

**BIOCOMPATIBILITY
EVALUATION OF NICKEL-
TITANIUM SHAPE MEMORY
METAL ALLOY**

**JORMA
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Department of Surgery

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JORMA RYHÄNEN

**BIOCOMPATIBILITY EVALUATION
OF NICKEL-TITANIUM SHAPE
MEMORY METAL ALLOY**

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Abstract

The shape memory effect, superelasticity, and good damping properties, uncommon in other implant alloys, make the nickel-titanium shape memory metal alloy (Nitinol or NiTi) a fascinating material for surgical applications. It provides a possibility to make self-locking, self-expanding and self-compressing implants. The purpose of this work was to determine if NiTi is a safe material for surgical implant applications.

The primary cytotoxicity and the corrosion rate of NiTi were assessed in human osteoblast and fibroblast cell cultures. Comparisons were made with 316 LVM stainless steel (StSt) and pure titanium. The metal ions present in the media were analyzed using atomic absorption spectrometry (GFAAS). Despite the higher initial nickel dissolution, NiTi induced no toxic effects, decrease in cell proliferation or inhibition in the growth of cells in contact with the metal surface.

The general soft tissue responses to NiTi were compared to corresponding responses to StSt and Ti-6Al-4V alloy in rats during a follow-up of 26 weeks. The muscular tissue response to NiTi was clearly non-toxic and non-irritating, as were also the neural and perineural responses. The overall inflammatory response and the presence of immune cells, macrophages and foreign body giant cells were similar compared to the other test materials. At 8 weeks, histomorphometry showed that the capsule membrane of NiTi was thicker than that of stainless steel, but at 26 weeks the membrane thicknesses were equal.

A regional acceleratory phenomenon (RAP) model was used to evaluate new bone formation, bone resorption and bone (re)modeling after periosteal implantation of NiTi, StSt or Ti-6Al-4V in rats using histomorphometry. Maximum new woven bone formation started earlier in the Ti-6Al-4V group than in the NiTi group, but also decreased earlier, and at 8 weeks the NiTi and StSt groups had greater cortical bone width. Later, no statistical differences were seen. NiTi had no negative effect on total new bone formation or normal RAP during a 26-week follow-up.

The ultrastructural features of cell-NiTi adhesion were analyzed with scanning electron microscopy (FESEM). Cell adhesion and focal contacts showed a good acceptance of NiTi.

Femoral osteotomies of rats were fixed with either NiTi or StSt intramedullary nails. Bone healing was examined with radiographs, peripheral quantitative computed tomography (pQCT) and histologically. The maximum follow-up was 60 weeks. There were more healed bone unions in the NiTi than the StSt group at early time points. Callus size and bone mineral density did not differ between the NiTi and StSt groups. Mineral density in both groups was lower in the osteotomy area than in the other areas along the nail. Density in the nail area was lower than in the proximal part of the operated femur or the contralateral femur. Bone contact to NiTi was close, indicating good tissue tolerance. Determination of trace metals from several organs was done by GFAAS or inductively coupled plasma-atomic emission spectrometry (ICP-AES). There were no statistically significant differences in nickel concentration between the NiTi and StSt groups in distant organs. The FESEM assessment showed surface corrosion changes to be more evident in the StSt implants.

On the basis of this study, the biocompatibility of NiTi seems to be similar to or better than that of stainless steel or Ti-6Al-4V alloy. NiTi appears to be suitable for further use as a biomaterial, because its biocompatibility is good. When NiTi is intended to be used in long-term implants, optimal surface treatment must consider.

Keywords: NiTi, biocompatible materials, bone and muscle response, corrosio.

Dedicated to my family

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Oulu, April 1999

Jorma Ryhänen

Abbreviations

A _f	Austenite finish temperature
AISI	American Iron and Steel Institute
AO/ASIF	Arbeitsgemeinschaft für Osteosynthesefrage/Association for the Study of Internal Fixation
A _s	Austenite start temperature
B.Ar	Bone area
B.Pm	Bone perimeter
BMC	Bone mineral content
BMD	Bone mineral density
Ct.Ar	Cortical bone area
CtBMD	Cortical bone mineral density
Ct.Wi	Cortical bone width
FB	Fibroblast
FDA	United States Food and Drug Administration
FESEM	Field emission scanning electron microscopy
GFAAS	Graphite furnace atomic adsorption spectrometry
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
LVM	Low vacuum melted
M _d	Highest temperature to strain-induced martensite
M _f	Martensite finish
M _s	Martensite start
Ni	Nickel
NiTi	Nickel-titanium shape memory alloy (also Nitinol)
N.Wo.B	New woven bone
OB	Osteoblast
OC	Osteoclast
pQCT	Peripheral quantitative computed tomography
RAP	Regional acceleratory phenomenon
StSt	Stainless steel alloy
Ti	Titanium
Ti-6Al-4V	Titanium - aluminum (6%) - vanadium (4%) alloy
TTR	Transition temperature

Definitions

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

Biocompatibility: The ability of a 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.

Biomaterials science: The study and knowledge of the interactions between living and non-living materials.

Bone bonding: The establishment, by physicochemical process, of continuity between implant and bone matrix.

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 from 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 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 μ M). It is a description of clinical performance devices and is not applicable to the description of biomaterial interactions.

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

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

Superelasticity (pseudoelasticity): 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 then 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.

Transition temperature: Temperatures at which changes of material phases occur

List of original publications

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

- I Ryhänen J, Niemi E, Serlo W, Niemelä E, Sandvik P, Pernu H & Salo T (1997) Bio-compatibility of nickel-titanium shape memory metal and its corrosion behavior in human cell cultures. *J Biomed Mater Res* 35: 451-457.
- II Ryhänen J, Kallioinen M, Tuukkanen J, Junila J, Niemelä E, Sandvik P & Serlo W (1998) In vivo biocompatibility evaluation of nickel-titanium shape memory metal alloy: muscle and perineural tissue responses and capsule membrane thickness. *J Biomed Mater Res* 41: 481-488.
- III Ryhänen J, Kallioinen M, Tuukkanen J, Lehenkari P, Junila J, Niemelä E, Sandvik P & Serlo W Bone modeling and cell-material interface responses induced by nickel-titanium shape memory alloy after periosteal implantation. *Biomaterials* (In press).
- IV Ryhänen J, Kallioinen M, Serlo W, Perämäki P, Junila J, Sandvik P, Niemelä E & Tuukkanen J Bone healing and mineralization, implant corrosion and trace metals after nickel-titanium shape memory metal intramedullary fixation. *J Biomed Mater Res* (In press).

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1. Introduction

The development of modern surgery is notably related to the development of biomaterials. Internal fracture fixation devices, articular prostheses, vascular prostheses and substitute heart valves are examples of breakthroughs in this area.

In the early 1960s, Buehler *et al.* discovered the shape memory effect in an equiatomic alloy of nickel and titanium (Nitinol, chemical symbol NiTi). Since that time, intensive metallurgical investigations have been made to explore the mechanics of its basic behavior. The use of NiTi for medical purposes was first reported in the early 1970s. In the early 1980s, the idea got more support and some orthodontic and experimental orthopedic applications were released. It was only in the mid-1990s, however, that the first widespread commercial stent applications made their breakthrough in medicine. Currently, NiTi seems to arouse notable interest in the medical and commercial sectors.

NiTi has unique properties that could be very useful in surgical applications. Thermal shape memory, superelasticity and good damping properties make it possible for such alloys to behave differently compared to ordinary implant metals (Buehler *et al.* 1967, Andreasen *et al.* 1987). Using its thermal shape memory property, a material sensing a change in external temperature is able to convert to a preprogrammed shape. While NiTi is soft and easily deformable in its lower temperature form (martensite), it resumes its original shape and rigidity when heated to its higher temperature form (austenite). The shape memory effect is based on this temperature-dependent austenite-to-martensite phase transformation on an atomic scale. Within a given temperature range, NiTi can also be strained several times more than conventional metal alloys without being plastically deformed. This superelastic property is also based on martensitic transformation (Shimizu *et al.* 1987).

When applied in certain surgical implants, NiTi is expected to provide radically new functional capabilities, improved performance and a possibility of using minimally invasive techniques. It provides a possibility to make self-locking, self-expanding and self-compressing implants activating at body temperature (Drugacz *et al.* 1995, Blum *et al.* 1997, Ryhänen *et al.* 1998).

Because of the high nickel content of NiTi, it is theoretically possible that nickel may dissolve from the material due to corrosion and cause unfavorable effects. The biocompatibility of NiTi must be very well confirmed before it can be safely used as an implant

material. At present, there are not enough conclusive biocompatibility data available on NiTi. Some previous studies revealed few or no biocompatibility problems, but unanswered questions are numerous. The consequences of surface conditions, the dissolution of nickel ions or compounds *in vivo* after a long period of implantation, the accumulation of trace ions, the responses in different tissues, carcinogenicity, the responses to fracture healing and bone formation, and the effects at the cellular and molecular levels are all issues which require further clarification.

The present studies approach the nickel-titanium shape memory alloy from the basic biocompatibility point of view. The primary acute cytotoxic effects of NiTi on human fibroblasts and osteoblasts, general soft tissue, and neural and perineural responses were evaluated. New bone formation and bone (re)modeling activity as well as the effect of NiTi on the osteogenesis and ossification of the osteotomy defect were studied. The surface corrosion properties and release of trace metals from NiTi were also examined. Common implant materials were used as control materials.

2. Review of the literature

2.1. Biomaterials science

Biomaterials science examines the mechanical, physical and chemical properties of materials as well as the complex host responses to introduced bulk material, material surface and biomaterial applications. Biomaterials science has been officially defined as “*the study and knowledge of the interactions between living and non-living materials*”, and biomaterial as “*a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body*” (Williams *et al.* 1992).

The development of modern biomaterials is related to the development of modern medicine and new materials. Stainless steel and cobalt chromium alloys were the first materials successfully used inside the body for fracture fixation. In the early 1960s, Sir John Charnley made the first attempt to link together a stainless steel hip prosthesis and high-density polyethylene with metachrylate bone cement. This can be considered the beginning of modern orthopedics, in which the development of better materials plays a central role. In the late 1960s, the excellence of titanium was discovered in medicine (Branemark *et al.* 1969). At that time, some materials began to be classified as “biomaterials”. Various materials (polymers, ceramics, composites and metals with improved properties) and applications (orthopedics, vascular and heart surgery, etc.) have been developed since then. Today, there are a great number of different professions dealing with the problems associated with biomaterials, and the cross-scientific approach is essential.

There are international organizations which give recommendations and standards for the manufacturing and testing of biomaterials (ISO= International Standards Organization, ASTM= American Society for Testing and Materials). There are also national organizations that supervise biomaterial applications in human use. One of the best known and most demanding control organizations is FDA (Food and Drug Administration of USA) (Brown *et al.* 1996). It must be pointed that FDA does not regulate the materials used in medical devices, but rather the devices themselves. The biocompatibility of the material is a central factor in devices intended for use inside the body. Biocompatibility has been officially defined as “*the ability of a 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. When evaluating the biocompatibility of the nickel-titanium shape memory metal alloy, both of these must be considered.

2.2. Host response to metal biomaterials

2.2.1. Nearly inert host response

On the basis of their interface reactions, materials may be classified as toxic, biologically inactive (nearly inert), bioactive, or resorptive (Hench 1996). Toxic materials are not used in implants. Metallic biomaterials are classified as nearly inert materials. Because of their mechanical strength and biocompatibility, metals are superior in load-bearing implants.

The biocompatibility of the implant material is closely related to the reactions between the surface of the biomaterial and the inflammatory host response (Thomsen *et al.* 1991). There are several factors that contribute to this. These may depend on individual patient characteristics, such as general health, age, tissue perfusion, immunological factors, or implant characteristics, such as surface roughness and porosity, chemical reactions at the surface, corrosion properties of the material, and the toxicity of the individual metals present in the alloy (Klinger *et al.* 1997). The surgery itself, the technique applied, and biomechanical considerations (stability) modify the inflammatory response.

The releasing agents from the cell may alter the characteristics of the material surface. The surface is also changed due to the influence of proteins absorbed from plasma (Anderson *et al.* 1990).

After implantation, a coagulating and vascularizing process takes place. The implant is covered by a blood clot containing leukocytes and erythrocytes, thrombocytes and coagulating proteins. The implant and the surgical trauma trigger an inflammatory reaction which eliminates the damaged tissue, clot and bacteria. Inflammatory cells, first polymorphonuclear granulocytes and later monocytes, arrive to expurgate the debris and foreign materials. If there is too much foreign substance for granulocytes, monocytes developed into macrophages. If there is a delay in removing the substance, the enzymes of activated macrophages affect the fibroblasts to make a fibrous capsule around the implant. As long as phagocytic activation is maintained, the capsule becomes thicker. In soft tissues, the inert material forms a thin, fibrous encapsule around the implant (Anderson 1996).

The implantation response in bone differs in some ways from that taking place in soft tissue. There is an inflammatory and a reparative response which occur one on the other. The reparative response starts 2-3 days after the implantation. The stem cells of bone develop into osteoblasts, which form a layer near the implant together with fibroblasts. Fibroblasts, osteoblasts and capillaries penetrate into the blood clot, replacing it, and fill the space between the implant and bone (Tarr *et al.* 1986). After the formation of a collagen-rich extracellular matrix (ECM), mineralization follows. Normally, there are vesicles in ECM and some of them include calcification focuses. The presence of vesicles with biomaterial in the early period is a sign of good primary acceptance. When the membranes of these vesicles rupture, the erupted apatite crystals unite and form calcifying structures (Davies 1991). Early trabecles grow and continue to mineralize, and some of

them reach the implant surface. In an optimal situation, the material is covered by bone tissue and not by fibrous capsule. The healing of bone tissue continues like fracture healing. Remodelation of bone tissue begins after two weeks and continues for the lifetime. Woven bone is replaced by functionally oriented lamellar bone.

The changes in the local environment, such as acidity, oxygen content, electric charge, ion concentration, enzymes, growth factors, etc., have effects on the differentiation and migration of stem cells. The attachment of osteogenic stem cells to substrate and the formation of mineralized ECM are essential for the differentiation of osteoblasts (Davies 1991). When the material is biocompatible, there is an abundance of ECM and osteoblasts. This is confirmed by the close attachment and fast proliferation of these cells (Vrouwenvelder *et al.* 1993). Brånemark *et al.* (1969) first suggested that titanium may form direct bone contact. He defined this "osseointegration" as direct metal-to-bone contact at the light-microscopic (0.5 μm) level. This definition appears to be somewhat inaccurate, and some clinically based definitions of osseointegration have been suggested (Albrektsson *et al.* 1993). In optimal situations, however, bone accepts the implant as part of its ECM, and clinically asymptomatic rigid fixation is achieved in bone during functional loading. Such fixation is possible with titanium implants. Other implant metals usually form some fibrous tissue between the bone and the implant and are often called "nearly inert". Because of their mechanical strength and biocompatibility, metals are superior in load-bearing implants. Even chemical bone bonding (*the establishment, by physico-chemical processes, of continuity between implant and bone*) is seen with bioactive glasses, but their mechanical properties are inferior to metal biomaterials (Hench 1996).

2.2.2. Signs of inferior tissue response

Highly toxic material causes tissue necrosis. The signs of sub-acute toxicity or low tissue tolerance may be manifested in several ways. A large amount of foreign-body giant cells is usually a sign of a prolonged stimulus. Also, the presence of phagocytes at a later time may signify a rejection of the implant. The propagation of lymphocytes or plasma cells may indicate the activation of the immune system against the material. Profuse accumulation of neutrophils is a sign of infection. Vacuolization and resorption of muscle are signs of an inferior tissue response (Williams 1986).

The response of individual cells to material can be considered to be dependent on how well the material mimics the natural (extracellular) environment of the cell. The physical structure of the surface may have an inferior influence on the biological response of the material, which is normally non-toxic and does not release any biologically active substance. Osteolysis, bone resorption and the formation of a thick fibrous layer between the implant and bone reflect poor biocompatibility. Also, microparticles of certain size of normally non-toxic materials may trigger an inflammatory response. These particles cause an irritation of phagocytic cells and activate them to produce and release cytokines, proteinases, growth factors and other proinflammatory factors, finally leading to chronic inflammation, fibrosis, osteolysis and porosis in bone (Shanbhag *et al.* 1994, Tang *et al.* 1996). In the case of aseptic loosening of the prosthesis, wear particles are expected to lead to the

formation of a poorly vascularized, synovial-like interface membrane between the prosthesis and bone (Santavirta *et al.* 1998). The formation of necrotic foci, granulomas and osteolysis may finally result in loosening of the prosthesis (Santavirta *et al.* 1996). The increase of metallic wear increases the surface of the metal material and the quantity of metal ions. The porous surface increases the surface area, but also particular wear.

2.3. Fundamental characteristics of nickel-titanium shape memory alloy

2.3.1. History of shape memory alloys

The first reported steps towards the discovery of the shape memory effect were taken in the 1930s. According to Otsuka and Wayman (1998), A. Ölander discovered the pseudoelastic behavior of the Au-Cd alloy in 1932. Greninger & Mooradian (1938) observed the formation and disappearance of a martensitic phase by decreasing and increasing the temperature of a Cu-Zn alloy. The basic phenomenon of the memory effect governed by the thermoelastic behavior of the martensite phase was widely reported a decade later by Kurdjumov & Khandros (1949) and also by Chang & Read (1951). In the early 1960s, Buehler and his co-workers at the U.S. Naval Ordnance Laboratory discovered the shape memory effect in an equiatomic alloy of nickel and titanium, which can be considered a breakthrough in the field of shape memory materials (Buehler *et al.* 1967). This alloy was named Nitinol (*Nickel-Titanium Naval Ordnance Laboratory*). Since that time, intensive investigations have been made to elucidate the mechanics of its basic behavior. The first efforts to exploit the potential of NiTi as an implant material were made by Johnson and Alicandri in 1968 (Castleman *et al.* 1976). The use of NiTi for medical applications was first reported in the 1970s (Cutright *et al.* 1973, Iwabuchi *et al.* 1975, Castleman *et al.* 1976, Simon *et al.* 1977). In the early 1980s the idea attained more support, and some orthodontic and mainly experimental orthopedic applications were released. It was only in the mid-1990s, however, that the first widespread commercial stent applications made their breakthrough in medicine. The use of NiTi as a biomaterial is fascinating because of its superelasticity and shape memory effect, which are completely new properties compared to the conventional metal alloys.

2.3.2. General principles

NiTi shape memory metal alloy can exist in a two different temperature-dependent crystal structures (phases) called *martensite* (lower temperature) and *austenite* (higher temperature or parent phase). Several properties of austenite NiTi and martensite NiTi are notably different.

When martensite NiTi is heated, it begins to change into austenite (Fig. 1A). The temperature at which this phenomenon starts is called *austenite start temperature* (A_s). The temperature at which this phenomenon is complete is called *austenite finish temperature* (A_f). When austenite NiTi is cooled, it begins to change onto martensite. The temperature

at which this phenomenon starts is called *martensite start temperature* (M_s). The temperature at which martensite is again completely reverted is called *martensite finish temperature* (M_f) (Buehler *et al.* 1967).

Composition and metallurgical treatments have dramatic impacts on the above *transition temperatures*. From the point of view of practical applications, NiTi can have three different forms: martensite, stress-induced martensite (superelastic), and austenite. When the material is in its martensite form, it is soft and ductile and can be easily deformed (somewhat like soft pewter). *Superelastic* NiTi is highly elastic (rubber-like), while austenitic NiTi is quite strong and hard (similar to titanium) (Fig. 1B). The NiTi material has all these properties, their specific expression depending on the temperature in which it is used.

2.3.3. Hysteresis

The temperature range for the martensite-to-austenite transformation, i.e. soft-to-hard transition, that takes place upon heating is somewhat higher than that for the reverse transformation upon cooling (Fig. 1A). The difference between the transition temperatures upon heating and cooling is called *hysteresis*. Hysteresis is generally defined as the difference between the temperatures at which the material is 50 % transformed to austenite upon heating and 50 % transformed to martensite upon cooling. This difference can be up to 20-30 °C (Buehler *et al.* 1967, Funakubo 1987). In practice, this means that an alloy designed to be completely transformed by body temperature upon heating ($A_f < 37\text{ °C}$) would require cooling to about +5 °C to fully retransform into martensite (M_f).

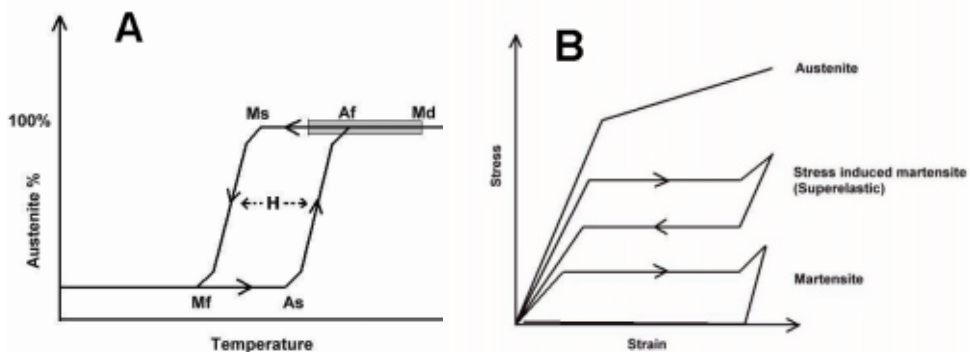


Fig. 1. A) Martensitic transformation and hysteresis (= H) upon a change of temperature. As = austenite start, Af = austenite finish, Ms = martensite start, Mf = martensite finish and Md = Highest temperature to strain-induced martensite. Gray area = area of optimal superelasticity. B) Stress-strain behavior of different phases of NiTi at constant temperature.

2.3.4. *Thermoelastic martensitic transformation*

The unique behavior of NiTi is based on the temperature-dependent austenite-to-martensite phase transformation on an atomic scale, which is also called *thermoelastic martensitic transformation*. The thermoelastic martensitic transformation causing the shape recovery is a result of the need of the crystal lattice structure to accommodate to the minimum energy state for a given temperature (Otsuka & Wayman 1998).

In NiTi, the relative symmetries between the two phases lead to a highly ordered transformation, where the displacements of individual atoms can be accurately predicted and eventually lead to a shape change on a macroscopic scale. The crystal structure of martensite is relatively less symmetric compared to that of the parent phase.

If a single crystal of the parent phase is cooled below M_f , then martensite variants with a total of 24 crystallographically equivalent habit planes are generally created. There is, however, only one possible parent phase (austenite) orientation, and all martensitic configurations revert to that single defined structure and shape upon heating above A_f . The mechanism by which single martensite variants deform is called *twinning*, and it can be described as a mirror symmetry displacement of atoms across a particular atom plane, the twinning plane (Buehler *et al.* 1967, Andreasen *et al.* 1987).

While most metals deform by slip or dislocation, NiTi responds to stress by simply changing the orientation of its crystal structure through the movement of twin boundaries.

A NiTi specimen will deform until it consists only of the correspondence variant which produces maximum strain. However, deformation beyond this will result in classical plastic deformation by slip, which is irrecoverable and therefore has no “memory effect”. If the deformation is halted midway, the specimen will contain several different correspondence variants. If such a specimen is heated above A_f , a parent phase with an orientation identical to that existing prior to the deformation is created from the correspondence variants in accordance with the lattice correspondences between the original parent phase and each variant (Fig. 1C). The austenite crystal structure is a simple cubic structure, while martensite has a more complex rhombic structure. This phenomenon causes the specimen to revert completely to the shape it had before the deformation (Andreasen *et al.* 1987, Gil *et al.* 1998).

The above phenomenon is the basis of such special properties as the shape memory effect and superelasticity.

2.3.5. *Shape memory effect*

NiTi senses a change in ambient temperature and is able to convert its shape to a preprogrammed structure. While NiTi is soft and easily deformable in its lower temperature form (martensite), it resumes its original shape and rigidity when heated to its higher temperature form (austenite) (Fig. 1C). This is called the *one-way shape memory effect*. The ability of shape memory alloys to recover a preset shape upon heating above the transformation temperatures and to return to a certain alternate shape upon cooling is known as

the *two-way shape memory effect*. Two-way memory is exceptional. There is also an *all-round shape memory effect*, which is a special case of the two-way shape memory effect (Shimizu *et al.* 1987).

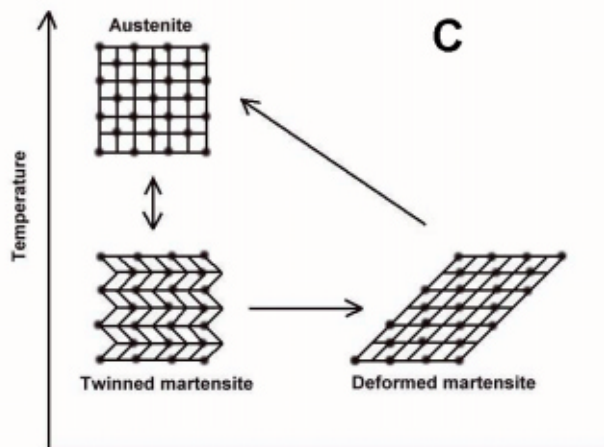


Fig. 1. C). Transformation from the austenite to the martensite phase and shape memory effect. The high-temperature austenitic structure undergoes twinning as the temperature is lowered. This twinned structure is called martensite. The martensitic structure is easily deformed by outer stress into a particular shape, and the crystal structure undergoes parallel registry. When heated, the deformed martensite resumes its austenitic form, and the macroscopic shape memory phenomenon is seen.

2.3.6. Superelasticity

Superelasticity (or *pseudoelasticity*) refers to the ability of NiTi to return to its original shape upon unloading after a substantial deformation. This is based on *stress-induced martensite* formation. The application of an outer stress causes martensite to form at temperatures higher than M_s . The macroscopic deformation is accommodated by the formation of martensite. When the stress is released, the martensite transforms back into austenite and the specimen returns back to its original shape (Fig. 1D). Superelastic NiTi can be strained several times more than ordinary metal alloys without being plastically deformed, which reflects its rubber-like behavior. It is, however, only observed over a specific temperature area. The highest temperature at which martensite can no longer stress induced is called M_d . Above M_d NiTi alloy is deformed like ordinary materials by slipping. Below A_s , the material is martensitic and does not recover. Thus, superelasticity appears in a temperature range from near A_f and up to M_d . The largest ability to recover occurs close to A_f (Duerig *et al.* 1996).

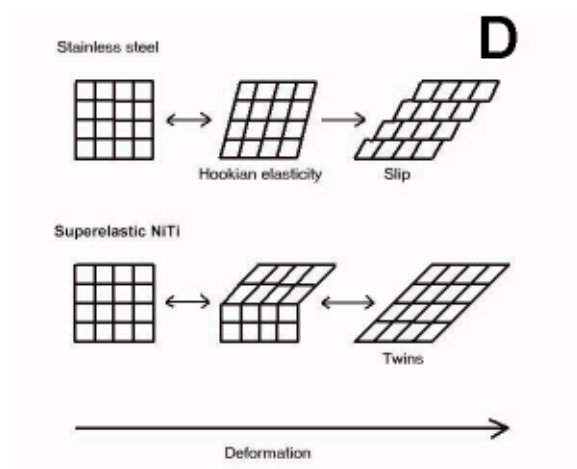


Fig. 1. D). Schematic presentation of lattice structure changes caused by outer stress in stainless steel or superelastic NiTi alloy. In stainless steel, outer stress first causes reversible Hookian type changes in the elastic area. In the plastic area, deformation takes place via a mechanism called slip. This deformation is irreversible. In superelastic NiTi alloy, outer stress causes a twinning type of accommodation which is recovered when outer stress is removed.

2.3.7. Limitations of shape memory and superelastic behavior

About 8% strain can be recovered by unloading and heating. Strain above the limiting value will remain as a permanent plastic deformation. The operating temperature for shape memory devices must not move significantly away from the transformation range, or else the shape memory characteristics may be altered. A shape memory NiTi implant must be deformed at a temperature below A_s (usually $< +5^\circ\text{C}$). Moreover, the deformation limit determined by distinctive implant design (sharp angles, etc.) and the intrinsic strain tolerance of NiTi material must not be disregarded (Otsuka & Wayman 1998).

