HEALING OF ENDOSSSEOUS IMPLANTS WITH DIFFERENT SURFACE CHARACTERISTICS IN GRAFTED AND NON-GRAFTED BONE
Clinical and experimental studies

Måns Jungner
Copyright © Måns Jungner 2014
This work is protected by the Swedish Copyright Legislation (Act 1960:729).
Healing of endosseous implants with different surface characteristics in grafted and non-grafted bone. Clinical and experimental studies.
New series No. 130
ISBN: 978-91-7459-783-7
ISSN: 0345-7532
The cover picture shows bone formation as solitary bone islets in the granulation tissue.
Printed by: Print & Media, Umeå University.
Umeå, Sweden, 2014
”Och ljuset lyser i mörkret, och mörkret har icke fått makt därmed”.

Joh 1:5
# Table of Contents

Table of Contents  
Abstract  
Abbreviations  
Original papers  
Introduction

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>1</td>
</tr>
<tr>
<td>Implant surface</td>
<td>1</td>
</tr>
<tr>
<td>Implant surface and the impact on peri-implant bone and mucosal health</td>
<td>2</td>
</tr>
<tr>
<td>Bone formation</td>
<td>4</td>
</tr>
<tr>
<td>Bone formation in grafted bone</td>
<td>6</td>
</tr>
<tr>
<td>Bone formation in guided bone regeneration</td>
<td>7</td>
</tr>
<tr>
<td>Maxillary sinus augmentation</td>
<td>8</td>
</tr>
<tr>
<td>Sinus augmentation procedures</td>
<td>9</td>
</tr>
<tr>
<td>Grafting materials</td>
<td>9</td>
</tr>
<tr>
<td>Sinus membrane elevation and simultaneous placement of implants</td>
<td>11</td>
</tr>
<tr>
<td>Early bone formation in the sinus elevation situation</td>
<td>11</td>
</tr>
<tr>
<td>Principles of histology and immunohistochemistry</td>
<td>12</td>
</tr>
</tbody>
</table>

| Aims                                                                    | 14   |
| General aims                                                           | 14   |
| Specific aims                                                          | 14   |

| Material and methods                                                   | 15   |
| Clinical studies                                                       | 15   |
| Paper I and II                                                         | 15   |
| Paper III                                                              | 18   |
| Experimental study                                                     | 21   |
| Paper IV                                                               | 21   |

| Results                                                                 | 24   |
| Clinical studies                                                       | 24   |
| Paper I and II                                                         | 24   |
| Paper III                                                              | 26   |
| Experimental study                                                     | 28   |
| Paper IV                                                               | 28   |
Abstract

Aims: This study uses radiological and clinical evaluations of the healing of endosseous titanium implants presented with different surface characteristics in the clinical situation (paper I-III) and experimentally to describe the early bone healing in maxillary sinus membrane elevation with and without the use of grafting material (paper IV).

Material and methods: In paper I, 136 patients were treated with 394 dental implants – 199 were oxidized titanium implants (Nobel Biocare TiUnite) and 195 were turned titanium surface implants (Nobel Biocare Mark III). Implant survival rates were retrospectively investigated after a minimum of five months after functional loading of the implants. At the five-year follow-up (paper II), eight patients were deceased and 128 were invited. Twenty-five patients refrained from participating in the study. The remaining 103 patients (287 implants – 133 with a turned surface and 154 with an oxidized surface) were examined after at least five years of functional loading. Clinical examinations of bleeding on probing (BoP) and pocket depth (PD) were performed. Intraoral radiographs were used to assess marginal bone levels (MBLs). In paper III, 28 patients were subjected to autologous bone graft and delayed implant placement, with a total of 92 dental implants. Thirteen patients received 47 implants with a turned surface and 15 patients received 45 implants with an oxidized surface. After a minimum of five years of functional loading, all patients were clinically examined regarding PD and BoP. The MBL was measured in intraoral radiographs. Cone beam computed tomography (CBCT) was used to evaluate the apical bone level (ABL) of the implants and intra-sinus conditions. The experimental study (paper IV) used nine adult male tufted capuchin primates (Cebus apella). Eight animals were subjected to bilateral maxillary sinus membrane elevation using a lateral replaceable bone window technique. One oxidized dental implant was placed in the residual bone of the sinus floor, protruding into the maxillary sinus cavity on both sides. In four animals, one sinus was left without any additional treatment, while the contralateral sinus was filled with autologous bone grafts from the tibia. In two animals, the implants were inserted under the elevated sinus membrane on both sides. In two animals, the sinus membrane was totally removed bilaterally before placement of implants. The animals were euthanized after 10 (n=4) or 45 (n=4) days. One non-operated animal representing pristine tissue conditions served as the control. The maxillary sinuses with implants were retrieved and further processed to prepare light microscopic ground sections or decalcified sections for immunohistochemical analyses.

Results: In paper I seven implants were lost in five patients – six in the maxilla and one in the mandible. All failed implants were Mark III turned implants. The overall implant survival rate was 98.2% with a survival rate of 96.4% for implants with turned surface after a minimum of five months after functional loading. In paper II, one additional oxidized implant failed, giving an overall cumulative survival rate of 94.7 and 99.4%, respectively, after at least five years of functional loading. There was no difference for BoP, PD, or MBL between turned and oxidized implants. A total of two implants, three oxidized and one turned, showed a PD > 3 mm, MBL > 4 mm, and BoP. However, none of these were associated with suppurative infection on examination. In paper III no difference was found between the two implants surfaces used in terms of PD, BoP, MBL, or ABL. Pathological reactions to the sinus membrane were seen in four
of the patients (14%). Radiographic signs of sinus pathology were not correlated to either survival rate of the implants or any of the investigated parameters. In the experimental paper IV, bone formation started from the bottom of the sinus floor, sprouting into the granulation tissue along the implant surface under the elevated membrane irrespective of time and surgical technique. Bone formation was not seen in direct conjunction with the sinus membrane. A distinct expression of osteopontin was observed in the serous glands of deeper portion of the lamina propria in direct connection with the elevated sinus membrane and close to the implant within all groups.

**Conclusion:** After more than five years of function in non-grafted patients, oxidized implants had a survival rate higher than turned implants, although this was not statistically significant. No difference was found in MBL, PD, or BoP. Grafting of the maxillary sinus floor with intra-orally harvested bone and delayed placement of either turned or oxidized implants resulted in equally high long-term survival rates, MBL, ABL, and BoP. Pathological findings in the maxillary sinus cavity, in terms of sinus membrane health, are few and not correlated to any of the other investigated parameters. In the experimental study bone formation after sinus membrane elevation with or without additional bone grafts started at the sinus floor and sprouted into the elevated space along the implant surface. Removal of the membrane resulted in less bone formation. The sinus membrane did not seem to present osteoinductive potential in sinus membrane elevation procedures.

**Keywords:** Dental implants, surface characteristics, bone graft, maxillary sinus, implant survival, oxidized surface, turned surface, sinus membrane elevation, bone formation, macrophages, osteocalcin, osteopontin, CD68.

**ISBN:** 978-91-7459-783-7
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>Avidin–biotin–complex</td>
</tr>
<tr>
<td>ABL</td>
<td>Apical bone level</td>
</tr>
<tr>
<td>BIC</td>
<td>Bone to implant contact</td>
</tr>
<tr>
<td>BoB</td>
<td>Bleeding on probing</td>
</tr>
<tr>
<td>CSR</td>
<td>Cumulative survival rate</td>
</tr>
<tr>
<td>DAB</td>
<td>Dimethyl-aminoazobenzene</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>GBR</td>
<td>Guided bone regeneration</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>MBL</td>
<td>Marginal bone level</td>
</tr>
<tr>
<td>MBLs</td>
<td>Marginal bone loss</td>
</tr>
<tr>
<td>MP</td>
<td>Macrophages</td>
</tr>
<tr>
<td>OC</td>
<td>Osteocalcin</td>
</tr>
<tr>
<td>OP</td>
<td>Osteopontin</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PD</td>
<td>Pocket depth</td>
</tr>
<tr>
<td>RANK</td>
<td>receptor activator of nuclear factor</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear factor-ligand</td>
</tr>
<tr>
<td>TiO₂</td>
<td>Titaniumdioxide</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
Original papers

The dissertation is based on the following papers, which will be referred to in the text by their roman numerals.


III. **M. Jungner, P-E. Legrell, S. Lundgren**, “Apical and marginal bone levels in patients rehabilitated with maxillary sinus augmentation using particulated mandibular bone graft and delayed placement of implants with two different surfaces. A minimum 5-year clinical and radiological follow up in 28 patients”. Submitted.

Introduction

Background

Osseointegration, the integration of titanium implants in bone, was first described by Brånemark and co-workers (Brånemark et al. 1969; Brånemark 1977). Experimentally, osseointegration is defined as the close and direct contact between bone and implant material in histological sections (Albrektsson and Johansson 2001; Brånemark 2005). In clinical terms, osseointegration is defined as the stability and ankylosis of an implant in bone (Albrektsson and Zarb 1993). Albrektsson and co-workers proposed six factors that have been generally accepted as especially important for the establishment of reliable osseointegration: (i) implant material; (ii) implant design; (iii) surface quality; (iv) status of the bone; (v) surgical technique; and (vi) implant loading conditions (Albrektsson et al. 1981). Good bone conditions – in terms of both vertical and horizontal width in the jaw, initial implant stability, biocompatible implant surface, favourable loads, and harmonious occlusion – are paramount to successful implant treatment (Buser et al. 1991; Ericsson et al. 2000a; Ericsson et al. 2000b). The original protocol with two-stage surgery, submerged healing of the implants under a reflected muco-periostal flap before final abutment connection, is well described in the literature (Adell et al. 1990; Lekholm et al. 2006).

To shorten the treatment time due to faster final prosthetic reconstruction and no need for a secondary abutment surgery, new treatment protocols for surgical techniques and healing times were introduced. Implants with modified surface topography were designed to improve and speed up the healing in bone. However, the rough implant surface carried the inherent risk of increased marginal bone resorption and peri-implant infections, complications seen as considerable by some studies and insignificant in other studies (Albrektsson et al. 2007; Francetti et al. 2012; Nicu et al. 2012; Sayardoust et al. 2013). During rehabilitation of the posterior edentulous maxilla, the mere lifting of the sinus membrane and simultaneous placement of implants can be an alternative to augmentation with bone grafts or bone substitutes (Lundgren et al. 2004; Cricchio et al. 2013). The mechanism of the bone formation under the elevated sinus membrane and the possible participation of the membrane itself is not fully understood (Srouji et al. 2010; Scala et al. 2011).

Implant surface

The original Brånemark titanium implant had a turned and minimally rough surface, and follow-up studies have reported predictable results with high survival rates and steady marginal bone levels (Lekholm et al. 1986; Brånemark et al. 1995; Nyström et al. 2009a). In an early long-term follow-up study, Adell and co-workers investigated 700 patients with 4,636 turned implants in 759 edentulous jaws. More than 95% of
maxillae had continuous prosthesis stability at five and ten years, and at least 92% at 15 years. The corresponding values for the mandibles were higher, showing 99% at all time intervals (Adell et al. 1990).

To enhance bone-to-implant contact (BIC) and therefore possibly obtaining stronger and faster bone healing, surface topography modifications with rougher surfaces were introduced in the 1990s. There are two main ways to improve an implant’s surface characteristics; (i) chemical and biochemical techniques that incorporate inorganic phases or organic molecules to the titanium dioxide (TiO$_2$) surface (Morra 2007; Morra et al. 2009) and; (ii) physical techniques that alter the surface topography, creating a rougher surface with a larger surface area, which improves bio-mechanical locking (Wennerberg and Albrektsson 2010). Modern dental implants often use combinations of chemical and physical surface processing, including titanium plasma spraying, acid-etching, grit-blasting, and electrochemical anodization of the titanium surface (Sul et al. 2009). Early studies of the surface characteristics and modification of the surface towards a rougher surface of the implants demonstrated stable and predictable results (Karlsson et al. 1998; Cordioli et al. 2000; Gotfredsen and Karlsson 2001). Histomorphometric studies (Kim et al. 2003; Sul 2003) found increased bone-to-metal contact to be positively correlated to enhanced surface roughness. Gotfredsen and co-workers found that more bone came into contact with the implant surface when using TiO$_2$-blasted implants rather than turned ones (Gotfredsen et al. 1995). A number of experimental studies have shown a better anchorage of titanium implants with a roughened surface compared with titanium implants with a turned surface (Gotfredsen et al. 2000). The increased surface roughness may enhance the mechanical interlocking between the macromolecules of the implant surface and the bone, resulting in increased resistance to compression, tension, and stress shear (Carlsson et al. 1988; Sul et al. 2002).

