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Thesis

The morselized and impacted bone graft

Animal experiments on proteins, impaction and load

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Contents

List of papers

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Introduction

Clinical background

The results of total joint replacement in hips and knees have improved since its introduction in the 1960s. The 10-year survival rates are now 97% for hips and 90–95% for knees in the Swedish multicenter national registers (Herberts and Malchau 1997, Knutson et al. 1994). The initial problems of infection, poor implant design and fatigue fractures of the implant have essentially been solved, and some authors even doubt the need for further research in these fields (Huiskes 1997). However, the problem of prosthetic loosening remains. The results of revision surgery are not as good as those of primary replacements (Kavanagh et al. 1985, Gustilo and Pasternak 1988). About one million THRs are performed annually worldwide, and approximately 2% of the population in the western world over the age of 60 have a THR (Murray 1998). This means that even with a low incidence, aseptic loosening affects many patients, and the younger and more active ones run a higher risk (Malchau et al. 1993, Knutson et al. 1994).

A loose prosthesis leads to changes in the surrounding bone with osteolysis and loss of bone stock. With minor bone loss and in elderly patients, satisfactory results have been reported for cemented revisions with 10–18% re-revision rates at 10 years (Marti et al. 1990, Estok and Harris 1994, Herberts 1994). In younger patients (Kershaw et al. 1991) and in cases with major bone deficiencies (Strömberg 1995) cemented revisions give less favorable results and the risk for re-revision is high. Even with the use of improved cementing technique the results are inferior (Katz et al. 1995, Mulroy and Harris 1996). At re-revision the prerequisites for stable fixation of a prosthesis are even worse. Other solutions have been tried with varying results. Cementless revision with complete hydroxyapatite-coated stems resulted in 90% survival at 9 years (Lawrence et al. 1993) while others have reported femoral survival in only 42% after 8 years (Berry et al. 1995). With a long-stemmed and fully-coated stem, the re-revision rate was only 6/287 at 7-16 years (Paprosky 1998). Long-stemmed, uncemented, coned and ribbed prostheses showed promising short-term results (Wagner and Wagner 1993, Hartwig et al. 1996) but no longer follow ups are available.

When major or massive bone loss occurs, bone grafts can be used in the proximal part of the femur or the acetabulum. Autogeneic and allogeneic structural grafts have been used in the acetabulum with good initial results (Harris et al. 1977), but resorption of the graft and subsequent loosening of the implant have been reported to occur later (Kwong et al. 1993, Shinar and Harris 1997). More favorable mid-term results have been reported with a similar technique (Garbuz et al. 1996). The better results in the latter were presumed to be due to better fixation of the graft by compression screws, which were placed in a more oblique- to vertical-position. On the femoral side, good short- and medium-term results have been obtained with structural grafts (Pak et al. 1993, Head et al. 1994, Gross et al. 1995)

In the late 1970s, the "Slooff-Ling technique", named after its inventors, was introduced (Figures 1 and 2), based on Hastings and Parker's (1975) operation for protrusio acetabuli in rheumatoid arthritis. They placed an autograft in the acetabulum and cemented a cup with a vitallium mesh between the graft and the cement. In 1978, Slooff started to use this method in cases of acetabular component loosening with osteolysis. Instead of the autograft, he used allograft chips, which were impacted into the acetabular cavity, and a cup was cemented directly onto the graft. The results were shown in 1984 (Slooff et al. 1984). One year later, Ling started to use the same technique for femoral reconstructions (Gie et al. 1993). With this technique, the bone chips are impacted with a phantom in the femoral canal. A cavity is produced, surrounded by a layer of tightly impacted allograft chips forming a compact lining of the thin cortical walls (Figure 1). The graft is contained within the

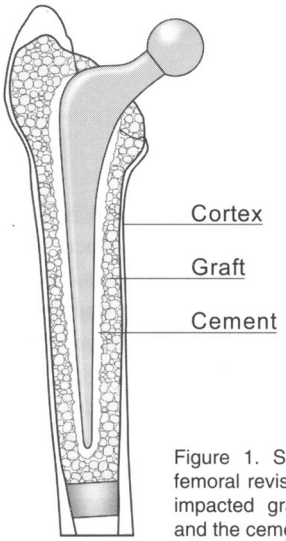
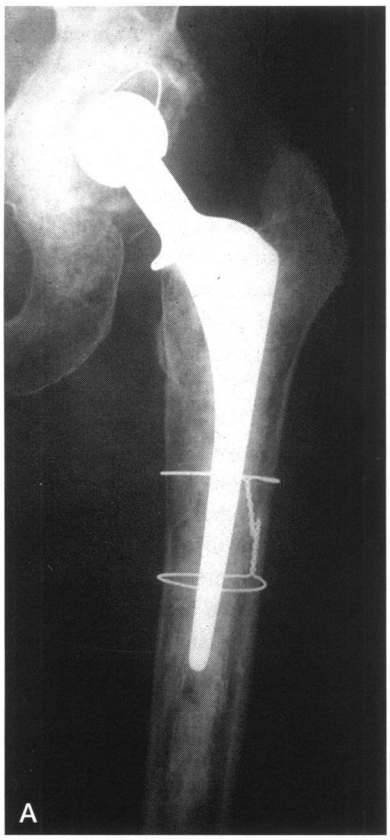


Figure 1. Schematic drawing of a femoral revision with morselized and impacted graft between the cortex and the cement.

cortex of the femur. A cemented prosthesis is then inserted in the same way as in a primary hip replacement with the cement pressurized into the graft during cementation. The method of using morselized and impacted allograft has also been applied to knee prosthetic revisions (Ullmark and Hovelius 1996, Whiteside and Bicalho 1998), to treat avascular necrosis of the hip (Gardeniers et al. 1997, Scher and Jakim 1999) and in primary hip (Keblish 1996) and primary knee (Bloebaum 1994) replacements.

Theoretically, the impacted allograft would be expected to fail. A large volume of necrotic tissue placed under high mechanical stress should resorb and collapse, just as the necrotic bone collapses after avascular necrosis of the hip or knee, without being able to maintain its volume during healing and remodeling. Moreover, the morselized and impacted graft is of allogeneic origin, and an immunologic reaction with activation of macrophages and osteoclasts would further enhance the resorption and lead to graft collapse and reloosening. Compared to the rather drastic introduction of a 0.5 to 1 cm thick layer of necrotic tissue, much more subtle changes around a prosthesis have been thought to be deleterious and even the cause of aseptic loosening, e.g. heat necrosis, fibrous layer development, and inflammatory cytokines.



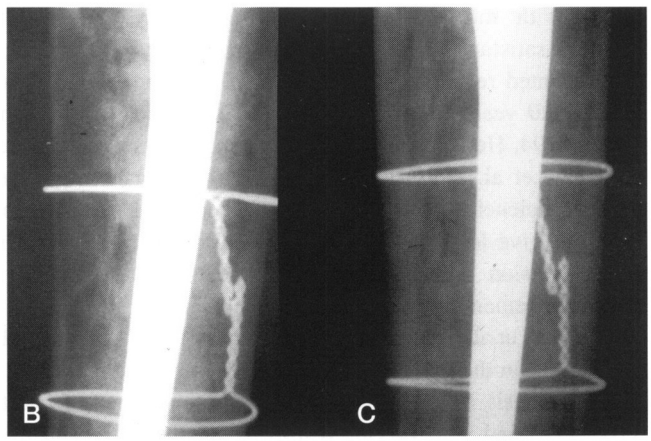


Figure 2. A. Hip revised with impaction grafting. The marrow cavity was cleansed of soft tissue and bony debris, and filled with morselized allograft, which was impacted with a phantom. A prosthesis was cemented into the cavity surrounded by a wall of impacted bone within the thin cortex. B and C. The postoperative radiograph (left) shown in higher magnification. Note the thin cortex. C (right) One year after the operation, the cortex looks thicker.

Table 1. Literature survey of series using impacted morselized bone graft in revision surgery

Author (year)	Site [a]	Implant	Numbers followed	Follow-up (years)	Revised due to reloosening	Comments
Slooff et al. 1996	A	Cemented cups	88 acetab. 10 femur	6	2/88	
Nivbrant and Kärrholm 1997	A	Cementless HA-coated ABG	25	2	0	RSA Small migration
Pitto et al. 1998	A		81	3–9	1	
Schreurs et al. 1998	A	Cemented Müller or Allopro	60	10–15 (11.8)	3	(6, 9 and 12 years)
Ornstein et al. 1999	A	Exeter	21	2	0	RSA migration 0.5–6 mm
Gie et al. 1993	F	Cemented Exeter	56	2–4	0	Migration; 5–10 mm, n=9 >10 mm, n=2
Capello 1994	F	Cemented	17	<2	0	
Elting et al. 1995	F	Cemented CPT [b]	67	2–5	0	Small migration; mean 2.8 mm
Franzen et al. 1995	F	Cemented Scan-Hip and Optima	5	1	0	RSA migration; 0.4–4.9 mm
Ling 1996	F	Cemented Exeter	400	0-9	1	
Eldridge et al. 1997	F	Cemented CPT [b] and Exeter	79	1 (0.5–3)	6	Large migration; 5–10 mm, n=9 >10 mm, n=9
Masterson and Duncan 1997	F	Cemented Exeter	35	0.5	1	Large migration; 0–10 mm, n=28 >10 mm, n=7
Meding et al. 1997	F	Cemented CPT [b]	34	2 (2–4)	2 (26 and 36 months)	Large migration; mean 10 mm (0–34)
Migaud et al. 1997	F	Cemented, long stemmed	20	5–10	0	Large migration in one patient (4.4 mm)
Kärrholm et al. 1999	F	Cemented	24	2	0	RSA small migration mean 0.3 mm
Leopold et al. 1999	F	Cemented Harris Precoat	29	5	1	
Masterson et al. 1999	F	Different	187	No data	No data	Cement analysis
Masterson et al. 1999	F	Cemented Charnley	50	0.5	No data	Cement analysis
Mikhail et al. 1999	F	Cemented CPT[b]	43	5–7		0–5 mm, n=29 5–10 mm, n=8 >10 mm, n=0
Ornstein et al. 1999	F	Exeter	18	2	0	RSA distal migration 1.4–4.3 mm
de Waal Malefijt 1998	K	Cemented, non-constrained		9	3	0
Whiteside and Bicalho 1998	K	Uncemented	63	No data	4	

[a] A Acetabulum, F Femur and K Knee
[b] CPT (Zimmer, Warsaw, USA) is a collar-less double tapered polished femoral stem.

The clinical results of the Slooff-Ling technique, however, are very good, with re-revision rates no higher than after a primary arthroplasty in the hands of the innovators (Ling 1996, Slooff et al. 1996, Schreurs et al. 1998). Others have also reported good short- and mid-term results with this method (Table 1), but poorer results have been noted, raising concerns over a high rate of subsidence (Eldridge et al. 1997, Meding et al. 1997, Masterson et al. 1997). Yet, this method must be considered successful.

Hypotheses

Why does the method of using morselized and impacted allograft work so well? As often, when the clinical need for a solution is great, clinical trials start before the theoretical basis of the method is clear. Ingrowth of host bone into a large, structural, non-morselized allograft is usually limited to 2–3 mm (Enneking and Mindell 1991, Bloem 1996, Gouin et al. 1996), whereas in the morselized and impacted graft a distance of at least 10 mm in the trochanteric region is thought to be remodeled. Why should a thick layer of morselized necrotic allograft bone become better incorporated, without causing the resorption and reloosening often encountered in structural grafts? We suggested three hypotheses to explain the excellent long-term results of impaction grafting at the start of this study:

1. The production of a large fracture surface area by fracturing the bone during morselization permits release and access to biologically active substances in the graft.
2. Impaction improves the osteoconductive properties of the graft.
3. The load-bearing capacity of the graft increases due to the impaction, and early mechanical load is possible. The compliance of the graft enables mechanical load to cause deformations, which stimulate bone formation.

