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Clinical and experimental studies of bone substitutes and dental implants in compromised bone sites

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Abstract

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Background: With an ageing population, an increase of more challenging implant treatments is expected. In this thesis, we evaluate the outcome of two faster implant protocols, in patients with compromised alveolar bone. We examine the bone integrating abilities of two new synthetic bone substitute materials and in another paper, we discuss the effects of nonsteroidal anti-inflammatory drugs (NSAID) on bone healing.

Aim: In paper I we investigate implant survival and effect of reduced implant-tooth distance. In paper II we evaluate the long-term implant survival and function of immediately loaded implants. In paper III & IV, we analyse if added NSAID reduce postoperative pain and if it has a reduced effect on new bone formation in a rabbit sinus lift model. We also investigate if a ceramic compound (CPC, granules) and hydrogel (HABP·CaP) result in a similar or larger bone amount, in comparison with bovine bone mineral. In Paper V we assess new bone formation adjacent to a hollow CPC implant.

Material & Methods: Paper I present a clinical and radiological follow-up, performed on subjects that previously received 3.0-3.3 mm diameter implants in the aesthetic area. In paper II, clinical and radiographic examinations were performed on subjects that had received six implants each with immediate loading in the maxilla 8-11-year ago. For paper III-IV, pain was assessed by clinical examination and scoring of facial expressions from photos. Histomorphometry and histology evaluations were performed. In paper V, a critical radius defect was created and either replaced by particulate autologous bone (AB) or a CPC implant. Qualitative and quantitative radiographic and histology evaluations were performed.

Results: In paper I, an implant survival of 97.2% up to 124 months was shown with a tooth-implant distance in many cases of <1.5 mm. Discoloration and recession of the buccal gingiva was the most frequent patient concern. In paper II a cumulative implant survival rate was 81.9 % at the final follow-up. In paper III and IV it was shown that NSAID had no effect on pain relief or bone formation. No difference was shown between CPC and control, but both showed larger bone amount and BIC than HABP·CaP. In paper V new bone was seen in sites throughout the entire CPC implant.

Conclusion: Satisfactory long-term dental implant results can be obtained without bone augmentation in most patients with atrophic alveolar bone, but there is still a minority in this group that may benefit of bone enhancement prior to implant treatment. To avoid the negative effects of autologous bone grafting, synthetic materials as the presented CPC, have shown promising results as a solution or alternative to existing bone substitutes in animal models.

Keywords: clinical follow-up, calcium phosphates, rabbit model, sinus lift, radius model, in vivo, bisphosphonates, narrow diameter implants, dental implants

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Till Johan & Lea

List of Papers

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

I Trbakovic A., Bongenhielm U., Thor A. (2018.) A clinical and radiological long-term follow-up study of narrow diameter implants in the aesthetic area. *Clin Implant Dent Relat Res.* 20:598–605

II Trbakovic A., Toljanic J., Kumar V. V., Thor A., 8-11-year follow-up of immediately loaded implants placed in edentulous maxillae with compromised bone volume and poor bone quality: A prospective cohort study (manuscript)

III Hedenqvist P., Trbakovic A., Thor A., Ley C., Ekman S., Jensen-Waern M. (2016) Carprofen neither reduces postoperative facial expression scores in rabbits treated with buprenorphine nor alters long term bone formation after maxillary sinus grafting. *Res Vet Sci.* 107:123-

IV Trbakovic A., Hedenqvist P., Mellgren T., Ley C., Hilborn J., Ossipov D., Ekman S., Johansson B.C., Jensen-Waern M., Thor A. (2017.) A new synthetic granular calcium phosphate compound induces new bone in a sinus lift rabbit model. *J Dent*. 70:31-39

V Trbakovic A. */ Mellgren T. *, Thor A., Ekman S., Ley C., Öhman Mägi C., Hammarström Johansson P., Jensen-Waern M., Hedenqvist P., Guided bone tissue regeneration using a hollow calcium phosphate based implant in a critically sized rabbit radius defect (manuscript)

* Authors contributed equally to this work and should be regarded as joint first authors

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Contents

Abbreviations

AB-Autologous bone
ATh- Andreas Thor
ATr- Amela Trbakovic
ATS- Astra Tech OsseoSpeed™ 3.0S Microthread or TX
BIC- Bone to implant contact
BMP- Bone morphogenic protein
BNP -Nobel Biocare, Brånemark system® Mk III 3.3Narrow Platform
Bup- Buprenorphine
Carp-Carprofen
CL- Cecilia Ley
CPC- Calcium phosphate cement
HABP•CaP- Bisphosphonate-hyaluronic acid-calcium phosphate nanocomposite hydrogel
NDI- Narrow diameter implant
NSAID- nonsteroidal anti-inflammatory drugs
NZW- New Zealand White (rabbits)
PH- Patricia Hedenqvist
PRP- platelet rich plasma
Sal-Saline
SDI- Standard diameter implant
SE-Stina Ekman
SNN-Straumann Standard Plus™ Narrow Neck 3.3 SLA/SLActive/Roxolid
3I- Biomet 3i Osseotite™
TM- Torbjörn Mellgren

Introduction

Scope of the thesis

With time, the edentulous jaw will be resorbed if no teeth or implants are present, affecting not only the bone in the respective jaw but also the sagittal relation between maxilla and mandible. Replacement of missing teeth with dental implants is an established treatment for rehabilitating the edentulous patient. The long-term survival rates of the implant treatment are predominantly over 90% in selected groups[1, 2] and implant treatment often result in good patient satisfactory scores. [3, 4] It is not always possible to place implants in the remaining alveolar bone because of reduced bone volume and bone quality (categories for this have been described by i.e. Lekholm & Zarb [5] and Cawood & Howell [6]). To improve the success of the treatment in challenging sites, as in the case of atrophic jaw in a totally edentulous patient, bone augmentation prior to implant placement has commonly been used. [7-10] For this purpose a variety of grafts are available. Autologous bone has been one of the most commonly used graft materials, but they are prone to resorption and complications connected to the donor site.[11] Usually, for small bone voids in the jaw, particulate or smaller blocks of autologous bone (taken from intra oral sites), bovine/porcine based minerals or synthetic calcium phosphate based bone substitute materials are used. Larger defects in need of augmentation, are preferably grafted by autologous pieces of bone, vascularized or non-vascularized, which are harvested from extra oral sites, i.e. anterior or posterior iliac crest or fibula.[10]

To lower patient morbidity, extra costs and prolonged healing time that comes with bone augmentation, other treatment options based on the adjustment of the implants to the bone has been looked into. As these might work for some patients, there is still a need in other patient groups for better synthetic bone substitute materials that could replace autologous bone grafts. This thesis is based on five studies, two, which evaluate the long-term survival and function of implants adjusted to compromised alveolar bone sites and three, that explore the *in vivo* possibilities of two new synthetic materials as bone substitutes.

Bone biology

The bones in a human skeleton consist of cortical bone (the cortex), which is the fundamental outer shell surrounding the cancellous (trabecular) bone. Most of the bone mass consists of cortical bone (80%), that is dense and gives the compressive strength, and the rest is cancellous (20%), which is filled with bone marrow and brings elasticity to the construction. The bone marrow is at first filled with hemapoetic bone marrow but turns into marrow adipose tissue (MAT) as we get older. The cortical bone has a fibrous membrane called periosteum on the outside, and a membrane, endosteum that divides the cortical from the cancellous bone. All mature bone consists of microscopic arranged lamellae with interspersed lacunae containing osteocytes. In the cortical layer, the circumferential lamellae make the outer shell lining and concentric lamellae create the bulk of the matured bone. The later build the osteon around a central canal, Haversian canal, which contains the required vessels and nerves. In the cancellous bone, the trabeculae are also arranged as concentric lamellae, but because of the inter-trabecular rich blood vessel and nerve innervation there is no need for Haversian canals.

Much of bone tissue is inorganic (50-70%) and made of mineralised hydroxyapatite crystals, $Ca1_0(PO_4)_6(OH)_2$, with small amounts of ionic substitutes such as carbonate, sodium, magnesium, strontium and potassium. The organic part of bone is around 20-40 % and contains mostly dense collagen type I (90% of the organic part), but includes also osteocalcin, osteopontin (OPN), bone sialoprotein and alkaline phosphatase. Water takes up 5-10% and < 3% lipids. [12]

The most important cells involved in bone formation and remodeling are osteoclasts, osteoblasts, chondroblasts and osteocytes.

Osteoclasts differentiate from monocyte/macrophage precursors. They are multinuclear specific macrophage cells with a main purpose to resorb mineralized bone matrix by secreting hydrochloride acid and collagenase by mainly proteases cathepsin K, TRAP and MMPs secretion.[13] Some important cytokines that are critical for survival, expansion and differentiation of the osteoclast precursor cells are RANKL and M-CSF that activates the osteocytogenesis, while OPG inhibits it. [14]

Osteoblasts have their origin in mesenchymal stem cells (MSC), which may differentiate into fibroblasts, chondrocytes, myoblasts and adipocytes. The differentiation into osteoblasts appears in four steps*:*

preosteoblasts, osteoblasts, osteocytes and bone-lining cells. They are activated by growth factors such as TGF- β, BMPs and PTH (which may in high doses have a reversed effect on bone matrix). Osteoblasts are bone forming cells that have several functions in the bone remodeling; production of RANKL, and M-CSF OPG, as well as synthesizing bone matrix proteins

(collagen type I). Osteoblasts are vital in bone mineralization. Osteocalcinin and ALP are good markers for the osteoblastic activity and thereby bone formation.

Chondroblasts are MSC progenitor cells in situ and are responsible for forming the cartilage matrix. They are vital for endochondral bone formation. When chondroblasts embed themselves in lacunae in the cartilage matrix they become *chondrocytes* and keep expanding the cartilage through the extracellular matrix.

Osteocytes are terminally differentiated osteoblasts that are trapped in the mineralized osteoid in lacunae. The communication between osteocytes, osteoblast lining cells and other osteoblasts, is made by dendrites in canaliculi canals. They are initiators of bone remodeling by signalling to the i.e. osteoclasts by RANKL release. They can also resorb bone through osteolysis. Osteocytes synthesize the sclerostin protein, which have a inhibitory effect on osteoblasts and thereby bone formation, calcitonin increases the production of the protein.

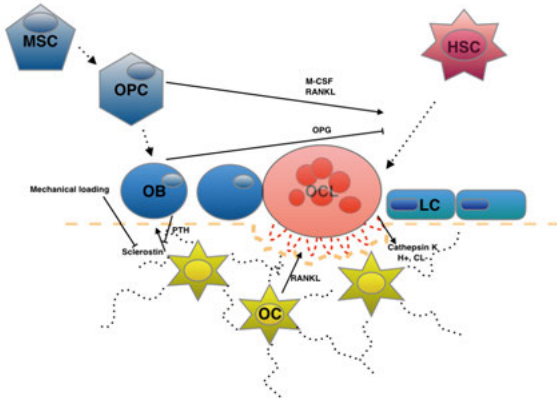

Figure 1. Simplified schematic image over bone remodeling

Bone formation and remodeling

Bone remodeling refers to the process of bone resorption and formation that is needed for maintenance of bone tissue and mineral homeostasis. It is equivalent to replacement without alterations of the bone morphology. Almost all bone in the adult skeleton is matured lamellar bone, but around 10% of new bone is formed every year.

Bone formation can happen by two major pathways, the first is called intramembranous and is initiated by the mesenchymal stem cells (MSC). These are joined together in groups and transformed into osteoprogenitor cells, which produce the extracellular bone matrix, spiculi. Other MSC turn into osteoblasts and create osteoid, containing mostly of collagen type I, which they join together, and form the woven bone. During the maturation process, the woven bone is replaced by lamellar bone by osteoclast resorption and osteoblasts production. The second, indirect bone formation is called endochondral, is found i.e. long bones, and forms the callus in fracture healing. The osteoprogenitor cells transform to osteocytes, which form fibrocartilage scaffold that with time is calcified. This becomes first woven bone and later matures into lamellar bone as well.

Fracture healing

Direct bone healing is rare in fracture healing and happens only in cases with less than 0.01 mm distance between fracture edges and 2 % strain. [15] It can be seen in cases with i.e. rigid surgical fixation or in stable unicortical fractures. It takes place without a callus and starts with a conical shape formed by osteoclast in osteons at the fracture site. These are followed by osteoblasts, which are repairing the osteons in bones longitudinal axis. For defects of up to 800 μm -1 mm a gap healing is possible were the direct formation of lamellae occurs but perpendicularly to the long axis. This requires rearranging of lamellae and another round of bone remodeling to acquire longitudinal osteons with vascularization and thereby a longer duration to complete healing.

