BIOCOMPATIBILITY AND BIOMECHANICAL ASPECTS OF NITINOL SHAPE MEMORY METAL IMPLANTS

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Abstract
Nickel-titanium shape memory metal Nitinol (NiTi) is a new kind of implant material, which provides a possibility to prepare functional implants activated at body temperature and withstands kinking better than conventional metals. Applications utilizing these unique properties are a target of active research interest. Host reactions to NiTi and to the forces created by functional implants should also be studied.

A functional NiTi intramedullary nail, which causes a bending force on the bone, was developed for correcting bone deformities. In the present studies, the action of the device was inverted to induce a bone deformity instead of correcting one, in order to test the hypothesis that bone modelling can be controlled using such functional nail. Implanting the nail into the medullary cavity of rat femur for twelve weeks caused bowing of the bone, retardation of its longitudinal growth, and thickening of the bone and the cortex. In another study the effects of functional and straight nails were compared. Bowing of the bone and significant overall thickening of the bone and the cortex were associated only with the functional nail, while the straight nail induced only minor thickening of the bone. Retardation of longitudinal growth was seen in both groups, and this may have been caused by perforating the distal epiphyseal plate during the nailing. Finite element model of the bone-nail combination was also created.

Porous NiTi was studied as a bone graft substitute by filling a bone defect in the distal femoral metaphysis of a rat bone with porous NiTi implants of different porosities. After 30 weeks, porosity of 66.1% (mean pore size (MPS) 259µm) showed the best bone-implant contact (51%). However, porosity of 46.6% (MPS 505µm) with 39% bone-implant contact was not significantly inferior in this respect and showed a significantly lower incidence of fibrosis within the implant and thus seemed to be the best choice for a bone graft substitute, out of the porosities tested here. The porosity of 59.2% (MPS 272µm) showed lower contact values.

NiTi tendon suture material was studied by implanting NiTi sutures into rabbit tendon and subcutaneous tissues for two, six, and twelve weeks. NiTi proved to be stronger than polyester, which served as control material. The encapsulating membrane was minimal with both materials, suggesting good biocompatibility in tendon tissue. The implantation did not affect the strength properties of either material.

On the basis of the present studies, NiTi provides a possibility to develop new kinds of implants for correcting bone deformities, for filling bone defects in weight-bearing locations and a good candidate for a tendon suture material.

Keywords: biocompatible materials, bone modeling, nickel-titanium alloy, porosity
Dedicated to my family
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>$A_f$</td>
<td>Austenite finish temperature</td>
</tr>
<tr>
<td>AO/ASIF</td>
<td>Arbeitsgemeinschaft fur Osteosynthesefrage/Association for the Study of Internal Fixation</td>
</tr>
<tr>
<td>AP</td>
<td>Anteroposterior</td>
</tr>
<tr>
<td>$A_s$</td>
<td>Austenite start temperature</td>
</tr>
<tr>
<td>BMD</td>
<td>Cortical bone mineral density</td>
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<tr>
<td>BMU</td>
<td>Basic multicellular unit</td>
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<tr>
<td>CSA</td>
<td>Cross-sectional cortical area</td>
</tr>
<tr>
<td>$D_{\text{MAX}}$</td>
<td>Maximum thickness of bone</td>
</tr>
<tr>
<td>$D_{\text{MIN}}$</td>
<td>Minimal thickness of bone.</td>
</tr>
<tr>
<td>FE</td>
<td>Finite element</td>
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<tr>
<td>CtTh</td>
<td>Cortical thickness</td>
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<tr>
<td>MDx</td>
<td>Microdamage</td>
</tr>
<tr>
<td>$M_d$</td>
<td>Highest temperature to strain induced martensite</td>
</tr>
<tr>
<td>$M_f$</td>
<td>Martensite finish temperature</td>
</tr>
<tr>
<td>$M_s$</td>
<td>Martensite start temperature</td>
</tr>
<tr>
<td>MPS</td>
<td>Mean pore size</td>
</tr>
<tr>
<td>Ni</td>
<td>Nickel</td>
</tr>
<tr>
<td>NiTi (or Nitinol)</td>
<td>Nickel-titanium shape memory alloy</td>
</tr>
<tr>
<td>pQCT</td>
<td>Peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>StSt</td>
<td>Stainless steel alloy</td>
</tr>
<tr>
<td>Ti</td>
<td>Titanium</td>
</tr>
<tr>
<td>Tot A</td>
<td>Total cross-sectional area</td>
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</table>
Definitions

Austenite
The high-temperature (parent) phase of material.

Biocompatibility
The ability of material to perform with an appropriate host response in a specific application.

Biomaterial
A material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body.

Finite element analysis
A computer based analysis method which calculates the theoretical response of the model by solving a set of simultaneous equations that represent the behaviour of the structure under loading.

Hysteresis
The difference between the temperatures at which the material is 50% transformed to austenite upon heating and 50% transformed to martensite upon cooling.

Implant
A medical device made of one or more biomaterials that is intentionally placed within the body, either totally or partially buried beneath an epithelial surface.

Martensite
Low-temperature phase of metallic material.

Martensitic transformation
A lattice transformation involving shearing deformation and resulting from cooperative atomic movement.
Osseointegration (or osteointegration)
Direct bone-to-biomaterial interface without fibrous tissue for a functioning implant at the optical microscopy limits of resolution (0.5 µm). It is a description of clinical performance devices and not applicable to the description of biomaterial interactions.

Osteoconduction
The ability to guide bone formation on a material surface in a bony environment.

Osteoinduction
The ability to induce bone formation in non-osseous tissues.

Superelasticity
The ability of an alloy specimen to return to its original shape upon unloading after a substantial deformation.

Shape memory effect
When an alloy in which some fixed shape has been stored is deformed at low temperatures and subsequently heated above the transition temperature, it reverts to its original shape.

Shape memory alloy
Material with an ability to return to some previously defined shape or size when subjected to an appropriate thermal procedure.

Strain
The proportional change in bone dimension upon loading.

Transition temperature
The temperatures at which changes of material phases occur.
List of original publications

This thesis is based on the following articles referred to in the text by their Roman numerals:


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References
1 Introduction

The skeleton is a mechanically optimized biological system, whose composition and organization are greatly influenced by mechanical forces. The geometry of the cortical compartments and the trabecular structure are the result of functional adaptation to normal physiological loads (Einhorn 1992). It has been shown that bone also adapts to externally applied forces (Raab-Cullen et al. 1994b). Another area of adaptive bone remodeling is the bone response to the insertion of orthopaedic implants.

Nickel-titanium shape memory metal alloy, Nitinol (NiTi), is a functional material whose shape and stiffness can be controlled with temperature. The metal undergoes a complex crystalline-to-solid phase change called martensite-austenite transformation. As the metal in the high-temperature (austenite) phase is cooled, the crystalline structure enters the low-temperature (martensite) phase, where it can be easily bent and shaped. As the metal is reheated, its original shape and stiffness are restored. (Buehler & Wang 1968.) NiTi has also been shown to have excellent super-elastic properties, which means that, within a given temperature range, it can be strained several times more than conventional metal alloys without being plastically deformed (Miura et al. 1986).

These unique properties make NiTi a very interesting implant material. Because of its shape memory property, it provides a possibility to prepare self-locking, self-expanding, self-compressing, and other functional implants activated at body temperature. Furthermore, because of its superelasticity, it can be used in implants exposed to continuous distortion or kinking (Duerig et al. 1996). Biocompatibility studies have shown NiTi to be a safe implant material, which is at least equally good as stainless steel or titanium alloys (Ryhänen 2000, Kapanen et al. 2001). The biocompatibility of NiTi in tendon tissue, however, has not been studied before.

The present studies evaluate the bone modeling response of bone tissue to functional and porous NiTi implants. A functional NiTi intramedullary nail, which causes a controlled force on the bone, was developed for correcting bone deformities. In the present studies, the action of the device was inverted to induce a bone deformity instead of correcting one, to test the hypothesis that bone modelling can be controlled using a functional bending nail. The bone modeling response to functional nailing was recorded. A simplified finite element analysis (FEA) of nail-bone combination was performed. The leading thought of this part of the study was, that ability to change bone shape with a
functional nail might be useful in correcting clinical bone deformities in the future. Osteointegration and bone ingrowth in porous NiTi implants of different porosities were also examined. In addition, the biocompatibility and mechanical properties of NiTi wire in tendon tissue were studied.
2 Review of the literature

2.1 Control of bone architecture

The skeleton has an ability to adjust its architecture in response to the variable loading environment (Einhorn 1992). Theories concerning these adaptive processes and the mechanisms related to them are here discussed in more detail.

2.1.1 History

For over a century, scientists have sought to understand the biologic response of skeletal tissues to mechanical stimuli and their effects on the control of bone architecture. In 1892, Wolff wrote: “Every change in the form and function of bone, or of their function alone, is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws” (Wolff 1892). In 1905, Roux generalized the notion that tissues and organs possess an ability to adapt their structure to the physical environment (Roux 1905). This alteration in response to mechanical stresses has been referred to as “functional adaptation” (Carter & Orr 1992). In 1917, D’Arcy Thompson proposed the idea of continuous interaction between muscles and bones: “between muscle and bone there can be no change in one, but it is correlated with changes in the other…” (Thompson D’Arcy 1917). By 1960, however, a paradigm of skeletal physiology presumed that “effector cells” (e.g. osteoblasts, osteoclasts, chondrocytes, fibroblasts) mainly cause skeletal health and disease, and that the causes and cures of diseases should hence be sought in those cells and in their regulation by non-mechanical factors (McLean & Urist 1961). That paradigm ignored skeletal adaptations to mechanical needs (Frost 1998b).

The controversy between the ideas of different research teams created a need to combine multidisciplinary evidence and ideas. This need created the still-evolving Utah paradigm of skeletal physiology and disease that combines variable ideas and pieces of
anatomical, clinical, pathological, and basic science evidence. According to this paradigm, many features of the skeletal architecture and the biologic mechanisms that can change it already exist at birth, and after birth these activities adapt the skeleton to its mechanical loads and strains, using such cells as osteoblasts and osteoclasts to do so. (Frost 2000.)

2.1.2 Bone modeling and remodeling

Bone tissue is under constant reconstruction. Bone has an ability to respond to mechanical forces and to gradually change its external geometry and internal structure, a process called bone modeling (Weinans et al. 1993). Bone modeling takes place mainly during growth and in conditions of changed mechanical loading, resulting in altered bone shape, since new bone is formed at a location different from the site of bone resorption (Frost 1990). Bone remodeling refers to the turnover of bone in small packets (Frost 1998a). Internal modeling can be expressed as a change of porosity (Carter et al. 1989). External, or surface, modeling, can be expressed as a displacement of the subperiosteal surface, which redefines the external geometry (Cowin 1987). Modeling contributes to skeletal strength homeostasis by adjusting bone mass and geometric properties to withstand the current loading conditions (Kimmel 1993).

The hypothetical adaptive modeling process can be explained as follows. A bone is loaded by external forces that cause stresses and strains in it. This local mechanical stimulus is assumed to be sensed by the bone. If the stimulus is abnormal, it affects the bone turnover rate by activating osteoblasts or osteoclasts. This results in adaptation of the geometry (external modeling) and the density (internal modeling). As a result, the stresses and strains in the bone also change, affecting the remodeling potential again. This process continues until the mechanical stimulus is normalized. (Weinans et al. 1993.) This “target strain” pattern of long bones, which reflects an appropriate match between loading and architecture, varies depending on the location (Biewener et al. 1986, Lanyon & Skerry 2001). Frost called this mechanism the “mechanostat” (Frost 1987). Apart from load bearing, other factors, such as factors associated with growth and the influence of calcium regulation, also contribute to bone modeling (Lanyon 1996). These factors are, however, not discussed here.

2.1.3 Cellular mechanisms of bone modeling and remodeling

In 1963, it was proposed that bone adaptation is controlled by basic multicellular units (BMUs) (Enlow 1963). A BMU makes and uses first osteoclasts and then osteoblasts to replace a small “packet” of old bone with new lamellar bone. This takes three months or longer, which time is called the “remodeling period”. Each BMU creates a small temporary hole in bone. All such holes together define the bone’s remodeling space, which normally occupies about 4% of the bone’s volume, but may exceed 25%, and is
greater in trabeculae than in compacts. Increased remodeling space temporarily weakens the affected bones. When completed BMUs make less bone than they resorb ("Disuse mode"), remodeling may cause osteopenia. When BMUs equalize their resorption and formation ("Conservation mode"), remodeling may stimulate bone turnover while simultaneously preventing osteopenia. (Frost 1998a.)

Apart from BMUs, modeling drift is another important mechanism of bone adaptation to stresses and strains. Formation drifts make and control new osteoblasts to add bone to some bone surfaces, while separate and independent resorption drifts make and control new osteoclasts to remove bone from other surfaces. (Frost 1966.) Through this non-adjacent activity of osteoblasts and osteoclasts, bone cell activity moves bone surfaces to determine the shape and diameter of the bone and the strength of trabeculae and whole bone. This modeling works best during growth and poorly on adult cortical bone, but it may affect trabeculae for life. (Frost 1998a.) Macromodeling strengthens bone by expanding both the periosteal and the endocortical diameters of bones (Kimmel 1993). Minimodeling refers to alignment of trabeculae within cancellous bone regions (Cowin et al. 1992).

Recent research has illuminated the biological responses of bone to mechanical loading at the cellular level, but the precise mechanosensory system that signals bone cells to deposit or resorb tissue has not been identified. Bone modeling has been proposed to be initiated by osteoclasts (Teitelbaum 1993). Contrary to this, however, several authors have proposed osteoblasts, osteocytes and lining cells to be the cells responding to strain and influencing modeling and remodeling, the activity of osteoclasts being indirectly controlled by them (Cowin et al. 1991, Kimmel 1993, Dodds et al. 1993, Lanyon & Skerry 2001). Furthermore, there is increasing evidence to suggest that osteocytes modulate the targeting of remodeling by their apoptosis, which is associated with locally increased bone resorption (Noble et al. 2003, Weyts et al. 2003). As high strains appear to decrease the presence of estrogen receptor alpha in osteocytes, estrogen receptor alpha has also been hypothesized to be involved in the bone cell responses to mechanical strain (Zaman et al. 2000, Ehrlich et al. 2002). It has been proposed that mechanical strain stimulates osteoblast proliferation through the estrogen receptor similarly in males and females (Damien et al. 2000). The most recent studies have also suggested osteocytes to respond to strain as a population rather than as individual strain-responsive cells (Ehrlich et al. 2002).

### 2.1.4 Stresses and strains

Loads on bone effected by weight bearing, muscle work, or other causes are called stresses. When a bone is loaded, it deforms until the internal forces holding the structure together are equal to the external forces derived from the load. The proportional change in bone dimension is called “strain”. A strain quantified as 1.00 represents a doubling of the dimension. Functional strains in bone are far smaller, falling between 0.0005 and 0.003. These values are sometimes expressed as 500 and 3000 microstrain. (Lanyon & Skerry 2001.)
Minimum effective strain (MES) reflects the borderline strain below which strain does not evoke adaptive architectural bone modeling, although greater strain does (Frost 1983). Stresses on bone must exceed the remodeling threshold strain range (MESr, 50-100 microstrain) in order to be able to initiate conservation mode remodeling (Frost 1998a). Whenever strains remain below that threshold, as in disuse, disuse mode remodeling results in removal of bone, usually next to marrow. This causes “disuse pattern” osteopenia, in which bones have less spongiosa, wider marrow cavities, and thinner cortices than before, but unchanged outside diameters and lengths. Strains above the modeling threshold range (MESm, near 1000 microstrain) can make modeling strengthen a bone, and reduce later strains towards the lower limit of that range. (Frost 1998a.)

Repeated strains may cause microscopic fatigue damage or microdamage (MDx) in bone (Burr et al. 1997). Microcrack accumulation impairs the mechanical properties of bone by reducing its elastic modulus (Burr et al. 1998). Normally, bones detect their MDx, and BMUs then repair it by removing damaged bone and replacing it with new bone. The strain range that begins to cause so much MDx that it can no longer be adequately repaired can be defined as the operational MDx threshold range (MESp, near 3000 microstrain) (Burr et al. 1997). For comparison, the ultimate strength of bone as strain is about 25000 microstrain (Martin RB & Burr 1989). Strains above 3000 microstrain may also stimulate woven bone formation instead of lamellar bone formation. A BMU needs three months or more to repair one locus of MDx, and excessive MDx will cause fractures (Frost 1966).

