ON THE INFLUENCE OF MICRO- AND MACROSCOPIC SURFACE MODIFICATIONS ON BONE INTEGRATION OF TITANIUM IMPLANTS

by

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To Magnus and Dante with love

This PhD thesis represents number 32 in a series of investigations on implants, hard tissue and the locomotor apparatus originating from the Department of Biomaterials/Handicap Research, Institute for Surgical Sciences at Göteborg University, Sweden.

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ABSTRACT

Background: Osseointegrated titanium implants are routinely used in clinical denstistry as anchorage units for dental prostheses. Although the overall clinical results are good, there are clinical situations when an optimized implant healing is desirable, for instance in order to shorten healing periods and to allow immediate loading.
Aims: The present work was undertaken to study the influence of some micro- and macroscopical surface modifications on the integration and stability of titanium implants in bone. In addition, the aim was also to study the influence of a bone growth factor and autogenous bone grafts on implant healing in bone defects.

Materials & Methods: The thesis is based on five experimental studies using a total of 12 mongrel dogs and 39 New Zealand White rabbits. In total, 327 screw-shaped implants were evaluated with histology and biomechanical tests. The implants had either a turned surface or had been treated with anodic oxidation to create a porous surface structure (microscopic modification). A groove with various sizes was added to one thread flank of oxidized implants (macroscopic modification) for comparison with implants without a groove. Ground sections of the intact bone-titanium interface were prepared for light microscopy and quantitative morphometry. A micro-CT technique was used for 3D visualisation of the bone in relation to the implant surface. Implant stability was measured with removal torque (RTQ) tests and resonance frequency analysis (RFA) measurements.

Results: Turned and oxidized implants were placed in the dog mandible with circumferential defects which were filled with dog BMP+ carrier, carrier alone, autogenous bone chips or nothing. No differences in histological response and implant stability were seen between the different materials and controls after 4 and 12 weeks of healing. However, oxidized implants shower a stronger bone tissue response and were significantly more stable than turned implants after 4 weeks. A rabbit study demonstrated direct bone formation at the surface of oxidized but not turned implants after 7, 14 and 28 days. A darkly stained layer became populated with osteoblasts which produced osteoid towards the implant surface whilst turned implants seemed to be integrated by approximation of bone from the surrounding bone and marrow tissues. In paper III, an increased resistance to RTQ was seen for oxidized implants with a 110µm wide and 70 µm deep groove as compared with control implants without a groove after 6 weeks of healing. This was not observed for 200 µm wide grooves. Histology showed an affinity of bone formation to the grooves. Paper IV evaluated the influence of three different groove sizes on implant stability as measured with RTQ and RFA. The results confirmed that 110 µm grooves resulted in better stability than implants with 80 µm or 160 µm wide or no grooves. Histology of RTQ specimens revealed an increased incidence of bone fracture at the entrance of the groove as opposed to a separation at the bone-implant interface with decreased groove width. Bone formation had an affinity to the grooves which increased with decreased groove width. In paper V, bone formation was seen to occur more frequently in grooves than on opposing flank surfaces after 7, 14 and 28 days of healing in implant sites with small bone volumes.

Conclusions: The present thesis shows that both micro- and macroscopical surface modifications have positive influences on the bone tissue response and stability of titanium implants. It is suggested that this is due to a combination of (i) contact osteogenesis as stimulated by the microtopography and (ii) guided bone formation as stimulated by the macrotopography, which resulted in an improved mechanical interlocking between bone and implant surface.

Keywords: titanium, dental implants, surface modification, bone tissue, biomechanics, BMP,

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LIST OF PAPERS

Studies on oxidized versus turned titanium implants

I. Salata LA, Miranda Burgos P, Rasmusson L, Novaes Jr AB, Papalexiou V, Dahlin C, Sennerby L. Osseointegration of oxidized and turned implants in circumferential bone defects with and without adjunctive therapies. An experimental study on BMP-2 and autogenous bone graft in the dog mandible. *Submitted*

II. Miranda-Burgos P, Rasmusson L, Meirelles L, Sennerby L. Early bone tissue responses to oxidized and turned titanium implants in the rabbit tibia. *Submitted*

Studies on grooved oxidized titanium implants

III. Hall J, Miranda-Burgos P, Sennerby L. Stimulation of directed bone growth at oxidized titanium implants by macroscopic grooves. An in vivo study. *Clinical Implant Dentisty and Related Research 2005;7(suppl 1):76-82*

IV. Miranda-Burgos P, Sennerby L, Meirelles L, Hall J. Influence of groove width on affinity for bone formation and stability of grooved oxidized screw-shaped titanium implants. A biomechanical and histological study in the rabbit. *Submitted*

V. Miranda-Burgos P, Schupbach P, Hall J, Sennerby L. Early bone formation at grooved oxidized titanium implants. A descriptive light microscopic and micro-CT study in the rabbit. *In manuscript*

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INTRODUCTION

Osseointegrated dental implants are routinely used as anchorage units for prosthetic crowns, bridges and overdentures. The original protocols prescribed a healing period of 3 to 6 months to allow osseointegration of the implant prior to loading, either using a submerged or non-submerged implant placement technique (Brånemark et al 1969, Schroeder et al 1976). Reviews of clinical follow-up studies show that survival rates around 95% can be expected on all indications over a 5-yer period of time (Esposito et al 1998, Berglundh et al 2002). From the same bulk of knowledge it is also evident that some of the risk factors which may lead to implant failures are soft bone, limited bone volumes, grafted bone, overload and smoking (Sennerby & Roos, 1997, Sennerby & Rasmusson 2001). In addition, there has been a gradual challenge of biologic limits for osseointegration; implant healing times have been shortened, implants are placed in extraction sockets and immediate loading protocols have been introduced. Whereas these approaches have certainly widened indications for osseointegrated implants and dramatically shortened the treatment time, they have at the same time presented increased risk for failure, at least for smooth surface implants placed in soft bone qualities (Glauser et al 2001, Rocci et al 2003). The higher failure rates may be overcome by optimizing the implant surface and design to promote bone integration and stability. One such possibility is change of the surface topography at the microscopic level. Numerous experimental studies have found a stronger bone tissue response and increased stability for moderately rough surfaced implants as compared to smoother control implants (Buser et al 1991, Wennerberg 1996). As a consequence, most implants systems of today have a moderately rough surface as opposed to the original Brånemark turned and smoother surface (Albrektsson & Wennerberg 2005). Macroscopical modification of the implant is another theoretical possibility to improve integration, stability and clinical function. Most dental implant system have some kind of interlocking geometry at the apical aspect of the implant where bone ingrowth may help to stabilize the implant. However, it would probably be more beneficial if a macroscopic modification could be made also near the coronal part of the implant placed in marginal bone. The mechanisms behind the observed positive bone tissue response to surface modifications and the consequences on implant stability in different clinical situations are not fully understood. A third way of improving the healing of implants would be to use bone growth factors. This would be especially beneficial in situations of placing implants in defects and tooth extraction sockets in order to facilitate bone growth towards initially uncovered implant surfaces. It is possible that the combination of bone growth factors and implant surface modification may amplify the bone tissue response due to the previously demonstrated stronger bone reactions to moderately rough surfaces.

Bone tissue

Bone tissue is a highly specialised form of connective tissue of mesenchymal origin. The extracellular matrix is mineralized giving the rigidity and strength to the skeleton maintaining some degree of elasticity. It actively participates also in maintaining calcium homeostasis in the body.

Morphology

Morphologically, bone tissue is divided into cortical/compact and cancellous/trabecular bone. The differences are both structural and functional although both have the same matrix composition. Cortical bone have higher density and less porosity compared to cancellous bone since mature cortical bone consists of densely packed sheets of collagen lamella, concentric, parallel and interstitial, whereas in cancellous bone the matrix is a meshwork of bars and spicules of bone lamella, thus more loosely organised. Cortical bone forms about 80% of the mature skeleton and surrounds the cancellous bone plates and marrow. Differences in distribution and arrangement are responsible for differences in mechanical properties.

Cortical and cancellous bone can be further divided into woven/primary and lamellar/ secondary bone. At healing, woven bone is formed and then replaced by mature lamellar bone. Woven bone has a rapid turn over rate, an irregular pattern of collagen fibrils and four times as many osteocytes per unit volume with different size, distribution and orientation. Lamellar bone is normally less active than woven bone, consist of predominantly mature cortical and cancellous structures , densely organised parallel collagen fibres with a uniform pattern around a central canal (the Haversian canal) containing blood and, sometimes, nerves. The Haversian system or osteon consist of a central canal, surrounding osteocytes and canaculi, this system is separated from other osteons with a cement line linning the outside of the osteon. The cell processes in the canaculi and the collagen fibrils of the osteon do not cross the cement line .The Haversian canals have an anastomosing network with transversly oriented canals, Volkmans canals. The network of canals connects the periostal and endosteal surface and the bone marrow enabling regulation of cell and bone metabolism.

The periosteum is a membrane covering the outer surface of the bone made up of an outer fibrous layer of dense irregular connective tissue with blood and lymphatic vessels and nerves passing into the bone, the inner layer or cambium layer consists of elastic fibres , blood vessels and bone cells. The periosteum is involved bone growth, nutrition and they can help to form an extraosseus callus during fracture healing. The endosteum is a membrane on the bone surface separating the bone from the marrow cavity; it contains osteoprogenitor cells and osteoclasts.

The bone marrow serves as a source of bone cells, the blood vessels of the bone marrow form a critical part of the circulatory system in bone.

Bone matrix

The bone tissue consists of cells and extracellular bone matrix, which makes up more than 90% of the volume of the bone tissue. The extracellular matrix contains 35% organic, 65% inorganic components and the water component is 10%.

The organic component, approximately 90% of the organic matrix, consists mainly of collagen type I (Buckwalter et al 1996a, Gerhon Robey et al 1993). The non collagenous proteins are composed of non-collagenous glycoproteins and bone specific proteoglycans, these proteins include osteocalcin, osteonectin, bone phosphoproteins, bone sialoproteins and small proteoglycans. There are also a large number of proteins hat are absorbed from the circulation, as albumin and áHS glycoprotein. The non collagenous proteins have different functions in the regulation of bone mineralization, cell-to-matrix binding and interactions with structural proteins. Less of 1% of the non collagenous proteins contains growth factors influencing the cells but also secreted by them.

The inorganic bone matrix performs two essential functions; as an ion reservoir and and a structure giving the bone tissue its stiffness and strength. Approximately 99% of body calcium, 85% of the phosphorous and 40-60% of total body sodium and magnesium are associated in mineral crystals in bone tissue. The physiologic concentrations of these ions in the extracellular fluid are thereby sustained. By forming hydroxyapatite-crystals ($Ca_{10}[PO_4][OH_2]$) of calcium and phosphate the bone tissue is provided with stiffness and strength. These crystals undergo important changes in composition with age, thus their biologic functions depend on the amount and the age of the mineral crystals (Buckwalter et al 1996a).

Bone cells

There are four different cell types, osteoblasts, osteoclasts, bone lining cells and osteocytes. Osteoblasts, osteocytes and bone linning cells originate from a mesenchymal stem-cell line, whereas osteoclasts arise from the fusion of mononuclear precursors, originating from hematopoietic stem-cell line. Osteoblasts, lining cells and osteoclasts are present on bone surfaces, whereas osteocytes permeate the mineralised interior.

Osteoblasts

The osteoblast originates from the proteoblast which is a mesenchymal cell found in bone canals, endosteum, periosteum, bone marrow and as vascular pericytes. The pre-osteoblast is present on the bone surface usually in the surfaces below the active mature osteoblast. The osteoblasts have rounded oval, polyhedral form at active state and are seen tightly packed as seams. The osteoblasts synthesise, secrete, and regulate the deposition of the extracellular matrix of bone. This is first seen as osteoid which consists of uncalcified bone tissue on the surface of the mineralised bone tissue. The cytoplasmic processes of osteoblasts extend through the osteoid to com into contact with osteocytes within the mineralized matrix.

Active osteoblasts may remain on the surface of the bone, became inactive and assume a flatter form, bone-lining cells. They may also surround themselves with matrix and become osteocytes or may disappear from the site of bone formation.

Bone-lining cells

Bone lining cells are also referred to as resting osteoblasts or surface osteocytes. The majotity of these cells are derived from osteoblasts that have become inactive but they could also be derived from other endosteal cells or stroma cells. They lie against the bone matrix and have an elongated, flattened form with cytoplasmic extensions penetrating the bone matrix in order to get in contact with the extensions of osteocytes (Miller et al, 1989).

Osteocytes

The osteocyte is the most common cell in bone and may have the potential to live as long as the organism itself. They are found with individual lacunae in the mineralized bone matrix. Each osteocyte communicates with its neighbours by means of gap junctions, extending processes through small channels in the bone matrix called canaculi. Osteocytes are therefore in close communication with bone lining cells, osteoblasts, and pericytes off capillaries and sinusoids supplying nutrients (Noble and Reeve 2000). Osteocytes derive from osteoblasts which become enclosed within the bone matrix during bone formation.

Osteoclasts

Osteoclasts are multinucleated cells which can resorb bone. They are formed by fusion of mononucleated cells derived from hematopoetic tissue (Lerner 2000).Once formed; the osteoclasts attach to bone and initiate the resorption process by creation of so called Howship's lacuna. The part of the cell facing the lacunae is characterized by the presence of a ruffled border membrane. Resorption of bone includes enzymatic dissolution of hydroxyapatite crystals and proteolytic breakdown of bone matrix proteins.