Indirect (secondary) bone formation in fractures is the most common method, and resembles mostly endochondral but also to some degree intramembranous bone formation.[15] It is dependent on an acute inflammation, a callus formation, vascularization, and bone remodeling cells. The process starts with a hematoma that is clotted with help of the acute inflammation, which has its peak after 24 h and is there up to a week. It recruits inflammatory agents and promotes angiogenesis. Some important factors in this process are TNF-α, IL-1, IL-6, IL-11, IL-18. TNF-α induces differentiation of MSC

cells (both osteoblasts and osteoclasts). The interleukins IL-1 and IL-6 are (presently considered) the two most important interleukins in bone healing. IL-1 is a product of macrophages and it induces production of IL-6 in osteoblasts, promote production of the primarily cartilage callus and angiogenesis. It also promotes the production of VEGF, which stimulates the angiogenesis and differentiation of osteoblasts and osteoclasts. MSCs are recruited from the surrounding soft tissue and bone marrow. The hematoma is followed by formation of fibrin rich granulation tissue and endochondral formation in-between the fracture ends and external to periosteal sites. At the same time an intramembranous ossification response occurs subperiosteal directly adjacent to the fracture ends building a hard callus. Here begins a molecular cascade including proteins such as collagen type I and type II. Revascularization and angiogenesis phase begins with i.e. chondrocyte apoptosis and degradation of the dense cartilage. One of the most important vascular regulators is the VEGF protein that is expressed by osteoblasts and hypertrophic chondrocytes. During the hardening of the callus, chondrocytes carry calcium in their cytoplasm before and deposit them to join phosphatase in the extracellular matrix, where hydroxyapatite crystals are formed. As the chondrocytes become hypertrophic and the extracellular collagenous matrix becomes calcified, a cascade of actions occur, orchestrated by M-CSF, RANKL, OPN and TNF-α. These factors recruit bone cells and osteoclasts to form woven bone. At this point, chondrocytes go into apoptosis. In animal models the hard callus peak is by day 14 and as the cartilage with time is being replaced by the woven bone, the stability of the callus will increase. The last step of the fracture healing is the remodeling phase, with resorption of the hard callus by osteoclasts and rebuilding of lamellar bone by osteoblasts. This phase starts 3-4 weeks from initiation and can last up to several years.[15]

Factors affecting bone healing

There are plenty of substances, which may affect bone healing and NSAID is one of them. The negative effects of NSAID drugs on bone healing are thought to be created by affecting the acute inflammation. PGE-2 is a prostaglandin that is involved in multiple actions in various organs and has been shown to be important in inflammation as well as bone formation. PGE-2 is synthesized by osteoblasts through arachidonic acid. This transformation is induced by COX-1 and COX-2 which NSAID drugs have been shown to inhibit. Non-selective NSAID inhibits both COX-1 (and sometime only COX-2) but it has been shown that only by inhibiting COX-1, the bone healing will not be stopped.[16] Plenty of animal studies show reduced bone formation and bone healing with NSAID administration.[17, 18] In a rabbits study by Goodman et al., a Cox-2 selective drug, rofecoxib, was shown to inhibit bone formation when administrated for 6 weeks, although no difference was

seen when the drug was administered shorter than 2 weeks post-surgery.[17] The study by Oh et al. however, showed that there might be a possible compensatory reaction that allowed bone healing in dogs.[17, 19] In these study designs, different NSAID doses and time frames (often prolonged up to 6 weeks), as well as sort of animals (often mice, rats and lagomorphs), is greatly varied in the different studies. Human data are inconclusive in this area of research of affected fracture healing because of NSAID drugs, with a lack of prospective randomized studies. [20]

There are also a lot of factors that are thought to enhance fracture healing and stimulate bone growth. In this introduction only two are related to the included studies. The first one is BMP-2 (bone morphogenic protein-2) and the second is bisphosphonates. BMPs are growth factors (also called cytokines) that are involved in many systems in the body including bone formation. In bone formation, BMP-2 is of most importance and is expressed during the whole fracture bone-healing period. It is strongly expressed during the acute inflammation and in lower degree throughout the rest of the process. It has been shown that mice lacking the BMP-2 also lack calcified callus. [21] MP-2 has been expressed in chondroid progenitor cells, periosteal lining cells, osteoblast lining cells and in secondary ossification by osteoblasts. [15] The clinical difficulty with BMP-2 is to administrate the right concentration and amount of the substance, as well as to steer the bone formation. It is also associated with strong inflammation reaction. [22]

Bisphosphonates (BPs) is the other substance we have tested. They are chemical analogues to pyrophosphates and binds strongly to hydroxyapatite of bone. BPs has been shown to inhibit osteoclast activity leading to inhibited bone resorption, which has been used as treatment for osteoporosis and some bone tumors.[23, 24]

Animal studies of fracture healing with BPs have shown larger callus and prolonged bone remodeling from woven to lamellar bone, but human studies with patients that used the drug long term, have only in few patients showed delayed bone healing and atypical fractures. [25] Bisphosphonate-coated implants have shown to induce better implant stability,[26, 27] but this might be only an effect of local administration. A study by Cardemil et al. revealed that ovariectomised rats treated with one dose of Zolondric acid showed improved BIC in tibia, but the opposite effect in the mandible, which may indicate a site specific effect. [28]

Alveolar bone

Both the mandible and maxilla are so-called irregular bones and have their origin in mesenchymal tissue. The maxilla consists mostly of cancellous bone with a thin cortical layer, whilst the mandible has a more thick cortex

and is more dense.[29] Schematically, the maxillary alveolar bone resorbs from the labial plate and the lower alveolar bone from the lingual plate, resulting in cases with total tooth loss, in a narrower maxilla and wider lower jaw. The vertical bone height is also reduced in areas of tooth loss, leading to less alveolar bone in the maxillary posterior part, beneath the sinuses, and closer distance to the mandibular canal in the posterior lower jaw area. The sagittal relation is also affected due to jaw atrophy, leading to an often retrognathic maxilla in relation to the wider mandible. In conclusion, both the width of the bone (volume) and bone quality (described by Lekholm & Zarb and Cawood & Howell) [5, 6], as well as the upper and lower jaw bone relation/ the toothless space must be taken into consideration before implant placement and bone augmentation from a prosthodontic point of view.

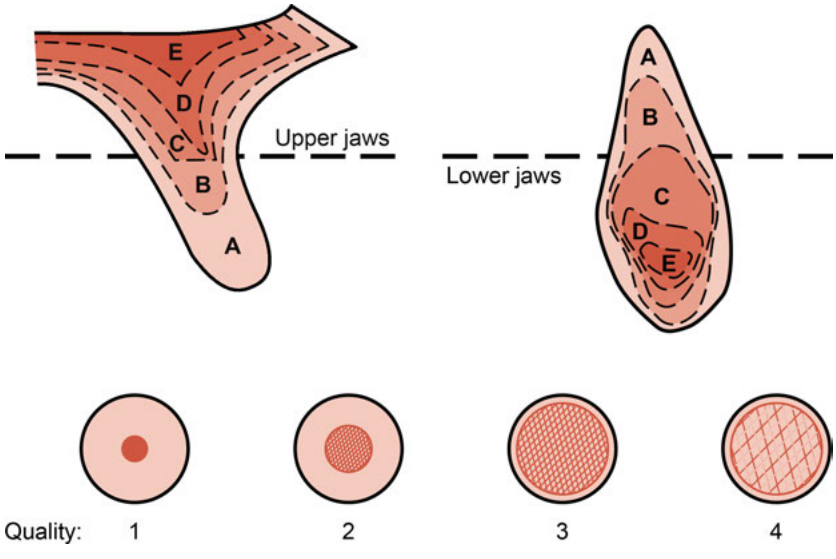

Figure 2. An adapted schematic image based on the Lekholm and Zarb classification of alveolar bone in the edentulous jaws (reprint from Tissue integrated prostheses: osseointegration in clinical dentistry, In: Patient selection and preparation. Quintessence Publishing Company, Chicago, 1985).

Implant integration

In the 1960s, Per-Ingvar Brånemark started to conduct studies with titanium implants and observed how the implants developed permanent attachment to bone [30]. He called this "osseointegration". During the years, the research has been focused on gaining better clinical results in survival and success of the implants, but also to try to find ways of helping patients with limited amount of bone to receive implants in edentulous sites. Different options, besides of

course to widen and augment the osseous volume, have been used to adjust the implant treatment to the remaining bone. Some of the options are to adjust the implant form, use narrow or short implants, [31, 32] or try to enhance the implant surface in different ways to gain a faster healing [33-35] and also to use a tilted implant insertion or to use a distant anatomical bone stock for anchorage with zygoma implants. [36-38]

Long term implant studies of implants with a diameter less than 3.3 mm in diameter over a period of 10 years, has shown a cumulative mean value of 94.6% survival and 1.3 mm in marginal bone loss, in a recent review study by Moraschini et al.[39]. In atrophic alveolar bone, the risk of implant failure increases if there is not enough bone around the implant to support it. [40] A general opinion is that there should be at least a distance of 1.5 mm between a tooth to an implant..[41] However, a recent study by Galindo-Moreno et al., were narrow diameter implants (NDIs) were followed up to 36 months, show that this might not be the case and that marginal bone loss was less in implants that were placed nearer to the adjacent teeth [42]. Narrow diameter implants have been introduced to solve the problem of narrow edentulous spaces mostly in the anterior jaw region. In the review study by Klein et al., in the studies that were included, the NDIs were categorized into three groups depending on the implant diameter. Group 1 (<3.0 mm) showed survival of 90.9%-100%, group 2 (3.0-3.25mm) displayed a survival rate of 93.8 %-100%, and group 3 (3.3mm-3.5mm) reached a survival of 88.9 %-100%. It was shown that group 2 and 3 were well documented and that there were no significant difference in survival between the 3.3mm-3.5mm and conventional implants. [43]

Different implant shapes with e.g. micro threads have been introduced to increase the primary bone stability. Clinically in studies, micro threaded implants have been shown to promote bone formation and reduce marginal bone loss during the first year. [44, 45] One research area, which keeps drawing attention, is implant surface modification. Turned implants with smooth surfaces have now evolved into roughened surface modified modern implants, such as the TiO_2-blastied or etched implant surfaces with i.e. fluoride ions. [34, 46] One of the earlier studies on this subject was performed by Wennerberg et al., were it was shown that more bone was found in contact to implants with blasted (with Al_2O_2) surfaces compared to the turned ones. A moderate rough surface that was blasted with 75 μm Al_2O_2 particles (compared to an machined surface, and surfaces blasted with 25μm and 250 μm Al_2O_2 particles) was the one that displayed the most bone contact. [47] These modified implants are designed to shorten the healing time and, in some cases of poor bone and little amount of bone, eliminate the necessity for bone augmentation. [48-50]

Tilting the implants is another way to use the remaining bone volume. Recent studies of long-term follow-ups of tilted implants, often with immediate or early loading, show high survival rates for this treatment and high

patient satisfaction scores. [3, 36, 51] This treatment of immediate loaded implants not only reduces the patient costs, morbidity and healing time it also facilitates further the implant treatment for the patients. Several recent long-term follow-up studies show comparable survival rates as conventionally loaded implants. [52, 53]

Bone augmentation

Bone grafts may be autologous, allografts (from a different individual), xenografts (grafts from animal origin) or synthetic bone substitute materials. For successful incorporation in the recipient site the material may be osteoconductive or even osteoinductive. *Osteoconductive* materials are materials that work as scaffolds for the osteogenic cells to adhere to and build new bone on their surface or inside pores. *Osteoinductive* materials on the other hand contain growth factors, such as BMP-2, and can stimulate new bone formation. To test the osteoinductive properties of a material, it can be inserted in non-osseous sites such as muscles or subcutaneous tissue, and if bone is induced the material is regarded as inductive. [54 55]

Guided bone regeneration

Membranes have been used for the guided bone regeneration solely or together with different grafting materials. They are divided into two major groups: resorbable and non-resorbable. From the 1980s the thought behind the guided bone regeneration has been to avoid soft tissue ingrowth and in that way, facilitate for the osteogenic cells and the underlying bone formation. [56, 57] For the non-resorbable membrane group, polytetrafluoroethylene (ePTFE) has been commonly used and are now also titanium enforced, which facilitate the modeling and guiding of the tissue. The surface allows no bone or soft tissue ingrowth in the material but the membrane requires a second operation for removal. Studies show good results for bone formation under these membranes, but also complications such as dehiscence and abscesses. [58, 59]

The resorbable membranes, such as the non-cross-linked collagen membranes, show fewer complications than the non-resorbable. [60] As these membranes are hydrophilic and made by collagen, they do not possess any strong up-holding abilities when applied in wet surroundings. They are applied more to shield off the soft tissue ingrowth into to bone healing site and need an underlying graft material. In recent years, the interest in collagen membranes have risen, as it has been shown that these membranes not only possess the ability to divide the soft/hard tissue, but also play a more active role than thought before in bone formation and remodeling of the defects they are outlining. [61, 62] In a study where Gultekin et al. compared resorbable mem-

branes to non-resorbable in a bilateral maxillary augmentation model with particulate autologous bone mixed with bovine bone mineral, less bone resorption was found with the non-resorbable membranes. [63]

Autologous bone grafts

Different solutions have been presented for the problem of limited amount of alveolar bone. As mentioned before, autologous bone grafts are still considered to be the golden standard. The autologous grafts fulfil most of the graft requirements (stated by Hollister et al. [64]), such as an optimal space filler and a stable load bearing space maintainer, as well as they are easily secured to the remaining bone and contains often both inductive bone growth factors and osteogenic cells which promote bone formation. [64, 65] The disadvantages are graft site morbidity and longer healing time as well as often the higher cost of the treatment that drives the researchers to other approaches.

The required amount of bone decides the site of the bone harvesting and often the grafting techniques. Autogenous bone grafts often consist of bone blocks or particulate bone grafts that can either be placed as onlays or in bone defects to uphold the bone volume. Smaller amounts of bone can be placed as onlays prior to, or at the same time as implant placement in cases with e.g. fenestration of the implant. These bone grafts can easily be obtained from intraoral sites from the mandible or maxilla.