2.3.8. Mechanical properties of NiTi

For orthopedic biomaterial applications, the two properties of major importance are strength (mechanical) and reactivity (chemical). Generally, there are two basic mechanical demands for the material and design of the implant. Service stresses must be safely below the yield strength of the material, and in cyclic loads the service stress must be kept below the fatigue limit (Fig. 1E).

The mechanical properties of NiTi depend on its phase state at a certain temperature (Buehler *et al.* 1967, Van Humbeeck *et al.* 1998) (Fig. 1B). Fully austenitic NiTi material generally has suitable properties for surgical implantation. The common mechanical properties of martensitic and austenitic NiTi are presented in Table 1. There are some exceptional properties that might be useful in surgery. NiTi has an ability to be highly damping and vibration-attenuating below A_s . For example, when a martensitic NiTi ball is dropped from a

constant height, it bounces only slightly over half the height reached by a similar ball dropped above the A_f temperature. From the orthopedic point of view, this property could be useful in, for example, dampening the peak stress between the bone and the articular prosthesis. The low elastic modulus of NiTi (which is much closer to the bone elastic modulus than that of any other implant metal) might provide benefits in specific applications. NiTi has unique high fatigue and ductile properties, which are also related to its martensitic transformation. These properties are usually favorable in orthopedic implants. Also, very high wear resistance has been reported compared to the CoCrMo alloy (Sekiguchi 1987). NiTi is a non-magnetic alloy. MRI imaging is thus possible. Electrical resistance and acoustic damping also change when the temperature changes.

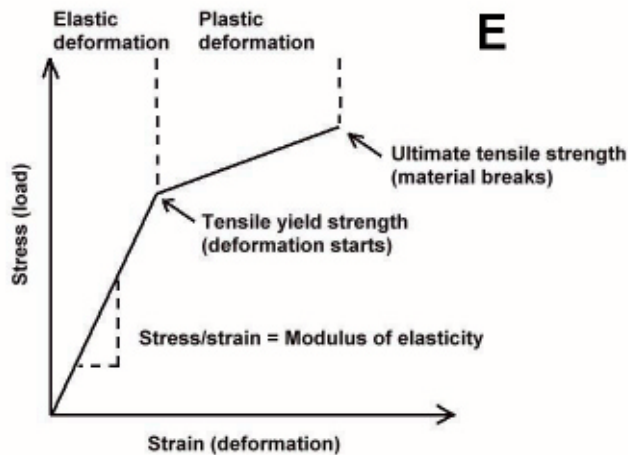


Fig. 1. E) Schematic presentation of the stress-strain behavior of ordinary implant metals. The material exhibits elastic behavior until sufficient stress is applied to reach the tensile yield strength, at which point permanent deformation occurs. In the elastic range, the stress/strain ratio determines the elastic modulus. The metal breaks when the stress exceeds the ultimate tensile strength.

Table 1. Selected mechanical properties of NiTi, implant stainless steel (316LVM), titanium (cp-Ti, grade IV) and Ti-6Al-4V alloy.

	NiTi		Stainless Steel	Titanium	Ti-6Al-4V
	Austenitic	Martensitic			
Ultimate tensile strength (Mpa)	800 - 1500	103 - 1100	483 - 1850	540 - 740	920 - 1140
Tensile yield strength (Mpa)	100 - 800	50 - 300	190 - 1213	390	830 - 1070
Modulus of elasticity (GPa)	70 - 110	21 - 69	190 - 200	105 - 110	100 - 110
Elongation at failure (%)	1 - 20	up to 60	12 - 40	16	8

* Lowest and highest values have been compiled from picked references (Buehler *et al.* 1967, Funakubo 1987, Breme *et al.* 1998, Van Humbeeck *et al.* 1998).

2.3.9. Effect of alloy composition, heat treatment and mechanical working on NiTi properties

It is feasible to vary the critical transition temperatures either by small variations of the Ti/Ni composition or by substituting metallic cobalt for nickel. Lowering of A_f is possible by adding nickel. If nickel is added above 55.6 Wt%, a stable second phase (Ti-Ni₃) forms and the NiTi properties are lost. To avoid this problem, the cobalt substitution can be used to lower the TTR. The properties of NiTi can also be greatly modified by mechanical working and through heat treatment (time and temperature) (Buehler *et al.* 1967).

2.3.10. Fabrication

Solid NiTi alloys are manufactured by a double vacuum melting process, to ensure the quality, purity and properties of the material. After the formulation of raw materials, the alloy is vacuum induction melted (1400°C). After the initial melting, the alloy transition temperature must be controlled due to the sensitivity of the transition temperature to small changes in the alloy chemistry. This is followed by vacuum arc remelting to improve the chemistry, homogeneity and structure of the alloy. Double-melted ingots can be hot-worked (800°C) and cold-worked to a wide range of product sizes and shapes (Andreasen *et al.* 1987).

Porous NiTi can be made by sintering or using self-propagating high temperature synthesis, also called ignition synthesis. The possibility to make composite SMA products (combination with polymers) is under investigation (Brailovski *et al.* 1996).

2.3.11. Programming

The use of the one-way shape memory or superelastic property of NiTi for a specific application requires a piece of NiTi to be molded into the desired shape. The characteristic heat treatment is then done to set the specimen to its final shape. The heat treatment methods used to set shapes in both the shape memory and the superelastic forms of NiTi are similar. Adequate heat treatment parameters (temperature and suitable time) are needed to set the shape and the properties of the item (Otsuka & Wayman 1998). They must usually be determined experimentally for the requirements of each desired part. Rapid cooling of some kind is preferred, such as water quenching or rapid air cooling.

The two-way shape memory training procedure can be made by SME training or SIM training. In *SME training*, the specimen is cooled below M_f and bent to the desired shape. It is then heated to a temperature above A_f and allowed freely to take its austenite shape. The procedure is repeated 20-30 times, which completes the training. The sample now assumes its programmed shape upon cooling under M_f and to another shape when heated above A_f .

In *SIM training*, the specimen is bent just above M_s to produce the preferred variants of stress-induced martensite and then cooled below the M_f temperature. Upon subsequent heating above the A_f temperature, the specimen takes its original austenitic shape. This procedure is repeated 20-30 times.

2.4. Corrosion and surface of metallic biomaterials

2.4.1. General

Various studies have shown that the metallic components of the alloys used in orthopedics may be toxic and dissolve in body fluids due to corrosion (Poehler 1983). Every metal has its own intrinsic toxicity to cells, but the corrosion mostly determines the existing concentration. Thus, the corrosion resistance of the alloy and the toxicity of individual metals in the alloy are the main factors determining its biocompatibility.

The corrosion of metals in aqueous solutions takes place via an electrochemical mechanism. Different metals have different intrinsic aptitudes to corrode. The more noble the metal, the lesser is its aptitude to corrode. Reactions taking place on the metal surface and in the specific environment may cause radical changes in this theoretical nobility. After implantation, the metal is surrounded by serum ions, proteins and cells, which may all modify the effect on local corrosion reactions. The corrosion behavior of a metal in non-physiological *in vitro* studies vs physiological *in vitro* studies vs *in vivo* studies may vary dramatically. Every implant metal corrodes inside the human body (Williams *et al.* 1996). After implantation, elevated metal concentrations are often measured even in distant organs. This is due to ionization, but also to the phagocytosing cells which circulate small metal and metal oxide particles.

Some forms of corrosion are typical of implant use. Corrosion focused to small points is called pitting corrosion. Galvanic corrosion may occur when dissimilar metals are used. The less noble metal becomes anodic and corrodes (stainless steel screws corrode when used with titanium plate). Fretting corrosion occurs when micromotion between two metals breaks their passivation layers (as with screws and plates) (Brown 1987).

There are numerous factors which affect metal corrosion. Porosity and rough surfaces increase the reacting surface area of the implant and thus the total amount of corrosion. The loading areas of the implant are more sensitive to corrosion compared to the less loading areas (Kruger 1983).

The structure, composition and thickness of the passive layer are highly dependent on the metal itself and its environment. Metals contain various elements, such as lattice defects, impurities and contaminants, which may affect the corrosion reaction. The different heat treatments and working processes change the grain size and energy state of the metal and cause surface heterogeneity (Poehler 1983). All these factors may affect the passivation layer.

2.4.2. Passivation and ionization

The corrosion resistance of metals and metal alloys is mainly based on a passivation phenomenon (Kruger 1983). The passivation of a metal is due to the compact coat, the passive layer, which contains hardly any original metal, but forms a metal-oxide layer, a “skin” on the metal. This oxide layer may be amorphous or crystal. The composition of the oxide layer also changes from its outer surface towards the metal. The oxide layer is thicker on implanted metal than on non-implanted metal. Contaminants of Ca and P are generally seen (Kasemo *et al.* 1991).

The human body is a very demanding environment because it is so salty. When metal ions are dissolved from the points where the oxide layer is not fully developed, they form metal hydroxide. This is immediately surrounded by water molecules and then attaches to the passive layer. When there are chloride ions present, as in human plasma, these replace the water molecules from the passive layer. If the passive layer is not fully developed, the dissolved metal ions form a metal-chloride complex which dissolves into body fluids. This impairs local passivity, and may lead to pitting corrosion (Williams *et al.* 1996). When the passive layer breaks locally, this anodic area is very small and the surrounding cathodic area is very large. This may lead to very rapid local corrosion and unexpectedly fast destruction of the material (Kruger 1983).

2.5. Corrosion of NiTi

The corrosion resistance of the implant alloy is a very important determinant of its biocompatibility. As pointed out above, the nature of the environment and the surface treatments have a marked influence on corrosion. Most of the knowledge on the corrosion behavior of NiTi is from studies of dental arch wires and *in vitro* conditions. In fact, the knowledge of the corrosion behavior of NiTi inside the body is very limited.

2.5.1. In vitro corrosion

The good corrosion resistance of NiTi in sea water was first reported by Buehler *et al.* (1967). The attempts to evaluate the corrosion in a simulated physiological environment and the comparisons with other implant alloys were made much latter.

Speck *et al.* (1980) found that, in Hank's solution, titanium materials, including NiTi, have better corrosion resistance than Co-Cr-Mo or 316L stainless steel. The addition of cysteine amino acid to the solution caused a lower breakdown potential for Ti-Ni, but did not affect the breakdown of Ti-6Al-4V.

Edie *et al.* (1981) compared the corrosion of used and unused NiTi and stainless steel orthodontic wires. They concluded that NiTi wires are no more subject to corrosion than stainless steel.

Sarkar *et al.* (1983) found NiTi to be more sensitive to corrosion than titanium in 1% NaCl solution. Pitting of the NiTi surface was observed, and they speculated that this pitting could be due to selective dissolution of nickel from NiTi.

When NiTi was tested in artificial saliva, the release rates of nickel from stainless steel and nickel-titanium arch wires were not significantly different (Barrett *et al.* 1993).

Better resistance to the chemical breakdown of passivity was found for the NiTi alloy compared to AISI 316 LVM (American Iron and Steel Institute, and low vacuum melted) (Wever *et al.* 1998).

When stainless steel (316L) was coupled with NiTi and subjected to an immersion corrosion test in 37° C, 0.9 wt% sodium chloride solution, 316L was found to suffer from crevice corrosion (Platt *et al.* 1997).

Contrary to the above studies, Rondelli (1996) found that the Ni ion release was three times higher for NiTi than for austenitic stainless steels when evaluated in physiological simulating fluids. NiTi had good resistance to pitting similarly to Ti6-Al-4V. Tests in which the passive film was abruptly damaged indicated that the characteristics of the passive film formed on NiTi are not so good as those on Ti6-Al-4V, but are comparable or inferior to those on austenitic stainless steels.

Montero-Ocampo *et al.* (1996) found annealed NiTi to be more corrosion-resistant than cold-worked material. Thus, the heat treatment and mechanical working had a significant influence on corrosion behavior. The same study also indicated that straining of NiTi led to significant improvements in corrosion resistance. This may be due to the development of a single martensite variant during deformation.

2.5.2. In vivo corrosion

Castleman *et al.* (1976) reported no generalized or localized corrosion on NiTi plates under microscopic examination at magnifications of 50x with a maximum follow-up of 17 months after implantation in dogs. Neutron activation analysis of distant organs in the same study showed no accumulation of trace metals from NiTi.

When Cragg *et al.* (1993) implanted forty-four NiTi intraluminal stents in the iliac arteries of 22 sheep, only minimal corrosion was seen at 6 months. Pitting was the predominant type of corrosion. They estimated the mean pit penetration rate to be approximately 0.0046 cm per year. Corrosion product analysis around the pit sites indicated that the main product of pitting was a titanium-bearing compound, probably an oxide. The clinical importance of this finding is not known, because no such corrosion studies have been performed on other stent materials in similar conditions.

2.5.3. Improving the corrosion resistance of NiTi

As there is some dissolution of nickel from NiTi, some surface treatments have been introduced to improve corrosion properties.

The titanium nitride coating of NiTi prepared by the arch ion plating method was found to improve corrosion resistance in 0.9% NaCl solution (Endo *et al.* 1994).

In the next two pioneering studies by the same author, the corrosion resistance of the NiTi alloy was enhanced by chemical modification with human plasma fibronectin via aminosilane (γ -APS) and glutaraldehyde as coupling agents. It was found that the corrosion rate decreased by approximately 50% with this surface modification in a 0.9% NaCl solution and a cell culture medium containing serum. The reduced corrosion rate was accompanied by a significant reduction in the release of nickel ions from the NiTi alloy substrate (Endo 1995b). The greatest insight of the above treatment was the idea to introduce biofunctional protein precoating to acquire desirable adhesion properties of the NiTi surface (Endo 1995a,b). The stability and durability of this surface remain unassessed.

A plasma-polymerized tetrafluoroethylene (PPFTE) coating has been used to improve the corrosion resistance of NiTi plates and corresponding NiTi stables. A PPTFE coating improved the pitting corrosion resistance. The passivation range increased from 35% to 96% compared to an untreated sample, and the pit diameter decreased from 100 microns to 10 microns (Villiermaux *et al.* 1996). The coating complies with the large deformations induced by the memory effect of the alloy without cracking. However, when the film is damaged, corrosion seems to increase in comparison to untreated samples. A surface of this kind may be suitable to stent applications, but its durability in orthopedic surgery may be insufficient because of the fretting surface loads.

The addition of Cu raises the repassivation potential of NiTi and may improve its corrosion resistance. This was not proved in a study by Wen *et al.* (1997), but the corrosion potential and corrosion rate of Ti50Ni50-xCux ($x = 1, 2, 4, 6, 8$) alloys are irrelevant to its Cu content and the values are almost the same as those of NiTi alloys.

The study by Iijima *et al.* (1998) showed that small amounts of Cr and Cu added to change the super-elastic characteristics do not change the corrosion resistance of the Ni-Ti alloy in simulated physiological environments.

The laser surface treatment of NiTi (i.e. melting of the surface) leads to an increase of the oxide layer, a decrease of superficial Ni and an improvement of the cytocompatibility of NiTi up to the Ti level (Villiermaux *et al.* 1997).

Surface chemistry may be a more important determinant of platelet behavior than surface topography. There were no cytotoxic or hemolytic effects of any of the surface-treated NiTi samples (annealed, polished or shot-peened). However, platelet spreading (size) after attachment showed dependence upon the NiTi surface treatment and was more abundant for NiTi compared to titanium and stainless steel (Armitage *et al.* 1997).

The covering of NiTi stents with biodegradable PLA material has been shown to be inferior in *in vivo* conditions. Elastic mismatch of the non-elastic coating and the self-expandable NiTi stent led to misplacement and vessel occlusion, probably due to PLA filaments fraying into the vessel lumen (Schellhammer *et al.* 1997).

In the most recent study by Trepanier *et al.* (1998), the effects on the corrosion resistance and surface characteristics of electropolishing, heat treatment, and nitric acid passivation of NiTi stents were studied. The results show that all of these surface treatments improve the corrosion resistance of the alloy. This improvement was attributed to the removal of the plastically deformed native oxide layer and its replacement by a newly grown, more uniform one. The authors concluded that the uniformity of the oxide layer, rather than its thickness and composition, seems to be the predominant factor to explain the improvement of corrosion resistance.

2.6. Surface of NiTi

The surface of NiTi consists mainly of titanium oxides (TiO_2) and smaller amounts of nickel oxides (NiO and Ni_2O_3) and metallic Ni, while nickel-titanium constitutes the inner layer (Hanawa 1991, Oshida *et al.* 1992, Endo 1995a,b, Shabalovskaya 1996, Yahia *et al.* 1996). The thickness of the oxide layer varies within 2-20 nm. Depending on the preparation method, the surface chemistry and the amount of Ni may vary over a wide range (Trigwell *et al.* 1997). The surface of untreated NiTi is mostly composed of oxygen, carbon, and titanium oxide with traces of nickel. Nickel may dissolve more easily than titanium because its oxide is not so stable. Surface layers of nickel-titanium arch wires have been found to have irregular features characterized by lengthy island-like structures, where selective dissolution of nickel may occur (Oshida *et al.* 1992).

Shabalovskaya (1996) found that when the surface was mechanically polished, the Ti/Ni ratio was 5.5, showing that there was five times more titanium on the surface. When the item was boiled or autoclaved in water, the concentration of Ni decreased and the Ti/Ni ratio increased up to 23.4-33.1. The findings of Hanawa (1991) were similar. The Ti/Ni ratio was 5.8 in polished samples, but increased even up to 91 when the sample was immersed for 30 days in a neutral electrolyte solution. In the above mentioned study by Hanawa, the Ti-6Al-4V surface had similar amounts of aluminum as NiTi had nickel, even though the bulk material of Ti-6Al-4V had only 6% aluminum and NiTi had 50% nickel. There was no nickel on the surface of stainless steel, but its surface had Cr and Fe.

Pure titanium and some of its alloys are considered to be among the most biocompatible materials (Albrektsson *et al.* 1981). The good biocompatibility is thought to be due to the stable titanium oxide layer. During implantation, the oxide layer formed on a Ti implant grows and takes up minerals and other constituents of biofluids, and these reactions, in turn, cause remodeling of the surface. Hanawa found that the surface oxide film on implants consisted two layers, calcium phosphate and titanium oxide. In other words, calcium phosphate formed on a passive oxide film. This film was thicker on pure titanium than on titanium alloys (including NiTi), and the Ca/P value of the film was close to that of hydroxyapatite. The calcium phosphates formed on NiTi or Ti-6Al-4V were less similar to hydroxyapatite. The presence of nickel in the surface film on NiTi and that of aluminum in the film on Ti-6Al-4V may have caused these results. Stainless steel also has a calcium phosphate layer of this kind. However, the formation of this layer is slower and differs in this respect from NiTi (Hanawa *et al.* 1991, Hanawa 1991, Hanawa *et al.* 1998). The good biocompatibility of NiTi and other titanium alloys may be the cause of the calcium phosphate film, while corrosion resistance is the cause of the passive oxide film (Hanawa *et al.* 1991). The findings of Hanawa were also supported by a recent study by Wever *et al.* (1998). As far as implantation is concerned, more surface studies are certainly needed to clarify the basic surface structures *in vivo*.

2.7. Biocompatibility aspects of NiTi alloy components

It is necessary to review the biocompatibility of NiTi alloy components for several reasons: 1) There is only little knowledge about the biocompatibility of NiTi. 2) Components may dissolve from NiTi due to corrosion. 3) Alloy components may form some compounds which have their own effects and toxicity. 4) Nickel may have deleterious effects. 5) Titanium may have some deleterious effects, especially in a particular form.

The corrosion resistance of the alloy and the toxicity of the individual metals that make up the alloy are the main determinants of biocompatibility. The properties and biocompatibility of NiTi have their own characteristics, which are different from those of nickel or titanium alone. Due to corrosion, however, nickel and titanium ions may dissolve from NiTi. To understand the possible host effects of NiTi, it is very important to understand the effects of its components. The local and systemic toxicity, carcinogenic effects, immune response, and teratogenic aspects of nickel will be reviewed in detail below. This matter is essential because of the high nickel content of the NiTi alloy. Titanium, the other component of NiTi, will be discussed briefly.

2.7.1. Nickel: absorption and elimination

Nickel is received into the body via the lungs, oral intake and skin. The average oral intake from the diet is estimated to be 150 microgram/person/day and may increase up to 900 micrograms/person/day or more (Flyvholm *et al.* 1984). Only a minor amount (1%) of the nickel from food is adsorbed into body from the intestine, but one fourth of the nickel from drinking water is adsorbed (Sunderman *et al.* 1989).

In blood, nickel is mainly bound to the albumin fraction, but also to many other proteins of serum (Nielsen *et al.* 1994). The serum and blood values vary within < 1-5 µg/l (Iyengar *et al.* 1994, Andreassi *et al.* 1998).

Most of the nickel is eliminated into urine (90%) and some into feces. The elimination half-life of nickel is quite rapid (Sunderman *et al.* 1989), but the elimination of different nickel compounds may be radically different (Oller *et al.* 1997).

2.7.2. Nickel in tissues

There has been great variation in the concentrations of nickel in human tissues reported in the literature. Standard reference values are still missing. The older methods of measurement and sample processing have involved many sources of error. There is also some variation in the concentrations between different animal species and humans due to metabolic and other factors. The suggested normal nickel concentrations in human tissues are (microgram/kg of dry weight): 173 in lung, 62 in kidney, 54 in heart, 50 in liver, 44 in brain, 37 in spleen and 34 in pancreas (Rezuke *et al.* 1987).

Increased nickel concentrations have been found in tissues adjacent to stainless steel implant materials (116 and 1200 mg/L) as well as in some distant organs (Michel *et al.* 1978, Bergman *et al.* 1980, Poehler 1983). The maximum rate of Ni release due to corrosion in patients with implants made of Ni alloys is estimated to be 20 µg/kg/day (Black 1981). Infection may raise the peri-implant nickel concentrations (Hierholzer *et al.* 1984).

2.7.3. Nickel as an essential trace element

Nickel is one of the trace elements essential for vertebrates, including humans. Nickel deficiency in goats, rats and chicks has been found to have many deleterious effects and pathological consequences. These include general disorders, such as reduced growth, weight loss and increased perinatal mortality (Anke *et al.* 1984). Skin changes, including altered skin pigmentation, parakeratosis and uneven hair development, have been reported (Szilagyi *et al.* 1991).

Nickel deficiency impairs the metabolism of iron, fats, glucose, and glycogen. It may disturb the incorporation of calcium into the skeleton and decrease the length:width ratios of chick tibias and femurs. Animals with nickel deficiency have been found to have depressed activity of several enzymes in the heart, liver and kidneys as well as degeneration of cardiac and skeletal muscle (Szilagyi *et al.* 1991). Changes in the liver have also been reported. These include differences in the rough endoplasmic reticulum, decreased liver cholesterol and triacylglycerol accumulation (Nielsen *et al.* 1975, Nielsen *et al.* 1984, Stangl *et al.* 1996).

2.7.4. Toxicity and carcinogenicity of nickel

The chemical toxicity of metal inside the body is closely related to the concentration of released ions and wear particles, the toxicity of these elements and the toxicity of the formed compounds. Even a poisonous substance has no toxic effects in small concentrations, while nutritious substances cause adverse responses when present in excessive amounts. It is difficult to know the exact concentrations of metallic compounds released from implanted material, because there are many factors affecting them, such as implantation time and the local conditions (PH, fretting, etc.).

The high nickel content of NiTi (54 % by weight) may cause biocompatibility problems if deleterious amounts of nickel dissolve from it. The toxicity of nickel has been studied using *in vitro* and *in vivo* nickel salts, solid nickel or particulate form nickel (Putters *et al.* 1992, Takamura *et al.* 1994).

The problem with using metal salts is that the toxicity of different nickel salts vary notably. The benefit of this method is that we know the exact composition of the nickel salt, and it also permits the use of very high concentrations. The benefits and weaknesses of using nickel powder are that the particle itself may have toxic, irritating and even carcinogenic effects. This has been documented with alloys normally non-toxic, such as titanium (Zhang *et al.* 1998, Maloney *et al.* 1998). Another problem associated with reading *in vitro* results is that different cells have different toxic responses. The benefit of using

solid nickel is that solid nickel *in vitro* usually correlates in situation *in vivo*, but we cannot be sure what kind of compounds have the effect we observe. The benefit of solid and particle material testing is that metal alloys can also be tested. Also, *in vitro* methods can never simulate the *in vivo* environment completely, and these results can only be considered suggestive.

Nickel is known to have toxic effects with cellular damage in cell cultures at high concentrations (Putters *et al.* 1992). It also appears to be harmful to bone in tissue cultures, but less so than cobalt or vanadium, which are also routinely used in implant alloys (Gerber *et al.* 1980). The toxicity of metal salts in cell cultures has shown decreasing toxicity in the order cobalt > vanadium > nickel > chromium > titanium > iron (Yamamoto *et al.* 1998). *In vitro* tests have also shown cobalt, nickel and chromium to have a potency for carcinogenicity.

Pure nickel implanted intramuscularly or inside bone has been found to cause severe local tissue irritation and necrosis (Laing *et al.* 1967) and to have high carcinogenic and toxic potencies. The tumors that retained nickel were malignant fibrous histiocytomas or fibrosarcomas (Takamura *et al.* 1994). Inhaled Ni₃S₂ caused adenomas and carcinomas of the lungs in rats, but nickel oxide and sulphate did not (Oller *et al.* 1997).

Due to the corrosion of the implants, small amounts of metal ions may also be released into distant organs. Systemic toxicity may be caused by the accumulation, processing, and subsequent reaction of the host to corrosion products (Bergman *et al.* 1980, Lugowski *et al.* 1991, Ishimatsu *et al.* 1995).

When high-dose nickel salts were injected into mice, accumulation and some deleterious effects were seen in the liver, kidney and spleen (Pereira *et al.* 1998).

We do not know what compounds form inside the body after the implantation of nickel-containing alloys. However, it is likely that NiCl and NiO compounds may form in the body environment, while the most toxic and carcinogenic compounds, e.g. Ni₃S₂, are not likely to occur. The underlying mechanism of the carcinogens of nickel is still unclear (Hartwig *et al.* 1994, Oller *et al.* 1997).

In vivo, Ni²⁺ ions may cross the cell membrane using the Mg²⁺ ion transport system. Since the concentration of Mg²⁺ inside and outside the cell is in the millimolar range, the levels of soluble nickel needed to compete with Mg²⁺ for its uptake must be at least in the millimolar range. Additionally, once Ni²⁺ is inside the cell, it binds to cytoplasmic ligands and it does not accumulate in the cell nucleus at the concentrations needed to have a genetic effect (Abbracchio *et al.* 1982a, Abbracchio *et al.* 1982b). In addition, soluble Ni²⁺ is rapidly cleared *in vivo*, which is why no direct efficient delivery of Ni²⁺ to the target site within the cell nucleus may occur to cause carcinogenic effects *in vivo* (Oller *et al.* 1997). Thus, carcinogenesis seems to be related to some nickel compounds rather than Ni²⁺ ions.

Another way in which nickel may be harmful is the effect of phagocytosed nickel compound particles. Some of the characteristics of nickel compounds that increase their ability to be endocytosed include crystalline nature, negative surface charge, 2–4 µm range particle size, and low solubility (Sunderman *et al.* 1987). Ni₃S₂ and NiO, which show otherwise low *in vivo* solubility may act by this mechanism (Dunnick *et al.* 1995). It was shown early on that endocytosis by target cells was likely to play an important role in the transforming potential of nickel compounds (Costa *et al.* 1980). When the nickel compound particles are endocytosed by the target cells, the endocytic vesicles are acidified by

fusion with lysosomes and Ni^{2+} is released. Deleterious changes, such as the formation of oxygen radicals and DNA damage and the inactivation of tumor suppressor genes, may occur (Klein *et al.* 1991a, Klein *et al.* 1991b).

Pathological alterations of nickel metabolism have been recognized in several human diseases. The diverse clinical manifestations of nickel toxicology include (1) acute pneumonitis from inhalation of nickel carbonyl, (2) chronic rhinitis and sinusitis from inhalation of nickel aerosols, (3) cancers of nasal cavities and lungs in nickel workers, and (4) dermatitis and other hypersensitive reactions from cutaneous and parental exposures to nickel alloys (Sunderman 1977).