Bone repair and bone graft incorporation

Bone graft remodeling

The incorporation of a bone graft is well described as imitating the process of fracture healing (Friedlaender 1991, Goldberg and Stevenson 1987). Various phases have been distinguished by the histologic appearance (Table 2). Growth factors are involved in the process (osteoinduction). Since the various growth factors peak early or late during bone graft incorporation, their gene expression over time can be used to divide bone graft healing into stages. Three stages have been proposed: an early inflammatory phase (1–3 weeks), a middle phase (week 4–5) and a late reparative phase with return of the gene expression of the growth factors to baseline (Morone et al. 1998).

Just as fracture healing includes primary union and secondary remodeling, graft healing or incorporation can be divided into graft union and graft remodeling. In graft union, the host tissue grows into the graft, ensuring mechanical stabilization. A few mms ingrowth of host tissue into a non-morselized graft is sufficient for the construct to achieve stability (Enneking and Mindell 1991). Graft union does not guarantee graft remodeling. The speed, extension and amount of graft remodeling differ due to various host and graft-related factors, unlike the bone remodeling after fracture. The incorporation process differs for cortical and cancellous bone grafts. The *cortical* grafts remodel at a slower rate and seldom completely, due to their dense structure (Burchardt 1983). The vascular penetration into the graft depends on osteoclastic resorption preceding the fibrovascular ingrowth (Enneking et al. 1975). The osteoclasts cut tunnels through the bone, which results in increased porosity and subsequent weakening of the graft during incorporation (Enneking et al. 1962). A *cancellous* bone graft, with its natural porosity and open architecture, can more easily be penetrated by ingrowing vessels, bringing differentiating osteoblast precursor cells into place and depositing osteoid directly onto the graft trabeculae, without preceding resorption (Goldberg and Stevenson 1987).

The source of the bone graft affects the various immunologic responses invoked by the graft. Allografts function more poorly than autografts (Bonfiglio and Jeter 1972, Burchardt and Enneking 1978, Ray 1972). Similarly, a lower expression of mRNAs coding for Type I collagen, osteonectin, TGFß-1 and decorin was found in allografts compared to autografts (Virolainen et al. 1993, 1998). Both allo- and autografts invoke an initial inflammatory response, which subsides by the second week (Goldberg and Stevenson 1987). In allografts, the immune system of the host at that time becomes sensitized to donor antigens. In dogs, the antigen titer peaks at six weeks,

Table 2. Stages of bone graft incorporation (adapted from White et al. 1977)

Stages of bone graft incorporation			
Stage 1	Inflammation	Hours	A hematoma forms in and around the graft. The fibrin clot attracts platelets, which release vasoactive mediators. Vasodilatation and exudation of plasma follow and leukocytes and macrophages start to invade the graft.
Stage 2	Consolidation	Days	Fibrovascular tissue starts to invade the graft. Osteoclast precursors are recruited and start to resorb the graft trabeculae. Osteoblasts are recruited locally from the surrounding host bone in the form of mesenchymal cells that differentiate to osteoblasts. Osteoid is deposited on the graft trabeculae, which serve as a mechanical support for the osteoblasts (osteoconduction).
Stage 3	Remodeling	Weeks	Further remodeling of the graft-host bone complex occurs.

when allografts are used, and thereafter declines. The immunologic response can be influenced by lipid extraction (Thorén and Aspenberg 1995) and freezing. In animal studies, allogeneic grafts function better when they have been frozen, which might depend on a reduced antigenicity (Bos et al. 1983b, Stevenson et al. 1997). Syngeneic grafts function better in general than allografts, but after freezing they revascularize less and more slowly (Stevenson et al. 1997), obviously due to the loss of living cells.

Bone response to proteins

Since bone graft healing appears to mimic fracture healing, local regulatory factors are probably important for sensitizing the local cells and regulating the release of biochemic messengers (Frost 1989). Hippocrates thought that an endogenous product that could heal bone, must be present in the human body (Reddi 1997). Levander (1938) was the first modern author to describe bone-inductive substances in bone tissue. He implanted bone segments subcutaneously or intramuscularly and found a surrounding layer of newly-formed bone. Aqueous and alcoholic extracts from bone injected extraosseously in rabbits caused bone formation also. Levander suggested that some specific agent in the bone, activated the non-specific mesenchymal tissue to produce bone. Later, Annersten (1940), Bertelsen (1944) and Lacroix (1945) reported induction of osteogenesis, and the latter named the hypothetical substance osteogenin. Urist (1965) found that demineralized bone matrix from adult human bone could induce the formation of ectopic bone. He coined the terms bone morphogenetic protein for the substance, and osteoinduction for the phenomenon of inducing bone at a non-skeletal site. The first, at least partially purified osteoinductive substance was named osteogenin, referring to the term of Lacroix (Sampath et al. 1987). At almost the same time, BMPs were purified (Wang et al. 1988), sequenced and synthesized recombinantly (Wozney et al. 1988). Up till now, more than 14 BMPs have been identified (Mont et al. 1998).

The extracellular matrix accounts for about 90% of the total weight of bone and is composed of 60% calcium phosphate, in a form resembling hydroxyapatite, and 27% fibrillar type I collagen (Hauschka et al. 1986). The remaining 3% consists of various types of collagen and other proteins such as growth factors. Bone matrix contains a mixture of bone active proteins (Mohan and Baylink 1991). The bone active proteins or bone-derived growth factors are synthesized by the osteoblasts and deposited in the matrix until trauma or remodeling causes them to be released. Bone growth factors function as chemoattractants for progenitor cells, mitogens for mesenchymal cells, cause differentiation of chondrocytes, calcification of the cartilaginous matrix, vascular invasion, bone remodeling and marrow differentiation (Reddi et al. 1998). Normal bone modeling and remodeling depend on a balanced action by growth factors with a sequential multistep cascade (Reddi and Huggins 1972).

The fibroblast growth factor (FGF) family consists of at least 9 members, FGF 1–9. They are primarily mitogens but also important for angiogenesis and revascularization. Insulin-like growth factors (IGF I and II) are the growth factors in bone, found in the highest concentrations. IGFs are regulated by growth hormone and stimulate proliferation of undifferentiated osteoblasts. Platelet-derived growth factor (PDGF) is an important chemotactic factor, and is also mitogenic. Transforming growth factor beta (TGF-ß) is found in 5 subtypes and is a potent regulator of bone metabolism. It is also found in high concentrations in blood platelets. TGF-ß belongs to a family of related proteins with similar amino acid sequences, the TGF-ß superfamily. Other members of this family include the GDFs or CDMPs and the BMPs.

BMPs

BMPs are low molecular weight polypeptides, which have been isolated from the bones of different species (Cook and Rueger 1996) and can be generated by recombinant DNA methods. In animal and in vitro experiments, the responses of BMP-containing implants resemble the sequence of events observed during endochondral bone formation in embryogenesis (Reddi and Huggins

1972). Cells condensate and proliferate around the BMP-implant and produce cartilaginous matrix. The cartilage cells hypertrophy and are replaced by bone, forming an ossicle, often with a central marrow cavity. In embryogenesis, BMPs are expressed at sites where early skeletal condensations are formed around developing cartilage and in the periosteum (Helder et al. 1995). BMPs are also expressed during fracture healing in adult animals (Bostrom et al. 1995, Nakase et al. 1994). A controversy exists as to whether the whole family of BMPs is needed (Lyons et al. 1995), with each member taking care of its own step in a cascade of events, or if they are interchangeable as triggers for cartilage and bone formation (Kingsley 1994). A combination of both theories seems plausible: some induce distinct responses while others are interchangeable, depending on the physiological situation. Subgroups with similar amino acid probably bind to similar cell surface receptors (Kingsley 1998).

BMPs cause differentiation of mesenchymal cells into osteoblasts (Yamaguchi 1991). The effect of BMPs on the osteoclastic cell lines has not been clarified. A bone graft with BMP, implanted subcutaneously, forms an ossicle with a central marrow cavity replacing the originally implanted material. This would suggest that a functional bone marrow is formed, at least indirectly, by BMP activity (Kanatani et al. 1995). In vitro, BMP-2 stimulated osteoclast-like cell differentiation in a culture of osteoclast-free bone cells (Kanatani et al. 1995). In vivo, the number of osteoclasts increased when grafts were treated with BMP-2 (Lamerigts 1998). With BMP-7, graft resorption reportedly increased in a large segment defect model in dog ulnas (Salkeld et al. 1997).

The BMPs present in a bone graft are thought not to be activated in experimental implants unless the bone is demineralized (Urist 1965). However, in Burwell's (1964) classical studies on bone grafting, some new bone formation may also be induced by non-demineralized allografts. Although it is often stated that the matrix of bone grafts contains several growth factors, it is not known whether they become activated and play a role in undemineralized bone graft. Exogenously applied growth factors in a carrier increased tissue ingrowth, bone formation and the ability to heal large segmental defects. (Cornell and Lane 1992, Gerhardt et al. 1993, Cook et al. 1994a, b, 1995). This effect could also be seen when BMPs are used with a mechanically bearing scaffold, such as hydroxyapatite (Horisaka et al. 1991, Aspenberg et al. 1996, Boden et al. 1999) or a bone graft (Johnson and Urist 1998, Schwartz et al. 1998). Fewer data are available on the use of growth factors combined with impacted morselized grafts, but clinical studies are already in progress. Increased ingrowth of soft tissue and bone was found in a bone chamber model with impacted bone graft and BMP-2 (Lamerigts 1998). The inhibitory effect of nicotine on the healing rate of an experimental rabbit spine fusion was reversed, using a morselized graft in combination with rhBMP-2 (Silcox et al. 1998).

Bone response to load—theoretical models

Apart from proteins, bone remodeling depends on mechanical stimuli, presumably mediated via signaling proteins. Wolff (1892) postulated that the bone is a dynamic organ, which responds to mechanical stimuli by an increase or decrease in the bone mass. Roux (1895) expressed similar views. Wolff regarded the stresses in the bone as the major stimulus, whereas Thompson (1921) suggested that strain, the result of the stresses, was a direct stimulus for growth itself. Frost (1964) proposed that a "minimum effective strain" threshold had to be surpassed before bone adaptation could occur. Later, he introduced the mechanostat hypothesis, in which he distinguished between modeling and remodeling, but also proposed thresholds for formation of lamellar and woven bone formation (Frost 1983). Lisskova and Hert (1971) showed that dynamic, but not static, strains would increase bone formation. Lanyon and Rubin (1984) confirmed this in the avian ulna model. In addition, frequency (Rubin and McLeod 1994) and strain rate (Turner et al. 1994, 1995) have been shown to be important for the remodeling of bone.

The first mathematical model of bone remodeling is known as the theory of adaptive elasticity (Cowin and Hedegus 1976), with an equilibrium

strain field related to an external load, and deviations in strain producing stimuli that increase or decrease bone density. This is referred to as internal remodeling, since no changes in shape appear. This type of remodeling applies to cancellous bone. In contrast, external remodeling refers to an alteration of the bone geometry, with resorption or deposition of bone periosteally and alteration of trabecular thickness (Cowin and Buskirk 1979). A general theory of remodeling including internal and external remodeling has been used (Weinans et al. 1993, van Rietbergen et al. 1993). No consensus exists about which stimuli are responsible for remodeling.

With the introduction of computers, various programs for calculating bone remodeling have been proposed, referred to as finite element models (FEM). On the basis of differences in the mechanical properties of various tissues, changes can be calculated after each modeled loading cycle. Two methods are currently available for such calculations, the single-phasic and the biphasic. Carter and co-workers (Carter et al. 1988, Beaupré et al. 1990) have used the first, based on hypotheses of Pauwels (1980). Mathematical predictions of skeletal morphogenesis, fracture healing and models of distraction osteogenesis have been presented. They suggested that the type of stress, caused different tissues to differentiate from the mesenchymal cells. High compressive stresses would make cartilage form, high shear stresses fibrous tissue, and low compressive and/or shear stresses would cause bone to form (Carter et al. 1988). In the biphasic model, the fluid flow between and in the different tissues are included in the calculations. However, the predictive value of the FEM analyses is not clear. Only a few studies have been done in which a predicted tissue response, based on the theoretical models, could be verified in vivo (Weinans et al. 1993, Prendergast et al. 1997, Carter et al. 1998).