*Implant surface and the impact on peri-implant bone and mucosal health*

The introduction of the modified implant surface lead to the expectation of higher clinical survival rates, but also pointed out the risk of an increased marginal bone loss with the inherent risk for peri-implant infection problems. *Paper I* and *paper II* address this issue in non-grafted situations by examining and comparing two implant surface characteristics from a clinical aspect. As much as 1.5-mm marginal bone (to the first thread) is normally remodelled during the first year of function (Laurell and Lundgren 2009; Laurell and Lundgren 2011), although some studies have shown only minor changes of the average marginal bone loss (Jemt and Johansson 2006). The consensus report from the 1st European Workshop on Periodontology suggested that a successful implant is characterised by “an average marginal bone loss of less than 1.5 mm during the first year after the insertion of the prosthesis and thereafter less than 0.2 mm annual bone loss” (Albrektsson and Isidor 1994). However, some
implants show more and sometimes continuous bone loss, which may lead to soft tissue problems and loss of the implant. The mechanisms behind such bone loss are most likely multifactorial and may be explained by remodeling as part of implant healing, the response to loading, on-going atrophy after tooth loss, infection, or by other factors such as surface topography. Several reports of marginal bone resorption around modified surface implants with a high incidence of peri-implantitis have been published. Experimental (Albouy et al. 2008; Albouy et al. 2009) and clinical studies on a one-piece implant design (Albrektsson et al. 2007) reported extensive marginal bone loss in conjunction with oxidized implant surfaces. Of 550 NobelDirect implants placed at 18 different centres and using a punch procedure, 59 implants failed. Of interest is the large difference in failure rates between the directly loaded implants (58 failures) and indirectly loaded implants (one failure). Albrektsson further concluded that an unusually high failure rate after one year is seen in NobelDirect implants placed with the punch procedure and loaded directly.

However, a number of follow-up studies have shown good conservation of the marginal tissue with no apparent differences compared with turned surfaces (Rocci et al. 2003; Friberg et al. 2005; Friberg and Jemt 2010). The large differences in numerical values reported, and thus a potential source of error, may be due to different definitions of peri-implantitis. In a review article by Berglundh and co-workers, a 2.5 mm peri-implant bone loss, probing depth (PD) >6 mm, and bleeding on probing (BoP) and/or suppuration were proposed as diagnostic criteria of peri-implantitis (Berglundh et al. 2002). Of great importance, however, is whether peri-implantitis is defined on a level of subject or evaluated from implant-based data (Fransson et al. 2005). By transferring periodontal principles to implants, which are of a different type of tissue than the natural tooth, generous and wide definitions for bone resorption during the first year of healing have been proposed. The consensus report by Zitzmann (Zitzmann and Berglundh 2008) from the 6th European Workshop on Periodontology described peri-implant mucositis as “the presence of inflammation at an implant site with no signs of supporting bone loss”. They further described peri-implantitis as “the presence of inflammation of the mucosa and loss of supporting bone”. Moreover, based on two study samples, findings of peri-implant mucositis in 50% of the implant sites were reported. Peri-implantitis was identified in 12% and 43% of implant sites, respectively. Roos-Jansaker and co-workers defined peri-implantitis as implants demonstrating BoP and/or pus combined with a total bone loss of 1.8 mm or more between eight and 14 years after the first annual check-up (Roos-Jansaker et al. 2006). And further reported a presence of peri-implantitis in 16% of the patients and in 6.6% of the implants, in a study based on 218 patients with 999 implants clinically and radiographically examined (Roos-Jansaker et al. 2006). Fransson and co-workers, based on radiographic examinations, concluded that 423 implants (12.4%) out of 3413 implants demonstrated progressive bone loss (Fransson et al. 2005). In a later study (Fransson et al. 2008), even higher values (28%) regarding peri-implantitis were exhibited, and an association between clinical
signs of pathology and marginal bone loss at implant sites was proposed. In 2010, Koldsland and co-workers in a study consisting of 109 patients and 374 implants reported a varying prevalence of peri-implantitis, between 11.3% and 47.1%. In the discussion, the author suggests that it may be more appropriate to assess bone loss in percentages of implant length rather than millimetres when determining the severity of peri-implant disease (Koldsland et al. 2010).

Recent studies regarding marginal bone loss, however, demonstrate considerably lower prevalence of peri-implantitis. In ten studies of three modern implant brands of moderately rough surfaces with ten year or longer follow-up times, Albrektsson and co-workers concluded that the frequency of implants with reported peri-implant infection and significant bone loss was on average 2.7% between seven and 16 years of function (Albrektsson et al. 2012b). Jemt and co-workers studied further progression of bone loss in a group of 182 patients and 1029 turned implants who had been diagnosed nine years earlier with marginal bone loss and peri-implantitis (Fransson et al. 2005). Less than 30% of the patients with progressive bone loss exhibited one or more implant failures or high MBLs (>0.2 mm) during follow-up. Interestingly, patients treated by an oral hygienist and/or by peri-implantitis surgery did not perform better than untreated patients with regard to bone loss or implant failure (Jemt et al. 2013). Based on the Estepona consensus meeting on peri-implantitis (Albrektsson et al. 2012a), the author listed the following main conclusions: (i) the great majority of well-documented oral implants show very good long-term clinical results with implant survival rate >95% over ten years and the incidence for peri-implantitis is less than 5%; (ii) a limited amount of crestal bone loss may be a biologic response to implant placement and can occur without the presence of an infection; (iii) peri-implantitis is defined as an infection with suppuration associated with clinically significant progressing crestal bone loss after the adaptive phase; (iv) peri-implant mucositis is defined as inflammation of the peri-implant mucosa without progressing bone loss; and (v) the periapical radiograph is an important clinical tool to be used at implant placement, implant loading, and repeatedly thereafter. Clinical examinations that include BoP and PD do not by themselves function as indicators of crestal bone loss. Qian and co-workers highlight combined causes including implant hardware, clinical handling, and patient-related factors. They also note that rigid control of these produce stable clinical results with a low prevalence of peri-implantitis (Qian et al. 2012).

Bone formation

Human bone is a living, biologically active tissue. It has its own blood supply and different active cells. The most important function of human bone is to; (i) build support and structure for the body; (ii) provide protection for internal organs; (iii) produce blood cells in the bone marrow; and (iv) participate in the mineral metabolism of the body. Bone tissue is composed mainly of two types of mineralized
structures: an outer hard structure called the cortical (compact) bone and an inner layer of trabecular (cancellous) bone with lower density. Other structures of the bone consist of an osteoid with non-mineralized matrix of collagen and protein as well as bone forming cells (osteoblasts and osteocytes) and resorbing cells (osteoclasts). The function of the specific bone cells is to participate in bone formation, bone healing, and bone remodeling. Osteoblasts develop and differentiate from mesenchymal stem cells and are involved in the synthesis of bone matrix and further in the mineralization of the bone. Osteocytes are osteoblasts that are involved in the metabolism of newly formed osteoid, which later becomes mineralized. Cells in the deeper layer of the bone communicate via specific channels (canalici) with new osteocytes in the osteoid and more mature cells on the bone surface. Because these osteocytes respond to physical loading of the bone, they can initiate both resorption and bone formation activities. The multi-nucleated cells in the bone, called osteoclasts, derive from the hematopoietic lineage. They are involved in bone resorption of mineralized tissue and are seen on the bone surface in locations with active resorption, with characteristic resorption lacunae where secretion of bone resorptive enzymes occur (Giannoudis et al. 2011). Osteoid consists mainly of type-I collagen and non-collagenous proteins (94%). The density of the osteoid depends on the extent of mineral salts, which are a complex of calcium and phosphatase (hydroxylapatite). Calcified bone consists of mineral (70%), water (5%), and organic matrix (25%). Two types of bone, woven bone and lamellar bone, are evident depending on the collagen pattern in the formation of osteoid. Woven bone develops when osteoblasts produce osteoid in a rapid sequence and can be seen especially in foetal bones and in fracture healing. The woven bone remodels and is later replaced with lamellar bone (Lundgren and Sennerby 2008).

Osteogenesis (bone formation) takes place as the result of two mechanisms; (i) an intramembranous mechanism, where bone formation occurs by replacement of connective tissue with bone and; (ii) an endochondral ossification, where hyaline cartilage is replaced by bone. Bone modeling is the process where resorption and bone formation occurs on different surfaces (the growth of long bones). Bone remodeling is the process where old bone tissue is replaced with new bone tissue. The regulation of bone resorption, bone modeling, and bone remodeling is regulated by the RANKL-RANK-OPG system. The osteoblastic stromal cells regulate osteoclast formation through expression of the members of the tumour necrosis factor (TNF) superfamily; (i) receptor activator of NF-κB ligand (RANKL); (ii) osteoprotegerin (OPG); and (iii) receptor activator of NF-κB (RANK) (Boyce and Xing 2008). This signal system regulates osteoclast formation, activation, and survival in normal bone modeling and remodeling and in several pathologic conditions. Bone remodeling can be divided into five phases; (i) activation: activation of pre-osteoclasts into mature cells stimulated under the influence of cytokines; (ii) resorption: resorption of old bone by osteoclasts; (iii) reversal: the termination of resorption; (iv) formation: development of new bone by osteoblasts; and (v) quiescence: osteoblasts turned into
resting cells on the newly formed bone surface (Marsell and Einhorn 2011; Bellido 2014). The term osteoinduction is the process by which osteogenesis is induced, whereas osteoconduction refers to bone growing on a surface, a phenomenon regularly seen in dental implants (Albrektsson and Johansson 2001). Different schemes for classifying bone quality in the clinical situation have been described. The most accepted is the one presented by Lekholm and Zarb. This scheme describes the shape of the bone using a five-degree scale (usually performed on preoperative radiographs) and the quality of the bone using a four-degree scale: type I (almost entirely composed of homogenous compact bone); type II (a thick layer of compact bone surrounds a core of dense trabecular bone); type III (a thin layer of cortical bone surrounds a core of dense trabecular bone); and type IV (a thin layer of cortical bone surrounding a core of low density trabecular bone of poor strength) (Lekholm et al. 1985). To describe the edentulous jaws, the commonly used classification is described by Cawood (Cawood and Howell 1988).