2.7.5. Nickel-containing biomaterial alloys in humans

Neoplasms associated with clinical implants are very rare. They may be related more to the physical than the chemical configuration of the implant. The mechanism of tumor formation is not understood, but it appears to be related to the implant fibrous capsule (Schoen 1996). Occasional reports on humans have been published, which report the development of malignant fibrous histiocytomas and osteosarcomas at the site of a prosthetic replacement or previous internal fixation. Most of these (> 80%) have been related to the cobalt-chromium alloy, some to stainless steel or other nickel-containing alloys, and none to titanium (Rock 1998).

The low toxicity of a constituent does not exclude the possibility of deleterious effects. As local or systemic toxicity is usually dose-dependent, reactions caused by the immune response may activate at much lower thresholds (Remes *et al.* 1992).

Nickel is the major cause of allergic contact dermatitis (Peltonen 1979). Epidemiological studies have shown a sensitization frequency up to 20 % in young females and 10 % in the elderly (Menne 1996). Two to four percent of males are sensitized. Most cases of nickel allergy may be related to skin contact with nickel-containing metallic items. The significant biological parameter is not the nickel concentration in the alloy or the coating, but the amount released to the skin during exposure to human sweat. A threshold of 0.5 microgram/cm²/week has been established, at which only a minor part of nickel-sensitive subjects will react (Menne 1996).

When implants containing perceptible amounts of nickel, for example, stainless steel implants (nickel content 10-14 %), are clinically used inside the body, no sensitization or immune disorders commonly occur (Christensen 1990, Gawkrödger 1993). Why could it be used even in patients with nickel contact dermatitis?

Allergic contact dermatitis is a cell-mediated immune response caused by Ni^{2+} ions. In fact, the nickel ion itself is too small to act as an antigen. It binds with a carrier protein and acts as a hapten. The nickel-protein complex activates Langerhans' cells in the skin, which presents an antigen to T-lymphocytes. Memory T-cells develop. When circulating in the body, these memory cells are able to start cell-mediated immune reactions upon meeting the same allergen again.

The antigenic determinants created by nickel as well as the mechanisms of recognition by specific T-cell clones have not been elucidated (Moulon *et al.* 1995). T-cells detect haptens as structural entities attached covalently or by complexation to self-peptides anchored in the binding grooves of major histocompatibility antigens (MHC proteins) (Weltzien *et al.* 1996).

Two major types of hapten-specific T-cell receptors have been identified: one reacting to hapten regardless of the chemical composition of the carrier peptide, and the other contacting hapten and peptide via two apparently independent contact sites (Martin *et al.* 1994). The present study suggests that the presence of specific CD8⁺ T-cells and a distinct pattern of cytokine release (e.g. augmented production of interleukin-10) by CD4⁺ T-cells may be important elements in determining whether a hapten induces allergy or a silent immune response (Cavani *et al.* 1998). T lymphocytes are critical effectors in the pathogenesis of contact hypersensitivity. Nickel-specific CD4⁺ T helper cells have been extensively characterized. The characterization of nickel-specific cytotoxic CD8⁺ T-cells with different requirements for nickel-specific target lysis may have important implications for the development or control of human contact hypersensitivity reactions to nickel *in vivo* (Moulon *et al.* 1998).

The intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1), and the endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin) are endothelial surface molecules that play a role in leukocyte recruitment to sites of inflammation during, for instance, contact hypersensitivity. NiCl₂ and, to a lesser extent, CoCl₂ were found to up-regulate ICAM-1, VCAM-1, and ELAM-1 expression on cultured human umbilical vein endothelium. Both Ni²⁺ and Co²⁺, which frequently induce simultaneous contact sensitivity, have the ability to directly up-regulate endothelial adhesion molecules. This shared property may represent an adjuvant mechanism that promotes sensitization and elicitation events in contact hypersensitivity to these haptens (Goebeler *et al.* 1993). It was observed recently that Ni ions can either promote or suppress the expression of the intercellular adhesion molecule 1 (ICAM-1) on endothelial cells, depending on their concentration and probably the time of exposure. ICAM-1 is known to be involved in the recruitment of inflammatory cells from the bloodstream. Ni ions could promote the expression of ICAM-1 at concentrations high enough to suppress cell metabolic activity. At lower concentrations, they suppress ICAM expression (Wataha *et al.* 1997).

Control of the allergic reaction also requires inhibitory systems which prevent the immune response from causing systemic damage. To control the reactions, several kinds of suppressor T-cells are generated at different levels (Barnetson *et al.* 1993). Unresponsiveness to oral exposure (oral tolerance) to nickel is due the action of these suppressor cells (van Hoogstraten *et al.* 1992, Ishii *et al.* 1993). This is also the presumptive explanation for why sensitization and immune disorders from metallic prostheses are very unusual, although, for example, the stainless steel used in implants contains perceptible amounts of nickel (Björholm *et al.* 1990, Gawkrödger 1993, Milavec-Puretic *et al.* 1998).

2.7.6. Titanium

It is generally accepted that pure titanium is extremely well tolerated by local tissues and induces neither toxic nor inflammatory reactions (Branemark *et al.* 1969, Toth *et al.* 1985, Linder *et al.* 1988, Pfeiffer *et al.* 1994). The normal tissue concentration of titanium in humans is 0.2 ppm. Around the titanium implants no clinical tissue toxicity has been observed even at local concentrations higher than 2000 ppm (Hildebrand *et al.* 1998). In optimal situations, titanium is able to osseointegrate with bone, thus forming a direct contact with bone at the light microscopy level (Branemark *et al.* 1969). The good bone contact may be due to the ability of titanium to form a Ca-P rich layer on its surface (Hanawa 1991). Titanium is bacteriostatic (Elagli *et al.* 1992) and does not significantly activate or inhibit different enzyme systems specific to toxic reactions, e.g. β -glucuronidase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and acid phosphatase (Elagli *et al.* 1995). The good biocompatibility and corrosion resistance are due to the naturally forming stable titanium oxide (TiO_2) film on titanium surfaces (Zitter *et al.* 1987, Kasemo *et al.* 1991).

Particles from titanium arise from the passivation layer of the implant, but they are not titanium ions, but mostly insoluble titanium oxides or suboxides, which are recognized to be biologically inert. Indeed, the passivation layer is immediately reformed after abrasion because of the high oxidizability of titanium. This behavior protects the alloy and prevents the formation of chemical compounds other than oxides (Hildebrand *et al.* 1998). Tissue discoloration due to titanium oxide particles is sometimes seen around pure titanium implants, but this seems to have no clinical consequences (Onodera *et al.* 1993, Rosenberg *et al.* 1993). Experiments with laboratory animals and some limited analyses of human tissues have also revealed evidence of titanium release into distant tissues (Schliephake *et al.* 1993, Jorgenson *et al.* 1997).

Wear particles produced by abrasion appear especially in the vicinity of articular prostheses and implants with certain mobility, e.g. uncemented total hip replacements. These particles may induce multiple tissue reactions, including osteolysis, degradation of normal bone structure, severe macrophagic reactions, granuloma, fibrotic capsules and chronic inflammation, which may cause destabilization and loosening of prostheses and implants (Santavirta *et al.* 1991, Santavirta *et al.* 1993, Rubash *et al.* 1998). Particle size and composition are of essential importance in that process. Deleterious reactions have been reported with Ti-6Al-4V based prostheses (Nasser *et al.* 1990, Rubash *et al.* 1998), but not with pure titanium implants.

In vitro, pure titanium particles have also been shown to have some effects on cells. Low concentrations may stimulate fibroblast proliferation, while high concentrations may be toxic. At high particle concentrations, titanium caused a decrease in proteolytic and collagenolytic activity in the culture medium. Titanium also elevated the lysosomal enzyme marker, hexosaminidase, except at high concentrations (Maloney *et al.* 1993).

Titanium osseosynthesis plates have been observed to be totally recovered by newly formed bone tissue after an exposure period of 3-4 years. The retrieval of such implants becomes particularly difficult (Hildebrand *et al.* 1998).

2.8. Biocompatibility of NiTi

2.8.1. Biocompatibility of NiTi *in vitro*

Only a few *in vitro* studies of cell response to NiTi have been reported. The results have been slightly contradictory. These differences may be due to differences in test protocols, including different cell types, different observed factors, variations in surface treatments, surface area, surface roughness, etc.

A preliminary report intending to evaluate the acceptance of NiTi *in vitro* was published by Castleman & Motzkin (1981). Human fetal lung fibroblasts were used in that study. The results were surprising: 316L stainless steel and Co-Cr alloy did not differ in cell growth from the control cultures, but NiTi and more titanium significantly reduced cell growth. The morphological changes of cells with NiTi and titanium were also more pronounced. As far as titanium is concerned, these findings are contradictory to most later studies. Titanium is considered one of the best accepted metals *in vitro* and *in vivo* (Trentz *et al.* 1997, Doran *et al.* 1998).

In a well-monitored study by Putters *et al.* (1992), the effects of increasing dose exposure to NiTi, nickel or titanium in cell cultures were examined. The results showed that nickel induces a significant inhibition of mitosis in human fibroblasts, whereas no significant effects of this kind were found for titanium or NiTi. NiTi was considered biocompatible and comparable to titanium.

More confusing results were also reported when direct contact and agar diffusion cytotoxicity assays were performed using Confluent L-929 fibroblasts. Cells were incubated in the presence of NiTi, titanium, Co-Cr-Mo and 316L stainless steel discs. The evaluation of cytotoxic reactions was done under light microscopy. Both assays indicated that all metals induced a mild biological reaction. The cytotoxicity of NiTi was found to be approximately equal to that of Co-Cr-Mo, both being more than that of pure titanium, Ti-6Al-4V or 316L stainless steel. The authors of that study also used NiTi samples with plasma surface treatment, which was found to increase the cytocompatibility of NiTi (Assad *et al.* 1994).

Endo *et al.* (1995) reported that human plasma fibronectin (pFN), an adhesive protein, can be covalently immobilized onto NiTi substrate. Fibronectin significantly improved human gingival fibroblast spreading, suggesting that this chemical modification enables the controlling of metal/cell interactions.

The results of a study in which various surface treatment effects were studied in rat splenocytes showed that cells exposed to NiTi are critically affected by the surface preparation. The hydrogen peroxide surface treatment of NiTi caused a toxic effect comparable to that of pure nickel. However, the situation changed tremendously when NiTi was treated by autoclaving in water or steam. The reaction with these NiTi specimens was clearly non-toxic. The explanation for this was that the Ni surface concentration may vary from 0.4 to 27%, depending on the specific surface treatments used (Shabalovskaya 1996).

In conclusion, earlier *in vitro* studies have neither established the position of NiTi among the metallic biomaterials nor confirmed its ultimate cytocompatibility.

Weaver *et al.* (1997) evaluated the short-term biological safety of the NiTi alloy. They used an end-point dilution minimal essential medium extract cytotoxicity test, a guinea-pig sensitization test and two genotoxicity tests: the Salmonella reverse mutation test and the chromosomal aberration test. The NiTi alloy showed no cytotoxic, allergic or genotoxic activity. The findings were similar to those on AISI 316 LVM stainless steel. They concluded that the NiTi alloy can be regarded as a biologically safe implant material.

The *in vitro* genotoxicity of NiTi has also been evaluated using human peripheral blood lymphocytes. A comparison was made with commercially pure titanium and 316L stainless steel. Cells were cultured in a semiphysiological medium that had previously been exposed to the biomaterials. An electron microscopy *in situ* end-labeling assay was performed to provide quantification of *in vitro* chromatin DNA single-stranded breaks. NiTi, titanium and stainless steel induced similar DNA strand breaks of interphase chromatin, but stainless steel induction on metaphase chromatin was more intense than with NiTi or pure titanium. The authors concluded that NiTi genocompatibility is promising in view of its biocompatibility approval (Assad *et al.* 1998).

The influence of the corrosion products of different orthodontic wires on the cytotoxicity of a fibroblast culture was investigated by Rose *et al.* (1998) using Mosmann's MTT test. Ion release was assessed by ICP-AES analysis. NiTi, stainless steel and beta-titanium alloy wires had no effect on the rate of cell proliferation. The most severe growth inhibition was induced by the Co-Cr-Ni alloy. The degree of growth inhibition depended upon the concentration of corrosive cobalt and nickel ions in the elute.

2.8.2. Muscle response to NiTi

The first published study on the reaction of tissue to 55-NiTi was reported in by Cutright *et al.* (1973). In that study, NiTi wire sutures were placed subcutaneously in forty-five rats, which were followed for 9 weeks. The tissue reaction was minimal at all checkup points. The reparative process was initiated within 1 to 2 weeks and resulted in a dense, relative avascular fibrous connective tissue capsule by 5 to 6 weeks, with little change beyond that. When compared to the tissue reaction to stainless steel seen in earlier experiments, NiTi was indistinguishable from stainless steel within similar time periods. It was concluded that 55-NiTi compares favorably with stainless steel and could be used in deep tissues. The lack of a simultaneous control group, the short implantation time (nine weeks) and the non-standardized (subcutis or muscle) implantation site may have caused some uncertainty to the results.

The first attempt at a profound biocompatibility evaluation of NiTi was made by Castleman *et al.* (1976). The methods of that study were versatile and the approach was well-advised. This study has often been used as a reference study when discussing the biocompatibility of NiTi. There were, however, some weaknesses in the study, which were also pointed out by the authors. These may have critically affected the final conclusions. First, the total number of test animals was quite small. There were three dogs in the NiTi implant group and one Co-Cr implant and one "sham" as a control at each killing point. The complete NiTi data consisted of 12 beagles examined after exposures of 3, 6, 12, and 17 months. The maximum follow-up time can be considered sufficient for the conclu-

sions, at least as far as implant use in fracture fixation is concerned. The NiTi alloy used in the experiment was laboratory-prepared and had no commercial counterpart. The analysis of scar capsule membrane thickness seems to be based on an invalid hypothesis. Statistical tests were used, expecting no significant differences between the mean thickness values of the scar capsules associated with NiTi and those associated with the Co-Cr alloy. However, the authors admitted earlier that “considerable variation was evident between the capsules of different specimens of same material and between the capsules of different metals and also depending on implantation time”. Thus, it seems that the statistical data in this case cannot be used as a basis of relevant conclusions. The muscle tissue in dogs exposed to NiTi implants for 17 months showed some variability. The areas adjacent to or overlying the screw head showed a looser arrangement of striated muscle fiber bundles with larger areas of areolar connective tissue between the muscle fibers. Overall, the gross clinical, radiological, and morphological observations of tissue at the implantation sites at autopsy revealed no signs of adverse tissue reactions resulting from the implants. The study warranted the conclusion that NiTi had no clearly toxic effects *in vivo*. The authors concluded that no significant differences were noted between the samples taken from the controls and those taken from the dogs exposed to the implants, and that NiTi alloy is sufficiently compatible with dog tissue to warrant further investigation of its potential as a biomaterial.

It is astonishing that no further comprehensive studies on the tissue reaction to NiTi have been published so far.

Recently, one comparative study was published, in which the corrosion resistance and tissue biocompatibility of NiTi and Ti50Ni50-xCux ($x = 1, 2, 4, 6, 8$) alloy were investigated. Electrochemical and quantitative histomorphometric methods were used. The connective tissue layer covering the Ti50Ni42Cu8 plates was statistically significantly thicker than that of Ti50Ni50, Ti50Ni48Cu2, or Ti50Ni44Cu6 plates after one month. The numbers of connective tissue cells, polynucleated cells, macrophages and round cells were higher for Ti50Ni42Cu8 plates than those of the other three types of plates, but no statistically significant differences were detected. There were no significant differences in the tissue reaction parameters after two and three months between the four alloys. After three months' implantation, no corrosion was observed on the plate surfaces. It was concluded that Ti50Ni50-xCux ($x = 2, 6, 8$) shape memory alloys also have good biocompatibility (Wen *et al.* 1997).

2.8.3. Bone response to NiTi

NiTi is one of the most innovative concepts introduced in the field of metallic biomaterials in the recent years, but its biocompatibility remains controversial, especially in bone.

The first attempts to study NiTi as a bone implant were made also by Castleman *et al.* (1976). A prototype of NiTi bone plates was made and implanted into the femurs of 12 beagles. Commercial cobalt-chromium (Co-Cr) alloy bone plates served as reference controls (1 per time period). The plates were removed from the animals and examined after exposure for 3, 6, 12, and 17 months. There was no evidence of either localized or general corrosion on the surfaces of the bone plates and screws. No signs of adverse tissue reac-

tions resulting from the NiTi implants were seen. Decalcified histological samples showed no evidence of bone resorption in specimens adjacent to the plate. Nor were any significant differences noted in the sham-operated controls. The data used in neutron activation analyses suggested that there is no nickel contamination in bone due to the implants. However, the authors suggested that there does appear to be some chromium contamination from the Co-Cr alloy implants in the adjacent bone. The results of neutron activation analysis implied some uncertainty associating with the contamination of samples during the cutting procedure. In the NiTi group, some high nickel concentrations were also observed, but these were attributed to contamination.

Yang *et al.* (1992) made their own internal fixing device of NiTi and applied it to fractured femoral shafts of dogs. Comparison was made with a 316L stainless steel plate-screw system. Osteotomy on both sides of the femoral diaphyses was performed in 15 dogs. One side was plated with a bone plate and the other with a NiTi device. Five animals in each group were killed at 4, 8, 12 weeks after operation. Radiographic examination, light microscopy and transmission electron microscopy methods were used. The fracture healing and the course of callus remodeling were similar in these two groups, but the cortical bone remodeling underneath the fixator near the osteotomized area was significantly different. The authors suggested that since the elastic modulus of the NiTi shape memory alloy is lower, the stress-shielding effect in the bone underneath the NiTi device is less. The axial compression stress of the fracture line is kept greater and the contact of that NiTi device with the bone was not so close. This might be beneficial for the recovery of blood supply and bone remodeling.

A preliminary report on the use of porous NiTi was published by Simske & Sachdeva (1995). The material has a controllable open structure that provides a possibility for the ingrowth of bony tissue into the body of the implant, resulting in desirable firm fixation to bone. Eight uncoated porous NiTi implants (average pore size 300µm; 50% average void volume) were placed to either side of the frontal bone of rabbits. In the other frontal location, a coralline hydroxyapatite implant of was fitted as a control. The animals were killed at post-surgical intervals of 2 (n=2), 6 (n=2), and 12 (n=3) weeks. The implants were evaluated for gross biocompatibility, bony contact, and ingrowth. Overlaying soft tissues and connective tissues readily adhered to the implants even after 2 weeks. No adjacent macrophage cells were seen for either implant type. Both materials made bone contact with the surrounding cranial hard tissue, and the percentage of ingrowth increased with the surgical recovery time. The bone histology and microhardness parameters showed that the bone in contact with the implants was similar in quality to the surrounding cranial bone. Porous NiTi implants appear to allow for significant cranial bone ingrowth after as few as 12 weeks. Compared to HA, the NiTi implants demonstrated a trend for less total apposition and more total ingrowth after 6 and 12 weeks of implantation. The authors concluded that porous NiTi appears to be suitable for craniofacial applications. The small number of animals used in this study can be criticized. It allows no quantitative conclusions. The implantation time was also quite short, but the bone response was still good. Further studies are needed for the conclusions on final biocompatibility and the value of porous NiTi in craniofacial or other bone-related applications.

A new type of ear stapes prosthesis made of nickel-titanium shape memory alloy wire was developed by Kasano & Morimitsu (1997). Its biocompatibility was examined in 24 ears of 12 cats. The prosthesis was implanted at the long crus of the incus and the incus

was examined 27-355 days after operation. In 23 ears, the prosthesis was found macroscopically well implanted at the intended position. In one ear, the prosthesis was found to be dislocated, and in another, it was slightly loosened. The incudes were removed, and five specimens were prepared for scanning electron microscopy, while the other specimens were observed under a light microscope. Histological studies revealed severe bone resorption of the long crus in the dislocated case and moderate bone resorption in the slightly loosened case. These instances of bone resorption were found to have been caused by inadvertent removal of the mucosal membrane during the implant operations. Slight bone resorption was seen at the contact area of the prosthesis in seven ears under a light microscope and in one ear under a scanning electron microscope. This bone resorption was induced by the mechanical pressure of the prosthesis and was not progressive due to the diminishing pressure. With the exception of pressure-induced bone erosions, there was no progressive bone resorption which was prosthesis-induced. The authors concluded that the biocompatibility of the nickel-titanium alloy stapes prosthesis with the long crus of the incus was hereby proven.

The above studies suggested that NiTi is quite well accepted into bone. However, there are two conflicting studies, in which NiTi has been found to have inferior properties compared to the other implant materials.

Berger-Gorbet *et al.* (1996) evaluated the biocompatibility of NiTi screws using immunohistochemical methods. The distribution of bone proteins during the bone remodeling process around a NiTi implant was observed. The control materials were screws made of Vitallium, c.p. titanium, Duplex austenitic-ferritic stainless steel (SAF), and stainless steel 316L. The test materials were implanted in rabbit tibias for 3 (n=2), 6 (n=2), and 12 (n=2) weeks. The embedding was done in hard resin, and undecalcified sections with bone-anchored implants were used for the immunohistochemical procedure. The authors concluded that the immunostaining method developed by them seemed to be a reliable technique for staining proteins in undecalcified sections. The biocompatibility results of the NiTi screws compared with the other screws showed a slower osteogenesis process characterized by no close contacts between the implant and bone, disorganized migration of osteoblasts around the implant, and a lower activity of osteonectin synthesis. The study included some uncertainties, however. The number of samples was too small to allow statistically significant histomorphometry. No characterization of the surface was done. Careful saline cooling was used while drilling the hole in the bone, but the material of the drill was not specified. Authors said that "on all NiTi sections black granules could be observed along the screws". Microparticles from the drill are possible and may affect the results. The authors used mouse anti-osteonectin and goat anti-collagen type III antibodies. There might be some problems in cross-reaction if rabbits are used as test animals. The assessment of osteonectin was good because it is an important protein in the bone remodeling process. The role of CIII was considered to be less useful even by the authors.

The bone reaction to NiTi implants inserted transcortically and extending into the medullary canal of rat tibiae was quantitatively assessed using an image-processing system by Takeshita *et al.* (1997). The control materials were composed of pure titanium, anodic oxidized titanium (AO-Ti), Ti-6Al-4V alloy and pure nickel. Three rats were killed 7, 14, 28, 84 and 168 days after operation (n=3). Essentially the same histological findings were made for NiTi, Ti, Ti-6Al-4V and AO-Ti implants. While NiTi and the other materials were progressively encapsulated with bone tissues, Ni was encapsulated with connective

tissues and showed no bone contact through the 168-day experimental period. Histometric analysis revealed no significant differences between the tissue reactions to Ti, AO-Ti and Ti-6Al-4V, but NiTi implants showed a significantly lower percentage of bone contact and bone contact area than any of the other titanium or titanium alloy materials. In terms of bone contact thickness, however, there were no significant differences between NiTi and the other three materials (Ti, AO-Ti and Ti-6Al-4V).

2.8.4. Bone response to NiTi in humans

NiTi has also be used as a bone implant material in humans, but worldwide medical applications have been hindered for a long time because of the lack of knowledge of the biocompatibility of NiTi. A bone anchor (Mitec G2®) which includes a small piece of super-elastic NiTi wire has been lately approved by FDA. In the USA, FDA limits the marketing of long-term implanted NiTi devices because their biocompatibility has not been proved. There are reports that NiTi material has been successfully used in bone-related human applications in Russia and China in a large number of patients (Yang *et al.* 1987, Kuo *et al.* 1989, Shabalovskaya 1996, Dai *et al.* 1996). Very few well-monitored studies have been published in peer-reviewed journals up until now. Also, no controlled or randomized studies have been published so far.

Drugacz *et al.* (1995) tested the clinical application of Ti50Ni48.7Co1.3 alloy shape-memory clamps for the fixation of mandibular fractures using transoral access. The clamps were used to treat all types of fractures occurring between the mandibular angles. The clamps were removed after a period of at least 6 weeks, and tissue samples were taken for microscopic examination. Seventy-seven patients with mandibular fractures were treated using the clamps. Altogether 93 fractures were treated, involving 124 clamps. There were 56 cases of single fracture and 21 cases of multiple fracture. In 72 patients the treatment progressed satisfactorily, while in five cases infections occurred. Tissue samples for histologic examination were taken from 58 patients after removal of the clamps. There were no pathologic or atypical tissue reactions or signs of disturbed cell maturation. The authors concluded that the application of shape memory clamps for the surgical treatment of mandibular fractures facilitates treatment while ensuring stable fixation of the bone fragments.

There are also two other studies in which NiTi implants were used in the surgical correction of maxillo-facial fractures. The results showed that the surgical treatment of these fractures by NiTi devices was simple, ensured a good stability of the fracture surfaces, reduced the time needed for operative procedures and rehabilitation, and allowed rapid bone healing (Sysolyatin *et al.* 1994, Itro *et al.* 1997).

The results of ventral intercorporeal lumbar spondylodesis with a NiTi implant were reported by von Salis-Soglio (1989). The operative technique was characterized by primary stabilization of the moving segment by means of a memory implant that was inserted intercorporeally following ventral removal of the intervertebral disc. The results included 51 cases of bony fusion within an average postoperative period of 9 months, one case of pseudoarthrosis and 11 cases of delayed bony fusion. The author concluded that, in view of the easier operative technique, the earlier mobilization of the patients and the

good fusion rate, the memory spondylodesis seems to have important advantages over the transplantation of bone chips only. The use of a NiTi staple to lock a tri-cortical iliac bone graft in cervical anterior fusion was used by Ricart (1997). Fifty patients with various clinical diagnoses were treated. Good and very good clinical results were reported in 80% of the cases and the average bone fusion rate was fast (7 weeks).

Silberstein (1997) reported a clinical study where 84 patients with fractures, tumors or intervertebral disc disease of the cervical and lumbar spine were treated with anterior fusion and porous NiTi implant grafts. They concluded that porous NiTi implants can be successfully used, probably because their mechanical properties are similar to those of the vertebral bodies, and the material itself shows a high degree of biocompatibility.

Thirty-six metatarsal osteotomies using internal fixation of a shape memory metal compression staple for hallux valgus were performed in a study by Tang *et al.* (1996). The recovery period preceding return to light work averaged 19 days, and normal work and normal walking were resumed an average of 41 days postoperatively. Twenty patients (35 feet) experienced complete pain relief. Only in one foot was the pain transferred under the second metatarsal head. Radiographic analysis of the feet showed that all the osteotomies united, and the average angle of hallux valgus and the intermetatarsal angle improved. The distal fragment during the healing of the osteotomy was stable. No external fixation by plaster splintage was needed. According to the authors, the benefits of this internal fixator were that the period of bone healing was shortened and the patients were allowed to bear weight earlier than usual.

Musialek *et al.* (1998) reported the fixation of small bone fragments with NiTi clamps in 64 patients. Clamps were used for compressive stabilization in several kinds of fractures. Three aspects were studied: bone union, wound healing problems and histology. Non-union occurred in 4 patients treated with only one fixative. Two clamps implanted in non-parallel planes seem to be advisable to exclude the need for longer immobilization. Neither toxic manifestation nor episodes of allergic reaction occurred. No suppuration appeared when a heat stimulus was applied by using a contact resistance heater. Histological evaluation of the tissue covering the implants in 22 patients did not reveal any adverse reactions. The study suggests that by using NiTi clamps in an appropriate way, satisfactory outcomes could be achieved with respect to both biofunctionality and biocompatibility.

In conclusion, on the basis of a few studies, it seems the NiTi material in itself has no deleterious effects in human use. The clinical relevance of the devices will not be discussed here.

2.8.5. Systemic response

In an early study by Castleman *et al.* (1976), neutron activation analyses were carried out on a small number of tissue samples from the liver, spleen, brain, and kidneys. The findings of the analysis suggest that there is no metallic contamination in the distant organs due to the implants.

Using NiTi paravertebral implants in 4 rabbits, Matsumoto *et al.* (1993) found that the blood Ni concentration after implantation reached a level twice the normal in 6-9 hours (28 ± 11 vs. 13 ± 5 ppb). After 4 weeks, the Ni concentration was 4-fold in the kidneys (140 ± 43 ppb), 2-fold in the liver (40 ± 18 ppb), and 10-fold in urine (90 ± 35 ppb). The authors concluded that Ni elution from NiTi alloy should be limited by, for example, using some coatings.