Strength of bone grafts

The mechanical properties of cortical and cancellous bone, have been shown to be related to its apparent density, which is the total mass divided by the total volume including the pores. Although

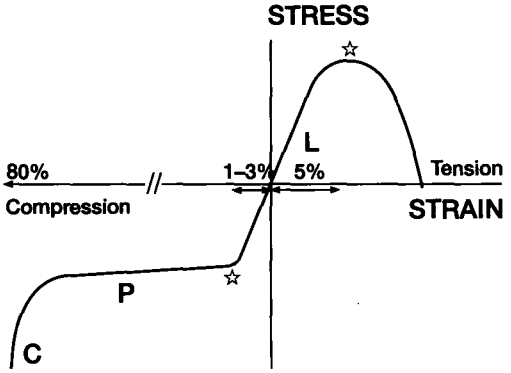


Figure 3. The stress-strain behavior of cancellous bone showing the linear (L), plateau (P) and consolidation region (C). Yield points (stars) in tension and compression. After the yield point, in the plateau region, the bone continues to collapse plastically by elastic buckling, plastic yielding and overt trabecular fractures. During this post-yield plateau region of cancellous bone, an impacted graft is literally being produced (Adapted from Taylor 1997).

challenged, some claim that the differences in mechanical properties between cortical and cancellous bone depend simply on variations in the apparent density which, in turn, depends on the porosity (Carter and Spengler 1978). The most commonly reported mechanical properties of cortical bone are Young's modulus, tensile and compressive strength, and of cancellous bone, compressive modulus and ultimate strength. The means and range of strength reported in the literature are fairly consistent but the variations between the studies are large, especially regarding cancellous bone, due to the material complexity. The wide variation is caused by the method of testing, like, uni- or multiaxial analyses, strain rate and viscoelastic behavior. It also reflects the complexity of the cancellous bone itself, the mechanical properties being influenced by its architecture, age, the geographic location, disease, etc.

Some authors have compared the stress-strain behavior of cancellous bone to rigid engineering foams (Carter et al. 1980, Gibson 1985) with three distinct regions: the linear, the plateau and the consolidation region (Figure 3). After the initial elastic linear behavior of the cancellous bone, yield occurs at 1–3% strain in the case of a single (monotonic) load (Rohlmann et al. 1980, Keaveney et al. 1994a). A long plateau follows, in which the cancellous bone behaves as a perfectly

plastic material until it becomes solid with no remaining porosity. The length of the plateau depends on the apparent density of the bone. In this plateau or post-yield region of the stress-strain curve, cancellous bone is literally transformed into an impacted cancellous bone graft.

Few researchers have studied the post-yield behavior of cancellous bone. Keaveny (1993, 1994) proposed, on the basis of post-yield behavior, that cancellous bone yields, because of microstructural damage to the individual trabeculae and that this was caused by damage to vertically aligned trabeculae. Investigation of the failure mechanisms in the human vertebral body by compressing it well above the yield point to 15% strain, Fyhrie and Schaffer (1994) found only a few broken trabeculae, mainly perpendicular to the load. The vertically aligned trabeculae showed substantial internal damage such as bulging, but were grossly intact. On the basis of this observation, they concluded that micro-callus formation is due, not to a healing of overtly fractured trabeculae, but more to repair of internally damaged trabeculae. This would permit the cancellous bone graft to maintain its structural integrity and increase its strength during remodeling, which presumably would also apply to an impacted or post-yield cancellous graft.

Bone graft strength during remodeling

According to some authors, bone necrosis does not change the strength of the bone unless or until it is being revascularized and remodeled (Burchardt 1983, Gardeniers 1988, Glimcher and Kenzora 1979). The strength of a bone graft after implantation thus depends on remodeling (Burchardt 1983) and is different in cancellous and cortical grafts. Cancellous grafts are incorporated via a process of osteoblastic deposit of osteoid directly onto the dead trabecula, without preceding osteoclastic preparation and the mechanical strength increases, as new bone is appositioned on the dead graft trabeculae (Burchardt 1983). This can be seen as increased bone density on radiographs (Goldberg and Stevenson 1987). Cortical grafts are incorporated more slowly and less completely, with an initial increased osteoclastic resorptive phase and a simultaneous weakening of the bone due to increased porosity. This can also be seen in radiographs, but as a decreased bone density. The strength of a bone graft thus depends on the rate and speed of the remodeling and either a decrease or increase will follow temporarily, depending on whether one chooses a cancellous or a cortical graft. A human autologous cortical graft loses about half of its strength during the first six months, maintains this strength for the next half-year and then it slowly increases (Enneking et al. 1980). At that time complete union between the host and the graft may have occurred, but the graft may not be fully remodeled in its deeper parts.

How large proportion of a large bone graft that remodels differs in cortical vs. cancellous grafts as well as in autografts vs. allografts. In canine cortical grafts, the proportion of viable to necrotic bone increased between two weeks and six months but remained unaltered at 60% between six months and two years (Enneking et al. 1975). In humans, the repair seemed to be similar, but slower, only half the rate, and only 20% new bone formed (Enneking et al. 1980). It is not known why this balance evens out, but the repair, gradually seems to stop. Instead of measuring the proportion of a graft that remodels, it would appear more relevant to study how far the remodeling reaches into the graft. Histologic observations in humans of retrieved large structural allografts reveal poor ingrowth of new bone into the graft, large areas remain unremodeled and even unrevascularized for many years (Enneking and Mindell 1991, Bloem et al. 1996, Gouin et al. 1996). The cortical bone is penetrated a few mm, just like the cancellous graft. In a few cases, involving trauma in the remodeling period e.g. fracture, autogenous bone transplantation or extraction of a medullar nail, rather extensive remodeling with marked transient osteoporosis of the cortical bone takes place. In the revascularized and remodeling areas, fracture is therfore predicted to occure within the first two years (Enneking et al. 1980). In avascular bone, fatigue fractures, due to stress accumulation, would occur later, and then lack the prerequisites to heal in the dead bone. Larger cracks and clinical fractures that extend to vascularized areas, however, can heal via ingrow-

ing vessels from surrounding soft tissues (Enneking and Mindell 1991). Fractures of large allografts reportedly occur in 16-50% (Berrey et al. 1990, Enneking and Mindell 1991, Muscolo et al. 1992, Thompson et al. 1993).

Biomechanics of an impacted graft

The biomechanics of an impacted morselized graft is largely unknown and how it remodels can only be speculated about. Does it remodel in the same way as a cancellous graft, with no loss of strength or even an increase during remodeling? Does it remodel at all, and finally, is remodeling necessary or is it a disadvantage?

The mechanical properties of a morselized impacted bone graft were analyzed using the technique of soil testing (Brodt et al. 1998). Due to its non-structural nature, simple tensile, compressive or shear tests that are normally performed could not be done on a morselized graft. Instead, the graft was contained in an elastic membrane and the pressure kept isobaric in a surrounding fluid to prevent the graft from barreling out. A bi-phasic curve was produced, initially rather stiff, followed by a "crush-point" at approximately 0.2 MPa. A linear more compliant domain then followed until the test stopped. The findings indicated that, although the morselized graft is a non-cohesive material, the graft morsels and trabeculae interlocked when sliding relative to each other in the stiff initial curve of the stress strain curve, and that the more compliant curve represented breakage of the trabeculae. In the above study, no clear difference in mechanical properties could be found that was related to differences in bone chip size. In another study, larger chips gave a more stable construct than smaller ones (Eldridge et al. 1997).

Comparing the initial stability of femoral stems in bench tests, cemented hip prostheses with an intact cancellous marrow cavity were more stable than femoral revisions using impacted morselized bone (Eldridge et al. 1997). Nevertheless, better stability was achieved with the morselized and impacted grafts than with cementless revisions (Berzins et al. 1996). In still another study, more subsidence was found in the revision operations using an impacted graft than with a primary prosthesis, but the rotatory stability was about the same (Malkani et al. 1996.). The authors' conclusion that "the operation restores the integrity of the proximal femur" was challenged by Ling (1997), who pointed out that the initial stability by no means ensures stability during remodeling. This distinction between initial stability and stability during and after remodeling is important and was exemplified in a study on acetabular cups, cemented into an impacted morselized graft in goats (Schimmel et al. 1998). The mechanical stability of the prostheses increased during remodeling. When the remodeling process was completed and the interface revascularized, a fibrous membrane developed between the remodeled graft and the cement. As a consequence, the prostheses became loose. This implies that remodeling is not always beneficial and can even be hazardous for longevity of the prosthesis.

Conversely, it was noted that no radiographic signs of loosening or radiolucencies were seen in the patients with a hip prosthesis in the series of retrieved large allografts (Enneking and Mindell 1991). The graft adjacent to the cement around the stems showed no signs of revascularization or resorption. In another study radiolucent lines were absent around prostheses cemented into large structural allografts (Rosenberg and Mankin 1986).

The impacted graft as a compromise between cancellous and cortical bone

Initially, when the need for hip prosthetic revisions emerged, it seemed desirable to use cortical grafts thanks to their load-bearing capacity. However, biologic events, such as decreased strength during remodeling, led to clinical problems. Other solutions for restoring the bone stock were tried. Perhaps the impacted cancellous bone graft can be regarded as intermediate between the cortical and the cancellous grafts? The purpose of using an impacted and morselized graft is certainly intended to give structural support. If the morselized and impacted graft were as resistant to load as a cortical graft, but could still remodel like a cancellous graft without transient mechanical weakening (Slooff et al. 1993), it would act as an ideal com-

Table 3. Histologic findings in impacted hip revisions

Author	Type of biopsy	Number	Follow-up time	Histologic findings
Ling et al. 1993	autopsy femur	1	3.5 years	Regenerated viable cortical bone. Mixed middle zone. Deep layer containing dead trabeculae.
Nelissen et al. 1995	biopsy femur	4	11–27m	Viable regenerated cortex. Middle zone with viable trabecular bone. Inner zone with bone cement, fibrous tissue and necrotic trabeculae.
Heekin and Engh 1995	autopsy acetabulum	1	18 and 53m	Vascular penetration to a depth of 4 mm. Partial resorption of graft trabeculae and living bone along allograft fragments.
Buma et al. 1996	biopsy acetabulum	8	1–72m	Gradual remodeling into trabecular bony structure, with few if any, remnants of graft bone.
Ullmark and Linder 1998	biopsy femur	1	6m	Most graft revascularized. Fibrous tissue embeds the graft pieces close to the cement.
Whiteside and Bicalho 1998	biopsy knee	14	3w–37m	Early revascularization. Less osteoclast activity after 2 years. Entombed graft surrounded by new bone, visible at 37 months.

promise. The initial mechanical demands are met if the graft is just as strong as the endofemoral cancellous bone in a primary prosthesis operation, which is less than the strength of cortical bone (Taylor 1997). Its particulate and non-cohesive structure, however, is non-structural and the whole concept relies entirely on the graft maintaining its volume during remodeling and not being resorbed. The morselized and impacted graft can still be regarded as a porous structure and ingrowth of vessels is thought not to be impaired (Stevenson 1998). Even after a fairly firm experimental impaction, 35% of the graft volume still consists of non-osseous material (I) for the fibrovascular tissue to penetrate.

There are no published studies on the maximal or mean ingrowth distances of new bone into impacted and morselized grafts in animals or humans. On the basis of radiographs that show increased radiographic density and load-oriented trabecular orientation, it is thought that most of the graft volume remodels into trabecular living bone (Gie et al. 1993). Some concern has been expressed that the remodeled areas are mainly found in certain load-bearing zones at the midshaft (Linder 1998), in the trochanter and distal to the tip of the prosthesis (Kärrholm et al. 1999). In humans, histologic studies of biopsied or retrieved, morselized and impacted grafts (Table 3) often reveal mixed areas of remodeled bone and necrotic graft bone. In animal studies, the volume of grafted morselized bone is often less than in humans. All of the morselized and impacted grafts were found to have remodeled into new bone in a hip prosthesis study in goats (Schreurs et al. 1996, Schimmel et al. 1998). In larger animals, also complete remodeling was found in the metatarsophalangeal joint of a horse (Buma et al. 1998). Although not mentioned, the ingrowth distance in both of these studies probably did not exceed the few mm ingrowth distance of the massive allograft in Enneking and Mindell's study or the 4–5 mms mentioned in osteonecrosis.