Bone formation

Bone formation requires the recruitment and /or migration of potentially osteogenic cell population and the differentiation of this population into mature secretory cells. Cells that differentiate before reaching the target surface will stop and secrete matrix. Osteoconduction will result in a bony spicule advancing towards the target surface. Bone formation during healing or remodelling comprises 2 phenomena, de novo bone formation and appositional bone formation (Davies and Hosseini).

The novo bone formation decribes the biological cascade of events that occur during the initiation of bone formation by newly differentiating population of osteogenic cells whereas appositional growth is a continuance of the synthetic activity of differentiated osteogenic cells. The novo bone formation is most important in fracture and implant-healing sites, as well as within potential tissue-engineering scaffolds. This mechanism comprises four stages when bone growth is located on a solid surface like an implant. Firstly the absorption of non collagenous bone proteins to the solid surface, secondly the initiation of mineralization by the absorbed proteins, thirdly continued mineralization resulting from crystal growth and finally the assembly of a collagenous matrix overlying the interfacial matrix with mineralization within the collagenous matrix. On a solid surface, de novo bone formation occurs in the zone where the differentiating osteogenic cells are in contact with the surface, whereas with in a soft 3-dimensional matrix it occurs in the zone where the front line of differentiating osteogenic cells precede the advancement of the growing bone spicule.

Appositional growth begins with cell polarization and the transition from migratory to secretory activity of the osteoblasts. As a result of matrix accumulation at their basal side, the cells will passively recede in apical direction. Individual cells will become buried in the matrix and become osteocytes, this will be compensated by limited proliferation. Slow and synchronous secretion will result in lameller bone whereas woven bone is the result of rapid and asynchronous secretion (Davies & Hosseini 2000).

On a solid surface, osteoconduction in conjunction with de novo bone formation leads to growth along the surface, whereas appositional growth leads to greater mass of bone in a direction perpendicular to the surface. These two mechanisms do also occur in a soft 3-dimentional matrix as in the process of intramembranous growth of a bone spicule, growth of newly forming bone on calcified cartilage during endochondral ossification, during bone remodelling or bone growth along an implant surface during contact osteogenesis. Osteoconduction permits s faster rate of bone growth than is possible by appositional growth because bone can be secreted simultaneously in many locations along an axis in the direction of growth. The growth rate in appositional bone growth have been reported as 0,6µm per day and in osteconductive growth as 30 to 50µm per day.

There is only one mechanism of bone formation but it may occur within cartilage (enchondral), within an organic membrane (intramembranous), or by deposition on existing bone (appositional) (Buckwalter et al 1996b).

The enchondral ossification mechanism begins with the aggregation of undifferentiated cells that secrete cartilaginous matrix and differentiate into chondrocytes. A periostal covering appears around the hyaline or hyaline-like cartilage and begins to form a thin collar of bone. Some regions of the cartilage mineralize, the chondrocytes enlarge, osteoclasts resorb the

central part of the cartilage crating a marrow cavity and vascular buds invade the marrow. The peripheral osteoblasts, coming from the periosteum, arrive with blood supply in order to carry out formation and turn over of bone. This type of bone formation occurs in long bones, short bones and epiphyseal centers of ossification until skeletal maturity. It also occurs during healing in some types of fractures, specially when there is motion at the site of fracture (Buckwalter et al 1996b).

The intramembranous process is initiated by the aggregation of undifferentiated mesenchymal cells into layers of membranes. These cells synthesize a loose organic matrix, containing blood vessels, fibroblasts and osteoprogenitor cells. The osteoprogenitor cells differentiate into osteoblasts and deposit spicules and islands of organic bone matrix than then mineralize (Buckwalter et al 1996b, Long 2001). Osteoclasts in the matrix become osteocytes and cytoplasmic extensions develop in order to maintain contact with other cells. This type of bone formation occurs during embryonic development of flat bones, during healing of some fractures as during distraction osteogenesis (Buckwalter et al 1996b).

Appositional formation of bone occurs during periostal enlargement of bones and during bone remodelling. This process begins with the alignment of osteoblasts on an existing bone surface, these cells then synthesize osteoid, thereby enlarging the bone layer by layer (Buckwalter et al 1996b).

Bone healing

Bone healing is a delicate and pre-programmed process which is governed by local and systemic factors and occurs in two main stages; formation of immature woven bone followed by remodelling and maturation. The first stage is signified by random and scattered formation of immature bone at numerous locations in the defect with the aim to quickly bridge a defect/gap with osteogenic tissue. During the second stage the immature bone is replaced with highly organised lamellar bone with re-established biomechanical properties as a result.

At the microscopic level, the early critical events of intramembraneous bone healing involve blood clotting and the establishment of a fibrin matrix in which cells from adjacent tissues can migrate and differentiate into angiogenic cells. The initial blood clot is probably of utmost importance and apart for providing a scaffold for early cell migration; its extension will probably determine the area possible for bone formation and regeneration. The formation of new vessels will provide adequate nutrition for mesenchymal stem cells to migrate from adjacent bone and marrow tissues and to differentiate into preosteoblasts and osteoblasts. The osteoblast will synthesize a collagen mesh which is gradually mineralized from osteoid to bone. This occurs at existing bone surfaces or as solitary islands in the granulation tissue. The osteoblasts become entrapped in bone and, by definition, turn into osteocytes. The osteoblast is not a

migrating cell per se and there is a need of a continuous influx of new stem cells to the wound area. Depending on the local conditions the mesenchymal stem cells can differentiate through different pathways and can also become fibroblasts or chondroblasts which in the case of intramembraneous bone healing would be undesirable. Osteoclastic activity is seen at the surface of pre-existing bone often but not always prior to new bone formation. Also different inflammatory cells and phagocytes take part in the early healing phase to remove debris and necrotic tissue. Remodelling involves coupled bone resorption and formation of lamellar bone by so called Bone Metabolizing Units (BMUs). In this way the woven immature bone is replaced by lamellar well-organized bone which in cortical bone is seen as the formation of secondary osteons. In trabecular bone, new lamellar bone is formed by appositional growth. The time frame for new bone formation and remodelling is depending on size of the defect and the healing conditions. Some authors have suggested the first stage of bone formation to occur for 3 to 4 months followed by a remodelling period of up to 12 month

Growth factors

Bone Morphogenetic Proteins

The potential of substances to induce bone formation in soft tissues was first described in 1938 by Levander. He implanted autogenous bone fragments and also injected aqueus and alcoholic extracts from bone into rabbits subcutaneusly or intramuscularly and found bone inductive activity. In 1965, Urist discovered a substance in bone responsible for bone induction which he namned *bone morphogenetic protein*, BMP. Today at least 15 different BMPs have been characterised (Kawabta and Miyazono 2000). The expression of BMP-2 has been correlated with the differentiation of osteoblasts and chondroblasts from mesenchymal stem cells (Urist 1983, Chen 1991, Yamaguchi 1991, Ahrens 1993, Amedee 1994, Si 1997) and plays a critical role in cell growth and bone formation (Lieberman 2002). BMPs have been reported to be expressed in early fracture healing (Nakase 1994, Ishidou 1995, Bostrom 1995), which indicate that release of BMP from traumatised bone tissue is an important part of the healing process. Several in vivo studies have assessed the efficacy of recombinant human BMPs (rhBMPs) in the healing of critical-sized bone defects and the acceleration of fracture-healing (Lieberman et al 2002).

Transforming Growth Factor â

The TGF-â family consists of at least five close related members. TGF-â1, -â2 and -â3 have been found in all mammalian species, â4 in chicken and â5 in amphibians (Centrella

1991:#71GZ).The name originates from its ability to transform the fibroblastic phenotype *in vitro*. The most abundant resource and pool of TGF-â is bone (Centrella 1991, Bonewald 1990), all three isoforms are present but TGF-â1 is the most predominant (Centrella 1994: #271GZ). In fracture callus from humans (Andrew 1993) and in experimental fracture healing (Bolander 1992, Si 1997) TGF-â1 has been detected in chondrocytes and osteoblasts during chondrogenesis and endochondral bone formation. The role of TGF-â in the repair of bone has been studied in experimental models involving subperiosteal injections in the femur and calvaria, critical-sized defects, and bone ingrowth into prosthetic devices (Lieberman 2002).

Fibroblast Growth factors

Two of the members of the FGF family have been detected in adult bone, FGF-1 and FGF-2. Both FGF-1 and FGF-2 promote growth and differentiation of different cells, including epithelial cells, myocytes, osteoblasts, and chondrocytes. Both FGF-1 and FGF-2 activity have been identified during the early stages of fracture-healing (Lieberman 2002).

Insuline growth factors Insulin-Like Growth Factors

Growth hormone and insulin-like growth factors (IGFs) play critical roles in skeletal development and participates in the regulation of skeletal growth (Lieberman 2002). Two IGFs have been identified: IGF-1 and IGF-2. IGF-2 is the most common growth factor found in bone but IGF-1 seems to be more potent and has been localized in healing fractures in rats and humans (Lieberman 2002). It is difficult to determine the potential role of either growth hormone or IGF in the enhancement of fracture-healing. A number of studies have been performed in different animal models with use of different doses and methods of administration to assess the influences of growth hormone and IGF on skeletal repair but the results have varied (Lieberman 2002).

Platelet-derived growth factor

PDGF is secreted by platelets during the early phases of fracture-healing and has been identified at fracture sites in both mice and humans (Lieberman 2002). In vitro studies have demonstrated PDGF to be mitogenic for osteoblasts (Lieberman 2002).
Nash et al (1994) evaluated the efficacy of PDGF in the healing of unilateral tibial osteotomies in rabbits. In the animals that had been treated with PDGFthere was an increase in callus density and volume compared with the controls. There was also a more advanced state of osteogenic differentiation both endosteally and periosteally in the animals that had been treated

with PDGF than in the controls No differences in strength between the tibiae that had been treated with PDGF and the intact, contralateral tibiae was demonstrated. This study suggested that PDGF has a beneficial effect on fracture-healing but only a small number of animals were analyzed, the mechanical testing data were equivocal and the study was of a small size could not give statistical significant differences.

The bone tissue response to titanium implants

Titanium

Titanium is the first element of group 4A in the periodic table; its atomic number is 22 and atomic weight 47.9. About 0.2 atomic % of the earth crust consist of titanium and titanium is commonly used as a construction material in air, space, marine and chemical industry. Titanium is suitable for machining and has a high strength/weight ratio, corrosion resistance in saline solutions and a resistance to acids.

Titanium has a well documented biocompatibility in bone and soft tissues as it does not provoke any negative tissue reactions. The reasons may be due to its ability to rapidly form a few nanometers thick oxide film in air which is of highly protective nature. The oxide layer has hydrophilic properties and a dielectric constant close to water which may facilitate the interaction with bioliquids and biomolecules.

Integration of turned titanium implants in bone

A direct contact between bone and screw-shaped titanium implants was first described by Brånemark et al (1969) and later by Schroeder et al (1976). Since then, numerous reports have demonstrated a direct-bone implant contact for clinically retrieved implants (Albrektsson et al 1993, Piattelli et al 1998).Sennerby (1991) studied the healing process in around screw-shaped machined titanium implants in cortical bone after 3 to 180 days and found that it was characterized by an early cellular response, a relative absence of inflammatory cells and a rapid formation of woven bone from the endosteal surface. Seven days after implantation, solitary bone formation was observed in the threads and at the endosteal surface of the cortical bone. With time both types of woven bone fused and increasingly filled the implant threads. The increased bone-titanium surface contact was thus a result of ingrowth of bone from the surroundings and did not start at the implant surface. The final mineralization of the interface bone could have been an acellular process; scattered crystals of mineral fused slowly and formed the interface bone. The implant surface not covered by bone was covered by multinuclear giant cells as also reported by Piattelli et al (1996).This phenomena was earlier found by Donath et al 1984 to correlate with the roughness of the implant surface. Johansson & Albrektsson (1987) demonstrated an increased resistance to removal torque with healing time. Moreover, more bone-implant contacts were seen with time which could explain the

observed stability increase. Sennerby et al (1993) found differences in implant integration in cortical versus cancellous bone. Differences in removal torque for tibial and femoral intraarticular implants were believed to depend on differences in mechanical properties of the surrounding bone tissue which may depend on the type of bone and the degree of maturation of the bone tissue. The amount of compact bone in contact with the implant correlated with the removal torque.

Ultrastructural studies of the bone-titanium interface have usually demonstrated that there is an unmineralized or partly mineralized zone separating the titanium surface from the bone (Albrektsson et al 1982, 1985, Linder et al 1989, Sennerby et al 1991, Nanci et al 1994). For instance, Sennerby et al (1991) described an amorphous layer separating the mineralized bone and the titanium surface. This zone lacked collagen and mineral. Rupture by unscrewing during removal torque testing occurred in the innermost amorphous zone. The stability of threaded machined titanium implants was therefore believed to be due to a mechanical interlock with surface irregularities at the microscopical level and/or geometrical deviations at the macroscopical level.

Influence of surface topography

The surface properties of an oral implant may be subdivided into mechanical, topographic, and physicochemical properties (Albrektsson & Wennerberg 2005). Different methods can be used to alter the topography of implants, for instance, electropolishing, grinding, blasting, plasma spraying, coating, photolithography, laser treatment, anodic oxidation, acid etching and combination of techniques. Surface topography can be characterized by many different methods which can be contact or non-contact methods. The former measures surface roughness with a contact stylus while non-contact methods use a laser beam for illumination. Non-contact optical methods are preferable since the surface is not altered and the same implants can be used for implantation. Examples of such methods are optical profilometers, interpherometers, autofocus detection systems, and confocal laser scanning microscopy (CLSM). Surface roughness parameters are usually separated as amplitude parameters describing height, spacing parameters describing the space between irregularities and hybrid parameters measuring height and space (Wennerberg & Albrektsson 2000).