2.8.6. Biocompatibility of NiTi intravascular stents

Most of the recent commercial NiTi applications are meant for cardiovascular solutions. The idea behind this is to provide minimally invasive treatment instead of major surgery. Since the first experiments by Cragg *et al.* (1983), several studies have provided further information on the biocompatibility of NiTi as vascular stent material.

In the experimental studies of Rabkin *et al.* (1986), altogether 66 endovascular NiTi prostheses were implanted in 36 dogs. Long-term results obtained over a period of 14 months demonstrated good and prolonged permeability of the NiTi prostheses. Morphological investigations showed that the endovascular prosthesis was separated in a ring-like fashion by a thin layer of connective tissue, while inside it was lined with a layer of endothelial cells.

The long-term effects of twelve intravascular NiTi endoprotheses implanted in the iliac and femoral arteries of six normal dogs were evaluated by Sutton *et al.* (1988). No migration, erosion, inflammation, surface thrombus, or stenosis of the side branches was seen. Nor were any histopathologic effects detected. The authors conclude that the good biocompatibility manifested as a completely endothelialized, thin and stable neointima, satisfactory delivery and long-term patency at 2 years.

Another study was carried out by Cragg *et al.* (1993) to test an expandable NiTi intraluminal stent for biocompatibility, corrosion resistance, and patency. Forty-four stents were implanted in the iliac arteries of 22 sheep. Follow-up was performed with angiography and histologic examination for up to 6 months. All but one stent remained widely patent during the follow-up period. Minimal corrosion was seen at 6 months, and the stent appeared to be biocompatible. The authors conclude that a stent can be reliably and safely deployed in the vascular system.

Wakhloo *et al.* (1994) assessed the efficacy of tantalum or porous, tubular self-expanding NiTi stents for the treatment of carotid aneurysms. A total of 14 experimentally constructed aneurysms in dogs were treated. No incompletely occluded aneurysms were visible after the implantation of NiTi stents. After nine months, significantly more abundant intimal fibrocellular tissue growth surrounded the tantalum filaments than the NiTi filaments, which were covered with a smooth, thin neointimal layer. It was concluded that NiTi stents may become the treatment of choice for broad-based and fusiform aneurysms of the internal carotid artery. Improvements in the introducing system, stent material, and stent shape are required for simple implantation and reduction of intimal hyperplasia.

Cwikiel *et al.* (1997) used 6 pigs to evaluate the early proliferative reaction of smooth muscle cells in the media of the iliac artery following percutaneous transluminal angioplasty (PTA) compared with the reaction on the insertion of NiTi stents. The cell reaction appeared to be more pronounced after PTA than after the insertion of a self-expanding stent.

NiTi stents were implanted into the vertebral arteries in six dogs to evaluate the response associated with stent placement in low flow velocity arteries. Throughout the observation period up to 9 months, five arteries remained patent without significant narrowing. The total mean thickness of the intima covering the stents showed no significant differences over time. The histologic findings on the stented vessels showed atrophic compression of the media, but intact endothelial cell linings without necrosis or perforation were observed. Thus, no significant risk of thromboembolic events exists after the implantation of NiTi stents in the vertebral arteries in dogs (Wakhloo *et al.* 1995).

Despite the improvements afforded by intracoronary stenting, restenosis remains a significant problem. In the present study by Carter *et al.* (1998), the vascular response of a NiTi stent was compared to a balloon-expandable stent in porcine coronary arteries. Eleven NiTi and eleven stainless steel stents were implanted. On histology at 3 days, the stainless steel stents had more inflammatory cells adjacent to the stent wires than their NiTi counterparts. After 28 days, the vessel response was similar for the NiTi and stainless steel designs. The mean neointimal area and the percentage of stenosis were significantly lower in the NiTi than in the stainless steel group. The authors concluded that a NiTi stent exerts a more favorable effect on vascular remodeling with less neointimal formation, than a balloon-expandable design. Progressive intrinsic stent expansion after the implantation does not appear to stimulate neointimal formation and may therefore prevent in-stent restenosis. The results were in accordance with an earlier study by Sheth *et al.* (1996).

Grenadier *et al.* (1994) investigated the acute and long-term patency rates and the histologic responses of coronary arteries to a self-expandable NiTi coil stent. Twenty-two stents were implanted in sixteen dogs. The animals were monitored for 1 to 2 weeks, 1 month, 3 months, 6 months, and 1 year and underwent subsequent angiography and histopathologic examination. Angiographic artery dimensions measured immediately after stent implantation did not differ from those noted at follow-up. A histologic examination showed outward stent pressure compressing the internal elastic membrane and the media in most cases. Intimal hyperplasia started at 2 weeks and was most apparent at 3 and 6 months. Therefore, the NiTi self-expandable stent provokes a moderate cellular proliferative response that reaches its maximum in 3 to 6 months without further progression.

Based on the above studies, the histopathological changes caused by vascular NiTi stents are associated with a mild inflammatory response, some atrophy of vessel media, acceptable fibrocellular tissue growth and endothelization. The biocompatibility of NiTi stents seems to be equal or better compared to stainless steel stents.

2.8.7. Inflammation associated with polyester-covered and polyurethane-coated NiTi stents

Nonspecific inflammatory reactions characterized by local tenderness, fever, and flu-like discomfort have been seen in patients undergoing endoluminal graft placement in the abdominal aorta or the femoral arteries.

In a study by Kellner *et al.* (1997), magnetic resonance imaging demonstrated perivascular inflammation in 79% of patients with polyester-covered NiTi stents. Clinical symptoms were seen in 57% of these patients. No reaction was evident among the controls with uncovered NiTi stents and the subjects who underwent peripheral percutaneous transluminal angioplasty. The polyester-covered NiTi stent may induce systemic and severe local reactions. These reactions seem to be specific to this type of stent. No definite cause has been established, although the phenomenon appears to be self-limiting.

Hayoz *et al.* (1997) undertook a study to assess the clinical and laboratory parameters of this inflammation. Ten patients with femoropopliteal artery or aortic lesions were treated with polyester-covered NiTi (Dacron) fabric and compared with eleven patients implanted with a bare NiTi stent. In the stent-graft group, four patients showed clinical signs of acute inflammation manifested as fever and local tenderness. The authors also did an *in vitro* analysis, which showed that individual components of the stent-graft did not activate human neutrophils, whereas the intact stent-graft itself induced a marked neutrophil activation. The component of the self-expanding stent-graft that caused the non-specific inflammatory reaction was not identified.

To improve hemocompatibility, heparin-coated Dacron-covered NiTi stent-grafts have been used. These were also found to cause severe inflammatory perigraft responses in sheep during up to 6 months of follow-up. MR images demonstrated contrast enhancement and edema. Macroscopic examination showed marked vascular wall thickening and adhesions around the Dacron fabric; microscopic examination showed a pronounced inflammatory foreign-body response. There was almost no response to noncovered NiTi stents. The authors concluded, on the basis of their two studies, that the use of noncovered stents should thus be preferred to the use of Dacron-covered stent-grafts (Schurmann *et al.* 1997).

The polyurethane coating has also been associated with perivascular inflammation. Six polyurethane-coated and six bare NiTi stents were implanted and compared in rabbit carotid arteries. At 4 weeks, all stent struts were endothelialized. Mild proliferative responses with some neovascularization around both stent types were seen. No differences in the degree of neointimal proliferation between the stents were found, but the polyurethane coating was associated with an inflammatory tissue response consisting of lymphocytic infiltration and foreign-body reaction and the appearance of multinucleated giant cells. This may indicate a low biocompatibility of polyurethane, which may thus not be an ideal material for coating intravascular devices (Rechavia *et al.* 1998).

2.8.8. Biocompatibility of other cardiovascular applications

To determine the biocompatibility and thrombogenicity of NiTi blood clot filters, Prince *et al.* (1988) inserted 27 NiTi wire devices into the venae cavae of 16 dogs and one sheep. The results were analyzed after periods of one week to four years. All the 18 cleaned NiTi wire filters remained patent, but some showed venographic filling defects caused by adherent organized thrombi. The filters in larger veins tended to have less thrombus formation. Surface polishing and filter shape had no observable effect on thrombogenicity. Histologic study revealed patchy chronic inflammation on the surface of uncleaned filters, but only a benign fibrous tissue reaction on cleaned filters. Neointimal tissue overgrowth was observed in the contact area of the vena cava. Platelet adhesion and plasma coagulation effects of NiTi wire were tested *in vitro* in human blood and found to be similar to those of stainless steel. The authors suggest that NiTi may be a promising material for human intravascular prosthetic applications.

Das *et al.* (1993) designed a superelastic NiTi-Dacron atrial septal defect closure device and studied its efficacy in a canine model. The defects were created surgically in 20 adult dogs. Percutaneous transcatheter closures were attempted using the new device. The closures were successful in 19 studies and unsuccessful in one. Light microscopy at 8 weeks in 3 dogs showed the devices to be covered by smooth endocardium enmeshed in mature collagen tissue, with minimal mononuclear cell infiltration. The authors concluded that this new device permits effective and safe atrial septal defect closure in a canine model.

2.8.9. Biocompatibility of NiTi urethral stents

Urethral stents have been used for the treatment of urethral strictures. Very few studies have been available to date on the compatibility of NiTi urethral stents.

To study the long-term effects of urethral NiTi stents, 18 dogs were implanted by Latal *et al.* (1994). The reactions of the mucosa, muscles and periurethral tissue were evaluated. The follow-up examinations performed after 1 week and 1, 3, 6, 12 and 18 months included urine, macroscopic, radiological, histologic and scanning electron microscopic analyses. The authors conclude that, despite the excellent biocompatibility of the material with no evidence of foreign body reactions or corrosion, there were no complete incorporations of the stent by epithelialization. Clinical application therefore appears to be problematic. On the contrary, one study has been published where 39 patients with benign prostatic hyperplasia had NiTi urethral stents implanted with a clinical success rate of 89%. Follow-up for 26 months showed no incrustation or migration of the spiral (Qiu 1993).

2.9. Applications of NiTi: current status in medicine

2.9.1. General

Since the first attempts to introduce this material into medical use in the early 1970s certain progress has taken place (Castleman *et al.* 1976). NiTi superelastic wires were first introduced into orthodontic use (Andreasen *et al.* 1971). Nowadays, there are some commercial products available worldwide. At the present, the breakthrough of self-expandable stents in gastroenterology, radiology and cardiovascular applications seems convincing. The idea of using NiTi stents was first reported separately by two authors (Cragg *et al.* 1983, Dotter *et al.* 1983). By using stents, major surgical operations can be avoided. Sometimes a stent may be the only choice in critically ill patients. Stents have shown NiTi with certain criteria to be a material with huge possibilities.

2.9.2. Cardiovascular

The first vascular NiTi device was the Simon Nitinol filter (SNF) used to treat pulmonary embolism (Simon *et al.* 1977). The filter is inserted as a straight thin wire via the small bore catheter used for angiographic diagnosis. Upon reaching the lumen of the inferior vena cava and sensing body temperature, it reverts to its preset complex filter shape and locks into place permanently, trapping any further thromboemboli from the pelvis or the lower limbs. SNF has also been accepted by the U.S. Food and Drug Administration FDA.

The general trend of stenting is towards self-expandable NiTi-based stents. The thin stent is placed in the narrowed artery, where it expands and dilates the artery. The carotid artery stents and the endoluminal polyester-covered NiTi stent-grafts for infrarenal abdominal aortic aneurysms have been proved to be efficient and technically successful, but a careful long-term evaluation is still necessary (Blum *et al.* 1997, Wholey *et al.* 1998). Intracoronary (de Jaegere *et al.* 1996, Oesterle *et al.* 1998) and peripheral vascular NiTi stenting (Schwarzenberg *et al.* 1998) also seems to be increasing. Their benefits include good radial expansion capabilities and flexibility. Despite the improvements, restenosis and reocclusion remain a significant problem and the optimal physical and surface properties of an arterial stent have not been defined yet (Schurmann *et al.* 1995). Endoluminal repair of infrarenal abdominal aortic aneurysms with the use of Dacron-covered NiTi stent-grafts is feasible, safe and clinically effective. The attempts to improve properties with a heparin-coated Dacron cover have shown only a pronounced inflammatory response (Schurmann *et al.* 1997). As pointed out by the same authors, the need to evaluate the biocompatibility of new vascular devices is evident. Polyurethane stent coating was also associated with an inflammatory tissue response (Rechavia *et al.* 1998).

A transcatheter approach to the occlusion of atrial septal defects has also been recently reported. The implant consists of two umbrellas placed over a long veno-arterial guide-wire. The system has been described to be technically feasible. It has been used in a few

cases of adults and children (Sievert *et al.* 1995, Hausdorf *et al.* 1996). However, much further evaluation and long-term data are needed before this technique can be recommended.

2.9.3. Gastroenterology

Self-expanding stents for esophageal strictures and the palliation of malignomas have been studied by several authors (Cwikiel *et al.* 1993, May *et al.* 1995, Acunas *et al.* 1996). Esophageal NiTi stents are easy to implant, provide effective palliation of malignant esophageal obstructions, and have a low risk of severe complications. The only disadvantage was that incomplete initial stent expansion as well as tumor ingrowth/overgrowth occurred in nearly one third of the patients. Covering the NiTi-based stent with a thin Gore-tex sheath may give a possibility to avoid ingrowth and to use the stent even in the case of fistulas.

Biliary stents are effective in achieving long-term palliation in patients with malignant obstructive jaundice. Recently (1999), FDA released on the market a stent for this purpose. The treatment of benign biliary strictures with metallic stents is associated with a low long-term patency rate (Bezzi *et al.* 1994, Rossi *et al.* 1994). The use of stents reestablishes bile flow in the occluded biliary tree. The stent may, however, be technically difficult to insert (Smits *et al.* 1995).

The insertion of NiTi stents in patients with rectosigmoidal carcinoma is technically feasible and effective in the palliation of malignant rectosigmoid obstruction; they provide an alternative to repeated palliative laser therapy or palliative surgery (Tack *et al.* 1998).

2.9.4. Urology

The use of NiTi prostatic stents has also increased since the first reported experiment by Lopatkin *et al.* (1989). For high-risk patients with subvesical obstruction caused by prostatic carcinoma, the insertion of a permanent metal stent system offers a useful alternative to transurethral resection (Gottfried *et al.* 1997).

NiTi stents are also used as an alternative method for the treatment of benign prostatic hyperplasia. Patients were considered suitable for treatment with the stent when they presented with a high operative risk (Gottfried *et al.* 1995, Gesenberg *et al.* 1998).

The use of urethral stents was found to considerably decrease the number of repeated dilatations and urethrotomies in recurrent urethral strictures (Yachia 1993). Despite the good biocompatibility of the material in a long-term study on dogs, there were no complete incorporations of the stent by epithelialization, and the authors concluded that clinical application might therefore be problematic (Latal *et al.* 1994).

2.9.5. Orthopedics and bone-related applications

According to Castleman *et al.* (1976) the first thoughts to exploit the potential of NiTi as an implant material were made by Johnson and Alicandri in late 1960s. Since that time, further studies have been carried out into the viability of the alloy for orthopedic operations.

Some of the first *in vitro* studies were carried out by Baumgart *et al.* (1978), who examined the NiTi distraction rod in the correction of scoliosis. In China Lu *et al.* (1986) implanted NiTi rods in 26 patients with scoliosis. Correction was reported to be good and there were no complications. Matsumoto *et al.* (1993) and Sanders *et al.* (1993) published further *in vivo* experimental studies. It seems that the scoliosis-correction system based on NiTi shape memory or superelastic property has quite complicated biomechanical problems related to compression and distraction control. NiTi may not provide any improvements compared to the traditional implant systems.

NiTi compression staples were first introduced in China. According to Dai (1983) a shape memory staple was first used inside the human body in 1981. After that, NiTi staples and clamps have been used in comminuted fractures of the short tubular bone (Yang *et al.* 1992), for fixation of mandibular fractures (Drugacz *et al.* 1995), for metatarsal osteotomies (Tang *et al.* 1996), for anterior cervical decompression and fusion (Mei *et al.* 1997, Ricart 1997, Silberstein 1997), for fixation of small bone fragments (Musialek *et al.* 1998), and for several other cursory applications (Iwabuchi *et al.* 1975, Kuo *et al.* 1989, Dai *et al.* 1996). The only NiTi-containing orthopedic implant widely used in the western world is the Mitek G2 suture anchor. It has superelastic NiTi wings which prevent the anchor from pulling out of the bone after insertion and secure the tendons or ligaments to the bone (Barber *et al.* 1996). Another promising application is a NiTi hook used to restore the dislocated acromio-clavicular joint (Ryhänen *et al.* 1998).

The problem of most published studies in the orthopedic field is that they rarely satisfy the quality criteria of scientific study. It is not enough to say that a certain NiTi implant can be used without harm. To be considered fully successful, it must be proved to be better than the existing competitors. At the present, there are no comparative clinical studies and the series have generally been small. Randomized prospective studies are needed to apply new NiTi implant devices for constant clinical use in humans.

2.9.6. Others

The indications of stenting increase rapidly. The use of self-expanding NiTi stents to prevent major airway occlusion was first reported by Rauber *et al.* (1990). According to the early tests, they seemed to be very useful and effective in inoperable tracheal or bronchial stenosis due to intraluminal tumor invasion (Yanagihara *et al.* 1997, Hauck *et al.* 1997).

A NiTi-based mesh-expanding prosthesis for laparoscopic hernioplasty significantly shortened the operating time in a study of Himpens (1993).

The good holding and atraumatic characteristics of the detachable clamp have been confirmed by use in laparoscopic and thoracoscopic surgery on the gastrointestinal tract (Frank *et al.* 1995).

Also, new type of NiTi stapes prosthesis to restore the ossicular fixation after stapedectomy has been introduced (Kasano *et al.* 1997).

3. Aims of the present study

Nickel-titanium shape memory metal alloy (NiTi) has unique thermal shape memory, superelasticity and damping properties of a kind not seen in other implant alloys. These properties make it potentially useful for surgical applications. The performance and biocompatibility of every new material must be very well confirmed before it can be accepted into use as an implant material.

The main purpose of the present studies was to evaluate the biocompatibility and corrosion of NiTi for further safe use as a surgical implant material and to compare it to the ordinarily used implant metals.

The specific aims of this experimental work were:

1. To evaluate the acute cytotoxicity of NiTi to human fibroblasts and osteoblasts *in vitro*.
2. To clarify the general soft tissue response to NiTi.
3. To evaluate the effects of NiTi to neural tissue after perineural implantation.
4. To evaluate the ultrastructural characteristics of the cell-NiTi interface.
5. To evaluate the bone response of NiTi after periosteal implantation.
6. To evaluate if NiTi has deleterious effects on osteotomy healing, bone mineralization or the normal remodeling response.
7. To determine the rate of metal dissolution from NiTi in a simulated physiological environment *in vitro*.
8. To evaluate if there is systemic release of trace elements from NiTi to distant organs.
9. To determine the effect of long-term implantation on NiTi implants.

4. Materials and methods

4.1. Test implants

Study I: The materials tested were NiTi (54%Ni, 46%Ti, Unitec, U.S.A), stainless steel AISI 316 LVM (12%Ni, 18%Cr, 68%Fe, 2%Mo, Sandvik, Sweden), ASTM Grade 2 commercially pure titanium (TISTO GmbH, Düsseldorf, Germany), white soft paraffin (white soft paraffin is a semi-solid mixture of hydrocarbons obtained from petroleum and bleached) and a composite material known as Silux Plus® (Bisfenol-A-diglycidylmetacrylate 15-20%, triethylglycoldimetacrylate 15-20%, aluminoxide <1%, amorphous siliconoxide 50-60%, 3- metacryloxidepropyltrimetoxisilan <7%, Silux Plus, 3M, USA) Metal test discs of 6x7 mm were taken from a large bar by turning on a lathe. The composite material was prepared using a rubber mould. The surface preparation consisted of electrolytic polishing for stainless steel and water sanding for titanium and NiTi (waterproof silicon carbide paper FEPA P#2400).

Studies II and III: The tested materials were vacuum-melted, drawn and fully annealed NiTi (56 % nickel by weight, 44 % titanium by weight, $A_f = -10^\circ\text{C}$, NiTi Development Co., USA), AO/ASIF stainless steel (Synthes GmbH, Switzerland), and AO/ASIF Ti-6Al-4V alloy (90 % titanium by weight, 6 % aluminium by weight, 4 % vanadium by weight, Synthes GmbH, Switzerland).

Identical round test implants were taken from a longer wire by mechanically cutting. The implants used in both studies were 1.8 mm in diameter and 6 mm in length. The cut ends of the implants were rounded using water-cooled grinding (silicon carbide paper FEPA P#800, Struers, Denmark). The material surfaces were as received: stainless steel was electrolytically polished, NiTi and Ti-6Al-4V were received in a mechanically ground condition.

Study IV: The NiTi and stainless steel materials and surface preparations were as in the studies II and III. Implants of 1.8x18 mm were taken from a longer wire by mechanically cutting, and the cut ends of the implants were rounded as previously.

The test discs and implants were all degreased with 70 % ethanol, washed with an ultrasonic vibrobath and autoclaved (30 min., 121°C) before the procedures.

4.2. *In vitro* human cell cultures

The cells in study I were human fibroblasts taken from healthy palatal gingiva and osteoblasts taken from alveolar bone. The histochemical analysis of osteoblasts was done by staining them for alkaline phosphatase using Sigma Diagnostics Procedure No. 85, where only osteoblasts stained blue (Rifas *et al.* 1989). The cell lines had been derived in our laboratory. Both cell lines were plated on three 60 x 15 mm diameter culture flasks (Nunc-clon®, Dorn Intermed, Denmark). The culture medium used was Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies LTD, Paisley, Scotland) containing 5 % new-born calf serum, 1 % ascorbine (Merck, Darmstadt, Germany), 100 IU/ml penicillin, 100 µg/ml streptomycin, 1 % L-glutamine and 0,1 % amphotericine B (Fungizone®, Life Technologies LTD, Paisley, Scotland). The cultures were maintained at 37 °C temperature in an atmosphere of humidified air and 5 % carbon dioxide. They were passaged routinely with medium changes every second day and allowed to reach confluence before subculturing. Cells between the 6th and 10th passages were used. Cells were removed from the three flasks with trypsin (Life Technologies LTD, Paisley, Scotland). They were collected into one plastic tube and counted. Equal amounts of cells (1×10^5 cells) were then seeded on 40 mm diameter test flasks, each containing one test disc applied to the middle of the plastic flask and glued to the bottom by using sterile white soft paraffin. There were also control flasks without test discs and flasks with only sterile white soft paraffin of the same size as the test discs. Each flask contained 1 ml of culture medium, as described in detail above. The incubation conditions were also the same as above. The cells were then allowed to grow with medium changes every second day. At the end of the study, the cells were trypsinized and counted by using a hemocytometer. The total number of cell culture flasks was 18 for fibroblasts and 18 for osteoblasts. The culture flasks were photographed after a week of incubation, using a Wild MPS 51 camera (Heerburg, Switzerland) and a photoautomation device MPS 45 with 108x magnification.

4.3. Animals

The animal tests were performed with the approval of 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.

All the animals used were female Sprague-Dawley/MOL rats from the Laboratory Animal Centre, University of Oulu (Oulu, Finland). In studies II and III same rats were used. The ages of the rats ranged between 20 and 24 weeks with a weight range of 320 to 380 g. Seventy-five rats were randomized into 3 groups, each consisting of 25 rats. In study IV, the ages of the test animals ranged between 32 and 36 weeks. Forty rats were randomized into 2 groups, each consisting of 20 rats. The mean weight was 486 ± 65 g (mean $\pm$ 1SD) in the StSt group and 473 ± 52 g in the NiTi group. All the animals were housed in groups of 3 - 6 in Macrolon IV polycarbonate cages in a thermostatically controlled room at 20 ± 1 °C with a relative humidity of $50 \pm 10\%$. The room was artificially illuminated on a schedule of 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*.

4.4. Surgical procedures

All rats were anaesthetized with a Fentanylcitrate (80 µg/kg) - Fluanisone (2.5 mg/kg) (Hypnorm®, Jansen-Pharmaseutica, Belgium) - Midazolam (1.25 mg/kg) (Dormicum®, Roche, Switzerland) blend injected intraperitoneally. After the induction of anaesthesia, they received Cefuroximinenastrum 5 mg/kg i.m. (Zinacef®, Glaxo Wellcome plc, Great Britain). Buprenorphin 0.3 mg/kg s.c. (Temgesic® 0.3 mg/ml, Reckitt & Colman) was used as a postoperative analgesic. The hair was shaven around the implantation site and the skin was sterilized by brushing it with chlorhexidin before operation. The rats were killed using carbon dioxide.

Study II: NiTi, stainless steel and titanium alloy (Ti-6Al-4V) implants (25 of each) were implanted, one implant per rat. A 2 mm incision was made with a knife, and a cannulated needle (Cathlon IV™, Jelco Laboratories, USA) was inserted via the incision into the right paraspinal muscle near the gluteal area toward the sciatic nerve. The needle was removed from inside the plastic cannula, the implant was placed into it and inserted to its final position using the needle. Cannula-assisted implantation was used to avoid prejudicial scar tissue formation due to the surgical procedure itself. Five animals in each group were killed at 2, 4, 8, 12 and 26 weeks after implantation. The implants were dissected with 5 mm of soft tissue around them.

Study III: For periosteal implantation, 25 NiTi, 25 stainless steel and 25 Ti-6Al-4V implants were used, one test specimen per rat. A 10 mm skin incision was made with a knife along the lateral side of the right femur. The muscles were bluntly separated to disclose the femoral bone periosteum. The periosteum was kept intact, to avoid the scar formation effects of surgical trauma. The test implant was placed in direct contact with the intact femur periosteum, but it was not fixed inside the bone. The muscles around the implant were approximated on it with resorbable sutures (Vicryl rapid®, Ethicon) to press the implant against the periosteum. The skin was closed with intracutaneous sutures. Five animals in each group were killed at 2, 4, 8, 12 and 26 weeks after implantation. The implants were dissected with femur bone and 3 mm of surrounding soft tissue.

Study IV: The right knee was shaven and a medial parapatellar incision was made. The patellofemoral joint was exposed and the patella was dislocated laterally. The muscles were bluntly separated and the lower portion of the femoral diaphysis was exposed. The intramedullary space was penetrated from the intracondylar space by drilling it manually with a 1.9 mm (TiN-coated) drill. Osteotomy was made with a diamond saw in the distal third of the femur. The osteotomy was then fixed with a NiTi or StSt intramedullary nail installed distally. The wound was closed in layers using resorbable sutures (Vicryl rapid®, Ethicon). Buprenorphin 0.3 mg/kg s.c. (Temgesic® 0.3 mg/ml, Reckitt & Colman) was used as a postoperative analgesic. The rats were allowed to move freely in their cages after the operation with no external support. Three rats in each group were killed at 2, 4, 8, 12, 26 and 60 weeks after implantation. All femurs with the implants were dis-

sected, as were also the contralateral femurs and the brains, livers, spleens, kidneys and muscles around the operated femur in the 26- and 60-week groups for further studies.

4.5. Specimen processing

Study II: The tissue specimens with implants were fixed in 10% PBS formalin for 7 to 14 days. After fixation, the soft tissue and fibrous capsule at one end of the implant were cut and the implant was extremely gently removed, leaving a tidy hole. The samples were processed and mounted in paraffin. The block was cut precisely perpendicular to the longitudinal axis of the implant hole. Several 4 μm sections were cut from each sample specimen at the midsagittal site of the implant hole. At least 2 sections of each sample were inspected.

Study III: The tissue specimens with implants were fixed in 10% PBS formalin for 7 days. After the fixation, one femur from each group was placed in a hard resin embedding process without removing the implant. These samples were dehydrated in a graded alcohol series and embedded in metacrylate (Technovit®, Kulzer GmbH, Germany) using the standard method (Donath *et al.* 1982). The specimens were further cured at 50°C overnight, and then cut with a low-speed diamond saw along a perpendicular plane at the middle of the implant. One half was cut into thin ground sections (30 μm) using a sandwich method and the Exact Cutting-Grinding and Micro-Grinding system (EXAKT Apparatebau, Germany). The sections were mounted and stained with Goldner-Trichrome and Haematoxylin-Eosin stains for histology and a morphometric light-microscopic computer-aided examination. The other half of each sample embedded in hard resin was used for field emission scanning electron microscopy (FESEM). The metacrylate-embedded thick sections were treated for 10 min. in saturated NaOH in alcohol solution to remove the plastic from the tissues on the surface of the block before coating with carbon. The other four femurs in each group were decalcified and routinely processed and mounted in paraffin. Before embedding, the bone, the soft tissue and the fibrous capsule were cut at one end of the implant bed and the implant was gently removed, leaving a clear-cut hole. The block was cut perpendicular to the longitudinal axis of the bone and the implant hole. Sections of 4 μm were cut from each specimen at different midsagittal sites of the implant hole and stained with Haematoxylin-Eosin.