Bone grafts from i.e. calvaria, tibia or iliac crest are often used for extensive atrophic alveolar bone defects in the form of onlay blocks or particulate bone grafts.[66] These are often linked to graft site morbidity and severe patient concerns such as pain, postoperative bleeding, superficial skin sensory impairment, and functional disorders (e.g. disturbed gait). [67-69]

Unfortunately, autologous bone grafts are often exposed to resorption. It is difficult to point out in advance which patients will be affected by graft resorption and the total extent of it. [70, 71] In a study by Sbordone et al., it was shown that iliac maxillary bone grafts had total vertical and horizontal mean resorption of 105.5% and mandibular iliac grafts an average of 87% after six years. The most resorption occurred the first two years [72]. Dasmah et al. presented extensive graft resorption in a two-year study of block and particulate autologous grafts placed in atrophic maxillae. [71]

Some studies discuss implant survival and marginal bone loss in grafted patients such as in the study of Corinaldesi et al. A 100% implant survival was reported in augmented alveolar ridges with a mean marginal bone resorption of 1.58 ± 0.48 mm after 3 to 8 years, and an implant success rate of 96.4%. [73] Autogenous grafts are usually placed in the anterior maxilla as onlays attached by titanium screws or plates. It has also been suggested that some growth factors in platelet-rich plasma (PRP) from patients own blood may promote the healing process of the grafted bone and eventually prevent resorption. [74] Thor et al. however, showed no difference in implant survival

and marginal bone loss in PRP augmented maxillary grafts compared to grafts without the addition of PRP.[75]

Bovine bone mineral

Bovine bone mineral (hydroxyapatite) has been extensively explored during the past decades as a bone substitute material together with implant placement.[76, 77] It is mainly used as bovine bone chips and applied in bone voids, sinus augmentation and to the remaining alveolar bone to increase vertical and horizontal bone volume. As it is made of bovine deproteinized bone it has been reported to be biocompatible with bone conducting properties and a well-functioning space maintainer [78-80]. The material resorption rate has been shown to be very little to none after 11 years reported in a study by Mordenfeld et al. [79] As it is a well-researched grafting material and extensively used in the clinic prior to implant placement, it could constitute as a control for testing other biomaterials, as was done in the study by Cordaro et al. [81]

Synthetic bone substitute materials

Calcium phosphates

There has been an interest in other bone substitute materials, especially in those that are synthetic. Researchers have been interested in calcium phosphates for a very long time and studies show that several of the calcium phosphate materials have abilities for bone conduction, bone induction, and resorption of the material itself. [55, 82-84] The different calcium phosphate material end-products can be divided into two main groups: one that is basic (soluble over a pH >4.2) including hydroxyapatite and calcium-deficient hydroxyapatite, and the other group where we can find Monetite and Brushite, which are acidic (soluble at a pH< 4.2). These materials are similar in their chemical composition but present quite different properties.

Hydroxyapatite is a bone conductive material and shows low, or no resorption rate *in vivo,*[79] whilst monetite demonstrate resorbable properties. [85] Brushite on the other hand, is quite reactive and transforms quickly into hydroxyapatite, but can also synthetically be made into monetite. Calcium phosphates are regarded as osteoconductive, but have shown osteoinductive properties, when the materials were made with porous macrostructure or well-defined concavities. [86, 87] Calcium phosphate resorption is mediated by both a cellular processes and passive dissolution. [88-91] In the presence of pyrophosphate, monetite and brushite have the tendency to stay in their active form until resorbed. [82] Pyrophosphate is produced by hydrolysis of extracellular ATP and is a well-known and important component in bone mineralization, where it acts as an inhibitor and regulator of hydroxyapatite formation. [82, 92, 93] The specific calcium phosphate compound (CPC) produced at Uppsa-

la University has been shown to have a slow resorption rate and is now in use as a commercially available solid scaffold for large skull defects in humans. [94-96]

Hyaluronic acid hydrogel

Collagen, fibrin, and hyaluronan are examples of polymers produced and degraded by the human body that may be made into hydrogels and be used as scaffolds for e.g. drug delivery. The advantages with these hydrogels are that they are tolerated well by the body and can be injected or applied with minimal invasive techniques. Hyaluronic acid is biocompatible and is found in many locations throughout the body. It is easily degraded in well-vascularized areas and therefore need to be made less disintegrating. Nejadnik et al. presented a non-covalent cross-linked Bisphosphonate-hyaluronic acid-calcium phosphate nanocomposite hydrogel (HABP•CaP). It displayed properties, such as self-healing abilities and more widespread new bone formation in bone defects in rats after 4 weeks, compared to a corresponding covalent cross-linked hyaluronan hydrogel. The authors of that study concluded that the HABP•CaP was as degradable as the corresponding covalent hydrogel and that in the CaP-particles and BP-groups reversible bindings were enough to hold the material together, and additionally but also stimulated to bone formation[97]. Hulsart-Billström et al, presented in their study a covalently linked BP hyaluronic acid (HA) hydrogel which exhibited reduced release rate of the in-situ encapsulated bone morphogenic protein (BMP) [98]. BPs effect here was believed to prolong the local activity of the morphogen and in combination with that and the anti-resorptive activities that it possessed helped in increasing bone formation. [23, 99]

Augmentation of the maxillary sinus floor

Sinus augmentation is a grafting method to gain bone volume in posterior maxillary sinus area before, or simultaneous with, implant placement in severely atrophic maxillae. The basic principle to gain access to the sinus with the lateral technique is to make a small window in the lateral maxillary sinus wall. Thereafter, the Schneiderian membrane or sinus mucosa is gently elevated from the maxillary sinus floor and an augmentation material (autologous bone chips, bovine mineral or other bone substitute material) is added beneath the lifted mucosa or onto the sinus floor. An alternative is also to elevate the mucosa and to support it with an implant placed in the remaining alveolar subantral bone. Thereby a space beneath the mucosa and around the implant can now be filled out by blood.

The surgically osteotomized bone can then, in some cases, be replaced in the created window that allowed access to the sinus floor, or it can be covered with a collagen membrane, or just only by placing back the full thickness mucosal flap. This method has been shown to have good results in stud-

ies were bone usually surrounds the implants after 6-12 months, and the stability that is needed around the implants is achieved. [100-103] Studies have shown presence of osteoprogenitor cells in the sinus mucosa not associated to any bone fragments. [104] This, together with the well-vascularized mucosa, can explain the successful bone formation in this region.

Sbordone et al. showed that autologous block and particulate bone grafts suffers from resorption, measured to an average of 39.2% for the particulate procedure and 21.5% for the block-procedure after 6 years from sinus augmentation. The grafted blocks also underwent a density change where the cortical bone was resorbed in the block graft and a gain in density was seen in the particulate bone. [105]

As mentioned above, sinus lift can also be obtained solely by blood. A recent study by Stefanski et al. shows an 100% implant survival, in one-stage surgery, after 40 months with a mean height gain of 4.75 mm and a mean marginal bone loss of 1.01 mm. [100] In addition, other studies with sinus augmentation with bone substitute materials have shown good clinical results.[78, 80, 106] This was shown in the study by Mordenfeld et al., were two different bone substitute materials were used (bovine mineral and a ceramic compound) were the implant survival was >90 % for both materials after five years. [107] The disadvantage of this method seems to be the prolonged healing time and extra costs for the surgery.

The sinus lift method has also been used in animal studies to test compatibility and new bone formation of dental implants and new biomaterials. [108, 109]

Rabbits as model for bone research

All mammals have different bone structure, anatomy, physiological pros and cons, which must be taken into consideration before deciding on preclinical animal models. The rabbit is not the first-choice experimental animal before testing of i.e. biomaterials in humans, but is still one of the most commonly used animal species in orthopedic research. This because of considerable advantages of low cost, easy handling and early skeletal maturity. [110, 111] The rabbits are not suited for external and internal fixation models, but are good for calvarial defects and their anatomy allows testing of several implants in the long bones as well as performing different maxillary sinus lift models. Rabbits have faster bone healing than humans and they reach their skeletal maturation at 28-34 weeks. [112] While the human skeleton consists of mostly secondary osteons the rabbit bone displays primarily osteon bone structure. It is shown to have similar bone mineral density but higher organic density than human bone. It also displays the same fracture toughness as human bone [113]. The long bones of rabbits consist of a thick cortical bone layer with adipose marrow tissue and in their maxillary sinuses, the anatomy and phys-

iology differs from humans. Despite these differences, rabbits are frequently used for implant evaluation and testing of new bone substitute materials in different models, i.e. bone defects in tibia, radius or skull but also in sinus lift models. [108, 109, 114]

Aims

The overall aim of these five studies have been to evaluate the outcome of long-term implant treatment in places of compromised alveolar bone and how two new biomaterials could measure up to autologous bone and deproteinized bovine bone mineral in applicability as bone substitute materials in the maxillofacial area.

I The aim of this clinical and radiological study was to evaluate narrow diameter implant function up to ten years after installation, evaluate the position of these "free-hand" placed implants in relation to adjacent teeth and to study whether reduced implant-tooth distance had negative effects on the neighbouring teeth and surrounding tissue.

II The aim of this study was to evaluate the long-term survival and success of immediately loaded implants in subjects with poor bone quality and limited amount of maxillary bone.

III The aim of the third study was to evaluate if the perioperative treatment with the NSAID carprofen reduces facial expression scores in the immediate post-operative phase in buprenorphine treated rabbits and if it interferes with long term formation of new bone in a sinus augmentation model

IV The aim of this study was to investigate if a synthetic granular calcium phosphate compound (CPC) and a composite bisphosphonate-linked hyaluronic acid–calcium phosphate hydrogel (HABP•CaP) induced similar or more amount of bone as bovine bone in a modified sinus lift rabbit model.

V The aim of the last study was to evaluate new bone formation in a critical rabbit radius defect replaced and reconstructed with implantation of a calcium phosphate cement moulded 3D replica

Material and methods

Subjects and experimental outlines

Paper I. This study is a retrospective follow-up on a cohort of subjects that received single NDIs in the maxillary incisive lateral region or mandibular incisive area in three Oral & Maxillofacial specialist clinics in Uppsala and Västerås (Sweden). The reasons for tooth loss were agenesis, irreparable dental fracture, caries, endodontic issues or periodontitis. In all, complete data from 27 subjects was presented with a total of 30 implants, additional clinical data of two subjects (with total of three implants) and data from 27 interviewed subjects with total of 36 implants. 17 women and 10 men with age between 22-83 years (mean 57.6 years) underwent the clinical and radiological examination, and two men participated in the clinical examination. The period from the implant installation to radiographic follow-up in the study was 20 to 124 months (mean 63 months ± 28 months). Ten different surgeons were responsible for the installations of implants in the study subjects.

Paper II. This study was an 8-11-year follow-up performed on subjects, that received six implants (OsseoSpeed implants, Dentsply Sirona, Mölndal, Sweden), which were immediately loaded with a screw retained prosthesis. 25 subjects with atrophic alveolar bone (Lekholm & Zarb, quantity 3 and 4, and quality C and D) were included from the beginning. The follow-up consisted of a clinical examination with removal of the prosthesis and registration of: plaque, pus, pocket depth, and bleeding up on probing, mobility and percussion testing, and a radiographic evaluation of marginal bone loss.

Paper III & IV. These two studies were designed as prospective, randomized, controlled experiments. Eighteen adult male New Zealand White rabbits received bilateral sinus augmentation with direct implant placement. Two materials were tested: first a hydrogel and second a calcium phosphate compound in granular form, both compared to bovine bone mineral as control (see Grafting materials). Randomization was performed to which side the test material would be applied, which test material would be placed and furthermore which rabbits would receive NSAID or just saline additional to buprenorphine post-surgery The healing period was 12 weeks. During the first week, the rabbits received buprenorphine as pain relief up to three days

and the test group received additional Carprofen (NSAID), or saline, 40 minutes before surgery and then daily for four days post-surgery. For paper III, digital photographs were taken for assessment of pain; 6, 7, 12 and 13 h postoperatively. The 6 and 12 h photographs were taken immediately before administration of buprenorphine. Photos were scored by five observers at a later time-point, evaluating the facial expressions according to Keating et al.[115] This was done to evaluate if the NSAID might have had an additional effect on pain relief. After rabbit termination, a CBCT was performed on the rabbit skulls to facilitate location of the augmented area before slicing of histological sections. These events were followed by preparations of the histological sections for histological and histomorphometric evaluations.

Paper V. This was also a randomized experimental study and was a continuance of the previous material study. An initial twenty, female adult New Zealand White rabbits were used in a unilateral critical radius bone defect model. New bone formation was studied, in the defect area in which a CPC 3D replica of the radius segment had been implanted for reconstruction of the defect. Autologous bone served as control. Randomizations were made for treatment and side. . The recovery period was three months, after which the rabbits were killed and μCT was performed on both the operated and intact foreleg. This was followed by preparation for histology and later radiographic and histological quantitative and qualitative evaluations.

Ethics

Ethical approval was obtained from the Regional Ethical Review Board, Uppsala University for **paper I** with D-no: 2012/153 and for **paper II** D-no: 2014/467, with patients signed participation consent. For the animal studies in **paper III, IV and V** the experiments were approved by the local ethics committee for animal experiments in Uppsala (C 70/13).

Inclusion & Exclusion criteria

In the first study (**paper I),** all patients were included that had received single NDIs in the site of tooth 12, 22 or 32-42, in one of the three Oral & Maxillofacial units in Uppsala and Västerås during the period January 2002 and January 2011. Further, inclusion criteria of subjects were that surgery was performed without bone augmentation prior the implant treatment and that subjects could participate in the study. In **paper II**, all Swedish subjects that had participated in the three earlier studies by Thor et al and Toljanic et al. [50, 116, 117] were included. The original inclusion criteria for the first study were: adult patients (>20 years) with an edentulous history of >3 months in the

upper jaw, and by surgeons rating after installation of the implants and on the basis of radiographic findings, of bone quality 3-4 and quantity of C-E (classification according to Lekholm & Zarb [5]). Subjects had further, to be included, to present mandibular teeth in region from last premolar to last premolar (5-5) and ability to return for the future outlined visits. The exclusion criteria were: inability to comply with all study procedures as outlined in the investigational plan, presence of uncontrolled systemic disease or dental disease, history of chemotherapy or head and neck radiotherapy, alveolar bone augmentation surgery within 6 months of study treatment, history of tobacco product use within 6 months of study treatment, and finally, subject pregnancy.