Of special interest for the present thesis are two techniques, machining and anodic oxidation. Machining is not a surface treatment method but it can be used for producing specific surface topographies and surface compositions. The surface topography is characterized by grooves oriented along the machining direction. The surface roughness values are in the range of 0.6 -0.8 μm. The surface consists mainly of TiO_2, small amounts of Ti_2O_3, TiO, and Ti nitride. The oxide thickness is of 3-6 nm depending on the sterilization method. In anodic oxidation, electrode reactions are combined with electrical-field drive metal and oxygen ion diffusion (Kurze et al 1986, Hazan et al 1991, Larsson 1997, Sul 2002, Hall & Lausmaa 2000). This leads to an oxide film at the anode surface. The structural and chemical properties

of the oxides on titanium depend on the anode potential, electrolyte composition, temperature and current. The properties of the anodic oxide films vary widely. One of the anodizing processes is the spark anodizing, it is performed at voltages of 150-200V and higher. This method produces an increased surface roughness and a three-dimensional oxide structure consisting of numerous open pores. The oxide film can be firmly adherent and up to 20µm in thickness. The chemical composition of the oxide layer can be alter by adding different material into the electrolyte solution as for instance magnesium ions (Sul et al 2005, J Lausmaa et al)

Different pathways of integration for smooth and moderately rough implant surfaces have been suggested. Direct bone formation or contact osteogenesis have been described in vivo for HA coated and implants with a moderately rough surface topography (Osborn & Newesly, 1980, Piattelli et al 1996, Davies & Hosseini 2000). In addition, numerous *in vivo* studies comparing implant surfaces have demonstrated a better resistance to removal torque or pull-out/push-out forces for rough as compared to smooth topographies indicating a better stability. Moreover, studies have also shown a stronger bone tissue response to implants with a moderately rough surface (Sa 1-2 µm) seen as formation of higher degrees of bone contacts more rapidly (Albrektsson & Wennerberg 2005). Wennerberg (1996) experimentally investigated the influence of surface roughness on bone formation around implants and established an optimal range of surface roughness. Six studies were undertaken and a total of 318 threaded implants were inserted in rabbit bone. The different surface roughness were produced by blasting using 25, 75 and 250µm sized particles of Al2O3 and TiO2. Turned implants served as controls and a confocal laser scanner was used for the surface measurements. After different healing times the implants were evaluated by the peak removal torque, the percentage of bone-to-implant contact and bone inside the threads. The results demonstrated higher removal torques and percentages of bone-to-implant contact for implants blasted with 25and 75µm sized particles than turned or 250µm blasted implants. 75µm blasted screws showed stronger bone fixation than 25µm blasted implants. The corresponding average surface roughness were 1µm, 1,5µm, 0,6µm and 2,1µm. Implants prepared with a isotropic surface structure and a average surface roughness of 1,5µm were found to have the firmest bone fixation.

In a clinical histological study using microimplants histomorphometric evaluation, Ivanoff et al (2003) demonstrated significantly higher degrees of bone-to-implant contact for oxidized implants, whether placed in the maxilla or in the mandible when compared to turned control implants. Significantly more bone was found inside the threaded area for the oxidized implants placed in the mandible and maxilla. Similar results were reported by Zeichner et al (2003a) who compared machined, HA-coated and oxidized implants in a minipig model. The oxidized and HA coated implants showed more bone in contact than the machined ones after 3, 6 and 12 weeks. By analysing clinically retrieved oxidized implants Rocci et al (2003) described high degrees of bone contacts for implants subjected to immediate or early loading for 5 to 9

months. Moreover, bone formation directly at the implant surface was demonstrated. Similar good bone responses to clinically retrieved oxidized implants have been reported by Degidi and co-workers (2002)

In spite of the biological and mechanical advantages of moderately rough implants, few comparative study has shown any significant differences in clinical outcome in routine patients. However, non-controlled studies indicate that rough implants may perform better in challenging situations such as when using short implants (Renouard & Nisand 2005, Hagi et al 2004), in bone grafting cases (Brechter et al 2005) and in immediate loading (Glasuer et al 2001, 2003, Rocci et al 2003).

Influence of macrogeometry

Endosseus implants can be classified according to their designs as pins, needles, blades, disks and root-formed. The most common type of implant has been the root-formed analogues in the form of screws, cylinders, hollow.implants, truncated cones or combinations forms (Esposito Ch 24, Titanium in Medicine). Modern implants used today are with few exceptions threaded screws.

Implant design

Numerous experimental investigations have demonstrated that screw-shaped implants are superior to cylindrical ones with regard to initial stability (Lundskog 1972, Brånemark et al 1969, Carlsson at al 1986, Gotfredsen 1992). Moreover, long-term studies have shown signs of continuous bone loss around cylindrical implants (Albrektson 1993a) while screws have given high survival rates and minimal marginal resorption (Adell et al 1981, Lekholm et al 1994, Henry at al 1996). The use of threads have many advantages which may explain the differences; (i) they engage the bone at the implant site during insertion and thereby the stability is not entirely depending on press-fit and rapid bone integration, (ii) an axial compression of the bone between the tread flanks and the head of an implant can be achieved to ensure firm stability, (iii) the implant threads ensured an even distribution of loading stresses over the whole interface during functional loading.

Most threaded implants used today have self-tapping features which results in a better primary stability than if using a screw-tap prior to insertion (O'Sullivan et al, 2000). The Brånemark self-tapping implant was introduced in 1983/84 (Lekholm 1992) primarily on the indication low density bone. The clinical outcome was reviewed at 3 and 5 years follow-up showing a better survival rate of the self-tapping than for standard implants (Lekholm 1992). It has been demonstrated that a slight tapering of the implant body results in a better primary stability due to higher lateral compression of the bone during insertion (O'Sullivan 2000, 2004a, 2004 b). However, a comparative clinical study involving three centers could not

reveal any differences in clinical outcome when comparing a tapered and a parallel walled implant, in spite of higher primary stability with the tapered one as measured with RFA (Friberg et al 2003).

Implant length

It is generally anticipated that shorter implants are less successful than longer ones at least when using implants with a turned surface and in situations of advanced jaw resorption and poor bone quality (Friberg et al 1991, Lekholm 1992, Lekholm et al 1994, Roos et al 1997). However, there is also evidence that short implants may work very well at least if having a surface structure for bone ingrowth. For instance, Deporter et al (2002) performed a prospective clinical trial to assess the performance of short sintered porous-surfaced dental implants with a mandibular complete overdenture with 10 years of follow-up. Fifty-two fully edentulous patients received three free-standing implants (7-10 mm in length, mean length, 8.7 mm) each in the mandibular symphysis region. After 10 weeks of submerged healing, these implants were loaded. The results indicated a 10-year implant survival of 92.7% and a mean annual bone loss after year 1 of 0.03 mm. The authors conclude from the data available that short free-standing dental implants with a sintered porous surface used for implant fixation show a predictable outcome.

In a review paper by Hagi el at (2004), the relationship between dental implant failure rates and their surface geometry, length, and location (maxilla versus mandible) were analysed based on 12 published studies. The authors concluded that (i) machined surface implants experienced greater failure rates than textured surface implants;(ii) with the exception of sintered porous-surfaced implants, 7 mm long dental implants appear to have higher failure rates than those > 7 mm length. Their conclusion is supported by the findings of Renouard & Nisand (2005) who evaluated 96 6 to 8.5 mm long implants with either a turned or oxidized surface in 85 patients. Only one of 42 oxidized implants was lost whilst 4 of 54 turned implants failed over a follow-up of at least 2 years.

Based on a literature search, Das Neves et al (2006) evaluated 16,344 implant placements with 786 failures (4.8%) They found that implants 3.75 mm wide and 7 mm long failed at a rate of 9.7% compared to 6.3% for 3.75 x 10-mm implants.

In a clinical study on 905 consecutive Brånemark implants Östman et al (2005) found decreasing primary stability stability with increasing implant length as measured with RFA. This may be explained by that long Brånemark implants have a reduced diameter in the coronal direction to reduce friction heat. A similar observation was made by Miyamoto et al (2005).

Ivanoff et al (1996) demonstrated higher removal torque for longer than for short implants in a rabbit model. The authors suggested a correlation between resistance to removal torque and the implant surface area in contact with cortical bone.

Implant diameter

Langer et al (1993) introduced 5.0 and 5.5 mm wide diameter screw-shaped implants to be used on special indications, such as poor bone qualities, posterior regions with reduced bone heights, and for replacement of non-integrated implants or regular implants without firm primary stability. Increased implant surface topography and the possibility of engaging more marginal and lateral cortical bone were the advantages suggested for the wider implants. Ivanoff et al (1997) studied the influence of different implant diameters on the integration of screw implants in the rabbit tibia. A significant increase of the removal torque followed increase of the implant diameter. The authors suggested that the implant resistance to shear seemed to be determined by the implant surface in supportive cortical bone.

The influence of implant diameters (3.75mm, 4.0mm and 5.0mm) was also studied in a 3 and 5-year retrospective report. A relationship between implant failure and implant diameter was found, however, with a higher failure rate for the 5.0mm wide implant. No relationship between implant failure and jaw type or quality and quantity was found. Neither was found a relationship between marginal loss and bone quality, quantity, implant diameter or jaw type. The authors suggested a learning curve, poor bone quality and changed implant design as possible reason for the less positive outcome for 5.0mm wide implants. An other plausible explanation was that the 5.0mm wide implant was more often used in challenging situations, for instance to immediately replace regular implants that did not reach good primary stability. Aparizio and Orosco (1998) reported on similar experiences with 5 mm implants; In he maxilla the cumulative success rate was 97.0% and in the mandible 83.4% with a mean follow up of 32.9 months. However, other authors have presented good clinical outcomes with wide implants (Bahat & Handelsmann 1996, Friberg et al 2002) and even when used for immediate loading (Calandriello et al 2003). Moreover, in a review of 16,344 implants placements with 786 failures, 4 mm implants were more successful than 3.75 mm ones.

Influence of addititive growth factors

Bone healing is a multi-step process where the release of an adequate amount of the right growth factor at the appropriate time would theoretically improve the kinetics of bone healing and integration of implants (Dard et al 2000). A review by Salata et al (2002) summarizes the knowledge about the use of BMPs in conjunction with dental implants based on 39 scientific reports. The results show that the osteoconductive capacity of BMP is well documented but their effects in implant dentistry are not. Preclinical and clinical studies have not shown good outcomes in comparison with conventional treatments or controls. For instance, in a series of animal experiments Stenport (2002) failed to demonstrate any significant positive effects of locally administered BMP, BMP-7 and FGF-4 on implant integration. Salata et al (2002) suggested that the introduction of new technology must incorporate measures of implant stability

in order to provide reliable and conclusive information on dental implant anchorage in BMP-induced bone and ask for controlled investigations to determine the right dosage and vehicles for BMPs in dental dentistry.

Platelets release substances that promote tissue repair and influence the reactivity of vascular and other blood cells in angiogenesis and inflammation. For instance, they contain different growth factors (PDGF, TGF-â, Vascular Endothelial GF), cytokines and chemokines. Dental implant surgery is one of the situations where guided secretion of autologous platelet products may promote healing. In a review article of Anitua at al (2004) several experimental studies are mentioned in relation to implant healing and platelet-rich plasma (PRP) but no clinical study with regard to implant stability and failure/success is presented. Two experimental studies performed by Anitua et al (2001a, 2001b) investigated the performance of adding platelet-rich clot (PRP) around titanium implants used to anchor prostheses. The implant surface was moistened by PRP and inserted into the alveolus. At 2 to 3 months the test implants had denser bone with better organized trabeculae. Micro-roughened surfaces have shown to have an improved interaction with platelets (Park et al, 2001, Zechner at al 2003) showed that additions of PRP-clot resulted in increased bone-to-implant contact at 3 and 6 weeks and was primarily effective during the early healing phase. A dog study performed by Stefani et al 2000 showed that a combination of recombinant PDGF and IGF-1 promoted peri-implant bone regeneration in the early phase of healing.

Wikesjö‘s review (2005) discuss the biological potential, clinical relevance and perspectives of recombinant human bone morphogenetic protein-2 (rhBMP-2) technologies for alveolar bone augmentation. Several studies show that rhBMP-2 may be used to augment alveolar bone when used as an onlay and as an inlay. It is of great importance to provide space for rhBMP-2 induced bone formation. Supraalveolar defects (onlay) may require the combination with suitable space-providing devices to optimize bone formation (Sigursson et al 1997, Caplanis et al 1997). In contrast, space-providing intrabony defects (inlay indications) may be treated successfully using rhBMP-2 constructs with lesser biomechanical properties (Wikesjö et al 2003a, 2003b, 2003c, 2003d, 2004). The addition of standard GBR membranes does not provide additional value to the rhBMP-2 technology. Occlusive GBR devices/ membranes may decelerate BMP-induced bone formation as well as readily become exposed thereby compromising overall wound healing (Cochan et al 1999, Jovanovic et al 2004, Zellin and Linde 1997). There are studies (Sigursson et al 2001, Wikesjö et al 2002) showing considerable benefit of rhBMP-2 for alveolar augmentation and osseointegration of titanium oral implants, rhBMP-2 supports significant re-osseointegration of titanium implants exposed to long-term peri-implantitis (Hanisch et al 1997). Jovanovic et al (2003) showed that rhBMP-2 induces normal physiologic bone, allowing osseointegration, and long-term functional loading of titanium oral implants. The author concludes that clinical studies optimizing dose, delivery

technologies, and conditions for stimulation of bone growth are in the near future and will certainly have a profound effect on implant dentistry.