Loading of bone around a hip prosthesis

According to Wolffs' law, the femur reacts to the changed stress after a hip prosthetic replacement. The distribution of the stresses changes because the prosthesis now bears some of the load. The cortical bone reacts by reducing its mass proximally. This is achieved by increased porosity or decreased cortical thickness and is called "stress-shielding" (Huiskes et al. 1990, Engh et al. 1992). However, the bone next to the cement or the prosthesis cannot be said to be stress-shielded. On the contrary, implantation of a hip prosthesis led to

increased cancellous bone stresses, as calculated by finite element analysis (Verdonschot and Huiskes 1991, Taylor 1997). The stress levels were calculated to be sufficient to cause permanent deformation of the cancellous bone, with higher stresses in uncemented than in cemented fixation (Taylor 1997). In the hip prosthesis patient, this would mean that trabeculae in the cancellous bone surrounding the prosthesis deflect, deform or fracture. A continuous repair process is initiated or increased by the prosthetic implantation, eventually leading to equilibrium of modeling and remodeling and a stable prosthesis. However most radiostereometric studies of implants show that continuous migration occurs to various extents with the migration pattern relating to the final outcome (Kärrholm et al. 1994, Ryd et al. 1995). If the prosthesis does not migrate excessively, repair may be able to keep up with the microdamage to the trabecular bone. Failure occurs only if migration exceeds a given threshold.

Osteonecrosis of the hip as a model for remodeling of cancellous bone grafts under load

The incorporation of cancellous bone grafts resembles the events following avascular necrosis of the hip (Gardeniers 1988, Ostrum et al. 1994). In animal studies of avascular necrosis of the hip, early cellular changes can be seen, and at two weeks only the osteocytes appear intact, although absent of functional activity (Fineschi and de Santis. 1982). The bony framework of organic matrix and mineral, however, remains unchanged (Chambers 1980) and may do so for a long time even during revascularization. In laboratory studies it remained intact at least six months after the initial ischemia (Fineschi and de Santis 1982).

A fairly extensive repair begins in adjacent areas. In the cancellous bone, capillaries and mesenchymal cells proliferate, osteoblasts differentiate and start to produce new living bone on the surfaces of the dead trabeculae (Glimcher and Kenzora 1979). In the subchondral compact bone, reached by the repair tissue, cells and tissue are resorbed.

The repair process declines with time. Complete healing may occur, but the necrotic bone is usually revascularized only within certain limits, 15–20 mm from the initial site of repair (Mont et al. 1998). Sometimes the mesenchymal tissues fail to differentiate to osteoblasts in the revascularized areas. Glimcher and Kenzora (1979) reported cases where formation of new bone on dead trabeculae advanced only 1–2 mm into the necrosis, in spite of a revascularization depth of 0.5–2 cm from the initial repair. Others have also noted that the revascularization with or without resorption penetrates deeper than the osteogenic front (Chambers and Path 1980, Fineschi and de Santis 1982). Moreover, in more distant regions away from the sites of repair, necrotic areas persist without capillary invasion or living proliferating cells. The mechanism, which stops osteogenesis in vascularized areas and seals off vessels from other regions is unknown but coupling factors, loss of chemotactic signals (Gardeniers 1988), fractures at the junction of dead and repairing bone (Kato and Glimcher 1974) may play a role. However, the predominance of resorption over osteogenesis undermines the mechanical resistance of the bone and leads to collapse. The overall histologic picture of the gradual collapse in osteonecrosis of the hip seem to resemble findings in biopsy studies of morselized impacted bone (Table 3), with a mixed pattern of different stages of remodeling bone. The stresses are high in both the cancellous bone in osteonecrosis and the morselized and impacted bone graft in hip revisions, but the impacted bone does not seem to collapse. The use of morselized impacted allografts to treat osteonecrosis has been tried in humans with good short-term results (Gardeniers et al. 1997). In 5 patients, the necrotic bone was curetted and the cavity filled with impacted allograft. The grafts all showed consolidation and no lysis at 14–43 months. The mechanical resistance to the high stresses may persist during remodeling, which would then demonstrate the superiority of the impacted graft in a high-stress environment.

Design of own experiments

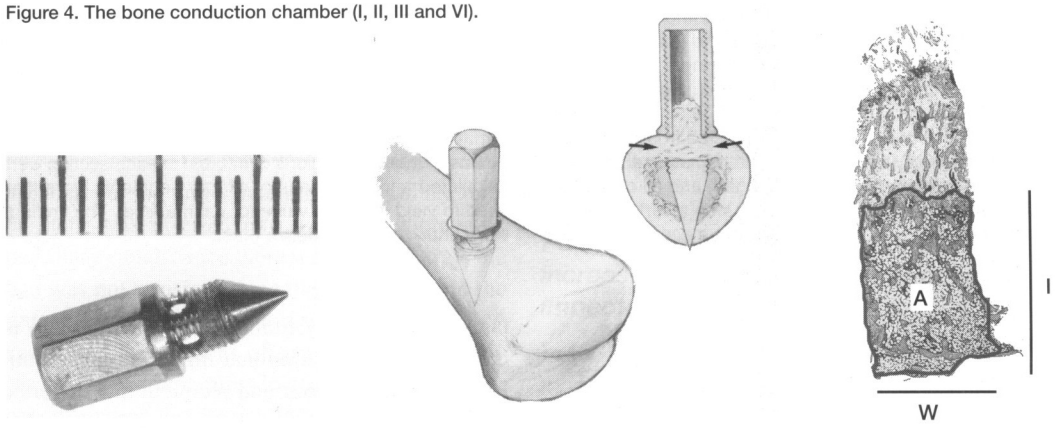
Paper I. *Bone graft proteins influence osteoconduction*

Do the endogenous proteins in a bone graft influence the ingrowth distance of new bone into the graft? Femoral and tibial diaphyses from rats were defatted and ground. One portion was slowly heated with water to 270 °C at an autogenic pressure of 55 bar for 4 hours, to destroy the proteins of the graft but leave the mineral phase of the bone unchanged. As a control, similarly ground but not heated bone powder was used and the ingrowth distance of new bone into the graft was studied, using the bone conduction chamber (BCC) in rats (Figure 4).

Paper II. *Impaction of cancellous bone grafts impairs osteoconduction in titanium chambers in rats*

Does impaction by itself influence the ingrowth distance of new bone into the graft? We developed an impacting device consisting of a hollow cylinder and an impacting piston. Two cancellous rat bone grafts were manually impacted into approximately the size of one. This procedure increased the volume fraction of osseous material in the graft from 35% to 65% (Figure 9). Impacted and unimpacted grafts were compared regarding ingrowth distances at 6 weeks in a BCC in rats.

Figure 4. The bone conduction chamber (I, II, III and VI).



The bone conduction chamber

The chamber screwed into the proximal tibia

The histomorphometric measurement

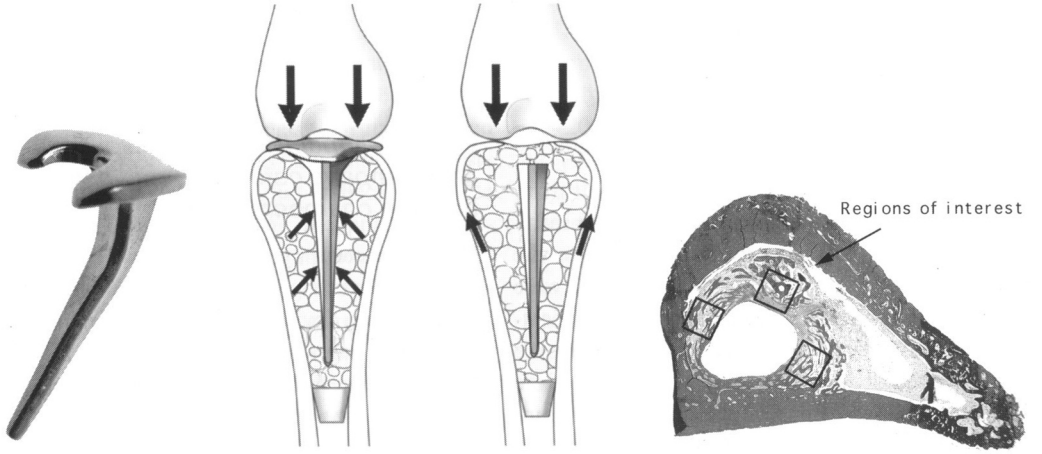
The bone conduction chamber (BCC, Aspenberg and Wang 1993) is screwed into the proximal tibia of a rat. The interior of the chamber is a standardized space of 2x7 mm. Tissue can grow into the chamber from the osseous compartment via ingrowth openings at one end, but not from the surrounding soft tissues. The interior can be left empty and the chamber fills with mesenchymal tissue, which gradually differentiates into bone. It can also be filled with an osteoconductive material, which can further be processed using growth factors, defatting, etc.

Outbred Sprague-Dawley rats were used for the graft experiments as donors and recipients, except in the immunology studies where inbred Sprague-Dawley and inbred Wistar-Furth rats were used to obtain controlled iso- and allografts. Bone grafts were prepared from donor rats by resecting a 2x6 mm cancellous bone rod, in the axial direction from the knee joint, after excising the epiphysis and the growth plate. The grafts were inserted into the chambers, which were then screwed into the proximal tibiae of recipient rats. After harvest, the grafts were taken out, decalcified, cut and stained with hematoxylin and eosin.

The area of newly-formed bone was measured histomorphometrically by circumscribing it on a digitizing table, using a computerized video system. The area (A) was divided by the width (W) of the specimen to obtain the mean ingrowth (I) distance of new bone in each specimen.

All rats had chambers implanted bilaterally; one side serving as the experiment side and one as the control side. Paired statistical tests were used to analyze the data.

Figure 5. Rabbit knee prosthesis model (IV).



The tibial prosthesis

The loaded (left) and the unloaded (right) prostheses implanted in the proximal tibia

The tibial segment with the regions of interest

A tibial prosthesis was designed for this experiment, and implanted in skeletally mature lop-ear dwarf rabbits. The prosthesis consists of a titanium plate replacing the tibial surface and a 25 mm long, conically shaped, unpolished stem. The articular surface is convex in the sagittal plane and tilted posteriorly. In the unloaded experiments, stems without a bearing surface were inserted into the graft bed. The femoral condyles then rested on the remainder of the tibial articular surface, without transferring a load onto the prosthetic stem and the impacted graft. No cement was used for fixation.

Cancellous bone grafts were harvested from donor rabbits and manually cut into 1 to 1.5 mm pieces and frozen. The bone marrow cavity was enlarged, and all cancellous bone removed. A distal rubber plug was inserted into the marrow cavity 25 mm down, and the space between the stem and cortex was filled with graft and impacted with a prosthesis. The complete prosthesis or only the intramedullary stem was then inserted and, in consequence, the bone graft surrounding the stems was either loaded or not. After harvest, the bone was sawed into segments, perpendicular to the tibial axis and were decalcified, cut and stained with hematoxylin and eosin. Four segments, at a distance of 4 mm, were blinded and analyzed from each animal. In all sections, the inner 0.9 mm (area of interest) at the three sides to the triangular-shaped stem void was examined by a Merz grid. The percentages of new bone, remaining dead graft and other tissues were recorded. The means of the three regions of interest in each section were calculated and the means of all four segments were then used to yield one final value for each animal. The findings were analyzed by Student's t-test.