Dental implants

In **Paper I**, patients received one of more single implants replacing tooth 12, 22 or teeth 32-42 with implants that were 3.0 mm to 3.4 mm in diameter and had a length of 8-15 mm. Different brands were identified and included: Astra Tech OsseoSpeed™ 3.0S Microthread or TX (ATS), Straumann Standard Plus™ Narrow Neck 3.3 SLA/SLActive/Roxolid (SNN), Nobel Biocare, Brånemark system® Mk III 3.3 Narrow Platform (BNP) and Biomet 3i Osseotite™ (3I).

33 implants were evaluated in the clinical examination (30 in both the radiographic and clinical examination) and 36 implants in the interview group.

In **paper II** the subjects had received six Astra OsseoSpeed ™ (Astra Tech Dental AB, Mölndal, Sweden) implants in the upper jaw, with implant diameter of 3.5-4.0 mm and implant length of 10-13 mm. These implants are sandblasted with spherical particles of TiO_2 and treated (acid etched) with fluoride ions, which has been shown to improve bone retention compared to untreated implants. [46] **Paper III &IV**. For the sinus lift rabbit model with direct implant installation, specially manufactured mini-implants (2.2 mm x 6 mm) were used. They were made by machined commercially pure titanium Grade 4, and cleaned after fabrication in a series of cleaning steps including detergent, butanol, and ethanol, (Enge Mikroteknik, Vittsjö, Sweden).

Grafting materials

Deprotenized bovine bone

In **paper III & IV**, Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland) was used as control. The material is made of bovine bone from cattle limbs, which has been deproteinized by long heat treatments, several chemi-

cal purifications processes and sterilized with γ-irradiation. [118] In our study, we used a particle size of 0.25-1 mm of the material.

Collagen membrane

In **paper III, IV and V** Bio-Gide® (Geistlich Pharma AG, Wolhusen, Switzerland) was used to hold the grafting material in place. The membrane is made of collagen from porcine origin and consists of two layers of non-cross linked collagen. Studies have shown that the membrane does not initiate any adverse reactions and that it may also assist in bone healing besides just keeping away the soft tissue ingrowth. [62]

Composite bisphosphonate-linked hyaluronic acid–calcium phosphate hydrogel (HABP•CaP)

Physically cross-linked HABP·CaP nanocomposite hydrogel was prepared according to the study by Nejadnik et al. [97] The nano-gel formation was accomplished by mixing 1.5 mL of 4% hyaluronan-bisphosphonate solution with 1.5 mL of 12% hydroxyapatite dispersion and taken into two separate 3 mL syringes, which were then connected through a plastic connector. Mixing of the components and forming the gel (se image below) was accomplished by pressing the syringes and moving the material back and forth several times. After completion, the nanocomposite hydrogel was collected in one of the syringes. Nine portions of approximately 325 mg weight were made and transferred into Eppendorf tubes.

Figure 3. Image of the ready to insert product of composite bisphosphonate-linked hyaluronic acid–calcium phosphate hydrogel (HABP•CaP)

Calcium phosphate cement

CPC granules: The cement was prepared by mixing mono-calcium phosphate monohydrate (MCPM), β-tricalcium phosphate (β-TCP), glycerol and deionized water (0.4 mL of water and 4.5 g material). The cement paste was

pressed through 1mm holed screen (Caleva bench-top screen extruder, Caleva process solutions Ltd., Dorset, UK) and shaped into 0.60mm and 1.18 mm granules (Spheronizer 120, Caleva process solutions Ltd. UK, Dorset). The granules were cured in 100% humidity at 37 °C for 24 h. The glycerol was removed by dissolution in a water bath for 48 h. The granules were then dried at room temperature for 24 h in a laminar flow cabinet. The final step was to autoclave the granules at 124 °C for 20 min. The phase composition of the granules was characterized by X-ray diffraction (XRD; D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany).

Phase composition of CPC granules from XRD analysis were:

β-CPP 6.1 wt.%
β–TCP 10.4 wt.%
Brushite 0.5 wt.%
Monetite 82.8 wt.%

followed by Rietveld refinement. Quantification of the different crystalline phases were done by Rietveld refinement analysis, using BGMN software (www.bgmn.de) with Profex interface (http://profex.doebelin.org).

CPC implant: The design of the CPC implant was based on a rabbit radius cadaver study that was executed as a preliminary surgery. The removed, 20 mm part of the radius was scanned using micro computed tomography (μCT, SkyScan 1172, Bruker Micro-CT, Kontich, Belgium). The images were reconstructed using software package NRecon (Bruker Micro-CT, Kontich, Belgium), a 3D-model was created with CTAn (Bruker Micro-CT, Kontich, Belgium) and exported to CAD software (Autocad, Autodesk Inc., San Rafael, California, USA). A replica of the radius bone was designed with the CAD software and could thereafter be 3D-printed. A 3D-model of the medullary cavity was also designed but the diameter was reduced by 20 %. The radius replica was 3D-printed (Makerbot 5th generation, Makerbot Industries LLC, Brooklyn, New York, USA) using a PLA filament (True White PLA, Makerbot). A casting mould using the radius replica was then produced using silicon rubber (Elastosil M4601 A/B, Wacker Chemie AG, Munich, Germany). The model of the reduced medullary cavity was 3D-printed (Makerbot Replicator 2) using a water-soluble polyvinyl alcohol filament (PVA water soluble, Makerbot). The CPC was prepared by mixing β-tri-Calcium phosphate ($Ca_3(PO)_4$) (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and monocalcium phosphate monohydreate (MCPM, - $Ca(H_2PO_4)_2{\cdot}H_2O$, Scharlau, Barcelona, Spain) in the mass ratio 55.2:44.8.

The powder mixture was then blended with glycerol (powder to liquid ratio of 3.9 g/ml). The cement was injected into the mould and the water-soluble replica was inserted in the middle of the cement-filled mould. The implant was left to cure, submerged in sterile and distilled water, at 37 °C for 4 days, whereby it was removed and checked for visible defects. It was then

left to dry in room temperature for 24 hours before it was sterilized in an autoclave for 20 minutes at 121°C. A similar phase composition was shown, determined by the Rietveld refinement (described above), for the CPC implants as previously for the granules: 83 wt.% Monetite, 10 wt.% β-TCP and 7 wt.% β-CP.

Excluded study subjects and animals

Paper I. We found, in the dental records of the three units investigated, 75 subjects that had received the described type of implants during the follow-up period. Of these 75, we reached 56 subjects. 27 subjects declined participation, but as an effort to find out more, we asked questions concerning the status of their implants and the adjacent soft tissue, which they all were willing to reply to.

In **paper II**, three subjects experienced early failures of three implants or more and were excluded from the study. Further, five patients declined partial or full examination at the last visit (three declined the full participation and two only the radiographic).

In the last study (**paper V),** six animals were lost, resulting in a total number of 14 rabbits that were evaluated at the end of the study. One loss was related to the experimental model: a fractured ulna on the side of the radius defect.

Surgical procedures

Paper I. All implants in this study were inserted in a conventional way on free hand with flap surgery by different surgeons at the three clinics in Uppsala and Västerås.

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Paper II. All implant surgeries were performed under local anaesthesia by one experienced surgeon (ATh), in collaboration with a present treating experienced prosthodontist. A continuous bilateral flap on the alveolar crest of the maxilla was extended from the nasopalatine papilla to the premolar area, distal of the anticipated last implant. Buccal release incisions were made at the distal end of the incision. An acrylic prefabricated surgical stent (made from a model of the patients own full denture) was used for the osteotomies at the chosen fixture sites. Because of the limited bone volume and poor quality, frequent under-preparations of the osteotomy sites had to be performed. When deficient width in the marginal bone, implant insertion was performed to the palatal side, resulting in buccal marginal coverage of threads, but palatal exposed marginal threads. Buccal, more apical, bone fenestrations were therefore in some cases encountered with several implant

threads exposed, which was left untreated. In the posterior premolar region, the implants were often placed in a distal angulated fashion, to maximize the use of the remaining bone and the support for the restoration. Three implants were inserted in each maxillary side and each implant received a straight or angulated transmucosal abutment chosen by the surgeon. The procedure ended with suturing of the soft tissue flaps around the abutments.

Paper III & IV. The sinus augmentation model used in these two papers is a modification of a previously presented rabbit model by Kim et al. [108]

The surgery was conducted under general anaesthesia by sufentanil (Sufenta®, Jansen-Cilag, Sollentuna, Sweden) and midazolam (Midazolam Actavis, Actavis AB, Stockholm, Sweden). Prior to this, the sedative medetomidine (2 mg/kg, Domitor® Orion Pharma Animal Health, Sollentuna, Sweden) was administered subcutaneously and the antibiotic ceftiofur (5 mg/kg, Exenel®, Orion Pharma AB, Animal Health, Sollentuna, Sweden) intramuscularly. The cheek area was prepared for surgery by shaving and washing with 2 % chlorhexidine. Thereafter, a subcutaneous injection of local anaesthesia, 4.5 mg (0.9 mL) of prilocaine (Citanest®, 5 mg/ml, AstraZeneca, Sweden), was administered bilaterally.

A skin incision was made in the cheek area, a few mm above the inferior border of the incisive bone and the maxilla, followed by dissection of the masseter muscle and periosteum. A bone window (10 x 10 mm) was cut with piezo-electric surgery (Mectron, Carasco, Italy) in the lateral wall of the maxilla and removed. The periosteum was elevated over the alveolar ridge and a fixture site prepared with a round burr, followed by a twist burr of diameter 2.0 mm (Astra Tech system drills, Dentsply Sirona, Mölndal, Sweden). A mini implant (2.2mm x 6 mm) was inserted, after which the grafting materials (0.5–1.0 mL) were applied under the sinus mucosa. Instead of the removed bone, a 25mm x 25mm collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland), was placed over the open lateral bone void, to hold the grafting material in place. The surgical site was closed with non-woven Monocryl sutures 5-0 (Ethicon, Johnson & Johnson AB, Solna, Sweden) and care was taken to suture tightly to hold the membrane in place. The surgery lasted 60–90 min.

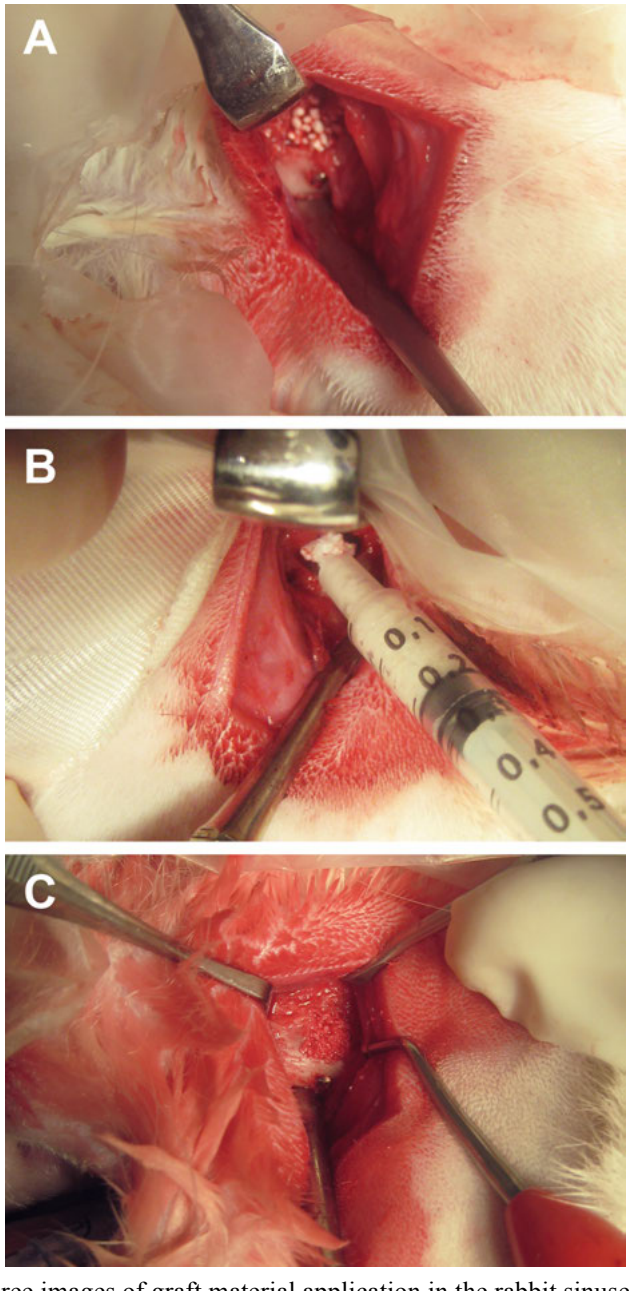

Figure 4. Three images of graft material application in the rabbit sinuses where A. shows CPC granules, B. HABP•CaP and C. bovine bone mineral (control)

Paper V.

