at the position of the bone surface were used to construct a local threshold matrix, which was used to segment the data set. An option in the algorithm was used to accurately represent thin trabeculae that have a size close to resolution limits.[16]
From each specimen a 1x1x1 mm^3 volume of interest was taken at the center of the bottom of the bone chamber. From the 3D-reconstructions we determined trabecular number (Th.N.), trabecular thickness (Tb.Th.) and structure model index (SMI). In all specimens the entire bone volume including bone marrow was measured using a (threshold-based) edge detection algorithm. First, the original thresholded data set (XYZ) was resliced in order to have slices parallel to the longitudinal axis of the chamber (XZY; similar to the sections used for histology and histomorphometry). Subsequently, for each slice the edge detection algorithm was used to find the actual surface of the bone, column by column, from top down. Finally, a line was fitted/drawn through the detected surface points, by which, for each slice, a bone ingrowth area including bone marrow was created and a bone ingrowth volume could be calculated for the entire specimen.

HISTOLOGY

Following µCT scanning the specimens were dehydrated using ethanol and embedded in polymethylmetacrylate (PMMA). The specimens were cut with a microtome (Leica RM 2155, Leica Microsystems Nederland BV, Rijswijk, the Netherlands) parallel to the longitudinal axis of the chamber. Sections were taken at 0, 300 and 600 µm from the center of the BCC specimens, and at 0, 900 and 1800 µm from the center of the larger specimens, each section 5 µm thick. All sections within each experiment were investigated in random order. The tests were blinded, but it was possible to see whether or not a specimen originated from the BCC or BCC+, ROU and BOT. The sections were stained with haematoxylin and eosin, Goldner-Masson trichrome for routine histology or left unstained for fluorescence microscopy.

HISTOMORPHOMETRY

Histomorphometric analysis was performed by using interactive computer controlled image analysis (analySIS, Soft Imaging System GmbH, Münster, Germany). The bone ingrowth distance in each slide was calculated by dividing the new bone area by the width of the specimen.[6] In all specimens marrow cavities surrounded by bone were included in the bone area. The mean of the three sections at 0, 300 and 600 µm from the center yielded a value for the BCC specimen. The mean of sections at 0, 900 and 1800 µm from the center yielded a value for the larger specimens. The total tissue ingrowth distance, which is the distance from the ingrowth end to the fibrous ingrowth frontier, was measured in the same way as bone ingrowth.

STATISTICS

Statistical analysis was performed using a univariate analysis of variance (SPSS Inc., Chicago, Illinois, USA) with factors goat, side, position and chamber type. To isolate the group or groups that differ from the others Tukey's multiple comparison procedure was used. Normality and homogenity of variance were tested using Kolmogorov-Smirnov's and Levene's test.

RESULTS

CLINICAL EVALUATION

No intra-operative complications occurred during surgery. All goats had good recovery after surgery, were standing within 1 day and had a normal gait pattern within 3 days after surgery. There were no signs of inflammation, skin ulceration, or wound healing problems. All bone chambers were strongly fixed into the tibia. In most cases the bone chambers were surrounded with a layer of callus and covered with fibrous tissue, irrespective of the contents of the chamber. No new bone formation was seen at the endosteal surface of the tibial cortex.

HISTOLOGY

Histologic examination of the specimens revealed cancellous bone in all 28 chambers. In the top of the chamber, a zone containing vascularized fibrous tissue was observed, followed by a zone with woven-fibered bone. Near the bottom, there was a zone with more mature, remodeled cancellous bone. Qualitatively, all specimens were similar although differences in ingrowth distances were found. A thin cortical bone plate, sometimes completely, closed the bottom of BOT model chambers.

HISTOMORPHOMETRY

Neither side ($p = 0.240$ and $p = 0.275$) nor position ($p = 0.628$ and $p = 0.512$) of the chamber did affect bone or total tissue ingrowth, respectively. None of the factors interacted. The BCC and the ROU showed similar bone ingrowth distances. No difference in amount of fibrous tissue ingrowth between the chamber types was found ($p = 0.362$). Bone ingrowth was significantly higher in the ROU ($p < 0.01$ and $p < 0.01$) and BCC ($p = 0.027$ and $p = 0.039$) compared to the BCC+ and the BOT, respectively (Fig. 3).

µCT

µCT showed ingrown bone in all chambers (Fig. 4). The new formed bone was not solely present around the ingrowth holes, but covered the entire bottom of the chambers (Fig. 5). Bone ingrowth was significantly higher in the BCC ($p = 0.009$ and $p = 0.036$) compared to the BCC+ and the BOT, respectively (Fig. 6a). No significant difference in bone ingrowth between BCC and ROU was found ($p = 0.529$). Furthermore, no significant differences in Tb.Th. ($p = 0.372$) (Fig. 6b), Tb.N. ($p = 0.503$) (Fig. 6c) and SMI ($p = 0.121$) (Fig. 6d) between the chamber types were found. Correlation indicated a good relationship between bone ingrowth measured using µCT and bone ingrowth measured using histomorphometry ($r^2 = 0.815$) (Fig. 7).

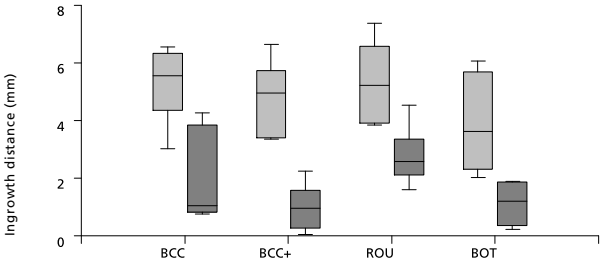


Fig. 3

Fig. 3
Boxplot of histomorphometric results. Fibrous tissue and bone ingrowth distance in all chamber types. There are no statistical significant differences in fibrous tissue ingrowth between the chamber types. There is significantly more bone ingrowth in the ROU ($p < 0.01$ and $p < 0.01$) and BCC ($p = 0.027$ and $p = 0.039$) compared to the BCC+ and the BOT, respectively.

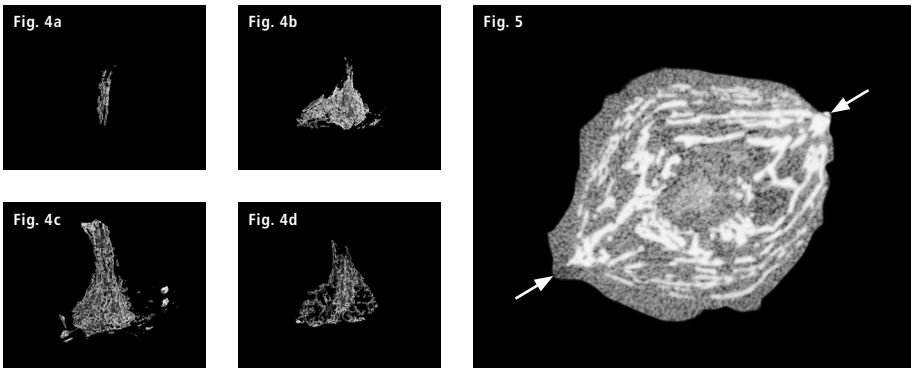


Fig. 4
µCT 3D reconstruction of typical examples of ingrown bone in bone conduction chamber and the three modified models. (a) BCC, (b) BCC+, (c) ROU and (d) BOT.

Fig. 5
µCT 2D cross-sectional image of a typical large bone chamber sample taken at the level of the ingrowth openings. Arrows pointing at ingrown bone at the ingrowth openings. Ingrown bone (white) is surrounded by fibrous tissue (gray).

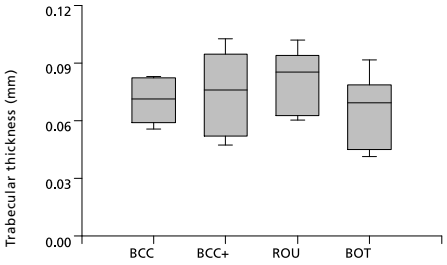


Fig. 6a Chamber type

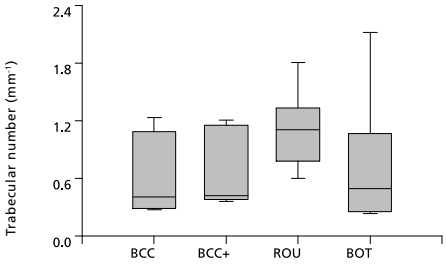


Fig. 6b Chamber type

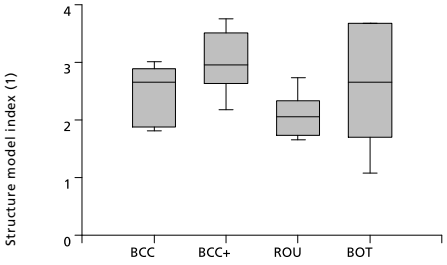


Fig. 6c Chamber type

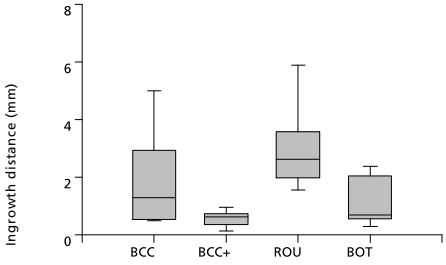


Fig. 6d Chamber type

Fig. 6
Boxplots of µCT-parameters. (a) Trabecular thickness (Tb.Th.), (b) Trabecular number (Tb.N.), (c) Structure model index (SMI) and (d) Bone ingrowth distance. There are no statistically significant differences between the chamber types for µCT-parameters measured.

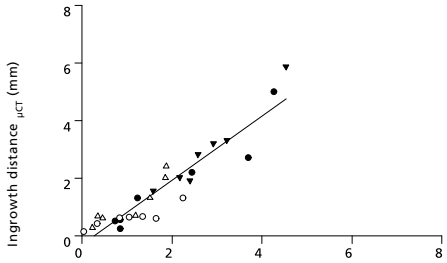


Fig. 7 Ingrowth distance $_{histomorphometry}$ (mm)
BCC ● BCC+ ○ ROU ▼ BOT △

Fig. 7
Correlation indicated a good relationship between bone ingrowth distance measured with µCT and histomorphometry ($r^2 = 0.815$) for all samples.

DISCUSSION

We adapted the bone chamber model used in rodents for use in a larger vertebrate in order to optimize the research of in vivo influences of biomaterials and growth factors on tissue and bone ingrowth, resorption and incorporation under reproducible, non-load bearing conditions. Empty chambers were used as a test of the ability of these chambers to evaluate the in vivo tissue- and bone ingrowth. Bone ingrowth did not completely fill any of the chambers, so the final amount of bone in the chambers can be used for measuring the effect to various attempts to decrease or increase bone formation. This seems to provide a satisfactory model for investigating the speed, amount and quality of bone ingrowth in the goat.

Several factors, including the distance between the ingrowth holes, the mechanical loading situation and, particularly in this study, the geometry of the ingrowth holes, may influence the formation of new bone inside the chambers. First of all, by placing the chambers into the bone, the surrounding tissue is traumatized. This stimulates the production of local trauma factors, which spread in space and stimulate the bone ingrowth.[17-20] Probably, the effect of these paracrine factors can only reach a diffusion-limited distance into the chamber and consequently bone ingrowth will stop at a certain level. The inner diameter (6 mm for the BCC+ and ROU vs. 2 mm for the BCC) and thus the distance from one ingrowth hole to the other differ. This distance can be important in the bone formation process. Bone could be present around the ingrowth holes only and could therefore be missed during histological and histomorphometrical evaluation. Therefore, the distribution of ingrown bone within the chamber was evaluated using µCT. We showed that histology and µCT-data correlated well and thus µCT is not a prerequisite, merely additional for the evaluation of bone ingrowth in bone chambers.

Secondly, mechanical loading conditions are important in bone healing and remodeling, both in the bone chamber but also in the human skeleton.[21, 22] Van der Donk et al.[7] found considerable amounts of bone ingrowth in the BCC after both 6 and 12 weeks, where bone ingrowth in the repeated sampling bone chamber (RSBC) was low or even absent.[23] Based on the differences in design and the differences in bone ingrowth between these bone chambers, Van der Donk et al.[24] hypothesized that in the BCC the bone formation process may be influenced by mechanical stimuli outside the chamber, either by stress-shielding or by soft tissue movement. The stiff and compact outer housing of the RSBC might protect the surrounding bone and inner chamber against mechanical stimuli. In contrast, the BCC consists of two thin titanium walls (at the level of the cap) and possibly some mechanical factors are involved in the bone forming process inside this chamber. Caps were placed over the chambers to provide stress shielding of the BCC. A loose cap was made to rule out that the surrounding soft tissue would result in motion of the chamber. However, no differences were observed for bone and tissue ingrowth between chambers, with or without a cap, either loose or tight fit. If stress shielding is not the reason for the difference in bone ingrowth, then other factors are involved that govern bone and tissue ingrowth into these chambers.

Finally, in the present study, the ROU model performed most similar to the BCC (gold standard) in both tissue and bone ingrowth. Bone ingrowth was less pronounced in the specimens from the BCC+. Remarkably, despite being subjected to the same cross-sectional area (2.25π), bone ingrowth was greater in the specimens from ROU having round, rather than the BCC+ with quarter of circle shaped holes. Moreover, the open bottom 'hole' in the BOT also provided a larger cross sectional area (6.25π), which might result in enhanced ingrowth of bone. However, a thin bone plate was formed, closing the ingrowth hole of the BOT, which seemed to inhibit the ingrowth of further bone into chamber. Only occasionally, tissue entered the chamber through a hole in this bone plate. Particularly the difference between the ROU and BCC+ was quite unexpected.

Previously, variable bone ingrowth was observed using different bone chamber designs (geometry of ingrowth holes) in one species.[24, 25] In a study using the micromotion chamber in rabbits, the chambers containing ingrowth cylinders with a round hole demonstrated less ingrowth as compared to cylinders with a square hole.[25] This observation might have been due to the (21.5%) greater cross sectional area of the square versus the round hole in the cylinder and the enhanced congruity provided by the square outer and inner holes versus a round outer and a square inner hole. When placed in the goat, different bone ingrowth in the BCC and the RSBC was found.[7, 23, 24] However, in the study of Goodman et al.,[25] the shape as well as the cross-sectional area of the ingrowth holes differed. Remarkably, the ingrowth holes of both the chamber types used in the study of Van der Donk et al.[24] had the same cross-sectional area but differed in shape and length of the ingrowth canal. In the present study, the differences in bone ingrowth are probably (solely) caused by the differences in shape of the ingrowth holes.

In designing porous coatings and bone regeneration scaffolds for tissue engineering there are similar debates on bone ingrowth and pore size. The optimal pore size for bone ingrowth often has been reported to be in the range of 150–600 μm.[26, 27] More recent, Hollister et al.[28] examined pore size and geometry issues using designed HA scaffolds in a minipig mandibular defect model. A total of nine designs were created, consisting of three shapes (circular struts, circular pores and square pores) and three pore diameters for each shape. Pore diameters for the circular and square pores were 400, 600 and 900 μm and for the circular struts 600, 900 and 1200 μm. Their results suggested that there is no optimal pore size or shape for bone regeneration. All pore sizes and shapes were conductive to bone ingrowth. In contrast, McGlohorn et al.[29] found that pore topography plays a critical role in the attachment of cells to a porous substrate. They concluded that changes in pore size and shape can radically affected the successful development of tissue. The optimal porosity or pore size for bone regeneration scaffolds and bone chambers remains an open question the answer to which may depend on the mechanical and vascular factors at the site of implantation.

The ROU model performed most similar to the BCC (gold standard) in both tissue and bone ingrowth. This new bone chamber offers new possibilities for studies of bone tissue reactions to various biomaterials (without crushing these biomaterials) and therefore is considered as a promising new tool in biomaterial research.

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CHAPTER THREE
RINSING BONE GRAFTS BEFORE AND AFTER IMPACTION

INTRODUCTION

Bone impaction grafting with a cemented prostheses is a biologically attractive technique for treating bone stock defects around failed total hip prostheses, with long-term clinical survival data available both on the acetabular and the femoral sides.[1, 2] Although the technique on the acetabular side has been well described by the original promoters,[3] a dispute has been raised about the pros and cons of rinsing the allograft bone chips. Both rinsing once (before) and twice (before and after) impaction are used. However, rinsing before impaction or before and after impaction might have quite different effects on the incorporation of the grafts.

Rinsing before impaction enhances the stability of graft reconstructions by creating a higher interparticle shear resistance.[4] Also, in more realistic acetabular testing models with bone impaction grafting, rinsing had a positive effect on the initial stability of the cemented cups.[5, 6] In addition, the use of allografts induces an immunogenic response to the graft. Since most antigenic cells are found in the marrow, rinsing the grafts to remove blood, marrow and fat have been shown to diminish this negative aspect.[7] Bone grafts rinsed before impaction are better incorporated compared to unrinsed allografts.[8]

Rinsing again after impaction is performed, however, only on the acetabular side, in the expectation of an additional effect on the initial mechanical stability through an increase in cement penetration. Although clinically practiced, there is no experimental data supporting the benefit of rinsing again after impaction. It is not known how the rinsing of morselized bone grafts again after impaction affects the incorporation process. Rinsing the grafts again after impaction might have a negative effect on bone induction if growth factors released by impaction are washed away.

By the impaction procedure, many microfractures are created, and biologically active factors in the bone matrix are, consequently, released. Recently, it was shown that a physiologically significant amount of transforming growth factor-β (TGF-β) was released by the strong compression of rinsed cancellous bone blocks from femoral heads.[9] Many contradictory reports have been published on the effects of TGF-βs on bone for mation,[10, 11] however, the effects of TGF-βs on bone graft incorporation are unknown.

Furthermore, it is not known if, other, more potent stimulators of bone induction, like bone morphogenetic proteins (BMPs), are released in physiologically relevant concentrations from the mineralized matrix during impaction. Therefore, we studied if TGF-βs and BMPs are released in physiologically relevant concentrations from the mineralized bone matrix by firm impaction, and if these released growth factors induce osteogenic differentiation in human mesenchymal stem cells (hMSCs).

MATERIALS AND METHODS

PRODUCTION OF "IMPACTION FLUID"

Cancellous allografts were collected from five human femoral heads obtained from the authors' institutional bone bank. Swabs were taken and tested for bacterial contamination. All specimens were clear of bacterial contamination. The allografts were cut to 5x5x5 mm pieces using a rongeur and were subsequently pooled. Blood and marrow were removed macroscopically by rinsing the grafts with saline for approximately 1 min, leaving only a white bone structure. Rinsing was done using a commercially available high-pressure pulsatile lavage system (SurgiLav Plus, Stryker Nederland BV, Waardenburg, the Netherlands). The grafts were in a sieve during rinsing. Allografts were dried overnight in a laminar flow cabinet (sterile airflow) at 4°C.

The bone grafts were inserted into a cylinder with a diameter of 30 mm. A piston, slightly smaller in diameter, was inserted into this cylinder. A rubber ring, sealing the piston, prevented the escape of fluid between the piston and the wall of the cylinder. Using an MTS loading device (MTS Systems Corporation, Minneapolis, MN, USA), the allografts were impacted. Fluid was evacuated through a canal in the bottom of the cylinder and were collected sterile. The applied pressure was calculated to be 12.5 MPa. The gathered fluid was centrifuged for 10 min at 3,000 rpm at 4°C. Any cellular and fatty debris was separated from the supernatant. The supernatant was called "impaction fluid".

ANALYSIS OF "IMPACTION FLUID"

TGF-β1 and TGF-β2 levels in the "impaction fluid" were measured by enzyme-linked immunosorbent assay (ELISA; Quantikine, R&D Systems Europe Ltd., Abingdon, United Kingdom) according to the manufacturer's instructions. The plate was read in an ELISA reader at 450 nm (Sunrise, Tecan Group Ltd., Männedorf, Switzerland). Experiments were performed in triplicate.

The presence of BMPs was determined using a modified BMP-selective reporter bioassay (BRE-luc) inducing luciferase expression.[12] This assay is based on 3T3 fibroblasts stably transfected with the BRE-luc reporter construct. In short, 3T3 BRE-luc cells were seeded in a density of 20,000 per well in microtiter plates in D-MEM (Gibco Invitrogen Corp., Grand Island, NY, USA) with 5% FCS. After 24 h, BMP-containing samples were added and the cells were incubated for a further 20 h. Luciferase activity was quantified using the Bright-Glo Luciferase Assay System (Promega, Madison, WI, USA). Luminescence was measured using a plate luminometer according to the manufacturer's protocol (POLARstar Galaxy, BMG Labtech GmbH, Offenburg, Germany). For the

standard curve, serial dilutions of rhBMP-2 (R&D Systems Europe Ltd., Abingdon, United Kingdom) to final concentrations of 0 to 100 ng BMP-2/ml were used. The lower detection limit of this assay is 1.6 ng/ml BMP-2. Experiments were performed in triplicate.
Almost all members of the TGF-β family, including BMPs, are secreted as latent complexes and need to be activated to exhibit their biological activity.[13] Therefore, both active and total growth factor levels were measured in order to have an impression of latent growth factor levels. The total growth factor levels were measured by activating the growth factors in the samples using hydrochloric acid and, subsequently, neutralizing the samples by sodium hydroxide, according to the manufacturer's instructions.

CULTURE STUDY

Human mesenchymal stem cells (Poietics hMSC, Cambrex Bio Science Verviers, S.p.r.l., Belgium) were cultured up to passage 7 on mesenchymal stem cell growth medium (MSCGM BulletKit (PT-3001), Cambrex Bio Science Verviers, S.p.r.l., Belgium). The cells were incubated in a humidified atmosphere of 92.5% air and 7.5% CO_2 at 37°C. After primary culture, cells were detached using trypsin/ EDTA (Cambrex Bio Science Verviers, S.p.r.l., Belgium), and the cells were seeded in 96-well plates at a density of 20,000 cells/cm². The cells were allowed to attach overnight. After cell attachment, the cells were cultured under static conditions for 7 days. Cells were cultured on osteogenic differentiation medium (hMSC Osteogenic Differentiation BulletKit (PT-3002), Cambrex Bio Science Verviers, S.p.r.l., Belgium) with and without 10^{-7} M dexamethasone (Sigma, Chemical Co., St. Louis, MO, USA). The supernatant ("impaction fluid") was lyophilized. Subsequently, the lyophilized "impaction fluid" was resuspended in osteogenic differentiation medium (without dexamethasone), equal to the original volume of the supernatant. The hMSCs were stimulated with 5 ng/ml TGF-β1 (rhTGF-β1, R&D Systems Europe Ltd., Abingdon, United Kingdom), 10^{-8} M vitamin D3 [$1,25(OH)_2D_3$], 100 ng/ml BMP-2 (rhBMP-2, R&D Systems Europe Ltd., Abingdon, United Kingdom) and 10%, 20%, 40% and 80% "impaction fluid". The culture medium was changed every three days. Experiments were performed in triplicate. Osteogenic differentiation medium without dexamethasone was used as the negative control, whereas osteogenic differentiation medium supplemented with dexamethasone and vitamin D3 was used as the positive control.[14]

ALKALINE PHOSPHATASE ASSAY

Alkaline phosphatase (ALP) activity was measured to obtain information about the osteogenic differentiation of the cells. Medium was removed and the cell layers were washed twice with phosphate buffer solution (PBS). The cells were fixed with 4% buffered formaldehyde and washed twice with PBS again.

The substrate solution was made by adding 1 ml of 10 mM $MgCl_2$ to 10 ml of 1 M di-ethanolamine (DEA) and dissolving 52.6 mg of para-nitrophenyl phosphate (PNPP) in 10 ml of this solution. Subsequently, 100 µl of substrate solution was added to each well and the plate was incubated for 15 min. The reaction was stopped by adding 100 µl of 0.5 M NaOH. The plate was read in an ELISA reader at 405 nm (Wallac VICTOR² 1420, PerkinElmer Life and Analytical Sciences, Inc., Boston, MA, USA).