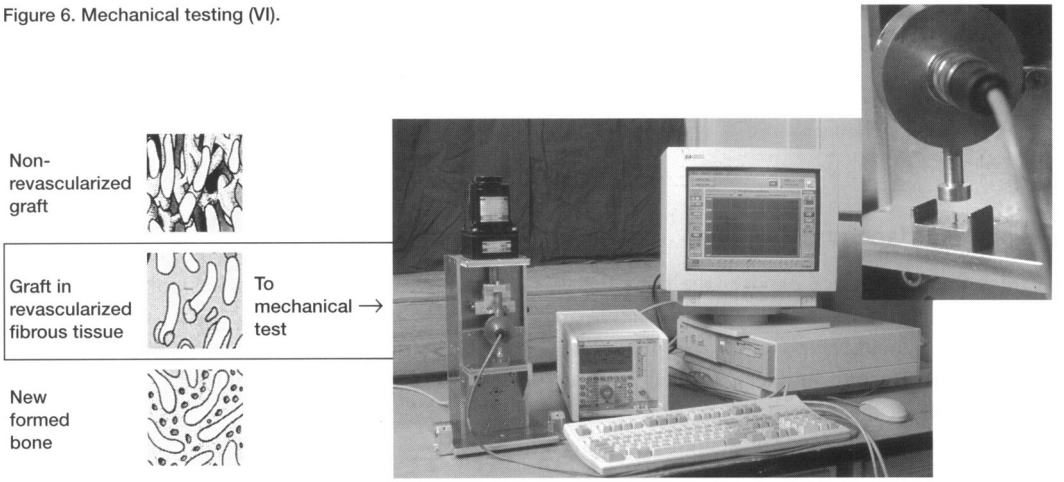
Paper III. *Bone graft incorporation—effects of osteogenic protein 1 (OP-1) and impaction*

How do time and immunologic compatibility influence new bone ingrowth into a graft, and will ingrowth increase if an exogenous growth factor is added? Three studies were done. First, impacted and unimpacted grafts were compared at 6 and 12 weeks to see whether the reduced ingrowth at 6 weeks, in the previous study, represented a delay or a permanent decrease. Secondly, allogeneic impacted and allogeneic structural bone grafts were compared, as well as syngeneic impacted and syngeneic structural grafts, the latter as a model for autogenous grafts. These series were performed to determine whether the ingrowth distance is affected by immunologic factors, and to rule out that any occasional immunological similarities between donor and recipient had distorted the results of the previous study. Thirdly, the influence of an osteoinductive substance (OP-1) on the ingrowth distance was studied. 1 μg OP-1 was adsorbed to a freeze-dried impacted graft and compared to an untreated, but also impacted graft at 6 weeks.

Paper IV. *Load bearing increases new bone formation in impacted and morselized allografts*

What effects do mechanical loads have on the remodeling of a morselized impacted graft? A rabbit knee prosthesis was developed (Figure 5). Six rab-

Figure 6. Mechanical testing (VI).



Impacted grafts were mechanically tested, either directly after impaction without being inserted, or after 4 weeks in the BCC. At 4 weeks, new bone and a marrow cavity starts to form at the bottom of the graft. Above that zone, fibrous tissue has grown into the graft but the uppermost zone is not yet vascularized. To test the bone graft with fibrous tissue ingrowth only, the proximal and distal parts of the harvested graft were cut off, leaving a middle cylindrical piece of 2 mm. The unimplanted impacted grafts were cut accordingly. A custom-made mechanical testing machine was used. The bone cylinders were placed between two metal plates and were compressed at a speed of 25 mm/min. A transducer connected via a digitalizer to a personal computer recorded the applied forces. The first reduction in resistance was regarded as the failure point, and the implanted and unimplanted grafts were compared with the Mann Whitney U-test.

bits had a stemmed uncemented tibial joint prosthesis inserted into an impacted graft in the tibial medullary canal. The load was transferred via the prosthesis to the graft. In another six rabbits, only the stem was inserted into a similarly prepared medullary canal. Since there was no tibial tray, the load was not transferred via the prosthesis and the graft was unloaded. The rabbits were killed after 6 weeks and the amount of graft bone and newly formed bone, remaining in the medullary canal, were measured histologically by Merz grid-counting in the two groups.

Paper V. *Incomplete incorporation of intravertebral impacted autogenous bone grafts in lumbar fractures—a histological study in 4 patients*

To what extent does an impacted graft remodel in humans? Four patients with lumbar fractures were operated on. An autologous iliac crest bone graft was packed into the fractured vertebral body via one pedicle, after the fractures had been reduced and stabilized with screws and plates. After 1.5 years, when the plates were removed, a biopsy was taken from the grafted area and analyzed histologically to evaluate the remodeling of the graft.

Paper VI. *Fibrous tissue-armoring increases the mechanical strength of an impacted bone graft*

In the chambers and in other animal and human models, fibrovascular ingrowth precedes the bone ingrowth temporally and spatially. Therefore, a region of dead graft in a fibrous tissue stroma will exist at some location throughout the graft incorporation. To determine whether this fibrous tissue-armoring contributes to the strength of a morselized graft, bone conduction chambers with impacted grafts were implanted in rats. After 4 weeks, the rats were killed and the middle portion of the specimen, consisting of graft and vascularized fibrous tissue, not yet reached by the ossification front, was removed. In a mechanical testing device (Figure 6) the yield points were compared to similarly produced impacted grafts, which had not been implanted.

Results of own studies and discussion

Fracture surface and proteins

A fresh fracture surface exposes the bone matrix to the surrounding tissues without a protective lining cell and osteoid layer. Our primary hypothesis was that the fracture surfaces created by morselization might have a growth-promoting effect by permitting the release or presentation of BMPs or other growth factors that would not have been released if the lining layer were intact. Bone matrix contains growth factors that modulate osteoblast differentiation (Mohan and Baylink 1991). It is not known whether the endogenous proteins from the graft play an active role in incorporation and remodeling, although their presence has been emphasized (Friedlaender 1987, Huo et al. 1992, Czitrom 1994). Exogenously added growth factors accelerate healing in numerous animal and human studies. Exogenously applied bFGF increased the ingrowth distance of new bone in the same BCC model as in the present study (Wang et al. 1996b) and we found that BMP-7 (OP-1) accelerates and increases ingrowth into an impacted graft (III). In the present study, the ingrowth of new bone into a structural graft was reduced when the proteins were destroyed by slow heating in a ceramic process (I, Figure 7, Table 4). The hypothesis that the morselization procedure would release or present growth factors at fracture surfaces is consistent with this finding. However, other effects of persisting proteins might be responsible, for example, proteins as a substrate for cellular attachment.

Impaction and ingrowth

In a comparison of impacted grafts with unimpacted ones in the BCC at six weeks, we found a striking reduction of bone ingrowth into the graft after impaction (Table 4, Figure 8). Two other series showed that both syngeneic and allogeneic grafts reacted with a reduction in ingrowth when impacted (Table 4).

It has been speculated that the amounts of immunogenic cells and cell remnants are minimized in the morselized and impacted graft, because most of the marrow is squeezed out (Slooff et al. 1996). We found reduced amounts of fat and marrow cells in the graft in our impacted bone pellets (Figure 9). In animal studies, cancellous bone containing marrow was more immunogenic than cortical bone, and removal of the marrow reduced the immunogenic-

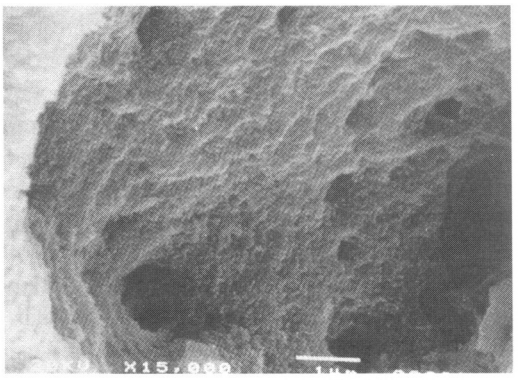
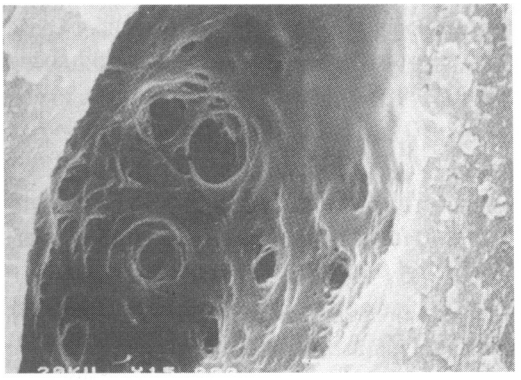


Figure 7 A. A morselized bone graft that has been deproteinized by slow heating, under high pressure. View from inside an osteocyte lacuna. The surface proteins are destroyed, but the mineral phase is left intact, visualized as a granular surface (EM).



B. Non-deproteinized morselized graft showing a smooth osteoid layer covering the mineralized bone.

Table 4. Results of studies using bone chambers (I, II and III)

Paper	Comparison	Ingrowth mm (SD)	p-value	95% C.I. for the difference exp–control	
I	Deproteinized morselized graft 6 w	1.4 (0.7)	0.008	0.2	1.0
	Non-deproteinized graft 6 w	2.0 (0.7)			
II	Impacted 6 weeks	0.6 (0.7)	0.005	0.5	1.5
	Structural 6 weeks	1.9 (0.5)			
III	Impacted 6 weeks	0.7 (0.4)	0.002	0.6	2.1
	Structural 6 weeks	1.7 (0.8)			
III	Impacted 12 weeks	2.0 (1.0)	0.5	–0.8	1.4
	Structural 12 weeks	2.3 (1.2)			
III	Isograft impacted 6 w	0.8 (0.7)	0.007	0.4	2.0
	Isograft structural 6 w	2.0 (0.7)			
III	Allograft impacted 6 w	1.4 (0.7)	0.02	0.2	1.1
	Allograft structural 6w	2.1 (0.7)			
III	Impacted with OP-1 6w	2.7 (1.1)	0.02	–2.7	–0.34
	Impacted 6w	1.3 (0.9)			

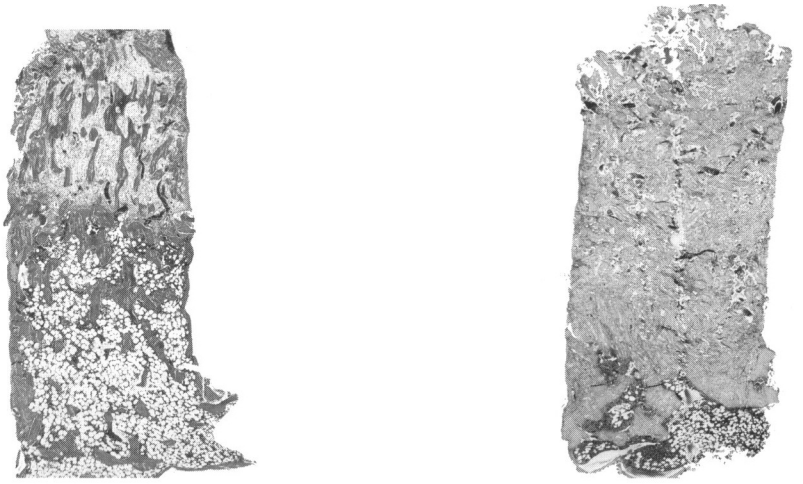


Figure 8 A. Unimpacted structural graft. Ingrowth from chamber openings at the bottom upwards. At the bottom, a marrow cavity has formed, followed by a border zone of new bone ingrowth. Above this, no new bone is found but only unresorbed graft in vascularized fibrous tissue. B. Impacted graft with a shorter penetration of new bone into the graft.

ity (Burwell 1963, 1964, Friedlaender et al. 1976, Musculo et al. 1976). In the same model as ours, chemical lipid extraction of structural grafts, using chloroform-methanol increased the bone ingrowth distance (Thorén and Aspenberg 1995). Perhaps the morselization and impacting procedure can be regarded as a mechanical defatting procedure comparable to a chemical one, which we know, is beneficial. As a speculation, defatting, regardless of how it is done, may reduce the differences between frozen allo- and autografts. It is striking, however, that in the clinical cases, the group with the lowest migration by radiostereometry, uses a mechanical defatting method in which the morselized wet graft simply was squeezed in a gauze towel before impaction (Nivbrant and Kärrholm 1997, Kärrholm et al. 1999).