Study IV: The sample fixation and hard-resin embedding process were as described above. After that, the specimens were cut longitudinally with a diamond saw at the middle of the implant. One half was then cut transversely at the osteotomy site and at a site outside the osteotomy, but in the nail area. Thin ground sections (30 μm) were made, as in study III. The sections were mounted and stained with Goldner-Trichrome stain for histology. Two femurs in the 26- and 60-week groups and one in each of the earlier groups were taken for pQCT measurements after removal of the nail. The nail was taken for a FESEM analysis of corrosion marks. After pQCT, the bones were decalcified and then routinely processed and mounted in paraffin. Several 4 μm vertical sections were cut and stained with Haematoxylin-Eosin. Organs for metal ion examination were dissected using a plastic spoon, quick-frozen, stored in polyethylene casks and kept at -20 °C until analysis.

4.6. Methods of analysis

4.6.1. Clinical and macroscopic observations

The animals in all studies were checked weekly for any abnormalities and to ensure that the wounds had healed. The dissected samples were first observed visually. After removal, the implants were studied for possible marks of macroscopic corrosion.

4.6.2. Light microscopy

The morphology and histology of the Goldner-Trichrome and Haematoxylin-Eosin stained sections were examined under a light microscope (magnification 12.8 – 320x in the studies II and III, 32-480x in study IV). Lamellar bone structures were studied in polarized light.

4.6.3. Graphite furnace atomic absorption spectrometry

Analysis of metal ion concentrations in cell culture media (Study I): Media collected every second day were stored in plastic tubes and kept at -20 °C until analysis. The media were collected from three flasks containing the same type of test material and mixed in a single tube. Corrosion analysis was done on this sample. Before the sample was analyzed, the media were burnt with acid. The nickel and titanium concentrations were assessed from the NiTi media. Nickel was determined from the stainless steel and titanium from the titanium flask media.

The analyses were performed using a graphite furnace atomic absorption spectrophotometry (GFAAS, Perkin Elmer SIMAA 6000, Rautaruukki Ltd., Raahe, Finland). Atomic absorption is a technique based on the unique spectrum of each element. For every element analyzed, characteristic wavelengths are generated in a discharge lamp (hollow cathode lamp) and then absorbed by a cloud or vapor of that element. The amount of absorption is proportional to the concentration of the element vaporized into the light beam.

Analysis of corrosion products in various organs (Study IV): The concentrations of nickel, chromium and iron in various organs were assessed at 26 and 60 weeks, to find out whether there had been release or accumulation of ions from the test nails. The samples were first freeze-dried for seven days. After that, they were weighed accurately (approximately to 0.1g) in Teflon decomposition vessels. Two milliliters of ultrapure nitric acid and two milliliters of hydrogen peroxide (pro analysis) were added. The samples were decomposed in a microwave oven (Milestone mls 1200) and diluted in 10 ml of pure water. Cr and Ni were determined by graphite furnace atomic absorption spectrometry (GFAAS) (Perkin-Elmer Zeeman/3030) and Fe by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (PU 7000). The concentrations were given as dry weights.

4.6.4. Soft tissue histomorphometry

Study II: The thickness of the reactive and fibrous capsule membranes around the implants were determined with a CCD camera-based digital image analysis system (MCID/M1, Imaging Research Inc., Canada). The system consisted of a microscope (Nikon Optiphot II, Japan), a videocamera (Dage MTI 72E, USA) and a personal computer with a digitizer (FG-100 AT; Imaging technology, USA). After several pilot stainings (Giemsa, Herovici, Van Gieson, Toluidine blue, Kossa), normal Haematoxylin-Eosin staining was chosen for subsequent use, since it gave the best distinction in the analysis of black-and-white video images. In order to randomize the points of measurement, we used an image overlay meshwork added to each picture. The screen area corresponded to 2.2 mm and the mesh size was 260 x 260 μm . The capsular thickness was determined in the orthogonal direction of each intersection point of horizontal and vertical mesh lines coinciding with the boundary between the capsule and the hole left by the implant. The capsule thickness was expressed as a mean value of 5-18 (average 12) hits. Two sections taken from different midsagittal areas of each sample were measured. There were a few ragged views, in which case no measurement was made. About 2000 single measurements were made during this study.

4.6.5. Bone histomorphometry

Study III: The real-color CCD camera-based digital image analysis system (MCID/M4, Imaging Research Inc., Canada) was used in bone histomorphometry. The system consisted of a microscope (Nikon Optiphot II, Japan), a videocamera (Sony DXC 930P, Japan) and a personal computer with a digitizer (Matrox Image 640 with CLD color board, Imaging Technology, USA). Goldner-Trichrome and Haematoxylin-Eosin stainings were used, since they gave the best distinction in the analysis of color video images. The measurement area of each slice was standard, corresponding to a screen of 2.2 mm². The mid-point of the bone cortex was adjusted to the middle of the screen, just under the cross-sectional radius of the implant (in the paraffin-embedded slices to the hole left by the implant). Whenever possible, the histomorphometric terminology recommended by Parfitt *et al.* (1987) was used.

The mean cortical width (Ct.Wi) was measured automatically from the given area as the median internal distance perpendicular to the maximum curved chord. The bone area (B.Ar) was measured in a defined area, excluding the trabecular spaces. The erosion area (E.Ar) of resorption pits was measured after reconstruction of the bone surface. The active erosion surface (erosion surface covered by osteoclasts and mononuclear cells) was not determined distinctly, but the active erosion surface perimeter was measured as a fraction of the total bone surface perimeter (E.Pm/B.Pm). The area of new woven bone (N.Wo.B) was measured like B.Ar. Two sections taken from different mid-sagittal areas of each sample were measured.

4.6.6. Analysis of callus size and osteotomy healing from the radiographs

Study IV: Medial radiographs of the operated femurs were obtained postoperatively at 1 week and along the killing schedule. The live animals were filmed under anesthesia. The maximum length (mm) and width of the callus area were measured from plain radiographs using a CCD camera (Dage MTI 72E), a light table and a PC with MCID/M4 software (Imaging Research Inc., St Catharines, Canada). The union of the osteotomy was judged visually as a consensus of two surgeons. Completely healed bone union with uniform, unbroken callus and no visible osteotomy line was classified as a "good" outcome. Marked but not uniformly overstepping callus formation with a blur osteotomy line was classified as a "satisfactory" outcome. Non-union with a clearly visible osteotomy line and no overstepping callus at the osteotomy site was classified as a "poor" healing outcome.

4.6.7. Peripheral quantitative computed tomography (pQCT)

Study IV: The structure of the callus and the changes in both total (BMD) and cortical bone mineral density (CtBMD) (mg/ccm) were assessed using a pQCT system (Stratec XCT 960A with software v. 5.20, Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany). An attenuation threshold of 0.700 cm^{-1} and a voxel size of $0.092 \times 0.092 \times 1.25 \text{ mm}^3$ were used. Nine sagittal cuts with a slice thickness of 1 mm were obtained from the operated femur. Three of these were in the callus area, three in the diaphyseal nail area outside the callus, and three in the area of proximal intact femur. Three cuts were performed on non-operated femurs corresponding to the osteotomy sites as controls.

4.6.8. Field emission scanning electron microscopy

Field emission scanning electron microscopy (FESEM) (Jeol JSM-6300F, Japan Electronoptics LTD, Japan) with a digital image processor (SemAfore pro-S, J. Rimppi Inc., Finland) was used.

In study III, FESEM was used to obtain high-quality magnifications (ad 170,000x) from the cell-implant interface. Direct cell contact or other signs of close attachment between cells and different materials were observed. The tissue-implant adhesion morphology at micro- and nanoscales was analyzed and compared with qualitative criteria.

In study IV: FESEM was used to evaluate the marks of corrosion on the surface of the retrieved nails. The differences between the implants and the influence of time were analyzed and compared with the qualitative criteria.

4.6.9. Statistical analysis

The values were expressed as mean $\pm$ standard deviation (SD). The data were analyzed using unpaired two-sample *t*-test in study I and one-way analysis of variance (ANOVA) with *t*-test in the study III. In study IV, the data on bone unions were analyzed using chi-square test and the trace metals using *t*-test. The differences were considered significant at a probability level of 95% ($P < 0.05$). All statistical analyses were performed with commercially available software.

5. Results

5.1. Cell attachment and proliferation in the presence of NiTi

5.1.1. Contact of single cells with test materials in vitro and in vivo

The contacts of single cells to material surface were quite similar between NiTi and the other implant materials in commercial use. The cell cultures showed that the cells had grown very close to the titanium and NiTi surfaces in the fibroblast cultures, but slightly less close to stainless steel in the osteoblast cultures. In this study, the toxic control composite material inhibited cell attachment prominently, as also did the white soft paraffin (Fig. 2).

**PETITIONER ARTHREX
EX. 1011**

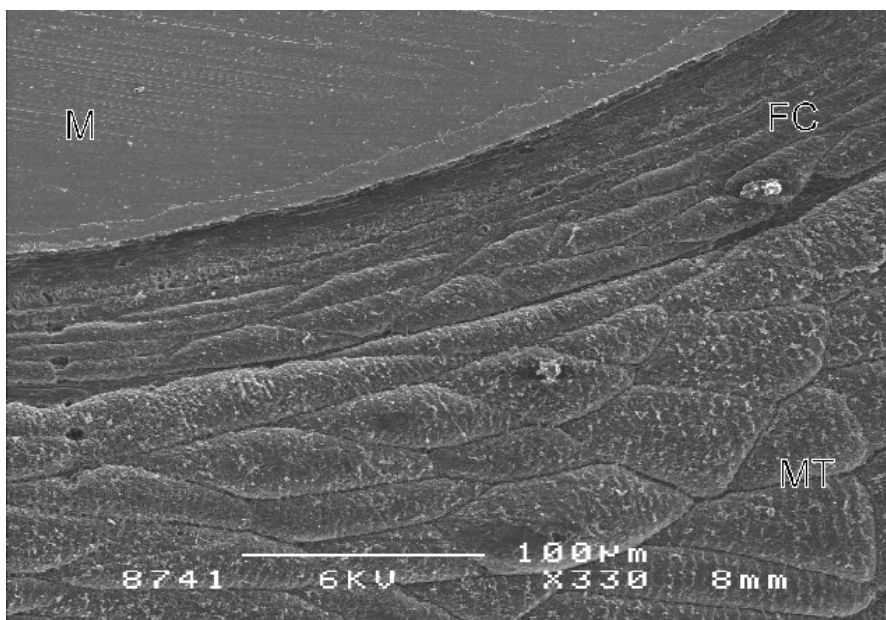


Fig. 3. A) A FESEM image of a hard resin embedded sample with a stainless steel implant 26 weeks after operation. M= metal implant, FC= fibrous capsule with collagen fibers and fibroblast type cells, MT= muscular tissue. A similar soft tissue reaction was seen with all materials at 26 weeks (magn. 330x).

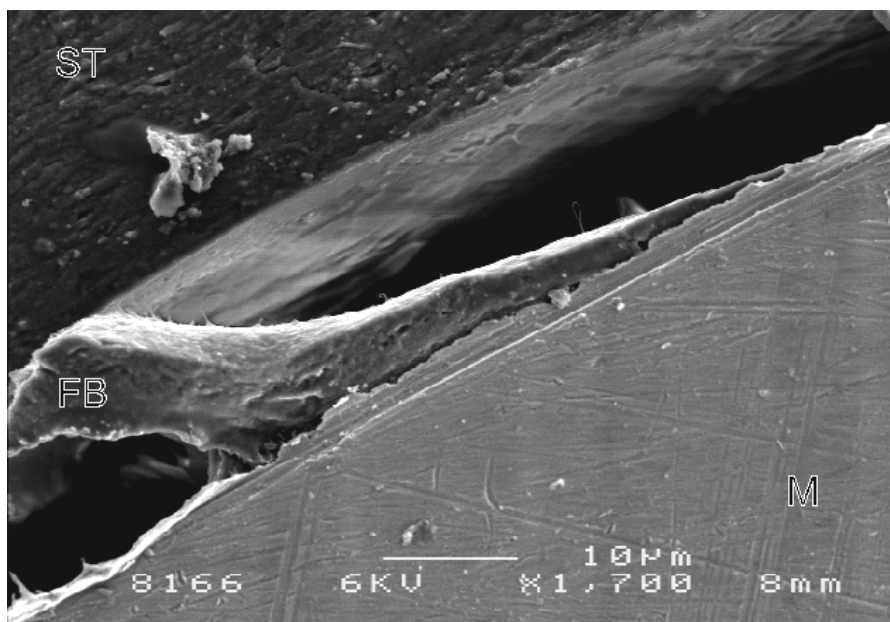


Fig. 3. B) Fibroblast (FB) attachment to a metal (M) surface. The 15 μ m gap between metal and soft tissue is due to sample preparation. A close connection, a slender cell shape and small filopodia are seen. Ti-6Al-4V 4 weeks after implantation. (FESEM, magn. 1700x).

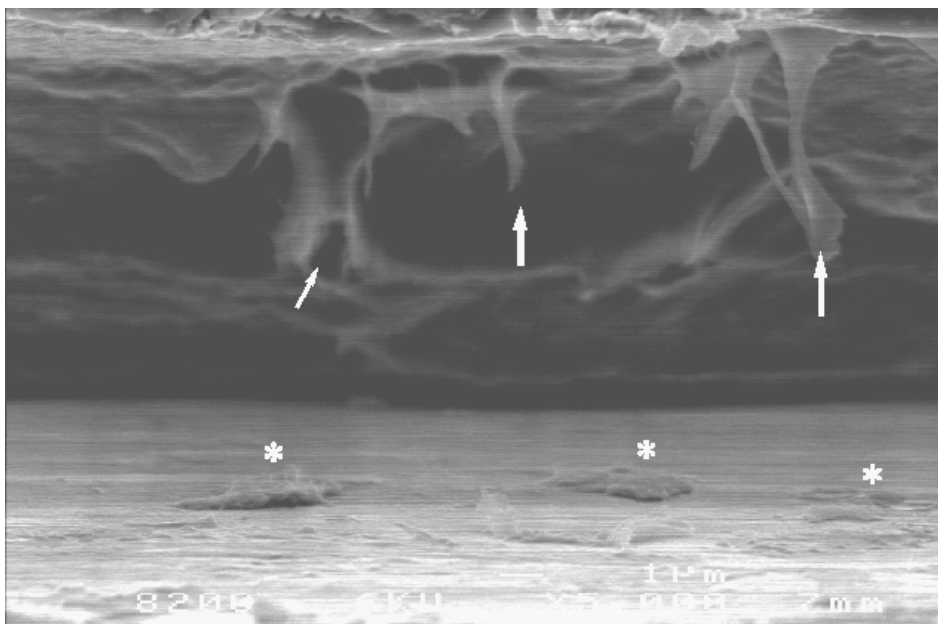


Fig. 3. C) Cell-metal interface of NiTi 4 weeks after implantation. Torn cell podia and membrane structures (arrows) can be seen in the under surface of the cell. Respective focal contacts to the metal surface (asterix) are also present (FESEM, magn. 5000x).

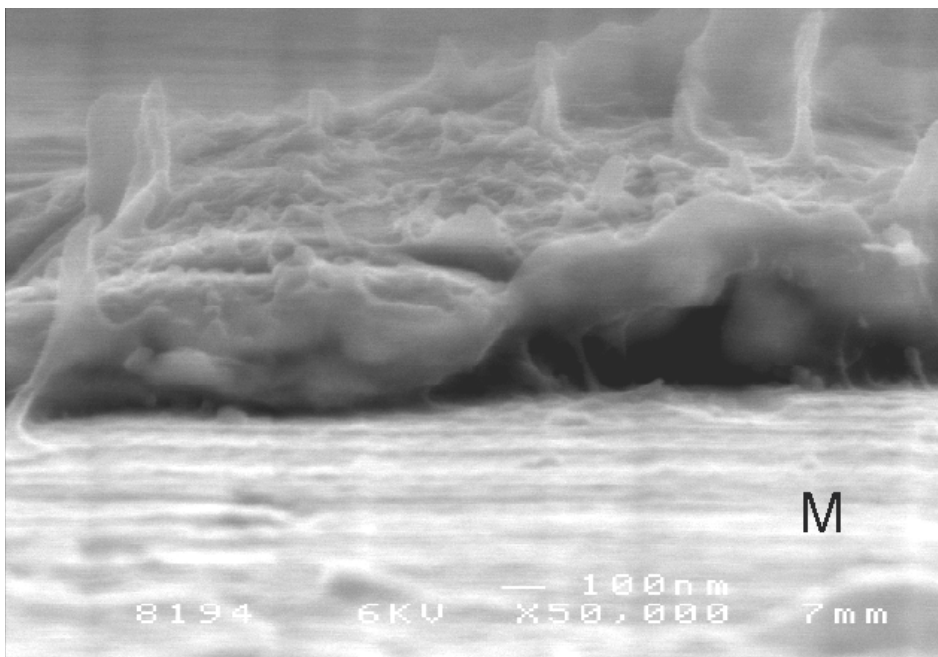


Fig. 3. D) A closely connected focal adhesion site with ruptured cell membrane structures. The gap to the metal (M) surface is under 30 nm. NiTi 4 weeks after implantation. (FESEM, magn. 50 000x).

In study III, a close contact was seen between the fibrous capsule layer, single cells and the NiTi, StSt and Ti-6Al-4V *in vivo* (Fig. 3 A and B). There was a thin interfacial "basal lamina like" zone over the surface of NiTi, Ti-6Al-4V and stainless steel materials. It consisted of an afibrillar layer of organic amorphous material with a wavy texture. This was generally thicker over Ti-6Al-4V than NiTi or stainless steel implants. Single cells seemed to adhere via this layer with clod-like, direct-contact adhesion structures (gap < 30 nm) (Fig. 3D). These were considered to be focal cell adhesion sites with proteins and cell membrane composition. The cell surfaces had respective fibril or torn cell membrane structures (Fig. 3C).

5.1.2. Cell proliferation *in vitro*

The rates of fibroblast and osteoblast proliferation in cell cultures correlated well with each other. The number of cells in the control group flask of fibroblast cultures at the end of the study was 1.6×10^5 (SD 1.2×10^4). The average proliferation of fibroblasts was 134.1% ($p < 0.02$) in the flasks containing titanium as compared to the control flasks, and 108.3% ($p < 0.136$) and 106.8% ($p < 0.174$) in the NiTi and stainless steel flasks. The proliferation of cells was only 63.2% ($p < 0.002$) in the white soft paraffin flasks and 48.3% ($p < 0.0001$) in the composite material flasks compared to the control group.

The proliferation of osteoblasts in the control group was 1.7×10^5 (SD 1.6×10^4). The values for titanium, NiTi and stainless steel were almost the same as those for the control group: 99.5% ($p < 0.483$), 100.5% ($p < 0.475$) and 104.7% ($p < 0.334$), respectively. In the white soft paraffin group, the number of cells was 81.5% ($p < 0.058$) compared to the control group. In the composite material flasks it was even less, 53.6% ($p < 0.025$) (Fig. 4).

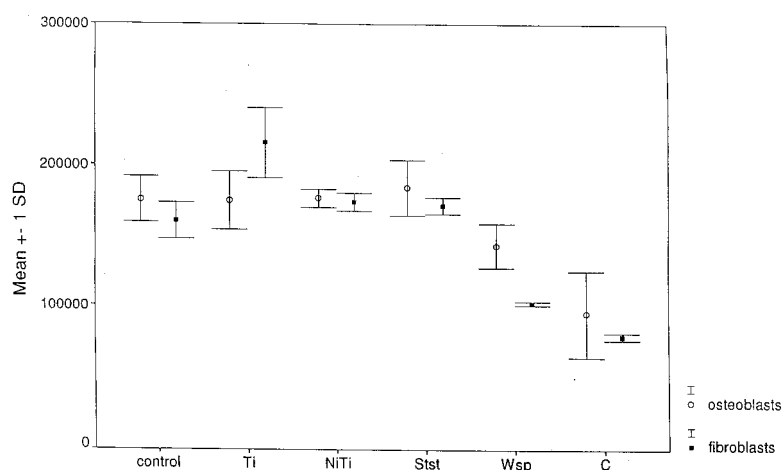


Fig. 4. Final number of osteoblasts and fibroblasts (cells/ml) at the end of the trial (10th day) in different test disc groups, mean $\pm$ 1 standard deviation. Control = no test disc, Ti = titanium, NiTi, Stst = stainless steel, Wsp = white soft paraffin, C = composite material Silux plus®.

5.2. Soft tissue response to NiTi

The general features of the soft tissue response in the studies II and III were similar. The tissue responses to NiTi as well as to the control materials were clearly non-toxic, regardless of the time point. No qualitative differences in histology between the NiTi, Ti-6Al-4V and stainless steel test materials could be seen at the light-microscopic level. Black-brown discolored wear was found in the extracellular space and inside some cells around four Ti-6Al-4V implants and one NiTi implant in study II. The discolored tissue was mainly located at the ends of the implant. There was no necrosis, granulomas or signs of dystrophic soft tissue calcification around any of the test materials.

The inflammatory reaction was most notable in the 2- and 4-week samples. Generally, the inflammatory reaction was mild and restricted to an area very near to the implants regardless of the material. At 4-12 weeks, the encapsulation membrane had two distinct zones: an inflammatory cell layer with a clearly milder inflammatory response than in the 2-week groups, and a distinctive fibrous capsule zone formed by fibroblasts and collagen fibers. In the 26 week group, there was only a thin fibrous layer with practically no macrophages or other inflammatory cells present (Fig. 5, A1-5, B1-5 and C1-5).

New capillaries were seen between the fibrous capsule layer and muscle tissue. These were more prominent after periosteal than intramuscular implantation, which may be related to different surgical procedures. The number of capillaries diminished over time. An avascular capsule was seen in the 26-week group regardless of the material.

Monocytes and macrophages were the main inflammatory cell types in the studies II and III. They were found very close to the implant-tissue interface. They were most prominent in the short-term implanted samples. After 4 weeks, macrophages began to disappear, and their numbers remained very low afterwards. No differences were seen between the different test materials.

Foreign body giant cells were observed at all time points, except in the 26-week samples. They were equally few in number in the 2- to 12-week samples and with different test materials. A few ring-shaped, small ($< 50 \mu\text{m}$), low-polarizing, non-metallic material particles were seen near the tissue-material interface in all time groups and materials.

Some polymorphonuclear and round cells (lymphocytes, plasma cells, mast cells) were also present in the 2- and 4-week samples, but cells of these types could only be seen incidentally at the later time points. However, separate mast cells and few eosinophils were seen in the 26-week samples. No signs of lymphocyte accumulation were visible as an indication of immune system activation by the materials, a finding similar in all test implants.

Active fibroblasts were seen in the 2-week samples. After 2 weeks, mature fibroblasts were found to form a distinct capsule between the soft tissue and the test material. The number of fibroblasts increased over time, but the fibroblast density also became higher. At 26 weeks, there were thin, well-defined, dense fibrous capsules between the soft tissue and the implant. The fibroblasts were flattened and elongated with wavy collagen fibers between them. Practically no macrophages or other inflammatory cells were present. Again, no differences were seen between the different test groups.

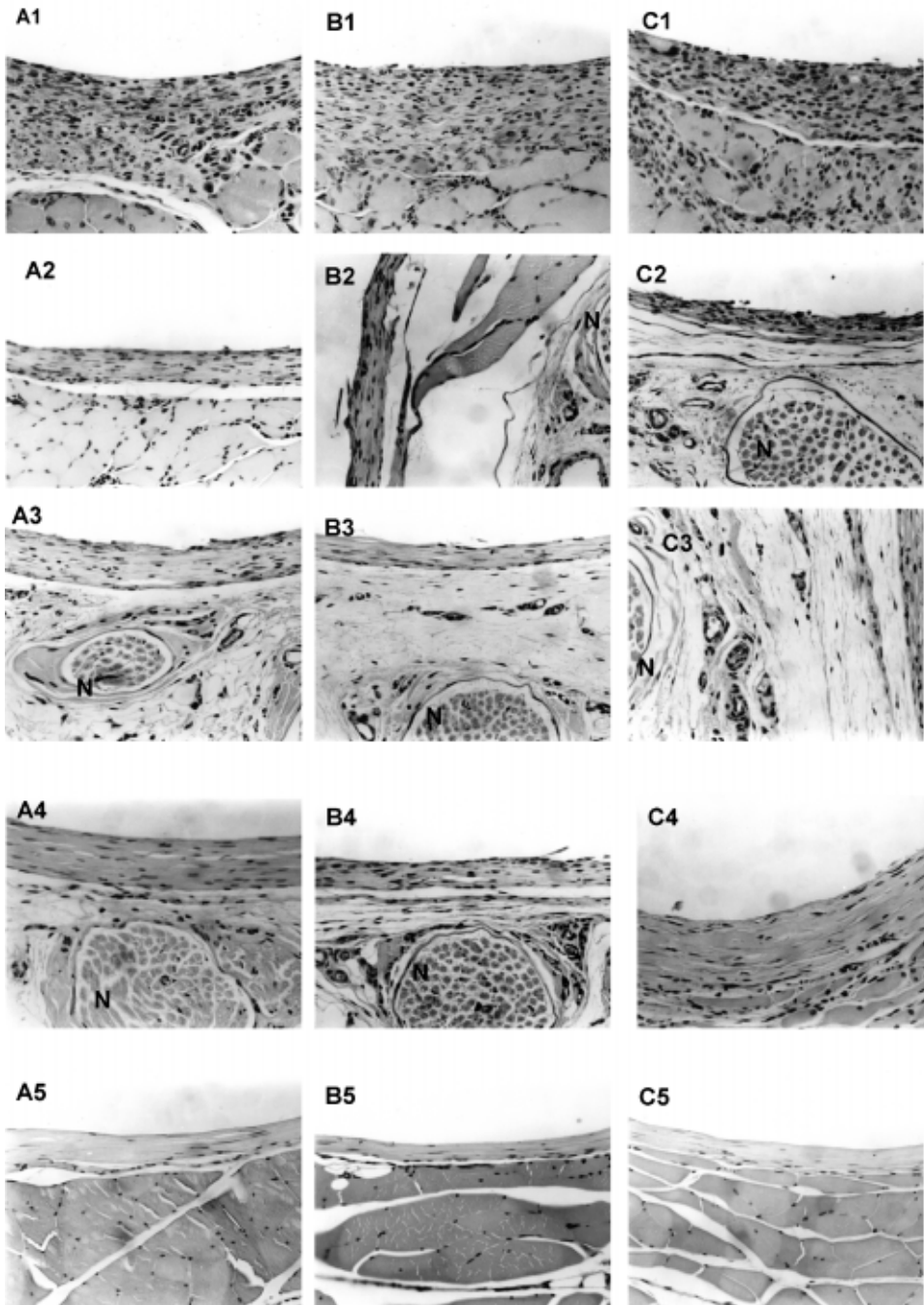


Fig. 5. Tissue response around tested materials after implantation with respect to time. A = Nitinol, B = stainless steel and C = Ti-6Al-4V. 1 = two weeks, 2 = four weeks, 3 = eight weeks, 4 = twelve weeks and 5 = twenty-six weeks. N = nerve.

5.3. Perineural response to NiTi

There were a total of 16 implants in very close contact with peripheral nervous tissue in study II. There were no perineural implants in the 2-week group. In the 4-week group, one stainless steel and 2 Ti-6Al-4V implant capsules were found very near to the nerve, but no NiTi capsules were found close to the nerve. In the 8-week group, there was one implant capsule in the stainless steel group, 5 in the NiTi group and one in the Ti-6Al-4V group with very close contact to the nerve. In the 12-week group, 2 stainless steel and 2 NiTi implant capsules were found next to the nerve. In the 26-week group, there were 2 NiTi and 1 stainless steel implant capsules near the nerve.