Experiments suggest that adaptive modeling and remodeling are sensitive to dynamic but not to static strain changes (Lanyon & Rubin 1984, Lanyon 1992, Hsieh & Turner 2001). A static load applied continuously produces an effect similar to disuse (Lanyon & Rubin 1984). Modeling responds to the typical largest strains of bones. Muscles (not body weight) have been shown to provide the largest loads on bone, and hence also the largest bone strains (Martin & Burr 1989, Burr 1997). Thus, muscle strength strongly influences bone strength and geometry. Although osteogenic responsiveness to load bearing is maintained into old age, it is proposed to decline with age (Raab et al. 1990, Rubin et al. 1992).

### 2.1.5 Externally applied forces

Functional adaptation of bones in response to artificial loading in vivo has been studied on many animal models. Four types of in vivo mechanical loading models are currently available: exercise (Järvinen et al. 1998), ulnar osteotomy (Burr et al. 1989b), external force application through implants (O’Connor et al. 1982, Rubin & Lanyon 1985) and external force application through muscle and soft tissue (Turner et al. 1991, Turner et al. 1992, Raab-Cullen et al. 1994a). Each model creates a unique loading environment. Ulnar osteotomy overloads the radius and alters the force distribution during all weight-bearing activities. Although the osteogenic response of the radius to an altered loading environment is demonstrated, the load magnitude, rate, and repetitions cannot be
controlled. External force application through implants provides a possibility to control these parameters, but requires surgery, which in itself may affect bone modeling (Raab-Cullen et al. 1994b). External force application through soft tissue, including application of force through pads contacting the external surface of the leg, does not require surgery, but the pads create local pressure on the leg at the contact sites (Raab-Cullen et al. 1994b). Most of the models utilizing external force application require immobilization of the limb in a bending device, eliminating the effect of normal weight bearing or load bearing during muscle work associated with normal movement of the animal. All of the available information on the effect of strain magnitude, rate, and repetitions on bone modeling have come from these external loading models (Raab-Cullen et al. 1994b).

The osteogenic response of bone to loading is dependent on numerous variables of the strain environment, including the number of applications of strain, the rate of strain, the magnitude of peak strain, and the direction and distribution of strain (Lanyon & Rubin 1984, Rubin & Lanyon 1985). The rate of strain change seems to be a major determinant of the adaptive osteogenic response to a mechanical load (O'Connor et al. 1982, Mosley & Lanyon 1998, Lee et al. 2002). Across the physiological range, a high rate of strain change provides a greater osteogenic stimulus than a corresponding peak strain achieved more slowly (Mosley & Lanyon 1998). Another important factor affecting the bone response to loading is the distribution of the load. Strains within the habitual range and distribution produce no osteogenic response (Mosley & Lanyon 1994, Skerry & Lanyon 1995). Strains within the habitual range presented in unusual distributions effect an osteogenic response (Rubin & Lanyon 1985). These unusual strains have also been called “error strains” (Lanyon 1996). The error strain distribution hypothesis suggests that bone cell populations maintain the skeleton’s structural competence by making architectural adjustments to eliminate or reduce perceived deviations from normal in the distribution of dynamic strains (Lanyon 1996). It has also been proposed that bones are sensitive to dynamic strain change at certain frequencies and that, if present at these frequencies, strains of very low amplitude may produce substantial osteogenic responses (McLeod & Rubin 1992, Hsieh & Turner 2001).

It has been suggested that externally applied dynamic forces stimulate bone formation as a result of increased bending strains, and that bending stimulates bone formation in the regions with maximum bending strains (Raab-Cullen et al. 1994b). During exercise-increased bone loading, bone formation accelerates only at the periosteal surface (Raab et al. 1991). Measured, mild external loading produces increased bone formation at the periosteal surface as well (Raab-Cullen et al. 1994a, Raab-Cullen et al. 1994b, Lee et al. 2002). The greatest increase in cortical bone area after axial loading seems to be near the midshaft of the bone (Lee et al. 2002). During major increases in loading, woven bone is added at the periosteal surfaces (Burr et al. 1989a, Frost 1992). Woven bone formation enables a rapid increase in strength because it forms more quickly than lamellar bone, but it is still placed at the periosteal surface. When such loading conditions persist, woven bone eventually remodels into lamellar bone (Rubin et al. 1995).

In artificial loading tests, mechanical loading has been shown to stimulate rapid changes in periosteal gene expression. It has been suggested that this change in the pattern of gene expression may signal cell proliferation. (Raab-Cullen et al. 1994c.) Growth rate, rather than gender, determines the size of the adaptive response of the growing skeleton to mechanical strain (Mosley & Lanyon 2002).
2.1.6 Adaptive bone modeling around surgical implants

Another area of adaptive bone modeling is bone modeling in response to the implantation of orthopaedic implants. The host response to implants placed in bone involves a series of cell and matrix events, ideally ending up with apposition of bone to the biomaterial (Puleo & Nanci 1999). Osseointegration (or osteointegration) refers to direct bone-to-biomaterial interface without fibrous tissue at the optical microscopy limits of resolution. For this contact to occur, the gaps between the bone and the implant must be filled, and the bone damaged during the preparation of the implant site must be repaired. In the case of porous implants, capillaries, perivascular tissues, and osteoprogenitor cells migrate into the porous spaces and incorporate the porous structure with newly formed bone. This process is called osteoconduction. (Cornell & Lane 1998.) Osteoinduction, in turn, refers to the ability of an implant to induce bone formation in non-osseous tissues. Further, a fracture fixation device or another orthopaedic implant in bone takes over part of the load that would normally be carried exclusively by the bone. This mechanism, called stress shielding, reduces the stress and strain in the femur relative to the natural situation and thus can be expected to affect bone modeling or remodelling. (Mølster 1986, Husby et al. 1989b, Husby et al. 1989c, Weinans et al. 1993.)

2.1.6.1 Influence of intramedullary nailing on bone

Most studies concerning the influence of intramedullary nailing on bone include fracture of the bone (Kessler et al. 1986). There are, however, some studies demonstrating the effects of mere intramedullary nailing, without fracture, on bone. The effects discovered in these studies are discussed here in more detail.

There are controversies in the literature as to the effect of intramedullary reaming of an intact bone on the cortical bone blood flow. Whiteside et al. found no change in the diaphyseal flow rate, using the hydrogen washout technique, after intramedullary reaming of the rabbit tibia (Whiteside et al. 1978). Indrekvam et al., however, using the microsphere method, found the diaphyseal flow to be reduced to one third after reaming of the femoral canal of a rat femur up to 1.8mm (Indrekvam et al. 1992). Grundnes and Reikerås evaluated the acute effect of an increasing degree of intramedullary reaming of the rat femur on bone blood flow using the microsphere method. Reaming up to 1.5mm caused only marginal reduction in total bone and cortical blood flow. Reaming up to 1.8mm halved total bone flow and reduced cortical blood flow by one third. Reaming up to 2.1mm reduced total bone flow to one third and cortical bone flow by one third. The authors concluded that modest intramedullary reaming has little effect on total and cortical blood flows, whereas reaming that involves destruction of the endosteal cortex reduces both total and cortical blood flows. (Grundnes & Reikerås 1993.) After the acute impairment of blood flow due to reaming, revascularization of the rat femur takes from a day to about a week, depending on the extent of reaming; the presence of the nail seems to be of no significance (Kessler et al. 1986, Indrekvam et al. 1992, Grundnes et al. 1994). After 12 weeks of nailing, the total blood flow of the rat femur is comparable to
that on the unoperated side, but the cortical blood flow is significantly increased (Grundnes et al. 1998). A complete intramedullary occlusion, however, seems to impair the endosteal blood supply and to induce a shift of diaphyseal circulation from a medullar to a more periosteal pattern, to compensate for the loss in endosteal flow (Rhineland 1974, Grundnes et al. 1998). The results on the effect of intramedullary nailing on bone thickness also seem to be conflicting. Indrekvam et al. reported a significant increase in the anteroposterior diameter and cross-sectional area of the rat femur 24 weeks after intramedullary nailing with a 1.8mm stainless steel (StSt) nail inserted from the trochanteric groove in a proximal to distal direction (Indrekvam et al. 1991). However, Grundnes et al. reported no differences in either the external or the internal anteroposterior or transverse diameters, in the cross-sectional area of the medullary cavity, or in the bone cortical area (CSA) after 12 weeks of implantation with a 2.0mm StSt nail implanted similarly as above (Grundnes et al. 1998). Husby et al. reported reduced cortical thickness (CtTh) 12 weeks after intramedullary nailing with a 1.8mm StSt nail inserted as described above (Husby et al. 1989a). In their series of studies, Husby et al. have shown that rigid intramedullary nailing causes strain shielding in rat femora (Husby et al. 1989b). Strain shielding immediately after intramedullary nailing of the rat femur was evaluated using unidirectional strain gauge units implanted on the anterior bone surface bilaterally, measuring the strains of the bones in vivo while the rat walked on a treadmill (Husby et al. 1989c). Steel nailing reduced the strain by 74% compared to the unoperated side. Strain shielding 12 weeks after nailing was similarly evaluated, showing the strain of the nailed femur to be 51% of the intact side (Husby et al. 1989b). There are, however, controversies in the literature about the changes in the porosity of a rat femur after intramedullary nailing. Grundnes et al. found no difference in the porosity of the rat femur between a group with reaming and nailing (from the trochanteric groove) and a control group 12 weeks after nailing (Grundnes et al. 1998). Husby et al. reported increased porosity (by a factor of 4.5) 12 weeks after intramedullary nailing with a 1.8mm StSt nail inserted as above (Husby et al. 1989a). Bjerkreim and Langård showed that damage to the central portion of the epiphyseal growth plate due to retrograde intramedullary nailing of the rat femur leads to significant inhibition of longitudinal growth (Bjerkreim & Langård 1983). In another study, where the nail was implanted from a proximal to a distal direction, with the nail entering the bone via the trochanteric groove, no difference in bone length was seen (Indrekvam et al. 1991).

2.1.6.2 Influence of the implantation of a porous implant on bone

The repair or regeneration process associated with the healing response to the surgical implantation of a porous implant has many phases (Spector et al. 1979). Immediately after implantation, an inflammatory response, characterized by increased vascular and cellular activity, is elicited, and a blood clot forms within the pores of the implant. Within
a few days of implantation, the blood clot becomes organized and comprises primarily red blood cells, fibrin, and platelets. Within a few days, the initial clot is replaced by loose fibrocollagenous tissue and capillaries. Following clotting, the pores become filled with osteoprogenitor mesenchyme. Approximately four weeks after the implantation, new bone trabeculae can be seen in the pores of the implant, completing the first phase of bone ingrowth. The second phase of the bone ingrowth response involves the stress-induced modeling and remodeling of the initial bony spicules into mature trabeculae. (Spector et al. 1988.) The factors that initiate the ingrowth from adjacent tissues, determine the completeness of incorporation, and affect the type of new tissue formed within the scaffold are only poorly understood (Cornell & Lane 1998).

Once formed as a consequence of the healing process following implantation, the bone undergoes stress-induced remodeling. The osseous tissue within and around porous implants in bone is influenced by the stress distribution altered by the very presence of the implant. (Weinans et al. 1993.) While bone ingrowth, in the first place, is dependent on the porosity and the structure of the framework of the material, the remodeling process is influenced by the mechanical properties of the material, particularly the modulus of elasticity (Spector et al. 1978). For example, the use of very stiff porous metallic femoral stems has been found to lead to bone atrophy in certain areas of the femur as a result of stress shielding effects (Engh et al. 1987).

### 2.1.6.3 Bone modeling around functional implants

The only study featuring a bone modeling response to forces caused by functional implants activated at body temperature is a report of the bone response to a new type of stapes prosthesis (Kasano & Morimitsu 1997). A NiTi alloy wire of 0.1mm in diameter shaped as a circle was opened after cooling in ice water and implanted at the long crus of the incus by warming with saline up to 50°C. The experimental study was done in 24 ears of 12 cats. The incus was examined 27-355 days postoperatively. Slight bone resorption in the form of bone erosion was seen in the contact area of the prosthesis, and this was assumed to be induced by the mechanical pressure of the prosthesis. Good biocompatibility of NiTi in the long crus of the incus was observed. (Kasano & Morimitsu 1997.) There is, to the authors' knowledge (apart from the present studies), no other reports of bone modeling responses to functional implants activated at body temperature.

### 2.2 Nitinol

In the early 1960s, Buehler et al. discovered the shape memory effect in an equiatomic alloy of nickel and titanium (Nitinol, chemical symbol NiTi) (Buehler & Wang 1968). The use of Nitinol for medical purposes was first reported in the early 1970s (Cutright et al. 1973). However, it was only in the mid-1990s that the first widespread commercial
stent applications of Nitinol were introduced. Nitinol was the first “functional biomaterial” to become available, and is now a subject of active interest in the medical field (Ryhänen 2000). The shape and stiffness of NiTi can be controlled with temperature (Buehler & Wang 1968). Nitinol has made it possible to prepare functional implants activated at body temperature. Furthermore, because of its excellent superelasticity, it can be used in implants exposed to continuous distortion or kinking (Miura et al. 1986). These unique properties make NiTi a very interesting implant material. Lately, studies on porous Nitinol have started (Simske & Sachdeva 1995).

2.2.1 Shape memory property

The shape memory effect means that when an alloy is deformed at low temperatures and subsequently heated, it reverts to its original shape (Buehler & Wang 1968). During this process, the metal undergoes a complex crystalline-to-solid phase change called martensite-austenite transformation (Fig. 1).

![Fig. 1. The martensite-austenite transformation. The crystal structure and the mechanical properties of the metal are altered as a result of the temperature change. $M_s$ = martensite start temperature, $M_f$ = martensite finish temperature, $A_s$ = austenite start temperature, $A_f$ = austenite finish temperature, $H$ = hysteresis.](image)

Cooling of the metal promotes the entry of the crystalline structure into the low-temperature (martensite) phase, where the metal is soft and easily deformable. The temperature at which this phenomenon starts is called the martensite start temperature ($M_s$), and the temperature at which the process is complete is called the martensite finish temperature ($M_f$). As the metal is reheated, its original shape and stiffness are restored (austenite). The temperature range needed for this process is somewhat higher than
above. The starting temperature of this process is called the austenite start temperature 
\( (A_s) \), and the temperature at which the process is complete is called the austenite finish 
temperature \( (A_f) \). The difference between these transition temperatures upon heating and 
cooling is called hysteresis (the difference between the temperatures at which 50% of the 
material is transformed into austenite upon heating and into martensite upon cooling). 
This difference can be up to 20-30°C. (Buehler & Wang 1968.) The crystal structure of 
Nitinol changes gradually in a non-linear fashion (starting slowly, proceeding fast 
halfway through, and getting slow again at the end) during the transition period (Moneim 
et al. 2002).

The temperature range of this transition can be modified by the metallic content, cold 
working, and annealing of the alloy (Shabalovskaya et al. 1995). By these procedures, a 
variety of implants can be prepared for different purposes.

### 2.2.2 Superelasticity

Superelasticity means that the material can be deformed much further than normal 
materials, but it still recovers its original shape upon unloading (Van Moorleghem et al. 
1998). The good superelastic properties of NiTi are based on stress-induced martensite 
formation (Miura et al. 1986), which means that an externally applied force deforming 
the metal causes martensite formation at temperatures higher than \( M_s \). When the stress is 
released, martensite transforms back into austenite, and the original shape of the metal is 
restored. With NiTi, superelastic deformations up to 8% are possible without permanent 
plastic deformation of the metal (Van Moorleghem et al. 1998). Furthermore, superelastic 
NiTi can be strained several times more than ordinary metal alloys without being 
plastically deformed, which reflects its rubber-like behaviour (Duerig et al. 1996).

Superelasticity is, however, observed only over a specific temperature area (Duerig et 
al. 1996). The highest temperature at which martensite can no longer be induced by 
stresses caused by externally applied forces is called \( M_d \), above which temperature NiTi 
alloy is deformed like ordinary metals. On the other hand, below \( A_s \), the material is 
martensitic and does not recover. Thus, the material is superelastic at temperatures from 
\( A_s \) to \( M_d \) (Duerig et al. 1996).

### 2.2.3 Biocompatibility

Biocompatibility refers to the ability of material to perform with an appropriate host 
response in a specific application (Williams et al. 1992). There are two main factors that 
determine the biocompatibility of a material: the host reactions induced by the material 
and the degradation of the material in the body environment. Because of the possibility 
of nickel (Ni) and titanium (Ti) ions to dissolve from Nitinol due to corrosion, it is 
important to understand the effects of these components.
It is currently accepted that pure titanium is very well tolerated by local tissues (Pfeiffer et al. 1994). The good biocompatibility and corrosion resistance of titanium are due to the stable titanium oxide (TiO$_2$) film that naturally forms on titanium surfaces (Zitter & Plenk, Jr. 1987). Good osseointegration of titanium implants in bone has also been observed (Branemark et al. 1969).