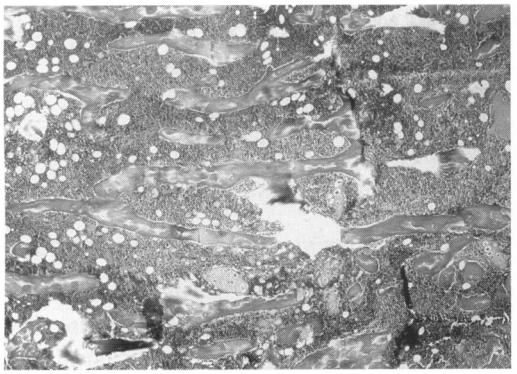
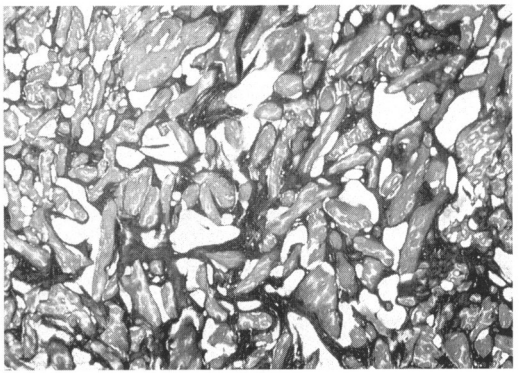


Figure 9. A. Structural graft before insertion into the chamber, with unfractured trabeculae, fat and marrow cells.



B. Impacted bone graft (same magnification) before insertion, with fractured trabeculae and reduced intertrabecular space. Smaller amount of fat and marrow cells are present than in A. (Reproduced by permission of Clin Orthop).

Contrary to the hypothesized increase, we found a reduction in the ingrowth of new bone into the impacted graft at six weeks (II). To determine whether the ingrowth was permanently reduced or only delayed, we studied the ingrowth of new bone into an impacted graft at six and twelve weeks (III). A reduction in ingrowth was again found at six weeks but no detectable difference between the impacted and the structural grafts at twelve weeks was found. We concluded that the ingrowth distance into an impacted graft is delayed, but tends to catch up later.

Impaction and growth factors

In a study of spinal fusion in rabbits using morselized autograft bone, the central grafted volume of the fusion mass was compared to a more peripheral one, and the extent of healing differed in relation to the distance into the graft (Boden et al. 1995). Peripherally in the graft, the healing was faster and the mechanical strength showed an earlier increase than in central parts of the graft, where healing was slower, incomplete and had stopped. The authors discussed whether the central graft is "compromised geographically" and concluded that, if the molecular events responsible for the delay in the central zone could be controlled, this might be the key to eliminate nonunions. Using RT-PCR, different gene expression patterns were found in the central and peripheral parts of the graft (Morone et al. 1998). The peaks of gene expression in the central zone lagged 1 to 3 weeks behind the peripheral parts of the graft. This correlated to the delay in bone formation, seen histologically, and the fact that nonunions, which occur in 35–45% of rabbits in this model, do so in the central fusion mass. Addition of a rhBMP-2 lowered the nonunion rate to 0% (Schimandle et al. 1995). Gene expression analysis of the BMP-treated fusion mass showed a marked increase in BMP-6 in the outer zone as well as elimination of the central lag of BMP-6, BMP-2, collagen and osteocalcin (Morone et al. 1998).

In our BCC model, the markedly decreased ingrowth caused by impaction was also reversed by letting OP-1 adsorb to the impacted graft (III, Figure 10, Table 4). The ingrowth was even greater than in the unimpacted grafts in the other groups. The effects of osteoinductive proteins on the osteoblasts have been studied extensively. In our study and in the rabbit spine fusion study (Morone et al. 1995), the osteoclastic resorption might have been increased by an osteoinductive substance as suggested by some studies (Kanatani et al. 1995, Lamerigts et al. 1998, Salkeld et al. 1997). An increased resorption would then compensate for a relative blockage of ingrowing tissue from intruding into the packed trabeculae. This blockage could be related to the reduced porosity of the graft. The effect of OP-1 might be to overcome this blockage by speeding up, not only the number of osteoblasts and the osteoblastic matrix synthesis, but also the osteoclastic resorption.

Figure 10. Impacted bone graft treated with OP-1. The ingrowth of new bone into the graft reaches farther into the OP-1 treated graft than in the un-treated one and the zone of newly-formed bone appears thicker. (Reproduced by permission of Clin Orthop).

Table 5. Results of rabbit prosthesis study (IV). The amounts of new bone and graft remnants were measured histologically by Merz grid-counting at various section levels from proximal to distal at 4-mm intervals. Row A is most proximal (metaphysis) and row D most distal (diaphysis). Percent of specimen surface area, mean (SD)

Section level	Loaded		Not loaded	
	new bone	unresorbed graft	new bone	unresorbed graft
A	40 (19)	7 (7)	14 (10)	16 (6)
B	32 (11)	7 (5)	11 (7)	17 (6)
C	22 (17)	6 (6)	2 (3)	4 (6)
D	23 (17)	5 (6)	2 (3)	8 (9)
Total all levels	30 (13)	6 (2)	7 (3)	11 (4)

This would permit the ingrowing new tissue to extend further into the graft at the time the healing cascade diminishes or stops.

In the lumbar fusion experiments (Morone et al. 1998, Schimandle et al. 1995), autogenous graft was used, just as in our lumbar vertebral fracture patients (V). In an autograft, no immunogenic response occurs, and some surface cells in cortical and cancellous grafts can survive to produce new bone (Basset et al. 1972, Bonfiglio 1958, Gray et al. 1979). No such cells can be expected in a morselized impacted allograft. However, in the impacted autograft, some cells may survive, for example, when used in protrusio acetabuli and spinal surgery. Freezing diminishes the biologic activity of an autograft by killing the grafted cells, but the handling of an unfrozen graft has also been shown to be important for the outcome (Albrektsson 1980). Vessels reenter the graft earlier with a careful surgical technique than with a more traumatic one (Albrektsson 1980). When using the impaction technique with a fresh autograft, as in some spinal operations, the graft cells may die because of the mechanical trauma. In consequence, the osteogenetic properties of the autograft would disappear.

The amount of ingrowing new bone in response to load

Our first studies were designed to separate various factors and mechanisms, in particular to find impaction-related factors that increase the osteoconductivity of the graft. Such an increase could be measured in the bone chamber model as an increased ingrowth distance of new bone into the graft, which would possibly equal the better penetration of new bone into the graft after impaction in clinical cases than that of the unimpacted structural grafts. Unexpectedly, we found a decrease or delay with our model and not an increase. We therefore had to find another explanation of the good clinical results with impaction grafting.

The better clinical results with the impacted grafts than with structural grafts have been ascribed to a better response to mechanical stimulation. Gie (1993) suggested that the load is "directed through the graft during healing." Load would increase remodeling just as an externally applied growth factor would. Indeed, mechanical stimulation of graft incorporation might be mediated by increased production of growth factors. A rabbit knee prosthesis model was designed to study the effect of a mechanical load on the remodeling process (IV, Figure 11). In that model, a loaded or unloaded tibial prosthesis stem was inserted into the impacted graft (Figure 5). In consequence, the graft into which the stems were inserted, was ei-

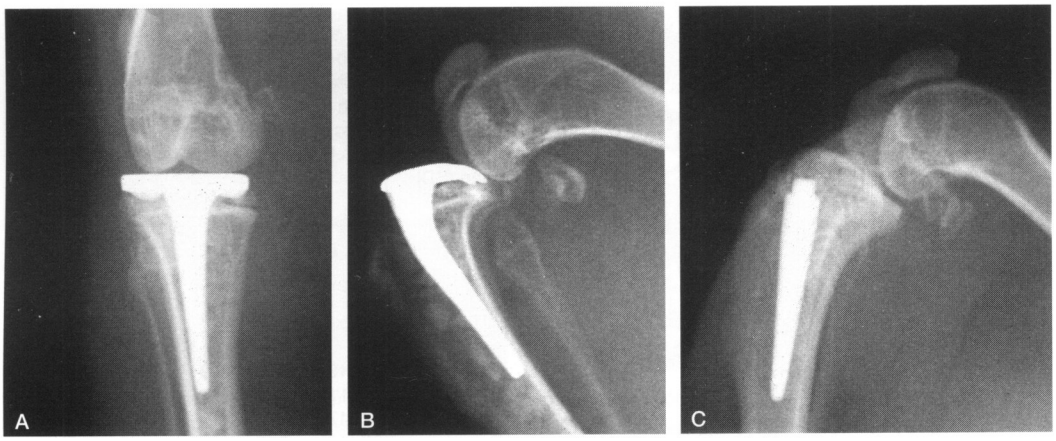


Figure 11. The rabbit tibial prosthesis. A and B. Complete prosthesis with tibial tray and a stem which transfers the load to the graft. C. The tibial tray has been cut off, the stem is not in contact with the articulating surfaces and the graft is therefore not loaded.

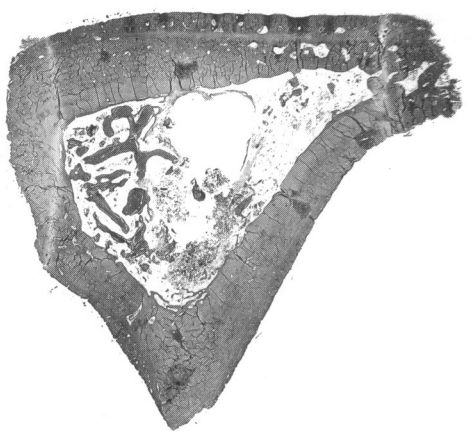
ther mechanically stimulated or not. In the loaded stems, the knee joint forces acting on the tibial plateau of the prosthesis loaded the graft surrounding the stems with each step. In the unloaded stems, the tibial tray was cut off, leaving only the stem, and the articulation took place between the remaining articular surfaces.

In this model, the load increased the remodeling of the graft. Both formation of new bone and resorption of the graft were increased. Around the unloaded stems, the proximal metaphyseal bone remodeled to some extent, but in the diaphyses, the graft was mostly resorbed without much formation of new bone (Table 5, Figure 12). We concluded that mechanical stimulation is important for the incorporation of a morselized impacted graft. However, just like the chamber model, this model can not detect an increased ingrowth distance or penetration of new tissue into the graft, exceeding the 2–3 mm mentioned previously (Enneking and Mindell 1991), because the distance from the cortex to the prosthesis is short.

The fate of the impacted graft during remodeling

Thus, it is of value for the graft to be subjected to a load during remodeling. Mechanical stability, however, is probably more important and essential for graft host union and further remodeling. In structural non-morselized grafts, some authors have stressed that instability may lead to fatigue fractures and nonunion can cause the graft to resorb (Emerson et al. 1989). In the morselized graft, stability, in spite of its non-structural appearance is also important. A morselized graft in a high-stress and unstable environment was uniformly resorbed when implanted into the acetabulum in bipolar hip prostheses (Brien et al. 1990).

Various bench studies have shown us what to expect from the impacted graft-prosthetic construct during the initial phase after surgery. It seems possible to achieve acceptable initial stability (Malkani 1996), eventhough morselized grafts have a non-structural nature (Brodt et al. 1998) and, not being contained, should theoretically be unable to withstand any mechanical load. During remodeling, the graft must maintain its volume and shape, not only during the initial weight-bearing by the patient, but also during the entire remodeling period, which involves osteoclastic resorption of the graft and simultaneous osteoblastic new bone formation. High stresses are excerted on the cancellous bone around a femoral prosthesis. In a finite element analysis, the stresses in the cancellous bone next to a hip prosthesis were near or above its yield point (Taylor 1997). This could explain the continuous small migrations (micromotion) with time, also found in stable, well-functioning prostheses as well as failure when a criti-



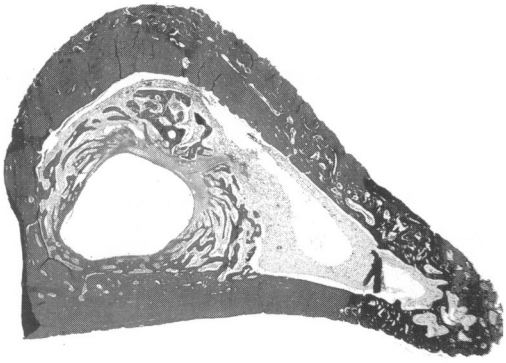


Figure 12. A. Histologic sections of a morselized and impacted graft surrounding an unloaded stem. Distal section around the tip of the stem, where most of the graft is resorbed and has hardly any newly-formed bone.