NEUTRAL RED ASSAY

A neutral red (NR) assay was performed to obtain an indication of the number of viable cells (proliferation). A 1:1 solution of neutral red stock (1 mg/ml milliQ water) and 1.8% NaCl solution was made and filtered. One hundred microliters (100 µl) of this solution was added to each well and the plate was incubated for 90 min at 37°C. Neutral red solution was removed and the cell layers were washed twice with PBS. Subsequently, 100 µl of elution buffer (0.05 M NaH_2PO_4/50% EtOH) was added to each well. The plate was read in an ELISA reader at 550 nm (Wallac VICTOR² 1420, PerkinElmer Life and Analytical Sciences Inc., Boston, MA, USA). The neutral red assay was used to correct the alkaline phosphatase assay for the number of viable cells.

RESULTS

ANALYSIS OF "IMPACTION FLUID"

Analysis of the "impaction fluid" revealed that TGF-β was abundant. Both TGF-β1 and TGF-β2 were present in the "impaction fluid" in physiologically significant amounts. The total levels of TGF-β1 and TGF-β2 present after activation were 5.39±1.12 ng/g bone and 1.20±0.12 ng/g bone, respectively. Before the samples were activated, an already active amount of 0.43±0.05 ng TGF-β1/g bone and 0.13±0.11 ng TGF-β2/g bone was measured (Fig. 1). No BMPs could be detected using the BMP-selective reporter bioassay. Luciferase expression did not exceed the detection limit of 1.6 ng BMP-2/ml.

CELL CULTURE STUDY

Adding dexamethasone to the osteogenic differentiation medium caused an approximately three-fold increase in ALP activity. Vitamin D3 added to osteogenic differentiation medium supplemented with dexamethasone showed the highest induction of ALP activity, whereas the addition of 5 ng/ml TGF-β1 strongly suppressed the induction of ALP activity. The "impaction fluid" dose-dependently suppressed the ALP activity induced by dexamethasone (Fig. 2). Both 40% and 80% "impaction fluid" showed similar suppression to 5 ng/ml TGF-β1.

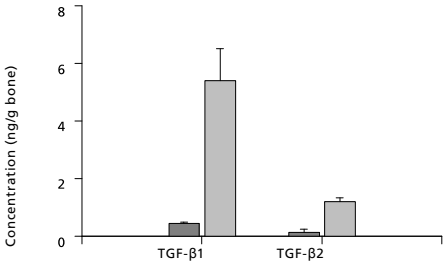

Fig. 1 ELISA
Active ■ Total □

Fig. 1
Levels of transforming growth factor-β1 and -β2 (TGF-β1 and TGF-β2) measured in the "impaction fluid". Both total and already active growth factor levels are shown. Data are mean±standard deviation of experiments performed in triplo.

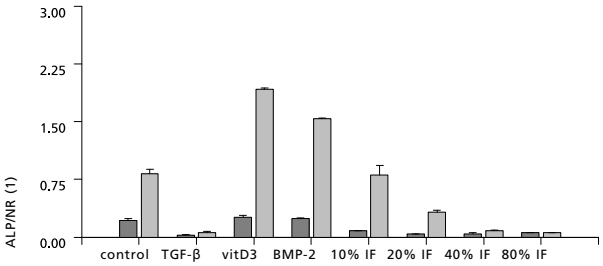

Fig. 2 Culture condition
Without dexamethasone ■ With dexamethasone □

Fig. 2
Data from cell culture. Human mesenchymal stem cells (hMSCs) were cultured with and without dexamethasone. As a measure for osteogenic differentiation, alkaline phosphatase activity (ALP) was corrected for the number of viable cells (NR). Data are mean±standard deviation of experiments performed in triplo.

DISCUSSION

The fracture surface created by impaction could have a growth promoting effect by enabling the release of growth factors. Rinsing morselized bone grafts again after impaction, therefore, might have a negative effect on bone induction if growth factors released during impaction are washed out. In our study, we examined if TGF-β and BMPs are released from the mineralized matrix by impaction, and whether these released growth factors induce osteogenic differentiation in hMSCs.

Both TGF-β1 and TGF-β2 were abundantly present in "impaction fluid". Corresponding to what is seen in demineralized bone, in our study, most TGF-β was present in a latent form. Bone grafts from five femoral heads were pooled to minimize variation to overcome possible bias. However, it should be noticed that a large variation of TGF-β exists in human bone tissue both in tissue location and between individuals.[15] Our results are in accordance with the results of Fyhrie et al.[9] who recently showed that strong mechanical compression of cancellous bone blocks from femoral heads accelerates the release of TGF-β2. In addition, they and others suggested that mechanical loading also might accelerate the release of other growth factors, like BMPs, from mineralized bone matrix.[9, 16] In our study, however, no BMPs could be detected. In the light of the overwhelming literature on BMPs in bone,[17, 18] we have to conclude from this experiment that BMPs cannot be released from the mineralized matrix by impaction. The BMPs in bone are considered to be activated by demineralization;[19] however, as described in Burwell's classical work on bone grafting, some new bone formation may be induced also from non-demineralized allografts.[20] If all BMPs are retained in the bone, this might explain the coupling of resorption and new bone formation during the incorporation of morselized cancellous bone in bone impaction grafting.[21]

Since TGF-β is abundantly present in the "impaction fluid", it is not surprising to see a suppression of osteogenic differentiation with an increasing concentration of "impaction fluid" in our culture experiment. TGF-β is known to inhibit the ALP expression of mesenchymal stem cells (MSCs), even when these cells are stimulated with BMPs.[11, 22] In contrast to BMPs, TGF-β is unable to induce osteogenesis in mesenchymal pluripotent cells.[11, 23] From this perspective, it seems that rinsing bone grafts again after impaction might even be beneficial if TGF-β is washed away.

Data from numerous in vitro experiments have demonstrated the role of TGF-β in every stage of bone formation.[11, 24] Despite conflicting results, most data support the following model:[11] TGF-β increases bone formation in vitro mainly by recruiting osteoblast progenitors and stimulating their proliferation, thus, expanding the pool of committed osteoblasts, in addition to promoting the early stages of differentiation (bone matrix production). On the other hand, it blocks later phases of differentiation and mineralization. These later stages are regulated by other growth factors, like BMPs. In other words, TGF-β generally inhibits the mineralization of the matrix it helps to produce.

However, TGF-β not only modulates bone formation, but it can also stimulate osteoclast recruitment, formation and function.[25, 26] Bone resorption is more detrimental for the outcome of impaction grafting than just suppression or lack of bone formation, because resorption may compromise implant fixation.[27, 28] In addition, little or no BMPs from bone can be released prior to demineralization. Only biological remodeling might free these factors (BMPs) to stimulate bone formation. Therefore, removing the excess of TGF-β from bone grafts might be a useful procedure.

On the contrary, TGF-β released after impaction might, indeed, be beneficial at an early stage postoperatively when chemotactic and proliferative signals might be needed. Furthermore, TGF-β promotes the early stages of osteoblast differentiation. However, the fluid released by the impaction procedure in clinical surgery will, probably, only be in situ for a short period of time at the very early postoperative stage, when osteoblast differentiation is not relevant.

It should be noticed that results obtained in vitro are often not in line or even contradictory to in vivo observations. In vivo, the presence of other growth factors, cytokines and hormones in the bone environment and the environment itself determine the exact outcome of TGF-β function.

In summary, by rinsing morselized bone grafts again after impaction, growth factors (TGF-βs) released during impaction are washed out. Although TGF-β appears to be a potent stimulator of chemotaxis and the proliferation of osteoblasts, but also of osteoclast recruitment, formation and bone resorption, more potent stimulators of bone formation (BMPs) are not released from a mineralized matrix during impaction. To our knowledge, there is only one study reporting in vivo data on the effects of rinsing and the impaction of morselized bone grafts on bone ingrowth.[8] Rinsing before impaction increased bone ingrowth in the allograft group to approach that of autografts. However, rinsing after impaction did not additionally alter bone ingrowth. The results from our study, and the dual effects of TGF-β reported in the literature, leave us only able to speculate whether rinsing grafts again after impaction has a beneficial effect on the incorporation process.

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CHAPTER FOUR
NO EFFECT OF IRRADIATION ON THE INCORPORATION PROCESS

INTRODUCTION

Impacted morselized bone grafts are used successfully to restore bony defects in revision hip surgery in both the acetabulum and the femur.[1-3] Despite promising clinical results with allografts, bone autografts remain the gold standard. However, the use of autografts is limited by the amount of graft available and additional donor site morbidity created by the harvest procedure. In order to obtain results that are more comparable with those of autografts, osteoconductivity is often promoted by rinsing allografts before impaction, thereby improving the incorporation process.[4, 5] However, one remaining major disadvantage of using allografts is the potential risk of disease transmission.

Gamma irradiation has been widely used for (terminal) sterilization of bone allografts due to its efficacy in preventing transmission of both bacteria and viruses.[6-9] Due to changes in mechanical properties of bone by gamma irradiation, it has been used with some caution in clinical practice, especially in load-bearing applications. Irradiated morselized bone was found to be less strong, less stiff and significantly more brittle than fresh-frozen control bone when tested in compression models.[10] However, it has been shown that irradiated freeze-dried impacted grafts provide a more stable femoral reconstruction than fresh-frozen grafts when tested in a hip simulator.[11] On the other hand, the higher degree of compactness caused by impaction of irradiated bone may reduce the speed of the incorporation process.[12]

Irradiation has also been found to alter proteins. This is favorable when it reduces immunogenicity, but appears to be undesirable when osteoinductive proteins are altered or damaged. Osteoinductive proteins within the graft may be important in the process of incorporation. These proteins may be released during the resorptive phase of bone graft incorporation, and can have a profound effect on bone cell physiology. Although studies have been performed to determine the effect of irradiation on bone morphogenetic proteins (BMPs),[13-19] the effect of irradiation on the incorporation process of morselized bone chips remains unclear. We studied the effects of sterilization by gamma irradiation on the incorporation of impacted morselized allografts. Using a bone chamber model in goats, the process of bone incorporation was studied in fresh-frozen bone grafts, rinsed bone grafts, and bone grafts that had been both rinsed and irradiated, at 12 weeks after surgery.

MATERIAL AND METHODS

ANIMALS

12 mature female Dutch milk goats (Capra Hircus Sana) (45–56 kg) were obtained from the Central Animal Laboratory, Radboud University Nijmegen, the Netherlands. The goats received two bone chambers at each side in the cortical bone of the proximal medial tibia. We implanted an empty chamber, a chamber containing fresh-frozen impacted allograft, a chamber containing rinsed impacted allograft, and a chamber containing allograft that had been rinsed and subsequently irradiated. The position of implantation among the chambers and the side for each chamber were randomized. All procedures were approved by the Animal Ethics Committee of the Radboud University Nijmegen.

PREPARATION OF GRAFTS

Cancellous allografts were obtained from the sternum of 6 donor goats. Familial bonds between donor and recipient goats were excluded. To prevent bias in terms of different immunological reactions, the allografts were pooled.

One-third of the pooled allograft was used as fresh-frozen allograft. Blood and marrow of the remaining two-thirds were removed macroscopically by rinsing the grafts with saline for approximately 1 min, leaving only a white bone structure. Rinsing was done using a high-pressure pulsatile lavage system (SurgiLav Plus, Stryker Nederland BV, Waardenburg, the Netherlands). Grafts were located in a sieve during rinsing. Half of the rinsed cancellous allograft was subsequently irradiated with a minimal dose of 25 kGy using a ^{60}Co gamma-ray source (Isotron BV, Ede, the Netherlands) at a temperature of -78.5 °C (on dry ice). Bacterial cultures from all three types of grafts were negative. The grafts were stored at -80 °C until use. Before implantation, they were thawed at room temperature and cut into pieces of 2x2x1 mm using a rongeur.

BONE CHAMBER

The bone ingrowth chamber used in this study was a modification of a large bone chamber (ROU),[20] which was in turn based on the bone conduction chamber.[21] The chamber consisted of two threaded half-cylinders held together by a cylindrical closed screw cap, and was made of commercially pure titanium (Fig. 1). The interior of the chamber had a diameter of 6 mm and a length of 8.5 mm. The outer diameter was 8 mm, and the overall length 13 mm. Two additional ingrowth openings were added to the orginal model,[20] resulting in a total of 4 round ingrowth openings, each 1.5 mm in diameter. All ingrowth openings were located at the same height, at endosteal level.

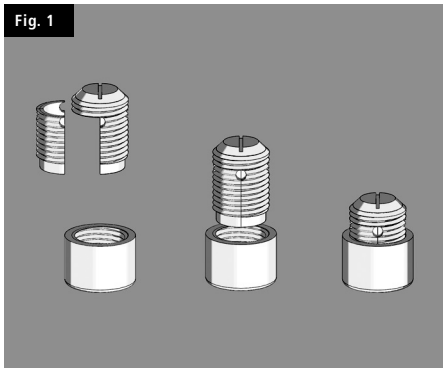

Fig. 1
The bone ingrowth chamber. The chamber consists of two threaded half-cylinders held together by a cylindrical closed screw cap. 4 round ingrowth openings are located at the endosteal level.

IMPACTION PROCEDURE

Impaction was performed by gradually filling the chamber with the allograft bone. A piston, slightly smaller in diameter, was used for impaction. The piston was guided by low-friction bearings, strictly limiting it to vertical movement. The chamber was clamped into a cylindrical holder. A constant force of 680 N was kept on the free end of the piston for 2 min. During this time, fluid could escape between the piston and the wall of the bone chamber and the ingrowth openings. The pressure applied was calculated to be 25 MPa. With this method of impaction, the mean volume fraction rises from about 35% in unimpacted grafts to about 65% in the impacted ones.[12] After impaction, the closed screw cap was screwed on.

SURGICAL TECHNIQUE

The goats were anesthetized by intravenous administration of sodium pentobarbital (0.5 ml/kg) and maintained after intubation with nitrous oxide, oxygen and isoflurane (1.5-2%). Under aseptic conditions, a longitudinal incision was made in the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, a hole was drilled through the medial cortex at approximately 4 cm from the joint cleft using a 7.3 mm watercooled hollow diamond-tipped drill (Surgical Diamond Instruments, Scientific Developments GmbH, Munich, Germany). The hole was tapped, and bone debris from drilling was removed. The bone chamber was screwed in manually. The second bone chamber was placed at a distance of 10 mm distally from the first one. This was repeated for the other side. The subcutaneous layer and the skin were sutured. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure, the animals received subcutaneous ampicillin (15 mg/kg/48 h) 3 times.

After 12 weeks, all goats were killed with an overdose of sodium pentobarbital (1 ml/kg). The tibiae were removed, and the bone chambers with surrounding cortex were fixed in 4% buffered formalin. After 1 day, the content was removed from the chambers and fixed additionally.

EVALUATION

All specimens were scanned in a µCT (µCT20, Scanco Medical AG, Bassersdorf, Switzerland.) with an isotropic spatial resolution of 9 µm to evaluate the bone volume fraction of the impacted graft remnants at the top of the chambers. Impacted irradiated bone could have a higher compactness, thereby influencing the incorporation process.

The scans were segmented into binary images. We used a local segmentation algorithm consisting of a standard edge-detection algorithm, extended to 3D, to find the location of the actual surface of the bone.[22] From each specimen, a 4x4x2 mm volume of interest was taken at the center of the top of the bone chamber. From the 3D-reconstructions, we determined the bone volume fraction (BV/TV) of the graft remnant.

Following µCT scanning, the specimens were dehydrated using ethanol and embedded in polymethylmetacrylate. The specimens were cut with a microtome (Leica RM 2155, Leica Microsystems Nederland BV, Rijswijk, the Netherlands) parallel to the longitudinal axis of the chamber. 5 µm thick sections taken at 0, 600, 1200, 1800 and 2400 µm from the center of each specimen, were used for histology and histomorphometry. All sections within each experiment were investigated in random order. All the tests were done blinded. The sections were stained with hematoxylin and eosin, and Goldner-Masson trichrome for routine histology. Histomorphometric analysis was performed using interactive computer-controlled image analysis (analySIS, Soft Imaging System GmbH, Münster, Germany). The bone ingrowth distance in each slide was calculated by dividing the area of new bone by the width of the specimen.[21] In all specimens, marrow cavities surrounded by bone were included in the bone area. The total tissue ingrowth distance, which is the distance from the ingrowth end to the fibrous ingrowth frontier, was measured in the same way as bone ingrowth. The mean of 5 sections yielded a value for each specimen.

STATISTICS

Statistical analysis was performed using a univariate analysis of variance (SPSS software) with the factors goat, side, position and chamber type. To isolate the group or groups differing from the others, Tukey's multiple comparison procedure was used. Normality and homogenity of variance were tested using the tests of Kolmogorov-Smirnov and Levene. When the assumption of normality or homogenity of variance was not met, a Friedman repeated-measures ANOVA on ranks was performed. P-values less than 0.05 were considered to be significant. The study was designed to have 80% power in detecting a difference of 0.5 mm between the means of all groups.

RESULTS

CINICAL EVALUATION

No intraoperative complications occurred. All goats recovered well after surgery. There were no signs of inflammation, skin ulceration or wound healing problems. All bone chambers were strongly fixed into the tibia. In most cases the bone chambers were surrounded by a layer of callus and covered with fibrous tissue, irrespective of the contents of the chamber. No new bone formation was seen at the endosteal surface of the tibial cortex.

HISTOLOGY

A layer of necrotic, nonvascularized graft remnant was present at the top of the chamber, either with fibrous tissue infiltration or only graft material as when inserted. Fibrous tissue was present between the new bone at the bottom of the chamber and the graft remnant at the top of the chamber. This fibrous tissue preceded the bone ingrowth front (Fig. 2a). The graft material was penetrated by a loose mesenchymal-like tissue with blood sinusoids and capillaries. In the fresh-frozen allografts, a denser fibrous tissue was observed. This group showed a more sharply defined ingrowth frontier, where the fibrous tissue was organized more loosely at the transition with the graft remnants in the rinsed allografts and those that were both rinsed and irradiated. No difference in amount of vascularization was seen. Newly formed bone was present in all bone chambers. New bone was formed by intramembranous ossification, growing upward into the chamber. The amount and appearance of the new bone varied between specimens, from young, woven bone surrounded by active osteoblasts to more mature lamellar bone with fatty marrow and trabeculae. If the resorption of the graft remnant was not complete, new bone was apposited on these remnants (Fig. 2b and 2c). This apposition of new bone was only observed in allografts that had been rinsed and those that had been both rinsed and irradiated.

HISTOMORPHOMETRY

Neither side ($p = 0.7$, $p = 0.5$, and $p = 0.4$) nor position ($p = 1.0$, $p = 1.0$, and $p = 0.7$) of the chamber affected bone ingrowth or total tissue ingrowth or amount of remaining graft, respectively. None of the factors interacted. Rinsing of allografts and subsequent irradiation had no added effect on total tissue ingrowth or bone ingrowth distance relative to rinsing only ($p = 1.0$ and $p = 1.0$) or no processing ($p = 0.7$ and $p = 0.2$), respectively (Fig. 3). However, no processing of allografts resulted in more graft remnant in the top of the chamber compared to rinsing ($p < 0.001$) and both rinsing and irradiation ($p < 0.001$) (Table 1). Irradiation after rinsing had no effect on the amount of graft remnant (resorption process) compared to rinsing alone ($p = 1.0$) (Fig. 3). Ingrowth of fibrous tissue in the empty chamber was significantly less than in the other groups ($p < 0.001$). However, there was significantly more ingrowth of bone in the empty chamber than in the unprocessed allograft group ($p = 0.01$), but this was no different from bone ingrowth in the group with rinsed allograft ($p = 0.4$) and in the group with allograft that was rinsed and subsequently irradiated ($p = 0.5$). Furthermore, no difference in bone volume fraction (BV/TV) of the graft remnants between the groups was found using µCT ($p = 0.6$) (Table 2).

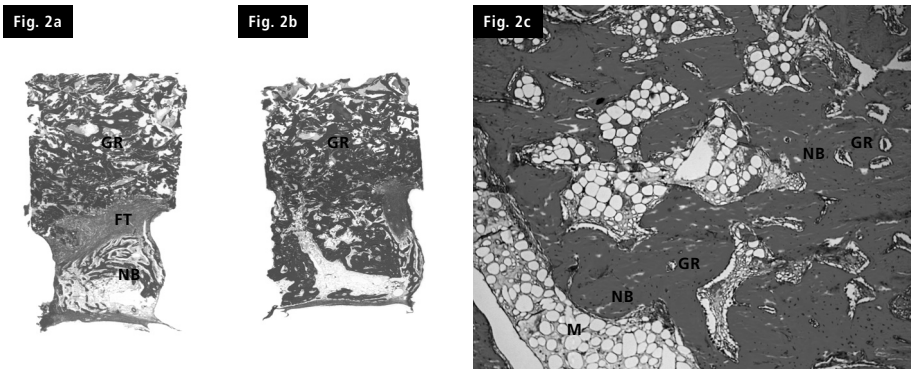

Fig. 2
Hematoxylin and eosin (H&E) stained sections. (a) Typical example of bone chamber content. Graft remnants (GR) are still present in the top of the specimen. New bone (NB) formation takes place from the ingrowth openings up to the top of the chamber. Fibrous tissue (FT) precedes the bone ingrowth front. 12.5x magnification (b) Overview of a rinsed and irradiated specimen. Unincorporated graft remnants (GR) are present at the top of the chamber. Incorporated graft remnants are present below the front of bone ingrowth. 12.5x magnification (c) Detail showing the area of new bone formation. Within this area, new bone (NB) is apposited on graft remnants (GR) at the bottom of the chamber and a marrow cavity (M) has formed. 50x magnification.

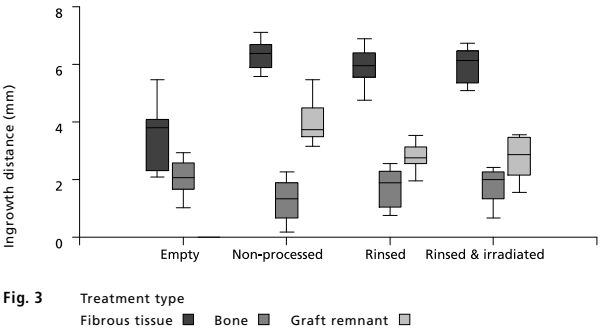

Fig. 3

Fig. 3
Boxplot of histomorphometrical results. Rinsing and subsequent irradiation had no effect on total tissue ingrowth or bone ingrowth compared to rinsing and no rinsing of allografts. No rinsing resulted in more graft remnant at the top of the bone ingrowth chamber.

Table 1

Histomorphometric results

	Implants	Empty	Allograft	Rinsed allograft	Rinsed & irradiated allograft
	(n)	(mm)	(mm)	(mm)	(mm)
Bone ingrowth	12	2.08±0.62	1.32±0.71	1.74±0.66	1.78±0.63
Soft tissue ingrowth	12	3.51±1.21	6.32±0.50	5.96±0.66	5.95±0.62
Graft remnant	12	-	3.99±0.74	2.79±0.49	2.77±0.70

Values are mean ± standard deviation

Table 2

Bone volume fraction of graft remnants

	Implants	Empty	Allograft	Rinsed allograft	Rinsed & irradiated allograft
	(n)	(%)	(%)	(%)	(%)
BV/TV	12	-	65.6±13.7	69.4±13.3	68.7±13.4

Values are mean ± standard deviation

DISCUSSION

During sterilization by gamma irradiation, highly reactive hydroxyl radicals are formed due to ionization of water molecules. These free radicals have been speculated to impair the integrity of collagen molecules.[23] In addition, radiation may also denature bone morphogenetic proteins, thereby reducing the osteoinductive potential of the allograft.