Although nickel is one of the essential trace elements for humans and animals, it is also well-known for its toxicity and propensity to cause allergies. In vitro studies have shown nickel to cause toxic effects, including cellular damage, at high concentrations (Putters et al. 1992). Pure nickel implanted intramuscularly or into bone has been shown to cause severe local tissue irritation, necrosis, and toxic reactions (Laing et al. 1967). Nickel is a major cause of allergic contact dermatitis (Peltonen 1979). Nickel has also been studied for its possible carcinogenicity (Oller et al. 1997). Although epidemiological evidence suggests an association between respiratory exposure to dust from a nickel refinery and an increased risk of respiratory cancers, recent evidence indicates that respiratory exposure to soluble nickel alone will not cause cancer (Oller 2002). Because of the high nickel content of NiTi, it is theoretically possible that, under certain conditions, nickel may dissolve from the material due to corrosion and cause adverse effects. Therefore, comprehensive biocompatibility studies of NiTi in different tissues are important before clinical use in these tissues.

The corrosion resistance of metal alloys is based on a passivation phenomenon (Kruger 1983), which arises as a result of the metal oxide layer that forms on the surface of the metal. The surface of NiTi consists mainly of stable titanium oxides (TiO$_2$) and lesser amounts of nickel oxides (NiO and Ni$_2$O$_3$) and metallic Ni, while nickel-titanium constitutes the inner layer (Endo et al. 1994, Shabalovskaya 2002). The surface chemistry and the amount of Ni on the surface of the NiTi alloy may vary over a wide range, depending on the methods of preparation and surface finishing that have been used (Shabalovskaya et al. 2003).

### 2.2.3.1 Muscle and tendon responses to Nitinol

The biocompatibility of Nitinol was first studied by implanting NiTi wires (15mm long, 0.76mm in diameter) into the subcutaneous tissue of 45 rats for nine weeks (Cutright et al. 1973). The tissue reaction was minimal. The reparative process ended up with a dense, relatively avascular fibrous connective tissue capsule around the implant. The results were considered comparable to the previous results with StSt, and it was concluded that NiTi could be used in deep tissues. (Cutright et al. 1973.) However, there was no control group, only the subcutaneous response was assessed, and the implantation period was relatively short.

In another study, the muscle tissue of dogs was exposed to Nitinol implants for three, six, 12, and 17 months, with three animals in each group (Castleman et al. 1976). Co-Cr implants were used as control. Gross clinical, radiological, and morphological analysis of tissue at the implantation sites showed no signs of adverse tissue reactions resulting from the implants. Although the implantation period was long enough, the small number of test
animals made reliable statistical analysis difficult, especially as considerable variation was evident between the capsules of different specimens of the same material. There was no difference between the test materials. No clearly toxic effect was seen. The authors concluded NiTi alloy to be sufficiently compatible with dog tissue to warrant further investigation of its potential as a biomaterial. (Castleman et al. 1976.)

In a recent study NiTi specimens were implanted into paravertebral muscle and near the sciatic nerve of rats for two, four, eight, 12, and 26 weeks for biocompatibility testing (Ryhänen et al. 1998). The encapsulating membrane thickness around the implants was measured using light microscopy. At eight weeks, the membrane encapsulating the NiTi implant was thicker than that of StSt (mean 62 ± 25µm versus 41 ± 8µm), but at the end of the study, the encapsulating membranes were of equal thickness. As there was no necrosis, granulomas, or signs of dystrophic soft-tissue calcification, and only a few foreign body giant cells were present, it was concluded that Nitinol has good in vivo biocompatibility in muscle and perineural tissue.

Although these studies have suggested NiTi to be a safe implant material in muscle tissue, the biocompatibility of Nitinol in tendon tissue has not been studied.

2.2.3.2 Bone response to Nitinol

In the first study aimed at evaluating the biocompatibility of NiTi in bone tissue, Nitinol bone plates were implanted into the femurs of beagles for three, six, 12, and 17 months (Castleman et al. 1976). No signs of adverse tissue reactions were seen. There was no evidence of bone resorption in the specimens adjacent to the Nitinol plate. (Castleman et al. 1976.)

Later, the biocompatibility of Nitinol screws was evaluated using immunohistochemistry to observe the distribution of bone proteins during a bone remodeling process around a NiTi implant (Berger-Gorbet et al. 1996). NiTi screws were implanted in rabbit tibia for three, six, and 12 weeks for biocompatibility testing. Vitallium, titanium, and StSt were used as controls. The biocompatibility results of NiTi screws compared well with the other screws, but NiTi showed a slower osteogenesis process characterized by poor contact between the implant and bone, disorganized migration of osteoblasts around the implant, and lower activity of osteonectin synthesis. (Berger-Gorbet et al. 1996.)

In another study histomorphometry was used to determine the bone response to cylindrical NiTi implants (1.0mm in diameter and 1.5mm in length) implanted transcortically (and extending into the medullary canal) into the proximal tibia of 15 rats for 168 days (Takeshita et al. 1997). In similarly sized control groups, Ti, AO-Ti, and Ti-6Al-4V were used. Histometric analysis revealed no significant difference between the tissue reactions to the tested materials, but Ni-Ti implants showed a significantly lower percentage of bone contact and bone contact area than any of the other materials. (Takeshita et al. 1997.)

In a recent study the bone response to Nitinol was evaluated using histomorphometry and scanning electron microscopy (FESEM) after periosteal implantation (Ryhänen et al.
A regional acceleratory phenomenon (RAP) model, in which a periosteal contact stimulus provokes an adaptive bone modeling response, was used. A test implant was placed into contact with intact femur periosteum, but was not fixed inside the bone. Nitinol was compared with StSt and Ti-6Al-4V. The maximum new woven bone formation started earlier (two weeks) in the Ti-6Al-4V group than in the Nitinol group, but after eight weeks the Nitinol and StSt groups had greater cortical bone width. At 26 weeks from implantation, no significant differences were seen. Cell adhesion and focal contacts were similar between the materials studied by FESEM. The authors concluded that Nitinol had no negative effects on total new bone formation on normal RAP. (Ryhänen et al. 1999b.)

In another study by Ryhänen et al. (Ryhänen et al. 1999a), femoral osteotomies of 40 rats were fixed with either NiTi or StSt intramedullary nails. Bone healing was examined with radiographs, peripheral quantitative computed tomography (pQCT), and histologically after two, four, eight, 12, 26, and 60 weeks of operation. There were more healed bone unions in the NiTi than in the StSt group at early (four and eight weeks) time points. Callus size was equal between the groups. Mineral density in the osteotomy area was lower than in the proximal part of the operated femur or the contralateral femur, but there was no difference between the groups. Close bone contact with NiTi was observed in this study. Apart from callus, a peri-implant lamellar bone sheet formed in the metaphyseal area after eight weeks, indicating good tissue tolerance. NiTi was concluded to be an appropriate material for further intramedullary use because it has good biocompatibility in bone tissue. (Ryhänen et al. 1999a.)

Lately, the effect of a NiTi implant on ectopic bone formation induced by decalcified allogenic bone matrix powder, was evaluated (Kapanen et al. 2001). Test implants of rat allogenic bone matrix with a cylindrical NiTi implant in gelatin capsules, were implanted under the fascia of the latissimus dorsi muscle in ten rats for eight weeks. NiTi was compared to StSt (n = 8) and Ti-6Al-4V (n = 8). Gelatin capsules containing allogenic matrix without any metal implants were used as control. Light microscopy showed no inflammatory or other pathological changes in the induced ossicle or its capsule. Densitometry showed the total bone mineral density (BMD) values of induced bone to be nearly equal in the NiTi samples and the control samples without any metal. The StSt and Ti-6Al-4V samples had lower BMDs. Histomorphometry showed no difference between the materials in a combined area of new fibrotic tissue and original implanted bone matrix powder around the implants. However, NiTi showed the largest areas of cartilage and new bone in the ossicles. The biocompatibility of NiTi was concluded to be good, as effects of NiTi on ectopic bone formation were found similar to those of StSt. (Kapanen et al. 2001.)

All studies concerning the bone response to porous Nitinol have suggested good biocompatibility properties of porous NiTi (Simske & Sachdeva 1995, Rhalmi et al. 1999, Ayers et al. 1999, Assad et al. 2002a, Assad et al. 2002b, Assad et al. 2003a, Assad et al. 2003b). These studies are discussed in more detail in the chapter titled “Porous NiTi.”
2.2.4 Functional NiTi implants

2.2.4.1 Compression bone staples

First compression bone staples made of NiTi for fixation of bone fragments were first introduced in 1983 (Dai 1983). A number of different designs of staples and clamps for the fixation of fractures and osteotomies have been introduced since (Kuo et al. 1989, Dai & Chu 1996). Most of them are based on the same basic principle: After cooling, the arms of the staple are opened and inserted into drill holes in each bone fragment, and at the body temperature the staple restores its programmed shape, causing compression between the fragments.

2.2.4.2 NiTi rods for the correction of scoliosis

One of the first functional NiTi implants introduced was a NiTi rod for the correction of scoliosis. The first preliminary studies were carried out with Dwyer instrumentation and NiTi wire using a transition temperature below body temperature (Baumgart et al. 1978). In China, NiTi rods were implanted in 26 patients with scoliosis and the rods were heated with warmed sponges. Good correction of scoliosis and no complications were reported. (Lu et al. 1986.)

In an experimental study six goats with experimental scoliosis were treated using NiTi rods (Sanders et al. 1993). The rod, which had fixed bends at both ends to prevent rotation, was, after cooling, bent to conform to the scoliotic curve and secured to the concave side of the spine with transspinous process wires. The implant was activated by heating with 450-kHz radio frequency induction. Good correction of scoliosis was reported. (Sanders et al. 1993.)

In 1997, a new type of NiTi device for the correction of scoliosis was introduced (Veldhuizen et al. 1997). In this system, the memory metal rod had a square cross-section of 6.35mm and was supported at its ends and in the middle. Anchors of two kinds were used to secure the rod to the vertebrae: An anchor based on a pedicle screw configuration and an anchor based on hooks in claw formation. After implantation, the system was activated by warming the rod with a special heating device based on high-frequency resistive heating. This device allows the surgeon to set the desired temperature, and the warming device automatically heats the rod to the desired temperature (a little above the body temperature). The temperature applied to the rod at operation determines the correction forces. In a cadaver study, the action of the device was inverted to induce a scoliotic curve instead of correcting one, and the system seemed to perform well. (Veldhuizen et al. 1997.)

Lately, an experimental study using this device in six pigs was reported (Wever et al. 2002). The action of the device was, once again, inverted. Immediately after surgery, radiographs showed scoliosis corresponding to the original curvature of the rod, and this curve remained constant during the follow-up of three and six months, after which the
device was almost overgrown with newly formed bone. The authors expected the device also to have the capacity to correct the scoliotic curve, but proposed extensive fatigue testing of the system before performing the clinical trials. (Wever et al. 2002.)

2.2.4.3 Suturing and anastomotic devices

Self-closing NiTi clips for suturing tissues in minimal-access surgery were introduced in 1999. The 5mm diameter clip closes when temporarily heated to a temperature slightly above the human body temperature and is intended to facilitate tissue suturing in endoscopic surgery. The results of the first experimental study were promising. (Xu et al. 1999.)

A NiTi double-ring colonic anastomotic device has been introduced recently. The double ring is inserted after cooling into both bowel lumens, and it contracts at body temperature into its original shape, tightly clamping the two intestinal loops together. The preliminary reports of experimental and clinical studies have been promising. (Nudelman et al. 2000, Nudelman et al. 2002.)

An expandable NiTi device for sutureless aortic anastomosis during the substitution of a prosthesis for the thoracic aorta was also recently introduced (Aluffi et al. 2002). The first experimental studies on microvascular anastomosis with Nitinol clips, using a rabbit model, showed promising results (Kuang et al. 2002).

2.2.4.4 Other functional NiTi implants

Nitinol has been used in various kinds of stents on arteries (Cho et al. 2003), esophagus (Riccioni et al. 2002), biliary obstruction (Bezzi et al. 2002), colon (Kang et al. 2002), urethra (Song et al. 2003), major airway obstruction (Prasad et al. 2002) and lacrimal system obstruction (Ko et al. 2003). One of the earliest functional NiTi implants was the Simon Nitinol filter (SNF) used to prevent pulmonary embolism (Simon et al. 1977). In 1993, a self-expanding prosthesis of Mersilene, strengthened with a cross- or star-shaped Nitinol wire, was introduced into laparoscopic hernioplasty (Himpens 1993). Another promising application is a NiTi hook used to restore the dislocated acromio-clavicular joint (Ryhänen et al. 2003).

2.2.5 NiTi implants utilizing superelasticity

A widely used implant utilizing the superelasticity of Nitinol is a Mitek® suture anchor, consisting of a titanium or NiTi body with two or more arcs of superelastic NiTi wire (Barber et al. 1995). The superelastic NiTi arcs bend at insertion, enabling the Mitek® anchor to be inserted into a hole drilled into the bone and preventing the anchor from
pulling out of the bone after insertion and securing the tendons or ligaments to the bone. In 1995, a detachable clamp with a Nitinol spring was developed for use in minimal-access surgical operations involving hollow visceral transsection and anastomosis (Frank et al. 1995). Lately, Nitinol wire has been introduced as a possible new tendon suture material (Moneim et al. 2002).

2.3 Porous materials

Porous materials have been used in surgical implant design (1.) to fabricate devices to replace or augment soft and hard tissues, (2.) as coatings on prostheses to accommodate tissue ingrowth for biological fixation, and (3.) as scaffolds to facilitate the regeneration of tissue (Spector et al. 1988). The basis for their application as implants lies in their ability to become incorporated into the soft or hard tissues into which they are implanted. Tissue ingrowth into the pores of the material during the process of repair or regeneration associated with the healing response to surgical implantation provides an interlocking, mechanical attachment of the porous material to the host. (Spector et al. 1988.)

2.3.1 Bone ingrowth

Initial tissue ingrowth into the pores of a porous material has been proposed to be independent of the type of porous material implanted as long as the material is sufficiently biocompatible. However, long-term survival of tissue around implants and within the pores of different materials is dependent on specific material properties, such as the elasticity of the material. (Spector et al. 1988.) Several factors have been associated with osteoconductivity and bone ingrowth into the pores of the implant. These factors include the porosity of the surface, the stability and degree of micromotion between the implant and bone, whether the host bone is trabecular or cortical, and the presence of gaps between the implant and the bone surface (Tisdel et al. 1994, Cornell & Lane 1998). The optimal pore sizes of porous hydroxyapatite have been studied before, and the estimates of optimal pore size vary from 150µm to 500µm (Daculsi & Passuti 1990, Chang et al. 2000, Blokhuis et al. 2000). Under non-load-bearing conditions, good bone ingrowth has been achieved even with pore sizes ranging from 50µm to 125µm by using porous titanium implants (Itälä et al. 2001). Apart from the porosity of the implant, the structure of the framework also affects osteoconductivity. It has been shown that porous implants must have interconnecting fenestrations to provide the space for vascular tissue required for continued mineralized bone ingrowth. Thus, both the pore openings of the material and the interconnecting channels between the pores must be of sufficient size to allow the infiltration of cells that are to form the matrix of the tissue within the pores of the material. (Hulbert et al. 1970, van Eeden & Ripamonti 1994) Relative movement of the implant can disrupt the stromal elements that form in the early stages of wound healing, preventing the regeneration of
osseous tissue within the porosity and leading to the formation of fibrous tissue inside the implant (Pilliar 1991, Puleo & Nanci 1999). On the other hand, it has been suggested that low-amplitude mechanical strain can enhance bone ingrowth into porous-coated titanium implants (Rubin & McLeod 1994).

### 2.3.2 Stress-induced remodeling

While bone ingrowth, in the first place, is dependent on the pore characteristics of the material, the bone remodeling process is influenced by the mechanical properties of the material, particularly the modulus of elasticity. For instance, stress-induced bone remodeling may cause changes around the implant in long-term implantation and is related to the compliance of the implant. (Spector et al. 1978.) It has been proposed that implant design should minimize microdamage in bone surrounding the implant (Frost 1995).