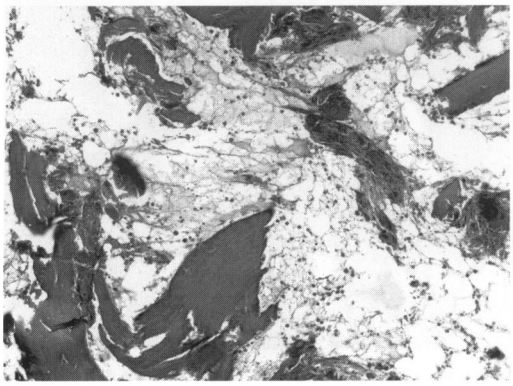
B. Morselized and impacted graft surrounding a loaded stem. Note, presence of more new bone which tends form a new endocortex. (Reproduced by permission of Clin Orthop).

cal value is exceeded (Taylor 1997). We do not know how the impacted graft reacts to these fairly high loads and stresses. Some hypothetical scenarios can be discussed.

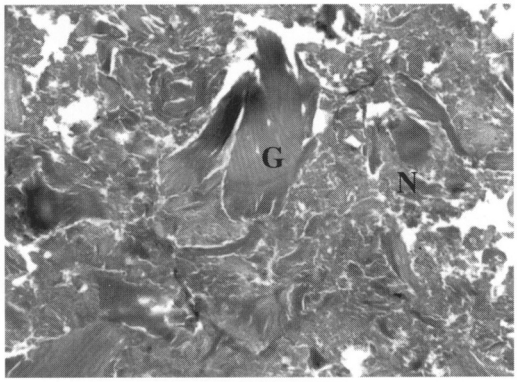
1. If the graft or part of the graft is revascularized and the osteoid apposition by the osteoblasts equals the resorption by the osteoclasts, equilibrium is achieved. A newly formed trabecular bone, adapted to the stresses during its formation, will ultimately replace the graft. This has been shown to happen in animal studies in the goat and horse, without mechanical weakening during remodeling (Schreurs et al. 1996, Schimmel et al. 1998, Buma et al. 1998). For this to occur, the grafted volume probably must be small and the stresses within certain limits. If at some stage the stresses exceed the yield-stress of the newly forming tissue, it will deform.
2. If the graft, or parts of it, do not revascularize, it will retain its mechanical properties, which we know roughly from bench studies. The stiffness is minimal, consisting mainly of trabecular interlocking and friction between fragments (Brodt et al. 1997). Creep, which in this case would be a sliding and packing of the bone chips relative to each other, is still possible. Fatigue fractures are not likely to occur, since the graft already consists of fractured bone
3. If the front of resorption extends further into the graft than the front of bone apposition, just as in osteonecrosis of the hip, the graft will collapse and lose its volume. If this affects a very thin layer of the graft, only a slow distal migration of the prosthesis will occur without clinical failure, which could be feared if the layer were thicker.
4. If the graft is revascularized and invaded by fibrous tissue embedding the trabeculae but with gradually less formation of new bone and reduced resorptive activity, as is thought to happen in some areas of the osteonecrotic hip (Glimcher and Kenzuro 1979), the mechanical properties will be preserved. Even improved resistance to torsional or shear stresses can be expected, due to the fibrous armoring of the trabeculae (VI), similar to the consolidation phase of fracture healing. During compression, the graft will act as a cushion, like a disc or meniscus, permitting stresses to be distributed over a larger area.

To speculate further, the secret of the morselized impacted allograft may lie in its being revascularized and remodeled more slowly than structural grafts. Perhaps one should be careful trying to enhance or accelerate the remodeling by adding growth factors, since so little is known about their effect on resorption. Some material other than bank bone could be used, such as hydroxyapatite granulae (Oonishi et al. 1997), thereby reducing the, at least emotionally important, risk of blood-borne diseases.

Figure 13. Biopsies from the central part of a grafted vertebral body obtained when the plates were removed 1.5 years after the operation. The graft was taken from the iliac crest and packed into the vertebral body via the pedicles.



A. Dead graft surrounded by vascularized fibrous tissue but no new bone apposition.



B. Dead graft surrounded by necrotic debris with no signs of revascularization or formation of any new bone.

In reality, these biologic scenarios probably occur in varying proportions throughout the graft. In patients operated on for a fractured vertebral body with morselized and impacted autograft, we found that large volumes of the graft still remained unremodeled as long as 1.5 years postoperatively, although the fractures were clinically and radiographically healed (V). Even if some parts of the grafts had become remodeled with an apparently normal marrow, other parts consisted only of dead graft trabeculae in necrotic avascular tissue. In yet other parts of the graft, the trabeculae showed no signs of remodeling, but were surrounded by revascularized fibrous tissue (V, Figure 13). Such incomplete remodeling occurred, despite the use of autologous cancellous bone.

This incomplete graft incorporation in human vertebras accords with published histologic findings in series using morselized and impacted grafts in patients with a hip revision (Table 3). In these studies also, areas in various stages of healing were found even after a long time. The composition in space and time of the various healing stages will predict the outcome of the grafting procedure. Since large volumes of graft are used, some parts of it will at a given time, not be revascularized and remodeled. In such graft parts, one might expect that the bone chips would slide relative to each other ("creep") when loaded and that the graft would lose height in the direction of the load. This probably explains why increased distal migration occurs initially in clinical series compared to primary prostheses. However, in the only two RSA studies available, this initial migration slows down after 1–2 years (Ornstein et al. 1999, Kärrholm et al. 1999) similar to stable primary prostheses (Kärrholm 1994, Ryd et al. 1995). At the time when the migration slows down, the remodeling stimuli may have come to an end, just as in large structural allografts or osteonecrosis of the hip, and the proportions of remodeled and unremodeled bone would not change for a long time. Other studies show such a large migration (Masterson et al. 1997), that it must be suspected that a considerable loss of graft volume occurs and future loosening has to be feared. This may be related to the surgical technique, notably if the degree of impaction is sufficient for the graft to carry an appropriate load.

The dead bone graft with fibrous tissue ingrowth—a composite material

Human histologic studies indicate that the graft remodels from the cortex towards the cement-graft interface. The part near the cement seems to be the least remodeled and often consists of dead graft in fibrotic tissue (Table 3). If the remodeling stops after a couple of years, the graft at its various healing stages must bear the load of the prosthesis for years to come. The strength of a necrotic graft, armored by fibrous tissue, might be sufficient to withstand the load of a femoral stem or an

acetabular cup. The largest loads on a prosthetic femoral stem are not the distally directed forces, thought to lead to subsidence. The rotatory forces encountered when rising from a chair or climbing stairs are much greater (Mjöberg et al. 1984, Bergmann et al. 1995). The radius of the femoral canal and of the femoral component is small compared to the moment arm from the femoral axis to the head ("the off-set"). An RSA study of rotational provocation showed that more than one third of the primary femoral stems were unstable during rotation (Mjöberg et al. 1984). Torsional moments measured in vivo are close to experimentally determined critical limits of implant fixation (Bergmann et al. 1995). Both stem design and fixation influence the resistance to rotational forces. In bench tests, straight stems with a rounded cross-section had the lowest torsional stability (Schneider et al. 1989). Cemented stems were 10 times stiffer than uncemented ones in the same study. In another test, cemented stems withstood a torque of 4.8% body-weight moment (BWm) and uncemented but ripped stems 2.4% BWm, a level at which smooth uncemented stems have already failed (Tanner et al. 1988). The resistance to torsion of a prosthetic stem, although cemented but lying in a tube of morselized and impacted bone chips, may be preferable to study than the resistance to subsidence. In a bench study, no difference was found in the resistance to rotational forces between a "primary" cemented femoral prosthesis vs. stems revised with morselized and impacted grafts (Malkani et al. 1996). If such a high torsional stability were also achieved in the patients, it might not be necessary for the graft to incorporate and remodel so long as the resorptive process were not too fast. We found that fibrous armoring of the impacted graft doubled the mechanical resistance to a compressive force in a non-constrained test set-up (VI, Table 6). Theoretically, the addition of fibrous tissue growing into the graft should enhance the coherence of the crushed bone and lead to an increased mechanical resistance, especially to shearing forces such as stem rotation. This could explain why the impacted and morselized grafts do not collapse during remodeling.

Table 6. Mechanical testing of the middle part of impacted standardized rat grafts (VI). We used freshly packed grafts hence without fibrous ingrowth, or impacted grafts that had been in the BCC in a rat for four weeks, and therefore invaded by fibrous tissue

Impacted graft	Yield (MPa)	SD	p
with fibrous tissue ingrowth	2.9	0.7	0.02
without fibrous tissue ingrowth	1.7	0.8	

Validity and reliability of used models

Validity

Rat models. 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. In the BCC model we used rats, because they are easy to handle and not expensive. Large series can be studied with just the intention to change one factor at a time and the model is advantageous for basic studies of bone physiology. 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 defatting or adding growth factors, have helped us to increase or reduce the ingrowth (Thorén 1994b, Wang 1996a). An osteoconductive material is usually inserted into the chamber, and tissue from the marrow cavity and/or the cortical bone can grow in through the ingrowth openings at the bottom (Figure 4). The interior of the chamber is stress-shielded and no deformations occur. This protects the ingrowing vessels, but also deprives the ingrowing tissues of mechanical stimulation. Any biologic effect that requires participation of a physical load, which bone remodeling presumably does, can not be adequately evaluated under these artificial unloaded conditions. However, the chamber model with its limitations has been used so extensively that we feel 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 (Wang 1996a). In our view, the BCC should be interpreted at that level in the hierarchy of experimental models.

Initially, we wanted to study the osteoconductive properties of a morselized and impacted graft as a special type of bone substitute. In such a pure material test the BCC model is adequate, with the ingrowth distance as a measure of osteoconductivity. We hoped to find some factor that would increase the ingrowth, paralleling the increased ingrowth into impacted grafts that is reported in the patients. The finding of a reduced or delayed ingrowth does not permit us to conclude that an impacted graft is inferior in patients. Instead, a model had to be used which allowed a mechanical load to be included as a test parameter. A load chamber similar to the BCC was designed that allowed mechanical loading of the tissue in the chamber during tissue ingrowth. With an empty load chamber at the start of the experiment, mechanical stimulation made the ingrowing tissue change its differentiation pathway and form cartilage instead of membranous bone and fibrous tissue that normally appear (Tägil and Aspenberg 1999). This model was also applied to the impacted graft, but we have not detected any clear effects at present. The stress distribution within the chamber, when it is loaded, is complex, and we may not yet have found the right loading parameters.

Instead, we developed a knee prosthetic model in the rabbit to show the effect of mechanical stimulation on the impacted graft under less standardized conditions (IV). A larger animal model is more clinically relevant and closer to the human than a rat model. The findings in that study are relevant to the way in which an impacted graft remodels in humans, although the rate, speed and extent of the remodeling might be different. As experimental model, more uncontrolled parameters were added, compared to the BCC, such as amount, direction and amplitudes of the forces acting on the impacted graft. Again, we were unable to measure the maximal distance of new bone ingrowth into the graft with the rabbit model, since the thickness of the graft layer was only about 1 to 2 mm and easily overcome by bone ingrowth. We were also unable to study what happens spatially and temporally further in the graft, whether the bone-forming stops and, if so, why.

Mechanical testing

It is not possible to perform a conventional compression test of a non-structural material, consisting of loose particulate bone, such as the morselized and impacted graft (Brodt et al. 1998). However, the individual pieces of the graft are held together by adhesion and/or interdigitation (Brodt et al. 1998) and the bone graft can be handled without falling apart. Many sources of measuring errors exist in our testing of this material, and the results are merely a rough estimate of the adhesive forces. We tested the grafts immediately, to avoid dehydration and denaturation of the proteins. Since no actual breaking point related to a breakage of bone exists, the "yield-point" corresponds to the first major sliding of the individual graft pieces. A fibrous tissue ingrowth between the graft trabecula would enhance the cohesive forces. The load was almost doubled before yield, allowing us to conclude that there is an effect as regards cohesive forces. Since our goal was simply to demonstrate a principle, we made no attempts to make stress-strain curves or material compliance estimates. Simply, the points were registered where the first drop in resistance to the force occurred. The results would be clinically relevant since there is an increased resistance to deformation in the zone where the graft has not yet been remodeled, but fibrous tissue has grown in between the graft trabeculae.