Influence of loading conditions

The routine loading protocol established by Brånemark et al (1985) was empirically based and used 3 month for the mandible and 6 months for the maxilla. At that time it was anticipated that premature loading may result in fibrous encapsulation and failure of the implant. In the 1970s several studies conducted by orthopaedic surgeons demonstrated that mechanical factors influence the bone-implant interface as loading could result in soft tissue encapsulation instead of bone healing. In the dental field, the similar observation was made by Brunski et al (1979). Experiments with titanium blade implants in a dog model gave evidence that fibrous tissue encapsulation of the implant was a consequence of early loading. Similar finding were registered for screw implants. From this and other papers it was demonstrated that micromovements like those induced by early loading of dental implants should be avoided and a stress-free situation during healing period is mandatory to achieve osseointegration. Cameron el at (1973) introduced a theory of a threshold micromovement at the bone-implant interface. The hypothesis was supported by the findings of Maniatopolous et al (1986) who suggested that micromovements do not automatically lead to fibrous tissue encapsulation, that a threshold exist and that this threshold is dependent of the design and surface of the implant. Displacements of 500 and 150µm were found to result in soft tissue encapsulation (Szmucler-Moncler et al 1998) whilst micromovements up to 50µm were well tolerated (Pillar et al 1995). Thus for implants with a bioinert surface the critical threshold lies between 50 and 150µm but it is determined by the surface topography and implant design.

Histology from experimental studies and clinically retrieved implants have demonstrated that implants can integrate under the influence of loading during the healing phase or immediately after implant placement (Piattelli et al 1993a, 1993b, 1997, 1998). Deporter et al (1990) found bone integration of dental implants loaded after 6 weeks in the dog mandible. Hashimoto et al 1988 and Piatelli et al (1993) loaded dental implants in a monkey model after 4 weeks of healing and found osseointegration. Immediate loading of a knee joint prosthesis in the rabbit (Rösttlund et al 1989) did not disturb bone repair and similar amount of bone apposition was found as in the non loaded implants. Immediate loading of conical, screw shaped implants in minipig jaw-bone revealed synthesis and deposition of bone related proteins by osteoblasts from day one, thus immediate loading of specifially designed implants do not disturb the biological osseointegration process (Meyer et al 2003). Rocci et al (2003) presented histology of oxidized implant that were clinically retrieved 3 to 9 months after being subjected to immediate or early loading. Direct bone apposition was demonstrated and high degreees of bone-implant contacts were reported. Similar results have been presented in a case report including histology of human dental oxidized implants by Degidi and co-workers (2002).

AIMS

The aims of the present study were:

1. To histologically and biomechanically compare bone integration of turned and oxidized titanium implants placed in bone defects.

2. To evaluate the effects of BMP and autogenous bone on implant integration in bone defects chips.

3. To histologically describe the early bone tissue response to oxidized and turned titanium implants.

4. To histologically and biomechanically study the influence of various widths of macroscopic grooves added to one thread flank on the integration of oxidized titanium implants.

5. To histologically describe the early bone tissue response to oxidized implants with a macroscopic groove on one thread flank.

MATERIALS & METHODS

Animals, anaesthesia and postoperative medication

Twelve mongrel male dogs weighing between 20 and 25 kg were used in study I. Before being admitted for surgery the animals were vaccinated, received anti-vermin drugs and were put into quarantine for clinical observation. The animals were pre-anaesthetized with xilazine (Ronpum®, Brazil, 20 mg/Kg I.M.) and ketamine 1g (Dopalen®, Brazil, 0,8 g/Kg I.M.) and anaesthetized with thionembutal 1 g (Tiopental®, Brazil, 20 mg/Kg I.V.). The animals were kept in intravenous infusion of saline during surgery. After surgery the animals were given vitamin compound (Potenay®, Brazil); anti-inflammatory/analgesic drug (Banamine®, Brazil); and antibiotic (Pentabiótico®, Brazil). The antibiotic was utilized in single doses immediatly after surgery, as well as 48 and 96 hours postoperatively. The study protocol was approved by the University of Sao Paulo´s Animal Research Ethics committee.

A total of thirthy-nine (39) female New Zealand white rabbits, at least 6 months old, were used in studies II-V. The animals were kept free in a purpose-designed room and were fed *ad libitum* with water and standard laboratory animal diet and carrots. Prior to surgery, the animals were given general anaesthesia by an intramuscular injection of fluanison and fentanyl (Hypynorm, Janssen Pharmaceutica, Brussels, Belgium 0.2mg/kg and intraperitoneal injection of diazepam (Stesolid, Dumex, Copenhagen, Denmark) 1.5mg/kg body weight. Additional Hyponorm was added when needed. Local anaesthesia was given using 1 ml of 2.0% lidocain/epinephrine solution (Astra AB, Södertälje, Sweden). After surgery the animals were kept in separate cages until healing of the wounds (1-2 weeks) and then released to the purpose-designed room until termination. Postoperatively, the animals were given antibiotics (Intenpencillin 2.250.000 IE/5 ml, 0.1 lm/kg body weight, LEO, Helsingborg, Sweden) and analgesics (Temgesic 0.05mg/kg, Reckitt and Colman, NJ, USA) as single intramuscular injections for three days. The study was approved by the local committee for animal research.

Implants

Titanium dental implants with either turned or oxidized (TiUnite™) surfaces were used in all studies. In Study I, 96 implants (48 turned, 48 oxidized), 10 mm long and 3.3 mm in diameter (Narrow platform, NP) were used.

In studies II-V, 276 slightly modified commercially available implants (7 mm long, and 3.75 mm in diameter, Nobel Biocare AB, Gothenburg, Sweden)) were used. (Figure 1) The implants had no cutting chambers or other apical features as have the commercially available ones. Four groups of test implants had a single

Fig.1 Implant design used in rabbit studies

groove positioned at the center of the inferior thread flank, i.e. facing the head of the implant (Studies II-V). The grooves were 70 µm deep and either 80 (S0), 110 (S1), 160 (S2) or 200 (S3) µm wide.(Figure 2) Implants without grooves were used as controls. All implants were subjected to surface modification by anodic oxidation (TiUniteä, Nobel Biocare AB, Gothenburg, Sweden) as described elsewhere. The implants had a special internal feature, which allowed for using a special connector (Stargrip, Nobel Biocare AB, Gothenburg, Sweden) to ensure firm grip when placing the implants and when performing removal torque measurements (see below).

Eighteen implants with a turned surface were used in Study II.

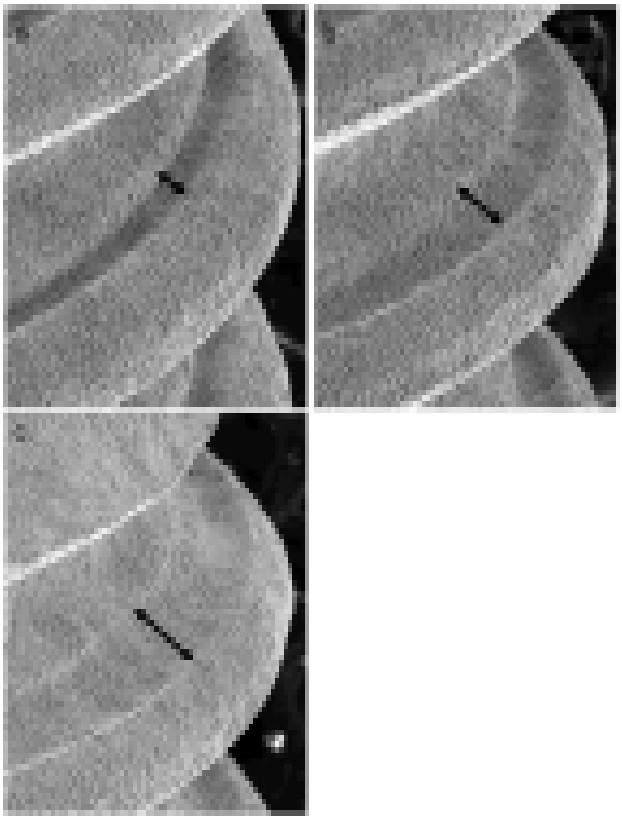


Fig.2 Scanning electron microscopy showing the test implants used in study IV. The grooves were 70 µm deep and either a/ 80 (S0), b/ 110 (S1) or b/ 160 (S2) µm wide .

Surface characterisation

Topographical analyses (Study II and IV) were performed using optical interferometry (MicroXAM™, PhaseShift, Tucson, USA) with measurement area of 60x190 μm^2 (50X objective, zoom factor 0.625) and the errors of form were removed with a digital Gaussian filter (size 50x50 μm^2). One implant from all groups except for implants with S3 groove was analysed. Images from the thread top, valley, inferior thread flank and groove, if present, were obtained at three different levels of implants: top, middle and bottom. Twenty seven or 36 areas (implants with groove) per specimen were analysed and the following 3D parameters were calculated: (Table 1)

S_a (µm) = the arithmetic average height deviation from a mean plane
S_{ds} (μm^{-2}) = the density of summits
S_{dr} (%) = the developed surface ratio
S_{ci} = core fluid retention index

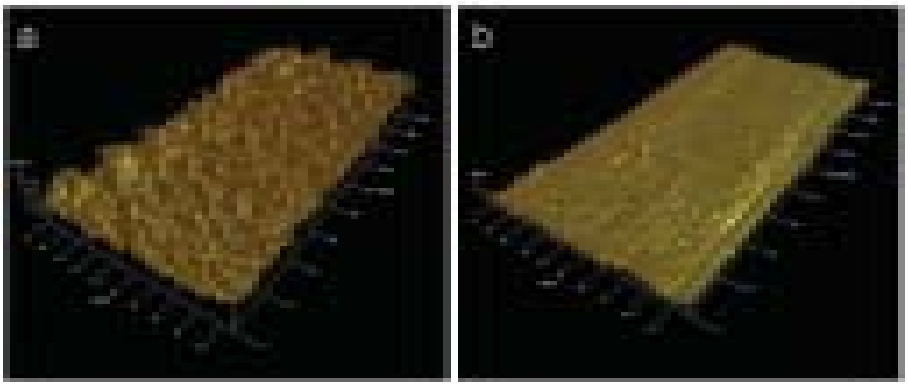


Fig.3 3D presentations of the two surfaces examined: a/ Oxidized implant, thread flank area. b/ Turned implant, thread flank area

Table.1

		SO		S1		S2		Ctr		Turned implant	
		Sa (µm)	Sdr (%)	Sa (µm)	Sdr (%)	Sa (µm)	Sdr (%)	Sa (µm)	Sdr (%)	Sa (µm)	Sdr (%)
Top	mean	1,2	53,3	1,1	48,3	1,2	52,5	1,0	44,5	0,4	8,1
	sd	0,2	8,1	0,1	5,1	0,1	6,7	0,1	5,7	0,1	2,3
Valley	mean	1,2	58,0	1,2	55,3	1,1	51,4	1,2	57,3	0,3	4,6
	sd	0,1	7,2	0,1	6,9	0,1	6,5	0,2	7,9	0,1	1,1
Flank	mean	1,1	47,5	1,2	53,2	1,3	58,2	1,3	56,3	0,5	6,8
	sd	0,1	5,3	0,1	6,5	0,1	9,0	0,0	2,4	0,1	0,5
Groove	mean	1,1	51,7	1,3	64,0	1,1	52,4	-	-	-	-
	sd	0,1	5,4	0,1	2,7	0,1	3,6	-	-	-	-

Surgery and experimental protocols

Dog study, Study I

The mandibular premolars were extracted five months prior to the experiment started. At the time of implant placement, crestal incisions were made and mucoperiosteal flaps raised bilaterally. Four 10 mm deep implant sites were prepared on each side using a 1.8 mm round drill and 2.0 and 2.7 mm twist drills. The crestal 5.0 mm of the implant sites were further widened to 6.3 mm using a guide and a trephine drill. (Figure 4) Four implants with a machined surface (10.0 mm long and 3.3 mm in diameter (NP) (Nobel Biocare AB, Gothenburg, Sweden) were randomly installed in one side and four implants of the same size but with an oxidized surface (TiUnite®), were placed on the other side. The implant heads were placed in level

with the uppermost contour of bone defect walls. Resonance frequency analysis (RFA) measurements were made on all implants (see below). Cover screws were attached to the implants. The four sites on each side were filled either with BMP-2 (RegenafilÔ, Regeneration Technologies ltd, Alachua, FL, USA) mixed in a collagen carrier; collagen alone; autogenous bone; or no treatment whatsoever (sham). The wounds were closed and sutured with interrupted stitches.

Re-entry surgery was made after 4 weeks of healing for RFA measurements in all dogs. Six dogs were killed for histology. The remaining six dogs were killed for histology 12 weeks after surgery when also RFA measurements were performed.

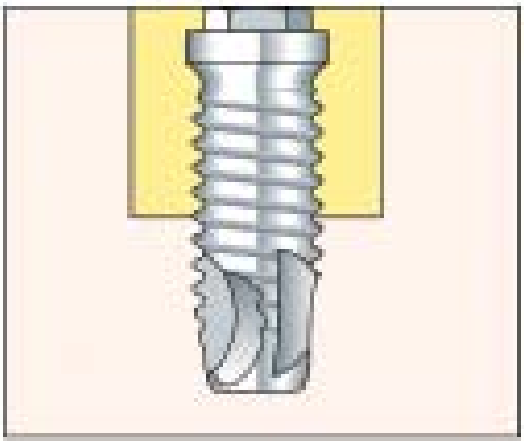


Fig.4. Schematic of the experimental site. A 10 mm implant 3.3 mm in diameter was placed in 6.3 mm wide and 5 mm deep defect.