Prior to general anaesthesia that was maintained with isoflurane, the rabbits received a mixture of medetomidine (0.25 mg/kg, Domitor 1mg/ml, Orion Pharma AB Animal Health, Danderyd, Sweden) and ketamine (15 mg/kg, Ketalar 50 mg/ml, Intervet AB, Stockholm, Sweden) subcutaneously. For analgesia, 5 mg/kg of the NSAID carprofen (Rimadyl, Orion Pharma, 50 mg/ml, Sollentuna, Sweden) was administered. Ceftiofur (Excenel 50 mg/ml, Orion Pharma, Sollentuna, Sweden), 10 mg/kg, was administered intramuscularly (IM) for infection control. Ropivakain (Ropivacain 10 mg/ml, Fresenius Kabi AB, Uppsala, Sweden), 3 mg/kg, was injected in the axillary area for a local nerve block. Aseptic preparation of the surgical site was performed after the fur was cut. A skin incision of 5 cm was made along the radius and the muscles were dissected to the bone. With the aid of a prefabricated metal template, removal of a mid-radius bone segment of 20 mm together with the periosteum was performed under saline cooling, using piezo surgery (Mectron, Carasco, Italy). The CPC implant, previously described, or particulate autologous bone (AB) (1 – 3 mm bone chips from a manual bone mill, the R. Quétin Bone-Mill, KLS Martin, Jacksonville, FL, USA) as control, was placed in the defect. A collagen membrane (25 x 25 mm, Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was positioned over, and wrapped around the AB for stability and separation from the soft tissue (figure 5 below). No additional fixation was used. Finally, the soft tissue was closed with resorbable Monocryl sutures 5-0 (Ethicon, Johnsons & Johnson AB, Solna, Sweden). The surgery lasted for 60-90 min.
For post-surgical analgesia, buprenorphine (Temgesic, 0.3 mg/ml, Indivior UK Ltd, Slough, Berkshire, UK) was administered for three days, complemented with carprofen (5 mg/kg, N-vet AB, Uppsala, Sweden), administered daily for two days.

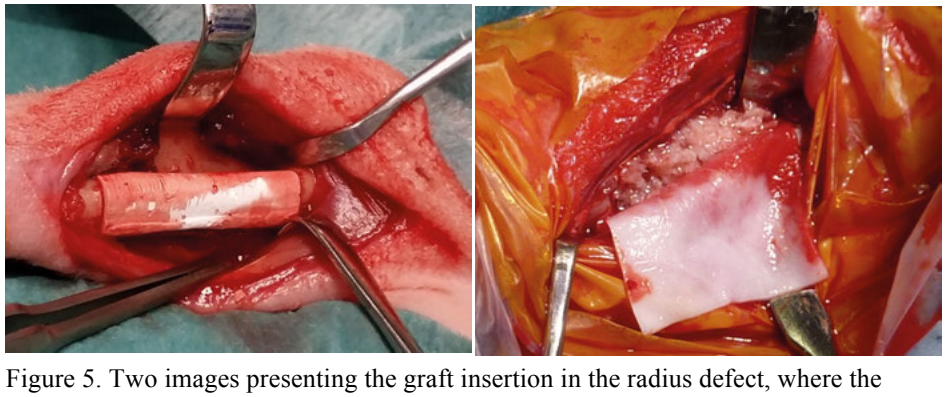

Figure 5. Two images presenting the graft insertion in the radius defect, where the left image is showing the CPC implant and the right AB graft (control).

Clinical examination

Paper I. The clinical examination started with subjects describing their perception of the prosthesis function and aesthetics, followed by the clinician's examination of the implant prosthetics and the periimplant mucosa. Registration of the implant survival time (months), pocket depth, bleeding on probing on four implant sites and the nearest sites at the neighbouring teeth (mesial and distal) were registered. Manual test of mobility and percussion was also performed to evaluate implant stability.

Paper II. The clinical examination was performed by two of the authors (ATc & ATh). Subjects gave their own view of their fixed prosthesis regarding function and aesthetics. Removal of the prosthesis followed and different parameters were registered: plaque around the implant, appearance of purulent exudate, bleeding following probing and measuring of pocket depth at four sites (mesial, distal, buccal and lingual) from the implant neck. The implant stability was assessed by registering tenderness to percussion, mobility and sound percussion test, which indicated success or failure of implant osseointegration.

In **paper III & IV** the rabbits were purchased from a specific pathogen-free colony (Lidköpings Kaninfarm, Lidköping, Sweden) and were 36 ± 4 weeks old and weighed in average 3.7 ± 0.2 kg at the start of the study. The animals were acclimatized to handling for two weeks prior to the experiments and a general clinical examination was performed on the day of surgery. Blood was collected before surgery to measure the acute phase protein serum amyloid A (SAA) by enzyme-linked immunosorbent assay (Tridelta Development Ltd, Maynooth, Ireland), as a marker of inflammation.

Postoperative pain assessment: Frontal and lateral face images were obtained with a digital camera (Panasonic Lumix DMC-T27, Osaka, Japan) before surgery, and at 6, 7, 12 and 13 h postoperatively. The 6 and 12 h photographs were taken just before administration of the opioid buprenorphine.

180 pictures were taken, numbered randomly and scored by five observers. The observers were instructed by viewing examples pictures from the publication on the Rabbit Grimace Scale by Keating et al. [115] The scoring system consisted of a scale of facial action units (FAUs); orbital tightening, ear position, cheek flattening, pointed nose and whisker change, each evaluated on a scale from 0 to 2 (0 = no change, 1 = moderate change, 2 = obvious change from normal). The pictures were also scored subjectively for the presence of pain, ranging from 0 (no pain) to 3 (severe pain).

In **paper V** the rabbits were obtained from a pathogen free animal farm (Lidköpings Kaninfarm, Lidköping, Sweden), and were 9-10 months of age and with a mean ± SD body weight of 3.9 ± 0.5 kg at study begin. Acclima-

tization to handling was performed during two weeks. The rabbits were clinically examined and weighed before surgery, during the first postoperative week daily and thereafter weekly.

Radiographic evaluation

Paper I. The radiographs were acquired with a CBCT (3D Accuitomo, J. Morita Corporation, Osaka, Japan) and evaluations of these were done with the radiology viewing software (KODAK CARESTREAM PACS, version 11.3.2.4051, Carestream Health, Inc., Rochester, New York, USA). All radiographs were evaluated, by an independent radiologist, in search for pathologies in the area before other measurements could be performed by one of the authors (ATr). The measurements consisted of the distance between the NDI and the adjacent roots at three different levels: at the implant cervix, middle and apex. The measurements were performed manually at both the mesial and distal side of the implant. Adjustment of the sagittal and coronal plane of each implant was needed before the interproximal bone measurements could be performed. Also, because of root angulation and longer implants some apex measurements could not be done.

In **paper II** the radiographic examination was done prior to the removal of the fixed prosthesis. A panoramic image was acquired for easier orientation. The measurements were performed on two intraoral ortoradial radiographs per implant with clear view of the implant threads. An independent blinded oral radiologist, evaluated the measurements in the same standardized protocol as previously reported in the earlier follow-ups. [50, 116, 117] The difference from earlier evaluations, were that all images were digitally acquired and different software (Kodak Carestream Vue PACS, Carestream Health Inc., Rochester NY, USA) was used. Marginal bone loss was measured from the reference point (the junction of the roughened and machined beveled surfaces) on the implant to the most apical implant bone site at two sides (mesial and distal). The changes of the marginal bone levels were documented as mesial and distal sites of each implant over time.

In **paper V** both fore limbs were scanned post-mortem with μCT (Bruker Micro-CT, Kontich, Belgium).

Radiographic quantitative evaluation: The amount of new bone and graft material was measured both inside the volume previously occupied by the radius (restricted volume) and in a volume without transversal restrictions (unrestricted volume). The ulna from the non-operated forearm was used to estimate and subtract the volume of the ulna on the defect side. The restricted volume was created by interpolation between the ends of the radius bone from both sides of the defect (CT An, Bruker Micro-CT, Kontich, Belgium).

The same methods were also used to evaluate the central 10 mm of the defect. The density was also measured.

Radiographic qualitative evaluation: Virtual 3D-models of the radii (CT vox, Bruker Micro-CT, Kontich, Belgium) were used to subjectively evaluate the degree of bridging (score 1-4) and degree of filling of the defect (score 1-4), as in the study by Bodde et al. [119] Three of the authors (ATr, TM, PH) individually performed evaluation.

Scoring system for the quantitative evaluation of CT images were as follows. (A) Degree of bridging. Values refer to the percentage of contact surface of bridge at each side of the defect. (B) Filling of the defect with bone + graft material. Values refer to the percentage of the defect area that is filled. A score of zero was used when no bone bridging was found.

Histology & Histomorphometry

Paper III & IV.

Sample processing: The samples were submerged in 10% neutral buffered formalin for 30-31 days, followed by water rinsing and thereafter dehydration with increasing ethanol concentrations (70% – 100%). The samples were then pre-infiltrated and embedded in pure resin (Technovit 7200, Kulzer, Hanau, Germany) and subsequently polymerized under UV light (ExaktR Apparatebau, Norderstedt, Germany). Non-decalcified cut and ground sections were prepared according to the Donath technique using the ExaktR equipment. [120] An initial thick section was prepared which then was ground (using SiC wet grinding papers starting with 800 grit up to 1200 grit papers) in water-cooled grinding equipment a thickness of 15 μm and finally the sections were stained with a mixture of toluidine blue and pyronin. [121]

Histology: In brief, two veterinary pathologists (CL, SE), blinded to treatment analysed the sections separately and then agreed on a consensus scoring. One section per animal from each side was examined for degree of implant incorporation in bone (rating as good, satisfactory or poor) and graft material in association with bone (yes or no). In addition, observations were made on the inflammatory changes in the sections.

Histomorphometry: A Nikon E600 light microscope was used together with Nikon DXM 1200 camera (Nikon Instruments, Melville, NY, USA) connected to a PC. All slides were photographed under same conditions for exposure time, light intensity, and camera gain. The analysis was performed with NIS-elements imaging software (NIS-elements Basic Research, Nikon, Tokyo, Japan). White balance adjustments, calibrations, and transformations of the photographs into binary images were performed for each captured image. The amount of new bone combined with graft material in the ROIs was counted and is presented as the fraction of the total region of interest (ROI). The new and old bone was divided visually by its colour, “dark pur-

ple" colour showed new bone and "pale purple" colour indicated old bone. Old bone was found in the cervical part of the mini-implant, i.e. around the first two-three implant threads where the insertion and stabilization of the implant initially took place at surgery. This was therefore not included in the measurements. New bone formation in contact to the materials was measured with two different ROIs: a) "free ROI (x10 magnification) and b) "rectangular ROI" (x40 magnification). Implant incorporation was evaluated by two different measurements at both sides of the implant (x40 magnification): implant ROI and BIC (bone to implant contact), see figure 6.

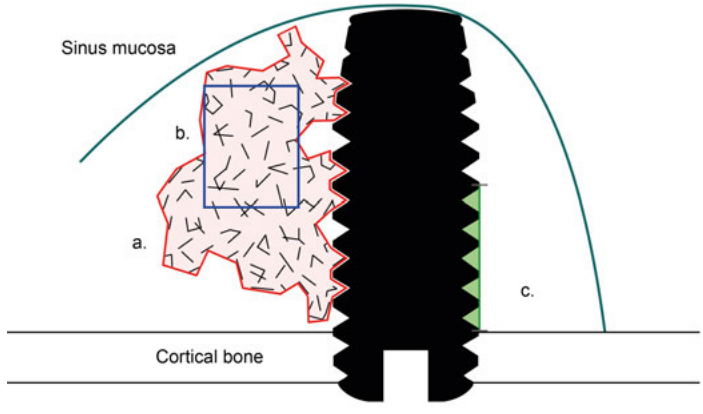

Figure 6. A schematic image of the different regions of interest (ROI) used for the histomorphometric measurements: a. The free ROI, b. the rectangular ROI, c. the implant ROI where also BIC was measured (both the last two measurements were performed at both sides of the implant.

Paper V.

Sample processing: The bones of the two front limbs were immersed in 10 % neutral-buffered formalin fixative. Routine and standard preparation of un-decalcified cut and ground sections were done according to Donath et al. [120, 122] and Johansson et al. [123] using the Exakt cutting and grinding equipment (Exakt Appartebau, Nordstedt, Germany). Dehydration of the sections was performed with increasing ethanol concentrations (>70 %), followed by resin infiltration (pre-infiltration in diluted resin concentrations, and finally pure resin (Technovit 7200/VLC light curing resin Kultzer, Hanau, Germany), polymerized in a light-curing unit. The specimens were first cut transversally in the middle of the defect. Thereafter two sections of 200 µm were cut from the surface of each side and ground with SiC papers (800 - 2400 grit), with continuous water-cooling, to reduce the thickness to 20-30 µm. [121] Further, the two remaining parts of the specimen were glued together (Loctite, super glue Henkel Norden AB, Stockholm, Sweden). The samples were

then cut in the longitudinal axis through the defect, i.e. one central section (of 200 µm thickness being thinned to 20-30 µm).

The sections were routinely. The procedure involved staining in a solution containing 1% toluidine blue dissolved in 1% borax and mixed with 1% pyronin G. The sections were stained in room temperature, and after rinsed in tap water to remove excess stain and dried before being cover-slipped with mounting media (Pertex, Histolab Products AB, Göteborg, Sweden).

Histomorphometry: All longitudinal sections were photographed with objective 1x (10x magnification) and in this study the same camera and procedure for imaging was used as in paper III/IV (*see previous section paper III/IV*). The measurements were performed on an area restricted radially by the defect ends, and laterally by the original ulnar bone and the outer a border of the material and/or bone. The amounts of filling (material + bone and bone) in the restricted area were presented as fractions and compared between the two materials, see figure 7 below.

Qualitative Scoring: A qualitative scoring of the sections was performed by the authors (SE, CL, ATr) using light microscopy (Nikon eclipse E600, Nikon Instruments, Melville, NY, USA). The longitudinal sections, one per animal, were examined and scored for; a) *new bone formation adjacent to the defect ends*, b) *cortical integrity (lateral surface)*, c) *cancellous bone*, d) *inflammation*, e) *filling of the defect*, f) *bony fusion with ulna in the defect area*, g) *bony fusion between ulna and radius adjacent to the defect*. For the transversal section, an average scoring of two sections per animal were evaluated for the categories b, c, d, f., see table 1 below, page 44.