### 2.3.3 Bone substitutes

The golden standard for the treatment of bone defects is autograft transplantation from the iliac crest or other donor sites (Block & Thorn 2000). Autograft is structurally competent, osteoconductive, osteoinductive, and osteogenetic (Resnick 2002). However, there has been growing concern about the morbidity associated with the practice of harvesting autologous tissue from another operative site (Laurie et al. 1984, Younger & Chapman 1989, Sasso et al. 1998). Moreover, the need to obtain an autogenous graft increases the operating time. A considerable research and development effort has been recently expended to test and validate alternative bone grafting materials (Bauer & Muschler 2000, Keating & McQueen 2001).

Allograft bone is often used as a substitute for autograft. It is osteoconductive, but not osteoinductive or osteogenetic. Furthermore, allograft bone is expensive, difficult to process, risks transmissible diseases, and is in short supply in many parts of the world. (Resnick 2002.) Common complication seen with the use of large allografts is allograft fracture (Thompson et al. 2000). Renal toxicity has also been reported to be associated with some commercially available demineralised bone matrix materials (Wang et al. 2001).

Demineralized bone matrix (DMB) is osteoinductive material, which is commercially available in a variety of products and is mainly indicated for management of nonunions or fractures. The preparations available are of limited structural strength since they are prepared as putty or paste-like material, and are not suitable for situations in which bony support may be required, such as certain metaphyseal fractures. Bone morphogenetic proteins (BMPs), the active components of DMP, combined with a suitable carrier collagen and osteoconductive material, has also been investigated as a bone graft substitute. The suitability of these products for situations in which immediate bony
support may be required depends of the mechanical properties of the osteoconductive material used in the composite implant. (Keating & McQueen 2001.)

Calcium sulfate (plaster of Paris) has been used for bone defect repair since 1892 (Dreesman 1892), and is perhaps the oldest osteoconductive material available. Its main drawback is the chemical reaction, which occurs during setting, resulting in consequent inconsistency in the material properties. It also resorbs very rapidly at a rate which may exceed the capacity of surrounding bone to regenerate. At present it has been superseded by more reliable osteoconductive materials. (Keating & McQueen 2001.)

Some natural sea corals, which have pore sizes and pore interconnectivity that allow for osteoconduction, have been harvested, cleansed, and then used as bone graft material (Biocoral®, Inoteb, Saint-Gonner, France). These corals may also be chemically treated to convert aragonite to hydroxyapatite without changing the three-dimensional structure of the coral. This graft material is called coralline hydroxyapatite and is one of the first ceramics to be used as an osteoconductive material (ProOsteon® 200R and 500R, Interpore Cross International, Irvine, CA, USA). Hydroxyapatite ceramic can also be produced synthetically. (Shors 1999.) Hydroxyapatite and other porous ceramics are widely used as bone graft substitutes because of their excellent bioadhesion, but the main disadvantage of these graft materials is their brittleness and low tensile strength making them difficult to handle (Uchida et al. 1984, Pilliar et al. 1987, Holmes & Hagler 1988, Blokhuis et al. 2000). The mechanical properties of hydroxyapatite grafts are more similar to cancellous than to cortical bone (Resnick 2002).

Tricalcium phosphate is a synthetically produced ceramic that has a porous structure suitable for osteoconduction. It has been used alone (Vitoss®, Orthovita, Malvern, PA, USA) and in combination with hydroxyapatite (Triosite®, Zimmer, Rungis, France) as a bone graft substitute. Also these ceramics are brittle and difficult to handle limiting their use in situations in which immediate structural strength is required. (Keating & McQueen 2001)

Calcium phosphate cements are growing in popularity as an osteoconductive bone graft substitute. After mixing of the cement it sets to a hard material in 10 to 15 minutes and after 24 to 48 hours has a compressive strength similar to normal cancellous bone. These materials are, however, weak in tension and they will not resist shear forces. (Keating & McQueen 2001) Therefore, according to numerous studies, injection of calcium phosphate cement alone is inadequate for stabilization of the fracture, but additional internal, or external fixation is also needed (Kopylov et al. 2002, Higgins et al. 2002). Soft tissue reactions to injectable calcium phosphate cements have also been reported (Welkerling et al. 2003, Kopylov et al. 2002).

Studies of porous titanium as a bone graft substitute has recently been reported (Pilliar 1987, Itälä et al. 2001).

Osteoconductive bone graft substitutes are often used to fill bone defects that require mechanical support (Keating & McQueen 2001). In situations, in which immediate structural strength is be required, such as certain metaphyseal fractures, sufficient strength of a bone graft substitute is warranted.
Recently, research on the use of porous nickel-titanium alloy as a bone graft substitute has started (Simske & Sachdeva 1995). The major benefits of porous NiTi compared to other bone graft substitutes include its better mechanical strength in weight-bearing applications and superelasticity. Elastic modulus of Nitinol is closer to that of bone than any other metallic material (Table 1) (Miura et al. 1986, Itin et al. 1994, Shabalovskaya et al. 1995). The process of manufacturing porous NiTi (self-propagating high-temperature synthesis) allows for a nearly 100% open pore structure, allowing good bone ingrowth, as well as a range of porosity of 30 – 70% and a controllable pore size of 60-300µm (Itin et al. 1994). These unique properties combined with good biocompatibility and shape memory characteristics make porous NiTi a promising new candidate material for a bone graft substitute.

Porous niti was first studied by implanting porous NiTi discs (5x5x 1mm, 50% porosity, MPS ~300µm) into the cranial bone of a rabbit for two (n = 2), six (n = 2) and twelve (n = 3) weeks. There was a trend toward increasing bone contact and ingrowth, but because of the small number of cases, no statistical analysis was possible. (Simske & Sachdeva 1995.)

Later, different porosities of porous NiTi were compared by implanting porous NiTi implants (5x5mm, thickness 305-676µm) with three different porosities (mean pore size 353±74µm (n = 7), 218±28µm (n = 6) and 178±31µm (n = 7)) into the cranial bone of a rabbit for six weeks. (Ayers et al. 1999). There were no significant differences in bone ingrowth between the different porosities at this early stage of bone ingrowth in the implant. The authors suggested that a long-term study might be appropriate to evaluate tissue ingrowth with porous NiTi implants. (Ayers et al. 1999.)

In another study porous cubic NiTi implants (5x3x3mm, 40 - 90µm pores) were implanted into the proximal diaphysis of rabbit tibias for three, six, and twelve weeks, with four animals in each group (Rhalmi et al. 1999). There seemed to be some direct bone-implant contact, but also fibrosis and bone marrow inside the implants. The results of histomorphometric measurements were not reported, and no statistical analysis was made. As further perspectives, the authors suggested an experimental model with implantation of porous nickel-titanium in the long-bone metaphysis. (Rhalmi et al. 1999.)

All previously mentioned studies have concluded by highlighting the good biocompatibility properties of porous NiTi. (Simske & Sachdeva 1995, Rhalmi et al. 1999, Ayers et al. 1999.)

Table 1. Elastic modulus of bone, Nitinol, and stainless steel.

<table>
<thead>
<tr>
<th></th>
<th>Elastic modulus (Gpa)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancellous bone</td>
<td>0.01 – 3.0</td>
<td>Hvid &amp; Jensen 1984, Claes et al. 1986, Ducheyne et al. 1977</td>
</tr>
</tbody>
</table>
Biocompatibility studies have shown porous NiTi to be well tolerated by cells and tissues. In a cytotoxicity and genotoxicity evaluation no signs of harmful reactions was detected in cell layers exposed to porous NiTi extracts (Assad et al. 2002a). Nor was any increase in the number of chromosomal aberrations, bacterial revertant colonies, or micronuclei observed in genocompatibility testing. In these studies, porous NiTi was considered cytocompatible and genocompatible by the authors. (Assad et al. 2002a) In an in vivo biocompatibility evaluation by Assad et al. (Assad et al. 2002b), three different methods were used: the classical skin sensitization assay (Buehler patch test) in guinea pigs, the rabbit intracutaneous test, and the systemic injection test in mice. No skin reactions or toxic symptoms were observed, and porous titanium-nickel was considered to be a non-sensitizing, non-irritant, and non-toxic biomaterial for medical applications. (Assad et al. 2002b.)

Porous NiTi was first introduced for intervertebral fusion in 1997 (Silberstein 1997). In 2003, Assad et al. reported a porous NiTi device for intervertebral fusion in a sheep model. Porous NiTi implants were compared to traditional intervertebral fusion cages packed with autologous bone. The two devices were implanted at two non-contiguous intervertebral lumbar sites for three, six, and twelve months. The ungrafted porous NiTi implant was considered to have significantly better fusion characteristics in comparison to conventional intervertebral fusion cages. In this study, porous NiTi osteointegration showed a time-dependent trend, increasing from 21.4% to 37.6% (3-12 months). (Assad et al. 2003b.) A surface analysis and nickel release assessment were additionally performed three, six and twelve months after the implantation (Assad et al. 2003a). Scanning electron microscopy (SEM) combined with backscattered electron analysis (BSE) showed no evidence of surface corrosion either pre- or post-implantation. The total nickel level in blood samples and tissues was assessed using inductively coupled plasma-mass spectrometry (ICP-MS). Following surgery, no significant increase in blood nickel content was observed. The nickel content in tissue adjacent to porous NiTi, as well as in remote organs, was equivalent in both porous NiTi and control groups. (Assad et al. 2003a.)

2.4 Tendon repair materials

2.4.1 General

Early passive or active motion programs after flexor tendon repair have been shown to be extremely beneficial (Gelberman et al. 1990, Stewart 1991, Wang & Gupta 1996). However, the application of early active motion increases the stress on the repair, leading to a significant number of repair ruptures (Becker et al. 1979, Ejeskär 1984, Small et al. 1989). Two factors are involved in producing strong mechanical repair: the suturing technique and the suture material used. A number of multi-strand methods have been described to increase the strength of suture repair (Dinopoulos et al. 2000, Xie et al. 2002, Angeles et al. 2002). However, the increased amount of suture material causes
thickening of the tendon at the repair site, which may restrict the gliding of the tendon in the narrow tendon sheath (Momose et al. 2001). Development of suture materials with a minimal cross-sectional diameter and sufficient strength to withstand the range of forces generated in the early rehabilitation protocol might provide a solution to the problem.

Nowadays, 3-0 and 4-0 braided polyester sutures are the most commonly used suture materials in flexor tendon surgery (Miller 1970, Strickland 1985, Trail et al. 1989). StSt wire has better tensile strength and good knot-holding security (Trail et al. 1989), but it is difficult to use and is weakened by kinking (Nyström & Holmlund 1983). Recently, internal stainless steel anchors (Gordon et al. 1998) and augmentation splints (Silfverskiold & Andersson 1993, Aoki et al. 1994) have been introduced to strengthen flexor tendon repair. Although the recent studies of using absorbable sutures for tendon repair have been encouraging (Bourne et al. 1988, O’Broin et al. 1995), the rates of material absorption and strength reduction are of some concern (Mashadi & Amis 1992).

2.4.2 Biocompatibility

Obviously, any suture material used in tendon repair should elicit a minimal inflammatory response. The biocompatibility of suture materials has been studied by several authors.

In order to access the inflammatory response of tendon tissue to the suture materials, Srugi and Adamson implanted several kinds of suture materials within the flexor tendons of dogs for four weeks. Histological analysis revealed that nylon produced the least inflammatory response of the different materials tested, and StSt, polyester, chromic catgut, and polyglycolic acid produced almost equal results, which were 2- to 2.5-fold compared to the response to nylon. The most marked reaction was produced by silk and silicone-treated silk. (Srugi & Adamson 1972.)

In a study by Postlethwait et al., the human tissue reaction to sutures was studied in 666 specimens obtained from patients one day to 23 years after operation. The tissue reaction was characterized by non-absorbable sutures being encapsulated by connective tissue membrane and histocytes, giant cells, and lymphocytes found near the suture. The most marked tissue reaction was produced by silk and cotton, while a lesser reaction was seen with dacron and the least marked reaction with nylon and StSt wire. (Postlethwait et al. 1975.)

Later, Postlethwait described the tissue reaction to sutures implanted in the abdominal wall muscles of rabbits for five years. Histology revealed a minimal tissue reaction around nylon and dacron. Teflon coating around dacron caused an enhanced tissue reaction. Polypropylene sutures showed fragmentation in 4% of the sutures examined and perisutural formation of bone, cartilage, or both in 2.6%. (Postlethwait 1979.)

Craig et al. studied the tissue reaction to two different absorbable sutures in rats. The tested materials were polyglactin (Vicryl®) and polyglycolic acid (Dexon®). Histologic examination suggested that both suture types elicited a minimal tissue response. (Craig et al. 1975.)
Sandz et al. evaluated several kinds of absorbable sutures in fascial closure in rats. Two hundred and ten rats were randomized into five groups to compare the tissue reactions elicited by polyglyconate (Maxon®), polyglactin (Vicryl®), catgut (Chromic Catgut®), and polydioxanone (PDS®). The authors concluded that polyglyconate and polydioxanone caused less chronic inflammation than polyglactin and catgut. (Sanz et al. 1988.)

In a study by Mashadi et al., flexor tendons of the third toe of 48 chickens were transected and sutured using absorbable polytrimethylene carbonate (Maxon®) sutures. The animals were evaluated on the day of surgery and at five, 15, and 45 days after the operation. In this study, the intense tissue reactivity of polytrimethylene carbonate during its dissolution was found to cause adhesions. (Mashadi & Amis 1992.)

In a study by Wada et al., 64 canine flexor digitorum profundus tendons were repaired using polydioxanone monofilament or control braided polyester. The animals were evaluated seven, 14, 28, and 42 days after surgery. Histologically, an inflammatory response was observed around the polydioxanone monofilament, and this reaction increased from day 14 to day 42. However, in this study, the inflammatory response did not cause large adhesions or extensive tendon callus formation. (Wada et al. 2001a.)

2.4.3 Strength properties

Trail et al. evaluated the breaking loads of the most common tendon suture materials (Table 2). StSt and monofilament polyglyconate appeared to be the most suitable in that they had high breaking loads and good knot-holding security. The only disadvantages were that StSt is difficult to use and monofilament polyglyconate is absorbable. Braided polyester and polypropylene were considered reasonable alternatives. (Trail et al. 1989.)

Wada et al. compared the strength properties of 5-0 polyvinylidene fluoride to 5-0 polypropylene monofilament suture materials and tendon repair made using them as epitendinous sutures combined with a 4-0 braided polyester core suture. The breaking loads of polypropylene sutures (9.4 ± 0.5 N) and polyvinylidene fluoride (9.2 ± 0.2 N) were comparable, but the latter had greater knot pull strength and less delayed extension in creep testing than polypropylene sutures. (Wada et al. 2001b.)

The strength properties of many suture materials, including non-absorbable materials, have been shown to decrease after implantation (Greenwald et al. 1994, Outlaw et al. 1998). Braided polyester seems to retain its strength exceptionally well after implantation (Greenwald et al. 1994). Numerous studies have been made to evaluate the strength properties of absorbable sutures after implantation (Sanz et al. 1988, Bourne et al. 1988, Outlaw et al. 1998, Kangas et al. 2001), but this topic will not be discussed in detail here.
Table 2. Breaking loads of 4-0 suture materials (Trail et al. 1989).

<table>
<thead>
<tr>
<th>Material</th>
<th>n</th>
<th>Load at failure (Newtons), Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>unknotted</td>
</tr>
<tr>
<td>Monofilament polyglyconate</td>
<td>10</td>
<td>23.95 ± 3.78</td>
</tr>
<tr>
<td>(Maxon®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monofilament stainless steel</td>
<td>10</td>
<td>21.12 ± 2.15</td>
</tr>
<tr>
<td>Braided polyester (Ticon®)</td>
<td>10</td>
<td>19.16 ± 1.09</td>
</tr>
<tr>
<td>Braided polyglycolic acid</td>
<td>10</td>
<td>18.97 ± 2.30</td>
</tr>
<tr>
<td>(Dexon®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multifilament stainless steel</td>
<td>4</td>
<td>16.37 ± 3.81</td>
</tr>
<tr>
<td>Monofilament polybutester</td>
<td>10</td>
<td>14.77 ± 1.03</td>
</tr>
<tr>
<td>(Novafil®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypropylene (Surgi®ne®)</td>
<td>10</td>
<td>13.76 ± 2.22</td>
</tr>
<tr>
<td>Multifilament nylon (Dermalon®)</td>
<td>10</td>
<td>12.23 ± 0.87</td>
</tr>
</tbody>
</table>

2.4.4 NiTi as tendon suture material

Despite their good strength, the present metallic suture materials are not widely used in tendon surgery, as they are difficult to use and are weakened by kinking (Nyström & Holmlund 1983). Nitinol suture is a new candidate for tendon suture material, with better manageability compared to stainless steel and good superelastic properties. NiTi can be deformed by about 8-10% without damage to the material. (Duerig et al. 1996, Van Moorleghem et al. 1998.) This is why NiTi sutures are not so easily weakened by the movements, strains, and kinking associated with early mobilization of the tendon. Tendon tissue itself has elasticity of up to 4-5% before permanent structural damage (Butler et al. 1978).