**Bone formation in grafted bone**

Autogenous bone grafts require special circumstances for healing and further bone remodeling as the graft is considered to be a free graft because its previous blood supply is cut off and some cell death will occur within the grafts. The graft may be harvested from intraoral and extraoral donor sites that include: (i) mandibular chin (Clavero and Lundgren 2003); (ii) mandibular ramus (Misch et al. 1992); (iii) zygomatic body (Kainulainen et al. 2005); (iv) iliac crest (Nyström et al. 1993); (v) tibial condyle (Louis et al. 2008); (vi) calvarium (Eichhorn et al. 2009); and (vii) fibula (Rohner et al. 2002; Rohner et al. 2003). Four factors for the healing of bone grafts are generally disclosed; (i) revascularisation of the graft (Goldberg and Stevenson 1987); (ii) rigid fixation of the graft (Lin et al. 1990); (iii) good mucosal coverage (Phillips and Rahn 1990); and (iv) healing without loading (Lundgren and Sennerby 2008). The bone graft, however, unlike bone substitute materials, contains growth factors, and inflammatory mediators that interact with the destination area in the healing process and initiate migration of bone formative cells and further revascularisation from the surrounding tissue. Therefore, ingrowth of new blood vessels in the graft is of the utmost importance and differences in re-vascularisation time can be seen between cancellous bone (due to its porous nature) and cortical bone. Lundgren and co-workers performed histological analysis of titanium micro-implants retrieved after six and twelve months from ten patients with severely resorbed maxillae and treated with iliac cortico-cancellous bone grafts. The results suggested that a delayed implant placement worked well for grafted situations due to a higher bone-to-implant contact. They also concluded that delayed implant placement contributed to the partly re-vascularized grafted bone, so it was able to respond to the surgical trauma, resulting in interfacial bone formation (Lundgren et al. 1999). Regarding particle size in relation to the early stages of bone formation, Pallesen and co-workers conducted an experimental study in rabbit calvarium. Four
bicortical clavarium bone defects were prepared in 15 rabbits. Three of the four defects were filled either with (i) small autogenous bone graft particles or (ii) large autogenous bone graft particles or (iii) no graft, serving as control. After one, two, and four weeks, histological and stereologic evaluations were performed. They found that the total volume of newly formed bone in defects with small particles was larger and more mature compared to defects with large particles after two and four weeks, concluding that the early stages of bone regeneration were strongly influenced by the particle size of the graft, a finding possibly related to an increased release of growth and differentiation factors from the larger surface of the small particles (Pallesen et al. 2002).

**Bone formation in guided bone regeneration**

Guided bone regeneration (GBR) describes the use of biocompatible membranes or scaffolds to avoid unwanted ingrowth of non-osteogenic tissue to the site of bone formation (Dahlin et al. 1988). Several possibilities, individually or in combination, can stimulate and facilitate bone formation: (i) osteoinduction by growth factors; (ii) osteoconduction with bone grafts or substitutes; (iii) the supply of progenitor cells that can differentiate into osteoblasts; (iv) distraction osteogenesis; and (v) GBR using membranes (Buser 2009). In a histological study of canine mandible, Schenk and co-workers evaluated the pattern of bone regeneration in membrane-protected defects in the mandibles of four dogs. After a healing period of two and four months, control sites (without membranes) exhibited incomplete healing with a persisting defect and the sites with membranes demonstrated significantly better bone healing. They further concluded “bone regeneration in membrane-protected defects progresses in a programmed sequence through a series of maturation steps, which closely resemble the regular pattern of bone healing” (Schenk et al. 1994). In a histological study of the mandibles of five foxhounds (Buser et al. 1995), inserted with 15 non-submerged titanium implants, bone regenerated in extended membrane-protected defects during a six-month healing period. After histological analysis, the authors drew two main conclusions; (i) bone regenerated in membrane-protected defects responds to implant placement as if it were non-regenerated bone with no apparent differences concerning bone remodeling activities; and (ii) placement of implants into regenerated bone stimulates bone maturation and bone remodeling.

Both resorbable and non-resorbable membranes with or without reinforcements are available on the market, but the most commonly used membrane in GBR procedures today is the non-cross-linked collagen membrane as it has the following desirable characteristics; (i) high hydrophilic quality and favourable clinical handling; (ii) low risk of post-operative complications; and (iii) the elimination of secondary surgery with retrieval of the membrane (Buser 2009). In an early clinical and histological study of sinus elevations with and without the use of a barrier membrane in twelve patients, Tarnow and co-workers noted that: (i) placement of barrier membrane
tends to increase bone formation; (ii) a barrier membrane has a positive effect on implant survival; and (iii) the use of membranes should be considered for all sinus elevation procedures (Tarnow et al. 2000). Surgical techniques using mere elevation of the sinus membrane can also be described as a form of GBR technique to create a space with a barrier against soft tissue, which in an undesirable manner can reduce the potential for bone formation (Cricchio et al. 2009a; Cricchio et al. 2009b). An recent study by Johansson and co-workers in 24 consecutive patients provided with 30 sinus elevation procedures resulted in statistically equal new bone formation in all three groups: (i) lateral sinus elevation with replacement of bone window without additional bone graft; (ii) lateral sinus elevation with covering of osteotomy site with a collagen membrane without additional bone graft; and (iii) lateral sinus elevation with autogenous bone graft. They further noted that most of the implants’ apices were not covered with bone at the time of retrieval (Johansson et al. 2013). Bone coverage of the apex area of the implant, however, may be of secondary importance as experimental studies have shown that the coronal cortical anchorage dominates (Pierrisnard et al. 2003), the bone stress is concentrated to this area, and no stress concentration occurs at the implant apex for axial and non-axial loading (Gross and Nissan 2001; Gross et al. 2001).

Maxillary sinus augmentation

The maxillary sinus is a pyramid-shaped, mucosal lined air-bearing cavity located in the bones around the nasal cavity. Several theories of the function of the maxillary sinus have been proposed, including its role as a resonant chamber, air conditioning chamber, pressure equalizer, and heat insulator (Bell et al. 2011). The maxillary sinus may also protect the skull base and brain from facial trauma. The average volume of a human adult maxillary sinus is 15 ml – 30 mm high, 23-25 mm wide, and 32 mm in the antero-posterior dimension (Karmody et al. 1977; Lawson et al. 2008). The ostium, a opening connecting the maxillary sinus with the middle meatus, is located high up on the medial wall with an average diameter of 2.4 mm (Bell et al. 2011). The mucosal lining within the maxillary sinus cavity consists of a specialized respiratory epithelium, referred to as ciliated pseudostratified columnar epithelium. The main components of this layer are basal cells, goblet cells, and ciliated cells (Mogensen and Tos 1977). Goblet cells are secretory cells that produce mucin, and ciliated cells are columnar epithelial cells that are coated with cilia and serve to move the mucin-containing trapped particles towards the ostium for removal. The combination of the periosteum-like membrane, the loose connective tissue (lamina propria (Figure 1) consisting of vessels, glands, etc.), and the pseudostratified epithelium is most often referred to as the Schneiderian membrane. In humans, an increase in numbers of the submucosal glands of the lamina propria is commonly associated with pathological conditions of the sinus mucosa (Fang 1994; Melgarejo-Moreno et al. 1996).
Sinus augmentation procedures

Insufficient bone volume in the edentulous posterior maxilla, due to normal alveolar bone resorption of the alveolar crest (Tallgren 1972; Cawood and Howell 1988) and pneumatization of the sinus cavity (Ariji et al. 1994; Ma et al. 2013), often creates a need for bone reconstruction so as to provide adequate stability before installing dental implants. Augmentation of the maxillary sinus with autogenous bone was first described by Tatum, Boyne, and James (Boyne and James 1980; Tatum 1986). This technique consists of the exposure of the alveolar bone by a muco-periosteal flap and creating access to the sinus cavity through an opening in the lateral sinus wall. The opening on the lateral wall is made with a round bur, creating a rectangular osteotomy. The bone window is then carefully removed from the underlying sinus mucosa. By means of a careful dissection of the sinus mucosa, such as a periosteal dissection, the membrane is elevated in the cranial direction to create a space where a particulated bone graft is placed. The space is then covered by the reversed muco-periosteal flap and sutured to a complete passive closure. Reconstruction of the atrophic posterior maxilla in conjunction with or before endosteal implant placement is well described and well documented (Lundgren et al. 1996; Johansson et al. 1999a; Wannfors et al. 2000; Sjöström et al. 2007; Nolan et al. 2014).

Grafting materials

Various materials can be used for sinus augmentation procedures. These can be divided into four main groups: (i) autologous bone (Raghoebar et al. 1997); (ii) allografts (Kassolis et al. 2000), which are usually harvested from human cadavers; (iii) alloplasts (Karabuda et al. 2001; Fischer et al. 2009), which are made of synthetic bone material; and (iv) xenografts (Hallman et al. 2002; Mozzati et al. 2013; Peñarrocha-Oltra et al. 2013), which are grafts from a non-human species. The use of autologous bone, however, is the gold standard for reconstruction in this area.
due to a high biocompatibility and osteoinductive properties (Thorwarth et al. 2005; Nkenke and Stelzle 2009). Several authors have described stable and good results with different grating materials, using both immediate and delayed implant placement (Morra et al. 2009; Cabezas-Mojón et al. 2012; Schmitt et al. 2012; Silvestri et al. 2013). Cho-Lee and co-workers demonstrated a cumulative implant survival rate of 93% after 12 years of loading, following sinus bone grafting procedures, using both autologous bone and a mixture of autologous bone and xenograft (Bio-Oss) (Cho-Lee et al. 2010). The use of autologous bone harvested from the iliac crest exhibited a cumulative implant survival rate after two years of 99.5% (Stricker et al. 2003). Nyström and co-workers demonstrated a long-term (mean 11 years) survival rate of 90% and stable marginal bone levels in 334 turned implants placed in maxillary onlay bone grafts harvested from the iliac crest (Nyström et al. 2009b). Becktor and co-workers reported a survival rate of 98.5% in a one-year follow-up study following bone augmentation with a particulate cortical bone graft harvested from the lateral part of the ramus/body of the mandible (Becktor et al. 2008). In a recent study, Dasmah and co-workers compared block vs. particulated bone grafts used for augmentation of the atrophic maxilla in 15 patients. In this prospective study, two implants were placed on each side of the midline in either block or particulated bone grafts. They found indications, although not significant, that the marginal bone loss of the side with block grafts was larger compared to particulated grafts. They further concluded that most of the resorption occurred during the first year (Dasmah et al. 2013). Lee and co-workers concluded in an experimental study consisting of maxillary sinuses in dogs that installation of implants under the elevated sinus membrane using simultaneously placed corticocancellous block bone grafts is superior to corticocancellous particulate bone grafts for bone healing around dental implants (Lee et al. 2007).

Comparisons and long-term follow-up studies of dental implants with different surface characteristics in the augmentation of the posterior maxilla with autogenous bone grafts are sparse as are studies evaluating the clinical outcome in grafted situations. In a systematic review, Del Fabbro and co-workers concluded that the performance of rough surface implants is superior to that of turned implants when placed in grafted maxillary sinuses. Survival rates were 85.64% for turned implants and 95.98% for rough surface implants (Del Fabbro et al. 2004). Findings that were later confirmed by Cabezas-Mojón and co-workers. Their survival rate for turned implants was 75.6% and for rough surfaces, 96.8% (Cabezas-Mojón et al. 2012). Implant stability and marginal and apical bone stability in grafted situations and the impacts of different surface characteristics in relation to the above parameters have not been fully investigated in previous studies, but these issues have been investigated from a clinical perspective in paper III.
Sinus membrane elevation and simultaneous placement of implants

To increase cost-effectiveness and to decrease invasive intervention in the maxillary sinus augmentation, elevation of the sinus membrane and simultaneous placement of implants was introduced. This method was found to be an alternative to traditional augmentation using different graft materials (Lundgren et al. 2004). An experimental animal study confirmed the results by histological means and verified bone formation and implant integration (Palma et al. 2006). Several other authors have demonstrated similar and predictable results even in cases with a reduced residual bone height (Sul et al. 2008; Borges et al. 2011; Cricchio et al. 2011; Cricchio et al. 2013). The technique and the steps for the sinus membrane lifting using a lateral sinus access technique are described below (Figure 2a-f).

**Figure 2a-f.** Membrane elevation with lateral sinus access technique. a) After a midcrestal incision and vertical releasing incisions mucoperiosteal flaps are raised and reflected to access the alveolar bone, b) a rectangular replaceable bone flap created using an oscillating saw under continuous saline irrigation, c) the sinus membrane dissected around the margins of the exposed window and extended inferiorly to expose the floor of the sinus, d) a dental implant, placed in the residual alveolar bone, protruding into the maxillary sinus cavity providing support for the elevated membrane, e) The bone windows repositioned using tissue glue, f) a blood clot formed under the elevated space of the sinus membrane. Finally, the muco-periosteal flap reversed and the incision closed.