There were no signs of necrosis, prolonged inflammation or irritation with the NiTi material. Also, no accumulation of specific cell types, cells with phagocytosed material, foreign particles or abnormal fibrous tissue responses were seen in the perineural tissue with any of the tested materials.

There was no edema, neuronal or glial reaction or inflammatory reaction in the nerve itself. Very few giant cells, PMNs, plasma or mast cells could be observed in the area between the nerve and the encapsulating membrane (Fig. 5, A3-4, B2-4 and C2-3).

The end-stage neural and perineural responses were nearly inert with NiTi and also with the other tested materials.

5.4. Encapsule membrane thickness

The overall capsule thickness was time-dependent, and generally decreased over time. There was, however, an increase in thickness with NiTi at 8 weeks (mean $\pm$ 1 SD: NiTi 62 ± 25 μ m vs. StSt 41 ± 8 μ m vs. Ti-6Al-4V 48 ± 12 μ m) and with Ti-6Al-4V at 12 weeks (NiTi 45 ± 10 μ m vs. StSt 38 ± 15 μ m vs. Ti-6Al-4V 57 ± 31 μ m) compared to stainless steel. These differences were not found to be statistically different, as the number of animals in each time group was not considered sufficient. The stainless steel capsule was thickest at 2 weeks (NiTi 70 ± 19 μ m vs. StSt 84 ± 28 μ m vs. Ti-6Al-4V, 69 ± 49 μ m). At 4 weeks, there were no clear differences (NiTi 52 ± 12 μ m vs. StSt 55 ± 16 μ m vs. Ti-6Al-4V 54 ± 16 μ m). At 26 weeks, all capsules around the test implants in the different groups were found to be equally thin, about 4 - 8 cell layers (NiTi 37 ± 9 μ m vs. StSt 35 ± 8 μ m vs. Ti-6Al-4V 37 ± 12 μ m), and the morphologies were also similar (Fig. 6). There were some variations in capsule thickness between the samples taken from a given material group at the same time point, as there were also between individual samples of different materials. The capsule was thicker at the ends of the implant. That was the reason for taking the measurements at mid-region.

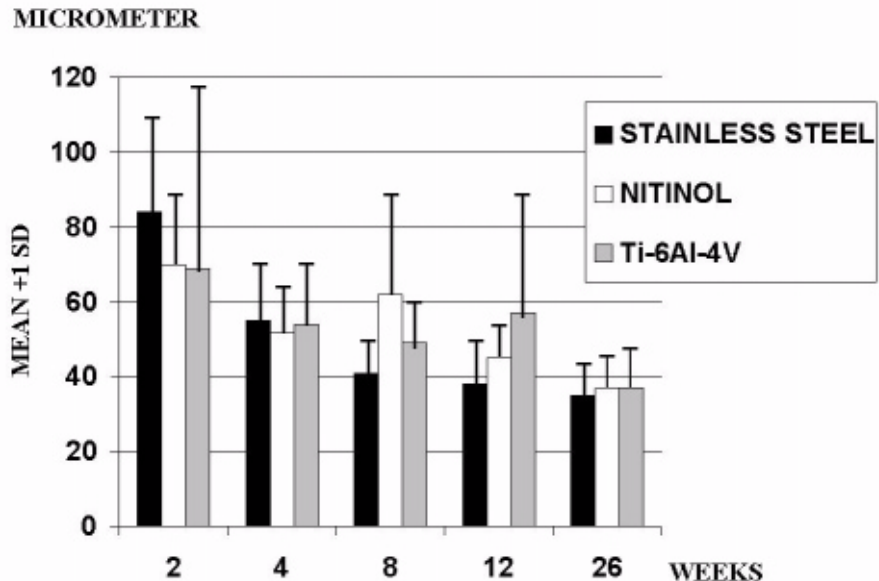


Fig. 6. The capsule membrane thickness after implantation. The Y-axis indicates thickness in micrometers. The X-axis shows the time elapsed after implantation. The columns depict the mean thickness +1 SD for the different material groups.

5.5. Bone response to NiTi in the regional acceleratory phenomenon (RAP) model

Some changes in new bone formation and cortical bone width were found after periosteal implantation. The results of histomorphometric measurements are shown in Table 2 and in the Figures 7 and 8. Figure 9 is a scheme illustrating the area of measurement and the different parameters. At 2 weeks, the new woven bone area (N.Wo.B) was larger with Ti-6Al-4V compared to NiTi ($p < 0.01$) or StSt, but the erosion area (E.Ar) and the erosion surface perimeter as a fraction of the bone surface perimeter (E.Pm/B.Pm) were also greater with Ti-6Al-4V compared to NiTi, indicating active early modeling. NiTi and StSt had their maximum new bone formation activity at 4 weeks, while the new bone formation of Ti-6Al-4V began to diminish at that point. There were no statistically significant differences between the materials at this point. At 8 weeks, Ti-6Al-4V had its highest erosion activity. Its Ct.Wi was lower than that of StSt ($p < 0.005$) or NiTi ($p < 0.05$). NiTi had higher N.Wo.B than StSt. The differences between the tested materials were smallest at 12 weeks. E.Ar was hardly measurable for all materials. At 26 weeks, there was still some new bone formation and bone resorption activity left, indicating that a steady state had not been reached yet. E.Pm/B.Pm remained higher with Ti-6Al-4V. Remodelation

occurred with all materials, as the perforated surfaces were partly filled with new bone. There were no statistically significant differences between the materials at 12 and 26 weeks.

Table 2. Histomorphometric measures of bone area (B.Ar), erosion area (E.Ar) and active erosion surface perimeter/ bone surface perimeter (E.Pm/B.Pm). Values are given as mean $\pm$ 1 SD.

Variable	Material	Time (weeks after implantation)				
		2	4	8	12	26
B.Ar (μm ²)						
	NiTi	1012±142	1063±298	1070±145	1011±350	1090±266
	Ti-6Al-4V	1009±64	953±156	933±140	981±70	890±131
	Stst	919±174	1156±130	1075±105	1064±158	1190±302
E.Ar (μm ²)						
	NiTi	10±9	28±30	16±13	–	10±22
	Ti-6Al-4V	26±16	21±18	30±25	–	–
	Stst	24±16	12±10	10±16	4±8	17±27
E.Pm/B.Pm (%)						
	NiTi	23	22	24	12	15
	Ti-6Al-4V	33	34	34	34	24
	Stst	26	17	25	12	10

The number of rats in each time group was five/ tested material (n=5). Value not measurable = (–).

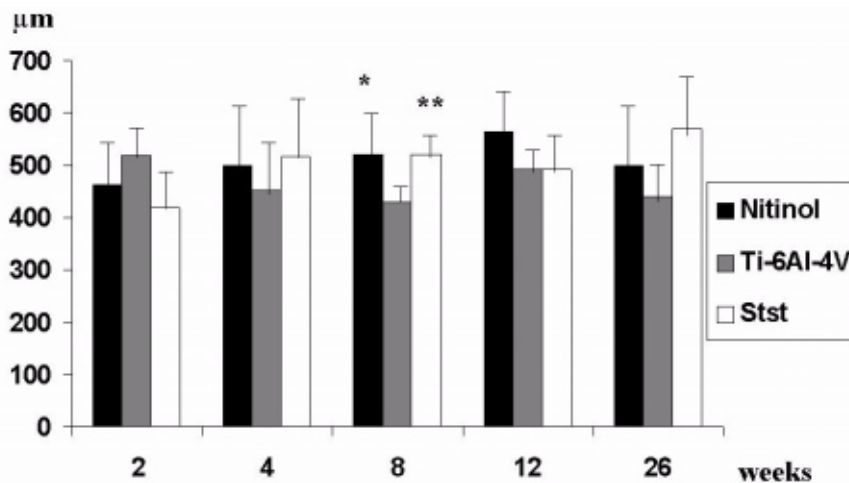


Fig. 7. Cortical width after implantation. The columns depict the mean area +1 SD for the different material groups. At 8 weeks, the cortical widths (Ct.Wi) were significantly greater in the NiTi ($p < 0.05 = *$) and Stst ($p < 0.005 = **$) groups than in Ti-6Al-4V group.

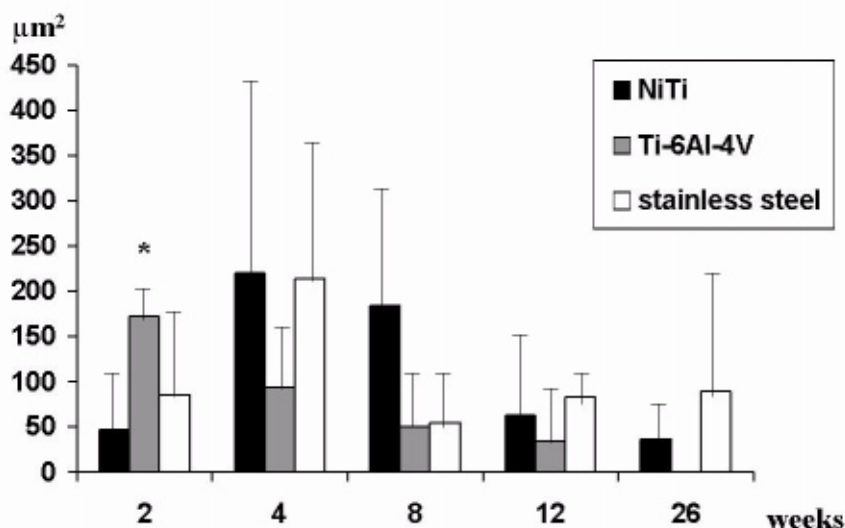


Fig. 8. The area of new woven bone (N.Wo.B) after implantation. The columns depict the mean area $\pm$ 1 SD for the different material groups. At two weeks, the new woven bone area N.Wo.B of the Ti-6Al-4V group was significant larger than that of NiTi. (* = $p < 0.01$).

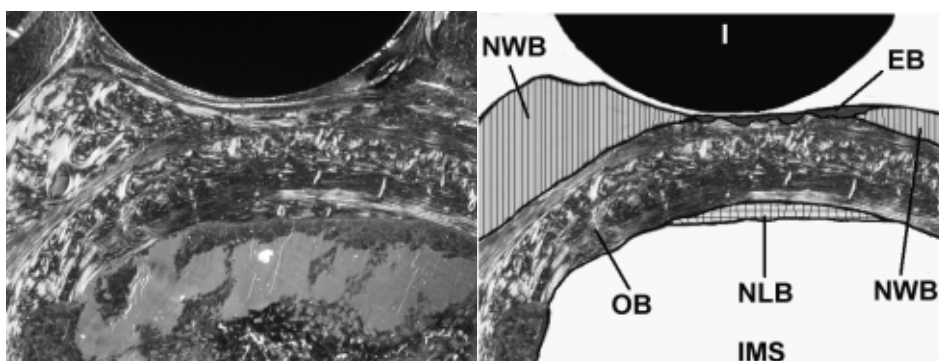


Fig. 9. A scheme illustrating the measurement area and the different parameters. Left: bone structures visualized in polarized light (NiTi 8 weeks after implantation). Right: various areas of measurement. NWB = new woven bone, EB = eroded bone, NLB = new lamellar bone, OB = original bone, I = implant and IMS = intramedullary space. The calculated total bone area (B.Ar) includes NWB + OB + NLB.

Certain common features in bone structure were observed over time in all samples. There was a thin fibrous ($< 20\text{--}200\text{ }\mu\text{m}$) layer present between the bone and the implant. The closest contacts were generally found in the 12- and 26-week samples (Fig. 10E and 10F).

The 2-week groups (Fig. 10A) showed formation of new woven bone, mostly at the verge of the closest implant contact area. Some chondral cells were incidentally seen. In the 4-week groups, the new bone lined the nearest implant contact area even more clearly. The outer lamellar bone began to be destroyed under the implant by osteoclasts, and there was

distinct bone porosis. In the 8-week groups, erosion already reached the deeper bone structures (Fig. 10D). Slightly U-shaped cross-sectional bone modelation was observed. The most radical modifications in bone structure and cortical width were seen at 8 weeks (Fig. 10C and 10D). The formation of endosteal callus with lamellar structure was also clearly discernible. In the 12-week samples, the erosion of bone was less abundant and the endosteal callus was thicker (Fig. 10E). In the 26-week specimen, this deformation was already reduced (Fig. 10F). Multiple layers of lamellar and woven bone under the implant were observed, and the lamellar structures under the implant were repaired, indicating remodeling.

In conclusion, an adequate regional acceleratory phenomenon with normal new bone formation was evident with NiTi. The new woven bone formation started earlier in the Ti-6Al-4V than the NiTi group, but at 8 weeks the NiTi and stainless steel groups had greater cortical width compared to the Ti-6Al-4V group. Later than that, no statistical differences were seen.

5.6. Effects of NiTi on fracture healing after intramedullary nailing

5.6.1. General findings

In study IV, NiTi was compared to stainless steel after intramedullary nailing. There were no infections in either group. At 1 week, four rats were seen to hobble. Radiographs showed two nails in both groups to have penetrated far to the knee joint in these rats. The rats were killed and excluded from the study. All the other rats used their legs normally after one week. One fast-growing tumor with skin laceration was found in one rat in the stainless steel group 5 weeks after implantation. The rat was killed and also excluded from the study. The histology showed the tumor to be a benign fibroblastoma.

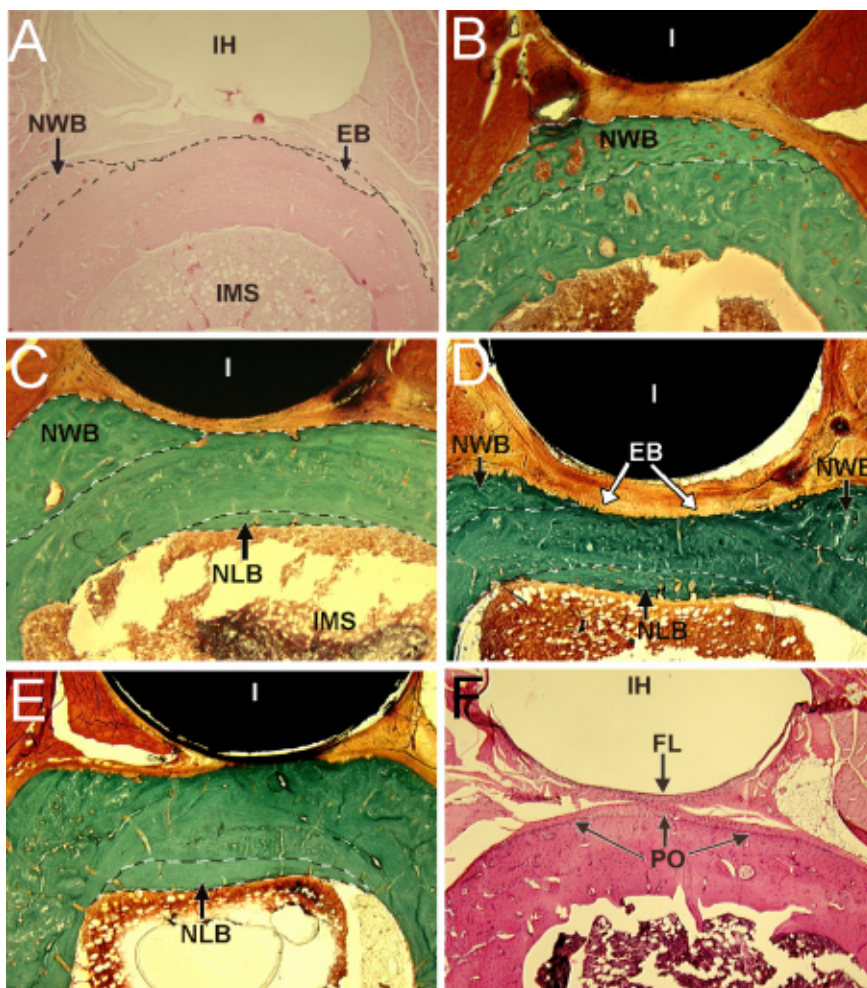


Fig. 10. The photographs show the bone-implant area (magn. 48x). The black disks on top are metal implants (I) in hard resin embedded samples. The white semicircular areas are holes left by removed implants (IH) in paraffin-embedded samples. The bone wall is located in the middle of each picture and the intramedullary space (IMS) is shown below. The areas of histomorphometric measurement are about the same as those seen in these pictures. The broken lines have been added to clarify the different areas. NWB = new woven bone. EB = eroded bone with resorption pits and rough surface. NLB = new lamellar bone in endosteal surface. PO = bone peristosteum. A) NiTi 2 weeks after implantation. There is a new woven bone area left of the outer cortical area. Some eroded bone is also visible, indicating the beginning of the modeling process. B) New woven bone formation was most apparent at 4 weeks in the NiTi group, which is also seen in this photograph. C) NiTi at 8 weeks. A slightly U-shaped cross-sectional bone modeling has developed when periosteal resorption under the implant is compensated by lamellar endosteal callus and lateral new bone bracing. D) Ti-6Al-4V at 8 weeks, when the cortical width value was found to be lower than in the NiTi group. The ragged cortex indicates active resorption, but some new bone can also be seen. E) NiTi 12 weeks after implantation. A close connection with bone is seen. F) NiTi 26 weeks after implantation. There is a thin fibrous layer (FL) between the bone and the hole left by the implant. There is no osteoporosis, continuous resorption or any other sign of harmful irritation.

5.6.2. Histology and morphology

The common histology and morphology of the healing process were similar in the two groups. The osteotomies healed via secondary bone union. At 2 weeks, the callus consisted mainly of fibrous granulation tissue. Chondral tissue was seen around and between the bone fragments and in the endosteal area. Some bony mineralization points were seen. The ends of the osteotomized bones were avascular. At 4 weeks, there was clearly mineralized callus (Fig. 11A) and woven bone formation. The chondral tissue was mostly seen between the bone heads. New woven bone was found in the peripheral area of the external callus and in the endosteal area. Fibro-cartilage tissue was seen in the middle of the external callus. At 8 weeks, there was clearly more mineralization. Woven bone bridged the fracture site as a mark of bony union. In the metaphyseal area, an intramedullary fibro-cartilaginous layer surrounded the implants (Fig. 12A and B).

At 12 weeks, there were complete bony unions (Fig. 11B; 13A, B and C), where lamellar bone structures were also evident in polarized light microscopy. A three-layer callus structure was sometimes seen, including outer peripheral callus bone, the original bone heads and separate endosteal bony callus. These layers were separated by bone marrow and loose trabecular spaces. At 26 weeks, most samples had a compact, thin, circular bone layer around the nail in the metaphyseal intramedullary area out of the osteotomy site. This formed a close contact with both NiTi and stainless steel. This structure was also clearly visualized by the CT scans. Some cortical ends with normal ossifying and vascularizing processes by osteons were visible (Figures 14A and B). Most osteotomies were completely healed. The replacement of woven bone by lamellar bone continued, and remodeling of the whole callus was obvious.

At 60 weeks, the callus contained well-organized lamellar structures. The osteotomy line had disappeared. A thicker peri-implant bone sheet was seen in the metaphyseal area of the intramedullary space (Fig. 12C). Bone and the NiTi implant formed very close contacts (Fig. 12D).

5.6.3. Callus size and the consolidation of osteotomy

The maximum width and length of the callus area in the NiTi and StSt groups during the study period are shown in table 3. The results were notably similar between the groups, and no statistical differences were seen. At 60 weeks, the callus was generally thinner and shorter than at the earlier time periods, indicating a normal remodeling and healing response.



Fig. 11. A) Radiograph of the right femur of a NiTi rat 4 weeks after intramedullary fixation of diaphyseal osteotomy. Cloudy callus has developed and the fixation is stable. B) The right femur 12 weeks after implantation. The osteotomy is well healed.

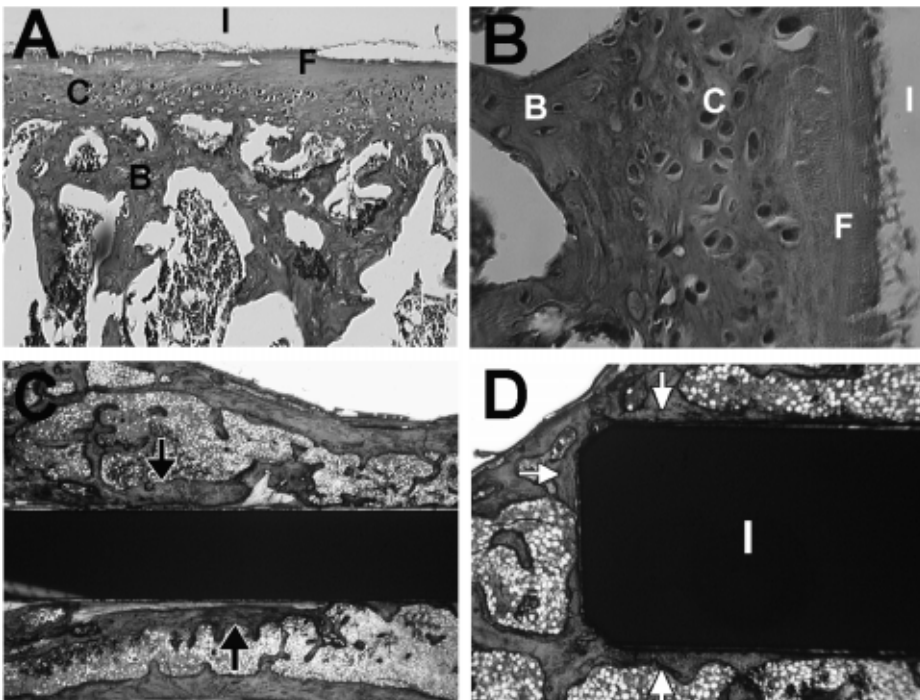


Fig. 12. A and B) A mineralized layer around the removed NiTi nail 8 weeks after implantation in the metaphyseal area. I = The hole left by the removed intramedullary nail. The closest layer is composed of a collagen-rich thin fibrous (F) layer. This turns into cartilaginous (C) and bone (B) tissue. HE staining. Magn. 120x and 480x. C) Metaphyseal femur area distal to the osteotomy site. A thick lamellar bone (arrows) sheet around the implant is seen. NiTi 60 weeks after implantation. Magn. 32x. D) Bone envelops very closely the implant head (arrows). Magn. 48x. I= implant. Hard resin embedded section with Goldner-Trichrome staining with the intramedullary nail in place. Slice thickness 30µm.

At 2 weeks, there was no overstepping callus formation and a clear osteotomy line was seen in all femurs. At 4 weeks, callus formation was observed in all rats (Fig. 11A). Most of the osteotomies were classified as showing “good” or “satisfactory” healing in both the NiTi and the stainless steel groups, but there were more “poor” results in the StSt group (27% vs. 20%). There was one rat in the NiTi group and two rats in the StSt group with a 15° antero-posterior malposition. At 8 weeks, more osteotomies were classified as “good” in the NiTi (50%) group than in the stainless steel (36%) group. The total number of rats at this time point was not the same in the two groups, because the rat with the tumor had been excluded from the StSt group. At 12 weeks, the rates of consolidation were equal between the groups. At 26 and 60 weeks, the percentile healing responses were also equal. The consolidation of the osteotomy line as revealed by radiographs is shown in table 4.

Table 3. Maximum callus dimensions measured from radiographs. Values are given as mean (mm) $\pm$ 1 standard deviation. There is not significant differences between the groups. n=number of the rats.

Fixation	Time (weeks after implantation)				
	4	8	12	26	60
NiTi	n=15	n=12	n=9	n=6	n=3
Max width of the callus	7,5 $\pm$ 1,0	7,4 $\pm$ 1,3	7,3 $\pm$ 1,0	7,8 $\pm$ 1,2	7,2 $\pm$ 0,5
Max length of the callus	10,5 $\pm$ 2,2	10,0 $\pm$ 2,3	11,4 $\pm$ 2,4	10,4 $\pm$ 2,5	9,9 $\pm$ 1,1
Stainless steel	n=15	n=11	n=9	n=6	n=3
Max width of the callus	7,6 $\pm$ 1,2	7,6 $\pm$ 1,6	7,5 $\pm$ 1,6	7,5 $\pm$ 2,1	6,6 $\pm$ 1,5
Max length of the callus	10,7 $\pm$ 2,7	10,5 $\pm$ 3,4	11,4 $\pm$ 3,5	11,0 $\pm$ 5,0	8,8 $\pm$ 3,4

Table 4. Union of osteotomy fixed with NiTi or stainless steel intramedullary rod. Values are expressed as percentages.

Fixation	Time (weeks after implantation)				
	4	8	12	26	60
NiTi	n=15	n=12	n=9	n=6	n=3
Good	40%	50%	55%	66%	100%
Satisfactory	40%	34%	33%	17%	0
Poor	20%	16%	12%	17%	0
Stainless steel	n=15	n=11	n=9	n=6	n=3
Good	33%	36%	55%	66%	100%
Satisfactory	40%	46%	33%	17%	0
Poor	27%	18%	12%	17%	0

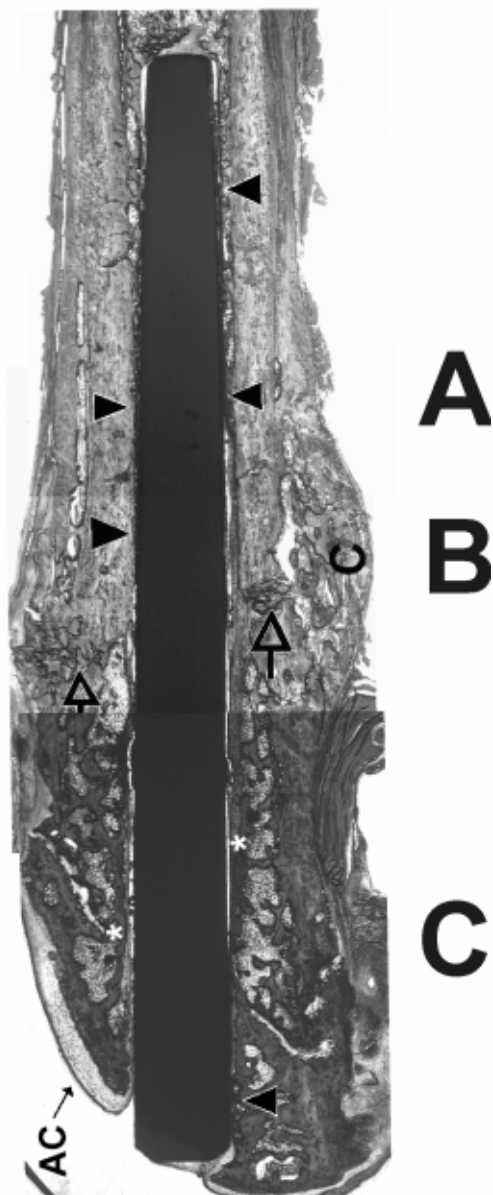


Fig. 13. Diaphyseal osteotomy fixed with a NiTi intramedullary nail 12 weeks after implantation. The three photographs are linked together (A, B and C). Good contact between the bone and the nail is evident (black arrows). B) Orderly callus with healed osteotomy (open arrows) is seen. C= callus. Hard resin embedded section with Goldner-Trichrome staining with the intramedullary nail in place. Slice thickness 30 μ m. Magn. 32x. C) In the metaphyseal area, a thin layer of lamellar bone (asterisk) demarcates the nail bed quite closely, indicating good tissue tolerance. AC= articular cartilage of knee joint.

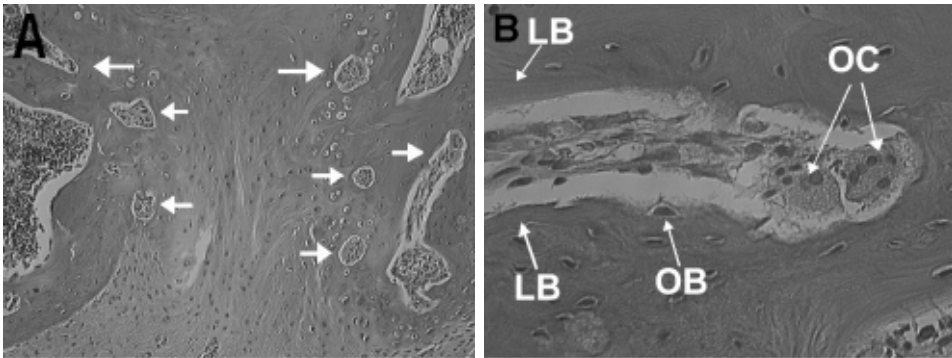


Fig. 14. A) Remodelation of the original bone ends. Mineralized chondral tissue between the bone ends has been replaced by secondary osteons (white arrows) at the osteotomy line. NiTi 26 weeks after implantation. Magn. 120x. B) Remodelation unit at work. A cutting cone with multinuclear osteoclasts (OC) resorbs the woven bone, and lamellar bone (LB) is formed by osteoblasts (OB). NiTi 26 weeks after implantation. Magn. 480x.