Controversial results concerning the effect of radiation sterilization on the osteoinductive potential of both bone and BMPs have been published. In some studies, irradiation has been found to inhibit or only partially destroy the osteoinductive capacity.[13, 16, 24-26] In others, no effect of irradiation on the osteoinductive capacity has been found.[14, 17-19]

There have been few clinical reports of impaction grafting with irradiated bone grafts in hip revision surgery on both the femoral and acetabular side,[27-30] using radiological and clinical scores as outcome measures. In one study evaluating femoral impaction grafting with the Exeter hip and unwashed irradiated (25 kGy) bone grafts, a lack of radiological incorporation and trabecular remodeling was found.[27] In contrast, in a 2-7 year follow-up study using rinsed fresh irradiated (25-30 kGy) allograft bone in 41 revision femoral arthroplasties, Bankes et al.[28] found no significant difference in clinical or radiographical outcome. Comparing fresh-frozen and irradiated allograft bone in impaction grafting of the acetabulum, Holt et al.[29] found no obvious difference in clinical performance with evidence of incorporation and remodeling after 6 months and 13 months for the irradiated graft, using 2 different radiographical criteria. Buckley et al.,[30] using unwashed irradiated (25 kGy) bone for acetabular revision surgery, showed an 88% survival rate at a mean follow-up time of 5 years, which is comparable with that of studies using unirradiated bone. In addition, Hamer et al.[31] histologically examined 5 cases of re-revision where irradiated cortical allograft had been used. All cases showed favorable histological features, with soft tissue attachment by fibrous adhesion, union of graft to host, and osseous remodeling.

One explanation for the good performance of the rinsed and subsequently irradiated allografts in our study may be that the allograft was irradiated on dry ice and not at room temperature. Recently, Hamer et al.[23] reported that irradiation of cortical bone at dry ice temperature gave partial protection against embrittlement compared to the more common practice of irradiating bone at room temperature. Freezing reduces the mobility of water molecules, and may therefore decrease the production of free radicals. Protection of the osteoinductive properties of bone allografts has been observed when irradiation was performed at low temperatures.[15] Furthermore, the removal of marrow by rinsing the allografts before irradiation may have been favorable, since gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds from osteoblastlike cells.[32] Another explanation may be that the osteoinductive capacity of bone allografts in impaction grafting may be less important than when implanted in a healthy bone bed. Observations on retrieved human allografts suggest that large frozen allografts in humans are osteoconductive rather than osteoinductive.[33]

In summary, we have found no negative effect on graft incorporation after impaction of rinsed and subsequently irradiated bone in an unloaded model. Further clinical studies are warranted.

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CHAPTER FIVE

HIGH DOSES OF OP-1 INHIBIT FIBROUS TISSUE INGROWTH

INTRODUCTION

Aseptic loosening is the main reason for total hip arthroplasty (THA) component failure.[1] The loosening process often is accompanied by progressive bone stock loss around the prosthesis. Several reconstruction techniques to deal with this problem have been suggested.[2, 3] One of these techniques, bone impaction grafting, has become a popular method to restore bone stock deficiencies. The intent of this technique is to obtain stable implant fixation, to restore normal hip biomechanics, and in the long term, to revitalize bone with restoration of normal bone anatomy. Several authors report satisfactory clinical results on the acetabular and femoral sides.[4-7]

The chance of a reconstruction failure is greatest immediately after the reconstruction when the bone graft is not yet incorporated. If the incorporation of bone grafts can be facilitated by growth factors, the critical period after a reconstruction with impacted bone grafts might be shortened.[8-10]

Growth factors, such as bone morphogenetic protein-2 (BMP-2), platelet derived growth factor (PDGF), and osteogenic protein-1 (OP-1 or BMP-7) are well known stimulators of bone formation.[11-13] However, BMP-2 and OP-1 also stimulate osteoclasts in vitro and PDGF has been associated with aseptic loosening of prostheses.[14-18] The role of OP-1 in stimulation of osteoclasts has not been studied in vivo. Preliminary results of a trial in humans with unstable thoracolumbar spine fractures, treated with transpedicular OP-1 transplantation, suggest increased bone resorption as a primary event.[19] Salkeld et al.,[20] using autograft and allograft with OP-1, found an accelerated resorption of the graft material but also substantial formation of new bone. Moreover, a study by Jensen et al.,[21] using impacted mixtures of allograft and OP-1 in a gap model around implants in dogs, showed an extensive effect on new bone formation by OP-1, but also accelerated graft resorption. The results from these studies suggest OP-1 initially enhances osteoclast activity.

Impacted morselized allografts around prostheses serve not only as a bone conductor, but also as a mechanical support for the prostheses. Accelerated bone graft resorption before the formation of bone may cause loss of stability of the prostheses, resulting in micromotion and ultimately failure. An often suggested solution would be an osteoconductive material providing initial stability

after reconstruction.[22-27] Synthetic ceramic calcium phosphate-based materials such as tricalcium phosphate (TCP), hydroxyapatite (HA), and biphasic mixtures of these two components are considered promising materials. TCP and HA are incorporated in a way similar to human bone grafts. However, the remodeling process, as being part of the incorporation process, generally takes place much slower in TCP/HA.[28] The role of BMPs in hip revision surgery then may be to serve as a promoter of bone formation when used in combination with slow resorbing or unresorbable graft materials, such as TCP/HA particles or bone grafts pretreated with bisphosphonates.[29, 30]
We attempted to determine if OP-1 promotes the incorporation of impacted morselized allografts and TCP/HA into host bone, if bone formation is preceded by an initial process of accelerated resorption, and if a dose-related bone graft remodeling response to OP-1 exists.

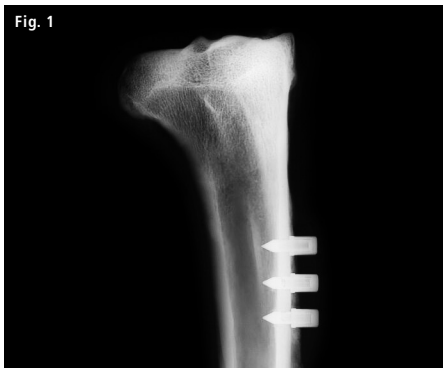
MATERIALS AND METHODS

We performed two experiments, an allograft experiment and a TCP/HA experiment (Table 1). In both experiments, we tested three doses of OP-1 combined with allograft or TCP/HA. In each experiment an allograft control was included. For each experiment, 12 mature Dutch milk goats (Capra Hircus Sana) (range, 46-55 kg) were obtained from the Central Animal Laboratory, University of Nijmegen, the Netherlands. The goats received three bone chambers at each side in the cortical bone of the proximal medial tibia (Fig. 1). The position of implantation among the six chambers and the side for each type of chamber were randomized. We observed the animals for 4 weeks. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen, the Netherlands. In the TCP/HA experiment, one of the groups was used for testing another osteoconductive material. Because the outcome was outside the scope of our hypothesis and the outcome of the other groups was not influenced by this particular group, it was excluded from the statistical analysis.
We used the bone conduction chamber (BCC), which is a model for membranous ossification (Fig. 2).[31] The BCC consists of a titanium screw with a cylindrical interior space. It is made up of two threaded half-cylinders held together by a hexagonal closed screw cap. The interior of the chamber has a diameter of 2 mm, and a length of 7.5 mm. There are two ingrowth openings for bone ingrowth located at the bone end of the chamber. Therefore, the ingrowing tissues enter the cylindrical space from the bone compartment. The chamber extends far out into the subcutaneous region and the ingrown bone-derived tissue can fill the chamber without competing with other tissues. Thus, the tissue ingrowth distance from the holes toward the other end of the chamber can be used to estimate tissue regeneration. The shape of the chamber makes it easy to distinguish areas for histomorphometry.[32] The tissue ingrowth distance can be increased by placing an osteoconductive material, such as a bone graft, in the chamber. Originally developed as a rat model, the BCC was adjusted for use in goats.[33] The threaded end of the implant is screwed into the bone, so that the ingrowth openings are in direct contact with the endosteal transition from marrow into bone. To accomplish this in goats, a 1 mm thick disk was inserted into the cap to lower the ingrowth openings through the cortex.

Table 1

Overview of experimental design

Experiment	Group	OP-1 (µg)	Collagen carrier (mg)
"Allograft experiment"	Allograft	-	-
	Allograft + OP-1 [1]	0.83	0.24
	Allograft + collagen carrier [1]	0	0.24
	Allograft + OP-1 [2]	2.5	0.72
	Allograft + OP-1 [3]	25	7.2
	Allograft + collagen carrier [3]	0	7.2
"TCP/HA experiment"	Allograft	-	-
	TCP/HA	-	-
	TCP/HA + OP-1 [1]	0.83	0.24
	TCP/HA + OP-1 [2]	2.5	0.72
	TCP/HA + OP-1 [3]	25	7.2



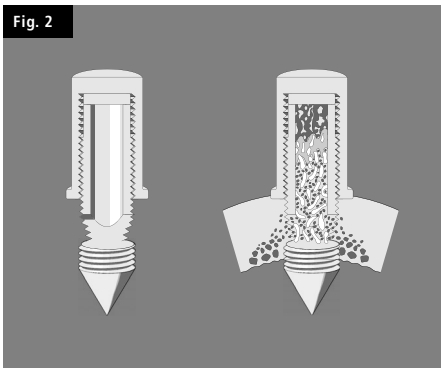


Fig. 1
A radiograph shows implanted bone chambers in the proximal medial tibia of the goat.

Fig. 2
A schematic drawing shows the bone conduction chamber. A 1 mm thick disk was inserted into the cap to lower the ingrowth openings through the cortex. New bone and fibrous tissue are shown growing into the graft. Unremodeled graft is seen at the top of the chamber.

We obtained cancellous allografts from the sternum of six donor goats. Familial bands between donor and recipient goats were excluded. To prevent bias from different immunologic reactions, the allografts were pooled. Most blood and marrow were removed by rinsing the grafts with saline for approximately 1 min, leaving only a white bone structure. Rinsing was done using a high-pressure pulsatile lavage system (SurgiLav Plus, Stryker Nederland BV, Waardenburg, the Netherlands). The grafts were in a sieve during rinsing, and then were stored at -80 °C until use. Cultures from the grafts were negative. Before implantation, the grafts were thawed at room temperature and cut in 2x2x1 mm pieces by using a rongeur.

The TCP/HA particles were composed of 20% HA [$Ca_{10}(PO_4)_6(OH)_2$] and 80% TCP [$Ca_3(PO_4)_2$] (BoneSave, Stryker Howmedica Osteonics, Limerick, Ireland). We used granules with a diameter of 2 to 4 mm. The TCP/HA granules have a 50% non-interconnected macroporosity (range, 300-600 µm), which is produced by burning sacrificial carbonaceous filler during sintering. The granules are also microporous (range, 5-80 µm) (porosity values derived from Stryker Orthopaedics). Before use, the TCP/HA particles were crushed to fit in the BCC and subsequently soaked in saline for 30 min.

The recombinant human osteogenic protein-1 (rhOP-1) device (Stryker Biotech, Hopkinton, MA, USA) was supplied sterile for implantation and consisted of 3.5 mg rhOP-1 combined with 1000 mg highly purified bovine bone-derived type I collagen. Immediately after warming to room temperature, the rhOP-1 device was mixed with a preweighed amount of allograft chips before impaction. The BCC volume was 23.56 mm^3, which allowed 0.0325 g of allograft chips and 0.0163 g of TCP/HA particles to be impacted in each implant.

We tested three doses of the rhOP-1 device, a low-dose OP-1 (0.83 µg/implant), a medium-dose OP-1 (2.5 µg/implant), and a high-dose OP-1 (25 µg/ implant). The medium dose (2.5 µg/ implant) would correspond to approximately one OP-1 device combined with one ordinary femoral head. According to the instructions of the manufacturer, this is the intended dose for bone impaction grafting. We tested two doses of collagen carrier, a low-dose collagen carrier (0.24 mg/ implant) and a high-dose collagen carrier (7.22 mg/implant), which equals the amount of carrier used in the low-dose OP-1 and high-dose OP-1 groups described above.

We performed impaction in the chambers by gradually filling the BCC with the allograft bone/ OP-1 mixes or TCP/HA particles/OP-1 mixes. We used a piston, slightly in smaller diameter, for impaction. The piston was guided by low-friction bearings, strictly limiting it to vertical movement.[32] The BCC was clamped into a cylindrical holder. A constant force of 40 N was kept on the free end of the piston for 2 min. During this time, fluid could escape between the piston and the wall of the bone chamber and the ingrowth openings. The applied pressure was calculated to be 12.5 MPa. The plate was placed in the hexagonal closed screw cap and the cap was screwed on the two threaded half-cylinders.

The goats were anesthetized by intravenous administration of sodium pentobarbital (Nembutal, CEVA Santé Animale, Maassluis, the Netherlands) (0.5 ml/kg) and maintained after intubation with nitrous oxide, oxygen, and isoflurane (1.5-2%). Under aseptic conditions, a longitudinal incision was made in the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, a hole was drilled through the medial cortex approximately 4 cm from the joint

cleft using a 3.1 mm drill. The hole was tapped and bone debris from drilling was removed. The bone chamber was screwed in manually. The other bone chambers were placed 10 mm from the others. This was repeated for the other side.

The subcutaneous layer and the skin were sutured. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure the animals received subcutaneous ampicillin (Albipen LA, Intervet International BV, Boxmeer, the Netherlands) (15 mg/kg/48 hours) three times.

After 4 weeks all goats were sacrificed with an overdose of sodium pentobarbital (Nembutal, 1 ml/kg). Tibiae were removed, and the bone chambers with surrounding cortex were fixed in 4% buffered formalin. After one day, the contents were removed from the chambers and fixed in 4% buffered formaldehyde, dehydrated using ethanol, and embedded in polymethylmethacrylate (PMMA). Specimens from the TCP/HA experiment were decalcified using 25% ethylenedinitrilo tetraacetic acid (EDTA) in 0.1 mol/l phosphate buffer (pH 7.4) before dehydration. The specimens were cut with a microtome (Leica RM 2155, Leica Microsystems Nederland BV, Rijswijk, the Netherlands) parallel to the longitudinal axis of the chamber. Sections were taken at 0, 300, and 600 µm from the center of the BCC specimens,[31] each section 5 µm thick. All sections in each experimental group were investigated in random order. The tests were blinded, but it was possible to see whether a specimen contained allograft or TCP/HA. The sections were stained with hematoxylin and eosin, Goldner-Masson trichrome, and tartrate resistant acid phosphatase (TRAP) for routine histology.

Histomorphometric analysis was performed by using interactive computer-controlled image analysis (analySIS, Soft Imaging System GmbH, Münster, Germany). The bone ingrowth distance in each slide was calculated by dividing the new bone area by the width of the specimen. In all specimens, marrow cavities surrounded by bone were included in the bone area. The mean of the three sections at 0, 300, and 600 µm from the center yielded a value for each specimen. The total tissue ingrowth distance, which is the distance from the ingrowth end to the fibrous ingrowth frontier, was measured in the same way as bone ingrowth.[31]

Statistical analysis was performed using a univariate analysis of variance (SPSS Inc, Chicago, IL, USA) with the factors goat, side, position, and graft type. To isolate the different groups, we used Tukey's multiple comparison procedure. Normality and homogeneity of variance were tested using Kolmogorov-Smirnov's and Levene's tests. When the assumption of normality or homogeneity of variance was not met, a Friedman Repeated Measures ANOVA on Ranks (nonparametric test) was performed. In both experiments an allograft control was included. Therefore, we were able to compare the difference in bone and fibrous tissue ingrowth between equal OP-1-dosed groups by comparing the differences between the OP-1 groups and their allograft controls.

RESULTS

No intraoperative complications occurred during surgery. All goats recovered fully after surgery, were standing within 1 day, and had a normal gait pattern within 3 days after surgery. There were no signs of inflammation, skin ulceration, or wound healing problems. All bone chambers were strongly fixed into the tibia. In most cases, the bone chambers were surrounded with a layer of callus and covered with fibrous tissue, regardless of the contents of the chamber. No new bone formation was seen at the endosteal surface of the tibial cortex.

The incorporation process of impacted morselized allografts and TCP/HA was not promoted by OP-1. A layer of necrotic, nonvascularized graft remnants or TCP/HA granules were present in the top of the chamber, with fibrous tissue infiltration or only graft material as was inserted. In the allograft and TCP/HA groups, a well vascularized loose mesenchymal-like tissue with numerous blood sinusoids and capillaries penetrated the graft material. In the OP-1 and carrier (collagen particles) groups, a denser fibrous tissue was observed. These groups showed a sharply defined ingrowth frontier, where the fibrous tissue was organized more loosely at the transition with the allograft and TCP/HA remnants. However, in general, no accumulation of plasma cells, lymphocytes, or polymorphonuclear cells was seen in OP-1 and non-OP-1 groups. After 4 weeks, newly formed bone was present in part of the bone chambers (Table 2). New bone was formed by intramembranous ossification, mainly at the level of the ingrowth openings, sometimes growing upward halfway into the chamber. If the resorption of the graft remnant was not complete (as seen with the TCP/HA cases), new bone was deposited on these remnants (Fig. 3). Active osteoblasts and osteoid were seen. No cartilage was seen. Regardless of group or OP-1 dose, in some sparse cases, we found a direct contact between newly formed bone and collagen particles. In the allograft experiment, bone ingrowth was higher in the allograft with low-dose OP-1 ($p = 0.019$) and the allograft with medium-dose OP-1 ($p = 0.038$) compared with the allograft with high-dose collagen carrier (Table 3, Fig. 4). However, we observed no difference in bone ingrowth between the groups tested in the TCP/HA experiment (Table 3, Fig. 5).

Bone formation was not preceded by an initial process of accelerated resorption. At the ingrowth front mainly osteoclasts were present as indicated by the TRAP staining. Thus, osteoclasts invaded the grafts clearly ahead of bone ingrowth. After the passage of the ingrowth front, resorbing cells disappeared from the stroma. Multinucleated giant cells were seen only occasionally, close to the resorption front, in the fibrous tissue. In the OP-1 and collagen carrier groups, the osteoclasts were aligning the sharp defined ingrowth front, where in the non-OP-1 groups (controls) the osteoclasts were distributed over the more loosely organized ingrowth front (Fig. 6). However, the number of osteoclasts per bone-resorbing surface seemed similar. In most cases, allografts totally resorbed behind the ingrowth front. In some sparse cases, regardless of group or OP-1 dose, allograft was not totally resorbed and new bone was deposited on the graft remnants. The TCP/HA granules showed no signs of resorption in the form of presence of resorption pits. However, TCP/HA granules/particles were surrounded by TRAP-positive cells (osteoclasts and multinucleated giant cells) (Fig. 6). In addition, very small TCP/HA particles (< 1 mm) were engulfed in mononuclear macrophage-like cells.

Table 2

Number of bone chambers showing bone formation after 4 weeks

Experiment	Group	Total (n)	Bone formation (n)	No bone formation (n)
"Allograft experiment"	Allograft	12	10	2
	Allograft + OP-1 [1]	12	9	3
	Allograft + collagen carrier [1]	12	9	3
	Allograft + OP-1 [2]	12	9	3
	Allograft + OP-1 [3]	12	5	7
	Allograft + collagen carrier [3]	12	5	7
"TCP/HA experiment"	Allograft	12	9	3
	TCP/HA	12	8	4
	TCP/HA + OP-1 [1]	12	11	1
	TCP/HA + OP-1 [2]	12	9	3
	TCP/HA + OP-1 [3]	12	5	7

Table 3

Histomorphometric results

Experiment	Group	Fibrous tissue (mm)	Bone (mm)
"Allograft experiment"	Allograft	3.02±0.83	0.20±0.25
	Allograft + OP-1 [1]	2.89±0.94	0.59±0.78
	Allograft + collagen carrier [1]	2.81±0.82	0.20±0.29
	Allograft + OP-1 [2]	2.17±0.86	0.44±0.42
	Allograft + OP-1 [3]	1.30±0.66	0.26±0.49
	Allograft + collagen carrier [3]	1.68±0.66	0.06±0.11
"TCP/HA experiment"	Allograft	4.43±1.10	0.46±0.74
	TCP/HA	4.75±1.36	0.56±0.59
	TCP/HA + OP-1 [1]	3.82±0.83	0.66±0.42
	TCP/HA + OP-1 [2]	2.47±0.80	0.42±0.41
	TCP/HA + OP-1 [3]	1.50±0.42	0.24±0.41

Values are mean ± standard deviation

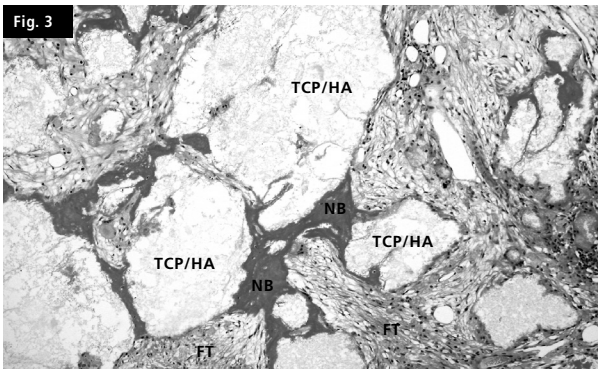


Fig. 3
A typical example of remodeling and incorporation of TCP/HA granules is shown. Newly formed bone (NB) was deposited on TCP/HA granules (TCP/HA) (Stain, hematoxylin and eosin; original magnification, x100).
FT = fibrous tissue.

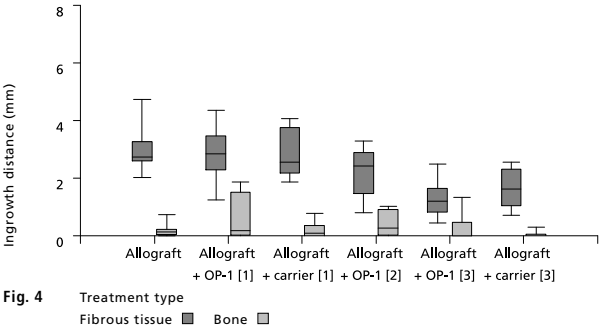


Fig. 4

Fig. 4
Boxplot showing the histomorphometric results of the allograft experiment including the ingrowth distances of fibrous tissue and bone for each treatment group. The OP-1 device doses included: [1] 0.83 µg OP-1 combined with 0.24 mg collagen carrier per implant; [2] 2.5 µg OP-1 combined with 0.72 mg collagen carrier per implant; and [3] 25 µg OP-1 combined with 7.2 mg collagen carrier per implant.
The collagen carrier doses included: [1] 0.24 mg collagen carrier per implant; and [3] 7.2 mg collagen carrier per implant.

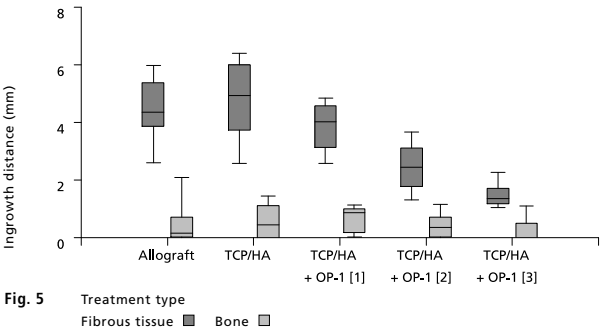


Fig. 5

Fig. 5
Boxplot showing the histomorphometric results of the TCP/HA experiment including the ingrowth distances of fibrous tissue and bone for each treatment group. The OP-1 device doses included: [1] 0.83 µg OP-1 combined with 0.24 mg collagen carrier per implant; [2] 2.5 µg OP-1 combined with 0.72 mg collagen carrier per implant; and [3] 25 µg OP-1 combined with 7.2 mg collagen carrier per implant.