Early bone formation in the sinus elevation situation

The mechanism behind the early bone formed after elevating the membrane is not conclusively described in the literature. One question of special interest is the role of the Schneiderian membrane in the early formation of bone under the lifted membrane. Paper IV attempts to further clarify the function and mechanism behind initial bone formation. It has been suggested that sinus-derived osteoprogenitors have an important role in bone formation when using sinus-lifting procedures (Srouji et al. 2008; Srouji et al. 2016). Studies both support and reject the notion that the
sinus membrane has osteoconductive potential. In an in vitro study, Srouji et al. (2010) observed indications of innate osteogenic potential of the maxillary sinus membrane, and thus adding a possible contribution to the bone reformation in sinus lifting procedures. On the other hand, a recent study on primates concluded lack of influence of the Schneiderian membrane in bone formation. Histological assessments have described bone formation at implants starting from the residual bone and extended coronally toward the apex of the implants with the most apical portions of the implants not covered with a bony coating (Scala et al. 2011).

Principles of histology and immunohistochemistry

Histology is the study of the cellular organization and embraces the study of both tissue and cells and the relationship between these structures with respect to physiological function. Many techniques have been developed that are designed to preserve the structural integrity of a specimen so that it can be viewed microscopically. In studies involving soft tissue, hard tissue, and metal components, the most commonly used technique is the embedding in plastic and further cutting and grinding of the preparations to a desired thickness (Donath and Breuner 1982; Gotfredsen et al. 1989). There are five common stages in the preparation of histology slides using the cutting and grinding technique; (i) stabilizing the proteins and nucleic acids by making them insoluble, which can be done by either chemical or by frozen fixation; (ii) processing (dehydration) by removal, for example, of water from the biological samples and adding another substance that may solidify, making the tissue more durable for cutting thin sections; (iii) embedding the tissue samples in a solid material (hard-grade acrylic resin) moulded with liquid-embedding material, which is hardened to a block; (iv) sectioning and grinding of the blocks containing the embedded tissue; and (v) staining with an appropriate histology stain (Donath and Breuner 1982).

To study the early bone formation observed in paper IV, immunohistochemistry (IHC) can be used to identify specific processes on a cellular level and at an early stage before histological signs and changes can be observed on sections. First described by Coons, IHC is a technique to label antibodies with a colour producing substrate that allows researchers to identify antigens in tissue sections (Coons 1954). The basic principle of IHC is the use of enzyme-linked antibodies to detect tissue antigens. A colourless substrate is converted by the enzyme into a collared product that can be identified on the slide at the tissue site of the reaction (Gao and Kahn 2005). With the continued development of IHC techniques, enzyme-labelling, using peroxidase and alkaline phosphatase, has been developed (Nakane and Pierce 1966). Four IHS methods are commonly used: (i) direct method with peroxidase-labelled primary antibody; (ii) indirect method with peroxidase-labelled secondary antibody; (iii) avidin–biotin method (ABC) in which avidin–biotin–peroxidase complex reacts with biotinylated secondary antibody; and (iv) peroxidase anti-peroxidase method in
which peroxidase-antiperoxidase complex reacts with a secondary antibody (same species). In addition to the above methods, multiple staining methods can be used to stain two or more antigens in the same tissue section. Avidin, a large glycoprotein, can be labelled with peroxidase or fluorescein for microscopic visualization and has a very high affinity for biotin. Biotin, a low molecular weight vitamin, can be conjugated to a variety of biological molecules, such as antibodies.

**Figure 3.** Schematic explanation of the immunohistochemical technique using avidin-biotin-peroxidase. a) Application of a primary antibody, binding to a specific epitope in tissue, b) application of a biotinylated secondary antibody, c) applying a complex of avidin–biotin peroxidase. The chromatogen, (DAB) is finally applied to the tissue and incubated with the avidin-biotin-peroxidase complex.

For the experimental study (paper IV), the ABC technique (Lunedo et al. 2012) was used to visualize four different antigens, a method by far the most sensitive and widely used technique for IHC staining (Figure 3). Primary antibodies against osteocalcin (OC), osteopontin (OP), macrophages (MP), and CD68 were used. Because OC is secreted by well-differentiated osteoblast and plays a role in early bone formation as well as binding and regulating calcium, it is used as a measurement of osteogenic maturation (Sato et al. 1998). OP may be an important factor in bone remodeling (Choi et al. 2008) and promote the attachment of osteoblasts to the extracellular matrix (Sodek et al. 2006). CD68 is a marker for cells of the macrophage lineage, including osteoclast, and in studies (Crotti et al. 2004) CD68 has shown to be closely associated with RANKL protein. The IHC technique involves three steps: (i) application of a primary antibody that binds to a specific epitope in tissue; (ii) application of a biotinylated (the process of attaching biotin to a molecule), a secondary antibody; and (iii) application of a complex of avidin–biotin peroxidase. The chromogen dimethyl-aminoazobenzene (DAB) is finally applied to the tissue and incubated with the avidin-biotin-peroxidase complex, producing a brown insoluble stain, permitting visualization in the microscope of the antigen-antibody binding (Murphy et al. 2008).
Aims

General aims

Using radiological, histological, and immuno-histochemical aspects, this study evaluates the healing of endosseous implants with two different surface characteristics in a grafted and non-grafted situation with respect to implant failure, peri-implant health, and soft tissue health. In addition, this study experimentally describes early bone healing after maxillary sinus membrane elevation with and without the use of grafting material.

Specific aims

I. To compare two surfaces of dental implants with respect to implant failure both in one-stage protocol with early functional loading and in two-stage protocol with traditional healing time (paper I).

II. To compare the two implant surfaces with respect to survival rate, marginal bone level, and whether the two surfaces act differently in one stage, two stage, or early loading protocols in the long-term with a minimum loading period of five years (paper II).

III. To evaluate the long-term implant survival rate, peri-implant soft tissue health, marginal and apical bone levels, and intra sinus conditions in patients subjected to sinus elevation procedures with autologous bone graft and delayed placement of implants with two surface characteristics (paper III).

IV. To describe the early bone formation in the maxillary sinus from a histological as well as an immuno-histochemical aspect using lateral access technique and sinus membrane elevation with and without bone graft to determine the role of the sinus membrane in the bone forming process (paper IV).
Material and methods

Clinical studies

Paper I and II

Objectives

Paper I compared two implant types with different surfaces (Nobel Biocare Mark III and TiUnite) with respect to implant failure. Paper II, a follow up study to paper I, intended to compare the clinical performance of turned and oxidized implants after more than five years of loading. Paper I and paper II used the same cohort of patients.

Patients

Paper I and II consisted of 136 consecutive patients treated with Nobel Biocare implants at a private clinic in Umeå, Sweden. These patients received a total of 394 implants – 199 oxidized titanium implants (Nobel Biocare TiUnite) and 195 turned titanium surface implants (Nobel Biocare Mark III). Sixty-three patients underwent a one-stage surgical protocol, and 24 of these object to early functional loading. The remaining 73 patients were treated with a traditional two-stage surgical technique (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Distribution of patients and implants. Paper I.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidized surface</strong></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Full arch</td>
</tr>
<tr>
<td>Partial</td>
</tr>
<tr>
<td>Single</td>
</tr>
<tr>
<td>Early loading</td>
</tr>
<tr>
<td>1-stage</td>
</tr>
<tr>
<td>2-stage</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

Inclusion criteria

All patients planned and scheduled for implant-supported fixed restorations between January 2001 and December 2002 were included in the study.
**Dropouts**

At the five-year follow-up study (paper II), eight patients had deceased and 128 were invited. Twenty-five patients refrained from participating in the study, resulting in a total of 103 patients included (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Oxidized surface</th>
<th>Turned surface</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Implants</td>
<td>Patients</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>82</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>72</td>
<td>29</td>
</tr>
<tr>
<td>Full arch</td>
<td>15</td>
<td>73</td>
<td>16</td>
</tr>
<tr>
<td>Partial</td>
<td>24</td>
<td>62</td>
<td>15</td>
</tr>
<tr>
<td>Single</td>
<td>16</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Early loading</td>
<td>7</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>1-stage</td>
<td>25</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>2-stage</td>
<td>23</td>
<td>80</td>
<td>34</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>55</td>
<td>154</td>
<td>48</td>
</tr>
</tbody>
</table>

**Surgical protocol**

The surgical procedures were performed following the Brånemark system protocol. Altogether, five surgeons placed the implants and strived to achieve high implant stability and a favourable placement for the prosthetic treatment. To achieve a good primary stability of the implants, the final drill diameter was chosen according to bone density. For soft bone, a 2.85-mm final drill was chosen; for dense bone, 3-mm final drill diameter was chosen. The primary implant stability was evaluated by clinical judgment of rotation stability. The patients were treated with a one-stage protocol with the inclusion criteria of good initial primary stability and rotation stability of all the implants and no interference with the temporary prosthesis during healing. All patients who did not fulfil the above criteria for the one-stage protocol were treated with the traditional two-stage protocol. In the group of 63 patients treated using the one-stage protocol, 24 patients were objects for early functional loading of the implants, all placed in the anterior totally edentulous mandible, with a mean implant healing time of 25 days. All other patients had a mean healing time of 17 weeks before loading the implants. The healing time was sometimes longer than planned because of patient logistics. In all patients, healing abutments were used during the healing time. In the edentulous mandibles, final abutments were inserted at the time of implant surgery.

**Prosthetic protocol**

Follow-up visits were performed one to two weeks after surgery. Sutures were removed and the patients received their temporary prosthesis and bridges after
adjustments. Patients undergoing the one-stage early functional loading protocol received their permanent fixed bridges after a mean time of 25 days. In the 112 remaining patients, the healing abutments were removed and replaced with the intended final abutments at the time of final impression. Patients then received a rigid fixed replacement.

**Survival and failure criteria**

Implants were classified as survivals when clinically stable and fulfilling purported function without any discomfort to the patient and with no clinical or radiological signs of infection or on-going pathologic process. All other implants were classified as failures (Roos et al. 1997).

**Clinical and radiographic examinations**

After more than five years of function, two clinicians clinically and radiographically examined the patients. A standardized protocol was used and comprised the following parameters; (i) bleeding on probing (BoP) at mesial and distal aspects of each implant (0 = no bleeding, 1 = bleeding at one surface, and 2 = bleeding at two surfaces), (ii) pocket depth (PD) in millimetres at distal and mesial aspects of each implant, and (iii) marginal bone level (MBL) in intraoral radiographs. The distance from the implant platform to the first bone contact was measured in tenth of millimetres at distal and mesial aspects of each implant using computer-based software. Each radiograph was calibrated using the known length of the implant as reference (Figure 4), and a mean value was calculated for each implant.

![Figure 4. Calibration and measurements on the distal and mesial aspect of the implant.](image)

**Statistics**

One-way ANOVA tests were used to compare turned and oxidized implants and loading protocols. A statistically significant difference was considered if p value ≤ 0.05.
Paper III

Objectives

Paper III compared the healing of endosseous implants with different surface characteristics in patients with sinus elevation procedures, autologous bone graft, and delayed implant installation. Implant survival, peri-implant soft tissue conditions, marginal bone level, intra-sinus apical bone level, and sinus health were studied.

Patients

Twenty-eight patients, all with remaining anterior maxillary dentition, were treated with a total of 92 dental implants (47 turned surfaced implants and 45 oxidized surface implants). Nine of the patients were treated in the right maxilla and sixteen in the left maxilla. The remaining three patients were augmented bilaterally before receiving implants. The autogenous bone was harvested from the mandibular symphysis in five patients, from mandibular symphysis and ascending mandibular ramus in three patients, and from the ascending ramus alone in the remaining twenty patients. None of the patients were smokers. All patients were treated at the Department of Oral and Maxillofacial Surgery, Umeå University Hospital, Umeå, Sweden, by the same surgeon (Table 3).