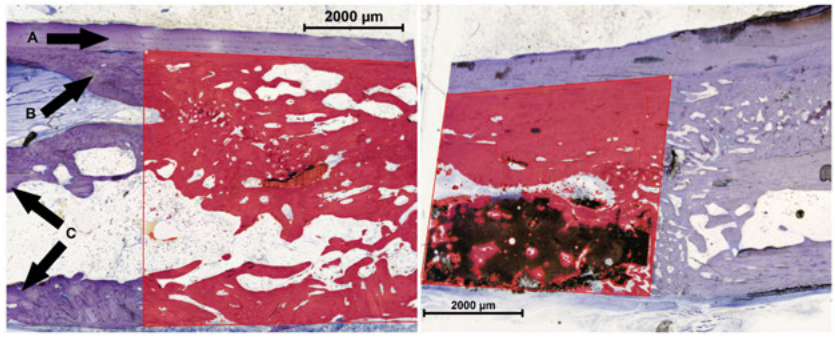

Figure 7. Two images showing the region of interest (limited by the red border). The left image is shows a defect grafted with AB and the right a defect grafted with CPC material (brown). The arrow A, points to the ulnar bone, B shows the new bone and C points to radius bone.

Table 1, showing the qualitative categories a-g and the scoring

	Score			
Categories	1	2	3	4
a) New bone formation associated with defect ends	No integration	One edge integrated	Both edges integrated	
b) Cortical integrity (peripheral surface	Periosteal fibrosis	Initiation of formation of lamellar bone	Reorganization in majority	Complete organization
c) Cancellous (trabecular) bone	Woven bone associated with graft	Lamellar and woven bone associated with graft	Lamellar and woven bone – no graft seen	Complete reorganization of trabecular bone
d) Inflammation	Multiple areas of > 20 monocytes in a high-power field (x400)	No inflammation	-	-
e) Filling of the defect	Filled with graft, bone and fibrous tissue	Areas of fibrocartilage in the bone	Only bone fill the defect	-
f) Bony fusion with ulna at the defect	No fusion	Fusion	-	-
g) Bony fusion between ulna and radius adjacent to defect	No fusion	Fusion	-	-

Statistical Analysis

Paper I. In this study only descriptive statistics were reported (tabulated data, mean values and range)

Paper II Descriptive statistics are presented as mean, median, SD, minimum and maximum, together with Kaplan Meier analysis of implant survival. Student t-test was used for the comparison of the different categories compared to marginal bone loss. The statistical analysis was done using SPSS® version 23.0 (SPSS Inc., Chicago, IL, USA). The statistical significance level was set at $p< 0.05$.

Paper III. In this study data was examined for normality and homogeneity of variance. Two-way repeated measures of ANOVA were used to compare body weight, subjective pain scores and facial expression scores, with group and time point as factors. A posthoc Bonferroni t-test was used (all pairwise multiple comparisons) to make comparisons between time points.
For the facial expression and subjective pain evaluation respectively, scores were calculated for each animal, time point and observer, by adding the scores from two pictures. From the scores from all observers means and standard deviations were calculated for the ANOVA. The area under the curve (AUC) for facial expression scores over time was compared with t-test between analgesia groups.

SAA levels were compared with repeated measures ANOVA on ranks within each group and with rank sum test between groups at all three time points. Fisher's exact test was used for the qualitative histology scoring to evaluate the distribution of scores in Group Bup + Carp and Group Bup + Sal in the sinus treated with control (bovine bone mineral). Morphometric results of bone formation in the sinus treated with HABP•CaP were compared by independent t-tests between groups with and without carprofen. The effect of carprofen on bone formation on the side with test material was evaluated by a two-way ANOVA, with material A/B and carprofen/saline as factors. The new bone amount measured in the ROIs was compared between materials A and B by Mann-Whitney rank sum test, and between the materials and the controls by signed rank test. A p-value < 0.05 was considered significant.

Paper IV.
Qualitative data was graded as ordinal data and measurements of implant incorporation was conducted by grading the data starting with good= 2, followed by satisfactory= 1 and poor= 0. The bone associated to the material was assessed as yes= 1 or no= 0. For the qualitative assessment, Mann Whitney Rank Sum test was used to compare the three different materials, and Wilcoxon Signed Rank Test for comparison of the test materials with controls. For the histomorphometric data, the Student's t- test was used to compare the test materials and paired t-test for test material against control. Statistical analyses were performed with Sigma Plot 13.x (SYSTAT, Chicago, Illinois). The significance level was set to $p<0.05$.

Paper V. Treatment and housing data were evaluated by two-way ANOVA. Following examination of interaction of factors, any main effect of treatment was further evaluated by pairwise comparison with LSD test (parametric data) or Mann Whitney U test (non-parametric data). Statistic evaluations were done with InVivoStat version 3.7.0.0. (Copyright 2008-2016. Simon Bate & Robin Clark). Statistical significance was set at the $p<0.05$ level. Values are presented as mean (SD) or median (range).

Results and Discussion

Paper I.
Results

Clinical group: In the examined group, no implants were lost nor were any implant fractured during the follow-up period and all implants functioned well as stated by patients and the examiners. The mobility and percussion test gave further confirmation of a well-integrated implant. One of the most frequent, but strongest pronounced concerns for the patients was the discoloration of the buccal periimplant mucosa. 13 implants had pocket depth of 5-7 mm, of those 11 implants showed one or more sites with bleeding on probing, including the two subjects that only participated in the clinical examination. Two subjects had implants that showed bleeding at one or two neighbouring teeth. One of these patients was assessed to suffer from localized periodontitis in the mandibular anterior region, while the other only had gingivitis. *For the interview group*: An overall good satisfactory level was stated by the interview group. Only one patient had experienced implant loss, but twice (two different brands), in the same region (region 12). However, he was now satisfied with the third try and the implant (third brand) and prosthetic crown. No other implant losses were seen or any reparations of the prosthetic construction had to be done during the follow-up. The patients in the interview group also stated buccal discoloration, retraction of the buccal gingiva together with inflamed periimplant mucosa as some of the most frequent concerns.

The radiologic examination showed that 13 implants were inserted very close or *less than one mm* to the neighbouring root at the cervical level. One of the upper lateral implants was positioned in a way that no interproximal bone was seen between implant and the root. In addition to this, thirteen implants were positioned one mm to neighbouring tooth (seven at the mesial surface and six at the distal surface). The average implant- root distance was at the cervical region 1.6 mm; in the middle region 1.8 mm, and at the apex 2.2 mm. 76% of the implants were placed closer to the mesial neighbouring tooth than to the distal one at the cervical level. The mean distance at the cervical implant level was 1.4 mm mesial and 1.9 mm distal. In the implant apex region, only 20 mesial sites and 26 distal site measurements could be made because of shorter adjacent root in some of the cases or an extreme implant angulation in comparison to the adjacent root. It was also seen that the mean mesial distance was 1,34 mm and the distal 1,92 mm. Very thin

buccal and lingual bone crest was noted in many implant sites and few implants lacked parts covered by bone.

Discussion:

This follow-up showed a high survival level of NDIs in the aesthetic area, reaching 97,2%; 94,7% in the interview group and 100 % in the examined group. This result is in accordance with other studies evaluating NDIs reviewed by Sohrabi et al. where they presented similar survival rate of NDIs as standard diameter implants. The most common NDIs survival rate in these studies were 95%-100 % with one study showing a low survival rate as 89%. [124] Two more recent prospective studies done by Maiorana et al. and Galindo-Moreno et al. show that early loaded NDIs replacing upper laterals and lower incisors confirm these results by showing cumulative survival rate of 95.9 % after 3 and 5 years. [31, 125]

The radiographic analysis of the tooth-implant distance in this study showed 17 implants placed very close to the adjacent teeth with 1 mm or less. Majority of the implants were placed closer to the mesial tooth than the distal. A common recommendation has been not to place the implant closer than at least 1.5 mm to the adjacent root [41] but results from studies are contradictory. [126] Follow-ups such as the study by Esposito et al have shown that reduced tooth-implant distance increased the marginal bone loss at the adjacent tooth. [127] Tarnow et al. demonstrated that the crestal bone loss was greater in the <3 mm implant-implant distance (loss of 1.04 mm) than in cases with distance of 3 mm or more (loss of 0.45 mm).[128] The effect of reduced bone width on the adjacent soft tissue in contact with implant has been discussed in studies by Buser et al. and Gunder et al. that suggested to take into consideration the biological width before implant placement and to secure a dimension of at least 2 mm buccal bone. [129, 130]

The clinical examination in our study showed most mesial and buccal pockets of 5-7 mm (at 13 implants including the two patients that only participated in the clinical examination), but results of the measurements and deepened pocket depths might have been distorted because of the prosthetic crowns which were cemented and not removable. Further, it is well known that cement residues may have induced an mucositis in some cases. [131]

A concern with the smaller implant diameters have been the fracture risk. Allum et al. evaluated the impact of fatigue in different diameter implants and advocated for caution when using implants of 3.0 mm in diameter or less. [132] In the clinical group of our study, no subjects were seen with implant fractures but in the interviewed group, one subjects experienced two earlier fractures in the same site.

This study has its limitations by having a retrospective set up, including different implant systems, different surgeons, no possibility of marginal bone loss measurements and a limited patient cohort that where interested in full participation in the study. However, the study still gives us an indication of

survival and function of these implants inserted by free hand without guides in compromised sites.

Obviously, and to the gain of the patients, the NDIs offer an easy restorative solution in complicated sites with less cost and faster healing time, compared to implant placement with prior or simultaneous bone augmentation or even pre-surgical orthodontic treatment, where the latter also brings along risks of root resorption. [133]

As shown in this study the close tooth-implant placement did not induce any major issues, such as resorption or endodontic problems. This has also been observed in a recent study by Galindo et al., where it was shown that reduced tooth-NDI distance did not affect the marginal bone height adjacent to the implant or the neighbouring tooth. [126] Even if the proximal bone and the neighbouring teeth are not affected; the reduced bone volume may later cause problems related to with the buccal bone plate and adjacent soft tissue resulting in gingival retraction and discoloration as a secondary complication as seen in this study. This was also observed by Cardaropoli et al. who saw a reduction in buccal bone following the remodeling with soft tissue alterations as consequences of this. [134] As pointed out in this study, not only is the aesthetic appearance of the prosthetic restoration important as noticed by the patients, (aesthetics is often difficult in this region because of the reduced mesial-distal distance), but the appearance of the soft tissue matters also. The patients should be made aware of the probability of this complication prior to the placements of NDIs in sites with higher risks of this occurrence in the aesthetic area.

Paper II

Results

At the beginning of the multicentre study that serves as a base for the Swedish cohort studied here, 51 subjects were enrolled for treatment of their edentulous maxillae, resulting in 306 implants included. The Swedish cohort contained 25 subjects with 150 implants and it was those 25 that were included in this study. The study had 10 early failures in 3 subjects during the first year, which lead to additional 8 censored with 22 remaining patients. All 25 patients are included in the three earlier published follow-ups of 1, 3 and 5-years. [50, 116, 117] In between the 5-year and 8-11-year visit, one implant failure occurred.

In this 8-11-year follow-up, 3 patients were satisfied with their implants and were not interested in travelling for the examination. This meant that 18 implants were censored, which resulted in 19 included subjects in the current follow-up. The mean age of these were 80.5 years (range 71-91 years) with a gender distribution of 13 females and 6 males. Two of these subjects were not willing to participate in the clinical examination because fear of complications when removing the fixed prosthesis, but they were willing to undergo the radiographic examination. These two subjects with 12 implants were only followed in the radiographic evaluation.

During the clinical and radiographic examination, 11 implants were assessed as failures according to criteria stated by Misch et al. [135] One of these implants was marked as notably mobile already at the 5-year examination.
The mean follow-up time from implant surgery to the study visit was 9 years 2 months (101-131 months). Radiographic evaluation showed a mean marginal bone loss of 1.29 mm (SD 2.47 mm, range 0-11 mm), see table 2, next page. A significant difference was observed between bone loss and bone quality ($p=0.021$), with less bone loss for category 3 (Lekholm and Zarb [5]), but no difference for bone quantity ($p=0.531$). No other differences could be observed between marginal bone loss and the different factors as the different implant positions or between the two sides of the implant (mesial and distal). Neither were there any differences between the implant survival and implant length.

At the last visit, the clinical examination included 101 implants, where plaque was detected in contact with 43 implants (42.5%) as well as bleeding upon probing at 73 of the implants (72.2%). As one of the implants had to be removed during the visit, pocket depth was measured on the remaining 100 implants (seventeen subjects) and resulted in a mean of 3.1 mm (SD 2.4 mm, range 1.5-13.5 mm). At the 8-11-year follow-up, the cumulative survival rate was 81.9 % and cumulative success rate was 74.7%, see figure 12, next page.

Only one patient smoked (>5 cigarettes per day for the last 50 years) and two patients used snuff daily. At the clinical examination, swollen gingiva in one patient and notable buccal gingival retractions in another patient was

observed. In addition, the clinical examination also revealed six fractured or chipped prosthodontic constructions in need of repair.

Table 2. Table presenting the marginal bone loss during the follow-up up to 8-11 years.