Fig. 6a

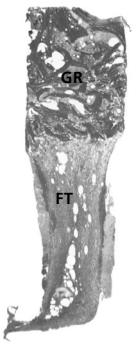


Fig. 6b

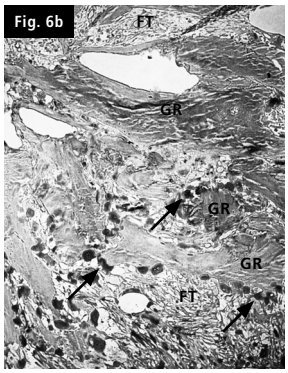


Fig. 6c

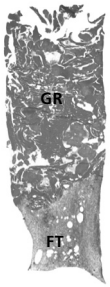


Fig. 6d

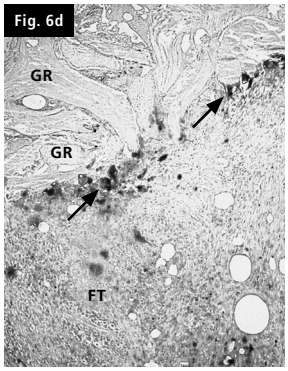


Fig. 6e

Fig. 6f

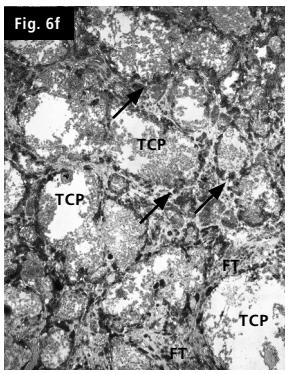


Fig. 6
Typical examples of bone chamber content after 4 weeks are shown (a,c,e). The details show the presence and position of TRAP-positive cells (b,d,f). (a) Loosely organized fibrous tissue (FT) can be seen penetrating the graft material (GR) in the allograft groups. (b) Osteoclasts (arrows) were distributed over the loosely organized ingrowth front. (c) In contrast, a sharply defined ingrowth front was shown in the OP-1 and carrier groups in allograft and TCP/HA groups. (d) Osteoclasts (arrows) aligned the sharply defined ingrowth front. (e) Loosely organized fibrous tissue penetrated the TCP/HA control group granules (TCP). (f) The TCP/HA granules (TCP) were surrounded by TRAP-positive cells (arrows) (Stain, hematoxylin and eosin; original magnification, x12.5 (a,c,e); Stain, TRAP; original magnification, x100 (b,d,f)).

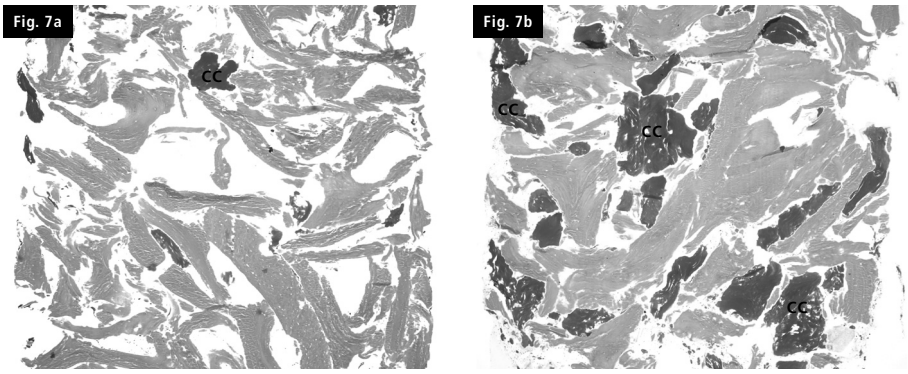


Fig. 7
A dose-dependent response to OP-1 on the incorporation process was found. Depending on the OP-1 dose tested, more or less collagen carrier was entrapped in the spaces between the impacted graft. Type I collagen carrier particles (CC) are shown trapped between the impacted allografts. Typical examples of (a) low-dose OP-1 and (b) high-dose OP-1 are shown (Stain, Goldner-Masson trichrome; original magnification, x50).

At the level of the ingrowth front, we found resorption of the collagen particles as indicated by the presence of osteoclasts adjacent to the particles. The collagen particles did not seem to behave different than the bone grafts.

We observed a dose-related remodeling response to OP-1. An increase in OP-1 dose resulted in an inhibition of fibrous tissue formation. Fibrous tissue penetration into the graft decreased with an increase in OP-1 or carrier concentration. Depending on the OP-1 dose tested, more or less collagen carrier particles were entrapped in the spaces between the impacted grafts or TCP/HA particles (Fig. 7). Fibrous tissue penetration into the graft seemed to be the highest in the allograft and TCP/HA groups without OP-1 added. This was confirmed by our histomorphometric data. The TCP/HA with the high-dose OP-1 (25 µg) showed less ($p < 0.001$) fibrous tissue ingrowth compared with the TCP/HA with the medium-dose OP-1 (2.5 µg). The TCP/HA with the medium-dose OP-1 showed less ($p = 0.003$) fibrous tissue ingrowth than the TCP/HA with the low-dose OP-1 (0.833 µg). The allograft, TCP/HA, and TCP/HA with the low-dose OP-1 (0.833 µg) showed similar fibrous tissue ingrowth (Table 3, Fig. 4). Similar results were found in the allograft experiment. Fibrous tissue ingrowth in the allograft with medium-dose OP-1 did not differ from the allograft with low-dose OP-1 ($p = 0.176$), whereas the allograft with the high-dose OP-1 showed less ($p < 0.001$) fibrous tissue ingrowth compared with the allograft with low-dose OP-1. The OP-1 carrier groups and their OP-1–supplemented counterparts were similar, as was the fibrous tissue ingrowth between allograft, allograft with low- and medium-dose OP-1, and allograft with low-dose collagen carrier (Table 3, Fig. 5). We observed no differences in bone and fibrous tissue ingrowth for allograft and TCP/HA with equal OP-1 doses.

DISCUSSION

The addition of OP-1 to allograft bone may hasten incorporation and remodeling, including an initial process of accelerated resorption. Accelerated resorption before the formation of bone may have negative consequences for bone impaction grafting because this may compromise implant fixation. An osteoconductive material, resistant to resorption and able to provide initial stability after reconstruction combined with growth factors would be a solution. We investigated the early effect of an OP-1 device on the incorporation process of impacted morselized allografts and TCP/HA. Because BMP-7 is combined with a collagen carrier in the OP-1 device, we also studied the effects of the carrier on the incorporation process.

The effect of growth factors on bone conductive materials usually is seen at an early stage during bone ingrowth. It is difficult to find the right time to measure these effects, if new ingrown bone rapidly fills the defect. Therefore, we used the bone conduction chamber, a bone chamber model that is never completely filled with ingrown bone.[31, 33, 34] The final amount of ingrown bone in the chambers can be used to measure the effects of growth factors on bone formation. Extrapolation of results of studies with bone conduction chambers to clinical recommendations should be made carefully, because factors like vascularity and composition of the host bone bed, its interaction with the bone graft, loading conditions, and surrounding tissue are not included in this study. However, the chamber with its limitations has been used extensively, therefore we think it is valid for detecting the effects of bone substitutes and signaling molecules that arise under unloaded conditions. It has been suggested that the BCC can be regarded as a bone tissue culture in vivo.[35, 36] In our opinion, the BCC should be interpreted at that level in the hierarchy of experimental models.

Osteogenic proteins require a viable cell source, vascularity, and mechanical stability to induce bone formation and remodeling.[37] In several studies with positive results from BMP-2 and BMP-7, considerable surgical trauma was present before the introduction of the BMP.[20, 38-42] Trauma such as a fracture or an osteotomy will release endogenous factors that activate migration of inflammatory cells and cells of mesenchymal origin, which can respond to the applied BMP and stimulate bone formation.[43] Our approach involved minimal surgical trauma which may explain the absence of this effect. This explanation also was suggested by Jeppsson and Aspenberg,[44] and Franke Stenport et al.[45] in similar studies using titanium implants. In addition, Bostrom et al.,[46] showed trauma (micromotion) switched the response to BMP-2 from inhibition toward stimulation of bone formation.

The migration of cells into the impacted allograft is compromised and vascularization is delayed for several weeks.[3] This delayed ingrowth after impaction may be related to physical factors, such as the size and numbers of pores for tissue intrusion into the material.[47] The available volume for tissue ingrowth might have been decreased by the impaction of TCP/HA particles, creating small TCP/HA particles between the larger ones.[48] Furthermore, by using higher doses of the OP-1 device, more collagen carrier material was impacted between the graft material, also filling up the space and thereby delaying tissue ingrowth and remodeling.

If the collagen carrier needs extensive exposure to macrophages and other cells to release the active substance in a proper way,[45] the compromised migration of cells into the impacted allograft and the delayed vascularization might have delayed the release of OP-1 from the carrier. This might explain the lack of difference in bone and tissue ingrowth between the bovine type I collagen carrier groups without OP-1 and their OP-1 added counterparts. An alternative explanation could be that a considerable amount of OP-1 was released from the carrier by the process of impaction. The amount of early release may be dependent on the carrier system used.[49] This makes it interesting to compare the results of this study with results of studies in which OP-1 was combined with graft without a carrier system. In a similar bone chamber model in rats, using a dose similar to our lowest dose without a collagen carrier (OP-1 solution) in impaction grafting, Tägil et al.[34] found an increase in bone formation. In contrast, studies using an OP-1 device (with collagen carrier) in combination with impaction grafting showed no or a very moderate effect on bone ingrowth.[50, 51] Moreover, using an OP-1 solution in a weightbearing rabbit knee impaction grafting model, Tägil et al.[26] showed no augmentation of morselized impacted bone graft. In addition, the influence of OP-1 on impacted allograft implants recently was investigated experimentally in loaded, primary, and impaired revision settings.[10] Under primary conditions, using OP-1 solution decreased mean implant fixation; in contrast, under the impaired healing conditions of the revision setting, OP-1 increased incorporation and fixation of the implants. In addition to the dose and carrier system, setting and/or application seems to be important.

In the impaction grafting setting, grafts also seem able to withstand the forces acting on them during revascularization and remodeling. Histologic studies show that full replacement of the grafts by new bone does not always take place.[52-53] Instead, a mixed pattern of living bone and areas of dead graft in a fibrous tissue stroma often is seen. The addition of ingrowing fibrous tissue, with collagen fibers winding between the graft chips might lead to a higher resistance to shear and affect the rate of prosthetic migration. It seems the delayed or reduced new bone ingrowth seen in experiments with impaction therefore, is less important and even may be beneficial, as long as the graft/fibrous tissue composite remains strong enough to withstand forces acting on it during remodeling.[54] In our case, using an OP-1 device also delayed the fibrous tissue ingrowth. Our data suggest the observed early failures in impaction grafting in combination with high doses of OP-1 device could be attributable to the lack of reinforcement by fibrous tissue and not necessarily because of an increase in resorption and remodeling.

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CHAPTER SIX

EFFECTS OF OP-1 DEVICE ON THE INCORPORATION PROCESS

INTRODUCTION

Growth factor-enhanced allograft incorporation could improve clinical outcome after hip revision surgery by accelerating new bone formation and thereby improving implant stability.[1] Bone morphogenetic proteins (BMPs) provide an opportunity to achieve faster and more extensive remodeling.[2] They stimulate bone formation and graft remodeling.[3, 4] However, also BMP stimulated bone resorption has been observed both in vitro and in vivo.[5-9] Accelerated resorption may have negative consequences for bone impaction grafting, since resorption before the formation of bone may compromise implant fixation. One solution would be an osteoconductive material providing initial stability after reconstruction.[10-12] The role of BMPs in hip revision surgery might then be to serve as a promotor of bone formation in combination with a slow resorbing or unresorbable graft material.[13, 14]

In a previous study, we investigated the early effects of osteogenic protein-1 (OP-1) device on the incorporation of impacted morselized cancellous bone and tricalcium phosphate/hydroxyapatite (TCP/HA) in an unloaded bone chamber in goats.[15] After 4 weeks, new bone formation was not promoted by the OP-1 device, and there were also no signs of accelerated resorption. However, a dose-related inhibition of vascularization and fibrous tissue ingrowth was found. In general, in impacted bone grafts, the migration of cells within the allograft is compromized and vascularization is delayed for several weeks.[16-18] In an unloaded bone chamber in rats, at 6 weeks the bone ingrowth into densely impacted allografts was delayed relative to bone ingrowth in allografts that had not been impacted.[19] By adding OP-1 solution to the impacted bone grafts in the same unloaded model, bone ingrowth increased dramatically.[4] In contrast, in our previous study the collagen carrier might initially have further delayed bone and fibrous tissue ingrowth into the impacted graft material by filling up the space between the impacted graft material.[15]

Thus, although the collagen type I carrier may not be optimal for use in bone impaction grafting, in clinical cases better late ingrowth may appear despite an early delay.[20] The present study was designed to determine whether the decrease in ingrowth represented a final loss or was just a delay. If the latter, could the OP-1 device overcome this initial delay and did this ultimately result in a better late ingrowth?

ANIMALS AND METHODS

EXPERIMENTAL DESIGN

12 mature Dutch milk goats (Capra Hircus Sana) (range, 40–56 kg) were obtained from the Central Animal Laboratory, Radboud University Nijmegen, the Netherlands. The goats received 3 bone chambers on each side in the cortical bone of the proximal medial tibia. The side and position of implantation of the 6 chambers were randomized. Two concentrations of OP-1 device were tested in combination with allografts and TCP/HA. Allografts not treated with OP-1 and TCP/HA served as controls (Table 1). The observation time was 8 weeks. All procedures were approved by the Animal Ethics Committee of the Radboud University Nijmegen.

Table 1

Overview of experimental design

Treatment groups	Dose OP-1 device (µg OP-1/mg collagen)	Implants (n)
Allograft	-/-	12
Allograft+OP-1[1]	0.83/0.24	12
Allograft+OP-1[2]	2.5/0.72	12
TCP/HA	-/-	12
TCP/HA+OP-1[1]	0.83/0.24	12
TCP/HA+OP-1[2]	2.5/0.72	12

BONE CONDUCTION CHAMBER

The bone conduction chamber (BCC) consists of a threaded titanium cylinder, formed from two half-cylinders held together by a hexagonal closed screw cap.[21] One end of the implant is screwed into the bone. The interior of the chamber has a diameter of 2 mm and a length of 7.5 mm. There are two ingrowth openings for bone ingrowth located at the bone-end of the chamber.

Thus, the ingrowing tissues enter the cylindrical space from the bone compartment. Originally developed as a rat model, the BCC was adjusted for use in goats.[22] Since the tibial cortex in rats is thinner than that in goats, a 1 mm disk was placed in the cap of the BCC to provide for location of the ingrowth holes of the chamber to be deeper down, just at the level of the endosteum after the implant is screwed in (Fig. 1).

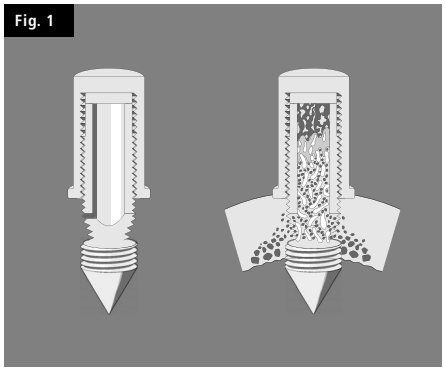

Fig. 1
The bone conduction chamber. The chamber consists of two threaded half-cylinders held together by a cylindrical closed screw cap. There are ingrowth openings at the endosteal level. A 1 mm thick disk was inserted into the cap to lower the ingrowth openings through the cortex. New bone and fibrous tissue are shown growing into the graft. Unremodeled graft can be seen at the top of the chamber.

IMPLANT MATERIALS

Cancellous allografts were obtained from the sternum of six donor goats. Familial bands between donor and recipient goats were excluded. To prevent bias because of different immunologic reactions, the allografts were pooled.

Most blood and marrow was removed by rinsing the grafts with saline for approximately 1 min, leaving only a white bone structure. Rinsing was done using a high-pressure pulsatile lavage system (SurgiLav Plus, Stryker Nederland BV, Waardenburg, the Netherlands). The grafts were in a sieve during rinsing. They were stored at -80 °C until use. Bacterial cultures from the grafts were negative. Before implantation, the grafts were thawed at room temperature and cut into pieces by using a rongeur.

The TCP/HA particles were composed of 20% HA ($Ca_{10}(PO_4)_6(OH)_2$) and 80% TCP ($Ca_3(PO_4)_2$) (BoneSave, Stryker Howmedica Osteonics, Limerick, Ireland). We used granules with a diameter of 2-4 mm. The TCP-HA granules have a 50% non-interconnected macroporosity (range, 300-600 µm), which is produced by burning sacrificial carbonaceous filler during sintering. The granules are also microporous (range, 5–80 µm) (porosity values obtained from Stryker Orthopaedics). Before use, the TCP/HA particles were crushed to fit into the BCC and subsequently soaked in saline for 30 min.

GRAFT PREPARATION

The rhOP-1 device (Stryker Biotech, Hopkinton, MA, USA) was supplied sterile for implantation and consisted of 3.5 mg recombinant human osteogenic protein-1 (rhOP-1) combined with 1 g of highly purified bovine bone-derived type I collagen.

Immediately after warming to room temperature, the rhOP-1 device was mixed with a preweighed quantity of allograft chips before impaction. The BCC volume is 23.56 mm^3, which allowed 0.0325 g of allograft chips and 0.0163 g of TCP/HA particles to be impacted in each implant. Two doses of rhOP-1 device were tested, a low-dose OP-1 (0.83 µg/implant) and a high-dose OP-1 (2.5 µg/implant). The high dose (2.5 µg/implant) would correspond to about one OP-1 device combined with one ordinary femoral head. According to the instructions of the manufacturer, this was the intended dose for bone impaction grafting.

IMPACTION PROCEDURE

Impaction was performed by gradually filling the BCC with the allograft bone/OP-1 mixtures or TCP/HA particles/OP-1 mixtures. A piston of a slightly smaller diameter was used for impaction.[23] The piston was guided by low-friction bearings, strictly limiting it to vertical movement. The BCC was clamped into a cylindrical holder. A constant force of 40 N was kept on the free end of the piston for 2 min. During this time, fluid could escape between the piston and the wall of the bone chamber, and the ingrowth openings. The pressure applied was calculated to be 12.5 MPa. The disk was placed on the two threaded half-cylinders and the hexagonal closed screw cap was screwed on.

SURGICAL TECHNIQUE

The goats were anesthetized by intravenous administration of sodium pentobarbital (0.5 ml/kg) and maintained after intubation with nitrous oxide, oxygen and isoflurane (1.5-2%). Under aseptic conditions, a longitudinal incision was made in the skin and fascia over the medial side of the proximal tibia. After raising the periosteum, the cortical bone was observed and a hole was drilled through the medial cortex at approximately 4 cm from the joint cleft using a 3.1 mm drill. The hole was tapped and bone debris from drilling was removed. The bone chamber was screwed in manually. The other bone chambers were placed 10 mm from the others. This was repeated for the other side. The subcutaneous layer and the skin were sutured. All animals were allowed unrestricted movement in their cages and had free access to water and food after the operation. After the implantation procedure, the animals received subcutaneous ampicillin (15 mg/kg/48 h) three times. After 8 weeks, all goats were killed with an overdose of sodium pentobarbital.

EVALUATION

All specimens were fixed in 4% buffered formaldehyde, dehydrated using ethanol, and embedded in polymethylmetacrylate (PMMA). They were decalcified using 25% EDTA in 0.1 mol/l phosphate buffer (pH 7.4) before dehydration. The specimens were cut with a microtome (Leica RM 2155, Leica Microsystems Nederland BV, Rijswijk, the Netherlands) parallel to the longitudinal axis of the chamber. From each specimen, a total of 5 sections were taken at 0, 300 and 600 µm from the center of the specimen, each section being 5 µm thick. All sections within each experimental group were investigated in random order. The tests were done blind, but it was possible to see whether a specimen contained allograft or TCP/HA. The sections were stained with hematoxylin and eosin (HE), Goldner-Masson trichrome, and tartrate-resistant acid phosphatase (TRAP) for routine histology. Histomorphometric analysis was performed by using interactive computer-controlled image analysis (analySIS, Soft Imaging System GmbH, Münster, Germany). The bone ingrowth distance in each slide was calculated by dividing the new bone area by the width of the specimen.[21] In all specimens, marrow cavities surrounded by bone were included in the bone area. The total tissue ingrowth distance, which is the distance from the ingrowth end to the fibrous ingrowth frontier, was measured in the same way as bone ingrowth. The mean of 5 sections yielded a value for each specimen.

STATISTICS

Statistical analysis was performed using a univariate analysis of variance (SPSS Inc., Chicago, IL, USA) with factors goat, side, position and graft type. To identify the group or groups that differed from the others, Tukey's multiple comparison procedure was used. Normality and homogenity of variance were tested using the Kolmogorov-Smirnov test and the Levene test. When the assumption of normality or homogenity of variance was not met, a Friedman repeated measures ANOVA on ranks (non-parametric test) was performed. This study was designed to have 80% power of detecting a difference of 0.5 mm between the means of all groups.

RESULTS

CLINICAL

No intraoperative complications occurred during surgery. All goats recovered fully after surgery, were standing within one day, and had a normal gait pattern within three days after surgery. There were no signs of inflammation, skin ulceration, or wound-healing problems. All bone chambers were strongly fixed into the tibia. In most cases the bone chambers were surrounded by a layer of callus and covered with fibrous tissue, irrespective of the contents of the chamber. No new bone formation was seen at the endosteal surface of the tibial cortex.

HISTOLOGY

A layer of necrotic, non-vascularized graft remnant was present at the top of the chamber, either with fibrous tissue infiltration or only graft material as it was inserted. Fibrous tissue was present between the new bone at the bottom of the chamber and the graft remnant at the top of the chamber (Fig. 2a). This fibrous tissue preceded the front of bone ingrowth. A loose mesenchymal-like tissue with blood sinusoids and capillaries penetrated the graft material.

Newly formed bone was present in all bone chambers. New bone was formed by intramembranous ossification, with growth upward into the chamber. The amount and appearance of the new bone varied between specimens—from young, woven bone surrounded by active osteoclasts to more mature lamellar bone with fatty marrow and trabeculae (Fig. 2b). No resorption of the graft material was observed in the TCP/HA-filled chambers (Fig. 2c). Apposition of new bone on the TCP/HA was observed in all TCP/HA-filled chambers (Fig. 2d). No collagen carrier material could be seen filling up the spaces between the impacted graft materials.

Fig. 2a

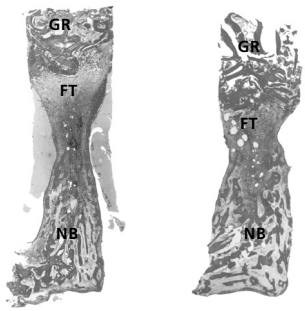

Fig. 2b

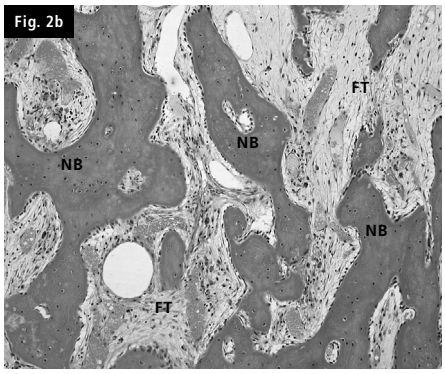

Fig. 2c

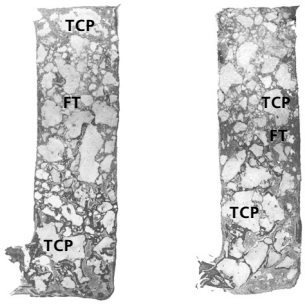

Fig. 2d

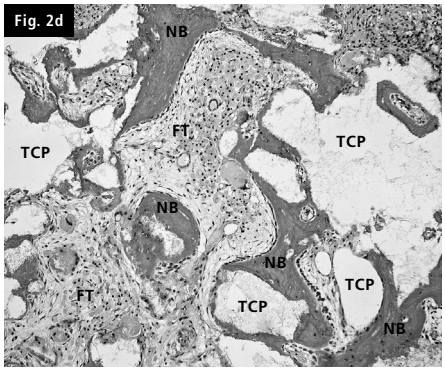

Fig. 2
(a) Typical examples of the bone chamber content of allograft control (left) and allograft with high-dose OP-1 (right). Graft remnants (GR) are still present in the top of the specimen. New bone (NB) formation takes place from the ingrowth openings upward to the top of the chamber. Fibrous tissue (FT) precedes the front of bone ingrowth. Hematoxylin-eosin stained section. Magnification: x12.5. (b) Detail of allograft with high-dose OP-1 specimen showing newly formed bone at the bottom of the chamber. There were no differences in appearance of newly formed bone between the controls and either low-dose or high-dose OP-1 specimens. Hematoxylin-eosin stained section. Magnification: x100. (c) Typical examples of the bone chamber content of TCP/HA control (left) and TCP/HA (TCP) with high-dose OP-1 (right). There are TCP/HA granules throughout the entire specimen. New bone (NB) formation takes place from the ingrowth openings upward to the top of the chamber. Fibrous tissue precedes the front of bone ingrowth. New bone is apposited on the TCP/HA granules at the bottom of the chamber. Hematoxylin-eosin stained section. Magnification: x12.5. (d) Detail of TCP/HA specimen with high-dose OP-1 showing new bone (NB) apposited on the TCP/HA granules at the bottom of the chamber. There were no differences in appearance of newly formed bone between the controls and either low-dose or high-dose OP-1 specimens. Hematoxylin-eosin stained section. Magnification: x100.