<table>
<thead>
<tr>
<th></th>
<th>Oxidized surface</th>
<th>Turned surface</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Implants</td>
<td>Patients</td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Right maxilla</td>
<td>5</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Left maxilla</td>
<td>10</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>Bilateral maxilla</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Mandibular symphysis</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Mandibular symphysis + Ramus</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ramus mandibulae</td>
<td>15</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>45</td>
<td>13</td>
</tr>
</tbody>
</table>

Inclusion criteria

Among patients treated with implant rehabilitation and sinus grafting of the atrophic posterior maxilla, two patient groups of the same size were established with a minimal follow-up of five years after loading of the implants. The two groups differed only in the surface characteristics of the implants used. Although 35 patients were identified, only 28 accepted to participate in the follow-up study.
**Surgical protocol**

After a mid-crestal incision and vertical releasing incisions, muco-periosteal flaps were raised and reflected at the edentulous posterior maxilla to access the alveolar bone. The lateral wall of the maxillary sinus was fully exposed and a window in the bone was created using a round burr or a reciprocating saw under continuous saline irrigation. The osseous window was freed by fracturing along the osteotomy lines, which were removed and kept in saline solution. Then the sinus membrane was carefully elevated with specially designed elevators. The bone graft from the chin was retrieved as described by Lundgren and co-workers (Lundgren et al. 1996). For a graft from the ascending ramus, the bone was harvested from the junction between the ascending ramus and the external oblique ridge (Lundgren and Sennerby 2008). The harvested bone was then particulated with a surgical bone mill and placed under the elevated sinus membrane. Next, the osseous window was repositioned, the muco-periosteal flap replaced, and the incision closed. The mean healing period between the bone transplantation and placement of implants was 28 weeks. The procedures of the implant surgery were performed following the Brånemark system protocol. To achieve a good primary stability of the implants, the final drill diameter was chosen according to the bone density. To estimate the height of the transplanted bone (i.e., the available bone in the planned implant site), the implant site was prepared by drilling through the bone into the sinus cavity with a 2-mm diameter twist drill. From the available bone, a suitable implant length was chosen. To use the full bone height, an implant with a corresponding length or slightly longer was selected, resulting in a penetration of the implant through the graft in most cases. For example, if the available bone height was 14 mm, a 15-mm implant was selected. If the bone were soft, a 2.85-mm diameter final drill was used. If the bone were dense, a 3.0-mm final drill was used. All the patients underwent a traditional two-stage protocol with a secondary surgical abutment surgery after a mean implant healing time of 20 weeks.

**Clinical and radiographic examinations**

After a minimum of five years of functional loading (mean ten years and range 5 - 19 years), all patients were scheduled for a follow-up visit at the clinic. One clinician, using a standardized protocol, performed a clinical examination to determine PD, BoP, MBL in intraoral radiographs. Smoking habits were recorded. A CBCT-examination with a volume of 10 x 5 cm was used to evaluate the apical conditions and bone coverage of the implants in the maxillary sinus. The CBCT images were evaluated using radiological software. The apical bone level (APL) surrounding the implants was measured from the most apical part of the implant on the anterior, posterior, medial, and lateral aspect on each implant (Figure 5a-b). The thickness of the sinus membrane was measured on reformatted CBCT images where the mean value of the membrane was measured at its thinnest and thickest points with tenths of a millimetre of accuracy.
Figure 5a-b. The apical bone level (APL), surrounding the implants was measured from the most apical part of the implant on the anterior-posterior (a), and medial-lateral (b) aspect, of each implant.

Ethical principles
The study was approved by the regional ethical review board in Umeå (DNR 2010-244-31M)

Statistics
The data were first analysed descriptively, and between-group differences in measurements were compared using parametric methods, including t-tests and Kruskal-Wallis 1-way ANOVA tests for comparisons between the groups of turned and oxidized implants. A statistically significant difference was considered if the p value ≤ 0.05.
Experimental study

Paper IV

Objectives

The aim of paper IV was to histologically and immuno-histochemically study the early bone formation events after membrane elevation in the maxillary sinus.

Experimental animals

Nine adult male tufted capuchin primates (Cebus apella) weighing between 2500g and 3050g were included in the study. The first, second, and third maxillary premolar were bilaterally extracted four months before the surgery, allowing for the healing of the dental socket.

Surgeries

Eight animals were subject to bilateral sinus membrane lifting using a lateral sinus access technique (Figure 6a-f).

Figure 6a-f. Surgical procedure of the experimental animals. a) mucoperiosteal flap raised and reflected on the posterior maxilla to access the alveolar bone, b) a rectangular replaceable bone flap was created using an oscillating saw, c) the bone window was detached and the sinus membrane was dissected around the margins of the exposed window, d) harvesting of particulated bone graft from the proximal tibia, e) one oxidized TiUnite implant, 3.75 mm wide and 8.5 mm long was placed in the residual alveolar bone, protruding into the maxillary sinus cavity, f) the bone window was repositioned using tissue glue, and cover screws placed.
In two of the animals in each time sequence, a total of four animals, one oxidized TiUnite implant, 3.75-mm wide and 8.5-mm long, was placed bilaterally in the residual alveolar bone and protruding into the maxillary sinus cavity. The right maxillary sinus in each animal was prepared with an additional bone graft harvested with a bone scraper from the surface of the proximal tibia and placed under the elevated membrane surrounding the implant, while the left side in the animals was left without any bone grafting material. In one animal in each time sequence, a total of two animals, the implants were inserted under the elevated sinus membrane bilaterally without any grafting material. In one animal in each time sequence, a total of two animals, the sinus membrane covering the implants was totally removed bilaterally (Table 4a).

<table>
<thead>
<tr>
<th>Primate</th>
<th>Time</th>
<th>Fixation procedure</th>
<th>Surgical protocol</th>
<th>Fixation procedure</th>
<th>Surgical protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left sinus</td>
<td>Right sinus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10 days</td>
<td>Immunohisto</td>
<td>Elevation and bonegraft</td>
<td>Immunohisto</td>
<td>Elevation</td>
</tr>
<tr>
<td>2</td>
<td>10 days</td>
<td>Histology</td>
<td>Elevation and bonegraft</td>
<td>Histology</td>
<td>Elevation</td>
</tr>
<tr>
<td>3</td>
<td>10 days</td>
<td>Immunohisto</td>
<td>Elevation</td>
<td>Histology</td>
<td>Elevation</td>
</tr>
<tr>
<td>4</td>
<td>10 days</td>
<td>Immunohisto</td>
<td>Removal of membrane</td>
<td>Histology</td>
<td>Removal of membrane</td>
</tr>
<tr>
<td>5</td>
<td>45 days</td>
<td>Immunohisto</td>
<td>Elevation and bonegraft</td>
<td>Immunohisto</td>
<td>Elevation</td>
</tr>
<tr>
<td>6</td>
<td>45 days</td>
<td>Histology</td>
<td>Elevation and bonegraft</td>
<td>Histology</td>
<td>Elevation</td>
</tr>
<tr>
<td>7</td>
<td>45 days</td>
<td>Immunohisto</td>
<td>Elevation</td>
<td>Histology</td>
<td>Elevation</td>
</tr>
<tr>
<td>8</td>
<td>45 days</td>
<td>Immunohisto</td>
<td>Removal of membrane</td>
<td>Histology</td>
<td>Removal of membrane</td>
</tr>
</tbody>
</table>

**Fixation procedures**

Four animals were sacrificed after ten days and four after 45 days. The primates were deeply anesthetized with Penthotal sodium and perfused via the ascending aorta with 0.9% saline at a pH of 7.0, followed by 2000ml of 4 % paraformaldehyde solution in 0.1M sodium acetate buffer, and finally with 2000ml of 4 % paraformaldehyde solution in 0.1M sodium borate buffer. From four animals in each time sequence, specimen were embedded in methyl methacrylate for processing decalcified sections. In these specimens, the histological and histomorphometrical analyses were evaluated. From the other four animals in each time sequence, the specimen were fixed in formaldehyde (4 %) and decalcified in EDTA (0.1M) until soft enough for sectioning. Next, the specimens were prepared for immunohisto-chemical staining. As the maxillary sinuses from the same species served as controls, they were cut and stained in the same way as the test specimens.

**Histology**

The preparation of the specimens for histological examination followed the protocol by Palma and co-workers. The specimens were dehydrated in a series of ethanol, embedded in hard-grade acrylic resin, and polymerized in a dry heat oven at 60°C.
The plastic blocks were mounted on slides, sawn to about 40-µm thick, ground manually to about 15-µm thick, and stained with toluidine blue/pyronin Y (Palma et al. 2006).

**Immunohistochemistry**

The specimens were embedded in paraffin, sectioned (horizontally and serially) in 5-µm sections, and collected on Superfrost Plus slides. Then the sections were deparaffinized in xylene and rehydrated in alcohol in decreasing concentrations. The sections were incubated for 90 minutes in 10 % horse serum and then incubated in a humidity chamber (4°C) overnight with primary anti-bodies against OC, OP, MP, and CD68, diluted 1:100 aside from MP which were diluted 1:10 (Table 4b). Primary antibody was not added to some sections as they served as negative controls for the immunohisto-chemistry procedure. After rinsing in PBS, the endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide. After another PBS rinsing, biotinylated secondary antibody (1:100) and anti-rabbit IgG incubated for 90 min were applied and these samples were again rinsed in PBS. The Avidin-biotin complex was added and the specimens were incubated for 30 min in darkness. Finally, the specimen was treated for two minutes with a DAB-Peroxidase substrate kit (Dimethyl-aminoazobenzene and 30 % hydrogen peroxide). The slides were stained with Mayers Hematoxylin, rinsed in tap water, and then analysed in a light microscope by two investigators on two separate occasions and finally compared on a third occasion.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Directed against</th>
<th>Catalog #</th>
<th>Host</th>
<th>Type</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>Osteocalcin (BGLAP)</td>
<td>ABIN105153</td>
<td>Rabbit</td>
<td>Polyclonal</td>
<td>1:100</td>
<td>Antibodies-online GmbH, Aachen, Germany</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Osteopontin</td>
<td>SAB4200018</td>
<td>Mouse</td>
<td>Monoclonal</td>
<td>1:100</td>
<td>Sigma-Aldrich Inc. St. Louis, MO, USA</td>
</tr>
<tr>
<td>Macrophage</td>
<td>Macrophage</td>
<td>LS-C124022</td>
<td>Mouse</td>
<td>Monoclonal</td>
<td>1:10</td>
<td>LifeSpan BioScience Inc, Seattle, WA, USA</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophage</td>
<td>M0876</td>
<td>Mouse</td>
<td>Monoclonal</td>
<td>1:100</td>
<td>DakoCytomation Denmark A/S, Glostrup, Denmark</td>
</tr>
</tbody>
</table>

**Ethical principles**

This animal study was carried out in accordance with the rules of the Brazilian Institute for Protection of the Environment and was approved by the Animal Ethics Committee at the Faculty of Dentistry of the University of the State of Sao Paulo – UNESP, Aracatuba, Brazil 2009 (FOA 087/95).
Results

Clinical studies

Paper I and II

Implant failure

A total of eight implant failures – seven with turned surfaces and one with an oxidized surface – were registered in six patients (three male and three female) from placement to the last follow-up. The overall cumulative survival rates were 94.7% for turned and 99.4% for oxidized implants. All turned surface implants – six in the maxilla and one in the mandible – were inserted following a two-stage protocol and were lost during the first year of function. Two of the failed implants were classified as early failures (i.e., before loading) and the remaining five implants were classified as late failures (i.e., after a mean loading time of 23 weeks). One maxillary oxidized implant inserted following a one-stage protocol was lost after four years of loading due to infection and marginal bone loss (Table 5).