Rabbit studies, Study II - V

The medial side of the femoral distal condyles and the tibial methaphyses were used as experimental sites. The sites were exposed via incisions through skin and fascia. Implant sites were prepared using a 1.8 mm round burr followed by 2mm and 3 mm twist drills during generous cooling by saline. No countersink drill was used. A screw tap was used to facilitate insertion of the implants. The fascia-periosteal flap and the skin were closed in separate layers with resorbable sutures.

Study II and V; One S1 implant and one control implant were inserted in the left and right femur respectively. One S1, control and turned implant were inserted in each tibial methaphysis. Three animals each were killed for histology after 7, 14 and 28 days. The left tibial implants were used for micro-CT analyses (Study V) (see below) whilst the other implants were analysed in histological ground sections (see below).

Study III; Each of nine rabbits received three S1 on one side (one in femur, two in tibia) and three control implants on the contralateral side. Another nine rabbits received S3 and control implants in the same way. The animals were killed after 6 weeks of healing when the implants in femoral and distal tibial sites were subjected to removal torque tests (see below). The implants in proximal tibial sites were retrieved for histology.

Study IV; Twelve rabbits received a total of eight implants each, two in each distal femoral condyle and two in each tibial methaphysis. Two S0, S1, S2 and control implants were inserted using a rotational scheme. After 6 weeks of healing all implants were subjected to RTQ tests and were thereafter retrieved for histology.

Biomechanical tests

Resonance frequency analysis (RFA) (Studies I, IV)

Implant stability was assessed using resonance frequency analysis measurements using an Osstell instrument (Integration diagnostics, Göteborg, Sweden) according to Meredith et al (1996). An L-shaped resonance frequency transducer was connected to the implant perpendicular to the jaw bone in dogs and to the tibia and femur and measurements are automatically made by the device. In brief, the transducer beam is excited over a range of frequencies which makes it vibrating. The amplitude of the vibrations is analysed and the maximal amplitude describes the resonance frequency of the system. The stiffer the system, the higher the stability and the higher is the resonance frequency. Measurements are given in ISQ units (Implants Stability Quotient) from 1 to 100 where the latter describes the highest degree of stability.

Removal torque (RTQ) testing (Studies III and IV)

The resistance to removal torque was tested with an electrical torque transducer consisting of a torsion rod. The rod was connected to each implant with the Stargrip™ connector (Nobel Biocare AB, Gothenburg, Sweden). An electronic motor ramped the torque to a maximum value, which was registered and stored by a micro processor . At the point of interfacial failure between the bone and the implant, the peak force dropped and a slight rotational movement of the implants was observed.

Histology

The implants and surrounding bone tissues were removed in blocks and fixed by immersion in 4% buffered formaldehyde. The specimens were dehydrated in graded series of ethanol an embedded in light curing plastic resin (Technovit 7200 VCL, Kulzer, Friedrichsdorf, Germany)(Donath & Breuner 1982). One section was taken through the longitudinal axis of each implant by sawing and grinding (Exakt Apparatebau, Norderstedt, Germany). The sections, about 10 m thick, were stained with toluidine blue and 1% pyronin-G.

Histomorphometry

Histological examinations were performed in a Leitz® microscope equipped with a Microvid system (Stuies I, III) or in a Nikon Eclipse 80i microscope (Teknoptik AB, Huddinge, Sweden) equipped with an Easy Image 2000 system (Teknoptik AB, Huddinge, Sweden) for morphometrical measurements (Studies II, IV and V). Morphometrical measurements were made in one section from each implant.

Study I

In study I, the degree of bone-implant contact (BIC) was measured and expressed as a mean total BIC, mean BIC within the defect area and mean BIC in the apical area. The bone area (BA) within the implants threads was measured and expressed as a mean total BA, mean BA in the defect area and mean BA in the apical area. Moreover, the vertical distance from the implant head to the first bone contact and to the level of the surrounding marginal bone was measured.

Study II

The degree of bone-implant contact and bone fill in implant threads were calculated.

Study III

The degree of bone-implant contact and bone fill in implant threads were calculated. The presence of bone in the groove and on the corresponding opposing flank surface (yes/no) was quantified within each implant thread.

Study IV

The histometric evaluation comprised: (i) Measurements of the thickness of the supporting bone. This was defined as bone tissue projected towards the implant surface from the surroundings. (ii) Quantification of number of grooves with and without bone tissue (iii) Quantification of the number of grooves showing direct contact with the bone. (iv) Quantification of the number of grooves showing separation between bone and the implant surface.

Study V

The histometric evaluation comprised: (i) Measurements of the degree of bone-implant contacts (ii) Measurements of the bone area occupying the implant threads (iii) Measurements of number of grooves with bone tissue in test implants (iv) Measurements of the number of surfaces opposing the groove showing bone formation, (v) Measurements of the number of surfaces, corresponding to the position of the groove in test implants, showing bone contact in control implants. (vi) Measurements of the number of surfaces opposing the corresponding groove surface showing bone formation in control implants.

Micro-CT

In study V, implants with and without grooves from the left tibia were analysed with a desktop cone-beam microCT scanner (mCT40, SCANCO Medical AG, Bassersdorf, Switzerland). The microfocus X-Ray-source had a spot size of 5 or 7 µm at 30-70 kVp / 20-50 keV (160 µA). The spatial resolution in the images was about 12 µm. The technique

includes software specifically designed for analyzing bone and similar structures were used which enable analysis of series of slices individually or as a 3D volume. The technique allowed for virtual 3D reconstruction of each specimen which could be rotated and studied from different angels. In addition, bone tissue and titanium could be color marked due to differences in opacity. The path of bone in relation to the implant surface and its geometry could thus be studied.

Statistics

The Wilcoxon Sign Rank Test was used to calculate differences between test and control implants. The Spearman Rank Correlation test was used for correlation statistics. A significant difference was considered if $p<0.05$.

RESULTS

Healing of turned and oxidized titanium implants implants in bone defects (Study I)

Histologic and histometric findings

A similar healing pattern was seen for all defects irrespective of treatment. Appositional bone formation starting from the bone walls towards the centre of the defect, which seemed more pronounced in the bottom, was observed for both types of implants. (Figure 5 and 6) More mature bone was present at 12 weeks as compared with 4 week specimens. New bone formation in BMP sites was observed in close relation to the BMP material. In higher magnification, lobular mineralized sites could be seen in the material with a higher concentration at the margins, indicating remineralization from the outside and inwards. The collagen sponge seemed to prevent bone formation from adjacent bone walls and and was often surrounded by loose connective tissue. The autogenous bone chips showed signs of remodelling and new bone formation on the surface.

Morphometric measurements regarding BIC after 4 and 12-week of healing showed no statistically significant differences when comparing sham and test sites. However, comparison between machined and oxidized implants revealed that oxidized implants showed higher total BA at 4 weeks. At 4 weeks the oxidized implants showed more BICs than machined ones when all sites were pooled for comparison. At 12 weeks, both BIC and BA at the defect area as well as total BIC values in all sites pooled showed statistic significance in favour of the oxidized surface. (Table 2)

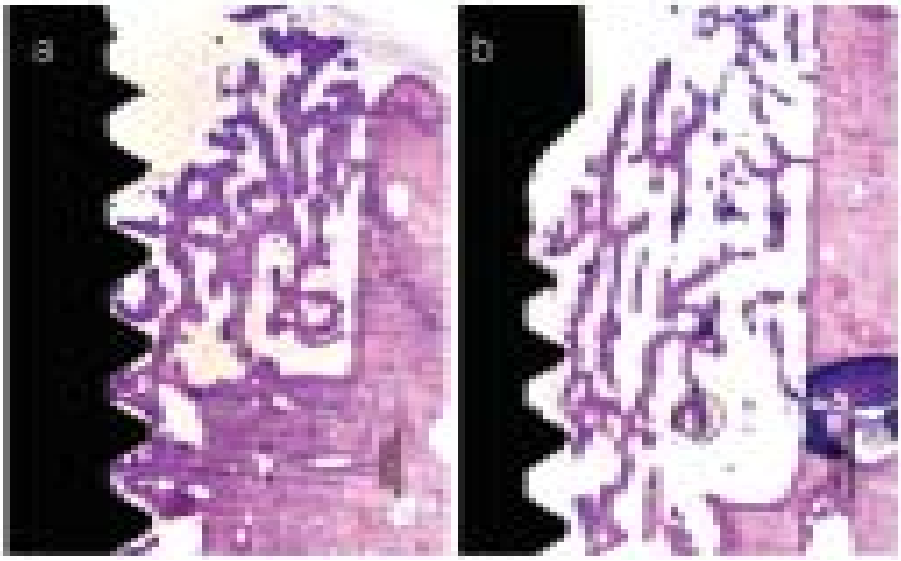
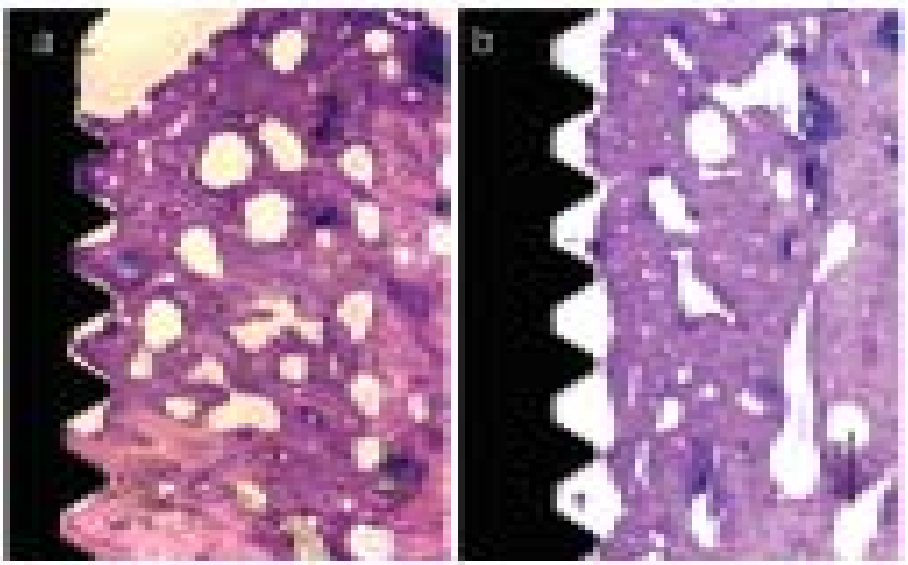


Fig. 5. Light micrographs of the defect areas at sham sites after four weeks of healing. A, Oxidized implant. Formation of new bone is seen in the defect and at the implant surface. Bar = 400 µm. B, Machined implant. Bone formation is seen in the defect but not at the implant surface. Bar = 400 µm.



Fig, 6. Light micrographs of the defect areas at sham sites after twelve weeks of healing. A, Oxidized implant. The defect is filled with new bone showing extensive remodeling. The bone is in contact with the implant surface. Bar = 400 µm. B, Machined implant. A similar bone fill as seen in A but in this specimen without bone contacts with the implant surface. Bar = 400 µm.

Implant stability measurements

The RFA measurements showed a significant increase of implant stability from placement to 4 weeks with 6.9 ± 5.1 ISQ for the oxidized implants in sham sites ($p<0.05$), compared with machined implants (0.4 ± 5.6 ISQ). When all sites were pooled at the same time period, the significance in favour of oxidized implants increased largely ($p<0.01$). The stability for all implants did not change as they were compared from 4 to 12 weeks in sham. (Table 2)

Table 2. Comparison between TiUnite vs Machined implants in sham and pooled sites, using BIC, BA and RFA values. Sham site = site with no adjunctive treatment; All sites pooled = based on mean values of all implants per surface and animal.

TiUnite vs Machined, histology 4 weeks		
	Sham site	**All sites pooled**
Area total	P?0.06	Ns
Area defect	Ns	Ns
Contact total	Ns	p?0.04
Contact defec	Ns	Ns

TiUnite vs Machined, histology 12 weeks		
	Sham site	**All sites pooled**
Area total	Ns	Ns
Area defect	Ns	p?0.005
Contact total	Ns	p?0.0008
Contact defec	Ns	p?0.0277

TiUnite vs Machined, RFA (ISQ)		
	Sham site	**All sites pooled**
Diff. 0-4 weeks	p?0.02	p?0.0061
Diff. 0-12 weeks	Ns	p?0.0187
Diff. 4-12 weeks	Ns	Ns

Early bone tissue responses to turned and oxidized titanium implants (Study II)

Light microscopy and morphometry

The histological analyses revealed a different pattern of bone integration for turned and oxidized implants. In essence, oxidized implants seemed to be integrated by bone formation directly on the surface, whilst bone formation was not seen at the surface of turned implants. (Figure 7) More bone was seen in contact with oxidized implants after 7, 14 and 28 days. On the other hand, higher values of bone area in the implant threads were seen for turned implants.

A darkly stained thin layer was observed at the oxidized surface as solitary spots or as continuous rims along several threads after 7 but also after 14 and 28 days. This material seemed initially to be acellular and had a globular appearance. (Figure 8a) In other areas, osteoblasts were seen on top of this layer producing osteoid towards the dark layer. (Figure 8b)At later stages, layers of mineralized bone with osteocytes were observed. For turned implants, bone formation was seen in the adjacent marrow tissue and always separated from the implant surface which with time reached the implant surface.