HISTOMORPHOMETRY

Neither side (p = 0.6 and p = 0.1) nor position (p = 0.9 and p = 0.2) of the chamber affected bone or total tissue ingrowth, respectively. There was no interaction between factors. The addition of OP-1 device to allografts at either low dose (p = 0.04) or high dose (p = 0.02) gave increased total tissue ingrowth distances compared to the allograft control. The use of TCP/HA either with or without the addition of OP-1 device did not result in any differences in total tissue ingrowth. However, total tissue ingrowth distance was significantly less in the allograft control than in the TCP/HA control (p = 0.02). OP-1 had no effect on bone ingrowth distance relative to the controls for both allografts and TCP/HA (Fig. 3). However, high-dose OP-1 mixed with allograft gave significantly more bone ingrowth than the TCP/HA control (p = 0.004).

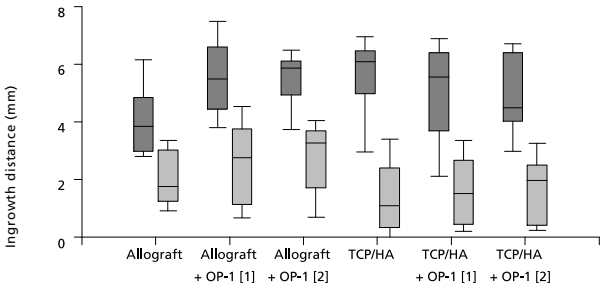

Fig. 3

Fig. 3
Ingrowth distances of fibrous tissue and bone for each treatment group, depicted in a boxplot. The doses of OP-1 device included: [1] 0.83 µg OP-1 combined with 0.24 mg collagen carrier per implant, and [2] 2.5 µg OP-1 combined with 0.72 mg collagen carrier per implant.

DISCUSSION

Within impacted bone chips, the migration of cells into the impacted allograft is compromised and vascularization is delayed for several weeks. It seems, however, that delayed or reduced new bone ingrowth seen in experiments with impaction is less important and may even be beneficial, as long as the graft/fibrous tissue composite remains strong enough to withstand forces acting on it during remodeling.[24] In a previous study, impaction of cancellous bone grafts and a TCP/HA bone substitute mixed with OP-1 device led to reduced fibrous tissue ingrowth as seen after 4 weeks.[15] Using higher doses of the OP-1 device, more collagen carrier material was impacted between the graft material, filling up the space between the impacted material and thereby delaying tissue ingrowth and remodeling. Similar observations, although not in the context of the impaction grafting setting, have been described previously by Franke Stenport and coworkers.[25] In addition, the volume available for tissue ingrowth was reduced by the impaction of TCP/HA particles, creating small TCP/HA particles between the larger ones.

From our present findings, the previously observed lack of ingrowth after 4 weeks appears to have been a delay rather than an inhibition. After 8 weeks, however, the delay was only partially overcome. Similarly to our results after 4 weeks, no differences in bone ingrowth between OP-1 device groups and their controls were observed. However, after 8 weeks significantly more fibrous tissue ingrowth was measured in allografts mixed with OP-1 device compared to the allograft control. In contrast, after 4 weeks significantly less fibrous tissue was measured when comparing the allografts mixed with OP-1 to the allograft control.[15] In the TCP/HA groups, no differences in fibrous tissue ingrowth between OP-1 device groups and their controls were observed, where there was a highly significant dose-dependent decrease in fibrous tissue ingrowth after 4 weeks. An observation time of 8 weeks might still be too short; however, no remnants of collagen carrier could be seen between the impacted allografts or TCP/HA granules, suggesting that all the OP-1 had been released from the carrier, thus no effect of the growth factor could be expected.

The data from both our previous study and the present work demonstrate the difficulty of using a biological enhancer of bone healing, such as an osteoinductive growth factor, in a situation where access to the blood supply and stem cells is limited and where bone healing is not optimal. Recently, Kärrholm et al.[26] showed that mixing of OP-1 with morselized allograft did not improve early fixation of either the acetabular or femoral component in revision surgery of the hip. In contrast, the influence of OP-1 on impacted allograft implants was recently investigated experimentally in loaded, primary, and impaired revision settings.[27] Under primary conditions, OP-1 reduced mean implant fixation; in contrast, under the impaired healing conditions of the revision setting, OP-1 increased incorporation and fixation of the implants. However, an OP-1 solution (without the collagen carrier) was used in that study.

The collagenous extracellular matrix of bone is considered an optimal delivery system for osteogenic proteins.[28] However, probably the collagen type I carrier is not the optimal carrier to use in impaction grafting. Ideally, a carrier should perform several important functions in addition to binding the protein;[29] it should not, however, obstruct the migration of cells into the impacted graft or vascularization. This makes it interesting to compare the results of the present study with the results of studies in which OP-1 was combined with graft without a carrier system. In a similar bone chamber model in rats, using a dose similar to our lowest dose without a collagen carrier (OP-1 solution) in impaction grafting, Tägil et al.[4] found an increase in bone formation. In contrast, a study using an OP-1 device (with collagen carrier) in combination with impaction grafting showed only very moderate effects on bone ingrowth.[1] Moreover, using an OP-1 solution in a weight-bearing rabbit knee impaction grafting model, Tägil et al.[12] showed no augmentation of morselized impacted bone graft incorporation.

The effects of BMPs have been shown to be concentration-dependent. The local concentration will be greatly determined by the release kinematics of the carrier.[30] The collagen carriers that have been used in clinical studies effect an initial bulk release. Such a release may result in excessive bone formation at locations with favorable conditions. At locations with less favorable conditions, however, such as within impacted bone grafts, the BMP clearance might be faster than the bone induction response of the host.[31]

Our data again indicate that the lack of effect when OP-1 is used in different clinical situations could be related to the problem that different biological environments require different dosages of growth factors and carriers for optimal stimulation. The challenge is to find ways to apply these drugs with consistent success in various applications in humans.

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CHAPTER SEVEN

COLLAGEN AND HEPARIN COATED TCP/HA AS A CARRIER FOR BMP-7

INTRODUCTION

Bone defects caused by congenital defects, malignancies, trauma, removal of failed implants, and orthognatic and preposthetic surgery require reconstruction for functional and aesthetic purposes. There are various ways to reconstitute these bone defects, each with its particular advantages and disadvantages. Although autografts show a considerable success rate because of their marked osteogenic potential, problems such as the limited availability of donor tissue and donor site morbidity exist for this type of graft. On the other hand, allografts are attractive alternative sources, however, there are also several problems encountered in using them, including the risk of disease transmission, immunogenicity, loss of biologic and mechanical properties secondary to its processing, increased cost, and non-availability world-wide due to financial and religious concerns.[1-4] Despite the benefits of both autografts and allografts, the relative concerns over the use of either autograft or allograft have led to the development of numerous synthetic and tissue engineered bone graft substitutes.

Calcium phosphate ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) have been investigated extensively because they are composed of materials similar to natural bone tissue.[5-8] Both TCP and HA are osteoconductive and biocompatible, however to achieve osteoconduction close proximity to the host bone is essential. However, these biomaterials are not osteoinductive. Therefore, their clinical application is limited to small bony defects or the areas of the defect directly facing the host bone.

To overcome these problems, a number of carrier systems have been designed and evaluated for the delivery of bone morphogenetic proteins (BMPs), such as collagen gel,[9] poly-L-lactic acid scaffolds,[10] hyaluronic acid,[11] fibrin gel,[11] porous HA,[12] and TCPs.[13] Although these delivery systems enhanced bone repair and accelerated fracture healing, there are still some problems in controlling release rate, often resulting in a high initial burst release.[9, 10, 13] Particularly in large voluminous defects where the repair might take considerable time, a controlled sustained release could prolong the action of BMPs to allow regenerative tissue forming cells to migrate to the area of injury and to proliferate and differentiate. Therefore, it would be ideal to develop a system for the sustained delivery of BMPs over an extended time period.

The attachment of heparin, a polysaccharide macromolecule associated with the cell surface and the extracellular matrix, to biomaterials may result in an appropriate matrix for the binding, modulation, and sustained release of biologically active BMPs.[14] Heparin avidly binds to a variety of bioactive components. These include basement membrane components, such as fibronectin, laminin and collagens, but also extracellular matrix proteins, proteases, cytokines, and growth factors.[15-18] Binding of growth factors to heparin stabilizes these growth factors and protects them from proteolytic degradation.[17, 19, 20] Furthermore, in the presence of heparin the half-life of BMPs in culture media is prolonged 20-fold.[21] In addition, sulfated polysaccharides including heparin, heparan sulfate, and dextransulfate have been shown to enhance the osteoblast differentiation induced by BMPs.[22] Recently, it has been shown that heparin sulfate-like molecules aid bone formation in-vivo.[15, 21, 23]

In order to create a carrier based delivery system with a localized sustained release, heparin was covalently attached to a cross-linked collagen type I coated TCP/HA bone substitute and subsequently loaded with BMP-7. The purpose of this study was to introduce and evaluate a new carrier system for BMP-7.

MATERIALS AND METHODS

PREPARATION OF GRANULES

Six different granules were prepared:

TCP/HA:	TCP/HA granules
TCP/HA+BMP-7:	TCP/HA granules with BMP-7
TCP/HA-COL:	TCP/HA granules coated with type I collagen
TCP/HA-COL+BMP-7:	TCP/HA granules coated with type I collagen and BMP-7
TCP/HA-COL-HEP:	TCP/HA granules coated with type I collagen and heparin
TCP/HA-COL-HEP+BMP-7:	TCP/HA granules coated with type I collagen, heparin and BMP-7

TYPE I COLLAGEN COATING

The TCP/HA granules were composed of 20% HA [$Ca_{10}(PO_4)_6(OH)_2$] and 80% TCP [$Ca_3(PO_4)_2$] (BoneSave, Stryker Osteosynthesis, Limerick, Ireland). We used granules with a diameter of 2 to 4 mm. The TCP/HA granules have a 50% non-interconnected macroporosity (range, 300-600 µm), which is produced by burning sacrificial carbonaceous filler during sintering. The granules are also microporous (range, 5-80 µm; porosity values derived from Stryker Osteosynthesis, Limerick, Ireland).

Insoluble type I collagen was isolated and purified from bovine achilles tendon as described previously.[24] A homogenous 0.5% (w/v) type I collagen suspension was made and swollen o/n at 4°C. The suspensions were homogenized using the Potter-Elvehjem on ice. The TCP/HA granules were put in the type I collagen suspension and frozen individually in liquid nitrogen. The granules were dried o/n in a Sublimator 500 II freeze dryer (Zirbus Technology, Bad Grund, Germany).

HEPARIN COUPLING

Cross-linking of type I collagen coated TCP/HA granules in the presence and absence of heparin (Sigma Chemicals Co., St. Louis, MO, USA) was performed using 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (ECD, Fluka Chemica, Buchs, Switzerland) and N-hydroxysuccinimide (NHS, Fluka Chemica, Buchs, Switzerland).[24, 25] Briefly, type I collagen coated TCP/HA granules were incubated in 40% (v/v) ethanol containing 50 mM 2-morpholinoethane sulfonic acid (pH 5.5) (MES, Fluka Chemica, Buchs, Switzerland) for 30 min at 20°C. Subsequently, the coated type I collagen was cross-linked by immersion in 40% (v/v) ethanol containing 50 mM 2-morpholinoethane sulfonic acid (pH 5.5), 66 mM ECD, and 6 mM NHS, with and without 0.5% (w/v) heparin for 4 h at 20°C. To stop the cross-link reaction, the granules were incubated twice for 60 min in 0.1 M Na_2HPO_4 and were washed twice with 1 M NaCl and six times with 2 M NaCl for 2 h. Finally, the granules were washed six times with demineralized water, frozen in -20 °C and dried overnight in a Sublimator 500 II freeze dryer.

BMP-7 LOADING ON COATED GRANULES

Granules were loaded with Chinese hamster ovary-derived recombinant human BMP-7 (R&D Systems, Minneapolis, MN, USA) by immerging the granules in 100 µl 1 µg BMP-7/ml phosphate buffered saline (PBS) solution for 2 h. As controls, granules were mixed with 100 µl PBS alone.

CHARACTERIZATION OF COATED GRANULES SCANNING ELECTRON MICROSCOPY (SEM)

To analyze the morphology, the granules were fixed on a stub with double-sided carbon tape. Granules were sputtered with an ultra thin layer of gold in a Polaron E5100 SEM coating system. Specimens were studied with a JEOL JSM-6310 scanning electron microscope (JEOL, Tokyo, Japan) with an accelerating voltage of 15 kV. The samples were examined using 1000x and 5000x magnifications.

IMMUNOFLUORESCENT ASSAY (IFA)

To determine the distribution of heparin and BMP-7, the granules were blocked for 20 min with 1% (w/v) bovine serum albumin (BSA) in PBS, washed 3 times with PBST and stained using specific antibodies. In case of heparin, the granules were incubated with anti-heparin antibodies (HS4C3 periplasmic fraction, monoclonal anti VSV glycoprotein clone P5D4 conjugate (Sigma Chemicals Co., St. Louis, MO, USA) and Alexa 594 labeled goat anti-mouse IgG (Invitrogen, Leiden, the Netherlands)) and washed with PBST. In case of BMP-7, the granules were incubated with rhBMP-7 antibodies (rhBMP-7 monoclonal antibody (MAB3541, R&D Systems Europe Ltd., Abingdon, United Kingdom) and Alexa 488 labeled goat anti-human IgG (Invitrogen, Leiden, the Netherlands)). Omission of the primary antibody was taken as a negative control.

TYPE I COLLAGEN CONTENT

To determine the amount of coated type I collagen, the collagen was isolated from the granules by using a 30 min 6 M HCl decalcification step at 21 °C. After six washings with milliQ water, the collagen remnants were freeze dried and weighted using a semi-microbalans (Simadzu AUW220D, Kyoto, Japan).

AMINO GROUP CONTENT

During cross-linking, an amine group reacts with a carboxylic group. Therefore, the extent of cross-linking was evaluated by analyzing the remaining free amine groups after a reaction with 2,4,6-trinitrobenzene sulfonic acid.[24]

HEPARINE CONTENT

The heparin content was determined by hexosamine assay using p-dimethylaminobenzaldehyde.[24]

BMP-7 BINDING AND ITS RELEASE

After loading the granules with 1 µg BMP-7/ml PBS for 2 h, the granules were withdrawn and the supernatant was analyzed with ELISA (Human BMP-7 Duoset, R&D Systems, Minneapolis, MN, USA).

The release from the granules was determined by immerging the granules in 2 ml microcentrifuge tubes containing 1.5 ml PBS and 0.05% (w/v) sodium azide. The tubes were incubated at 37 °C with continuous agitation. At various time points, the supernatant was withdrawn and the tubes were replenished with fresh buffer. The amounts of BMP-7 in the supernatant were determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequent staining with 0.2% $AgNO_3$ (Drijfhout, Amsterdam, the Netherlands), 0.2% NH_4NO_3 (Sigma Chemicals Co., St. Louis, MO, USA), 5% Na_2CO_3 (Fluka Chemica, AG, Buchs, Switzerland) and 1% tungsten acid (Fluka Chemica, AG, Buchs, Switzerland).[26] Gene Tools (Syngene, Cambridge, United Kingdom) was used to analyse the intensity of the bands with a standard curve of 0-100 ng BMP-7. TCP/HA, TCP/HA-COL, and TCP/HA-COL-HEP granules were taken as controls.

RESULTS

CHARACTERIZATION OF GRANULES

Type I collagen was coated on TCP/HA granules and subsequently cross-linked in the presence and absence of heparin. To determine the total amount of coated type I collagen on the surface of the granules, the collagen was isolated and weighted. The amount of coated collagen was 8.3±1.1 mg collagen/g granule (Table 1). The thickness of the type I collagen coating was measured by SEM and was approximately 100 µm (Fig. 1). The morphology consisted of a highly porous interconnecting network with a pore size of approximately 5 µm (Fig. 2a). The collagen was chemically cross-linked with EDC and NHS to covalently bind heparin. The addition of heparin did not change the porous structure (Fig. 2b). During cross-linking, an amine group reacts with a carboxylic group and hence the extent of cross-linking was evaluated by analyzing the remaining free amine groups. About 50-60% (167.7±10.6 from 331.4±22.2 nmol/mg collagen) of the amine groups were used for cross-linking (Table 1). The amount of heparin covalently bound to the scaffold was 193.5±51.1 µg heparin/mg collagen, as determined by hexosamine assay (Table 1).
The distribution of the different components on the granules was visualized using immunofluorescence, which revealed that collagen, heparin, and BMP-7 were present on and were evenly distributed over the granules (Fig. 4). The heparin staining confirmed the highly incorporated heparin in the TCP/HA-COL-HEP granules (Fig. 3). These heparin incorporated granules showed also a higher rhBMP-7 signal compared with the TCP/HA-COL granules (Fig. 4).

BMP-7 BINDING IN TIME

Immersion of the granules in BMP-7 solution to load BMP-7, resulted in the binding of 54±3% (62.9±5.4 ng BMP-7/mg granule) to the TCP/HA granule, 64±8% (69.0±9.6 ng BMP-7/mg granule) to the TCP/HA-COL granules, and 78±1% (92.9±4.8 ng BMP-7/mg granule) to the TCP/HA-COL-HEP granules (Table 1). TCP/HA granules showed a burst release of BMP-7 within the first 4 h. After an initial burst, the TCP/HA-COL granules showed a more gradual release of BMP-7. In contrast, BMP-7 release from the TCP/HA-COL-HEP granules was sustained up to 21 days (Fig. 5).

Table 1

Biochemical properties of granules

Granule	Cross-linked with ECD/NHS	Collagen content (mg/mg granule)	Amino group content	Heparin content (µg/mg collagen)	BMP content (ng/mg granule)
TCP/HA	-				
TCP/HA with BMP	-				62.9±5.4
TCP/HA-COL	+	8.3±1.1	331.4±22.2	25.4±10.1	
TCP/HA-COL with BMP	+	8.3±1.1	331.4±22.2	25.4±10.1	69.0±9.6
TCP/HA-COL-HEP	+	10.5±0.7	167.7±10.6	193.5±51.1	
TCP/HA-COL-HEP with BMP	+	10.5±0.7	167.7±10.6	193.5±51.1	92.9±4.8

Values are mean ± standard deviation

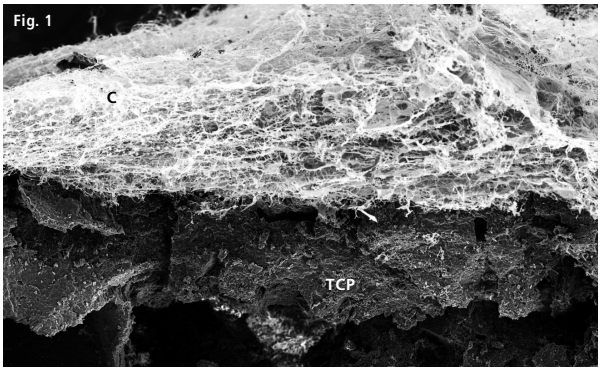
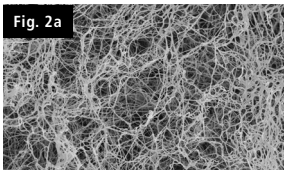
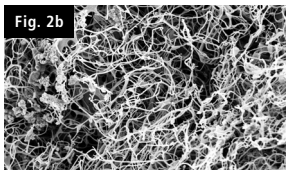
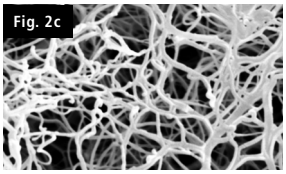


Fig. 1
Scanning electron microscopy picture of a cross-sectioned type I collagen coated TCP/HA granule. The highly porous interconnective type I collagen (C) network is clearly visible on top of the TCP/HA granule (TCP). Original magnification 150x.







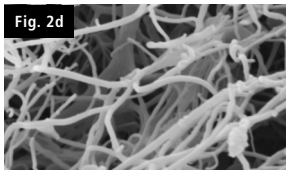
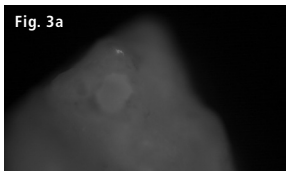


Fig. 2
Scanning electron microscopy picture of the highly porous interconnecting network of type I collagen cross-linked in the absence (ac) and presence (bd) of heparin. Original magnifications (ab) 1000x; (cd) 5000x.



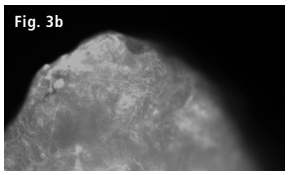
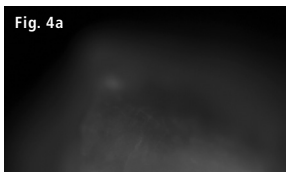
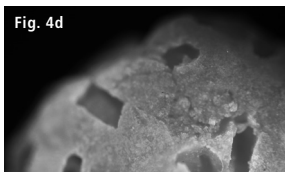
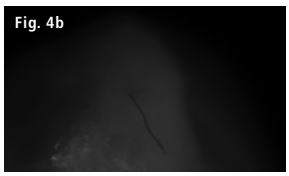
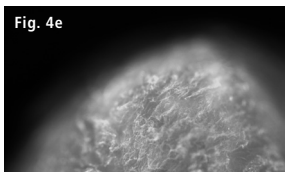


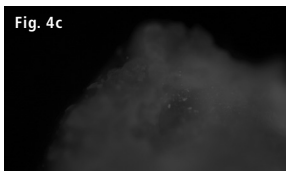
Fig. 3
Immunolocalization of heparin on the type I collagen coated TCP/HA granules cross-linked without (a) and with (b) heparin.











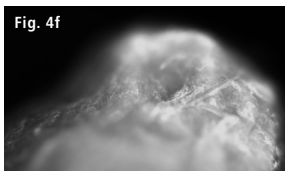


Fig. 4
Immunolocalization of BMP-7 on TCP/HA (ad), TCP/HA-COL (be) and TCP/HA-COL-HEP granules (cf). (abc) Control granules, (def) Granules loaded with BMP-7.

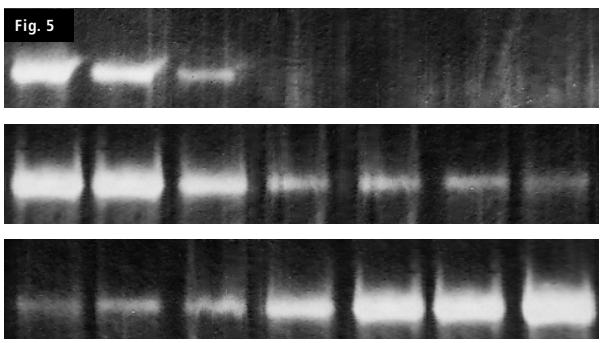


Fig. 5
The amounts of BMP-7 in the supernatant at different time points were determined using SDS-PAGE. TCP/HA granules showed a burst release of BMP-7 within the first 4 h. After that only very little BMP-7 was released. TCP/HA-COL granules showed an initial burst release, followed by a more gradual release. In contrast, BMP-7 release from the TCP/HA-COL-HEP granules was sustained up to 21 days.