Table 5. Specification of lost implants. Paper I and II

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (y)</th>
<th>Surface</th>
<th>Position</th>
<th>N implants lost</th>
<th>Length (mm)</th>
<th>Time of loss (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>76</td>
<td>Turned</td>
<td>21</td>
<td>1</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>61</td>
<td>Turned</td>
<td>25</td>
<td>1</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>Turned</td>
<td>12,11,22</td>
<td>3</td>
<td>15,15,13</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>Turned</td>
<td>44</td>
<td>1</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>Turned</td>
<td>25</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>66</td>
<td>Oxidized</td>
<td>15</td>
<td>1</td>
<td>13</td>
<td>51</td>
</tr>
</tbody>
</table>

Soft tissue health

BoP was recorded in 28 out of 133 turned implants (21%) and in 34 out of 154 oxidized implants (22%). There were no differences for BoP scores (0.5 ± 0.7 vs. 0.4 ± 0.6) and PD measurements (1.7 ± 0.8 vs. 1.8 ± 1.0 mm) when comparing turned and oxidized implants, respectively (Table 6). Neither were there any differences when comparing BoP scores and PD measurements between the different loading protocols. Four implants (1.4%), three oxidized and one turned, showed PD > 3 mm, MBL > 4 mm, and BoP. On examination, however, none of these were associated with suppuration.

Marginal bone level

After more than five years of function, the mean MBL was positioned 1.8 ± 0.8 and 2.0 ± 0.9 mm below the implant abutment junction for turned and oxidized implants,
respectively (Table 6). The difference was not statistically significant. Frequency distribution of marginal bone loss showed no statistically significant differences between the two surfaces, although more oxidized implants presented a MBL of more than 3 mm below the implant abutment junction (Figure 7). There was no correlation between BoP and MBL. Smokers with oxidized implants showed significantly more bone loss than non-smokers (p = .046). No differences were seen between smokers and non-smokers in the turned implant group. No differences in MBL could be seen between the loading protocols (Table 6).

![Figure 7. Frequency distribution of marginal bone level between the two surfaces.](image)

<table>
<thead>
<tr>
<th>Type and location</th>
<th>Oxidized surface</th>
<th>Turned surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginal bone level (mm)</td>
<td>2.0 ± 0.9</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Bleeding on probing (index)</td>
<td>0.4 ± 0.6</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>1.8 ± 1.0</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>Marginal bone level in relation to location (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full arch</td>
<td>2.2 ± 1.0</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Partial</td>
<td>2.2 ± 0.9</td>
<td>2.1 ± 1.2</td>
</tr>
<tr>
<td>Single</td>
<td>1.5 ± 0.6</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Marginal bone level in relation to loading protocol (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early loading</td>
<td>2.0 ± 0.5</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>1-stage</td>
<td>1.8 ± 1.0</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>2-stage</td>
<td>2.3 ± 0.9</td>
<td>1.8 ± 0.9</td>
</tr>
</tbody>
</table>

Table 6. Descriptives of marginal bone level in relation to surfaces. Paper II. (5-year follow up)
Paper III

Implant failure

A total of three implant failures (two turned and one oxidized) were registered in three patients (one male and two females). The overall cumulative survival rates were 95.7% for turned and 97.7% for oxidized implants, compared to 96.7% for the whole group (Table 7).

Table 7. Specification of lost implants. Paper III

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (years)</th>
<th>Surface</th>
<th>Position</th>
<th>Length (mm)</th>
<th>Donor site</th>
<th>Time of loss (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>62</td>
<td>Turned</td>
<td>24</td>
<td>13</td>
<td>Mandibular symphysis</td>
<td>158</td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>Turned</td>
<td>14</td>
<td>15</td>
<td>Ramus mandibulae</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>Oxidized</td>
<td>25</td>
<td>10</td>
<td>Ramus mandibulae</td>
<td>23</td>
</tr>
</tbody>
</table>

Peri-implant soft tissue health

Bleeding on probing (BoP) was recorded in 14 out of 47 turned implants (30 %) and in 10 out of 45 oxidized implants (22 %). There were no differences for BoP scores (0.4 ± 0.4 vs. 0.4 ± 0.6) and pocket depth (PD) measurements (2.3 ± 0.6 vs. 2.0 ± 0.8 mm) when comparing turned and oxidized implants (Table 8).

Marginal bone level

The mean MBL was positioned 1.4 ± 0.7 and 1.7 ± 0.7 mm below the implant abutment junction for turned and oxidized implants after more than five years of function (Table 8). The difference was not statistically significant.

Apical bone level

The mean apical bone level (ABL) was 1.2 ± 0.9 mm for the turned surface implants and 0.9 ± 0.8 mm for oxidized implants, with no significant differences (Table 8). In each of the implants, the corresponding ABL and MBL often showed a similar level. If the MBL values were small, the same values were found for ABL and vice versa.

Table 8. Marginal bone level, bleeding on probing, pocket depth and apical bone level in relation to surfaces.

<table>
<thead>
<tr>
<th></th>
<th>Turned surface</th>
<th>Oxidized surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginal bone level (mm)</td>
<td>Mean 1.4 SD 0.7 Min 0.6 Max 3.2</td>
<td>Mean 1.7 SD 0.7 Min 0.5 Max 3.1</td>
</tr>
<tr>
<td>Bleeding on probing (index)</td>
<td>0.4 Mean 0.4 SD 0.4 Min 0</td>
<td>0.6 Mean 0.4 SD 0.4 Max 1.5</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>2.3 Mean 2.3 SD 0.6 Min 1.3 Max 3.3</td>
<td>2.0 Mean 2.0 SD 0.8 Min 0.2 Max 2.4</td>
</tr>
<tr>
<td>Apical bone level (mm)</td>
<td>1.2 Mean 1.2 SD 0.9 Min 0.3 Max 3.5</td>
<td>0.9 Mean 0.9 SD 0.8 Min 0 Max 2.4</td>
</tr>
</tbody>
</table>
**Sinus conditions**

Thickness and anatomic characteristics of the sinus mucosa were recorded in the CBCT images. The thickness of the sinus membrane was 0.6 ± 1.3 mm for turned implants and 0.6 ± 0.7 mm for oxidized implants. The impact on the sinus membrane thickness, by different donor sites used for the bone grafting, exhibited the following overall measurements: 1.2 ± 1.9 mm in the symphysis only group; 0.2 ± 0.4 mm for bone grafts from both the symphysis and the ascending ramus groups; and 0.5 ± 0.7 mm for the patients with grafts from only the mandibular ramus. The differences were not statistically significant (Table 9). Using the classification described and modified by Schneider (Schneider et al. 2013), we found that four of the patients (14%) showed radiographic signs of sinus pathology. None of the four patients reported discomfort or awareness of the above-described conditions. Patient number one had a complete opacification and was one of the patients who lost an implant. Patient number four had undergone periodontal surgery/cleaning of the implant in position 26, and showed the highest value of the apical bone level (penetration of the implants into sinus without bone cover) with a mean of 3.2 mm. A correlation or higher levels of MBL or ABL was not seen in the other three patients.

<table>
<thead>
<tr>
<th>Table 9. Thickness of the sinus membrane (mm) in relation to surfaces and donor site of the bonegrafts.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidized surface</strong></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Mandibular symphysis</td>
</tr>
<tr>
<td>Mandibular symphysis + Ramus</td>
</tr>
<tr>
<td>Ramus mandibulae</td>
</tr>
</tbody>
</table>
Experimental study

Paper IV

*Histology of ground sections*

A typical ground section of the experimental area comprised the implant, which penetrated a thin marginal bone with most of the threaded part protruding into the sinus cavity. In some specimens, vertical cortical septa were present close to one of the implant sides. The sinus cavity of the experimental animals showed, in addition to the presence of vertical bone septa, a relatively narrow width of the palatal-lateral aspect, however, with favourable vertical space. Therefore, the placement of the implants resulted in a very steep angle of the elevated sinus membrane, and the following bone formation was almost pyramidal, with a pointed appearance along the implant surface (*Figure 8a-c*). For conformity of results, the findings from the side without near septa were used. A sinus membrane and various amounts of granulation tissue, bone, and bone marrow tissues were observed in this area depending on treatment and time of follow-up.

*Figure 8a-c.* Appearance and anatomy of the sinus cavity and sinus membrane in the experimental animals. a) Steep angle of the elevated sinus membrane, b) blood clot formed in the relatively small space, under the elevated membrane, c) pyramidal, pointed appearance of the bone formation along the implant surface.

*Sinus membrane elevation*

*10-day specimens.* A successful elevation of the sinus membrane formed a secluded area bordered by the implant, the endosteal surface of the marginal bone, and the sinus membrane on at least one side of the implant (*Figure 9a*). The secluded area was mainly filled with granulation tissue and bone fragments. Bone formation was seen close to the endosteal surface near the implant and sprouting into the granulation tissue (*Figure 9b*). In higher magnification, osteoblasts could be observed forming mineralized tissue at existing bone and bone fragments and near vessels as solitary islets (*Figure 9c*). No bone forming activities could be seen at the elevated sinus membrane near the top of the implant (*Figure 10a*). However, solitary
bone formation could be seen in the distal parts of the specimen where the sinus membrane had been separated from the endosteal bone surface (Figure 10b). Osteoblastic activity was also observed at the denuded endosteal bone surface. New bone formation was seen directly on the implant surface in the threads located in and just above the marginal bone (Figure 9b).

Figure 9a-c. Light micrograph of a specimen 10 days after membrane elevation. a) The implant (i) is penetrating marginal bone and protruding into the sinus cavity (SC). The region of interest (ROI) of the study is bordered by the implant, sinus membrane and marginal bone. Arrows = sinus membrane. b) Close up of figure 1a, new bone formation (dark blue) is seen at existing bone (B), bone fragments (BF) and as solitary islets (arrows pointing to some) into the granulation tissue (GT). Bone is formed at the implant (I) surface. c) Higher magnification of figure 1b, showing islets of osteoblasts and bone formation (arrows) near vessels (V).

Figure 10a-b. Details of specimen taken 10 days after membrane elevation. a) Sinus membrane (arrow) at the most apical implant thread (i). There are no signs of bone formation neither near the membrane nor in the granulation tissue (GT). b) Solitary bone formation (arrows) between the elevated sinus membrane (SM) and the marginal bone (MB). New bone formation (dark blue) is seen on the endosteal surface of the marginal bone.

45-day specimens. Well-developed bone and marrow tissues in the elevated space were observed (Figure 11a). The centre of the specimen included slender bone trabeculae, loose connective tissue, and fat cells. Newly formed bone lined the sinus membrane. The area between the respiratory epithelium, facing the sinus cavity, and the bone lining contained darkly stained glandular tissue (Figure 11b). Bone also
contacted the implant surface in the apical area. However, the tip of the implant was in contact with a dense fibrous tissue and finally with only the sinus membrane.

![Figure 11a-b](image)

**Figure 11a-b.** Light micrograph of a ground section 45 days after membrane elevation. a) New trabecular bone (arrows) and loose connective tissue (LCT) with fat cells is filling the area under the elevated sinus membrane. The new bone is following the contour of the sinus membrane (SM). I = implant, MB = marginal bone. b) Detail of figure 3a showing the relation between the sinus membrane (SM) and newly formed bone (B) and bone marrow (BM). Darkly stained glandular tissue is observed beneath the membrane.

**Removal of membrane**

**10-days specimens.** The specimen showed a similar morphology as the sinus elevation specimens but without a visible sinus membrane. Thus, granulation tissue filled most of the area lateral to the implant, and bone formation was seen at the endosteal bone and implant surface.

**45-days specimens.** A new sinus membrane lined the implant. Only a small area near the endosteal surface and coronal threads were filled with new bone. The sinus membrane touched the tip of the more apical threads and the top of the implant.

**Sinus membrane elevation and bone graft**

**10-days specimens.** Bone graft particles in a granulation tissue filled the area beneath the elevated membrane. Bone formation was seen at the endosteal bone and implant surface as in the other groups. No bone forming or resorptive activities could be observed at the grafted bone particles.

**45-days specimens.** A similar morphology as in the membrane elevation group was seen. However, in these specimens more dense fibrous tissues were observed near and at the apical part of the implant. Moreover, marginal bone resorption had occurred, so the area with newly formed bone and marrow tissue was small.
Immuno-histochemical observations

Osteocalcin (OC)

OC was clearly expressed in both the 10-day and 45-day specimens. A more substantial staining of the bone was observed in elevation and elevation + bone graft specimens, compared to the removed membrane specimens. Generally, the presence of OC was stronger in the 45-day group.