Reliability

Rat models. The methodological or analytical error for histomorphometric measurements of new bone ingrowth into a graft in the bone conduction chamber, i.e., the intraindividual error with repeated measurements, has been reported to be 6% (Thorén 1994). In these studies, just as in the present ones, we had to decide what is dead graft bone and what is living newly-formed host bone. Due to the impaction of the graft in the present studies, it was more difficult to define the bone as living or dead. All measurements were made by one laboratory technician. The author repeated the measurements in Paper II, and the interindividual error was calculated to be 8%. When the biologic variation between chambers was included, based on side comparisons, the error in bone ingrowth was reported to be 20% (Wang 1996a). In that study, the effect of growth factors on unimpacted structural bone was measured, and an increase in new bone ingrowth was found. In the present studies, the ingrowth was reduced, which could affect the error differently. The mean values were lower, but the biologic variations were perhaps similar. The coefficient of variation in the present studies was 75% (0.61/0.80) for the impacted grafts and 38% (0.72/1.84) for the unimpacted ones. Thus, fairly large variations were found between individual rats, and the coefficient of variation was greater for the impacted grafts. Previously, this variation caused us to do only paired comparisons. However, comparisons between groups have not been made, since differences in bone ingrowth distances between rat batches have been reported. In the present study, no difference was found between batches (6-week groups, 6 batches, n=46, Anova). To reduce systematic errors due to surgical technique on the right and left legs, we changed sides for experiments and controls throughout the studies. The mean difference between the right and left legs in the 6-weeks specimens (n=46) was about 2%.

The risk of a type 1 error corresponded to the chosen level of significance (=0.05 two-tailed analysis). The risk of a type 2 error occuring in future studies of impacted grafts was calculated to be 20% with the data in the present series, 10 rats and an expected reduced ingrowth of 50 %.

Conclusions

1. Endogenous bone proteins play a role in the incorporation of a bone graft.
2. Impaction of a frozen bone graft, regardless of allo- or autogenous origin, delays the ingrowth of host bone into the graft.
3. New bone ingrowth into an impacted graft can be increased by adding an exogenous growth factor, such as OP-1.
4. Mechanical loading of an impacted morselized graft increases graft remodeling.
5. In patients, impacted autografts were not entirely remodeled at 18 months after surgery, although clinically functional.
6. Fibrous ingrowth increases the mechanical strength of an impacted graft.

Acknowledgements

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Summary

The results of primary hip replacements are good. However, dealing with a loose prosthesis has been problematic, especially when major bone deficiencies are encountered. These problems appear to have been solved by the introduction of the Slooff-Ling method of using morselized and impacted allograft chips. The clinical results are excellent in the hands of the innovators. However, it remains confusing that a thick layer of dead, broken, immunogenic tissue taken from another individual does not resorb and collapse during remodeling. Still harder to understand is the impression, as judged by radiography, that this thick layer seems to incorporate and remodel up to a distance of perhaps 10 mm or more from the host bone, whereas the ingrowth distance into a non-morselized graft is limited to a few mms. To clarify the biological basis of the morselized and impacted grafts better, the present study was started. Three hypotheses were initially proposed to explain the good clinical results:

1. Morselization releases growth factors present in the graft (osteoinduction).
2. Impaction makes it easier for the ingrowing bone to climb up into the graft (osteoconduction).
3. The compliance or elasticity of the graft allows the load to produce deformations that stimulate bone formation (mechanical load).

In the first studies, bone chambers were implanted in rats and the distance of new bone ingrowth into a graft in the chamber was measured. In Paper I, a morselized graft was deproteinized by slow heating under high pressure. Ingrowing bone did not reach so far into the deproteinized graft as into a non-treated one. We concluded that the proteins present in the graft partly determine how far ingrowing new bone will extend into a graft.

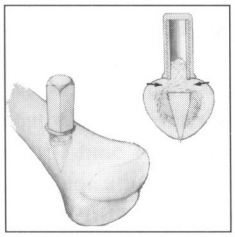
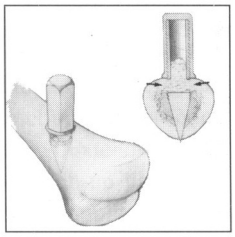
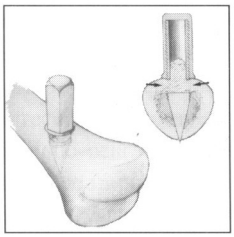
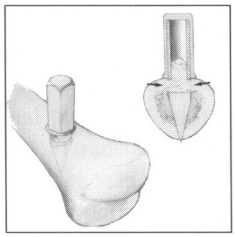
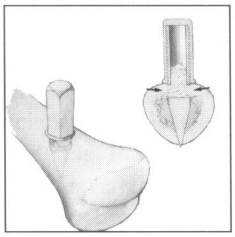
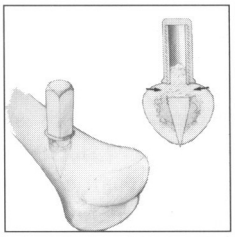
In Paper II, a cancellous graft was impacted so that the bone volume fraction of the graft rose from 35% in the unimpacted to 65%. The impacted grafts were compared to unimpacted ones and it was shown that impaction *reduced* the ingrowth of new bone into a graft in the chamber at six weeks. In Paper III, this somewhat unexpected finding was further studied. Syngeneic and allogeneic grafts showed a reduced ingrowth distance at six weeks when impacted, compared to unimpacted controls. However, the reduction was not found when the time for ingrowth was extended to 12 weeks, indicating a possible catch-up phenomenon. Moreover, an exogenously applied growth factor, osteogenic protein-1, was found to have *increased* the ingrowth distance of new bone into impacted grafts at six weeks.

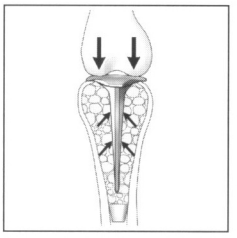
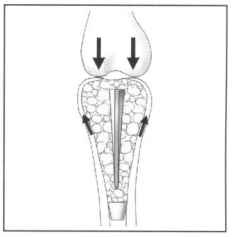
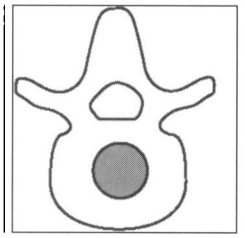
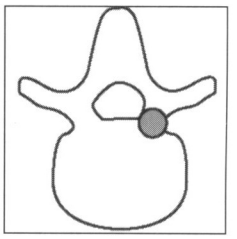
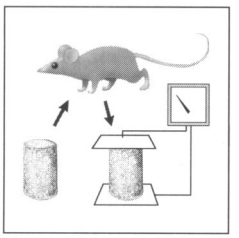
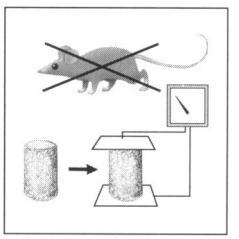
In Paper IV, a rabbit knee prosthesis was developed to study the effect of a mechanical load on the remodeling of a morselized and impacted graft. All rabbits had their tibial marrow cavity cleansed of cancellous bone, which was replaced by a morselized and impacted bone graft. Six rabbits received a complete tibial prosthesis with a tibial load-bearing tray and a stem transferring the load to the impacted graft with each step made by the rabbit. Another six rabbits had only the stem, without the tibial tray, inserted into the impacted graft. With this design, the load from walking was not transferred to the graft, since there was no joint surface replacement to transfer the load to the stem and the graft. Thus, the graft was loaded in rabbits receiving a full prosthesis, whereas it was unloaded in the animals receiving only the stem. New bone formation and resorption of the graft were increased in the loaded grafts, and we concluded that a load increases the rate or speed of remodeling. In Paper V, four patients were operated on for vertebral fractures. The fractures were stabilized by plates and the vertebral bodies packed with autogenous morselized graft. After 1.5 years, when the fractures were clinically and radiographically healed, a biopsy was taken. It was found, that even after such a long time, large areas remained unremodeled and sometimes even unrevascularized. In some parts, necrotic graft trabeculae were embedded in fibrous vascularized

scar tissue. In Paper VI, we studied the influence of this fibrous tissue armoring of the necrotic graft morsels. Impacted cancellous graft cylinders were inserted into bone chambers in rats and the part of the graft, which was embedded in a vascularized fibrotic tissue, was excised. Such parts were compared to freshly impacted cancellous grafts in a simple compressive test. The mechanical strength was almost doubled when fibrous tissue had armored the fractured graft trabeculae.

In conclusion, endogenous bone proteins from the graft affect the ingrowth distance of new bone growing into a morselized graft and load seems to enhance the remodeling of a morselized and impacted graft. Impaction, on the other hand, seems to *reduce* the ingrowth. In clinical cases full remodeling and incorporation can not always be expected, but the ingrowth of fibrous tissue between graft trabeculae may increase the mechanical strength.

Comparisons in this thesis

Study		Comparisons
Paper I		**Endogenous bone proteins**
Hypothesis	Proteins in a graft influence ingrowth.	
Method	Ground graft slowly heated to destroy the proteins but leave the mineral phase intact.	 *Deproteinized bone powder*
Time	6 weeks	
Animal	Outbred Sprague Dawley (SD) rats	
Graft	Morselized	
Model	Bone Conduction Chamber (BCC, Figure 4)	 *Non-deproteinized bone powder*
Conclusion	Deproteinization reduced ingrowth.	
Paper II		**Impaction**
Hypothesis	Impaction per se influences the ingrowth into a graft.	
Method	Cancellous graft was impacted and the bone volume fraction rose from 35% to 65%	 *Impacted graft*
Time	6 weeks	
Animal	Outbred SD Rats	
Graft	Impacted and unimpacted	
Model	BCC	 *Structural graft*
Conclusion	Impaction reduced ingrowth.	
Paper III		**Immunology, time, and OP-1**
Hypothesis	Difference between allo- and autografts. Catch-up of ingrowth with time and increase by an exogenous growth factor.	
Method		 *A Impacted allo* *B Impacted iso* *C 6 w impacted* *D 12 w impacted* *E Impacted with OP-1*
Time	6 and 12 weeks	
Animal	Outbred SD Rats Inbred SD Rats (isografts) Inbred Wistar-F Rats (allografts)	
Graft	Impacted and unimpacted	
Model	BCC	 *A Structural allo* *B Structural iso* *C 6 w structural* *D 12 w structural* *E Impacted*
Conclusion	Reduced ingrowth into impacted graft regardless of histocompatibility. Perhaps catch-up with time. Increased ingrowth into impacted grafts with OP-1.	

Study		Comparisons
Paper IV		**Load**
Hypothesis	Load increases remodeling	
Method	Proximal tibia impacted with morselized graft. Loaded or unloaded stems. Histological measurment of the amount of new bone and remaining graft.	 *Loaded graft*  *Unloaded graft*
Time	6 weeks	
Animal	Lop-ear rabbit (n=12)	
Graft	Morselized and impacted.	
Model	Tibial prosthesis.	
Conclusion	Load increases remodeling.	
Paper V		**Remodeling**
Hypothesis	Morselized and impacted graft remodel with time.	
Method	Patients operated on for vertebral fractures with plates and morselized graft impacted into the vertebral body. Biopsy after 1.5 years.	 *Histology from the grafted vertebral body*  *Histology from the pedicle of the grafted vertebra*
Time	18 months	
Animal	Humans (n=4)	
Graft	Autograft from iliac crest.	
Model	Vertebral fractures	
Conclusion	Even morselized and impacted autografts remain unremodeled after long time.	
Paper VI		**Strength by fibrous ingrowth**
Hypothesis	An unremodeled graft is strengthened by fibrous ingrowth.	
Method	Part with graft in fibrous tissue stroma after 4 weeks in a BCC, compared to freshly impacted graft without fibrous ingrowth. Compression test registering yield point.	 *Impacted graft in chamber 4 weeks before mechanical testing*  *Graft directly to mechanical testing after impaction*
Time	Fresh vs 4 weeks in BCC.	
Animal	SD Rats	
Graft	Impacted cancellous	
Model	BCC and mechanical test.	
Conclusion	Fibrous tissue ingrowth enhances the mechanical strength of an unremodeled morselized impacted graft.	

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