DISCUSSION

Many systems have been designed and evaluated for the delivery of BMPs.[9-13] Although these delivery systems enhanced bone repair and accelerated fracture healing, there are still some problems in controlling release rate, often resulting in a high initial burst release.[9, 10, 13] Therefore, it would be ideal to develop a system for the sustained delivery of BMPs over an extended time period. In order to create a suitable carrier based delivery system with a localized sustained release, heparin was covalently attached to a cross-linked collagen coated TCP/HA bone substitute and loaded with BMP-7.

The sustained delivery system in the present study is based on the inherent capacity of heparin to bind various growth factors, such as BMPs, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and transforming growth factor beta (TGF-β), via electrostatic interactions between heparin's negatively charged sulfate groups and the proteins positively charged amino acid residues.[14, 21, 27, 28] It is well established that BMP-2, BMP-4 and BMP-7 bind to heparin.[16, 22, 29-31] However, the location of the heparin binding site within BMP-7 remains to be established, as do the consequences of such GAG interactions. In addition, different types of receptors have been identified for the BMP-2 and BMP-7 subgroups.[21]

In the present study, the attachment of heparin to type I collagen coated granules amounted the binding of BMP-7 for up to 92.9±4.8 BMP-7/mg granule, whereas the TCP/HA and TCP/HA-COL granules bound 62.9±5.4 ng and 69.0±9.6 ng BMP-7/mg granule, respectively. The amounts of BMP-7 in the supernatant at different time points were determined using SDS-PAGE. TCP/HA granules showed a burst release of BMP-7 within the first 4 h. After that only very little BMP-7 was released. TCP/HA-COL granules showed an initial burst release, followed by a more gradual release. In contrast, BMP-7 release from the TCP/HA-COL-HEP granules was sustained up to 21 days. Recently, the BMP-2 release from a heparin conjugated PLGA scaffold was compared to that of an unmodified PLGA scaffold.[14] The delivery system of the heparin conjugated PLGA exhibited a sustained release of BMP-2, while BMP-2 was rapidly released from the unmodified PLGA scaffold. The slow BMP release can be attributed to the specific interactions between the immobilized heparin and the BMP. Similar release patterns were observed for various heparin conjugated hydrogel systems, such as, hyaluronic acid, fibrin gel, and alginate.[32-34]

The discrepancy in results between these studies may be due to differences in carrier materials, or in BMP subgroups (BMP-2 versus BMP-7). However, in-vivo release kinetics and drug diffusion will depend on a multitude of factors including materials permeability, shape, surface area, surface roughness, thickness, and other external modifications.[35]

For BMPs, it has been shown that these proteins exhibit a sustained release from both bovine type I collagen and heparin,[14] however, there is a strong binding affinity to hydroxyapatite mineral.[5, 36] Winn et al.[37] showed that after ectopic implantation in rats a hydroxyapatite based material initially had a burst release at the first collection interval, but thereafter, BMPs appeared to bind irreversibly to the hydroxyapatite. Since the hydroxyapatite carrier did not resorb, they suggested that the lack of BMP released from the carrier implies a strong affinity to the hydroxyapatite. In

contrast, a study evaluating five different ratios of hydroxyapatite to tricalciumphosphate as carriers for BMP-2, showed that more new bone was formed using ceramic with high HA ratio.[38]
Even though quite a large number of biomaterials based approaches have been developed for bone defect repair, none of them proved ideal. One major advantage of the carrier system presented in this study is that it might be used in load bearing applications. In early attemps, synthetic materials, such as HA and TCP, were only conformable to fill non-load bearing bone cavities. However, improved processing techniques have allowed the production of biologically and mechanically stronger ceramics that can withstand loading conditions of weight-bearing bones.[39-41] In addition, it was found that the presence of a collagen coating on TCP/HA ceramics increased the biocompatibility of the TCP/HA, increasing osteoblast infiltration and proliferation.[42] This may indicate that collagen coating of calcium phosphate ceramics may also increase their compatibility and osseointegration in-vivo. In addition, cross-linking of collagen decreased the biodegradability, and increased retention of BMP at the implant site.[43] An additional benefit to using heparin in the protein delivery system is its ability to enhance growth factor stability.[21] Furthermore, heparin may potentiate the BMP bioactivity.[22]
The delivery system developed in the present study could probably be applied to deliver dual or multiple growth factors that have affinities for heparin such as VEGF, BMPs and PDGF, which could synergistically enhance osteogenesis by increasing vascularity. The delivery system presented in this study may provide a powerful tool for bone regeneration.

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CHAPTER EIGHT

BEHAVIOR OF A NOVEL INJECTABLE CALCIUM PHOSPHATE CEMENT

INTRODUCTION

More than 500,000 bone grafting procedures are performed annually in the United States and 2.2 million worldwide to repair bone defects in orthopedics, neurosurgery, craniofacial surgery, and dentistry.[1] Bone grafts are often used to provide support, enhance biologic repair of skeletal defects and fill voids. Although autograft bone is the gold standard that alternatives must try to meet or even exceed, autograft has some serious disadvantages, including the limited quantity and associated donor site morbidity.[2, 3] Though successful clinical results have been reported using allografts, there are also several problems encountered in using them, including the risk of disease transmission, immunogenicity, loss of biologic and mechanical properties by its processing, increased cost, and limited availability worldwide.[4] In addition, some religions do not allow the use of allograft materials. Despite the benefits of both autografts and allografts, the relative concerns over the use of either autograft or allograft have led to the development of numerous synthetic bone graft substitutes.

One of the more promising groups of synthetic graft materials for augmenting acute fracture healing are the calcium phosphate based cements (CPCs).[5] There has been great interest in CPCs as bone substitute materials because they can be molded to fill a void or defect and are osteoconductive. Most of the research performed on CPCs has dealt with the improvement of bone cement formulations. Additives, such as accelerants, cohesion promoters, and fluidificants have been used to improve workability, and setting and hardening properties.[6-8] In particular, the development of injectable bone cements is still of great interest.

Injectable CPCs can be used in treating distal radius fractures, femoral neck fractures, vertebral body compression fractures, and reinforcement of pedicle screw fixation.[9] Recently, the suggestion has been made that they might also compete with the polymethylmethacrylate (PMMA) bone cements and the apatite coatings for fixation of metal endoprostheses in orthopedics and oral implantology.[10-12] In addition, the possibility to use CPCs not only as bone substitutes, but also as carriers for local and controlled supply of drugs, such as antibiotics, anti-inflammatory drugs, and growth factors, is very attractive and can be useful in treatments of different skeletal diseases, as well as for accelerating the rate of bone fracture healing.[13]

Commonly utilized calcium phosphate cements currently available for use in the United States are BoneSource (Stryker Osteosynthesis, Freiburg, Germany) and Norian SRS (Synthes, Monument, CO, USA).[14-16] Calcium phosphate transformations in vitro have been well described for both BoneSource and Norian SRS. However, only limited in vivo data are available.[17, 18] Recently, a novel calcium phosphate cement, HydroSet (Stryker Osteosynthesis, Freiburg, Germany), was developed to have improved (intrinsic) qualities as workability, injectability and cohesion. To date, no study has evaluated this new synthetic material and compared its crystallographic properties, biological activity, osteoconductive properties, and resorption rate with other commonly used CPCs.

MATERIALS AND METHODS

ANIMAL MODEL/EXPERIMENTAL DESIGN

The three bone cements were tested in a modified version of an established rabbit distal femoral defect model (Fig. 1).[19-22] Thirty-six adult female New Zealand White rabbits were operated bilaterally. The CPCs were randomly assigned to the right or left limb. All animals were skeletally mature and weighed between 3200 and 4900 g (mean 3770±403 g). The rabbits were acclimatized to their environment for 4 weeks prior to surgery and housed in groups of maximal six animals in a controlled environment with a normal light-dark cycle and a temperature between 23 and 26°C. The rabbits had access to water and rabbit chow ad libitum. All procedures were approved by the Animal Ethics Committee of the Radboud University Nijmegen, Nijmegen, the Netherlands. The biological activity, osteoconductive properties, and the resorption rate of the CPCs were evaluated using histology, histomorphometry, X-Ray Diffraction (XRD), and Fourier Transform Infrared Spectrometry (FTIR) at three different time points (Table 1).

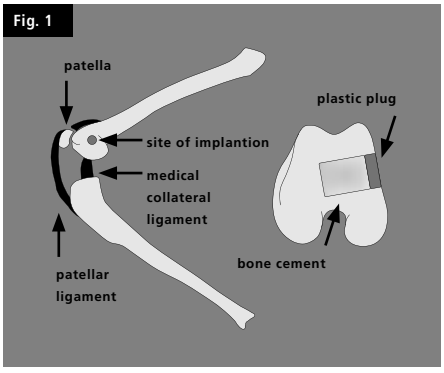


Fig. 1
Schematic drawing showing the location of the implantation site in the medial aspect of the medial femoral condyle (left), and an overview of experimental defect with CPC implant (right). The defect is closed with a press-fit plastic plug.

Table 1

Overview of experimental design

Histology & histomorphometry		XRD & FTIR	
Observation time	**Animals (n)**	**Observation time**	**Animals (n)**
-	-	**powder**	-
6 weeks	9	**24 hours**	3
26 weeks	9	**26 weeks**	3
52 weeks	9	**52 weeks**	3

FILLING MATERIALS

HydroSet powder consists of three powder components: dicalcium phosphate dihydrate (DCPD) [$CaHPO_4 \cdot 2H_2O$], tetracalcium phosphate (TTCP) [$Ca_4(PO_4)_2O$], and tri-sodium citrate [$C_6H_5Na_3O_7 \cdot 2H_2O$]. The liquid used for HydroSet is water based with polyvinylpyrrolidone (PVP) [$C_6H_9NO]_x$, and sodium phosphate [Na_2HPO_4] added (cement formulations obtained from Stryker Osteosynthesis, Freiburg, Germany).

Norian Skeletal Repair System (SRS) powder consists of three powder components: monocalcium phosphate monohydrate (MCPM) [$Ca(H_2PO_4)_2 \cdot H_2O$], α-tricalcium phosphate (α-TCP) [$Ca_3(PO_4)_2$], and calcium carbonate (CC) [$CaCO_3$]. The liquid used for Norian SRS is a sodium phosphate solution.[9, 23]

BoneSource powder consists of two powder components: tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) [$CaHPO_4$]. The liquid used for BoneSource is water based with 0.25 mol/l sodium phosphate solution added.[24]

SURGICAL PROCEDURE

Preoperatively, all animals received antibiotics (15 mg/kg) (Excenel, Pharmacia & Upjohn Animal Health BV, Woerden, the Netherlands) and analgetics (1.1 mg/kg) (Fynadine, Schering Plough Animal Health Benelux, Utrecht, the Netherlands). All animals were anesthetized by intravenous administration of 0.2 ml/kg medetomidine (Dormitor, Pfizer Animal Health BV, Capelle a/d IJssel, the Netherlands) and maintained after intubation with nitrous oxide, oxygen, and isoflurane (2%). Under aseptic conditions, a 2-3 cm longitudinal skin incision was made and the distal medial femur was exposed. A 2 mm diameter hole was drilled in the medial aspect of the medial femoral condyle. A guide pin was inserted and, using a custom made drill, a final drill hole with a diameter of 5.5 mm and a depth of 10 mm was created. The defect was cleaned from bone debris using a sharp spoon and saline. All CPCs were prepared according to the manufacturers instructions. The defect was filled with cement and a 2 mm thick polyethylene plug with a diameter of 5.55 mm was press-fitted on top of the implanted material to seal the defect. The area surrounding the defect was cleaned using saline to remove any remaining bone and cement debris. Soft tissues were closed in layers and the procedure was repeated on the other side.

After the implantation procedure the animals received subcutaneous injections of antibiotics (15 mg/kg) (Excenel, Pharmacia & Upjohn Animal Health BV, Woerden, the Netherlands) and analgetics (1.1 mg/kg) (Fynadine, Schering Plough Animal Health Benelux, Utrecht, the Netherlands) two times. Post operative X-rays were made to ensure proper placement and filling of the implant locations and to exclude fractures. The animals were allowed to walk freely. At the set time points, the rabbits were killed with an overdose of sodium pentobarbital (1 ml/kg) (Nembutal, CEVA Santé Animale, Maassluis, the Netherlands). The distal femora were immediately harvested, cleaned of soft tissue, and fixed in 4% buffered formaldehyde.

HISTOLOGY AND HISTOMORPHOMETRY

After localizing the PE plug, the defect with surrounding tissue was dissected from the host bone with a diamond coated saw. All sections were cut in a plane perpendicular to the long axis of the specimen. The specimen was split 4 mm underneath the plug, also perpendicular to the long axis of the specimen. All specimens were dehydrated using ethanol, and subsequently embedded in PMMA. Specimens from the lower part of specimen were decalcified using 25% ethylenedinitrilo tetraacetic acid (EDTA) in 0.1 mol/l phosphate buffer (pH 7.4) before dehydration. These specimens were cut with a microtome (Leica RM 2155, Leica Microsystems Nederland BV, Rijswijk, the Netherlands) perpendicular to the longitudinal axis of the specimen, each section 5 µm thick. These sections were stained with hematoxylin and eosin (HE), Goldner-Masson trichrome, and tartrate resistant acid phosphatase (TRAP) for routine histology. The superior part, directly underneath the plug, was used for undecalcified histology. Serial slices of 20 µm were made using a saw-microtome (Leica SP 1600, Leica Microsystems Nederland BV, Rijswijk, the Netherlands). These sections were stained with HE for routine histology (and histomorphometry). All sections were investigated blinded and in random order.

Histomorphometric analysis was performed by using interactive computer-controlled image analysis (analySIS, Soft Imaging System GmbH, Münster, Germany) and consisted of the following two measurements:[25, 26] (1) Percentage of bone contact at the interface. The amount of interfacial bone contact was defined as the percentage of cement perimeter at which there is direct bone to cement contact without intervening soft tissue layers. The total perimeter and the total length of sites with direct bone-to-cement contact were measured in the sections at 2.5x magnification. (2) Cement area. The total area of cement present in the transversal sections was determined and compared between the different implantation periods to determine the rate of cement resorption.

All quantitative measurements were performed for three different sections per specimen. The three sections were equally distributed over each specimen, each at a distance of ~1200 µm from the next. The presented data are based on the average of these three measurements.

X-RAY DIFFRACTION

Crystallographic analyses were made with powder XRD after the samples were ground to a fine powder in an agate mortar and pestle. CuK_α X-rays ($\lambda = 0.154$ nm) were diffracted off the sample plane and intensities recorded in steps of 0.02° from 20° to 40° 2θ (40 kV; 40 mA) (PW3710, PANalytical BV, Almelo, the Netherlands). The resultant XRD patterns were compared to the International Center for Diffraction Data standards for phase identifications. (HydroSet JCPDS file 9-432, 25-1137, and 2-85 for HA, TTCP and DCPD, respectively; Norian SRS JCPDS file 9-432, 9-348, and 5-586 for HA, α-TCP, and $CaCO_3$, respectively; BoneSource JCPDS file 9-432, 25-1137, and 9-80 for HA, TTCP, and DCPA, respectively.)

The relative proportions of the phases in the products were semiquantitatively determined with the use of the following relation:[27-29]

$$\% I_i = \left[\frac{I_i}{I_1 + I_2 + I_3} \right]$$

where I_i is the phase of concern, i = 1, 2, 3,

I_1 corresponds to the intensity of the HA (211)-peak at $2\theta = 31.8°$ for all three CPCs,
I_2 corresponds to the intensity of the TTCP (013)-peak at $2\theta = 29.2°$ for HydroSet and BoneSource, and corresponds to the intensity of the α-TCP (034)-peak at $2\theta = 30.8°$ for Norian SRS, and
I_3 corresponds to the intensity of the DCPD (021)-peak at $2\theta = 20.9°$ for HydroSet, the $CaCO_3$ (306)-peak at $2\theta = 29.4°$ for Norian SRS, and the DCPA (110)-peak at $2\theta = 26.6°$ for BoneSource

These peaks were selected because they occur at 2θ values, which do not overlap with other peaks of the various phases present.

FOURIER TRANSFORM INFRARED SPECTROMETRY

To characterize the molecular structure of the cements, infrared spectra of the cement powders were obtained from 4000 to 450 cm^{-1} by FTIR (Spectrum One, Perkin Elmer, Groningen, the Netherlands) with a resolution of 4.00 cm^{-1}.

STATISTICAL ANALYSIS

Statistical analysis was performed using a two-way ANOVA. To isolate the different groups, we used Tukey's multiple comparison procedure.

RESULTS

CLINICAL

No intraoperative complications occurred during surgery. All animals were able to walk within 48 h after surgery. There were no signs of inflammation, skin ulceration, or wound healing problems. All X-rays showed closed implant sites without plug displacement or plug loosening. No femoral fractures or fractures at the implant site were found.

HISTOLOGY

The CPCs were clearly visible in the created bone defects (Fig. 2). A very uniform bone reaction for all implantation times and CPC materials was observed. No clear differences in healing response to the three CPCs could be observed. Differences in morphological appearance were only found between the various implantation times. However, a large variation in remodeling was observed between animals. Bilateral a similar response was observed. Penetration of the CPCs into intertrabecular voids was seen, causing irregular contours of the cement plugs.

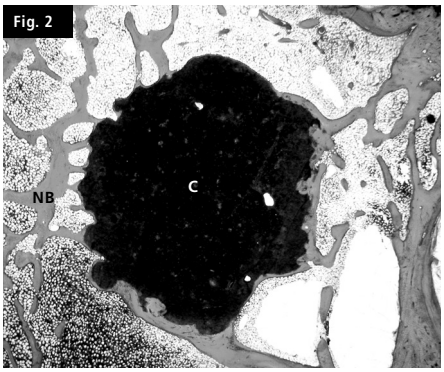


Fig. 2
Norian SRS in defect after 6 weeks of implantation.
The CPC (C) is almost totally covered with new formed bone (NB). HE stained section; original magnification x12.5.

IMPLANTATION TIME 6 WEEKS

After 6 weeks, the CPC surfaces were almost completely covered by newly formed bone. The remodeling process of this newly formed bone had already started (Fig. 3ab). A tight contact existed between the new bone and the cement surface, without signs of an intervening fibrous tissue layer. At locations where no bone was formed, fibrous cell dense tissue was formed with vascular invasion and osteoclastic resorption of the cements. Resorbed cement was replaced by new bone or by normal looking medullar tissue. No inflammatory cells were seen. The surface of the CPCs had a granular appearance. The surface zone of the CPCs had a less dense appearance compared with the central part. Occasionally, pores and/or cracks were seen in the CPCs. When the pores and/or cracks had an opening to the outer environment, the surface inside the pores and/or cracks was completely covered with a thin layer of bone.

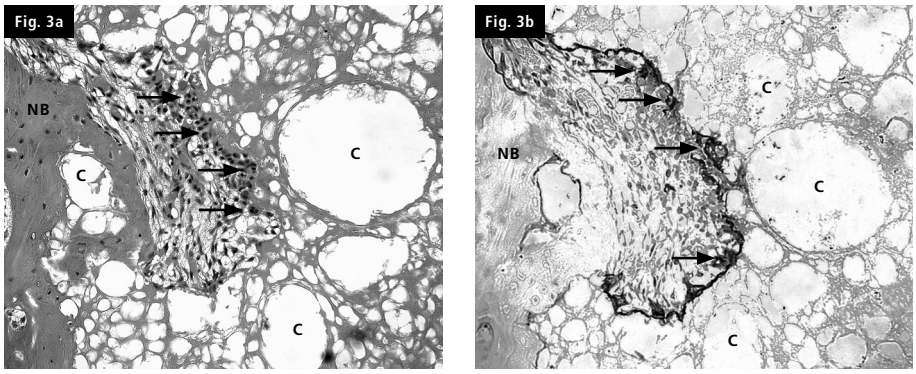


Fig. 3
(a) Detail of BoneSource after 6 weeks of implantation. Intense remodeling with active osteoclastic resorption (arrows). HE stained section; original magnification x200. (b) Adjacent section showing acid phosphatase positive cells (osteoclast-like cells) resorbing BoneSource after 6 weeks of implantation. TRAP stained section; original magnification x200. NB = new bone; C = cement; M = marrow.

IMPLANTATION TIME 26 WEEKS

After 26 weeks, an ongoing remodeling of the CPCs into (new) bone was seen. Remodeling activity in the interface, characterized by the presence of remodeling lacunae and osteoclast-like cells, was still seen (Fig. 4ab). Numerous remodeling lacunae could be seen close to the cements. Frequently, the cutting cone of these remodeling lacunae was directional and penetrated into the cement. In these situations, osteoclast-like cells were present at the cement outside, whereas bone apposition occurred at the other side of the lacunae. The bone was mature and could not be discerned from original trabecular bone. The major part of the CPCs was still present in the produced bone defects. Only occasionally larger parts of the cements had disappeared and became subsequently completely replaced by bone. Sometimes, cement remnants were intertwined within new bone.

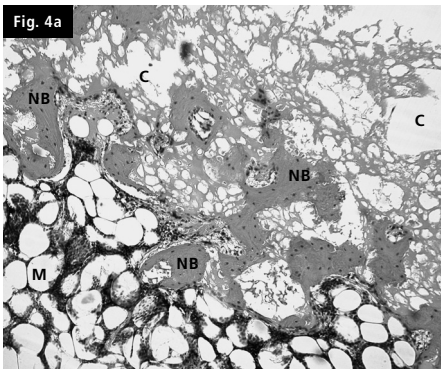
IMPLANTATION TIME 52 WEEKS

An ongoing remodeling of the CPCs into (new) bone was still seen (Fig. 5a). More often, larger parts of the cements had disappeared and were replaced by bone or marrow. Cement remnants intertwined within new bone were still seen (Fig. 5b).

HISTOMORPHOMETRY

PERCENTAGE OF BONE CONTACT AT THE INTERFACE

After 6 weeks of implantation, the CPC surfaces were almost completely covered by newly formed bone. No significant differences in percentage of bone contact existed between the three CPCs and their respective implantation times were found (Fig. 6a).



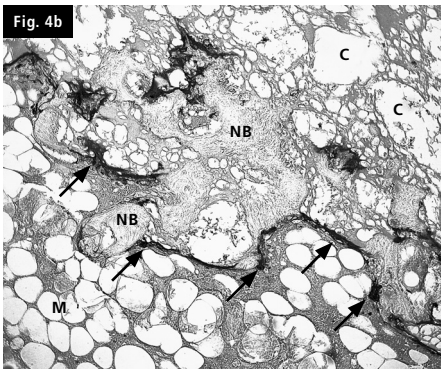
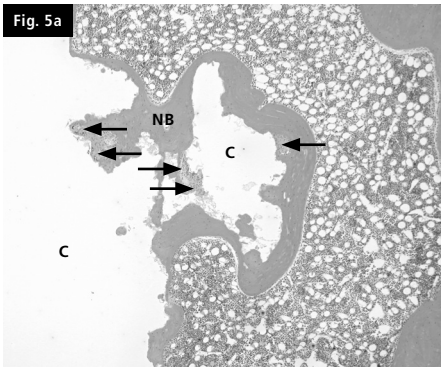


Fig. 4
(a) Continued remodeling of HydroSet after 26 weeks of implantation. HE stained section; original magnification x100.
(b) Adjacent section showing acid phosphatase positive cells (osteoclast-like cells; arrows) resorbing HydroSet after 26 weeks of implantation. TRAP stained section; original magnification x100. NB = new bone; C = cement; M = marrow.