A recent study suggested the NiTi suture material as well as tendon repair made with it to be stronger than 4-0 braided polyester (Ethibond®) and similar repair made with polyester. The breaking load of a shape memory alloy monofilament suture 0.144 mm in diameter (23.9 ± 0.2 N) was greater by 28% than that of 0.156 mm thick 4-0 braided polyester suture material (18.7 ± 0.3 N). The predominant mode of failure was pulling out of the sutures from the tendon (NiTi 85%, Ethibond 45%). The authors also pointed out that, with the metal in its soft and flexible phase, it is easier for the surgeon to do proper suturing and knotting. There are so far no other reports of NiTi used as a tendon suture material. (Moneim et al. 2002.)
3 Aims of the present studies

The aims of the present research were:

1. to test the hypothesis that bone modeling can be controlled with a functional intramedullary nail made of nickel-titanium shape memory alloy (I)

2. to find out if the changes observed in bone after functional intramedullary nailing are caused by the bending force effected by the curved nail or if they are due to the intramedullary nailing itself (II)

3. to study porous NiTi as a weight bearing bone graft substitute and to evaluate the effects of porosity on the osteointegration of NiTi implants in the rat long bone metaphysis (III)

4. to evaluate the biocompatibility of NiTi in tendon tissue and to compare the effect of implantation on the strength properties of NiTi suture material and conventional non-absorbable tendon suture material (IV)
4 Materials and methods

4.1 Implants

Study I: We fabricated a set of intramedullary nails with different thickness and curvature characteristics, to generate a variety of force ranges in order to perform preliminary testing of the effects of functional nailing on rat bone (Table 3). The material used was NiTi (55.7% Ni and 44.3% Ti by weight). After surface finishing, the ultimate intramedullary nails 26 mm in length and 1.0-1.4 mm in thickness, with curvature radii in the range of 25-37 mm, were cut from a longer wire. The chemical compound and the technological itinerary of the alloy resulted in implants that could be deformed at about 0°C (fully martensitic state) and that restored their initial shape at about 30°C (fully austenitic state).

Table 3. The characteristics of the functional intramedullary nails used in study I. Curvature of the nail given as the radius of curvature (mm).

<table>
<thead>
<tr>
<th>Rat nr</th>
<th>Thickness</th>
<th>Curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>1.4</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>1.4</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>1.25</td>
<td>29</td>
</tr>
</tbody>
</table>

Study II: A set of pre-shaped intramedullary nails (length 25 mm, thickness 1.3 mm) with a curvature radius of 25 mm were fabricated as above. The chemical compound and the technological itinerary of the alloy resulted in implants that could be deformed at about 0°C (fully martensitic state), and that restored their initial shape at about 25-30°C (fully austenitic state). Another set of straight intramedullary NiTi nails (length 25 mm, thickness 1.4 mm) were fabricated for control purposes.

Study III: Cylindrical implants (diameter 3 mm and length 5-7 mm) were cut from larger pieces of NiTi samples of three different porosities (Fig. 2). The porosities (average void volume) and the mean pore size (MPS) were 66.1% and 259 ± 30 µm for group 1 (n = 14), 59.2% and 272 ± 17 µm for group 2 (n = 4), and 46.6% and 505 ± 136 µm for
group 3 (n = 15), respectively. The cross-sectional porosities were 64.5 ± 10.2%, 46.8 ± 8.9%, and 65.7 ± 9.2%, respectively.

Fig. 2. A photograph of porous NiTi implants, a sample of human trabecular bone from the femoral neck (upper right), and a cross-section of the site of implantation in distal rat femur (bottom). The porosities (average void volume) and the mean pore size (MPS) of the implants from left to right are 66.1% and 259 ± 30 µm (group 1), 59.2% and 272 ± 17 µm (group 2), and 46.6% and 505 ± 136 µm (group 3), respectively. The cross-section is from the contralateral femur from the same experiment. All samples are in the same scale, the dimensions of the NiTi implants being 3 x 7 mm.

Study IV: Nitinol monofilament suture material (56.2% Ni and 43.8% Ti by weight) (Orfix®, Raah, Finland) with a diameter of 250µm (according to the manufacturer) and a length of 10 cm was used for biocompatibility testing, and another Nitinol suture with a diameter of 150µm (according to the manufacturer) and a length of 10 cm was used for strength evaluation. 4-0 braided polyester suture (Ethibond® excel, Johnson&Johnson, New Jersey, USA) was used as a control material (156µm diameter) (Moneim et al. 2002).

Before implantation all NiTi implants were degreased with 70% ethanol, washed in an ultrasonic vibrobath, and autoclaved (30 min, 121 °C).
4.2 Animals

The animal tests were performed after approval by the ethical committee of the University of Oulu. All aspects of animal care complied with the Animal Welfare Act and the recommendations of the NIH-PHS Guide for the Care and Use of Laboratory animals.

The animals utilized as an animal model in the studies II-III were male Sprague Dawley/MOL rats from the Laboratory Animal Center, University of Oulu (Oulu, Finland) (Table 4). In study I, eight rats were used. Their ages ranged between 12 and 13 weeks and weights within 420 - 460 g. In study II, twenty-four rats were randomized into 2 groups, each consisting of 12 rats. Their ages ranged between 11 and 12 weeks. The mean weight (±SD) was 384 ± 28 g in the study group and 392 ± 24 g in the control group. In study III, thirty-five rats were utilized as an animal model and randomized into 3 groups (consisting of 15, 5, and 15 rats). Their ages ranged between 27 and 28 weeks. Their mean weight was 492 g ± 31 g. The rats were housed in groups of 4 - 6 in Macrolon IV polycarbonate cages in a thermostatically controlled room at 20 ± 1 °C with a relative humidity of 50 ± 10%. The room was artificially illuminated with 12 h of light and 12 h of darkness. Aspen chips (Fintapway, Finland) were used as bedding. Pelleted rat feed (SDS R3 (E), Special Diet Services Ltd., Great Britain) and tap water were available ad libitum.

Table 4. Summary of the animals, groups, implants and implantation sites in the present studies. MPS = Mean pore size.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal</th>
<th>Group</th>
<th>N</th>
<th>Implant</th>
<th>Implantation site</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Rat</td>
<td>8</td>
<td>Functional intramedullary NiTi nail</td>
<td>Femoral medullary canal</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Rat</td>
<td>1. 12</td>
<td>Functional intramedullary NiTi nail</td>
<td>Femoral medullary canal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 12</td>
<td>Straight NiTi nail</td>
<td>Femoral medullary canal</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Rat</td>
<td>1. 15</td>
<td>Porous NiTi 66.1% porosity, MPS 259 µm</td>
<td>Distal femoral metaphysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 5</td>
<td>Porous NiTi 59.2% porosity, MPS 272 µm</td>
<td>Distal femoral metaphysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. 15</td>
<td>Porous NiTi 46.6% porosity, MPS 505 µm</td>
<td>Distal femoral metaphysis</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Rabbit</td>
<td>15</td>
<td>NiTi suture material, braided polyester</td>
<td>tendon, subcutis</td>
<td></td>
</tr>
</tbody>
</table>

In study IV, fifteen New Zealand White rabbits were utilized as an animal model (Charles River Laboratories, Inc., MA, USA). They were 12 weeks of age and within a weight range of 2.6-3.3 kg. The animals were housed in rabbit cages in a thermostatically controlled room at 20 ± 1 °C with a relative humidity of 45 ± 10%. The room was artificially illuminated with 12 h of light and 12 h of darkness. Pelleted rabbit feed (Lactamin K1, Lactamin AB, Stockholm, Sweden) and tap water were available ad libitum.

4.3 Surgical procedures

All rats were anesthetized with a blend of Fentanyl/citrate (80 µg/kg) - Fluanisone (2.5 mg/kg) (Hypnorm®, Janssen Pharmaceutica, Inc., Beerse, Belgium) and Midazolam
(1.25 mg/kg) (Dormicum®, Roche, Basel, Switzerland) injected intraperitoneally. After
the induction of anesthesia, they received cefuroximinenatrium 5 mg/kg i.m. (Zinacef®,
Glaxo Wellcome Ltd., Uxbridge, Great Britain). Before surgery hair was shaved around
the implantation site, and the skin was scrubbed with chlorhexidin.

In studies I and II, intramedullary nails were implanted into the medullary canal of the
right femur via medial parapatellar arthrotomy and lateral dislocation of the patella. The
medullary canal was approached distally via a hole drilled through the intercondylar
notch and reamed carefully using a 18-gauge needle with rotating motion. All nails and
all the instruments used during the implantation were submerged in sterile iced saline to
reach and maintain the martensite form of NiTi throughout the implantation process. In
their martensite form, the bent nails were straightened and inserted inside a 14-gauge
needle with the sharp edge of the needle cut off. The needle was approximated at the site
of the hole drilled into the medullary canal, and an additional 18-gauge needle with the
bevel end of the needle cut off was used to push the nail into the medullary canal. This
method offers a quick way of inserting the nail before it warms up and restores its
original shape. The nails were allowed to settle in an incidental position. The patella was
relocated and the extensor mechanism was reconstructed. The rats were killed at 12
weeks after the implantation. All femurs with implants were dissected, as were also the
contralateral femurs, which served as control. The lengths of both the operated and the
control bones were measured using a digital vernier caliper. In study I, the maximum and
minimum thickness of bone was also measured at 45% of the bone length from the distal
end of the bone (D_MAX and D_MIN, respectively). The mean of three measurements was
used in the calculations.

In study III, a longitudinal incision was made medially over the distal part of the
femur, and the muscles were slightly opened. A cavity 3 mm in diameter and 5-7 mm in
depth was drilled from the medial to the lateral direction in the distal condylar part
(metaphysis) of the right femur using a diamond drill irrigated with sterile saline (0.9%
wt/vol). The implants, sized to press-fit in such a cavity, were pushed into the cavity. The
rats were killed at 30 weeks after the implantation. All femurs with implants were
dissected, as were also the contralateral femurs.

After implantation all wounds were closed in layers using resorbable sutures (Vicryl®,
Ethicon, Inc., Somerville, New Jersey). Buprenorphin 0.3 mg/kg s.c. (Temgesic® 0.3
mg/ml, Reckitt & Colman Pharmaceuticals, Inc, Richmond, England) was used as a
postoperative analgesic. Carbon dioxide was used to kill the rats after the desired follow-
up time.

In study IV, all rabbits were anesthetized with ketamine (20mg/kg) (Ketalar®, Pfizer,
NY, USA, Parke-Davis, Sweden) and medetomidine (0.3mg/kg) (Domitor®, Orion-
Pharma, Espoo, Finland) injected intramuscularly. After the induction of anesthesia, they
received cefuroximinenatrium 20mg/kg i.m. (Zinacef®, Glaxo Wellcome Ltd., Uxbridge,
Great Britain). The back and medial aspects of both legs were shaved, and the skin was
scrubbed with chlorhexidin. 250µm NiTi sutures were implanted in the right medial
gastrocnemius tendon via a small medial incision over the tendon. A thin injection needle
was inserted through the tendon, the NiTi suture was pushed through the needle and the
needle was removed. The excess suture protruding from the tendon was cut off. For
strength measurements, another NiTi suture 150µm in diameter and 10cm in length was
implanted into the subcutaneous tissue of the right side of the rabbit’s back. The control
sutures for both biocompatibility evaluation and strength measurements were similarly implanted on the contralateral side. The wounds were closed using non-absorbable polyamid sutures (Ethilon®, Johnson & Johnson, New Jersey, USA). Buprenorphin 0.03mg/kg s.c. (Temgesic® 0.3 mg/ml, Reckitt & Colman Pharmaceuticals, Inc, Richmond, England) was used as a postoperative analgesic. The rabbits were killed using pentobarbital 180mg i.v. (Mebunat®, Orion-Pharma, Espoo, Finland) at two, six and twelve weeks after the implantation, with five rabbits in each group. All gastrocnemius tendons with implants were dissected. The sutures implanted in subcutaneous tissue were removed for strength measurements.

4.4 Radiography

In studies I and II, standard plain radiographs of the dissected bones were taken in anteroposterior (AP) and lateral projections (Fig. 3). The radiographs were digitized on a light table with a ccd camera (Dage MTI 72E, Dage-MTI, Inc., Michigan City, IN, USA) using a Micro Nikkor 55 mm objective (Nikon, Tokyo, Japan). The angle between the distal articular surface and the long axis of the femur (a line drawn from the intercondylar space to the end of trochanter major) was measured from AP radiographs using a digital image analysis system MCID M4 with the software version 3.0, rev. 1.1 (Imaging Research, Inc., Brock University, St. Catharines, Canada). The mean of three measurements was used in the calculations. The main bending direction of the nail was visually observed from the radiographs, and the main direction in the AP radiographs was considered to be the positive angle. In study III, standard plain radiographs of the femurs were taken after killing the rats to verify implant position and to exclude fractures and other possible complications (Fig. 4).

Fig. 3. Anteroposterior radiographs of all operated and control femurs of study I at 12 weeks after the implantation of the functional intramedullary NiTi nail.
Fig. 4. A radiograph 30 weeks after the implantation of a porous NiTi implant showing the location of the implant in the distal metaphysis of the rat femur.

4.5 Peripheral quantitative computed tomography (pQCT)

Study I: After radiography, the nails were removed for three-dimensional densitometry by excavating the necessary amount of bone around the distal end of the femur to expose the tip of the nail and to allow the nail to be grasped with forceps and pulled out. The bones with implants and all the instruments were submerged in iced saline to reach the martensite state of NiTi before removal of the nail. The diaphysis of each femur was scanned with a peripheral quantitative computed tomography (pQCT) system, Stratec XCT 960A, with the software version 5.20 (Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany). The bone was inserted with the anterior surface upwards into a plastic tube adapter for measurement. Fifteen consecutive cross-sections with a slice distance of 2 mm and voxel size of 0.148 x 0.148 x 1.25 mm$^3$ were measured, adjusting the first scan line at the distal end of the femoral caput. Cross-sectional cortical area (CSA), cortical thickness (CtTh), and cortical bone mineral density (BMD) were measured by using an attenuation threshold of 0.93 cm$^{-1}$ to define cortical bone. Another set of 40 consecutive cross-sections with a slice distance of 1 mm from one femur were scanned to obtain necessary data of the bone geometry for finite element analysis, which is more closely discussed in the next chapter. The maximum axial scanning length of the pQCT device is only 30 mm. Therefore, the sample adapter was here axially moved after 20 cross-sections, to enable all the 40 scans.

Study II: The nails were removed and densitometry performed as described above, with the exception that, in all cases, the whole femur was scanned starting from its distal end. Forty consecutive cross-sections with a slice distance of 1 mm were measured, adjusting the first scan line at the distal end of the femur. The sample adapter was axially
moved after 20 cross-sections, to enable all the 40 scans, as above. Total cross-sectional area (Tot A), CSA, CtTh, and cortical BMD were measured.

4.6 Finite element analysis (FEA)

Finite element analysis (FEA) is a computer based analysis method for predicting the theoretical response of structures and materials to forces, before any physical setting occurs. The process starts with the creation of a geometric model. Then, the model is subdivided into small pieces (elements) of simple shapes connected at specific node points. In this manner, the stress-strain relationships are more easily approximated. Finally, the material behavior and the boundary conditions are applied to each element. As a result a finite element model (FEM) is created. It allows the designer to examine any number of “what if” scenarios in optimising the design. (Perry 2002)

A Finite element analysis was performed to examine the initial response of the bone-nail combination after the nail has reached body temperature and tries to restore its initial curvature, which, however, is restrained by the bone surrounding it (Study I). Linearly elastic behavior was assumed for this analysis.

The cross-sectional 128 by 128 pQCT images of the bone were saved as 8-bit gray levels, the gray level being calibrated for density against the calibration phantom delivered by the device manufacturer. The images were entered into the pQCT data analysis software Bonalyse, ver. 1.3 (BonAlyse Oy, Jyväskylä, Finland) and exported as a set of 40 tagged image file format (TIFF) files. A 3D model of the bone was obtained using the I-DEAS software (Structural Dynamics Research Corporation, Milford, OH, USA). The surface image of the femur without bone ends is shown in Fig.5.

Fig. 5. A 3D image of the femur used in the finite element analysis (FEA) (left). The 3D image was retrieved by a set of quantitative densitometry scans with a slice distance of 1 mm (right). The image was used as a geometric model of the bone for the FEA.