	1 y	2 y	3 y	5 y	8–11-y
Number of implants	129	130	132	132	100
Mean	0.19	0.39	0.75	0.61	1.29
SD	0.40	0.47	0.92	1.19	2.47
Minimum	0.00	0.00	0.00	0.00	0.00
Median	0.00	0.28	0.50	0.25	0.35
Maximum	2.55	2.60	5.95	8.85	11.00

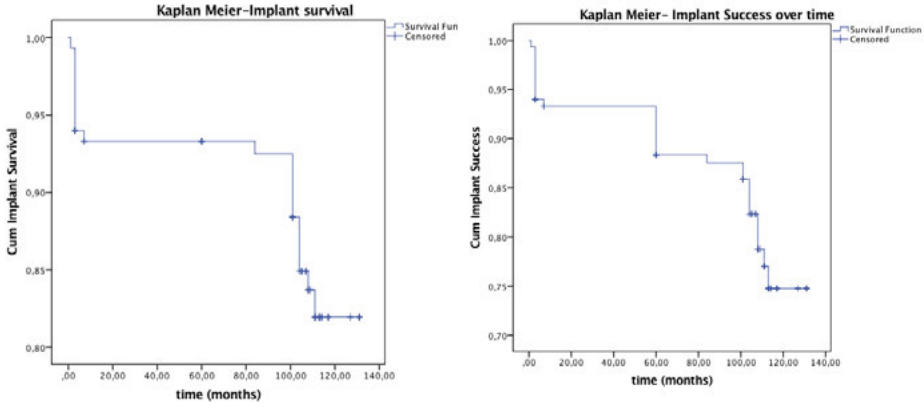

Figure 12. The left image showing implant survival Kaplan Meier curve and the right the implant success Kaplan Meier curve during a follow-up period of 11 years.

Discussion:

This prospective study was an 8-11-year follow-up of subjects with totally edentulous atrophic maxillae that received six immediately loaded implants without any additional bone augmentation. The outcomes of this cohort was included in previous published follow-ups of 1-, 3- and 5- years. [50, 116, 117] In this cohort we found 1- and 3- year cumulative survival and success rate of

93.3% and 93.3%, respectively, and at the 5-year visit cumulative survival of 93.6 % and success of 88.4 % with a marginal bone loss of 0.61 mm (SD 1.19 mm, range 0 - 8.85 mm). The implant survival rates of this study correspond to those that were published at the 1-, 3- and 5-year follow-up. At the last visit at 8-11-years the cumulative survival rate had dropped to 81.9 % and success rate to 74.7 %, with marginal bone loss of 1.29 mm (SD 2.47 mm, range 0 -11 mm).

The immediate loading treatment option is attractive for patients, because it offers a faster, less costly treatment with minimal morbidity. It has shown similar success rates in carefully selected patients as conventional loading. [53] However, the current study included only demanding patient cases with poor bone quality and quantity (as defined by Lekholm and Zarb [5]), which would have been proven challenging cases to treat even with extensive bone augmentation.

To improve the primary stability in poor bone quality, the implant sites were prepared with an under-dimensional drilling protocol, which resulted in acceptable stability, and temporary splinted and loaded implants within 24 hours. In addition, implants with self-tapping engaging threads and a fluoridated surface were used to potentially enhance the bone healing. The fluoride acid etched surface have been evaluated in studies such as Ellingsen et al., who found a higher degree of bone contact with fluoridated implants as compared to non-surface modified implants in a rabbit tibia study. [46] Collaert et al. presented a 2-year survival of 100 % with fluoridated implants placed in the mandible with bone loss of <1 mm after 24 months. Verveake et al. showed less marginal bone loss in implants that were TiO_2 blasted and fluoridated than in implants with only TiO_2 blasted surface, when used in edentulous mandibles.[136, 137] Widael et al. observed a survival rate of 100 % after 10 years and bone marginal loss of 0.49 mm (SD 1.08, range 0-7), in mandibles with predominantly bone quantity of A and B.[138] However, the results of our long-term follow-up indicate that implant surface modification might not compensate for compromised host bone.

This study shows lower implant survival results than reported in studies of conventional loading protocol in edentulous maxillae. Rasmusson et al. reported a cumulative implant survival rate of 96.6 % in 36 patients (with 199 implants inserted in the mandibles and 108 in maxillae) after 10 years from implant placement, as well as Mertens et al., who observed a cumulative survival rate of 99 % after 8 years for 17patients (with 106 implants in the maxillae).[139, 140] Also, Jemt et al. showed a cumulative survival of 90.9 % after 15 years for 76 patients with 450 turned Brånemark implants placed in the upper jaw. [141]

Studies with immediately loaded implant protocol show high implant survival, such as the study conducted by Cassetta that presented a 10-year implant survival of 97.9 % for 16 patients with an average of 7.37 implants in the maxillae and 5.83 implants placed in the mandible. [52] Testori et al. observed in one study of 21 patients (35 implants) that immediate loading had similar long-term results as the delayed loading, in different fresh extraction sites in both maxilla and mandible. In another study, they reported a 10 -year implant survival rate of 95.1 % for a patient cohort of 27 patients (162 implants). [36, 142]

When considering the results from these studies the low implant survival result in our study is unlikely caused by the immediate loading, but rather due to the poor bone quality, which has been described before in studies as a predictor for implant loss. Chrcanovic et al. showed that a bone quality of 4 was significantly more represented with lost implants than in any other quality criteria group 1-3 (criteria according to Lekholm & Zarb). [143]

The reason of this decline from the 5-year to the 8-11-year follow-up is difficult to explain. One reason we could think of is the lack of regular dental follow-ups after the 5-year visit. In between of the 5-year and 8-11-year follow-up, the subjects were asked to be enrolled and followed at the nearest dental clinic of their choice, but many of them declined this, as they did not experience problems.

We also observed an implant cluster behavior (including three lost implants or more) in five of the seven subjects with failed implants. Factors as limited bone support and/or poor bone quality are well-reported risk factors as well as heavy smoking and overload problems or bruxism.[144 145 146]

Paper III & IV.

Results:

Surgeries and post-surgeries were uneventful. SAA levels were below the detection level (<4.7 ng/ml) in all but two rabbits before surgery, and had increased three days post-surgery, to 37 ng/ml (range 12–182 ng/ml) in Bup + Carp group, and to 51 ng/ml (range 23–199 ng/ml) in Bup + Sal group. 21 days later, the SAA had dropped to preoperative levels.

For the Bup + Carp group mean weight loss was 0.28 ± 0.13 kg (8%) and for the Bup + Sal group 0.24 ± 0.26 kg (7%), but by day 21, the body weight had returned to the preoperative level. No significant differences were seen between the two groups for SAA levels or weight.

Facial expression scoring:

Only two of the five FAUs (facial action units) could be evaluated. These were the eye tightening and ear positioning, (nose pointing and cheek flattening were not detected and whisker position was not possible to score). Mean subjective scores increased from 0.0 to 2.6 ± 0.6 in Bup + Saline group and to 2.3 ± 0.2 in Bup + Carprofen group. No differences were found

between the NSAID group (Bup + Carprofen) and Saline group (Bup + Saline) in regard of the eye and ear scores at any time or before and after opioid (buprenorphine) administration.

Qualitative assessment (Figure 13, next page*):* Bovine bone mineral was identified as "bone chips" and was incorporated in new bone in 17/18 animals. The implant incorporation in new bone was scored as good or satisfactory in 28/35 sections. In the CPC sections, brown granular pieces of the material could be observed and all of them 8/8 were associated with new bone. The implant incorporation score was determined as good or satisfactory in all sections. The HABP·CaP sections displayed multifocal deposits of pale yellow-brown fine granular material with poor bone incorporation in all 9/9 sections as well as in degree of implant incorporation in 6 of 9 animals. An inflammatory reaction with multinucleated giant cells, macrophages, compatible with foreign body reaction was observed in 1/18 control sections, 0/8 CPC sections and in 9/9 HABP·CaP sections. One CPC section displayed evidence of a mild purulent sinusitis and another one of a moderate purulent sinusitis, which was associated with rupture of the sinus mucosa and displacement of the CPC material.

Histomorphometry: No difference was seen between control and CPC sections in any of the ROIs regarding new bone formation or BIC. There was however, a difference in BIC between control and HABP·CaP ($p=0.05$). Further, a difference was seen comparing new bone formation between CPC and HABP·CaP in the Free ROI ($p<0.001$), in the rectangular ROI ($p < 0.001$) and in the implant ROI ($P=0.004$). CPC showed more new bone than control in the implant ROI (<0.05). There was no difference between new bone formation in the NSAID (carprofen) group or control (saline) when compared between the two groups of bovine bone mineral, nor was it seen when tested between the two test materials (CPC and HABP·CaP). (Figure 14)

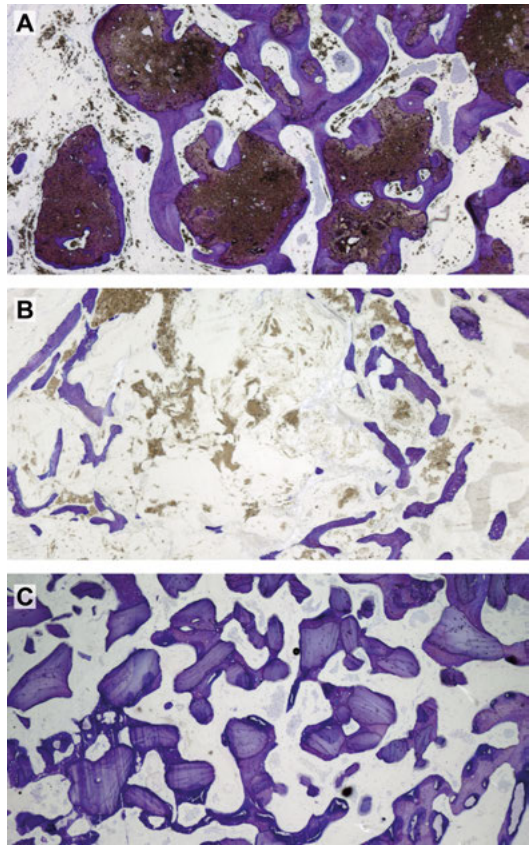

Figure 13. Image showing the three different grafting materials used in the sinus augmentation study with magnification of x40, a. CPC granules (brown colour), b. HABP·CaP (colourless and brown material), c. bovine bone mineral (the bovine bone mineral chips coloured light purple)

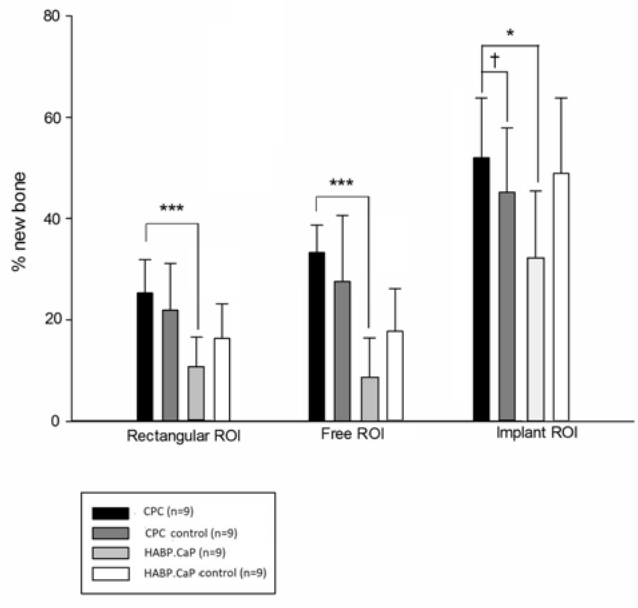

Figure 14. Image above presents percentage of new bone formation at the three regions of interest with: CPC granules (n=9), HABP·CaP (n=9) and bovine bone mineral (control) (n=18).

Paper III:

Discussion

This study was conducted to assess if carprofen had any additional effect on pain relief in buprenorphine treated rabbits and if it affected new bone formation in a sinus augmentation model.

The pain assessment was based on a facial expression scoring described earlier by Keating et al., [115] which revealed increased scores for eye tightening and ear position. This suggests that these two parameters may be most useful for assessing pain. The rest of the three FAU-categories (flattening of the cheeks, pointy nose and change in whisker position) were not used because they were either not observed or difficult to evaluate.

However, the results did not show any differences between rabbis that were treated with carprofen treated rabbits and those that were not. There were also no differences seen before or after buprenorphine administration. Neither was any difference of bone healing observed between the carprofen group and the control (saline) group.

There is no validated pain scoring system for postoperative orthopaedic pain in rabbits. The assessment needs to be sensitive enough and pain as-

sessment is difficult in rabbits. Keating et al. presented facial expression scoring as a good method for assessment of nociceptive pain in rabbits, which is different from postoperative pain. Assessment methods as described by Leach et al. take very long and are adjusted for abdominal surgery. [147]

There can be different reasons why no added analgesic effect was shown in the carprofen group. It could be that the scoring method is not sensitive enough, or that carprofen did not add any supplementary analgesia to that of buprenorphine, or that the dose was too low. Lower doses than the 5 mg/kg used in our study have been shown to reduce pain [148]. We also used a higher dose of carprofen (5 mg/kg) than recommended in other studies (2-4 mg/kg). Eye tightening can be also be caused by sedation as a residue of anaesthesia. [149] The fact that no difference was seen between before and after buprenorphine administration was also peculiar, but may be explained by the fact that the analgesic peak occurs later than one hour after buprenorphine administration. Readministration every 8-12 hours has been proposed, while the rabbits in this study were re-administered after 6 hours.[148, 149] The inflammatory acute phase protein SAA did not show any difference between groups, which could indicate that the carprofen dose was not optimal. Another type of study of the efficacy of pain relief could be a comparison between carprofen and buprenorphine. It has been shown that sinus elevation procedure does not induce high level of pain in humans compared to other surgical treatments. [150]

The bone healing seemed not to be affected by the additional administration of carprofen in either the control or the tested material groups. This goes against several animal studies that have shown that NSAIDs interfere in prostaglandin signalling between cells involved in bone healing, during the inflammation phase by inhibiting COX-1 and COX-2, which have been shown to be important in bone healing. [151]Also, this NSAIDs can disturb other mechanisms by modification of IL-1 and IL-6 release.[152] Zhang et al observed that bone formation in knocked out mice, that lacked COX-2, displayed disturbed endochondral and intramembranous bone formation.[16] Although, Oh et al. showed in a vitro study, that bone marrow-derived mesenchymal stem cells from dogs have a compensatory mechanism for NSAID that regulates the levels of PGE2 and helps to normalize the osteogenesis. [19]

Discussion

Paper IV.