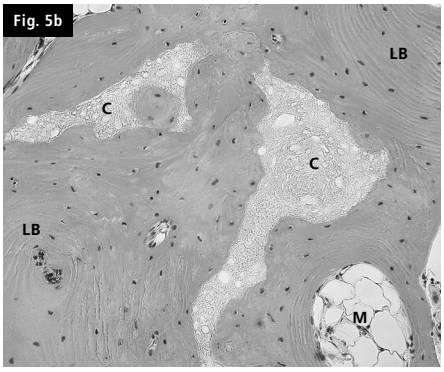


Fig. 5
(a) Ongoing remodeling of HydroSet after 52 weeks of implantation. The CPC is totally covered with bone (NB). Remodeling lacunae with cutting cone (arrows) headed towards CPC (C). Osteoclast-like cells (arrows) resorbing the cement. (b) Incorporation of HydroSet remnants (C) into lamellar bone (LB) after 52 weeks of implantation. HE stained sections; original magnification x200.

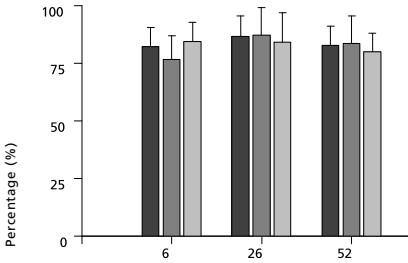


Fig. 6a

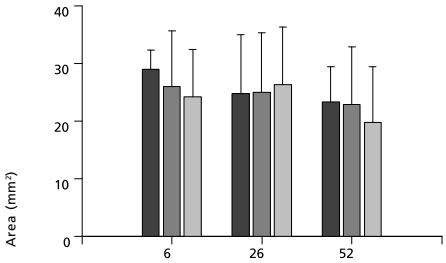


Fig. 6b

Fig. 6
Histomorphometrical results showing (a) Percentage of bone contact, and (b) Cement surface area.

CEMENT AREA

A large variation in CPC cement areas between animals was measured. The CPCs had penetrated into intertrabecular voids, causing a larger surface area than the area of the drill hole. The measurements suggest a decrease in CPC surface area at 52 weeks compared with the CPC surface area at 6 and 26 weeks (Fig. 6b). However, this could not be confirmed by statistical analysis ($p > 0.05$). Also, no significant difference existed between the three CPCs within an implantation period ($p > 0.05$).

X-RAY DIFFRACTION

Diminution of base peak intensities was observed and accompanied by a continuous increase in the HA peak intensity. All three CPCs converted towards HA with time (Fig. 7abc). Conversion towards HA was visible at 24 h. After 26 weeks no further changes were observed. The relative proportions of these phases over the time of this study are shown in (Figure 8abc). These observations are consistent with the formation of HA during this period.

FOURIER TRANSFORM INFRARED SPECTROMETRY

The FTIR spectra demonstrated the transformation to carbonated hydroxyapatite over time. FTIR measurements showed for all three CPCs two clusters of peaks, that is, from 900 to 1150 and from 550 to 600 cm^{-1}. These peaks could be attributed to the major absorption modes associated with the presence of phosphate (PO_4^{3-}).[30] Also, carbonate groups were observed at 1416, 1460, and 1540 cm^{-1}, indicating transformation of the CPCs into carbonated hydroxyapatite (Fig. 9abc).[31, 32] The spectra have the characteristic features of carbonated hydroxyapatite and show that the CPCs appear similar to bone apatite.[17, 32]

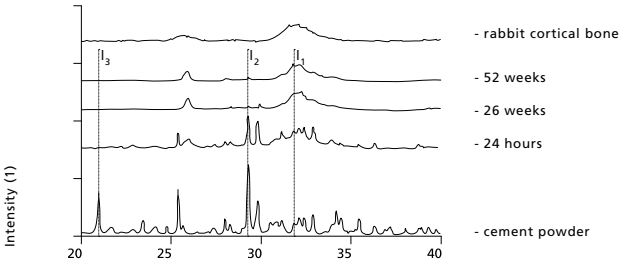


Fig. 7a 2 Theta (°)

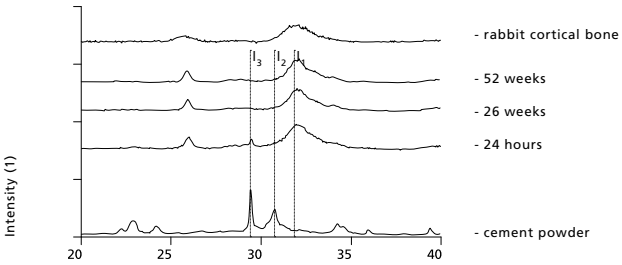


Fig. 7b 2 Theta (°)

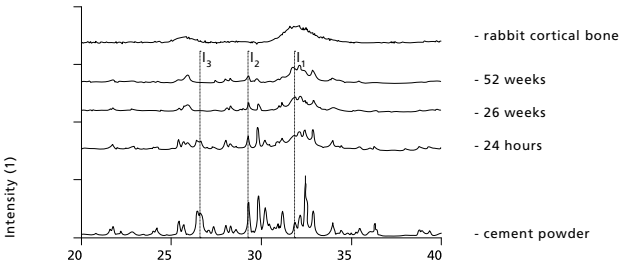


Fig. 7c 2 Theta (°)

Fig. 7
XRD patterns of (a) HydroSet, (b) Norian SRS, and (c) BoneSource after different evaluation periods. All CPCs converted towards HA with time. The positions of peaks I_1-I_3 are marked with a dotted line. An XRD pattern of rabbit cortical bone is added on top of each plot for comparison.

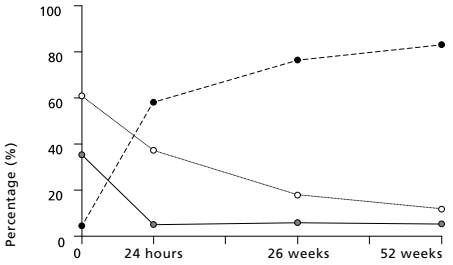


Fig. 8a Implantation period
TTCP DCPD HA

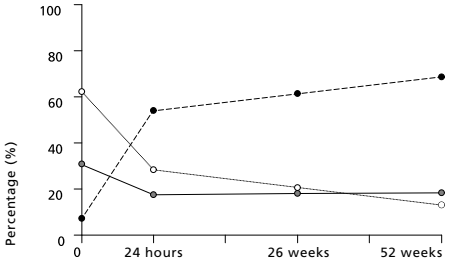


Fig. 8b Implantation period
$CaCO_3$ α-TCP HA

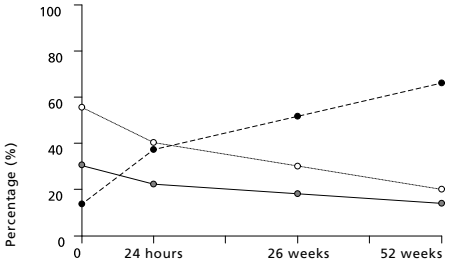


Fig. 8c Implantation period
TTCP DCPA HA

Fig. 8
Relative proportions of different phases present in the products. (a) HydroSet. Percentage of HA, TTCP and DCPD phases present as a function of time. (b) Norian SRS. Percentage of HA, **α**-TCP and $CaCO_3$ phases present as a function of time. (c) BoneSource. Percentage of HA, TTCP and DCPA phases present as a function of time.

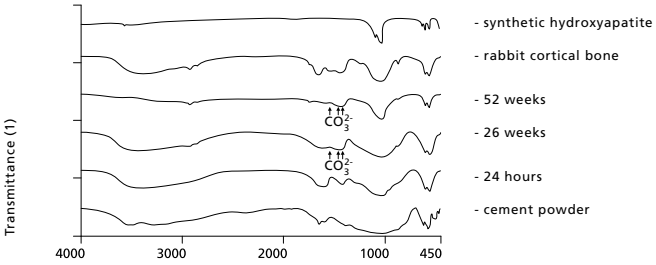


Fig. 9a Wavenumber (cm^{-1})

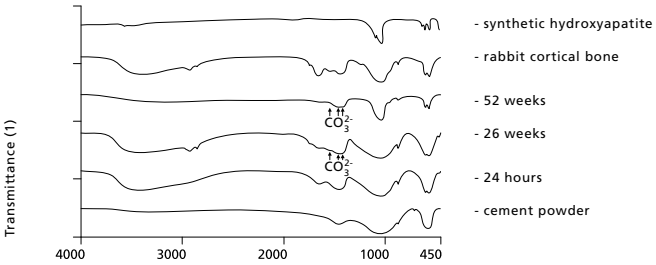


Fig. 9b Wavenumber (cm^{-1})

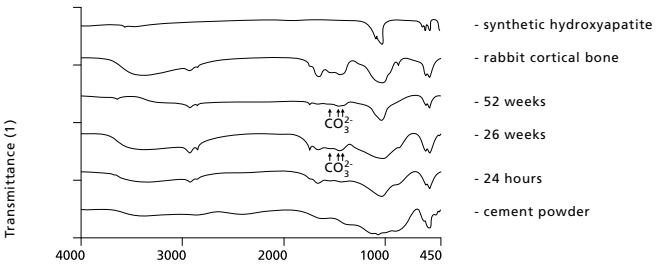


Fig. 9c Wavenumber (cm^{-1})

Fig. 9
FTIR patterns of (a) HydroSet, (b) Norian SRS, and (c) BoneSource after different evaluation periods. The FTIR patterns shows the absorption bands of the hydroxyl (OH), carbonate (CO_3^{2-}), and phosphate (PO_4^{3-}) groups. The FTIR patterns of these CPCs change with time to have the characteristic features of carbonated hydroxyapatite. The characteristic CO_3^{2-} bands are marked with arrows. FTIR patterns of rabbit bone and synthetic hydroxyapatite are added on top of each plot for comparison.

DISCUSSION

HydroSet is a novel, injectable, calcium phosphate cement that hardens to form hydroxyapatite and remodels to natural bone through osteoclastic resorption and new bone formation. It is a fast-setting cement indicated to fill bony voids or gaps that are not intrinsic to the stability of the bony structure.

Calcium phosphate transformations in vitro have been well described.[32, 33] However, most of this work has been carried out in vitro under standard laboratory conditions. More recently, the phase and morphological properties of CPC samples that were placed in living tissue for various periods were studied to evaluate whether the conversion and rate of transformation of CPCs in vitro is similar to that in vivo.[17, 18] It is important to know how these CPCs change chemically when implanted, as this will affect their resorption rate, osteoconductivity, and biocompatibility. The results from the present study showed that under in vivo conditions all three CPCs largely converted to carbonated HA after 24 h, and remained stable over time. XRD analyses showed that the carbonated HA formed in vivo from the present CPCs were comparable in crystallinity to that of the cement products reported by Constantz et al.,[17] and Takagi et al.[18]

The histological and histomorphometrical results confirmed the excellent bone biocompatibility of all three CPCs used. None of the materials evoked any inflammatory response. Excellent osteoconduction was observed in all three materials. Already after 6 weeks, the cement surface was almost completely covered with new deposited bone. These findings correspond with many other studies dealing with CPCs.[26, 34, 35] However, occasionally unfavorable bone healing of CPCs is reported, as characterized by the presence of inflammatory cells and fibrous encapsulation.[36, 37] Such a reaction is supposed to be due to the use of acidic cements or to the use of additives to improve the handling properties of the cement.

The CPCs were applied into the created bone defects by using a syringe. Injection of the CPCs offers the opportunity to fill defects in a retrograde way, which can prevent (significant) air entrapment. Furthermore, pressure can be generated during the injection.[26] This provides a good cement fill of the bone bed and a tight initial contact with the defect walls.[6, 9] Our histological results confirmed these advantages of the injection technique. However, as it is injected the CPCs interdigitated with the adjacent trabecular bone, causing an irregularity in the contour of the cement plugs, which may explain the large surface areas measured.

Although, an ongoing remodeling was observed, none of the materials did resorb significantly over the time of this study as shown from histology and histomorphometry. After 52 weeks, most of the cement material was still present in the bone defects. The kind of cells involved in the degradation/resorption of CPCs differs between the variously studied cements. Generally, for fast degrading cements, macrophages, and giant cells are the cell types involved in the resorption process.[38] This is in contrast to very slow degrading cements (months to years). Then osteoclast-like cells are held responsible for the degradation.[26, 34, 35] Evidently, this is true for CPCs tested in the present study. Eventually, the cements will completely disappear and be replaced by bone. However, this will take considerable time. The rate at which the material finally degrades varies between weeks to years.[35, 39] More than one year postoperatively, BoneSource still was

visible on radiographs of feline cranial defects.[24] Norian SRS was still present in the center of the metaphysis on 3 and 4 year follow-up radiographs.[5] However, complete degradation of CPCs is not only dependent on physicochemical properties, like crystallinity, density and porosity, but also on animal model and implantation site.[17, 35, 40, 41] Although we do not feel that it is a clinical problem to have the HA cements remaining, this still may be a concern at longer follow-up for certain surgical defects/locations.[5, 39]

Most CPCs, when implanted as a paste which is just starting to set, tend to disintegrate upon early contact with blood or other fluids. For this reason cohesion promoters must be added.[6] Some authors improved the cohesion of a cement consisting of tetracalcium phosphate and dicalcium phosphate by dissolving sodium alginate in the cement liquid.[42, 43] Recently, hydroxypropyl methylcellulose, carboxymethylcellulose, chitosan acetate, and chitosan lactate have been tried for the same type CPC cement.[44] All of these additives improved the handling properties with respect to the cohesion of the cement paste, whereas the setting was retarded by the cellulose derivatives and the mechanical strength was reduced by the chitosan derivatives. Furthermore, the disintegration of CPCs in circulating blood does not only compromise the mechanical properties, but also represents a risk of cardiovascular complications.[45-47] Therefore, a reliable cohesion of CPCs in an aqueous environment is essential for clinical applications. Unlike BoneSource, Norian SRS can set in the presence of fluid.[5, 48] HydroSet was specifically formulated to provide exceptional wet-field environment characteristics. As a cohesion promotor, PVP was added.

These wet-field environment characteristics were designed to make HydroSet particularly useful to stabilize fractures that communicate with the joint space, although it should be demonstrated first that HydroSet does not have any negative effects on articular cartilage if introduced in the joint space. Clinical application of CPCs in comminuted fractures revealed penetration of the viscous paste into the joint space.[49-51] Recently, Braun et al.[52] investigated the potential hazard of the presence of cement particles intra-articularly with observation periods from 1 week up to 24 months. Norian SRS cement was injected into one knee joint, the contra-lateral side received the same volume of Ringers' solution. No signs of pronounced acute or chronic inflammation were visible. Injected cement became surrounded by synovial tissue and there were signs of superficial resorption. In some cases, bone formation was seen around the cement. Degeneration of the articular surface showed no differences between experimental and control side, and no changes over time became present. Prolonged presence of cement still seems to make it advisable to remove radiologically visible amounts from the joint space.

HydroSet has been developed to set fast. Surgeons should be aware that for cements with a short setting time, this might result in application problems. The combination of a short setting time and a complicated surgical technique might affect a proper delivery of the CPC.

Although a large variation was observed between animals, HydroSet has been shown to be both biologically safe and effective as bone void filler. In this experimental model, no differences in biological activity, osteoconductive properties, and resorption rate compared with other commonly used calcium phosphate cements were found.

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CHAPTER NINE
SUMMARY, CLOSING REMARKS AND FUTURE PERSPECTIVES

CHAPTER ONE

Total hip arthroplasty (THA) is one of the most successful medical procedures. The success of THA in combination with the increasing possibilities in hip surgery has led to extension of indications for THA. Unfortunately, the survival of hip prostheses is not unlimited. The main reason for loosening of hip implants is aseptic loosening. Loosening of hip implants necessitates revision surgery. One of the major problems encountered in hip revision surgery is the loss of bone stock due to the loosening process and the removal of failed implants. Bone impaction grafting in combination with cemented implants for revision surgery of the hip has appeared to be a successful technique for dealing with bone loss. The clinical results are good and support recommendations for the continued use and development of the technique. The success of this method depends on achieving adequate initial stability followed by a biological response of graft incorporation and remodeling. Manipulation of the graft to improve one of these factors (biomechanical versus biological) may be detrimental to the other. The most appropriate production and processing techniques of the graft in order to generate a mechanical stable construct advantageous to biological incorporation and remodeling still has to be unraveled. In chapter 1, an overview is presented on the basic science of the bone impaction grafting technique. In this chapter relevant items, like materials, preparation and processing, impaction, cementing, alternative materials and growth factors, are discussed.

CHAPTER TWO

Various materials and factors have been tested for their effect on bone graft incorporation and bone formation using the bone conduction chamber. However, biomaterials often have to be crushed to fit in this small chamber. Since cellular responses to biomaterials are influenced by the size and shape of particles, research concerning the evaluation of biomaterials is limited by the dimensions of this bone chamber. In chapter 2 we developed and tested three new large bone chambers, based on the bone conduction chamber model, in order to be able to investigate the in vivo influences of biomaterials and growth factors on tissue and bone ingrowth. Empty chambers were used as a test to evaluate the in vivo tissue and bone ingrowth. The results showed that one of the enlarged models performed most similar to the bone conduction chamber. This new bone chamber offers new possibilities for studies of bone tissue reactions to various biomaterials.

CHAPTER THREE

Bone impaction grafting with a cemented prosthesis is a biological attractive technique to treat bone stock defects around failed total hip prosthesis, with proven long term clinical results. Although the technique on the acetabular side has been well described by the original promoters, a dispute has been raised about the pros and cons of rinsing the allograft bone chips. Both rinsing before and before and after impaction are used. However, rinsing before impaction or before and after impaction might have quite different effects on the incorporation of the grafts. Rinsing the grafts again after impaction might have a negative effect on bone formation if growth factors released by the impaction process are washed away. In chapter 3 we studied if TGF-β and BMPs are released from the mineralized matrix by impaction, and if these released growth factors induce osteogenic differentiation in human mesenchymal stem cells (hMSCs). The results showed that abundant TGF-β was present in the fluid released during impaction. No BMPs could be detected. Osteogenic differentiation of hMSCs was inhibited by the fluid. No detrimental biological effects of rinsing morselized impacted bone grafts prior to cementation were found. However, there are no in-vivo data to support whether rinsing after impaction is good for the incorporation process or not.

CHAPTER FOUR

Gamma irradiation has been widely used for sterilization of bone allografts. However, gamma irradiation alters proteins. This is favorable when it reduces immunogenicity, but is undesirable when osteoinductive proteins are damaged. Although the effect of gamma irradiation on BMPs has been studied, the effect of gamma irradiation on the incorporation process of morselized bone chips remains unclear. Therefore, the effects of sterilization by gamma irradiation on the incorporation of impacted morselized allografts were investigated in chapter 4. The results showed that the incorporation process proceeded in a similar way in unprocessed, rinsed, and both rinsed and irradiated bone grafts. We concluded that sterilization with gamma irradiation does not influence the incorporation of impacted rinsed allografts.

CHAPTER FIVE

Bone morphogenetic proteins have the potential to improve clinical outcome after revision surgery by improving graft incorporation and implant fixation. However, a major concern in using growth factors in impaction grafting is the potential stimulation of the osteoclastic lineage. A solution would be using an osteoconductive material resistant to resorption and providing initial stability after reconstruction. In chapter 5 we determined whether OP-1 would promote the incorporation of morselized allografts and tricalcium phosphate/hydroxyapatite (TCP/HA) into host bone, whether bone formation would be preceded by an initial process of accelerated resorption, and whether the response to OP-1 would be dose-related. The results showed that the incorporation process of impacted morselized allografts and impacted TCP/HA granules was not promoted by OP-1. No signs of accelerated resorption preceding bone formation were observed. Remarkably, an increase in OP-1 dose resulted in a reduction of fibrous tissue formation and OP-1 did not promote bone formation. Within impacted bone chips, the migration of cells into the impacted allograft is compromised and vascularization is delayed for several weeks. The collagen carrier might have additionally delayed bone and fibrous tissue ingrowth into the impacted graft material by filling up the space between the impacted graft material. Using higher doses of the OP-1 device, more collagen carrier material was impacted between the graft material, filling up the space between the impacted material and thereby delaying tissue ingrowth and remodeling.

CHAPTER SIX

In chapter 6 was analyzed whether the decrease in fibrous tissue formation observed in chapter 5 represented a final loss or just a delay, and if the decrease can be overcome and ultimately result in a better late ingrowth. The results revealed evidence of bone graft incorporation which proceeded in a similar way in both allografts and TCP/HA, with and without the addition of OP-1. No difference in bone ingrowth was found between OP-1 groups and their controls. The addition of OP-1 resulted in more fibrous tissue in the impacted allograft. We concluded that the previous observed delay in fibrous tissue ingrowth can be overcome only partly.

CHAPTER SEVEN

Data presented in chapter 5 and 6 demonstrated the difficulty of applying a biological enhancer of bone healing, such as an osteoinductive growth factor, in a situation where the access to blood supply and stem cells are limited and bone healing is not optimal. In chapter 7 we developed and evaluated a carrier based delivery system with a localized sustained release. Heparin was covalently attached to a collagen coated TCP/HA granules and loaded with BMP-7. The results showed that TCP/HA granules coated with collagen in the presence of heparin exhibited a localized sustained release of BMP-7. BMP-7 was released more rapidly from the uncoated TCP/HA granules and the TCP/HA granules coated with collagen only. This delivery system could probably also be applied to deliver dual or multiple growth factors that have affinities for heparin such as VEGF, BMPs and PDGF, which could synergistically enhance osteogenesis by increasing vascularity. The delivery system presented in this study may provide a powerful tool for bone regeneration. However, before advocating this it should be evaluated in-vivo first.

CHAPTER EIGHT

One of the more promising groups of synthetic graft materials for augmenting acute fracture healing are the calcium phosphate cements. Recently it has been suggested that they might also compete with the PMMA bone cements and the apatite coatings for fixation of metal endoprostheses in orthopaedics and oral implantology. In chapter 8, the physicochemical and biological properties of a new developed calcium phosphate cement (CPC) were investigated. The novel calcium phosphate cement was compared with two other commercially available CPCs. XRD and FTIR indicate that the cements convert to carbonated hydroxyapatite and remain stable over time. Histological and histomorphometrical analyses did not show any significant differences between the three implanted cements. The results indicate that the investigated cement is biocompatible, osteoconductive, and seems to be both biologically save and effective as a bone void filler.

VALIDITY AND RELIABILITY OF BONE CHAMBERS

Many animal models have been used to study bone repair. Our group has used bone chambers in vivo to create models where we have tried to change a single factor at a time. This factor can be modified and studied under standardized conditions. As an outcome variable, we used the distance of new bone ingrowth into a bony material or bone substitute. Various ways of treating a graft, like irradiation, rinsing, or adding growth factors, have helped us to increase or reduce the ingrowth. An osteoconductive material can be inserted into the chamber, and tissue from the marrow cavity and/or cortical bone can grow in through the ingrowth openings at the bottom. The interior of the chamber is stress-shielded and no deformations occur. This protects and deprives ingrowing vessels and tissues of mechanical stimulation. This means that any biologic effect that requires participation of a physical load, which bone remodeling presumably does, cannot be adequately studied under these artificial unloaded conditions. However, the bone chamber models with their limitations have been used so extensively that we feel they are valid for detecting the effects of bone substitutes and signaling molecules that arise under unloaded conditions. It has been suggested that bone chambers can be regarded as bone cultures in vivo. In our view, bone chambers should be interpreted at that level in the hierarchy of experimental models. However, extrapolation of results of studies with bone chambers to clinical recommendations should be made carefully, because factors like vascularity and composition of the host bone bed, its interaction with the bone graft, loading conditions, and surrounding tissues are not included in these studies.