5.7. Peripheral quantitative computed tomography

5.7.1. Callus morphology

The cross-section of the callus at the osteotomy site appeared to have distinct mineralization zones in pQCT. This callus structure with three layers developed in 12 weeks. The high-density outer zone consisted of periosteal callus. At 26 weeks, it was almost equally thick as the original cortex. The original bone cortex formed the middle layer. The inner high-density layer consisted of the thinner endosteal callus (Fig. 15B). In the distal metaphyseal area outside the osteotomy zone, a separate peri-implant high-density layer first appeared at 4 weeks and was distinct at 26 weeks and later (Fig. 15C). There were no morphological changes between the two groups. At 60 weeks, the periosteal callus was thick and well mineralized (Fig. 15A).

5.7.2. Bone mineral density

The total (BMD) and cortical (CtBMD) bone mineral densities did not differ between the NiTi and StSt groups. Distinct differences were seen in various parts of the femur. Mineral density was clearly lower in the osteotomy area than in the nail area out of the osteotomy, which, in turn, showed lower mineral density values than the proximal operated femur. The proximal femurs had no differences compared to the non-operated femurs of the same rats (Fig. 16A and B). The mean CtBMD in the osteotomy zone increased slightly from 26 weeks (NiTi = 972 ± 78 mg/ccm, StSt = 982 ± 85 mg/ccm) to 60 weeks (NiTi = 1038 ± 195 mg/ccm, StSt = 1100 ± 62 mg/ccm).

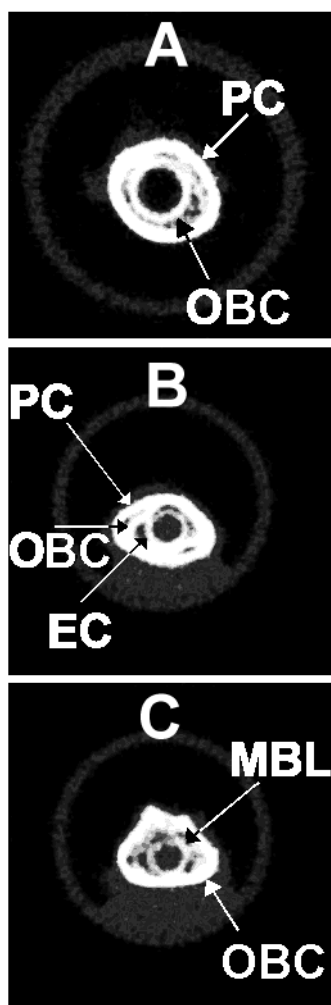
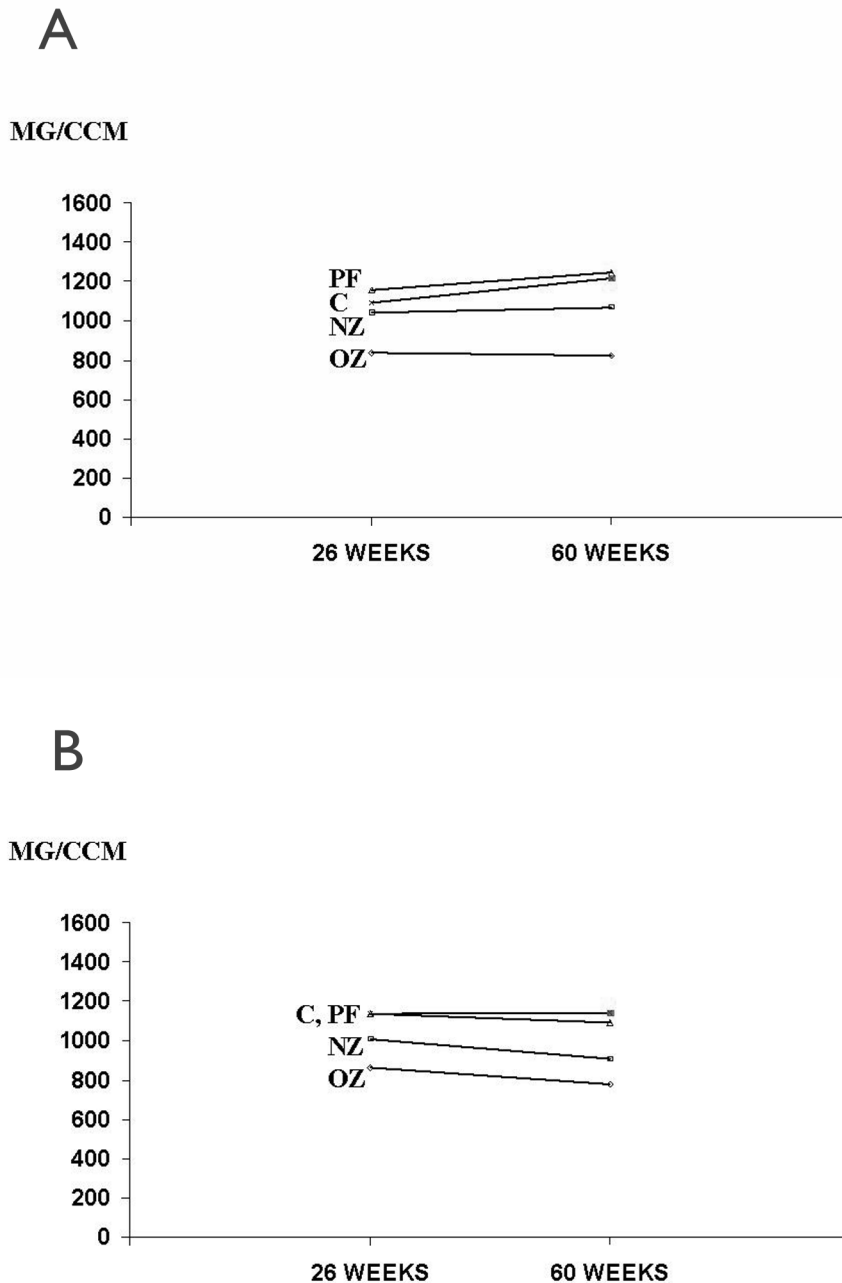


Fig. 15. pQCT scans of various sites of femur after intramedullary nailing (nail removed). PC = peripheral callus, OBC = original bone cortex, EC = endosteal callus, MBL = mineralized endosteal peri-implant bone layer. The scans refer to the areas in the figures 12A, B and C, respectively. A) Callus area proximal to the osteotomy site. NiTi 60 weeks after implantation. B) Osteotomy site 26 weeks after NiTi implantation. A three-layer structure can be seen. C) Metaphyseal area distal to the osteotomy site 26 weeks after NiTi implantation. A mineralized peri-implant layer has developed.



Figs 16 A and B. Mean bone mineral density (BMD) in the different zones of the operated femurs 26 and 60 weeks after operation. A) NiTi group and B) stainless steel group. OZ = osteotomy zone(♦), NZ = nail zone proximal and distal from osteotomy (□), PF = proximal area of the operated femur(Δ). The contralateral intact femur served as control = C (X) .

5.8. Corrosion of NiTi

5.8.1. Corrosion *in vitro*

In the fibroblast cell culture media, the amount of nickel in the NiTi group was 129 $\mu\text{g/l}$ on the second day (first analysis), 23 $\mu\text{g/l}$ on the fourth day (second analysis), 9 $\mu\text{g/l}$ on the sixth day (third analysis) and 8 $\mu\text{g/l}$ on the eighth day (fourth analysis). The titanium concentration was below 20 $\mu\text{g/l}$ in all cases, and there were no measurable concentration variations.

Nickel release from stainless steel was 7 $\mu\text{g/l}$ on the second day, 3 $\mu\text{g/l}$ on the fourth day, 2 $\mu\text{g/l}$ on the sixth day and 2 $\mu\text{g/l}$ on the eighth day. The titanium concentrations assessed from the titanium cell culture media were below 20 $\mu\text{g/l}$ in all samples and showed no variations.

Metal dissolution measured from osteoblast media correlated quite well with the results of fibroblast media. Again, the most pronounced increase in nickel ion concentration in the media of the osteoblast cultures was observed in the first NiTi medium analysis, 87 $\mu\text{g/l}$. It then decreased rapidly: 14 $\mu\text{g/l}$ (second sample), 5 $\mu\text{g/l}$ (third sample), 5 $\mu\text{g/l}$ (fourth sample). The titanium concentrations were all below 20 $\mu\text{g/l}$ in both the NiTi and the titanium medium samples. The nickel concentrations in stainless steel exposed culture media were 7 $\mu\text{g/l}$ (first sample), 1 $\mu\text{g/l}$ (second sample), 11 $\mu\text{g/l}$ (third sample) and 1 $\mu\text{g/l}$ (fourth sample) (Fig. 17).

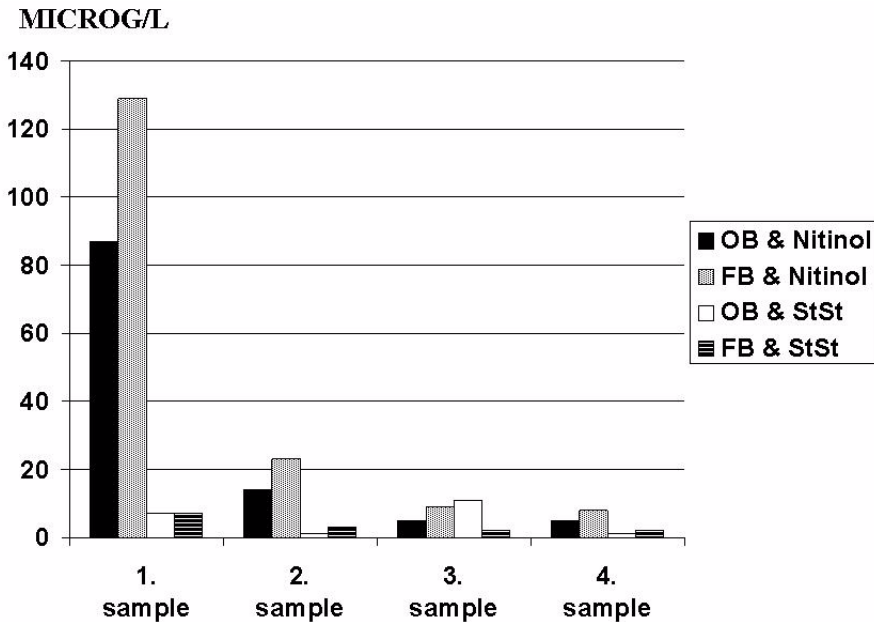


Fig. 17. Nickel release from NiTi and stainless steel test discs ($\mu\text{g/l}$) due to corrosion in osteoblast (OB) and fibroblast (FB) cell culture media. The 1st, 2nd, 3rd and 4th samples were measured on the 2nd, 4th, 6th and 8th days from the beginning of the assay.

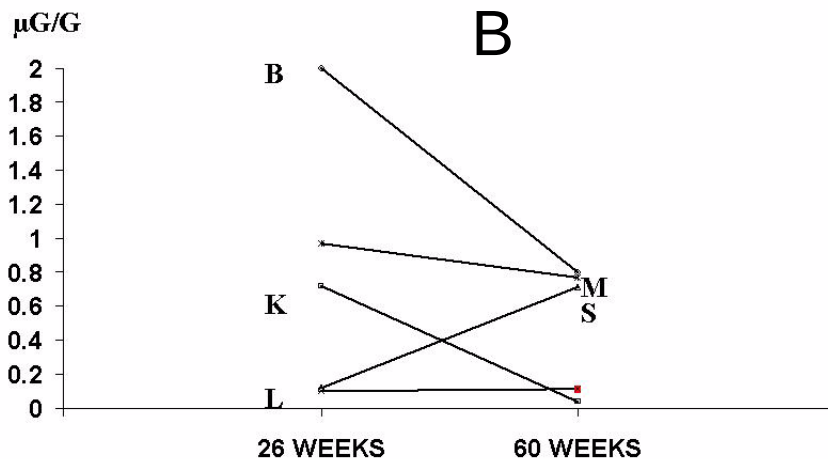
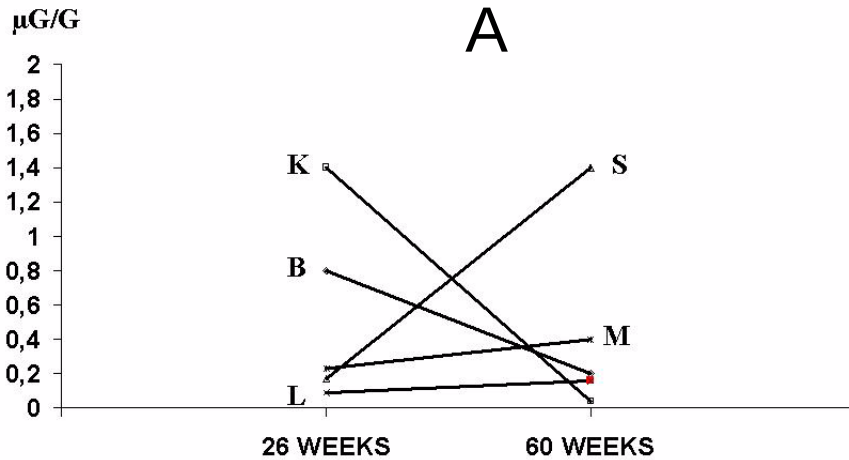
Although the amount of nickel in both fibroblast and osteoblast cell culture media was found to be higher in the NiTi than in the stainless steel cell culture media at the baseline, it had no effect on or correlation with cell proliferation or cell growth near the implant surface at the concentration levels measured in this study.

5.8.2. Trace ions in various organs

In study IV, the amounts of nickel measured in various organs of NiTi rats did not stand out in the stainless steel rats. The overall levels were very low ($< 2 \mu\text{g/g}$ /dry weight) and near the detection limit of the GFAAS-based sensitive method. In the NiTi group at 26 weeks, the highest Ni levels were observed in the kidney ($1.4 \pm 1.0 \mu\text{g/g}$). In the StSt group at 26 weeks, the highest Ni concentration was observed in the brain tissue ($2.0 \pm 0.1 \mu\text{g/g}$). The values decreased by 60 weeks. In the spleen, the Ni ion concentration slightly increased from 26 (NiTi $0.17 \pm 0.06 \mu\text{g/g}$, StSt $0.12 \pm 0.07 \mu\text{g/g}$) to 60 (NiTi $1.4 \pm 1.1 \mu\text{g/g}$, StSt $0.7 \pm 1.1 \mu\text{g/g}$) weeks in both groups (Fig. 18A and B). There were no statistically significant differences in the Ni concentration between the NiTi and StSt groups or between the 26- and 60-week time points in any of the organs. The spleen Fe concentrations at 26 weeks ($6010 \pm 1950 \mu\text{g/g}$) vs. 60 weeks ($14200 \pm 4370 \mu\text{g/g}$) were statistically significantly ($p < 0.05$) different in the StSt group, but not in the NiTi group. Comparing 26 to 60 weeks, an increased Fe content in the kidneys of both groups (NiTi 502 ± 95 vs. $915 \pm 71 \mu\text{g/g}$, $p < 0.005$) (StSt 572 ± 83 vs. $886 \pm 153 \mu\text{g/g}$, $p < 0.05$) was noted. The concentrations of Cr in organs were similar in both groups ($< 0.4 \mu\text{g/g}$).

5.8.3. Corrosion analysis of retrieval implants

In study II, only one stainless steel implant had rust streaks and some minor pitting corrosion visible to the bare eye. There was no evidence of corrosion on visual inspection in any of the NiTi, Ti-6Al-4V or stainless steel implants the studies III or IV. Overall corrosive changes, as shown by FESEM analysis in study IV, were more evident in StSt implants. Some corrosion pits were observed in the StSt surface after 60 weeks of implantation at magnifications of 200x or higher. The pits were uniformly distributed over the implant. No such pits were seen in the NiTi implants, which, in turn, contained small, longitudinal, irregular enlarged microstructures. Some surface contaminants were present in both materials, especially at 60 weeks (Fig. 19A, B, C and D).



Figs 18 A and B. The mean concentrations of nickel ($\mu\text{g/g}$ of dry weight) in organs 26 and 60 weeks after (A) NiTi and (B) stainless steel implantation measured by GFAAS. K= kidney (□), B= brain (◆), L= liver (X), S= spleen (△) and M= muscle (*).

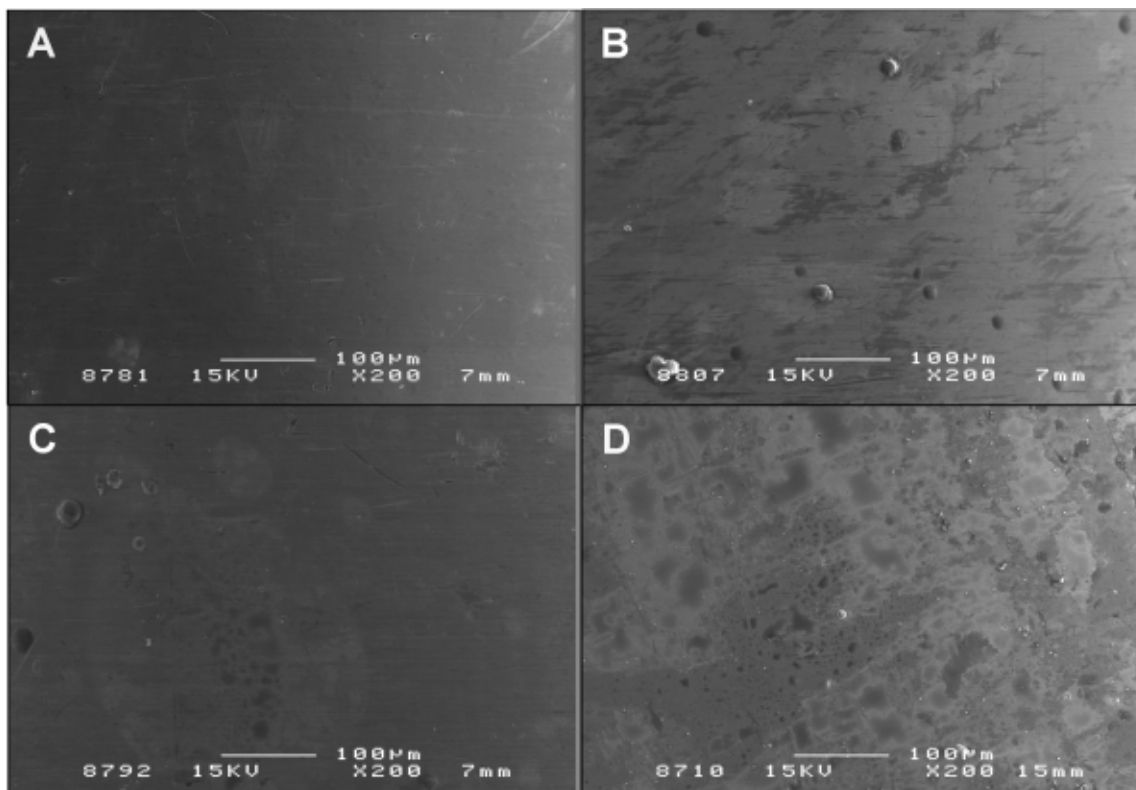


Fig. 19. FESEM images of retrieval intramedullary nails, magn. 200x. A) NiTi 4 weeks after implantation. No marks of corrosion. B) NiTi 60 weeks after implantation. Enlarged longitudinal irregularities due to corrosion and some organic debris are seen. C) Stainless steel 4 weeks after implantation. Smooth surface with some cell debris is seen. D) Stainless steel 60 weeks after implantation. Surface irregularities and corrosion pits as well as some organic debris can be seen.

6. Discussion

6.1. Cell proliferation and connection with NiTi in vitro

The *in vitro* responses of human fibroblasts and osteoblasts to Nitinol in an environment simulating physiological conditions were evaluated in cell cultures. No responses of human osteoblasts to NiTi have been published before.

The toxic effects of different metals can be well quantified *in vitro*, although cell culture studies cannot directly mimic the cellular response and environment *in vivo*. Generally, the cell culture method is considered as a very sensitive method of toxic screening (Rae 1986). This method also provides a possibility for direct observation of living cells.

The main question was whether the amount of nickel dissolved from NiTi is high enough to affect cell proliferation in a cell culture and what the dissolution rate is compared to another nickel-containing implant alloy, stainless steel. At high concentrations, nickel itself is known to have toxic effects in cell cultures and in tissues, but less so than cobalt or vanadium, which are also routinely used in implant alloys (Gerber *et al.* 1980).

The volume of media and cells were quite small compared to the volume and surface area of the tested metal disks. Thus, the corrosion effect on cells is expected to be much more prominent than *in vivo*. Also, the normal clearance of tissue fluids is missing, and the cultured cells are isolated from the normal detoxification pathways of the body. These factors enhance the effect of corrosion products on cells. For the above reasons, cell cultures can be considered a good basic screening method before animal tests.

Human mesenchymal cells were used in the cultures, because they are the cells present on the site of implantation. Osteoblasts were used as an *in vitro* model in order to determine the cytotoxicity of NiTi as an orthopedic material. The interesting point was if there is a difference in behavior between fibro- and osteoblasts in the presence of the test materials. The responses of both cells were similar, however, confirming the validity of the selected method.

Titanium was chosen as one of the experimental materials for two reasons: it has been widely used in traumatology and orthopedics and it is the other main component of NiTi. It is known to be one of the best tolerated metal biomaterials (Albrektsson *et al.* 1981, Serre *et al.* 1994).

Stainless steel is the most commonly used implant material in surgery. It is very well tolerated by human tissues. It contains 10-14% nickel. Composite material (Silux Plus®) is used as a tooth filling material in dentistry. It is known to have shown some toxicity *in vitro* (Peltola *et al.* 1992). That was the reason for choosing it as a toxic positive control for the other tested materials. White soft paraffin was used as a fixative in testing discs. Its effect was also analyzed separately.

Fibroblasts appear to be more sensitive to the growth-inhibiting effect of composite material and white soft paraffin or the growth stimulation of titanium than osteoblasts. The responses of both cells were similar, however. Parallel findings were made by Morrison *et al.* (1995). The proliferation of cells was undisturbed and similar in NiTi, stainless steel and the control group (Fig. 4).

The osteoblast proliferation with titanium was not more abundant than that with NiTi, stainless steel or control, but it gave some proliferatory stimulus to fibroblasts. Enhanced fibroblast proliferation is not always a good thing. It may contribute to the fibrous membrane, which may act as a conduit for the transport of polymeric debris produced at the articulating surfaces of hip prostheses and contribute to loosening or osteolysis (Maloney *et al.* 1993). In present study, NiTi caused neither inhibitory nor stimulatory effects with fibroblasts.

The nickel concentration was higher in the NiTi media than in the stainless steel media at the beginning of the test. However, this had no effect on the proliferation and the total amount of fibroblasts and osteoblasts. There are two possible explanations for this: the maximum concentration was well below the toxic level, or the high concentration was present for only a short time. Cells formed in quite close contact with the NiTi test discs (Fig. 2), which is also a sign of good biocompatibility. A clearly different reaction was seen in the case of composite material and white soft paraffin. The observed toxic influence of white soft paraffin on cultured cells was rather surprising.

6.2. Cell and soft tissue response to NiTi

The local tissue response is the most important aspect of biocompatibility (Vince *et al.* 1991). The cell and soft tissue responses to NiTi have not been elucidated in detail. The biocompatibility of a material *in vivo* can be evaluated by analyzing the cell population present, measuring the mediator and metabolite cells excreted, or analyzing the morphologic characteristics of the tissue around the implant (Hunt *et al.* 1993, Hunt *et al.* 1995, Anderson 1996, Anderson *et al.* 1996, Harada *et al.* 1996). If the material is clearly toxic, it induces a strong local response. Normal histological and morphological *in vivo* examinations with animals give reasonably reliable results and belong to the basic protocol used to estimate the biocompatibility of a new material. However, it must be kept in mind that if there are toxic elements in the material which dissolve slowly, they might give symptoms many years after implantation. When a material is intended for safe use inside the body, its *in vivo* performance and biocompatibility must be very well verified.

Based on a very small number of studies carried out earlier, it has been suggested that the biocompatibility of NiTi in muscle tissue is at least equivalent to Co-Cr and stainless steel alloys, and comparable to titanium (Cutright *et al.* 1973, Castleman *et al.* 1976).

Pure nickel implanted intramuscularly has been found to cause severe local tissue irritation and necrosis (Laing *et al.* 1967). On the other hand, nickel-containing alloys are notably well tolerated. However, there has been a substantial lack of evidence on the biocompatibility of NiTi and the role of nickel in the alloy. Thus, the aim of the intramuscular implantation study was to clarify the detailed soft tissue response to NiTi.

The host reaction after implantation is not a normal wound-healing process. It has certain specific features that ultimately depend on the bulk material and its surface properties as well as on biomechanical considerations (Thomsen *et al.* 1991, Anderson *et al.* 1996). From the standpoint of biocompatibility, the important tissue reactions are mainly related to the inflammatory reaction (Anderson 1988). The general peri-implant soft tissue reaction that took place in the present study is shown in Fig. 5.

Surgical trauma initiates an acute non-specific inflammation, which is characterized by vascular changes, including capillary dilatation, enhanced permeability and increased blood flow. The implant is surrounded by a blood clot containing white blood cells, erythrocytes, platelets and fibrin (Anderson *et al.* 1996).

Polymorphonuclear granulocytes, and later monocytes/macrophages, migrate into the damaged tissues, where their phagocytic activity removes tissue debris, deleterious foreign material and pathogens. The presence of phagocytes later than immediately after surgery may signify problems with the material, while a large number of neutrophils usually indicate infection. There were PMNs present in the NiTi capsule in the 2-week samples, and occasionally even in the 26-week samples, but similar findings were seen with stainless steel and Ti-6Al-4V materials.

The presence of lymphocytes may be anticipated in the cases where an immune response or type IV hypersensitivity reaction has occurred (Thomsen *et al.* 1991). Lymphocytes can be discerned by typical morphology, but the identification of different lymphocyte cell types is not possible with conventional microscopy and stainings. Furthermore, we cannot say much about their functional activity. The numbers of such cells observed in this study were, however, similar in all the materials.

Monocytes migrate to peripheral tissues, where they assume the role of macrophages (Burkitt *et al.* 1993). Macrophages play a very important role in acute inflammation and probably in final biocompatibility (Anderson *et al.* 1984). They may release mediators, which can, in turn, activate other cells. Macrophages are found to affect such processes as fibroblast and lymphocyte activity, the complement system and angiogenesis (Anderson 1988, Bonfield *et al.* 1991). The amounts of macrophages at different time points did not reflect differences between the materials tested.

Macrophages form multinucleated foreign body giant cells (Murch *et al.* 1982). The presence of these cells is significant, because they represent a specific inflammatory response evoked by the foreign substance (Thomsen *et al.* 1991). The giant cells were very few in number, and most were present together with the previously mentioned ring-shaped debris particles. Judging by the giant cells, NiTi is well tolerated.

Fibroblasts migrated to the injury site in the early phase of healing and gathered around the implants. Other granulation tissue was slowly replaced by fibroblast proliferation and collagen deposition. In optimal situations, the inert biomaterial forms a thin, relatively avascular and acellular fibrous scar capsule at the interface (Williams 1986). Such capsules were present with the NiTi implant capsules, demonstrating good acceptance.

After implantation, a number of cellular and humoral factors, such as chemotactic and growth factors, complement, cytokines, hormones, enzymes, adhesion molecules and a large number of other factors may be involved in the deleterious soft tissue reaction (Anderson 1988, Tsunawaki *et al.* 1988, Bonfield *et al.* 1991, Cardona *et al.* 1992, Tang *et al.* 1996). The long-term result of how an implant is accepted is the result of all these variables. The findings of this study support NiTi as being equally good as stainless steel and Ti-6Al-4V when implanted in muscular tissue. The results are in agreement with some earlier studies that complement them. To determine the final consequences of the biocompatibility of Nitinol, it may be necessary to analyze more cellular factors involved in the long-term implantation process.