The material properties of the NiTi nail were determined by performing tensile strength tests and three-point bending tests of the nails at three different temperatures (0°, 20°, and
A simplified 2D finite element model of the bone-nail combination was developed using the ANSYS software, ver. 5.5.1 (ANSYS, Inc., Southpointe in Canonsbur, PA, USA). A linear, asymmetric 2D beam element model (ANSYS BEAM 54) was applied to the bone, using 10 elements 3 mm in length. The bone ends were not considered in the beam model. Information of the cross-sectional area, the moment of inertia, and extreme fiber distances from the neutral axis were obtained from the model constructed with the I-DEAS software. A linear, symmetric beam element model (ANSYS BEAM 3) was used for the NiTi nail by dividing the nail length into 10 elements. Nail length was set to 26 mm, nail thickness to 1.4 mm, and the radius of curvature to 37 mm. The three contact areas between the nail and the bone were modeled by using artificial rod elements between the bone and the nail in the middle and at both ends. The clearance between the bone and the nail was described by using a suitable combination of temperature difference and the coefficient of thermal expansion for these artificial rod elements.

A 3D finite element analysis of the nail-bone combination was also developed by using ABAQUS software for calculations, and I-DEAS software for creating the graphics and demonstrating the results.

4.7 Histological analysis

Study I: The bones were fixed in 10% buffered formalin. After the fixation, the bones were decalcified in 5% formic acid and embedded in paraffin. Histological 6µm sections were stained with hematoxylin-eosin. The images were digitized with a Sony 930 DXC color camera (Sony, Tokyo, Japan) coupled to the MCID M4 image analysis system by using a Nikon Optiphot II microscope, a 1x plan objective, and a polarization filter set (Nikon, Tokyo, Japan).

Study III: The bones with the implants were fixed in 10% buffered formalin. After the fixation, they were placed in a hard-resin embedding process without removing the implant. The samples were dehydrated in a graded alcohol series and embedded in methylmethacrylate (Technovit®, Kulzer GmbH, Germany) using the standard method. (Donath 1985) The specimens were cut longitudinally with a low-speed diamond saw at the middle of the implant. Thin ground sections (20 µm) were made using a sandwich method and the Micro-Grinding system (EXAKT Appartebau, Germany). The sections were stained with Masson-Goldner-Trichrome stain for histological evaluation and histomorphometry.

Study IV: All gastrocnemius tendons with implants were fixed in 10% buffered formalin. After the fixation, they were placed in a hard-resin embedding process without removing the implant. The samples were dehydrated in a graded alcohol series and embedded in methylmethacrylate (Technovit®, Kulzer GmbH, Germany) using the standard method (Donath 1985). The specimens were cut transversely with a diamond saw at the point where the suture was inside the tendon. Ground sections (30 µm) were made using a sandwich method and the Micro-Grinding system (EXAKT Appartebau,
Germany). The sections were stained with haematoxylin-eosin stain for histological evaluation and histomorphometry.

4.8 Histomorphometry

Study III: The hard tissue sections were evaluated by a digital image analysis system MCID-M4 (Imaging Research inc, Canada) using a Nikon Optiphot II microscope and a 2.5x objective. Images were obtained with a Sony DXC930P 3CCD color camera. From the tissue slides, online interactive measurements were performed. The total implant area and the perimeter were automatically determined after interactive thresholding and segmentation of the image for the presence of metal structures. Thereafter, the bone contact perimeter was determined manually by tracing the bone-lined implant surfaces with the cursor of the computer. The proportional contact perimeter was calculated from the measurements. Based on the appearance and selective staining of fibrotic tissue, the area of fibrosis within the implant was determined interactively. The area of fibrotic tissue was calculated as the proportion of fibrous tissue out of the void space inside the implant.

Study IV: The hard tissue sections were digitized using a Nikon Coolpix E950 digital camera (Nikon, Tokyo, Japan) and a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) and a 4x objective. The thickness of the encapsulating membrane around the implants was measured using computer-assisted histomorphometry. The distances from the implant surface to the outer border of the connective tissue were measured as two-point distances at eight points around the implant, using the image analysis software UTHSCSA 2.0 ImageTool (University of Texas, San Antonio, USA). In order to ensure that the measurements were made randomly around the implant, a clock-like transparent template grid (with beams at 45-degree intervals) was centered over the tendon cross-section image on the computer screen, and the thickness of the encapsulating membrane was measured at the points where the beams of the template randomly crossed the membrane. Spatial calibration was done by digitizing a 2000µm test object (Ernst Leitz, Wetzlar, Germany). Any obvious adverse effects visible inside the tendon tissue were registered.

4.9 Strength measurements

In study IV, the mechanical testing of the sutures implanted in subcutaneous tissue for two, six, and twelve weeks was carried out on an Instron 5544 computer-controlled material-testing machine (Instron Ltd, High Wycombe, UK). The test suture was stretched tight between two clamps at both ends (Fig. 6).
Fig. 6. Strength test device used to determine the strength properties of Nitinol sutures and 4-0 braided polyester sutures as control material. The suture was stretched tight at both ends. (F = force)

The cross head speed on the material-testing machine was set at 5 mm/min, and the test device pulled the sutures until breakage. The breaking load was measured in terms of the breaking force in Newtons. A stress-strain curve for the sutures was drawn. Three measurements were performed for each suture at different points. All measurements were carried out in air at room temperature.

4.10 Statistical analysis

Statistical analysis was done by using the SPSS software, ver. 10.0 (SPSS Inc., Chicago, IL, USA). The values \( p < 0.05 \) were considered significant. The summary values are expressed as mean ± standard deviation (SD).

Study I: The differences between the nailed femur and the control femur of each rat were calculated for all parameters. The mean differences in femoral length, \( D_{\text{MAX}} \) and \( D_{\text{MIN}} \), and articular surface angle were calculated. The mean differences in BMD, CSA, and CtTh were calculated both slice by slice and for the average difference of all slices. Slice number 15 was excluded from statistical analysis because the defect at the distal end of the bone due to nail removal reached this area in some bones. The differences between the operated and control femurs were tested versus zero by one-sample t-test, to evaluate the statistical significance of all changes.

Study II: The differences between the nailed femur and the control femur of each rat were calculated for all parameters. The mean differences in femoral length and articular surface angle were calculated. The mean differences in Tot A, CSA, CtTh, and BMD were calculated both slice by slice and for the average difference of all slices from the
diaphysis of the femur. The slices number 1-6 from the distal end were excluded from statistical analysis because of the defect due to nail removal. The caput of the femur was also excluded, by taking the slice next to the caput as the last slice included. To compensate for the differences in bone size between the nailed and the control bones, 25 anatomically equal slices, starting from the distal and proximal ends of the diaphysis, were defined for statistical analysis. The differences between the operated and contralateral femurs were tested versus zero by one-sample t-test, to evaluate the statistical significance of all changes. These differences were also used in the comparison of the two groups. The independent samples t-test was used here to evaluate statistical significance.

Study III: The percentage of bone-implant contact out of the total implant perimeter was calculated. Analysis of variance (ANOVA) was used to evaluate the statistical significance of the differences in the means of this parameter between the three porosity groups. Whenever a significant difference was seen, the t-test was utilized. The differences in pore size and the porosity of the cross-sectional areas of the implants between the three groups were similarly determined. The percentage area of fibrous tissue out of the total void space inside the porous implant was calculated. Because most histological slides showed no signs of fibrosis, the analysis was divided into two parts (Lachenbruch 2002). First, a new parameter was created to express the presence or absence of fibrosis inside a specific implant. Fisher’s exact test was used to determine the differences in this parameter between the three porosity groups. Secondly, the cases with fibrosis were analyzed as the bone-implant contact above.

Study IV: The mean thickness of the encapsulating membrane around each individual suture was calculated from the eight measurements performed for each suture. The mean breaking load of each individual suture was calculated from the three measurements performed for each suture. The mean of these means were calculated for both materials at each time point. The significance of the differences between the materials at each time point was determined by using the Mann-Whitney test. The significance of the differences between the different time points of each material was determined by using the Kruskal-Wallis test followed by the Mann-Whitney test.
5 Results

5.1 General observations

The only complications in these series were the loss of the results of two rats in study III because one rat died and the specimen of the other rat broke. There was no other loss of material in these studies. No adverse effects, such as luxations, were seen from the arthrotomies. The implants did not cause any adverse effects, such as skin irritation or infection. The test animals recovered well from the operations.

Measurements performed using a digital vernier caliper showed significant retardation of longitudinal growth in all the femurs operated using functional NiTi nails compared to the contralateral normal femurs in study I ($p < 0.001$) (Table 5). In study II, significant retardation of longitudinal growth was observed after the insertion of both curved and straight nails compared to the contralateral normal femurs (Table 6). The operated femur ended up to be 4.5 % shorter on an average in the curved nail group ($p < 0.001$) and 5.1 % shorter in the straight nail group at the end of the study compared to the unoperated side ($p < 0.001$). There were no significant differences between the two groups. Significant thickening of the rat femurs operated using functional NiTi nails was observed in study I ($p = 0.001$ and $p = 0.004$ for $D_{\text{MAX}}$ and $D_{\text{MIN}}$, respectively). These changes appeared to be more obvious when the thickest (1.4 mm) nail was used.

The weight gain of the rats operated using functional intramedullary NiTi nails in study II was slightly inferior compared to the rats operated using the straight NiTi nail. The rats in the functional nail group weighed less by an average of 21g at the end of the study ($p = 0.021$) (Table 6). In study III, no significant differences in weight gain were seen between the three groups.
5.2 Radiography

Anteroposterior radiographs showed significant bowing of the rat femurs operated using the functional intramedullary NiTi nail, towards the direction of the bending force of the nail (Fig. 3). The bend averaged 6.7 degrees in study I \((p = 0.003)\) (Table 5), and 3.3 degrees in study II \((p = 0.026)\) (Table 6) compared to the contralateral normal femur. In some cases, there was also some posterior bending of the nail seen in lateral radiographs. The bending of bones into this direction was not measured, however, because in many cases there was no bending of the nail in this plane. In study II the straight nails showed 1.1-degree bending of the bone in the direction opposite to that seen in the study group compared to the contralateral normal femur, but the result was not significant (Table 6).

In study III, the radiographs showed that the implants remained in their original location inside the bone, and there was no dislocation or loosening of the implants after press-fit implantation. There were no fractures of the operated bone, although the implant occupied a major proportion of the weight-bearing bone structures in the distal femur (Fig. 4).

Table 5. Results of measurements performed using a digital vernier caliper and bowing of the bone in study I. Femoral length and mid-shaft thickness \((D_{MIN} \text{ and } D_{MAX})\) at the end of the study and the change in bone curvature after treatment measured as the distal articular surface angle. Curvature of the nail given as the radius of curvature (mm).

<table>
<thead>
<tr>
<th>Rat nr</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur length (mm)</td>
<td>control</td>
<td>40.56</td>
<td>41.43</td>
<td>41.69</td>
<td>40.92</td>
<td>40.20</td>
<td>40.50</td>
<td>41.01</td>
<td>41.45</td>
</tr>
<tr>
<td></td>
<td>operated</td>
<td>39.45</td>
<td>40.18</td>
<td>38.92</td>
<td>38.97</td>
<td>38.10</td>
<td>37.63</td>
<td>38.50</td>
<td>38.70</td>
</tr>
<tr>
<td></td>
<td>difference</td>
<td>-1.11</td>
<td>-1.25</td>
<td>-2.77</td>
<td>-1.95</td>
<td>-2.10</td>
<td>-2.87</td>
<td>-2.5</td>
<td>-2.75</td>
</tr>
<tr>
<td>DMIN (mm)</td>
<td>control</td>
<td>3.40</td>
<td>3.69</td>
<td>3.85</td>
<td>3.53</td>
<td>3.78</td>
<td>3.60</td>
<td>3.82</td>
<td>3.29</td>
</tr>
<tr>
<td></td>
<td>operated</td>
<td>3.52</td>
<td>3.84</td>
<td>4.13</td>
<td>3.72</td>
<td>3.78</td>
<td>4.00</td>
<td>4.14</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>difference</td>
<td>0.12</td>
<td>0.15</td>
<td>0.28</td>
<td>0.19</td>
<td>0.00</td>
<td>0.40</td>
<td>0.32</td>
<td>0.11</td>
</tr>
<tr>
<td>DMAX (mm)</td>
<td>control</td>
<td>4.40</td>
<td>4.72</td>
<td>4.77</td>
<td>4.84</td>
<td>4.93</td>
<td>4.54</td>
<td>4.86</td>
<td>4.31</td>
</tr>
<tr>
<td></td>
<td>operated</td>
<td>4.58</td>
<td>4.95</td>
<td>4.91</td>
<td>4.99</td>
<td>4.94</td>
<td>4.78</td>
<td>5.23</td>
<td>4.56</td>
</tr>
<tr>
<td></td>
<td>difference</td>
<td>0.18</td>
<td>0.23</td>
<td>0.14</td>
<td>0.15</td>
<td>0.01</td>
<td>0.24</td>
<td>0.37</td>
<td>0.25</td>
</tr>
<tr>
<td>Curvature change</td>
<td>difference</td>
<td>6.1</td>
<td>2.3</td>
<td>15.3</td>
<td>3.7</td>
<td>3.7</td>
<td>6.2</td>
<td>5.5</td>
<td>10.5</td>
</tr>
</tbody>
</table>
Table 6. The effect of 12-week treatment with a curved or straight intramedullary NiTi nail on rat femur (study II). The differences between the operated and contralateral femurs are given for femoral length, articular surface angle, and densitometric parameters. The increase of total cross-sectional area (Tot A), cortical cross-sectional area (CSA), cortical thickness (CtTh), and cortical BMD as averages of the whole set of 25 pQCT scans along the femoral diaphysis. The operated femur compared to the contralateral femur:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Curved nail</th>
<th>p</th>
<th>Straight nail</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>96 ± 21</td>
<td></td>
<td>117 ± 22</td>
<td></td>
</tr>
<tr>
<td>Femoral length</td>
<td>-4.5%</td>
<td>&lt; 0.001</td>
<td>-5.1%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Articular surface angle (degrees)</td>
<td>-3.3°</td>
<td>0.026</td>
<td>1.1°</td>
<td>N.S.</td>
</tr>
<tr>
<td>Densitometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tot A</td>
<td>5.4%</td>
<td>(p = 0.001)</td>
<td>3.5%</td>
<td>(p = 0.326)</td>
</tr>
<tr>
<td>CSA</td>
<td>8.5%</td>
<td>(p &lt; 0.001)</td>
<td>5.6%</td>
<td>(p = 0.067)</td>
</tr>
<tr>
<td>CtTh</td>
<td>6.7%</td>
<td>(p &lt; 0.001)</td>
<td>4.6%</td>
<td>(p = 0.062)</td>
</tr>
<tr>
<td>BMD</td>
<td>0.4%</td>
<td>(p = 0.245)</td>
<td>1.1%</td>
<td>(p = 0.016)</td>
</tr>
</tbody>
</table>

5.3 pQCT densitometry

Quantitative densitometry showed a statistically significant overall increase in CSA (p = 0.001 in study I, p < 0.001 in study II) and in CtTh (p = 0.002 in study I, p < 0.001 in study II) in the femurs operated using the functional NiTi nail compared to the contralateral femurs, when calculated from the whole set of slices (Table 6). Tot A was similarly increased in study II (p = 0.001) (Table 6). These changes were most obvious in the mid-diaphyseal area (Fig. 7). The femurs operated using a straight nail (study II) also showed some increase in Tot A, CSA, and CtTh, but these changes were smaller and only significant in the midmost diaphyseal slices, with no significant change in the means of the whole set of slices.
Fig. 7. Percentage differences in mean cortical cross-sectional area (CSA), cortical thickness (CtTh), and BMD as measured from 15 pQCT scans along the femoral diaphysis (Study I). The femur operated with the functional intramedullary NiTi nail compared to the contralateral femur (N = 8; + \( p < 0.05 \), ++ \( p < 0.01 \), +++ \( p < 0.001 \)). The big difference in slice 15 is explained by the defect at the distal end of the bone sustained upon nail removal.

Cortical bone mineral density (BMD) increased in the most distal slices after intramedullary nailing with either functional or straight NiTi nails. In study I, BMD seemed to decrease at the proximal end of the bone, while study II revealed only minor changes of BMD in the most proximal slices. No significant overall change of BMD was observed in study I when the whole set of slices were compared. In study II, only minor changes in overall BMD were observed in both nail groups (Table 6).