CLOSING REMARKS AND FUTURE PERSPECTIVES

Total hip arthroplasty (THA) remains one of the most successful procedures performed by any surgeon, as measured by quality adjusted life years with more than 90% clinical success rate at 10 years. Each year in the US there are over 200,000 THAs performed at a yearly cost of approximately $ 5 billion. With the demographics of an ageing population, this number is set to rise by as much as 174% to roughly 550,000 by 2030. At the opposite end of the age spectrum, the combination of younger and more active patients, with higher expectations and surgeons prepared to perform surgery on these patients, further increased the growing demand for THA surgery. These younger patients are likely to live longer and place higher demands on their joint replacements, which further increases the likelihood of revision surgery. In consequence, revision hip surgery has increased by 100% due to implant failure since 1991, and estimates from the American Academy of Orthopedic Surgeons (AAOS) show that this number is expected to double by the year 2026. Moreover, there are a number of issues surrounding revision hip arthroplasty, including high costs of revision operations, higher complication rate, longer operative time, significant blood loss in comparison with primary procedures, and outcomes are often inferior to the primary operation.

The causes for many of these difficulties are the extensive bone loss associated with loose implants requiring revision surgery. When an implant fails, bone is lost through a combination of stress shielding, osteolysis, instability and/or infection. Several techniques are available for dealing with bone loss and bone defects in hip revision surgery. With primary THAs and subsequent revision THAs being performed on younger patients, restoring rather than replacing bone stock will be the option of first choice. Of the many methods of reconstruction available, only two have the aim to restore the bone stock, namely bone impaction grafting and the use of structural allograft. In this thesis was focused on bone impaction grafting.

The objective of bone impaction grafting is to achieve stability of an implant with the use of impacted, morselized bone and subsequently to allow the restoration of living bone stock by bone ingrowth. This is an attractive option, and the aim of restoring the bone stock to a condition close to what was present during the primary arthroplasty may be achievable. There is, however, a delicate balance between the mechanical demands of achieving initial stability and the biology of long-term incorporation. During incorporation, the mechanical and biological properties of the reconstruction change with the progress of the process, which might disturb this balance. As the graft used in bone impaction grafting plays such an important mechanical role when initially implanted, it is important that the initial stability achieved through impaction is not compromised by stimulation of early graft resorption.

Recently, with demand outweighing the limited supply and increasing concerns regarding transmission of pathogens associated with the use of allograft, interest in synthetic materials has increased significantly. A prerequisite of these materials is that they must be able to endure the forces applied during the impaction procedure. Synthetic materials must initially also provide sufficient mechanical support for the prosthesis. Thereafter, the material is required to provide a framework onto which the host bone and vascular network can regenerate and heal. These synthetic materials are resorbed or degraded at various rates, altering the mechanical properties of these materials with time. Although the initial mechanical stability may be improved with the use of these materials, the biological aspects of the graft incorporation have not been fully studied yet. A denser synthetic graft, which may be the result of crushing of the synthetic material due to impaction, has been shown to reduce cement penetration. This will lead to reduced ingrowth with a higher chance of long-term failure. A further concern is the possibility of third body wear of the articulating surfaces by the synthetic particles.

Although impaction is mechanically beneficial, it has been shown to be detrimental to bone ingrowth and remodeling. This has led to widespread interest in graft additives and, in particular, the application of bone morphogenetic proteins (BMPs). Growth factor enhanced allograft incorporation could improve clinical outcome after revision surgery by accelerating new bone formation and thereby improving implant stability. However, BMPs not only stimulate new bone formation but also simultaneously stimulate osteoclastic activity. Therefore, BMPs might be particular useful in hip revision surgery as a promoter of bone formation in combination with a slow resorbing synthetic graft material, which provides initial stability. Maintenance of growth factors at the site of implantation and delivery of an optimal dose by an appropriate carrier material during the incorporation process are essential for successful application of growth factors.

A possible exciting way to improve both the biological and mechanical aspects of allograft in bone impaction grafting for revision hip surgery is the expanding emerging discipline of tissue engineering. However, for stem cells to play a role in bone impaction grafting it is necessary first to determine if, when combined with allograft or synthetic graft, these cells could survive the impaction process.

Areas of particular interest for the future of bone impaction grafting include the potential use of stem cells, and scaffolds containing multiple growth factors, resulting in sustained and controlled release over time, providing greater control of the delicate balance between initial stability and long-term incorporation. The current drive in tissue engineering and regenerative medicine energizes the further development of the bone impaction grafting technique, in particular the development of composites that provide enhanced biological, mechanical, and hopefully ultimately, improved clinical outcome for patients requiring revision hip surgery.

CHAPTER TEN

SAMENVATTING, AFSLUITENDE OPMERKINGEN EN TOEKOMSTIG ONDERZOEK

HOOFDSTUK EEN

De totale heupvervanging (THV) is één van meest succesvolle medische ingrepen. Het succes van de THV in combinatie met de toegenomen mogelijkheden binnen de heupchirurgie, heeft geleid tot een uitbreiding van de indicaties voor deze ingreep. Toch kunnen implantaten na verloop van tijd falen. De belangrijkste reden voor het falen van heupprothesen is aseptische loslating. Het falen van prothesen maakt een revisieoperatie noodzakelijk. Eén van grootste uitdagingen binnen de revisiechirurgie is het omgaan met botdefecten die worden aangetroffen tijdens een revisieoperatie. Botdefecten kunnen ontstaan door het loslatingproces zelf, maar kunnen worden verergerd door het botverlies dat optreedt bij het verwijderen van een gefaalde prothese. "Bone impaction grafting" in combinatie met het plaatsen van een gecementeerde prothese is een succesvolle techniek gebleken om met deze botdefecten om te gaan, zowel bij primaire heupvervangingen met botverlies als bij patiënten die een revisieoperatie moeten ondergaan. De klinische resultaten zijn goed en ondersteunen het verdere gebruik en de ontwikkeling van deze techniek. Het succes van de "bone impaction grafting" techniek berust op het bereiken van een adequate initiële stabiliteit, gevolgd door een biologische ingroei en remodellering van het bottransplantaat op de langere termijn. Het behandelen van bottransplantaten om één van deze factoren, mechanisch dan wel biologisch, te verbeteren kan nadelige gevolgen hebben voor de ander. De meest optimale productie- en behandelingstechnieken van de bottransplantaten om een mechanisch stabiele reconstructie te creëren welke biologisch incorporeert en remodelleert is nog niet gevonden. In hoofdstuk 1 wordt een overzicht gegeven van de huidige status van het wetenschappelijke onderzoek naar de "bone impaction grafting" techniek. In dit hoofdstuk komen relevante onderwerpen, zoals materialen, preparatie- en behandelingstechnieken, impactie, cementering, alternatieve materialen en groeifactoren aan bod.

HOOFDSTUK TWEE

In de "bone conduction chamber" zijn effecten van verschillende materialen en factoren op de incorporatie van bottransplantaten en botformatie onderzocht. Wanneer biomaterialen worden getest, moeten deze vaak fijngestampt worden om in deze kleine botkamer te passen. Aangezien de reactie van cellen op biomaterialen wordt beïnvloed door de omvang en vorm van de partikels wordt het onderzoek naar biomaterialen beperkt door de afmetingen van deze botkamer. In hoofdstuk 2 hebben we drie nieuwe typen grote botkamers ontwikkeld en getest om de in-vivo invloeden van biomaterialen en groeifactoren op bot- en weefselingroei te kunnen bestuderen. De drie nieuw ontwikkelde botkamertypen zijn gebaseerd op de kleinere "bone conduction chamber". Kamers zonder inhoud werden gebruikt om de in-vivo bot- en weefselingroei te onderzoeken. De resultaten laten zien dat één van de drie nieuwe typen botkamers bot- en weefselingroei vertoonde vergelijkbaar met de "bone conduction chamber". Deze nieuwe botkamer biedt mogelijkheden om de reactie van botweefsel op verschillende biomaterialen te bestuderen, zonder deze biomaterialen fijn te hoeven stampen.

HOOFDSTUK DRIE

De "bone impaction grafting" techniek is een biologisch aantrekkelijke techniek om botdefecten rond gefaalde heupprothesen te behandelen. Hoewel de operatietechniek aan de acetabulaire zijde goed beschreven is door de oorspronkelijke bedenkers van de techniek, is er toch een discussie ontstaan over de voor- en nadelen van het spoelen van bottransplantaten. Zowel het spoelen van bottransplantaten vóór impactie als het spoelen vóór én na impactie worden in de (klinische) praktijk gebruikt. Echter, spoelen vóór of spoelen vóór én na impactie van de bottransplantaten zou verschillende effecten kunnen hebben op de incorporatie van deze transplantaten. Het nogmaals spoelen van de transplantaten na impactie zou een negatief effect op de botformatie kunnen hebben wanneer de groeifactoren, afgegeven tijdens de impactieprocedure, weggespoeld zouden worden. In hoofdstuk 3 werd onderzocht of transformerende groeifactor-bèta (TGF-β) en bot morfogenetische proteïnen (BMPs) vrijkomen uit de gemineraliseerde matrix tijdens de impactieprocedure. De resultaten laten zien dat er overvloedig TGF-β aanwezig was in de vloeistof die vrijkwam tijdens impactie. In deze vloeistof konden geen BMPs worden aangetoond. De osteogene differentiatie van humane mesenchymale stamcellen werd geremd door de vloeistof. Er werden geen nadelige biologische effecten van het spoelen van geïmpacteerde bottransplantaten gevonden. Echter, er zijn geen in-vivo data beschikbaar die ondersteunen of nogmaals spoelen na impactie het incorporatie proces stimuleert of niet.

HOOFDSTUK VIER

Gammastraling wordt veel gebruikt voor de sterilisatie van bottransplantaten. Echter gamma straling verandert de structuur van eiwitten. Dit heeft een gunstig effect wanneer de immunogeniciteit verminderd wordt, maar is juist nadelig wanneer osteoinductieve eiwitten, die de ingroei van bot bevorderen, beschadigd worden. Hoewel het effect van gammastraling op BMPs bestudeerd is, blijft het effect van gammastraling op het incorporatieproces van bottransplantaten onduidelijk. In hoofdstuk 4 is het effect van gammastraling op de incorporatie van geïmpacteerde bottransplantaten bestudeerd. Het incorporatieproces verliep op een soortgelijke manier in onbehandelde transplantaten, gespoelde transplantaten en gespoelde en vervolgens bestraalde transplantaten. We concluderen dat bestralen met gammastraling de incorporatie van geïmpacteerde bottransplantaten niet beïnvloedt.

HOOFDSTUK VIJF

BMPs hebben theoretisch de potentie om klinische resultaten na revisiechirurgie te verbeteren, door het incorporatieproces te versnellen en de fixatie van de prothese te verbeteren. Echter, BMPs kunnen ook osteoclasten stimuleren. Een oplossing zou zijn om deze BMPs in combinatie met een langzaam resorberend osteoconductief materiaal, dat tevens initiële stabiliteit biedt, te gebruiken. In hoofdstuk 5 werd onderzocht of osteogeen proteïne-1 (OP-1 = BMP-7) het incorporatieproces van geïmpacteerde bottransplantaten en geïmpacteerde tricalciumfosfaat/hydroxyapatiet (TCP/HA) korrels versneld. Tevens werd onderzocht of botformatie voorafgegaan wordt door een proces van versnelde resorptie en of de respons op OP-1 dosis gerelateerd is. Het incorporatieproces van geïmpacteerde bottransplantaten en geïmpacteerde TCP/HA korrels werd niet versneld door OP-1. Er werden geen tekenen van versnelde botresorptie voorafgaand aan botformatie gevonden. Opvallend was dat een toename in de OP-1 dosis resulteerde in een afname van de ingroei van fibreus weefsel en dat er geen versnelde botformatie werd gevonden. In geïmpacteerde bottransplantaten is de migratie van cellen beperkt en de vascularisatie meerdere weken vertraagd. In deze studie zou het collageen dragermateriaal van OP-1 voor een extra vertraging in bot- en weefselingroei kunnen hebben gezorgd door het opvullen van de ruimte tussen de geïmpacteerde bottransplantaten waardoor de ingroei van weefsels extra vertraagd werd.

HOOFDSTUK ZES

In hoofdstuk 6 werd onderzocht of de in hoofdstuk 5 beschreven reductie van fibreus weefselingroei een definitief verminderde ingroei oplevert of dat het een slechts een vertraging van de ingroei tot gevolg heeft. Verder werd onderzocht of een mogelijke vertraging van de ingroei ingehaald zou kunnen worden en uiteindelijk zou kunnen resulteren in een betere ingroei op de lange termijn. De incorporatie van geïmpacteerde bottransplantaten en geïmpacteerde TCP/HA korrels verliep op soortgelijke wijze, zowel met als zonder toevoeging van OP-1. Er werd geen verschil gevonden in botingroei tussen OP-1 groepen en hun controle groepen. Het toevoegen van OP-1 leidde wel tot meer fibreus weefselingroei in de geïmpacteerde bottransplantaten. We kunnen concluderen dat de in hoofdstuk 5 geobserveerde vertraging in fibreus weefselingroei slechts gedeeltelijk kan worden ingehaald.

HOOFDSTUK ZEVEN

De resultaten gepresenteerd in de hoofdstukken 5 en 6 laten de moeilijkheid zien van het toepassen van een biologische stimulatie van botgenezing in een situatie waarin de bloedvoorziening beperkt is, en stamcellen verminderd aanwezig zijn. In hoofdstuk 7 wordt de ontwikkeling en evaluatie van een op een dragermateriaal gebaseerd afgiftesysteem met een lokale, langdurige afgifte van een groeifactor beschreven. Heparine werd covalent gebonden aan met collageen gecoate TCP/HA korrels en vervolgens geladen met BMP-7. TCP/HA korrels gecoat met collageen in de aanwezigheid van heparine lieten een lokale, langdurige afgifte van BMP-7 zien. BMP-7 werd aanzienlijk sneller afgegeven door de niet gecoate TCP/HA korrels en de TCP/HA korrels die alleen met collageen waren gecoat. Het beschreven afgiftesysteem kan mogelijk ook gebruikt worden voor de afgifte van twee of meer groeifactoren die affiniteit hebben voor heparine, zoals VEGF, BMPs en PDGF, zodat deze synergistisch osteogenese kunnen stimuleren door een toename van vascularisatie. Het afgiftesysteem beschreven in deze studie zou een krachtig gereedschap kunnen vormen voor botregeneratie. Echter, het afgiftesysteem moet om dit aan te tonen eerst in-vivo worden getest.

HOOFDSTUK ACHT

Eén van de veelbelovende synthetische materialen om fracturen te verstevigen en te herstellen zijn de calciumfosfaatcementen (CPCs). Recentelijk is zelfs gesuggereerd dat deze materialen zouden kunnen concurreren met PMMA botcementen en apatietcoatings die gebruikt worden voor de fixatie van metalen implantaten. In hoofdstuk 8 werden de fysico-chemische en biologische eigenschappen van een nieuw ontwikkeld CPC onderzocht. Het nieuw ontwikkelde cement werd vergeleken met twee commercieel beschikbare CPCs. Met Röntgendiffractie (XRD) en Fourier getransformeerde infraroodspectroscopie (FTIR) werd aangetoond dat alle drie de cementen converteren naar gecarboneerd hydroxyapatiet en stabiel blijven in de tijd. Histologische en histomorfometrische analyse lieten geen verschillen zien tussen de drie geteste cementen. Het nieuw ontwikkelde cement is biocompatibel, osteoconductief en lijkt biologisch veilig en effectief als botdefectvuller.

VALIDITEIT EN BETROUWBAARHEID VAN BOTKAMERS

Er zijn veel verschillende diermodellen gebruikt om botherstel te bestuderen. Onze groep heeft botkamermodellen gebruikt waarin we geprobeerd hebben steeds één enkele factor per keer te veranderen. Deze factor kan aangepast en bestudeerd worden onder standaard condities. Als uitkomstmaat gebruiken we de afstand waarover nieuw bot ingroeit in de bottransplantaten of de botvervangende materialen. Verschillende manieren van transplantaatbehandeling, zoals bestralen, spoelen of toevoegen van groeifactoren, hebben ons geholpen de ingroei van nieuw bot te vergroten of juist te verminderen. Een osteoconductief materiaal kan in de botkamer worden geplaatst waarna weefsel vanuit de mergholte en/of het omliggende corticale bot door de ingroeiopeningen onderin de kamer kan ingroeien. De binnenzijde van de botkamer is "stress shielded" en er treden geen deformaties op. Dit beschermt en depriveert ingroeiende bloedvaten en weefsels van mechanische stimulatie. Dit betekent dat elk biologisch proces dat deelname vereist van belasting, wat bij botremodellering het geval is, niet adequaat bestudeerd kan worden onder deze artificiële onbelaste condities. Echter, de botkamermodellen met hun beperkingen zijn zo intensief gebruikt dat we denken dat ze valide zijn om effecten te detecteren van verschillende botvervangende materialen en signaleringsmoleculen die ontstaan onder onbelaste condities. Botkamers kunnen gezien worden als botculturen in-vivo. In hiërarchie van experimentele modellen moeten de botkamers dan ook geïnterpreteerd worden op dat niveau. Het extrapoleren van resultaten van studies met botkamers naar klinische aanbevelingen moet daarom met enige voorzichtigheid worden gedaan. Factoren zoals bloedvoorziening, samenstelling van het botbed, interactie van het botbed met het transplantaat, belastingscondities en omliggende weefsels maken immers geen deel uit van deze studies.

AFSLUITENDE OPMERKINGEN EN TOEKOMSTIG ONDERZOEK

De totale heupvervanging blijft één van de meest succesvolle medische ingrepen, gezien de overleving van de totale heupprothese met meer dan 90% na 10 jaar postoperatief. In de Verenigde Staten worden ieder jaar meer dan 200.000 heupvervangende operaties verricht met ruim $5 miljard aan jaarlijkse kosten. Met het bevolkingsbeeld van een toenemende vergrijzing wordt een stijging verwacht van 174% naar een aantal van 550.000 heupvervangende operaties in 2030. Aan de andere kant van het leeftijdspectrum zorgt de combinatie van jonge, actieve patiënten en chirurgen die bereid zijn om deze jonge patiënten te opereren voor een verdere toename van de totale heupvervangende operaties. Deze jonge patiënten hebben een hogere levensverwachting en stellen hogere eisen aan hun prothesen wat de waarschijnlijkheid van een revisieoperatie in de toekomst doet toenemen. Mede hierdoor is, als gevolg van het falen van prothesecomponenten, het aantal revisie heupvervangingen toegenomen met 100% sinds 1991. Schattingen van de American Academy of Orthopaedic Surgeons (AAOS) laten zien dat dit aantal waarschijnlijk opnieuw zal zijn verdubbeld in 2026.
Er zijn een aantal issues rondom de revisie totale heupvervangingen, waaronder de hoge kosten van een revisieoperatie, de hogere kans op complicaties, de langere operatieduur, het grotere bloedverlies in vergelijking met primaire ingrepen en de vaak mindere klinische resultaten in vergelijking met de resultaten bij een primaire totale heupvervanging. De oorzaak van veel van deze problemen is het omvangrijke botverlies wat optreedt rondom falende prothesecomponenten. Wanneer een implantaat faalt, treedt botverlies op door een combinatie van "stress shielding", osteolyse, instabiliteit en/of infectie. Verschillende technieken zijn beschikbaar om met botverlies en botdefecten bij heup revisieoperaties om te gaan. Tijdens primaire totale heupvervangingen en eventueel latere revisieoperaties bij jongere patiënten heeft het herstellen van het botbed, eerder dan het vervangen van het botbed, de voorkeur. Van de vele beschikbare reconstructiemethoden hebben er slechts twee het doel het botbed te herstellen, namelijk de "bone impaction grafting" techniek en het gebruik van structurele allologe bottransplantaten. In dit proefschrift ligt de focus op de "bone impaction grafting" techniek. Het doel van de "bone impaction grafting" techniek is het bereiken van stabiliteit van de prothese met behulp van geïmpacteerde bottransplantaten en vervolgens het herstellen van het beschadigde botbed door middel van botingroei. Er is echter een delicate balans tussen de mechanische eisen van het bereiken van initiële stabiliteit en de biologische eisen voor incorporatie op de lange termijn. Tijdens het incorporatieproces veranderen de mechanische en biologische eigenschappen van de reconstructie, wat deze balans zou kunnen verstoren. Het is belangrijk dat de initiële stabiliteit die bereikt wordt door het impactieproces, niet gecompromitteerd wordt door een stimulus van vroege resorptie van het bottransplantaat.
Steeds vaker overschrijdt de vraag naar bottransplantaten de beperkte voorraad van deze transplantaten. Bovendien is er een toenemende ongerustheid over de transmissie van pathogenen die kan optreden bij het gebruik van allologe bottransplantaten. De belangstelling voor synthetische botvervangende materialen is hierdoor sterk toegenomen. Een eerste vereiste van deze botvervangende materialen is dat ze de krachten die tijdens de impactieprocedure uitgeoefend

worden, moeten kunnen weerstaan. Verder moeten de materialen een matrix leveren waarop bot en bloedvaten kunnen regenereren en herstellen. Deze synthetische materialen worden geresorbeerd met verschillende snelheden, wat zorgt voor een verandering van de mechanische eigenschappen in de tijd. Hoewel de initiële mechanische stabiliteit verbeterd kan worden door het gebruik van deze materialen, zijn de biologische aspecten van de incorporatie van deze materialen nog niet volledig bestudeerd. Een meer compact synthetisch transplantaat als gevolg van het verpoederen van een synthetisch materiaal door impactie, reduceert cementpenetratie en kan leiden tot een gereduceerde ingroei en een hogere faalkans van de reconstructie op de lange termijn. Een belangrijk punt van zorg is de mogelijkheid van het optreden van zogenaamde "third body wear", een proces waarbij synthetische partikels tussen de articulerende gewrichtsoppervlakken komen en daar slijtage veroorzaken.

Vanuit mechanisch oogpunt gezien is het impacteren van bot voordelig, maar voor de botingroei en remodellering is het juist nadelig. Dit heeft geleid tot een grote belangstelling voor transplantaatadditieven, met het gebruik van osteoinductieve groeifactoren, zoals BMPs, in het bijzonder. Incorporatie van allologe bottransplantaten met behulp van groeifactoren, zou de klinische resultaten na revisiechirurgie kunnen verbeteren door de botformatie te versnellen en daardoor de initiële stabiliteit van het implantaat te verbeteren. Echter, BMPs stimuleren niet alleen botformatie maar kunnen tegelijkertijd ook de activiteit van osteoclaststen stimuleren. BMPs zouden daarom bijzonder geschikt kunnen zijn voor gebruik in de heup revisiechirurgie om botformatie te versnellen, waarbij een langzaam resorberend synthetisch transplantatiemateriaal de initiële stabiliteit waarborgt. Behoud van groeifactoren op de plaats van implantatie en de afgifte van een optimale dosis door een geschikt dragermateriaal tijdens het incorporatieproces zijn essentieel voor het succesvol toepassen van groeifactoren.

Een veelbelovende manier om zowel de biologische als de mechanische aspecten van transplantaten in de "bone impaction grafting" techniek te verbeteren, zou kunnen voortkomen uit de zich sterk ontwikkelende discipline van de tissue engineering. Echter, voordat stamcellen een rol kunnen spelen in de "bone impaction grafting" techniek is het eerst noodzakelijk om vast te stellen of deze cellen, gecombineerd met bottransplantaten of synthetische botvervangers, het impactieproces kunnen overleven.

Bijzondere aandachtsgebieden voor de toekomst van de "bone impaction grafting" techniek zijn het gebruik van stamcellen en het gebruik van matrices die meerdere groeifactoren bevatten. Door middel van een langdurige en gecontroleerde afgifte van deze groeifactoren zou controle over de delicate balans tussen initiële stabiliteit en incorporatie op de lange termijn verkregen kunnen worden.

De huidige drive in tissue engineering en regeneratieve geneeskunde stimuleert de verdere ontwikkeling van de "bone impaction grafting" techniek. De ontwikkeling van synthetische materialen met verbeterde biologische en mechanische eigenschappen staat bijzonder in de belangstelling. Uiteindelijk moet dit in de toekomst leiden tot een beter klinisch resultaat voor patiënten die een revisie totale heupvervanging moeten ondergaan.

CHAPTER ELEVEN
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"Goed, dit doen we dus voorlopig even niet meer."
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CHAPTER TWELVE
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