10-day specimens. OC was mainly seen in endothelial cells in the superficial part of the lamina propria as well as in the stratified epithelial layer (Figure 12a). Strong staining of the bone was found in elevation and elevation + bone graft specimens, whereas weak staining was seen for removed membrane specimens.

45-day specimens. Except for the stratified epithelium, no staining of the lamina propria was seen. A more pronounced expression was observed in the deeper part of the residual bone (Figure 12b-c). The presence of stained osteocyte lacunae and bone marrow and the presence of lymphatic plaques were consistent for all three surgical interventions. The negative control showed no staining. In the pristine tissue sections, a small number of cells in the bone exhibited uptake of OC.

![Figure 12a-c](image)

**Figure 12a-c.** Osteocalcin (OC) staining. a) Expression of OC (arrows) of endothelial cells in the superficial part of the Lamina propria (LP) as well as the stratified epithelium (10-day, removal of membrane). b-c) OC staining (arrows) is not present in the Lamina propria, except for the stratified epithelium, but a more pronounced expression in the deeper part of the residual bone (RB). b) 45-day removal of membrane and c) 45-day elevation with bonegraft.

Osteopontin (OP)

10-day specimens. A strong expression of OP was seen in all 10-day specimens. Glands in the lamina propria and the periosteum-like membrane showed a marked uptake (Figure 13a). The expression of OP seemed to increase close to where the implant was placed.

45-day specimens. The expression and uptake of OP was stronger and more distinct in the 45-day specimens compared to the 10-day specimens for all surgical techniques. Glands in the lamina propria showed a marked staining adjacent to the implant site (Figure 13b).
In other locations in the same slide not adjacent to the implant (e.g., the non-implant site), no uptake in the glands or elsewhere was seen (Figure 13c). The negative control showed no staining. The pristine tissue sections expressed few solitary cells within the lamina propria with uptake of OP and exhibited a significantly thinner lamina propria (Figure 14a).

**Macrophages (MP)**

A discreet expression of single isolated cells was seen in the 10-day specimens. Isolated cells were seen interspersed in the lamina propria and in the underlying bone (Figure 14b). No staining was detected in the 45-day group or the negative control. Interestingly, the pristine tissue specimens from the non-operated animal showed a picture of local MP accumulation with secretion, a relatively strong MP staining of the epithelium that indicates the presence of a chronic sinusitis.

**CD68**

CD68 was expressed in a few cells of the lamina propria in the 10-day specimens (elevation protocol) and in the residual bone in the 45-day specimens (elevation with bone graft and removal of the membrane) (Figure 14c). The negative control and the pristine tissue specimens expressed no staining.
Discussion

**Clinical studies**

The present thesis has several methodological limitations that should be noted. *Paper I* is a follow-up study that evaluated implant survival rate after at least five months of functional loading (according to medical records). At the time for the evaluation, no clinical examinations were performed. Implants were classified as survivals when clinically stable and fulfilling purported function without any discomfort to the patient and with no signs of infection or on-going pathologic processes. Implant survival rate in *paper I* as well as the cumulative survival rate (CSR) after a minimum five years of functional loading in *paper II* were both high, with few lost implants, and exhibited no significant differences. In *paper I*, seven implants – six in the maxilla and one in the mandible – were lost in five patients (two males and three females). All failed implants were turned implants inserted following the traditional two-stage protocol. The implant survival rate for the whole-patient group was 98.2%: a 100 % survival rate for oxidized surface and a 96.4% survival rate for turned surface. At the five-year follow-up (*paper II*), an additional implant (a maxillary oxidized implant inserted following a one-stage protocol) was lost after four years of loading due to infection and marginal bone loss. The cumulative survival rate after five-years of loading was 94.7% for turned and 99.4% for oxidized implants, results that correspond with other recent studies. For example, in a ten-year follow-up study on immediately loaded TiUnite implants, Degidi and co-workers followed 59 patients with a total of 210 oxidized implants placed in both postextractive and healed sites. The implants placed in healed and postextractive sites achieved a 98.05% and a 96.52% cumulative survival rate, respectively (Degidi et al. 2012). The authors further reported an accumulated mean marginal bone loss (MBLs) and probing depth (PD) of 1.93 mm and 2.54 mm for the implants placed in healed sites and 1.98 mm and 2.63 mm for the implants placed in postextractive sites. Rocci and co-workers reported similar results. In a randomized open-ended nine-year follow-up study, 44 patients received 66 oxidized (TiUnite) and 55 machined implants, which were all immediately loaded. Very similar to *paper II*, the authors reported a failure of three oxidized and eight machined implants, resulting in a cumulative survival rate of 95.5% (oxidized) and 85.5% (eight machined) after nine years of loading. MBLs in the first year were 0.9 mm (oxidized) and 1.0 mm (machined); at the third year, MBLs were 0.4 (oxidized) and 0.5 mm (machined). After nine years, there was negligible further loss in height. Furthermore, the number of failed implants in the machined group was significantly higher in smokers and in sites with poor bone quality, findings not seen in oxidized implants (Rocci et al. 2013). The findings on smoking in relation to the surface, however, is in direct contrast to *paper II*, where smoking did not affect marginal bone level (MBL) at turned implants. In the oxidized group, however, there was a significant difference between smokers and non-smokers (p = .046): smokers had a lower bone level (mean difference -0.6 mm). Shibli and co-
workers showed similar findings, indicating that smoking had negative effects on early bone tissue responses to oxidized implants as assessed by histomorphometry, suggesting a slower wound repair (Shibli et al. 2010). These findings may be partly due to the relatively small study group used in the studies.

With the introduction of modified implant surfaces, experimental (Albouy et al. 2008; Albouy et al. 2009) and clinical studies (Albrektsson et al. 2007) reported extensive marginal bone loss in conjunction with modified implant surfaces. However, a large number of follow-up studies have shown good conservation of the marginal tissue with no apparent differences compared with turned surfaces (Brechter et al. 2005; Friberg et al. 2005; Friberg and Jemt 2010; Finne et al. 2012; Schliephake et al. 2012). Modified implant surfaces integrate significantly faster (Wennerberg et al. 1995) and with higher survival rates and more predictable osseointegration (Jemt et al. 2011). In a recent five-year follow-up study by Friberg and Jemt investigated the clinical outcome between two implant surfaces in relation to different surgical protocols in the edentulous mandible. Four groups were used: (i) two-stage surgical protocol with 338 turned Brånemark implants; (ii) one-stage surgery with 750 turned Brånemark implants; (iii) one-stage surgery with five implants placed in the mandibular intra-foraminal region (450 oxidized TiUnite implants); and (iv) one-stage surgery with four implants placed in the mandibular symphyseal region (300 oxidized TiUnite implants). In one-stage surgery, significantly more turned surface implants were lost, as more patients showed bone loss >1.8mm in the midline when using oxidized implants and especially in one-stage surgery. Complete failures (2.1%) and obvious more bone loss at several implants were mostly observed in younger patients (Friberg and Jemt 2013), survival rates confirmed by the findings in the present thesis. In papers I and II, failure rates for implants with turned surfaces (seven implants) were higher than in similar situations with an oxidized surface (one implant) after at least five years of functional loading. However, there were no statistical differences between the two surfaces. This result could be explained by the fact that rougher oxidized surfaces actually have better surface characteristics in relation to long-term survival rate versus the turned surface or that the relatively small size of the study group and the number implants included does not reflect a true statistical difference. That is, we conducted a retrospective power calculation using the small clinical differences between the two surfaces in relation to survival rate, MBL, PD, and BoP (as the actual results of paper I and II demonstrate). The results show that the groups should be twice as large before statistical difference without fail could be detected within a 95% confidence interval.

As with many retrospective reports, another obvious weakness to be discussed in paper II is the lack of baseline radiographs, which made it impossible to calculate marginal bone loss (MBLs). Instead, the analysis was based on the position of the MBL in the last follow-up radiograph. If all implants were fully submerged in bone at time of their placement, the baseline level would theoretically have been level with
the prosthetic platform and bone level data from the follow-up would reflect true marginal bone loss. However, some implants were probably not fully submerged; for example, in thin marginal bone, bone loss would be less than what the bone level data would suggest. Thus, one drawback is that bone level data cannot be compared with marginal bone loss data published in other studies. Nevertheless, MBL data can be looked on as a worst-case scenario. Regarding differences in loading protocols and particularly early functional loading, all implants included in papers I and II were placed in the edentulous mandible with no implant failures within the group. To achieve significant differences in terms of survival rates and clinical parameters between the surfaces and loading protocols used, a much larger group is needed as the bone quality and quantity is especially favourable in the area between the mental foramina and generally high cumulative implants survival rates are commonly seen (Eliasson et al. 2009; Vervaeke et al. 2013). In summary, many studies, including studies that this thesis is based on, show a numerical trend that dental implants with oxidized surfaces, compared to turned surface implants, would result in increased long-term cumulative survival rate. However, many studies cannot detect statistical differences, probably due to relatively small sample groups, in relation to differences found.

After studying the outcome of two surface characteristics in non-grafted situations in the first two clinical studies, a natural corollary was to investigate the outcome of the two implant surfaces in the grafted situation. Paper III compared the healing of dental implants with different surface characteristics in sinus elevation procedures, autologous particulated bone grafts and delayed implant installation, from a retrospective aspect with an additional tool, cone beam tomography (CBCT). Although many studies, even in immediate loading conditions (Lin et al. 2011; Cricchio et al. 2013; Johansson et al. 2013), have shown good predictable results using mere sinus elevation without additional bone substitutes or bone grafts, it is of great interest to study the outcome of different surface characteristics in terms of clinical survival rate, peri-implant soft tissue conditions, marginal bone level, intrasinus apical bone level, and sinus health in a long-term follow-up study. The overall cumulative survival rates were 95.7% for turned and 97.7% for oxidized implants compared to 96.7% for the whole group, results consistent with previous studies (Wallace and Froum 2003; Yamamichi et al. 2008; Cho-Lee et al. 2010; Schmitt et al. 2012). Of a total of 96 implants, two implants with turned surface and one with oxidized surface were lost. Thus, a pattern regarding implant survival rate, MBL, PoB, and PD is found, which is also consistent with the results from the non-grafted situations in paper I and II. To measure and describe the apical bone level (APL) and sinus health, we used a CBCT to describe the intra sinus conditions from a three-dimensional perspective and with a relatively small dose of radiation (Gupta and Ali 2013). After a minimum of five years of functional loading, none of the patients described subjective signs or symptoms of pathology in the maxillary sinus. Four out of 28 patients (14%) showed radiographic signs of sinus pathology, although with no
suppurative infections, according to the classification described and modified by Schneider (Schneider et al. 2013).