The changes in CSA, CtTh, and BMD measured slice by slice along the diaphyseal axis are shown in Fig. 7 and Fig. 8.
Fig. 8. Percentage differences in mean total cross-sectional area (Tot A), mean cortical cross-sectional area (CSA), cortical thickness (CtTh), and BMD as measured from 25 pQCT scans along the femoral diaphysis (Study II). A. A femur operated with a functional intramedullary NiTi nail compared to the contralateral non-operated femur (N = 12). B. A femur operated with a straight intramedullary NiTi nail compared to the contralateral femur (N = 12). + p < 0.05, ++ p < 0.01, +++ p < 0.001.

5.4 FEA

The prediction of distribution of stress at the lower surface of the nail and the bone yielded by linear finite element analysis (FEA) is shown in figure 9. The maximum values of stress were 635 MPa and 15 MPa, respectively. FE analysis predicted 1.09 degrees of theoretical bowing of the diaphysis immediately after implantation. A three-dimensional presentation of the distribution of stresses in the bone and in the nail after the implantation of a functional intramedullary NiTi nail is shown in the figures 10 and 11.
Fig. 9. Theoretical distribution of stress (A) at the lower surface of the NiTi nail and (B) at the bone after the implantation of a functional intramedullary NiTi nail, as predicted by the simplified 2D finite element analysis. A linear, symmetric beam element model for the nail and a linear, asymmetric beam element model for the bone. Wire length 26 mm, thickness 1.4 mm, and radius of curvature 37 mm.
Fig. 10. Theoretical distribution of stresses in bone after the implantation of a functional intramedullary NiTi nail, as predicted by the 3D finite element analysis. The maximal stresses are directed to the mid-diaphysis of the bone.

Fig. 11. Theoretical distribution of stresses in the functional nail after implantation into the femoral canal of a rat femur, as predicted by the 3D finite element analysis. The maximal stresses are directed to the mid-portion of the nail.
5.5 Histology

Study I: Polarized light microscopy revealed birefringence to be reduced in the nailed bones. Cortical bone was thickened on the concave side of the nail, where the nail was not in direct contact with the cortex. The thickened cortex consisted mainly of woven bone with irregular birefringence and only a narrow rim of more lamellar periosteal bone (Fig. 12D). The convex or compressed side also had less birefringence, but the lamellar structure was better preserved throughout the diaphyseal cortex (Fig. 12C).

Study III: Histological evaluation showed that the implants were well tolerated, and hard-tissue preparations revealed, at the level of light microscopy, that bone grew into direct contact with the implant surface and into the void spaces inside the implants with all porosities. The new bone was structurally normal, and it was connected with the surrounding bone. Normal-looking bone marrow occupied part of the implant pores and was continuously integrated into the surrounding marrow. In some cases, ectopic fibrous connective tissue within the implant was seen, but most of the samples had no fibrosis at all. Descriptive low-power micrographs from various groups are shown in figure 13.

**Fig. 12.** The untreated control mid-diaphyses showed high birefringence in polarized light microscopy (A, B). The mid-diaphyses in functionally nailed bones had decreased birefringence, indicating reorganization of collagenous fibers (C). Woven bone formation was seen in the thickened cortex (D). bm = bone marrow; * = space of the intramedullary nail. Scale bar 250µm.
Fig. 13. Histological slides of three different porosities of porous NiTi implants implanted in the distal metaphyseal area of the rat femur for 30 weeks. In group one, an implant with 66.1% porosity and a mean pore size (MPS) of 259 ± 30 µm showed good bone-implant contact (A), but suffered from fibrosis in many cases. In group two, an implant with 59.2% porosity and 272 ± 17 µm MPS showed worse bone-implant contact with fibrosis (B). In group three, an implant with 46.6% porosity and 505 ± 136 µm MPS showed good bone-implant contact and minimal fibrosis in some samples but not in this one (C). (b = bone, i = implant, f = fibrosis, bm = bone marrow)
Study IV: Light microscopy showed the samples of both Nitinol and braided polyester to be surrounded by thin collagen membranes (Fig. 14). Polarized light microscopy showed the encapsulating membrane around NiTi to have slightly higher birefringence, than the membrane around braided polyester (Fig. 15). Both materials seemed to initiate a similar, mild inflammatory response of a normal range. However, reliable counting of cells around the materials was not possible in the 30 µm thick ground sections. No tissue discoloration was seen near any of the Nitinol samples.

Fig. 14. A cross-sectional light microscopy image of a 4-0 braided polyester suture material (A) and a Nitinol suture (B) implanted in the gastrocnemius tendon of a rabbit for 12 weeks. There were no significant differences between the two suture materials in the thickness of the encapsulating membrane. Scale bar 100µm.

Fig. 15. A cross-sectional polarized light microscopy image of the material-tendon interface of a 4-0 braided polyester suture material (A) and a Nitinol suture (B) implanted in the gastrocnemius tendon of a rabbit for 6 weeks. There were no significant differences between the two suture materials in the thickness of the encapsulating membrane, but the membrane around NiTi showed higher birefringence than the membrane around braided polyester. The arrows show the encapsulating membranes around the materials. pe = braided polyester, n = Nitinol. Scale bar 50µm.
5.6 Histomorphometry

Study III: The best mean bone-implant contact was 51% in group 1 and 39% in group 3, but there were no significant differences between these two porosity groups ($p = 0.63$) (Table 7). In group 2, the mean bone-implant contact was 29%, which was significantly worse than that seen in group 1 ($p = 0.038$). Fibrosis was present significantly more often in group 1 than in group 3 ($p = 0.021$) (Table 7).

Table 7. Characteristics of porous NiTi implants and occurrence of mean bone-implant contact and fibrosis after implantation in the distal metaphysis of the rat femur for 30 weeks. Values are given as a mean ± standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 14)</th>
<th>Group 2 (n = 4)</th>
<th>Group 3 (n = 15)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity (average void volume) (%)</td>
<td>66.1</td>
<td>59.2</td>
<td>46.6</td>
<td></td>
</tr>
<tr>
<td>Cross-sectional area porosity (%)</td>
<td>64.5 ± 10</td>
<td>46.8 ± 8.9</td>
<td>65.7 ± 9.2</td>
<td>&lt; 0.01 (between 1 and 2)</td>
</tr>
<tr>
<td>Mean pore size (µm)</td>
<td>259 ± 30</td>
<td>272 ± 17</td>
<td>505 ± 136</td>
<td>&lt; 0.01 (between 1 or 2 and 3)</td>
</tr>
<tr>
<td>Bone-implant contact (%)</td>
<td>51 ± 18</td>
<td>29 ± 13</td>
<td>39 ± 15</td>
<td>&lt;0.05 (between 1 and 2)</td>
</tr>
<tr>
<td>Fibrosis present (yes / all rats)</td>
<td>8 / 14</td>
<td>3 / 4</td>
<td>2 / 15</td>
<td>&lt;0.05 (between 1 and 3)</td>
</tr>
</tbody>
</table>

Study IV: There were no significant differences in the mean thickness of the encapsulating membrane around the sutures between the test materials at any time point (Table 8). There were no significant differences in the thickness of the encapsulating membrane around the Nitinol wire at different time points, either. However, there was a significant increase in the thickness of the encapsulating membrane around the braided polyester suture between two and six weeks ($p = 0.021$). The encapsulating membrane seemed to be thickest around the sutures close to the epitenon or paratenon areas. The sutures located in the collagen-rich matrix area of the tendon seemed to be surrounded by a very thin membrane.

Table 8. Encapsulating membrane thickness around NiTi suture and 4-0 braided polyester control suture material after implantation in the gastrocnemius tendon of a rabbit for two, six, and twelve weeks. The encapsulating membrane thickness (±SD) is given.

<table>
<thead>
<tr>
<th></th>
<th>NiTi suture</th>
<th>Braided polyester</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>28.7µm (± 19.7) (n =5)</td>
<td>6.5µm (± 2.9) (n =4)</td>
<td>N.S.</td>
</tr>
<tr>
<td>6 weeks</td>
<td>27.5µm (± 13.2) (n =5)</td>
<td>35.3µm (± 12.0) (n =4)</td>
<td>N.S.</td>
</tr>
<tr>
<td>12 weeks</td>
<td>20.3µm (± 12.6) (n =5)</td>
<td>21.2µm (± 10.5) (n =5)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

5.7 Strength measurements

Study IV: The breaking load of the shape memory alloy suture was greater by an average of 40.4% than that of the control suture material after two weeks of implantation in rabbit
back subcutis ($p = 0.014$) (Table 9). Correspondingly, the breaking load of NiTi at six weeks was greater by 30.5% ($p = 0.053$) and that at twelve weeks greater by 29.2% ($p = 0.025$) than that of braided polyester. Calculated from the mean of all measurements, the Nitinol suture was 34.7% stronger on an average than the braided polyester suture. At six weeks, nitinol showed slightly greater breaking load values compared to its breaking loads at two weeks ($p = 0.032$) and twelve weeks ($p = 0.036$) (Table 9), but there was no significant difference between two and twelve weeks. There was no significant difference in the breaking loads of braided polyester at the different time points. Some of the test samples were excluded from the measurements because they were found to be attached to the muscular fascia of the muscles in the rabbit’s back and could therefore not be dissected out without causing possible harm to the test material. This explains the small number of measurements at some time points.

Table 9. Breaking loads of Nitinol suture and 4-0 braided polyester suture material after implantation in the subcutaneous tissue of rabbit back for two, six, and twelve weeks. The breaking load was measured in terms of the breaking force in Newtons (N). Implantation did not have a significant effect on the strength properties of either material. Breaking load ($\pm SD$) is given. ($n = number$ of cases).

<table>
<thead>
<tr>
<th></th>
<th>NiTi suture strength (N)</th>
<th>Braided polyester strength (N)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>25.5 ($\pm 0.57$) (n = 4)</td>
<td>18.2 ($\pm 0.98$) (n = 5)</td>
<td>$p = 0.014$</td>
</tr>
<tr>
<td>6 weeks</td>
<td>26.6 ($\pm 0.33$) (n = 5)</td>
<td>20.4 ($\pm 1.17$) (n = 2)</td>
<td>$p = 0.053$</td>
</tr>
<tr>
<td>12 weeks</td>
<td>24.2 ($\pm 1.37$) (n = 3)</td>
<td>18.8 ($\pm 1.23$) (n = 5)</td>
<td>$p = 0.025$</td>
</tr>
</tbody>
</table>
6 Discussion

6.1 Bone response to functional NiTi intramedullary nailing

These are the first studies evaluating the bone-modeling effects of a functional device implanted inside a tubular bone. The bone-modeling effect observed here can be presumed to be the combined effect of many factors associated with the surgical procedure, the bending force of the implant, and other forces directed to the bone.

6.1.1 Effects associated with surgery

There were no complications in these series that could interfere with the final results. The medullary canal was reamed using a 1.2 mm (18-gauge) needle with rotating motion, which can be considered modest reaming, at least on the basis of prior studies, and should not cause any severe problems in bone blood flow (Grundnes & Reikerås 1993). Perforation of the distal epiphyseal plate may have affected the growth of bone. This will be discussed in more detail in the section titled “Retardation of longitudinal growth” below.

6.1.2 Bone-modeling effects

Study I showed that bone modeling can be controlled with a functional intramedullary nail made of nickel-titanium shape memory alloy. All bones were bent in the direction of the nail, as shown by the AP radiographs, but the degree of bending varied individually. This is explained by the different thicknesses, curvatures, and locations of the nails used. Thicker nails seemed to cause more bending. There was a trend towards more bending
when the nail crossed the epiphyseal plate. If a smaller radius of curvature of the nails had been used, more marked changes in the bending angle might have been seen.

In previous studies concerning the modeling effects of strains on rat bone, the force has been applied through external pads to the rat lower limb. The model has the disadvantage that the pads create local pressure on the leg at the contact sites. (Raab-Cullen et al. 1994b.) The functional nail introduced here might provide a new alternative to apply force to bone in studies on artificial loading of rat bone.

In the present studies, the functional intramedullary NiTi nail caused a static bending force inside the rat femur, but as the leg was not immobilized, the normal weight-bearing activity by the rat also produced a dynamic strain. Further, the stress-shielding effect caused by an intramedullary nail implanted into the medullary canal of the femur, which takes over part of the load that would normally be carried exclusively by the femur, can be presumed to affect bone modeling (Mølster 1986, Husby et al. 1989b, Husby et al. 1989c, Weinans et al. 1993). Presumably, the final adaptive bone modeling or remodeling reaction seen in our study was due to the combined effect of an osteogenic response to the static strain and stress shielding caused by the nail and the dynamic strain caused by weight bearing and the loads induced by muscle action.

Study II: Here, we found similar bone bowing in femurs with curved nails. As expected, the straight nails resulted in no significant bowing of the bone. This confirms that the bending force caused by the nail rather than factors associated with the intramedullary nailing itself induces this bone-modeling effect. The average bowing in this study was 3.3 degrees, while bowing of 6.7 degrees was seen in study I, and there was remarkable individual variation in the bowing angle. This is partly because of the different positions of the nail inside the femoral cavity, which produces variable forces in the AP plane at which the radiographs were taken. In order to make the nailing operation easier and to make sure that the nails can be similarly implanted into each subject in this larger series of rats, the nails used here were 1 mm shorter than those in the previous study, and they hence produced less bending force. As in the previous study, there was a trend towards more bending when the nail crossed the epiphyseal plate, while the nails inserted deeper into the medullary cavity caused less bowing of the bone. In this study, most of the nails were located deeper in the medullary cavity, thus producing less bending.

### 6.1.3 Retardation of longitudinal growth

Significant retardation of longitudinal growth was observed in the rat femur after intramedullary nailing using either functional or straight NiTi nails compared to the contralateral normal femur. There was no difference between the groups in this respect. Because retardation of growth was associated with both nails, the growth effect seems to be due to the intramedullary nailing itself rather than the bending force. This effect might be caused by some damage in the epiphyseal growth plate during nailing. The thicker nails seemed to affect longitudinal growth more than thinner ones. The most marked shortening seemed to occur in bones with the nail crossing the epiphyseal plate.
Numerous descriptions of the effects of trauma to the growth plate have been reported before. Some studies have reported no growth disturbance after moderate central epiphyseal trauma (Siffert 1966). On the other hand, it has been reported that the nails passing longitudinally through the epiphyseal plate, resulted in definite growth retardation, whereas, if the pins were removed immediately after insertion no loss of growth occurred (Haas 1950). In a later study, drilling a 1mm thick Kirchner wire through the distal epiphyseal plate of a rat femur was found to cause significant retardation of longitudinal growth in 14 weeks, even if the wire was immediately removed after drilling (Bjerkreim & Langård 1983). Leaving the pin inside the femur caused similar retardation of growth, and so did leaving the pin penetrating the epiphyseal plate (Bjerkreim & Langård 1983). Recently, it has been found out that the extent of the trauma to the epiphyseal growth plate affects the occurrence of growth disturbance. In growing rabbits, destruction of 7% of the cross-sectional area of the distal femoral growth plate by drilling, caused permanent growth disturbance and shortening of the femur, while destruction of only 3% of the growth plate caused no growth disturbance. (Mäkelä et al. 1988.) Conflicting results in the studies concerning the effect of trauma to the epiphyseal plate may be partly caused by different methods of applying trauma to epiphyseal plate. Drilling using a Kirchner wire, for example, may cause more thermal effect, than drilling using a diamond drill of the same diameter.

6.1.4 Thickening of bone

Significant thickening of the operated bones compared to the contralateral normal femurs as well as increases in Tot A, CSA, and CtTh were observed after intramedullary nailing using the functional intramedullary nail. These changes seemed to be most significant at the mid-diaphysis. The femoral medullary cavity has a proximally narrowing shape. Therefore, the middle one of the three leaning points of the nail is not located in the middle of the nail but more proximally, causing the maximum strain to be applied to the proximal part of the bone. This might explain the axially non-symmetric increase of cortical thickness and area (Fig. 7). More thickening seemed to occur in the bones with the nail left to cross the epiphyseal plate.

In the femurs nailed using straight nails, some increase in Tot A, CSA, and CtTh was also observed. However, this increase was only seen in the midmost slices with no statistical difference in the whole set of slices compared to the contralateral unoperated femur. This suggests that the bending force of the functional nail seems to primarily induce these changes.

6.1.5 Effect of stress shielding

Stress shielding, i.e. the implant taking over part of the load that would normally be carried by the bone (Mølster 1986, Husby et al. 1989b, Husby et al. 1989c), can be
presumed to be present in femurs nailed with both curved and straight nails. In study I, BMD showed a statistically significant decrease in the proximal slices and an increase at the distal end. In study II, a significant increase in BMD at the distal end of the diaphysis was observed in both groups, but the changes were smaller than in the previous study. According to the present results, it seems that only minor changes in BMD are seen after intramedullary nailing with a NiTi intramedullary nail. The reason might be that NiTi has an elastic modulus closer to that of bone than any other metal, which diminishes the stress shielding effect compared to the other implant metals (Buehler & Wang 1968).