Influence of a macroscopic groove on integration of oxidized titanium implants (Study III - V)

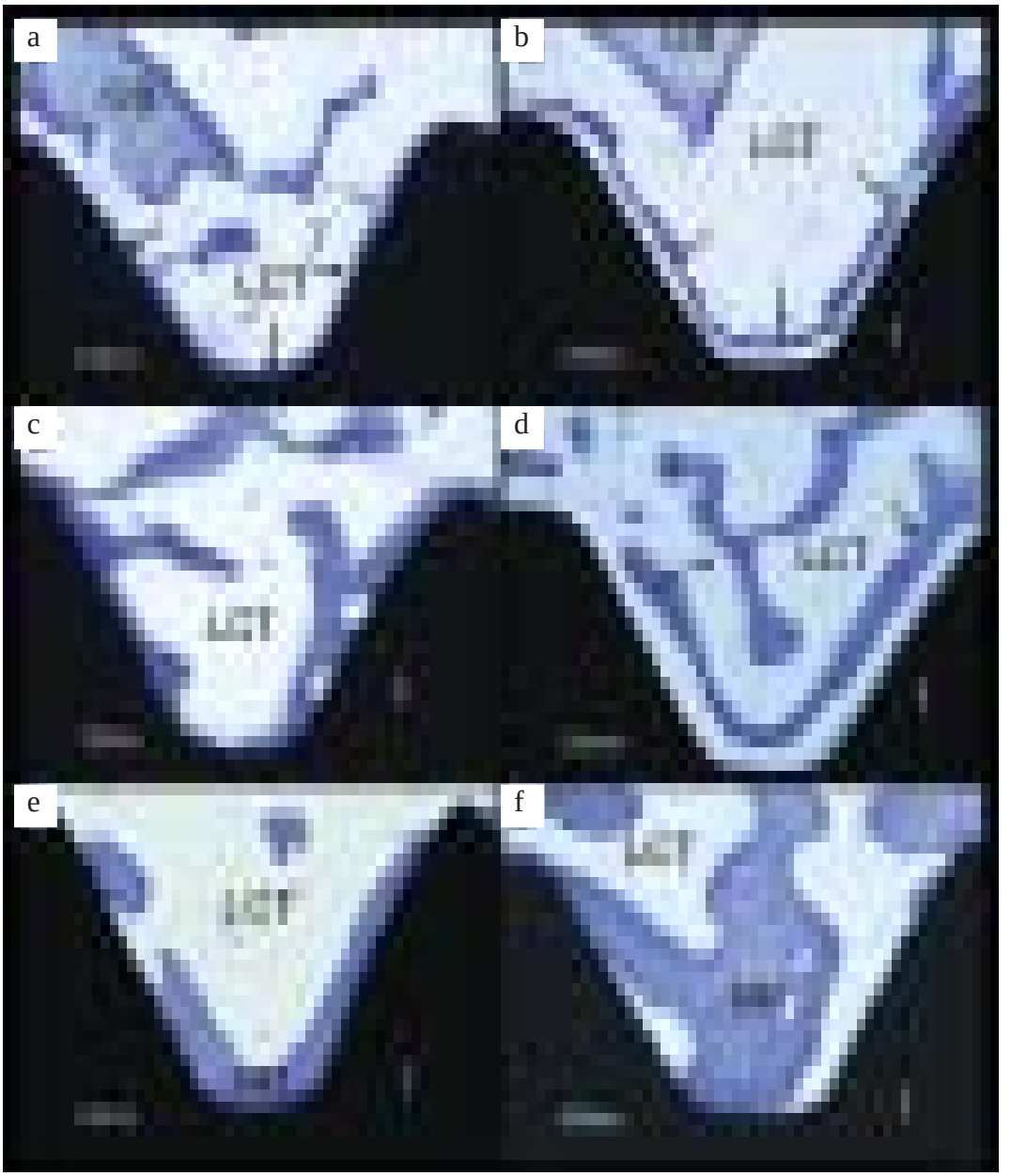
RTQ measurements

In study III, S1 implants were statistically more stable in tibial sites and in pooled sites. The differences were 30.4% (SD 33.8) (tibia) and 26.6% (28.1) (femur) higher RTQ for implants with the (S1) compared with implants without a groove. (Table 3) A similar but smaller and not statistically significant effect, 8.3% (SD 25.8) (tibia) and 7.7% (SD 16.1) (femur), was measured for S3 implants.

In study IV, the femoral implants showed statistically significant higher values for S1 but not for S0 and S2 implants compared to controls. (Table 4) The mean percentage difference between test and control implants were 22.0 % (SD 28.2) for the S1 implants, 8.5 % (SD 19.3) for S0 implants and -3.7%(SD 22.2) for the S2 group. For tibial implants there were no statistically significant differences between test and control implants. The mean percentage difference between test and control implants were 19.6 % (SD 50.2) for S2 implants, 0.3% (SD 36.5) for S1 implants and -1.9 % (SD 28) for S0 implants.

RFA measurements

RFA measurements in study IV revealed an increased implant stability for all implant groups. The increase was statistically significant for all femoral sites except for S1 implants. Tibial implants showed a significant increase for control and S0 implants. There were no statistically significant differences when comparing the primary or secondary stability for test and control implants.



Fig, 7. Light micrographs of oxidized (left) and turned (right) implants after 7 (a and b), 14 (c and d) and 28 (e and f) days of healing.

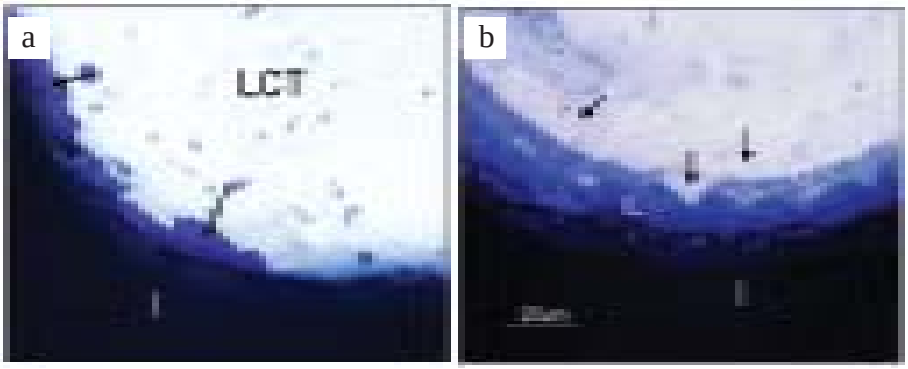


Fig.8 Light micrographs of oxidized implants after 7 days. Toluidine blue. a/ Example of darkly stained and acellular layer (arrows) frequently seen along the oxidized implant surface after 7 days. LCT = loose connective tissue. b/ Showing a darkly stained interface layer (IL) on the oxidized surface with globular appearance. Osteoblast (arrows) and osteoid (o) are seen on top of the layer, indicating bone formation from the surface.

Table 3. Results from removal torque measurements. Mean values in Ncm and differences in % ($(Sx + C_{sx})/C*100$)

	S1	**C_{S1}**	**Diff (%)**	**P-value**
Tibia	37.3± 10.2	30.4 ± 10.5	30.4 ± 33.8	0.04
Femur	63.1 ± 17.0		26.6 ± 28.1	0.12
Pooled	46.7 ± 12.9	37.2 ± 10.2	25.5 ± 21.2	0.04
	S3	**C_{S3}**	**Diff (%)**	**P-value**
Tibia	34.7 ± 10.1	32.3 ± 6.7	8.3 ± 25.8	ns
Femur	63.3 ± 12.8	59.2 ± 12.2	7.7 ± 16.1	ns
Pooled	49.0 ± 10.2	45.8 ± 7.9	7.3 ± 14.6	ns

Table 4. Results from RTQ measurements of femoral implants.

Group	**Peak value Ncm (SD)**	**Difference to control % (SD)**	**Statistics**
Control	72.4(18.5)	-	-
S0	76.6 (13.5)	8.5 (19.3)	NS
S1	87.1 (23.4)	22.0 (28.1)	0.03
S2	69.1 (19.4)	-3.7 (22.2)	NS

Histology and morphometry

The histology of the implants from study III showed that bone formed predominantly within the grooves with no apparent difference between S1 and S3 implants.(Figure 9) For S1 implants, 78.7 ± 15.8% of the grooves were filled with bone, whereas only 46.2 ± 27% of the corresponding flank surface showed the presence of bone ($p < .05$). The corresponding values for S3 and control implants were 72.7 ± 25.1% and 48.5 ± 13.6%, respectively ($p < .05$). Similar amounts of bone in the threads and similar degrees of bone-implant contact were seen for the S1, S3, and control implants.

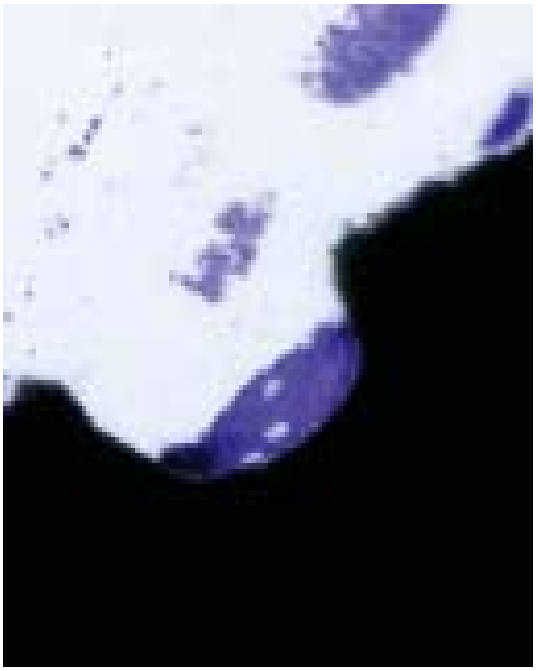


Fig.9 Light micrograph of a S1 implant demonstrating bone formation in the groove.

In study IV, the morphometric evaluation showed an increased degree of bone fill of the grooves with decreasing width. The fracture made by the RTQ measurements was more often located within the bone at the groove entrance for the S0 and S1 implants, whereas the fracture location at the S2 implants was more often located at the bone-implant interface.(Figure 10) Statistical analysis showed a significant correlation between decreased groove size and bone fill as well as between decreased groove size and the number of fractures at the groove entrance for both tibial and femoral implants.

Histology of S1 implants after 7, 14 and 28 days showed an identical bone formation pattern as described for oxidized implants in study II, which were also used as control implants in this study (V). As previously described, bone formation was evident directly on the surface in and outside the grooves.(Figure 11) Morphometry of the tibial implants revealed that new bone formation occurred more often in grooves than at opposing surfaces, which was more obvious after 7 than after 14 and 28 days.(Figure 12 and 13) Also control implants showed a higher incidence of bone formation at the inferior thread flank (corresponding to the location of the groove at S1 implants) as compared with the opposing superior flank. A similar pattern was observed for femoral implants.

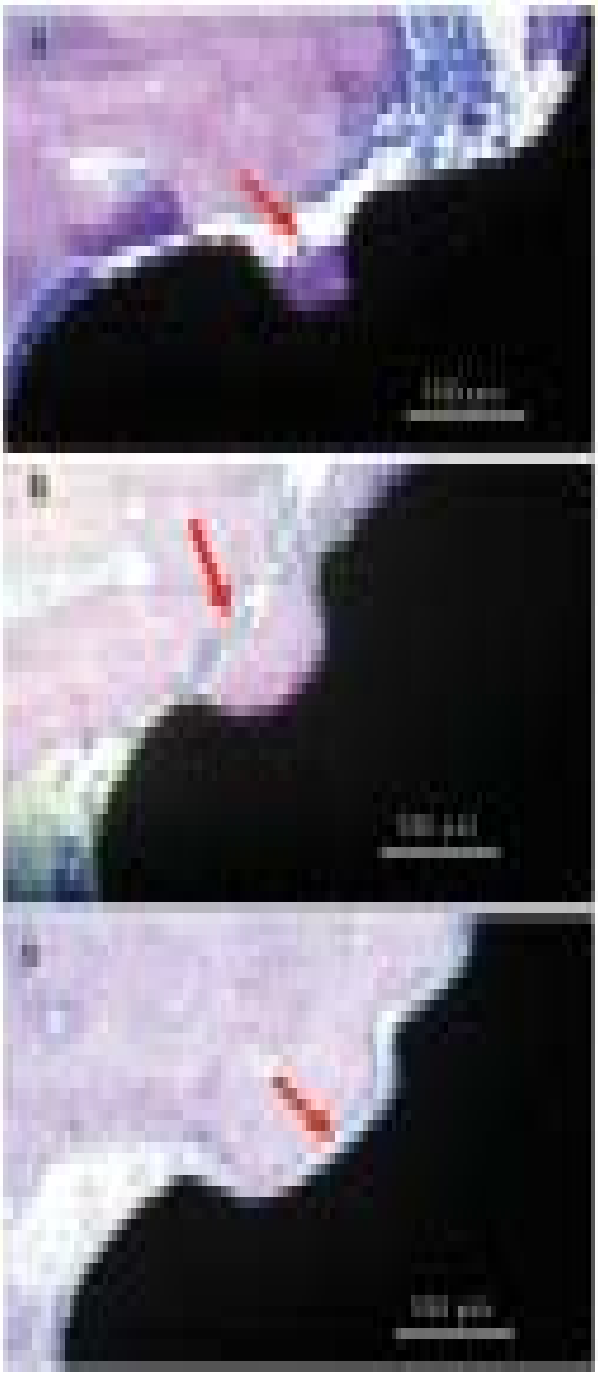


Fig.10 Light micrographs of specimens after RTQ testing showing a/ S0 groove with bone fracture (arrow) , b/ S1 groove with bone fracture (arrow) and c/ S2 groove with separation between bone and the implant surface (arrow).