Our results also agreed with Kim (Kim et al. 2013) and Lee's findings (Lee et al. 2013). Using a lateral window approach, Lee evaluated 86 patients (a total of 151 implants) with 100 consecutive sinus elevation procedures and alloplastic grafting. The one-year implant survival rate was 98.7% (149 of 151 implants). One patient exhibited an active sinus infection one week after augmentation, so the infected graft material was removed. Eight patients showed some degree of wound infection up to two weeks after surgical procedures and were all successfully treated with antibiotics, results that corresponded with the results in paper III. In paper III the apical bone level (APL) was evaluated by radiological measurements on four sides of the implant, and thus describes the amount of the implant not having bone coverage and hence protruding into the sinus cavity. Of all 92 implants installed, 37 implants (19 turned surface and 18 oxidized surface) showed an ABL measurement of 0 mm, and hence bone covered the implant or was at the level with the apical tip of the implant. As all implants were installed to use the entire (or more) vertical height of the bone, so in most cases initially penetrating the sinus cavity, the findings are interesting. An obvious shortcoming of the paper III, however, is the lack of baseline x-rays, both in terms of the marginal bone level (MBL) and the apical bone level (APL). The problem with the absence of marginal baseline values and radiographs has already been discussed in relation to paper II, and the same conditions exist for paper III. Therefore, the description of the apical bone level can not be discussed in relation to possible bone resorption of the graft or with respect to its extent, but should be used to compare the current apical bone coverage between the two implant surfaces and grafting material used as a cross-sectional description after a minimum of five years and with a span of up to 19 years. The thickness of the sinus membrane was measured on reformatted CBCT images where the mean value of the membrane was measured at its thinnest and thickest points with tenths of a millimetre of accuracy. The thickness of the sinus membrane was 0.6 ± 1.3 mm for the turned implants and 0.6 ± 0.7mm for the oxidized implants. No significant differences were found between surfaces or the origin of the grafting material used. However, Pommer and co-workers evaluated changes in membrane thickness in 65 maxillary sinus floor augmentation procedures with a lateral approach and bone grafting in 35 patients using CT scans. They found that the maxillary sinus membrane undergoes morphologic modifications after sinus floor elevation, and the membrane reactions may differ significantly depending on the graft material used (Pommer et al. 2012a). On the other hand, in a retrospective study including 37 sinuses corresponding to 37 consecutive patients undergoing sinus membrane elevation and deproteinized bovine bone grafts, Anduze-Acher and co-workers reports no significant change in the membrane thickness between pre- and postoperative CT scans, suggesting that sinus floor augmentation via a lateral approach has no impact on the natural sinus physiology (Anduze-Acher et al. 2013). Both studies suggest, however, that how the
sinus membrane reacts to different elevations techniques and different grafting materials is not fully understood. Therefore, it may be hard to summarize and fully evaluate the significance of the measurements regarding the thickness of the membrane in paper III. This lack of understanding could form the basis for future studies that focus on reactions and changes in the sinus mucosa in relation to sinus elevation surgery.

To summarize, because the relatively small number of patients and implants included, it was difficult to detect statistical differences between the implant surfaces used. However, the results from paper III indicate that the use of turned and oxidized surface implants in autologous bone grafted situations with delayed implant installation produces equally long-term results and few pathological reactions in the maxillary sinus.

**Experimental study**

*Paper IV* was an experimental study conducted on nine tufted capuchin primates (*Cebus apella*) to describe the early bone healing in maxillary sinus membrane elevation with and without the use of bone grafting. In these experiments, we observed bone formation that started from the sinus floor close to the implant. At ten days, woven bone trabeculae had projected into the granulation tissue, which occupied the space secluded by the elevated sinus membrane, implant surface, and the cortical bone of the sinus floor. At 45 days, well-developed bone and marrow tissues filled the elevated space and newly formed bone lined the sinus membrane and contacted the implant surface. For all three surgical techniques (sinus elevation alone, sinus elevation with bone graft, and removal of the sinus membrane), no bone forming activities could be seen at the elevated sinus membrane near the top of the implant. Furthermore, there was no histological evidence that the sinus membrane itself induced bone formation, although the immuno-histochemical analysis showed distinct expression of osteopontin in the serous glands of the deeper portion of the lamina propria in direct connection to the elevated sinus membrane and close to the implant.

The histological results and appearance of the healing process at the different time sequences of the present study confirms the results of an experimental study by Scala and co-workers conducted also on *Cebus apella*. Scala used an experimental model with mere elevation of the sinus membrane after a lateral approach where the bone window was reflected into the sinus cavity and simultaneously installed implants without the use of additional grafting materials. Healing times of 4, 10, 20, and 30 days were used. That study concluded that the sinus membrane probably has no direct influence on osteoinductive properties regarding bone formation in sinus membrane elevation surgery (Scala et al. 2011). On the other hand, Srouji and co-workers used an animal model to study sinus lift procedures and demonstrated an
increase of alkaline phosphatase enzyme activity and different osteogenic markers in the sinus membrane, a finding that supports the notion that the sinus membrane plays a role in bone formation (Srouji et al. 2008; Srouji et al. 2010). In an in vitro study using pristine pig sinus mucosa, Gruber and co-workers determined the amount of STRO-1 (cell surface protein), bone morphogenetic proteins (BMP-6 and BMP-7), alkaline phosphatase activity, and osteocalcin expression in frozen sections. Furthermore, stimulation of the cells of the sinus membrane with BMP-6 and BMP-7 increased osteogenic differentiation. These results indicated that the sinus mucosa holds mesenchymal progenitor cells, which theoretically can differentiate into osteoblasts and participate in bone formation (Gruber et al. 2004).

Two main methodological shortcomings can be identified in paper IV: (i) the limited amount of commercial available antibodies for immunohistochemical analysis in primates and (ii) the embedding of the specimen for the immunohistochemical analysis in paraffin, which does not permit sectioning of metal implants, making it impossible to describe the interface between the implant surface and the surrounding tissue. Moreover, as implants were not present on the slides, location of structures becomes more difficult and the overview somewhat lost. Embedding techniques that use methyl methacrylate and cut and ground sections with subsequent removal of the plastic base have been used (Johansson et al. 1999b; Schwarz et al. 2008), but technique sensitivity and maintaining viable tissue is difficult, so a more established and predictable method was selected in our study. In case of commercial availability, it would be desirable to include specific markers of cells directly involved in the angiogenesis: VEGF types, one of the key regulators of angiogenesis during bone formation (Gerstenfeld et al. 2003); alkaline phosphatase (ALP), which is involved in the mineralization processes and calcification of bone and has further been found to be increased just before mineralization is initiated (Piattelli et al. 1996); and members of the tumour necrosis factor (TNF) receptor ligand family, such as osteoprotegerin (OPG) and receptor activator of nuclear factor-IB ligand (RANKL). Both members shown to play important roles in bone remodelling while regulating osteoclast differentiation and function (Takahashi et al. 1999; Enhos et al. 2013). A simple measure that would improve the study significantly regarding the description of the early bone formation activity, and above all the localization of this, is the administration of a fluorescent dye, such as Calcein (Palma et al. 2006). A principal difference between the anatomy and appearance of the maxillary sinus of human compared to primates used in the study is the relatively shorter distance in the lateral-medial aspect of the sinus, making the implant site narrow, resulting in steep surrounding walls in the experimental animals (Figure 8a-c). It us unclear whether the steep bone formation in primates is a result of the actual anatomical appearance or a result of sinus pneumatisation and a high intra-sinus air pressure.

In human studies, sinus pneumatisation is identified after extraction of maxillary posterior teeth and the greater expansion following extraction of teeth enveloped by a
superiorly curving sinus floor (Sharan and Madjar 2008). In some specimens, a vertical cortical septum was present close to one of the implant sides. Several studies have described and classified the prevalence and structures of septa in human sinuses, and their presence is considered one of the most common causes of membrane perforation during sinus augmentation (Wen et al. 2013). Pommer reported that septa are present in 28.4% out of 8923 sinuses investigated (Pommer et al. 2012b) and Maestre-Ferrín and co-workers reported a prevalence between 13% and 35.3% (Maestre-Ferrín et al. 2010). Compared to humans, the size of the implants is relatively larger in relation to the primate sinus volume. This size difference means that a blood clot could (possible) stabilize in place, particularly when the membrane is removed. It appears that regardless of surgical intervention, a key element in sinus elevation surgery is the initiation of bone formation via the blood clot around the implant site (Kim et al. 2010). It is therefore likely that the sinus membrane only acts as a barrier that keeps the blood clot at the desired site.

The histological picture of the conditions within the space consisting of the elevated sinus membranes agrees with similar findings from an experimental study by Lundgren and co-workers. In nine rabbits, two implants were inserted in the tibia, so the five most marginal treads were not covered with bone. A particulated autologous bone graft from the skull was placed over the exposed surfaces. On one tibia in each animal, the bone graft was covered with a bioresorbable barrier, while the bone graft on the corresponding contralateral tibia served as the control. After a healing period of 12 weeks and retrieval of the specimen, a significant larger volume of bone and increased amount of bone marrow was seen in the sites covered with a barrier (Lundgren et al. 1997). Despite the fact that the study was conducted on the tibia of rabbits, it is of great interest to study the histological appearance of the sites covered with the bioresorbable barrier in the longitudinal slides. Two distinct cortical layers are seen, with an inner layer facing the implant surface and the outer layer facing the surrounding soft tissue. In between these layers, bone marrow and bone trabeculae were observed, an image that is basically identical to the appearance of the slides from paper IV. In a similar experimental study on twelve rabbits, Rasmusson and co-workers found similar findings and concluded that the bone formed under the barrier decreased in volume during a 16-week follow-up period after barrier removal. They also found that a solid surface (implant) would have a stabilizing effect on the bone (Rasmusson et al. 1997).

In summary, paper IV presents histological and possibly partially immuno-histochemical evidence that the sinus membrane does not seem to hold osteoinductive properties, but rather has an osteoconductive function in primates. For future experimental studies, it is of utmost interest to interpret and understand the strong expression of OP in the mucosal glands of the lamina propria close to the implant and further incorporate additional markers for early bone formation in sinus elevation surgery.
Conclusion

Based on papers I-IV, the following conclusions have been formed.

Paper I-III

I. A higher but not statistically significant survival rate was found for oxidized compared to turned implants after more than five years in function in non-grafted patients. No significant differences in marginal bone level, pocket depth, or bleeding on probing between turned and oxidized implants were seen.

II. Patients treated with sinus elevation, intra oral bone grafting, and delayed implant placement for the rehabilitation of the atrophic posterior maxilla showed an overall high long-term implant survival rate with no difference in outcome between oxidized or turned titanium implant surface.

III. No significant difference was found between oxidized and turned implants regarding pocket depth, marginal bone level, bleeding on probing, or apical bone level/bone coverage in bone grafted situations.

IV. Pathological findings in the maxillary sinus cavity following sinus elevation procedures with bone grafts, in terms of sinus membrane health, were few and not correlated to any of the investigated parameters.

Paper IV

I. For all time frames and for all surgical techniques, bone formation was seen close to the residual bone surface near the implant and sprouting into the granulation tissue.

II. For all three surgical techniques, no bone forming activities was seen at the elevated sinus membrane near the top of the implant.

III. For all groups, a distinct expression of osteopontin was seen in the serous glands of the deeper portion of the lamina propria in direct connection to the elevated sinus membrane and close to the implant.

IV. The maxillary sinus membrane did not seem to present osteoinductive potential in membrane elevation procedures in this study.
Acknowledgements

I would like to express my sincere gratitude to the following people.

Stefan Lundgren. You have always been there and believed in my ability. Your social events and your talent to get together various people is outstanding.

Malou Hultcrantz. Because you always had time and were always courteous, encouraging, and positive.

Carina Lundqvist. For constructive comments and interesting discussions.

Lars Sennerby. For time you’ve spent, your elegant solutions, and showing research is fun.

Peter Lundqvist. For all your help and time in the clinic.

Luiz S. Salata. For making the experimental study possible and for kindly showing me Brazil.

Per-Erik Legrell. For your radiological observations and help.

Giovanni Criccio. For help with language, travel, and research.

Paulo Faria, Vinicius Palma and José Américo de Oliveira. For your kind welcome, help, and assistance.

Elisabeth Nyström. For your wise comments and interest in my work.

Lina Holmström and Lillemor Hägglund. For all the help I’ve received.

Paula Mannström and Anette Fransson. For guidance on Gustav V research lab.

Lena Lundgren. For all your help and participation.

Anders R. Eriksson. For the creation of opportunity and time to initiate this thesis.

Göran Gynther. For giving the resources to complete the work.

Daniel Danielsson, Payam Farzad, Ali-Reza Badii and Johanna Ahlén-Swartling. For the times you have set-up when I was absent, and for your contribution to our friendly community.

Ulf Larsson-Flink. For your commitment and support, as a friend.

Jakob and Fanny. For being in my life

Viktor. For being my son

Johanna. For your tireless support, love, understanding, and being my wife.
References