6.1.6 Histology

The nailed bones had lower birefringence under a polarized light microscope compared to the unoperated contra lateral femurs. The non-lamellar nature of the thickened side of the cortex showed that the modeling response was mainly that of woven bone. Rat bone is not osteonal or Haversian bone, but highly organized and birefringent. This feature was affected in the bones operated with a functional intramedullary nails, resulting in less regular structure of the collagenous ground substance. The thickening of the bone was achieved by woven bone formation similar to the regional acceleratory phenomenon previously reported (Ryhänen et al. 1999b).

From previous reports, it is known that strains may cause microscopic fatigue damage or microdamage (MDx) in bone (Burr et al. 1997). This damage is normally repaired by the BMUs of bone. According to the literature, strain in the range of about 3000 microstrain begins to cause so much MDx that it can no longer be adequately repaired (operational MDx threshold range, MESp), and strains above 3000 microstrain may stimulate woven bone formation instead of lamellar bone formation (Martin & Burr 1989). Woven bone formation in the present study suggests that the strain created by the functional nail exceeded this point.

A BMU needs three months or more to repair one locus of MDx (Frost 1966). As the follow-up time in the present studies was 12 weeks, it is probable that the MDx created by the nail during the first few days after the implantation would have nearly healed during the follow-up. However, as the nail continues to cause the strain on the bone, new MDx will occur until the bone reaches a more bent shape through bone modeling and the MESp is no longer exceeded. After this, bone modeling may still continue until the force created by the nail on bone remains below the modeling threshold range (MESm, near 1000 microstrain), after which no modeling effect should occur.

Based on the above, much longer follow-up times would be warranted in the future to highlight the long-term changes caused by the functional NiTi nail on tubular bone.
6.1.7 FEA

Finite element analysis (FEA) is a valuable tool in the design and development of functional devices. It offers an alternative method for the trial and error techniques commonly used in the past. The primary benefit of this tool is reduced development cycle time, as FEA predicts real-world results of a setting without having to build numerous prototypes.

We performed a simplified FEA to examine the response of the wire-bone combination immediately following implantation. The analysis showed a stress distribution very similar to the changes seen in the bones of the experimental study. The maximum values of stress were 635 MPa for the wire and 15 MPa for bone, justifying the linearly elastic analysis, because both appear to be below the yield strength of the respective materials. The FEA indicates that the bone tries to adapt to this new situation by bending. The changes in the dimensions of the cross-section may be a result of this adaptation to diminish stress. The adaptation is not immediate, however. The theoretical bowing angle just after implantation was only 1.09 degrees in the FEA, while the experimental results after 12 weeks were 6.7 degrees on an average. This difference may indicate gradual modeling effects in the bone.

This was a simplified two-dimensional FE analysis, where non-linearity and anisotropy were not considered. In the future, a more detailed analysis might yield additional information about the non-linear changes of the nail-bone combination. A precise finite element analysis of the functional nail-bone combination is, however, complicated. It is known that microdamage (MDx) accumulation impairs the mechanical properties of bone by reducing its elastic modulus (Burr et al. 1998), thus affecting the nail-bone combination. On the other hand, as the crystal structure of Nitinol changes in a non-linear fashion during the transition period (Moneim et al. 2002), the forces created by the NiTi nail does obviously not decrease linearly either, as the nail recovers its original shape. Additionally, in vivo, forces and strains on the bone are created by not only the strain caused by the nail but also by the forces created by weight bearing and muscle work. Finally, all these factors cause different strains in different parts of the bone.

6.2 Porous NiTi osteointegration and bone ingrowth

A lot of research has been done to determine the optimal pore sizes of various porous materials. Evidently, there also exists an optimal pore size for porous NiTi, but it has not been determined yet, which is why the present study was carried out.

Osteoconductive bone graft substitutes are generally used to fill bone defects that require mechanical support (Keating & McQueen 2001). Porous NiTi offers a metallic alternative for a bone graft substitute, with an elastic modulus closer to that of bone than any other metal. Because of its elasticity, it can be assumed to cause less stress shielding than more rigid metals. Therefore, porous NiTi might provide a good alternative in applications requiring strong mechanical support.
In the present study, the porous NiTi implant occupied a major proportion of the weight-bearing bone structures in the distal femur and thus presumably provided mechanical support for the weakened bone. As no fractures were seen, the mechanical support can be postulated to have been sufficient.

6.2.1 Bone ingrowth

In the present study, both mineralized bone matrix and bone marrow were observed in the pores of the implant. They filled the total void space of the implants in most cases. In some cases, fibrosis was observed as well, and this will be discussed later in this book. As the implant was 3 mm thick, the bone had grown at least 1.5 mm inside the implant from each bone-implant contact area after the implantation. In a previous study, in which 11x20mm porous NiTi implants were implanted in sheep vertebrae, bone had grown through the whole implant in 3 of the 4 cases in 12 months (Assad et al. 2003b). To determine how deep in porous material bone would grow, even larger implants would have to be used. This would naturally be impossible in a rat model, but another animal model should be considered. Bone ingrowth could also be different at different locations (metaphyseal area, vertebrae, diaphyseal bone).

In some studies, the percentage bone ingrowth was measured by excluding bone marrow (Assad et al. 2003b). This was, however, not done here, as the bone-implant contact and the presence of fibrosis were felt to be more important.

6.2.2 Bone-implant contact

One of the goals of the present study was to evaluate the effect of porosity on osteointegration of porous NiTi in metaphyseal bone, and statistically significant differences in bone-implant contact were actually found between different porosities. According to these results, it seems that the contact values of 29% in group 2 were significantly worse than the contact values of 51% of group 1. However, there were only four rats in group 2, which makes the results of this group more vulnerable to incidental variation. The contact values of group 3 (39%) seemed lower than those of group 1, but no statistical difference was found.

In a previous study, in which porous NiTi was implanted in sheep vertebrae, bone apposition (percentage of the exterior and interior perimeter of the implant) showed a time-dependent trend, increasing from 11% to 24% (3-12 months) (Assad et al. 2003b). Compared to this, the bone-implant contact values of 51% in group 1 and 39% in group 3 in this study at 30 weeks of implantation seem good. However, as porous NiTi was implanted in the rat femur metaphysis instead of sheep vertebrae in the present study, the results are not fully comparable. In a study by Ayers et al., the bone contact of porous NiTi implanted in the cranial bone of a rabbit for six weeks was separately measured from the outer implant surface and within the implant, making it difficult to compare the
results with those obtained in the present study (Ayers et al. 1999). The exterior bony apposition varied from 32% to 47%, depending on the porosity of the implant, while the interior bony apposition varied from 36% to 42%. In many previous studies, no statistical data of the bone-implant contact were obtained, because of the low number of test animals. In a study by Simske et al., bone contact of porous NiTi implanted in the cranial bone of a rabbit was 35% at six weeks (n = 2) and 40% at 12 weeks (n = 3) (Simske & Sachdeva 1995). Rhalmi et al. used histomorphometry, but reported no contact values (Rhalmi et al. 1999).

Movements of the porous implant after implantation may interfere with the bone ingrowth and bone contact and might theoretically affect the results. In this study, tight press-fit of the implant into the osteotomy cavity was aimed at, to minimize gapping and micromotion of the implant. On the basis of good bone contact values it seems that the press fitting of the implants was sufficient in these cases, and no additional fixation was necessary.

6.2.3 Fibrosis

The occurrence or extent of fibrosis inside the porous NiTi implant in bone tissue has not been assessed before. Rhalmi et al. reported some fibroplasia and bone marrow within the pores of the porous NiTi implant, but no quantitative or statistical analysis was made (Rhalmi et al. 1999). In the present study, fibrous tissue inside the implant was present significantly more often with group 1 than with group 3 (p = 0.021). Most cases showed no fibrosis at all, but among the ones that did, the extent of fibrosis varied considerably. Therefore, no reliable quantitative analysis of the extent of fibrosis was possible.

Fibrosis was found only in two out of fifteen cases in group 3, thus meaning that the pores of the implant were completely filled with bone and bone marrow in thirteen cases of this group. Significantly more cases were associated with fibrosis inside the implant pores in group 1, which in turn seemed to show better bone-implant contact, as mentioned above. As no statistical difference in bone-implant contact between the two porosities was found, however, the porosity of group 3 would seem to be the best porosity for the use as a bone graft substitute out of the porosities tested here.

6.3 NiTi suture in rabbit tendon

6.3.1 General

The idea of using NiTi wire as a tendon suture material is very new. Only one study of this topic has been reported, focusing on the biomechanical aspects of the NiTi suture (Moneim et al. 2002). Several factors make NiTi suture a very tempting candidate for
tendon surgery. It is stronger than the conventional tendon suture materials of similar diameter. Compared to conventional steel wire, Nitinol is much more elastic, and it can therefore withstand kinking better (Duerig et al. 1996). It is a metal wire that feels like nylon. With the metal in its soft and flexible phase, it is easy for a surgeon to do the suturing and knotting. (Moneim et al. 2002.)

6.3.2 Biocompatibility

Although biocompatibility studies have shown NiTi to be a safe implant material, the biocompatibility of NiTi in tendon tissue has not been studied before. The healing of tendon may require a long time, and the sutures will remain permanently. Thus, the materials for tendon surgery should be chosen with care. These factors motivated the present biocompatibility testing study.

In the present study, as no harmful reactions were observed and the encapsulating membrane around the suture was found to be thin, the host response to Nitinol in tendon tissue was considered to be well within the acceptable range of biocompatibility and quite similar to that of the control material. The previous studies have shown the encapsulating membrane around Nitinol in perineural and muscle tissue to be 35-89µm in thickness (Ryhänen et al. 1998). Much thinner membranes (20.3-28.7µm) were found in the present tendon tissue study. There was marked individual variation, which seemed to be dependent mostly on the location of the suture inside the tendon. This might be explained by the two different cellular environments inside the tendon. The epitenon and paratenon areas are well vascularized and rich in cells, while the collagen matrix area contains only a few cells. In the collagen-rich matrix area of the tendon, only a very thin encapsulating membrane around the suture was observed with both materials, whereas near the epitenon and paratenon areas, the encapsulating membrane around the suture seemed to be thicker.

6.3.3 Strength properties

The present results of the mechanical testing of Nitinol suture material support the previous findings of Nitinol tendon suture material being stronger, than conventional tendon suture materials (Moneim et al. 2002). In addition, the effect of implantation was studied here.

The strength properties of many suture materials, including non-absorbable materials, have been shown to decrease after implantation (Greenwald et al. 1994, Outlaw et al. 1998). To assess the properties of the NiTi suture material in this respect, strength measurements were performed, along with the biocompatibility testing, after two, six, and twelve weeks of implantation in rabbit subcutis. Braided polyester suture was chosen as control material for strength measurements because it is the routinely used non-resorbable tendon suture material (Strickland 1985). The thickness of 4-0 braided polyester, reported to be 0.156 ± 0.014 mm, is also close to that of the NiTi suture
material used here (Moneim et al. 2002). Nitinol was, as expected, stronger than the braided polyester control material. The period of twelve weeks did not affect the strength properties of either tested material. The strength values of Nitinol at six weeks were slightly higher than those at two or twelve weeks, but there was no statistical difference between the strength values at two and twelve weeks. This difference might be due to the relatively low number of measurements per time point, which causes the small variation of the individual wires to influence more easily the mean of the measurements. A higher number of sutures tested might provide more uniform results. The breaking load of the braided polyester measured here was close to the materials reported before (Trail et al. 1989). The mean breaking load of the Nitinol suture material tested here (25.5N) was greater compared to the reported breaking load of the 4-0 monofilament stainless steel suture (21.1N) (Trail et al. 1989).

6.4 Future possibilities

The present studies were the first to demonstrate that bone modeling can be controlled by using a functional implant. In the present studies, a functional intramedullary NiTi nail was used to bend a normal diaphyseal bone. The method could also be applied the other way round, i.e. to straighten a deformed bone. Malunited fractures and especially frequent fractures associated with osteogenesis imperfecta lead to angular deformity and bowing of long bones. Operative treatment has usually consisted of cortical osteotomies with cast, internal fixation, or external fixation (Sofield & Millar 1959, Steen & Fjeld 1989, Yadav 1993, Ring et al. 1996). However, osteotomies are relatively large operations with much postoperative pain and a risk for complications. Implantation of an intramedullary bending rod would be a far smaller operation for the patient with short postoperative recovery. It might even be possible to insert the nails percutaneously using mini-invasive techniques, without perforating the epiphyseal plate. Thus, the functional intramedullary nail presented here might provide an easier, quicker, cheaper, and less painful way to correct such bone deformities in the future. Future studies are needed to highlight the detailed effects of nail thickness and curvature and to obtain more knowledge about the forces needed in different applications. The effect of the nailing direction on rat bone should also be studied to confirm that the growth retardation problem observed here is caused by the perforation of the growth plate, as assumed here. Proximal-to-distal nailing would spare the growth plate and presumably solve the growth retardation problem.

Porous Nitinol showed good osteointegration and bone ingrowth inside the pores of the porous NiTi implant in the present studies. This can be assumed to strengthen the implant even more as the void spaces are filled with bone. Good bone ingrowth can also be assumed to provide firm mechanical fixation of the implant in the bone. Studies on the mechanical properties of different porosities of porous NiTi implants before and after implantation, the strength of anchorage in bone, and further studies on weight bearing applications are warranted. Studies with larger implants and a suitable animal model for them would provide information on how deep in the porous material bone would grow.
Bone ingrowth could also be different in different locations (metaphyseal area, vertebrae, diaphyseal bone) and the determination of such differences would be interesting. Mechanical properties of the different porosity implants should be studied. Porous NiTi might provide a mechanically stronger alternative to the current bone graft substitutes for weight-bearing applications.

NiTi seems a very promising candidate for a tendon suture material. Good biocompatibility and no loss of strength at implantation were observed in the present study. Further experimental studies should be performed to compare the strengths and the tendencies for adhesion formation of tendons operated using NiTi tendon suture material and ones operated using conventional tendon suture materials at different time points.
7 Conclusion

1. Study I was the first study to use an intramedullary bending device to control the shape of the bone and to demonstrate that bone modeling can be controlled by using a functional implant. It showed that the implantation of a functional nickel-titanium shape memory alloy intramedullary nail in the rat femur causes changes in the external shape and the internal structure of the bone, and that the curvature of the bone increases in the bending direction of the nail. Statistically significant changes in bone thickness, cortical area, thickness, and BMD and retardation of longitudinal growth in the nailed femurs were also observed.

2. In study II, the bone-modeling effects caused by straight and curved intramedullary nails were compared. The bending force of the curved nail resulted in more evident bone thickening and increase in the cortical area of the femur. Bone bowing was observed after intramedullary nailing with a curved functional NiTi nail, as in study I, but not when straight nails were used, indicating the bending force of the nail to have induced this change. Retardation of longitudinal growth was present in both nail groups, and may be caused by some damage in the distal epiphyseal growth plate during the intramedullary nailing procedure. Further studies are warranted to confirm this hypothesis by saving the epiphyseal growth plate at implantation by implanting the nails in the proximal-to-distal direction in the case of the rat femur, for example. Using functional intramedullary nails might provide an easy method for correcting bone deformities in the future.

3. In study III, porous NiTi implants of different porosities were studied as weight-bearing bone graft substitute. Porosity of 66.1% (mean pore size (MPS) 259 ± 30µm) showed the best bone-implant contact (51%). However, porosity of 46.6% (MPS 505 ± 136µm) with 39% bone-implant contact was not significantly inferior in this respect and showed a significantly lower incidence of fibrosis within the implant and thus seemed to be the best choice for a bone graft substitute, out of the porosities tested here. The porosity of 59.2% (MPS 272 ± 17µm) showed lower contact values. Porous NiTi might provide a mechanically stronger alternative to the current bone graft substitutes for weight-bearing applications.
4. In study IV, the biocompatibility and strength properties of NiTi tendon suture material were evaluated. Encapsulating membrane formation around the sutures was minimal and quite similar to braided polyester, which was used as control material, indicating good biocompatibility in tendon tissue. The breaking load of Nitinol was significantly greater compared to braided polyester. Implantation did not affect the strength properties of either material. These results suggest NiTi to be a promising new tendon suture material with good biocompatibility in tendon tissue and better strength properties than those of the conventional materials used in tendon surgery.
References