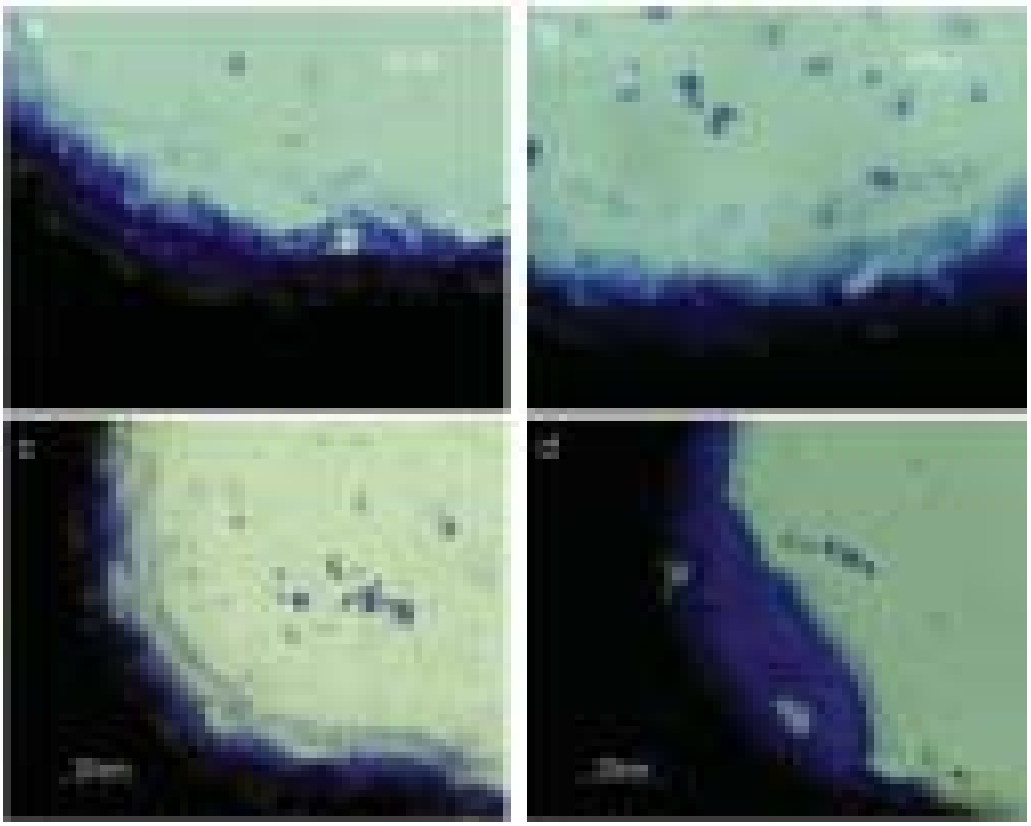


Fig. 11
A series of light micrographs of grooved implants demonstrating formation of bone directly on the implant surface. a/ A darkly stained layer about 10-15 um thick with no or few cells can be observed. Osteoblasts and osteoid can be distinguished on top of this layer (7 days). b/ Osteoblasts are becoming entrapped in mineralized matrix (7 to 14 days). c/ Osteocytes can be seen in the interfacial bone layer. Active osteoblasts are seen to producing osteoid on the layer (7-14 days). d/ A thicker and more mature bone is observed after 28 days.

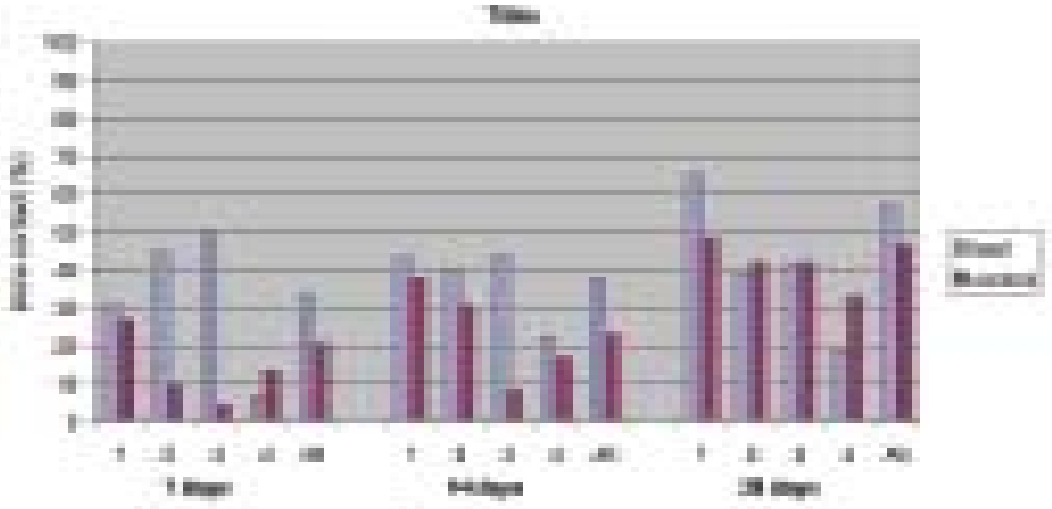


Fig.12 Graphs showing results from a/ bone-implant contact measurements and b/ bone area measurements for tibial implants

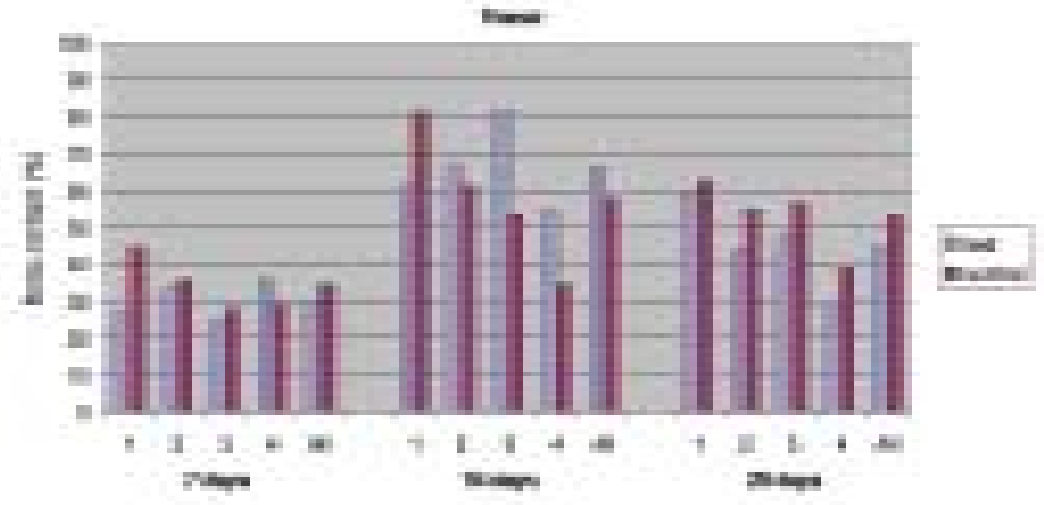


Fig.13 Graphs showing results from bone-implant contact measurements for femural implants

Micro-CT

3D color-coded reconstructions showed that new bone formation followed the path of the thread in apical direction for both test and control implants and especially in the bottom of the thread and along the inferior flank. In addition, bone was observed as rims in the groove of S1 implants. (Figure 14)

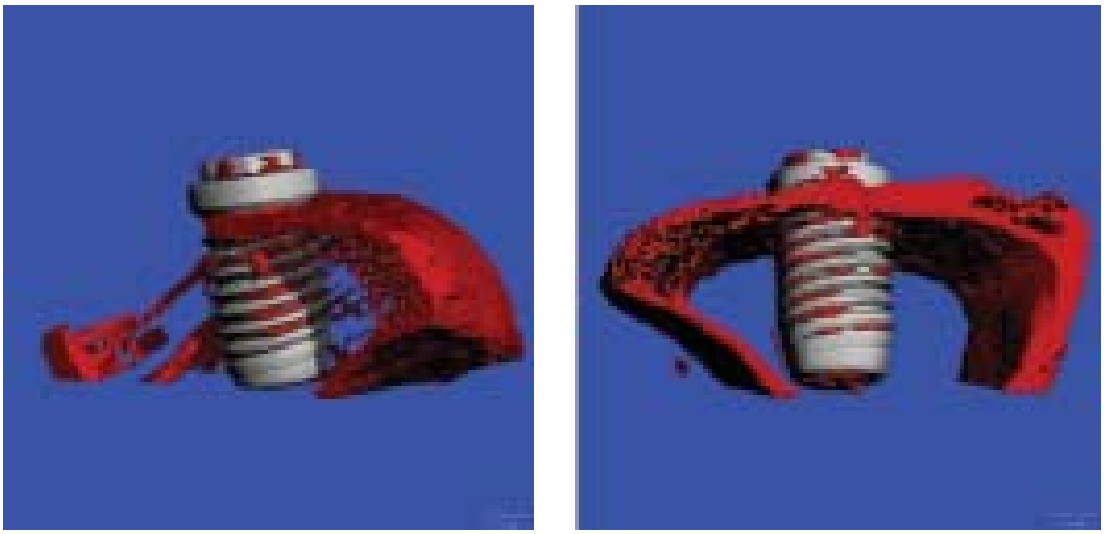


Fig.14 3D reconstruction of tibial implants a/ test implant after 7 days and b/ control implant after 7 days

DISCUSSION

Methods

Animal models

The rabbit model used in the present study is well known and has been widely used in implant research (Johansson 1991, Sennerby 1991, Gottlander 1994, Wennerberg 1996, Meredith 1997, Rasmusson 1998, Ivanoff 1999, Friberg 1999, Höstner 2001, Sul 2002, Franke-Stenport 2002, Slotte 2003) The model has some similarities with the dental clinical situation, with a cancellous femoral bone similar to the maxillary bone and a cortical tibial bone with similarities to the mandible. According to Roberts et al (1994) the rabbit is 2-3 times faster in bone formation, remodeling and maturation . Lamellar transformation of the peri-implant woven bone requires about 4-6 weeks after implant placement (Roberts et al 1984, 1993, Sennerby et al 1993a). Several scientists have reported that remodeling in rabbit requires 6-18 weeks (Roberts et al 1984, 1993, Sennerby et al 1993a, Piattelli et al 1995:). Johansson et al (1987) found continuous increase of removal torque up to one year after implant placement but the curve flatted out after 3 months indicating that remodeling was near completion. Meredith et al (1997a) studied implant stability in the rabbit tibia over a 5 months period and found an increase in resonance frequency up to 40 days (about 6 weeks) of healing and thereafter only small changes took place.

One disadvantage with the rabbit model is that the implants were not loaded and therefore the histological outcome may differ from the clinical situation. Another drawback is t that the rabbit tibia and femur are bones of endochondral origin and may differ from the intramembranous bone tissue in the healing process. However, it is though believed that the bone healing pattern of endocondral and intramembranous bone are similar.

The dog mandible is another well documented and used model for implant research both when placed in healed sites or in extraction sockets and defects (Akimoto et al 1999 Abrahamsson et al 2002, Cardaropoli et al 2003, Botticelli 2006). The jaw bone is similar to human bone and is of course of intramembraneous origin. A draw back is that the areas used for implant placement most probably is subjected to chewing and biting forces which may have negative influence on the healing. In the present study I, some of the cover screws became exposed. Moreover, an extensive remodelling/modelling of the buccal bone plate was observed which in part may be due to chewing forces.

Histology and morphology

Histomorphometry was carried out on undecalcified ground sections prepared as originally described by Donath and Bruner (1982) and Donath (1988). All samples were divided, sectioned and ground to a about 10µm thick samples controlled by a micrometer before staining. Johansson and Morberg (1995) demonstrated the importance of the section thickness in order to avoid overestimation of bony contacts, a thickness above 30µm gave

significant overestimation. Johansson (1991 thesis) found the smallest error of a "shadow effect" caused by a spherical implant with 10µm thick section cut through the center axis of the implant. Our routine staining method mentioned earlier permitted an intact analysis of the bone-implant interface and an overview of the gross anatomy and estimation of the maturation of the bone tissue.

The morphometrical readings were performed by one person in order to improve the accuracy. Previous work has not revealed any significant intraexaminer differences when repeateing measurements of the same specimens three times (Slotte et al 2003). The histomorphometry measurements and the descriptive analysis were performed on one section of each specimen. Serial sections would of course have given a more accurate result. However, the purpose of the technique was often to compare pairs of test and control implants and not to determine the absolute values of bone-implant contact and bone area in each specimen. Therefore, a single section is believed to give reliable and comparable information.

Removal torque measurements

The removal torque technique in the present studies measures the strength of the bone-implant-interface in shear. This method depends on different properties such as the implant design, implant surface and the properties of the surrounding bone tissue. Several studies have studied the relation between morphometric parameters and removal toque (Johanson & Albrektsson 1987, Ivanoff et al 1996, 1997)) and the method have been found to be a valuable instrument in biocompatibility studies (Johansson et al 1991). The correlation between the clinical performance of implants and removal torque is not yet known. The removal torque would correspond to rotational movements in the clinical situation which do take place in single-tooth restorations but it is believed that the most critical loading directions for the long term function are axial and specially bending loads. It is important to unscrew the implant with no deviation or tilting from the longitudinal axis of the transducer. This could be achieved by using a special jig for the torque instrument.