6.3. Encapsule membrane thickness around NiTi

Measurement of the capsule membrane thickness around the implant is widely used for estimating biocompatibility. There are many variations in the measuring techniques used by different investigators (Taylor *et al.* 1983, Ellies *et al.* 1988, Christel *et al.* 1989, Limberger *et al.* 1991, Benghuzzi 1996). The semiautomatic image analysis technique used here was a modification of the normal histomorphometric point calculation method developed for this study. Utilization of the thickness of the scar capsule around the implant alone is problematic, because there are other factors than the material itself that can affect capsular thickness. These include movement, location, implant surface texture and morphology, some animal-dependent factors and the surgical procedure used (Taylor *et al.* 1983, Ansbacher *et al.* 1988, Barone *et al.* 1992). The effect of surgical trauma was minimized using a cannulated needle. The implantation was done far from the skin incision, and no sutures were made. The overall capsule thickness was found to be time-dependent, and it generally decreased over time (Fig. 6). There was, however, an increase in thickness with Nitinol at 8 weeks and with Ti-6Al-4V at 12 weeks compared to stainless steel. The stainless steel capsule was thickest at 2 weeks. These differences were not found to be statistically different. The morphology of the capsule was equal with all materials. There was no accumulation of special cell types, for example, to explain the change. In optimal situations, the inert biomaterial forms a thin, relatively avascular and acellular fibrous scar capsule at the interface (Williams 1986). A capsule of this kind was present with the NiTi implant capsules, demonstrating good acceptance (Fig. 5, A5).

6.4. Perineural response to NiTi

The present study is the first to report neural and perineural responses to NiTi. Some NiTi applications intended to be used in contact with the central or peripheral nervous system have been suggested and further systems might be developed in the future (Iwabuchi *et al.* 1975). Also, the osteosynthesis material intended for use in cranial surgery must not cause any harmful effects on brain tissue.

In the present study, a total of 16 implants were in very close contact with peripheral nervous tissue (Fig. 5 A3- 4, B2-4, C2-3). Nine of these were NiTi implants. No signs of necrosis, prolonged inflammation or irritation, accumulation of a specific cell type, cells with phagocytosed material, foreign particles or abnormal connective tissue response were seen in the perineural tissue with any of the tested materials. There was no edema, irritation, glial hyperplasia or inflammatory reaction in the nerve itself. Very few giant cells, PMNs, plasma or mast cells could be found in the area between the nerve and the encapsulating membrane of the implant.

In conclusion, the observed neural and perineural responses were clearly non-toxic and non-irritating with Nitinol at 26 weeks follow-up. More studies are needed before application made of NiTi is intend to use inside the neural tissue

6.5. Ultrastructural features of cellular adhesion and morphology at the interface of NiTi

The general ultrastructural features of cellular adhesion and morphology at the interface of NiTi have not been studied so far. In the present study, they were analyzed using FESEM. The number of adhesion plaques, the morphological features of cells and their podia as well as the adhesion areas of single cells to the material surface have been accepted into use in the evaluation of biocompatibility (Puleo *et al.* 1992, Ushida *et al.* 1992).

Knowledge of the structure and composition of tissue-material interfaces and adhesion phenomena is important for several reasons, including the need to modify the surface of biomaterials to control and accelerate their interaction with the host tissues. The surface and substratum characteristics of materials are reflected in single cells and their structures (Sinha *et al.* 1996). Cell adhesion is a prerequisite for further cellular functions, such as spreading, proliferation, migration and biosynthetic activity. It is probably the most important aspect of cell interaction with a non-toxic biomaterial. The adhesion to material is mediated through preadsorbed proteins, which form focal contacts with the cell membrane (Anderson *et al.* 1990, Groth *et al.* 1994). A certain amino acid sequence of these proteins binds to cell membrane integrins, which are connected to the cytoskeleton (Pytela *et al.* 1985, Ruoslahti *et al.* 1987).

The present study demonstrated that fibroblast-like cells formed close connections with NiTi, showing good adhesion. The first organic layer on the metal surface with afibrillar wavy structures was probably made up of proteoglycans and other similar extracellular matrix components. The layer was slightly thicker with Ti-6Al-4V. The exact composition of this layer and its potential value for biocompatibility need to be clarified in the future. Within and above this layer, there were bigger adhesion clots with gaps of less than 30 nanometers, which were considered to be direct contact-forming focal cell adhesion sites with proteins and cell membrane composition (Fig. 3D). The number and composition of adhesion sites of NiTi did not differ from the control materials, and it thus appears to be well tolerated, judging by the criteria established in the FESEM study.

6.6. Effects of NiTi on new bone formation, bone remodeling and erosion

The biocompatibility data from limited studies in which NiTi has been used as a bone implant are slightly contradictory. NiTi bone plates have been found to be well tolerated and comparable to Vitallium in the dog femur (Castleman *et al.* 1976). Porous NiTi and NiTi clamps have also appeared to be suitable for craniofacial surgery (Simske *et al.* 1995, Drugacz *et al.* 1995). Nevertheless, NiTi appears to have less bone contact than pure titanium or other titanium alloys (Takeshita *et al.* 1997). NiTi showed a slower osteogenesis process and lower activity of osteonectin synthesis compared to Co-Cr alloy, pure titanium and stainless steel in a study by Berger-Gorbet *et al.* (1996). Unfortunately, the number of animals was insufficient for statistical analysis. The follow-up time was 12 weeks, which can also be considered quite short.

The biologic effects of NiTi on the normal regional acceleratory phenomenon (RAP), new bone and callus formation, (re)modeling and the fracture healing process are mainly unexplained, and they were a focus of research in the present study. Biologic failures in bone healing after surgery include inadequate callus formation or the lack of a normal regional acceleratory phenomenon (RAP), normal (re)modeling, or maldifferentiation of the healing tissues, plus combinations. One of the most common failures is the inability to form callus or normal RAP. When such failures co-occur, clinical problems, such as non-union and failed fixation, may ensue. Most biologic failures stem from problems attributable to mitogens, differentiating and priming agents, growth factors, and other labile biochemical and biophysical messengers and signals in the fracture region (Frost 1989). Toxic materials may cause biologic failures by affecting these factors.

6.6.1. RAP model

The effects of NiTi, Ti-6Al-4V and stainless steel on bone were examined in the RAP model developed for this study. An interesting point suggested by the results of the previous fracture healing studies is that an optimum level of loading and relative micromovement could be beneficial in stimulating bone formation around implants (Goodship *et al.* 1985, Kenwright *et al.* 1986, Kenwright *et al.* 1989). The added influence of trace ions released from the biomaterials and the possible biomaterial surface effects on cell activity must be considered in view of bone formation around implants (Pilliar 1991).

Typical RAP is induced by periosteal stimulation. In the RAP model used here, muscle movements caused intermittent micromotion at the contact surface between the non-fixed implant and the bone cortex. This dynamic loading gives an adaptive (re)modelation stimulus to bone. If the test material itself has toxic or unfavorable biocompatibility properties, it disturbs the normal RAP more than other materials do in the presence of the same mechanical stimulus. This is manifested as decreased new bone formation and increased bone resorption, i.e., a more negative bone balance compared to a more inert material. Thus, if bone formation with NiTi is disturbed by some means (necrosis/ no normal bone formation), or if there is severe disturbance in structure (porosity), maturation (woven to

lamellar bone) or modelation (organization of lamellar bone) compared to the controls, we can expect this to be also reflected in the biocompatibility of the material used in similar specific applications.

The differences between the responses to the implants may theoretically be due to two factors other than the implant material itself. The densities of NiTi, Ti-6Al-4V and StSt differ, and the slightly different weights of the test implants identical in size may possibly explain the changes to some extent, but we consider this to be of minor importance. Differences caused by surface roughness are not likely, because the surface was shown by the SEM examination to be similar in all materials. Since the mechanical factors affecting the tested implants were quite similar in all cases, we find it justifiable to suggest that the differences in bone parameters are due to the implant material itself (trace ions, wear particles and other physicochemical factors).

6.6.2. New bone formation, bone (re)modeling and erosion after periosteal implantation of NiTi

Nickel from NiTi and stainless steel and vanadium and aluminium from Ti-6Al-4V may be deleterious at high concentrations. *In vitro* nickel appears to be harmful to bone, but less so than cobalt or vanadium, which are also routinely used in implant alloys (Gerber *et al.* 1980). Aluminium may impair the osteoblastic functions and diminish bone collagen synthesis and mineralization (Rodriguez *et al.* 1990, Kasai *et al.* 1991).

The RAP model was used in study III. The subperiosteal resorption under the implants was found to be compensated for by endosteal bone thickening and lateral new bone bracing. This general adaptive bone modeling and normal RAP were seen with all implants (Fig. 9), but certain differences between the tested materials occurred. New bone formation started sooner in the Ti-6Al-4V group (2 weeks), but also decreased earlier in this group than in the others, which was clearly seen at 8 weeks, when the cortical width of Ti-6Al-4V was thinnest (Fig. 10D). No significant differences were seen at the later time points. Thus, similar normal RAP was observed with all the tested materials, but the culmination of the modeling process occurred and disappeared earlier in the Ti-6Al-4V group than in the NiTi or StSt groups. Our findings confirm and complement the previous results (Berger-Gorbet *et al.* 1996). NiTi may have a slower initial osteogenesis process compared to Ti-6Al-4V, but this has no negative effect on the total amount of new bone formation, normal RAP, or normal (re)modeling.

In the present study I, NiTi initially released some nickel *in vitro*. The amount of released nickel decreased rapidly within a few days. It is assumed that, after an initial release of surface nickel ions, a titanium oxide layer develops, which gives good protection against further corrosion. There is a possibility that the initial nickel ion release from NiTi and stainless steel contributes to the slower osteogenesis process. On the other hand, could the aluminium ions from Ti-6Al-4V have a diminishing impact on the modelation process earlier? Since the different surface treatments have evident influences on metal ion release (Buchanan *et al.* 1990, Browne *et al.* 1994), the treatments should be tested in the future to find out the optimum processing for NiTi used in bone surgery. The RAP model described here may be of potential value in this testing.

Based on histomorphometric measurements, the results of the present study show that the bone response to NiTi seems to resemble more the response to StSt than that to Ti-6Al-4V.

6.7. Bone healing after NiTi intramedullary nailing

Various implant-related factors, such as composition, mechanics, design, surface treatments, corrosion or wear properties, may underlie healing failure (Tarr *et al.* 1986, Frost 1989). In the present study (IV), normal secondary bone union and remodeling took place in both the NiTi and the stainless steel groups.

Secondary bone union is the normal healing mechanism of fractures treated by intramedullary nailing. The fracture heals through the formation of stabilizing callus without direct union of the cortical bone ends (Tarr *et al.* 1986). The fibrous granulation tissue surrounding the fracture differentiates further into cartilaginous tissue. At the periphery of the callus, the osteogenic cells of the periosteum directly differentiate into osteoblasts and produce new bone. A similar phenomenon takes place in the endosteal bone surface, resulting in the formation of endosteal bony callus (Kessler *et al.* 1986). In the present study, the three bone layers at the diaphyseal cross-section of the osteotomy site were seen in pQCT and histological analyses at 12 weeks (Fig. 15B).

After the formation of bone callus, the original cortical bone heals through secondary osteons, revascularizing the necrotic bone heads and enabling end-to-end cortical bone healing. Finally, the formed woven bone is remodeled into lamellar bone (Kessler *et al.* 1986). One relevant finding was a thin, circular, peri-implant bone sheet layer situated in the distal metaphyseal area. It formed a close contact with the NiTi and StSt nails and it was like an extension of the endosteal callus far from the osteotomy zone (Fig. 15C). This layer is probably composed of the small reaming particles with a bone-inductive potential. The development of this layer into lamellar bone demonstrates good tissue tolerance towards the implant material (Fig. 12A - D). It may also have an important stabilizing effect.

The fibrous-to-bone healing response is biomechanically logical in situations where some motion occurs between the bone ends. Elastic tissue is replaced by more rigid tissue during healing. Also, the shape of the periosteal callus rapidly results in an optimal cross-sectional geometry to resist bending or torsion loads (Tarr *et al.* 1986, Kessler *et al.* 1986). Large callus is usually a sign of instability (Sturmer 1996). The similarity of callus formation, callus size and normal remodeling in the present study indicated stability of the fixation and good biocompatibility.

Fixation materials with an elastic modulus close to that of bone may prevent stress protection atrophy and weakening of bone compared to more rigid materials (Viljanen *et al.* 1995). Austenite NiTi ($E = 83\text{--}110$ GPa) is more elastic than stainless steel ($E = 190$ GPa) (Breme *et al.* 1998). However, it had no positive effect on osteotomy healing or mineralization compared to stainless steel.

The earlier suggestions that NiTi may cause slower osteogenesis compared to stainless steel were not confirmed in the case of fracture healing. On the contrary, there were more “good” bone unions in the NiTi than the stainless steel group at early (4 and 8 weeks) time points. The present findings imply that NiTi is better or at least equally well accepted in intramedullary fixation as stainless steel.

6.8. Bone mineral changes after NiTi intramedullary nailing

The possible effects of NiTi on bone mineral changes have so far remained unknown. pQCT has proved to be an efficient and precise tool in evaluating the geometric and densitometric properties of bone (Takada *et al.* 1996, Augat *et al.* 1997). Both density and geometry have been shown to conduce essentially to bone strength (Ferretti *et al.* 1993, Augat *et al.* 1996). At the line of the former osteotomy, the bone mineral density (BMD) correlates with flexural rigidity (Augat *et al.* 1997). The pQCT finding of a lower mineral content in the nail zone compared to the proximal or control femur can be explained by stress protection atrophy commonly seen with bone implants (Uthoff *et al.* 1971). The lowest BMD in the osteotomy zone might be partly due to stress protection atrophy, but more probably to the fact that the increasing cortical diameter of the callus (Fig. 15A) is more important in resisting loads than bone density (Ferretti *et al.* 1993). Thus, the mineral content is sufficient for the existing loading conditions. Some criticism might be directed toward the relatively small number of animals measured in the pQCT studies. The results was, however, in agreement with the previous studies (Viljanen *et al.* 1998).

6.9. Fundamental aspects of implant corrosion

Various studies have shown that some metallic components of the alloys used in orthopedics are toxic and dissolve in body fluids due to corrosion (Ferguson *et al.* 1960). The corrosion resistance of the alloy and the toxicity of individual metals in the alloy are the main factors determining biocompatibility. The physical properties of the metal surface and the microparticles that dissolve from it also have an important role (Kononen *et al.* 1992, Maloney *et al.* 1993, Shanbhag *et al.* 1994).

The corrosion properties of metals depend on the surface reaction taking place in the respective environment. In many practical applications, corrosion resistance is due to passivation of the metal surface, i.e. the metal atoms on the surface react with the oxygen (and nitrogen) present in the environment to form a stable, non-reactive surface layer of oxide. This *in situ* process protects the metal from further reaction. In the presence of oxygen, rapid self-healing takes place if the surface film is mechanically damaged (Kruger 1983, Brown 1987). Oxides with a high dielectric constant have been hypothesized to cause less protein denaturation than surfaces with a low dielectric constant. Titanium, which forms a stable oxide with a high dielectric constant and exhibits intimate bonding to bone, is consistent with this suggestion (Klinger *et al.* 1997).

6.10. Surface of NiTi

In the present studies, it was considered important to test *in vivo* implanted NiTi with no special surface treatment. The reason for this was that when the material surface is damaged by a scratch, for example, only the natural, bulk-dependent oxide protects the implant against further corrosion.

The surface characteristics of NiTi have not been properly studied. It is evident that a NiTi surface is mostly composed of titanium oxides and, to a lesser extent, nickel oxides, while nickel-titanium constitutes the inner layer. Nickel may initially dissolve more easily than titanium because its oxide is not so stable. After that, the surface contains more titanium oxide. The good corrosion resistance of NiTi is thought to be mainly due to its ability to form a stable Ti oxide layer (Hanawa 1991). Surface layers of NiTi arch wires have been observed to have irregular features characterized by lengthy island-like structures, where selective dissolution of nickel may occur. The surface may also be electrochemically modified to selectively etch nickel away, leaving a titanium-enriched surface layer and forming a uniformly distributed porous surface (Oshida *et al.* 1992).

Some special treatments have been reported to improve NiTi's corrosion behavior. It has been demonstrated that thermal oxidation and titanium nitride coatings improve the corrosion resistance of NiTi (Endo *et al.* 1994, Trepanier *et al.* 1998). The clinical importance of these coatings is unknown.

6.10.1. Surface preparation of tested materials

The surface preparation was different for stainless steel and NiTi in the present studies. NiTi and pure titanium were polished by mechanical water sanding in study I, while stainless steel was electrochemically prepared. The surface treatments the studies II-IV were as received from the manufacturer. Stainless steel was electrochemically prepared. This is a normal treatment, which improves corrosion resistance. The surface of steel is mainly composed of Cr, Fe and Ni oxides. The Ti-6Al-4V and NiTi implants were in a mechanically ground condition. Such "clinical" titanium alloy implants are known to have a very thin, amorphous, homogeneous and non-porous oxide layer composed essentially of TiO₂ with some impurities depending on environmental handling (Kasemo *et al.* 1991). The surface of Ti-6Al-4V also contains minor amounts of aluminum. Since the Ti-6Al-4V material was processed in the same way, it was a good control material. Electrolytic surface preparation of stainless steel was justified, because it is never used inside the body without this treatment. Hence, a histological comparison *in vivo* between these materials was considered justifiable.

6.11. Corrosion of NiTi in vitro

The results of the *in vitro* study revealed that the nickel concentrations in both the osteoblast and the fibroblast cell culture media were higher for Nitinol than for stainless steel in the first samples (2nd day), but decreased rapidly to equal levels in the later samples (4th, 6th and 8th day) (Fig. 17). The titanium concentrations were at the same level in the Nitinol and the titanium media. It may thus be that after some initial dissolution of nickel from the surface, the nickel-titanium alloy may form a surface containing mainly titanium oxides in the outer layer and nickel-titanium in the inner layer. This may be a rapid process, and the decreasing concentration might have been found earlier in present study if the first sample had been taken earlier than on the second day.

The difference in surface preparation may cause some difference in the corrosion behaviors of the tested alloys. Mechanical polishing leaves some microscopic roughness, which increases the effective surface area in contact with the surrounding tissue. Consequently, electrochemical polishing could be used to minimize the initial release of nickel from the surface of NiTi specimens, i.e. to passivate the metal surface when intend to use clinically. In other words, the performance of NiTi in physiological environment is likely to be better if an electrochemical polishing method applicable to this alloy can be introduced.

6.12. Corrosion of NiTi in vivo

The present results show that the amounts of nickel measured in various organs of the NiTi rats after intramedullary implantation at 60 weeks' of follow-up were not higher than those measured in the stainless steel rats (Fig. 18A and B). The concentrations were very small ($< 2 \mu\text{g/g}$ dry weight) and near the detection limit of the GF-AAS-based sensitive method. The significantly increased Fe concentrations in the kidneys between 26 and 60 weeks in both groups may be due to aging. The significant increase of spleen Fe in the StSt group between 26 and 60 weeks may be due to the corrosion products of stainless steel.

Due to the corrosion of the implants, small amounts of metal ions may also be released into distant organs. Systemic toxicity may be caused by accumulation, processing, and the subsequent reaction of the host to corrosion products (Bergman *et al.* 1980, Lugowski *et al.* 1991, Ishimatsu *et al.* 1995). Systemic toxicity after implantation in humans is extremely rare. When high-dose nickel salts have been injected into mice, accumulation and some deleterious effects are seen in the liver, kidney and spleen (Pereira *et al.* 1998). The normal Ni concentration in the human liver was $10\text{-}50 \mu\text{g/kg}$ (wet weight), while the serum and blood values varied within $< 1\text{-}5 \mu\text{g/l}$ (Iyengar *et al.* 1994). In tissues adjacent to stainless steel 316L plates and screws, the Ni concentration ranged between 116 and 1200 mg/L (Poehler 1983). The maximum rate of Ni release due to corrosion in patients with implants made of nickel containing alloys has been estimated to be $20 \mu\text{g/kg/day}$ (Black 1981). Two contradictory studies have been published on systemic effects after NiTi implantation. Castleman *et al.* (1976) found no increase in the Ni concentrations in several distant organs of dogs after NiTi implantation at 17 months' follow-up, whereas 2- to 4-fold concentrations in the liver and kidneys of rabbits after 4 weeks' implantation were reported by Matsumoto *et al.* (1993). The total concentrations were, however, small.

NiTi without any surface treatment may initially release some nickel ions at early periods of implantation before the titanium oxide surface is dominant. This assumption is supported by the previous *in vitro* observations (study I).

6.13. Analysis of retrieved implants

In the studies II, III and IV, the retrieved implants were first visually analyzed. Including all studies, there was only one implant (stainless steel in study II) in which clear rust streaks and minor pitting corrosion spots were seen. There was no evidence of corrosion on visual inspection in any of the implanted intramedullary nails.

No high-magnification studies on the effects of long-term implantation on surface corrosion of NiTi have so far been available. In the present study, as shown by electron microscopy, overall corrosive changes were most evident in the stainless steel implants. After 60 weeks of implantation, a few corrosion pits were observed in the StSt surface at magnifications of 200x or higher (Fig. 19D). The pits were uniformly distributed over the implants. No such pits were seen in the NiTi implants, but they contained small, longitudinal, irregular enlarged microstructures (Fig. 19B). Some surface contaminants were present in both materials, especially at 60 weeks. The retrieved NiTi intramedullary implants were in a mechanically ground condition. Despite that, NiTi appeared to have equal or better *in vivo* surface corrosion resistance.

6.14. Value of NiTi as a biomaterial

NiTi alloy is quite new in medical use. It provides possibilities to make applications that no other implant material has offered before. A few commercial applications have been successfully developed since the 1970s, when Nitinol was first reportedly used for medical purposes. These applications include dental arch wire, vena cava filter and suture anchor for orthopedic surgery (Simon *et al.* 1977, Pelizzoni *et al.* 1989, Barber *et al.* 1993). In the 1990s, further development has been carried out with markedly increasing interest. Urethral, esophageal and intracoronary stents, aneurysm prostheses, and some orthopedic implants seem promising.

The idea behind shape memory applications is to introduce an item with a suitably small cross-section into the body, where its shape changes into a different, much larger structure upon application of heat. This thermal shape change phenomenon can be arranged to have a beneficial impact within the body, such as to restrict, dilate, push apart or pull together body components. Applications based on superelasticity, such as guide wires or laparoscopic tools, are another benefit of this material.

The biocompatibility of NiTi has long been a puzzle. Earlier studies are in agreement with our present findings and support the conclusion that NiTi is a safe material. Multi-center biocompatibility data must be available, including *in vitro* and *in vivo* experimental studies with several animal species and in several tissues. Surface treatments, compositional changes in the alloy, and various metallurgical and thermal treatments may all affect notably the biocompatibility of a specific implant application. In orthopedic appli-

cations, the amount of material inserted inside the body is usually considerable. The large amount of bulk material and the often larger surface area may adduce some harmful properties of the material due to corrosion. The implantation time is often long, even decades.

Many other problematic issues apart from biocompatibility may also arise when certain applications are manufactured for surgical use. This is especially true of orthopedic implants. The very important and complicated biomechanical considerations of the musculoskeletal system introduce extra puzzles.

The application of NiTi to orthopedic implants has involved problems with such aspects as implant fabrication, design and testing (Brailovski *et al.* 1996). It is the author's opinion that some of the prototypes that have been made have been subjected to human tests without due concern. To apply a new material or implant to surgical use, several demanding criteria must be met. In fact, the new implant must be better than the existing ones, or it must solve a clinical problem that the others fail to solve. This means it has to be better for the patient or the surgeon or society. Advanced technical properties, easy use, faster healing, less tissue damage, shorter operation time or lower total costs of operation must be behind every new application.

Some of criteria of a successful NiTi implant can be listed:

1. The design and function of the implant must be based on careful biomechanical considerations.
2. The compressive or other effective force caused by the implant must be known in every specific application.
3. The working time must be sufficient for proper implantation.
4. In case the implant is positioned wrongly, it must be able to remove it immediately during the operation or, if necessary, later.
5. There must be high-quality control and standardization of the procedure at every step from raw material to final product.
6. The sterilization method must not affect negatively the implant.
7. The learning of the new operative technique must be considered.

To fulfill these requirements, the new NiTi implants must be developed in close co-operation between surgeons, material scientists and mechanical engineers. The surgeon is rarely able to understand all the limitations of a material, while engineers are unable to evaluate the clinical validity. Computer-aided FEM-based implant design may be of great value for the design of optimal constructions.

Finally, the introduction of NiTi to human trials must fulfill every criterion of the normal protocol of implant testing. The conclusions concerning the superiority of every single NiTi application must be based on large series, multicenter studies and, preferably, randomized trials. The precondition for commercial availability and use is that sufficient background data are available and official national approvals are obtained.

The use of NiTi as a biomaterial has several possible advantages. Its shape memory property and superelasticity are unique characteristics and totally new in the medical field. The possibility to make self-locking, self-expanding and self-compressing thermally activated implants is fascinating. As far as special properties and good biocompatibility are concerned, it is evident that NiTi has a potential to be a clinical success in several applications in the future.

7. Conclusion

On the basis of the present study, the following conclusions can be made:

1. NiTi has good *in vitro* biocompatibility with human osteoblasts and fibroblasts. There were no toxic effects, decrease in cell proliferation or inhibiting effects on the growth of cells in contact with the NiTi surface *in vitro*.
2. The muscular response to NiTi was clearly non-toxic at 26 weeks' follow-up. The overall inflammatory response of NiTi was very similar to that of stainless steel and Ti-6Al-4V alloy. There was no necrosis, granulomas or sign of dystrophic soft tissue calcification. The immune cell response to NiTi remained low. The mean capsule membrane thickness of NiTi in the 8-week group was greater than that of stainless steel. At the end of the study, the mean capsule thickness was equal with all the materials tested.
3. The neural and perineural responses to implanted NiTi were clearly non-toxic and non-irritating at 26 weeks' follow-up. No qualitative differences in histology between NiTi, stainless steel and Ti-6Al-4V could be seen.
4. NiTi showed no differences in the ultrastructural characteristics of cell-metal adhesion constructions compared to stainless steel or Ti-6Al-4V.
5. The biocompatibility of NiTi in rats was good during a 26-week follow-up after periosteal implantation. The bone and soft tissue histology findings showed good acceptance, the end-stage healing response being nearly inert. A normal regional acceleratory phenomenon (RAP) with normal new bone and callus formation was evident. The histomorphometric findings showed that new woven bone formation started earlier (2 weeks) in the Ti-6Al-4V than the NiTi group. At 8 weeks, NiTi and Stst had greater cortical width compared to Ti-6Al-4V. At 12 and 26 weeks, no differences were seen. The developed RAP model may be a potential candidate in further biomaterial testing.
6. NiTi material had no delayed effect on the osteotomy healing response, consolidation, mineralization or remodeling response compared to stainless steel at 60 weeks' follow-up in rats. The mineral density in both groups was lower in the osteotomy area than in the other rod area, being lower than in the proximal operated femur or the opposite femur. The higher elasticity of austenite NiTi offers no benefits in view of stress protection atrophy. Bone contact with NiTi was close. Apart from callus, a peri-implant

lamellar bone sheet was formed in the metaphyseal area after 8 weeks, indicating good tissue tolerance.

7. At the beginning, NiTi with a water-sanded surface released more nickel in the cell culture medium than electrolytically polished stainless steel. After two days, however, the concentration decreased to about the same level. Further studies are needed to show whether electrochemical passivating treatment or some other surface treatment as a finishing treatment in nickel-titanium implant fabrication can be used to minimize the initial nickel release.
8. Systemic nickel ion release to the brain, liver, kidney, spleen or muscle due to the corrosion of NiTi intramedullary implants was not higher compared to stainless steels at 60 weeks' follow-up in rat.
9. The NiTi intramedullary rod showed slight marks of surface corrosion after 60 weeks' implantation, but less than stainless steel.

8. References

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Original publications