Resonance frequency analysis

The RFA is a non-invasive test that measures the interfacial stiffness between implant and bone tissue. Any entrapped soft or bone tissue may result in a false reading. However, this could be easily controlled by checking the morphology of the resonance frequency peak on the display of the instrument. No peak or double peaks were usually signs of entrapped tissue. In these cases the transducer was removed, the implant head cleaned and a new measurement was made. In general, RFA showed an increase for all implants with time, which indicates an increased stiffness due to bone formation and maturation. In the rabbit experiment, there were no obvious differences between test and control implants. This can be explained by the fact there was no major differences in the histological response as observed in Study

III. In the dog experiment there was a difference between turned and oxidized implants after 4 weeks. This can be explained by that bone easily formed contact with the oxidized implants and thereby better supported the implant.

Surface characterization

Dental implant surface measurements should comprise measurements of height, density and orientation of the surface irregularities because it is important to evaluate the implants mechanical interlocking properties (Wennerberg 1991). Wennerberg (1991) suggested that different parts of the implant screws show different roughness values and that measurements should be performed at different locations. In the present thesis, implants were analysed at flank, top and valley at the tip as well as in grooves at three levels of the implants. Between screws with the same surface modification a very low standard error was found and a high probability to detect differences in surface roughness and different positions when measuring 10 screws with the same surface modification and 9 measurements of each screw (Wennerberg 1991). In order to obtain significant measurements in surface roughness a minimum of 8 implants have to measured, giving a standard error of 0.0486 and an expected confidence interval of 0.10. We measured only one screw of each type and therefore no statistical differences could be investigated.

Micro-CT

Micro-CT was used in study V as a complement to histology. This non-destructive technique allows for a 3D reconstruction of the specimens which can be rotated and also sectioned in any direction at the computer screen (Van Oossterwyck et al 2000, Sennerby et al 2001). Moreover, different materials with different densities can be color coded to further facilitate the analysis. In the present study the technique was useful to describe the bone formation pattern over the implant body. It may be possible to used the technique also for morphometrical analysis since a good correlation has been found between micro-CT and histological parameters with exception for bone-implant contact measurements (Stoppie et al 2005)

Healing of turned and oxidized titanium implants in bone defects (Study I)

In the present thesis, healing of titanium implants was studied in a defect model in dogs. The aim of the study was to evaluate the influence of implant surface topography on integration as well to analyse the possible effect of adding either a growth factor (BMP-2), its carrier or autogenous bone chips into the defect. The apical 5 mm of the implant was stabilized in the alveolar bone whilst the upper 5 mm did not have any primary bone contacts but a void of about 1.5 mm existed between the implant and the bone surfaces. Histologically, the defect

region was increasingly filled with new bone from 4 weeks to 12 weeks postoperatively. Bone deposition began typically at the lowest vertical level in the defect area and extended upwards in contact with both types of implants surface, as also reported by Botticelli et al (2003). In the present study, ground sections were taken in buccal-lingual direction and extensive resorption of especially the buccal bone wall was evident. This can be explained as an reaction to the defect surgery, which often resulted in a thin buccal bone wall which may be more vulnerable due to the lack of vascularisation and possibly outer forces acting on the buccal bone wall during chewing. A similar resorption has previously been described when placing implants in extraction sockets in the dog mandible (Cardaropoli et al 2003, Araùjo et al 2005, Botticelli et al 2006). However, based on the studies by Botticelli et al (2005, 2006) it seems that implant placement in surgically created defects result in less resorption than implant placement in extraction sockets. The reason may be that the defects were created in healed bone when dimensional changes already have been taking place as a result of modelling and remodelling after extraction. In the present study, 5 months of healing after extraction was allowed before starting the experiment. It is also possible that the morphometrical outcome of the present study had been different if also mesio-distal sections had been used. It can be speculated that the integrity of the defect was better preserved and thereby the possibility for bone formation and implant integration.

No differences in implant integration could be seen when comparing the use of BMP-2, carrier or autogenous bone in the defects surrounding the implants as compared to empty control defects. However, it was likely that the BMP induced bone formation locally. This was seen as remineralisation of the bone chips in the mixture. However, it is possible that a higher dosage or repeated administration is required to have an effect on the defect at large. It is also possible that the collagen sponge not is an optimal carrier as this was seen to prevent bone formation and to be soft tissue encapsulated when used alone. Bone resorption and formation of the autogenous bone chips was seen as well as the particles became integrated with newly formed bone with time.

The only difference that could be revealed in our dog study was when comparing oxidized and turned implants with morphometry and stability measurements. Although the differences were not marked in all regions, the overall picture indicate a stronger tissue response to the oxidized implants. This is in line with the findings of Botticelli et al (2005) who found a better bone fill and integration of surface modified implants (SLA) in comparison with minimally rough control implants after placement in defects in a dog model. In the present study, RFA measurements showed a significantly higher increase in stability from placement to 4 weeks and a tendency after 12 weeks. At 4 weeks all 12 dogs were evaluated, whilst only 6 dogs were analysed after 12 weeks of healing which may explain the lack of significance. Our results regarding the strong bone response to oxidized implants corroborate with the results

from other studies comparing turned and oxidized implants (Albrektsson et al, Henry et al, Ivanoff et al 2003, Zeichner et al 2003a, Sul 2002).

Early bone tissue responses to turned and oxidized titanium implants (Study II)

Previous experimental and clinical histological studies as well as study I of the present thesis have demonstrated a more rapid formation of bone with higher degrees of direct contact at oxidized titanium implants in comparison with turned control implants. In addition, oxidized titanium implants have demonstrated a higher resistance to removal torque forces. Osborn & Newesly (1980) used the terms distance and contact osteogenesis to describe different patterns of implant integration in bone. Study II of the present thesis was undertaken in order to study the early bone tissue response to turned and oxidized implants. Using the same rabbit model, Sennerby et al (1991) described the kinetics of the integration of turned titanium implants using light and electron microscopy. It was evident that the integration process occurred by formation of bone from existing bone surfaces and as solitary island in the adjacent bone marrow which with time contacted the implant surface. The present study confirmed the results by Sennerby et al with regard to the bone tissue formation at turned implants, which showed an identical picture. However, the oxidized implants exhibited a complete different integration pattern.. It was evident that bone formation also occurred directly on the implant surface. In the early phase this was seen as a darkly stained granular layer with seemed at first to be acellular. In other areas and at later stages, osteoblasts were seen to produce bone towards this layer and later on osteoblasts became entrapped as osteocytes in this matrix. Our findings are in part similar to those reported by Davies et al (1991) who studied the interaction between osteoblasts and titanium in vitro. They described the formation of an afibrillar layer on titanium which evolved by the fusion of single calcified globulae less than 1 um in diameter formed by the osteoblasts. Collagen fibers produced by the cells attached to this layer and the matrix became mineralized with time. These events were later discussed by Davies and Hosseini (2000). A similar bone response as observed at oxidized implants has been reported for other surface topographies (Osborn & Newesly 1980, Piattelli et al 1996, Berglundh et al 2003, Ivanoff et al 2002)

Since only ground sections with limited resolution were used in the present study, ultrastructural techniques are needed to further investigate the interface at oxidized implants in vivo. It is possible that the electropolishing technique as used by Ericsson et al (1991) can be used for this purpose. With that technique the bulk part of the titanium implant can be electrochemically dissolved just leaving the surface oxide layer and thereby allowing for ultrathin sections for TEM to be produced.

The mechanisms behind the different integration patterns are not fully known but surface topography most certainly plays an important role although other factors such as surface chemistry should not be underestimated. The anodic oxidation process results in a surface

topography with pores with a size of 2 um and less (Hall & Lausmaa 2000). It can be speculated that the pores may serve as a reservoir for bone promoting factors from the blood clot. Davies and Hosseini (2000) suggested that the initial blood clot and its retention to the implant surface is an essential prerequisite for the migration of osteogenic cells. The early blood contact and establishment of a fibrin matrix through which osteogenenic cells can migrate to the surface is of importance. An acid-etched titanium surface with rough topography has been shown to better retain a blood clot compared to a smoother turned titanium surface (Park & Davies 2000). Moreover, a recent in vitro study demonstrated an increased trombocyte activation with increased surface roughness of calclum-sulphate coated surfaces (Kikuchi et al 2005).

Influence of a macroscopic groove on integration of oxidized titanium implants

The stability of a dental implant and its resistance to rotational, axial and lateral forces is in part due to a mechanical interlock between bone and implant surface. From an experiment using turned titanium implants, Sennerby et al (1992) suggested that the resistance to removal torque was depending on the amount of cortical bone contacting the implant surface. This was later further confirmed in rabbit experiments by Ivanoff et al (1996, 1997) using different lengths and diameters of turned titanium implants. The interlock theory can also in part explain the increased resistance to removal torque as seen with implants with an enhanced surface topography. However, Wennerberg (1996) suggested that there is an optimal surface roughness and that too rough surfaces may result in a decreased bone tissue response and less resistance to removal torque. Another way of increasing the interlock would be to add macroscopic features providing with an undercut for bone ingrowth. Commercially available titanium implants usually have bone cutting chambers at their apical aspect which probably contributes to implant stability after bone ingrowth. However, in soft bone qualities it would be more desirable to have such features in conjunction with the marginal cortical bone. Previous research has shown that surface structures may influence cellular response in vitro. For instance, Boyan and colleagues showed that forces acting on cells as they migrated on topographically modified surfaces stimulated matrix production. In vitro studies on surfaces with grooved structures in the range of tens of micrometers showed that it was possible to promote directed cell migration. Apart from serving as an undercut for bone interlocking, it was also speculated if the presence of a groove could stimulate and guide bone formation. In studies III to V, the influence of a macroscopic groove added to the inferior thread flank was biomechanically and histologically evaluated.

The results from study III, IV and V demonstrated that bone was preferentially formed in the groove as compared with the opposing and corresponding surface. It was also found that this preference increased with decreased width of the groove. However, any

correlation between resistance to removal torque and the presences of bone in grooves could not be observed. Instead, implants with 110 µ wide grooves showed a statistically significant increase compared with control implants, but this was not seen for implants with 80, 160 or 200 µ wide grooves. Histological analysis of implants after RTQ testing showed an increased incidence of fracture of the bone at the entrance of the groove as opposed to a separation at the bone implant interface. The occurrence of the maximum can therefore be explained as follows: For wider grooves, i.e. the 160 mm wide (S2) in study IV and the 200 mm wide (S3) in study III, the measured RTQ values are determined by a fracture located at the bone-implant interface. For narrower grooves, the number of fractures within bone at the groove entrance increases and the interface fractures decreases. If the force required fracturing bone is larger than the force required to fracture the interface, than the RTQ values should increase. However, as the grooves become narrower, the forces required to fracture the bone at the groove entrance decreases, and decreased RTQ values should be expected and approach the values for the control implants without grooves. Therefore, the curve of RTQ values as a function of groove width must have a maximum, which seems to peek close to the 110 mm wide groove (S1) in this experiment .

Study V was conducted in order to evaluate the early bone tissue response to S1 implants, which in Study III and IV showed a significant increase to removal torque. The implants were inserted in the rabbit femoral condyle , which represents a site rich of canncellous bone, and in the tibial methaphysis which normally consists of a thin cortical but no cancellous bone. It can thus be anticipated that the femoral implants had more primary contacts with bone along the surface as compared to implants in tibial sites. Interestingly, the histological evaluation after 7, 14 and 28 days showed a preference of bone formation in grooves as compared to the opposing flank in the same thread for tibial but not for femoral implants. Moreover, the difference was more marked after 7 days than later. The data indicate that the presence of the groove facilitated bone integration over and along the implants placed in a site with small bone volumes, whilst the effect of the groove on bone integration was not significant in a site with large bone volume. This is favorable from a clinical point of view since any enhancement of integration in poor bone situations may improve the clinical outcome.

CLINICAL IMPLICATIONS.

The findings from the present study show that surface modification by anodic oxidation results in a stronger bone tissue response and a more rapid formation of direct bone-implant contacts as compared to turned control implants. This difference was more marked during the early healing. In addition, the presence of a groove at one thread flank was shown to significantly increase the resistance to removal forces. It was further demonstrated that bone was formed more often in the groves than on opposing surfaces in

sites with small bone volumes. From a clinical point of view the use of oxidized implants with a groove would be especially beneficially in situations of low bone density and low primary stability when the survival of the implant may be depending on a rapid integration and stabilization in bone. The use of such surfaces may also be beneficial when using immediate loading protocols since the bone-implant interface will be biomechanically challenged from the day of placement. The findings of this thesis also indicate a more favourable integration and higher stability when placing implants in bone defects with no primary bone contacts. From a clinical point of view this would suggest an improved healing in extraction sockets and in maxillary sinus lift situations when placing bone grafts and implants simulataneously. However, in cases of dense bone and a high degree stability the effect may not be clinically significant and especially not if using a two-stage procedure with submerged healing of the implant prior to loading. Controlled clinical trials are obviously needed to confirm these suggestions of clinical use of the tested implant designs.

CONCLUSIONS

1. Oxidized titanium implants showed a stronger bone tissue response and better stability than turned implants when placed in marginal bone defects.

2. The addition of BMP or autogenous bone did not influence the healing of marginal defects around oxidized and turned titanium implants.

3. Oxidized titanium implants were integrated by direct bone formation on the surface whilst turned implants were integrated by appositional bone growth from the vicinity and towards the surface.

4. Oxidized titanium implants with a 110 µm wide and 70 µm deep groove showed a significantly higher resistance to removal torque than implants with 90 µm, 160 µm, 200 µm width or no groove. Bone formation occured preferentially in grooves as opposed to thread flanks without a groove. The incidence of bone formation increased with decreased groove width.

5. Early bone formation at oxidized implants occured preferentially in grooves and along the inferior thread flank when placed in bone sites with few primary bone contacts.

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