In this sinus lift model with simultaneous implant placement, the CPC granular material showed similar new bone formation and implant osseointegration, as did bovine bone mineral (control). However, the HABP·CaP material displayed less than both the control and the CPC material in both categories.

Differences were also noticed during surgical application of the different materials in the sinus cavity. The CPC granules and bovine bone mineral

were solid and easily applied whilst the HABP·CaP had gel-foam consistency that disintegrated into smaller pieces in contact with blood, which made it more difficult to apply at the intended site. The HABP·CaP materials load-supporting abilities are questionable, but as the sinus mucosal membrane was supported by the implant this cannot be evaluated. One advantage of the HABP·CaP material over the other two, is that it can be injected and thereby could be less invasive.

Some difficulties were encountered with this experimental model. It was difficult to place all the implants in the same angle, which complicated the slicing of the histological sections. We tried to facilitate the location of the implant and material with the help of CBCT. The individual rabbit anatomy with different variations, e.g. sinus septae, made it difficult to apply the same amount of material. We solved this by inserting as much material as possible around the titanium implant.

Bone formation was expected in all sections, as the sinus lift model without augmentation (only blood clot) together with a direct implant placement is a well-documented procedure. [100, 103, 153] The histology and morphometric evaluation showed bone integrated CPC granules that displayed osteoconductive properties. New bone was also seen in contact to the bovine bone mineral chips. The HABP·CaP on the other hand, displayed bone formation in the periphery of the material and a thin bone deposit adjacent to the peripheral material border. Small discarded pieces of the test materials were exhibited in the adjacent soft tissue, but only inflammatory cells could be seen in the HABP·CaP sections. The calcium phosphate materials are known to degrade both with dissolution and cell phagocytosis. It is unclear why inflammation cells were seen in the HABP·CaP sections and not in the CPC sections, as we believe that both display small discarded calcium phosphate pieces.

The CPC material presented in this study has been tried in severe cranial skull defects and has been a successful scaffold, in that it does not induce any adverse reactions in either bone or adjacent soft tissue. The cranial vault scaffold that was built of hexagonal ceramic tiles, also displayed bone-healing properties onto, and in between these tiles after 50 months from insertion. [94 96] The authors stated that the specific blend of the calcium phosphates together with β-calcium pyrophosphate, is the key to the bone healing and the slower, but still theoretically, resorbable properties of the material. For facilitating sinus augmentation, the CPC material was adjusted to granular form of sizes between 0.60mm–1.18 mm, which was like the bovine bone with sizes between 0.25mm–1.0 mm. The granular size may be a factor that affects the resorption of the material and affects theoretically the space maintaining time of the material, which could lead to less bone formation. Longer follow-ups with different termination points and different granular sizes would give us more information about the *in vivo* resorption rate of the material.

The hyaluronic acid hydrogels, especially with added BMP-2, have shown promising results of new bone formation in animal studies when injected in different locations. [154] [155] A study by Martinez et al. conducted on rats, showed that a hyaluronic acid-based hydrogel with added hydroxyapatite and BMP-2, induced notable bone formation when injected subperiosteal in a mandibular model, and also that the bone formation was dependant on the BMP-2 concentration. [156] Hulsart-Billström et al. observed in vivo that a bisphosphonate linked hyaluronic acid hydrogel with added BMP-2, released 10% of BMP-2 compared to the same hydrogel without bisphosphonates, that released 100% over two weeks. They argued that this slower release depends on the added BP. [98] In a recent study by Shi et al., two hyaluronan hydrogels, one with BP and the other without, were tested in vitro in a phosphate buffered saline, with or without Ca^{+}. The study showed that the additional BP reduced notably degradation of the hydrogel, but only in the Ca^{+} solution.[157] This could have been the reaction between Ca^{+} and BP that we saw in our study, resulting in both slow degradation of the material and low release of BP in the surroundings.

The rabbit as described earlier is a well-established animal for testing biomaterials and dental materials in bone healing research. Several methods like the sinus lift have been tested earlier and are comparable to other studies.[108, 110, 158] The fast bone healing, easy handling and cost for up-keeping makes these animals an attractive alternative.[111] In our study, the animals tolerated the procedure, and recovered quickly, with no clinical complications in the experimental area.

Paper V.

Results

Animals: 14 animals were left until the end of the study. The groups were as follows: CPC+ floor housing (n=3); CPC + cage housing (n=3); AB + floor housing (n=4); AB + cage housing (n=4). These animals remained healthy throughout the study.

Radiographic analyses: Quantitative μCT evaluation showed no difference between CPC implant and AB in bone+material volume, in neither the restricted nor the unrestricted volumes. The restricted volume was higher in density in the CPC group than in AB group but no difference was found for the unrestricted volume. Good correlation (0.83-1) could be observed between observers for *the defect scoring*. No effect of treatment on the bridging score was seen [CPC: 3.3 (1-4); AB: 4 (3.7-4), p=0.06]. A higher score for the filling of defect was seen in the CPC group [4.0 (3.3-4)] than in AB group [3.2 (1-4)], (p=0.0495).

Histological observations (Figure 12): The CPC material was observed as brown/black/dark purple coloured and it took up a major part of the defect area. Bone formation was found in multiple areas throughout the entire implant in all CPC sections. Both woven and lamellar bone was observed in

contact to the material. Internal disruptions/fractures of the implant could be seen in all CPC sections, but all except one implant held their form. This one displayed a dislocated implant fracture from the middle of the defect, but seemed to be stabilized by new bone formed. Adjacent to the larger material segments, smaller detached material pieces were seen. They were non-coloured/brown particles embedded in soft tissue and in larger magnifications looked like they were intracellular. The AB fragments were not easy to distinguish from the new bone, which meant that estimation of new bone formation was not possible.

The AB sections showed varying cortical and cancellous maturation ranging from woven bone to complete reorganization of cortex and trabecular bone. Fibrocartilage was found mostly in AB sections and resulted in transverse areas without bone fusion. Both CPC and AB sections revealed an ulnar periosteal reaction but with less fusion in the CPC sections between the bones (radius and ulna), especially in the middle parts of the defects. Inflammation was observed in multiple areas and in the periosteal fibrous tissue. This area contained mononuclear round cells of macrophage/monocyte characteristics, including multinucleated giant cells either in association with the test material or near bone tissue. The CPC defects often showed for multiple areas of more macrophages/monocytes when compared to the AB, but AB sections displayed a fibrous periosteum-like formation that often-contained macrophages/monocytes diffusely distributed in the collagen tissue.

Histomorphometry: The CPC (0.54 ± 0.13 cm^2) filled less of the defect area compared to AB (0.66 ± 0.08 cm^2, $p=0.034$) when both bone and material was included. No difference was seen in the total ROI area of the two materials (CPC: 31.3 ± 2.3 mm2; AB: 26.5 ± 2.6 mm2, $p=0.21$). The CPC group displayed a bone area fraction of 47.7 % whilst AB the group showed a bone area fraction of 66.9 %.

Histology scoring is presented in figure 11: The AB group showed a higher score on b.) *cortical integrity (longitudinal: $p=0.03$ and transversal: $p=0.01$ sections), c.) cancellous bone (longitudinal and transversal sections $p<0.01$), e.) the filling of the defect (longitudinal section: $p=0.03$), and on f.) bony fusion with ulna in the defect area (transversal section $p< 0.05$)*

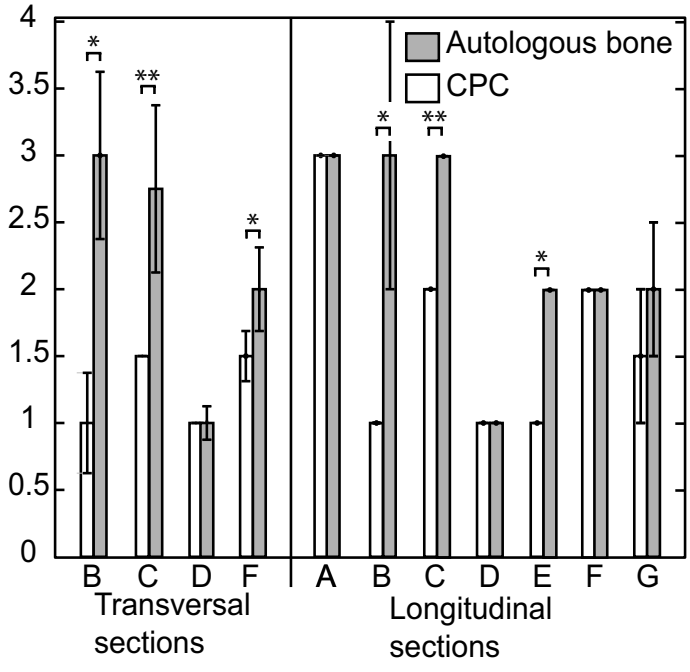

Figure 11 . The qualitative histology scoring for the transversal and longitudinal sections.

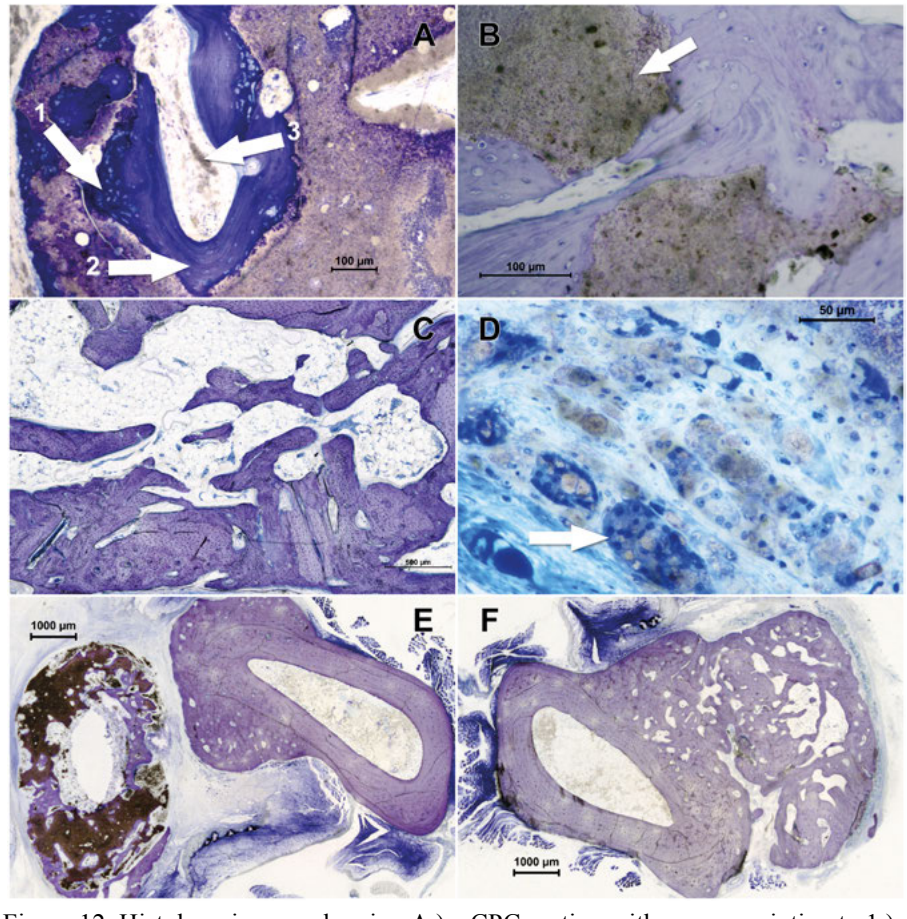

Figure 12. Histology images showing A.) a CPC section with arrows pointing to 1.) woven bone, 2.) lamellar bone formation and 3.) detached pieces of the graft material. B.) Shows a magnified CPC section with arrow pointing at the material. C.) This is an AB section presenting integrated lighter purple coloured pieces in darker coloured bone D.) A multinucleated cell is seen containing/in contact with small pieces of graft material. E). shows a transvers cross section of CPC material were bone can be seen in contact to the CPC implant and a soft tissue space between ulna and radius, F.) is an AB section and presents the ulnar bone and the new bone formation in contact to it.

Discussion

The purpose of this study was to evaluate the properties of an implant replica made of a specific calcium phosphate compound in a critical radius bone defect in rabbits. Bone was found mostly in contact to the fracture ends but also in places throughout the entire defect, in the defects with the replica, which indicates that the material possesses bone-conducting capabilities. Particulate autologous bone was used as control. It showed, in some cases, good regeneration of the defect area with complete cancellous maturity but

was variable in respect of bone formation and maturity in between the AB specimens.

To attain the best possible evaluation of bone formation in the different defects, the investigations were performed by both radiographic and histological analysis, including both qualitative and quantitative measurements. The μCT was a helpful tool in quantifying the grafted volume but a drawback of the method was the difficulty to differentiate bone from the test material because of the similar attenuation. What we could quantify was the total volume of new bone together with the graft material, using four different regions of interest. The results showed no differences in the filled defect volume between the two materials but a large part of the volume (CPC 30%, AB 45%) was found outside the previous radius segment. The qualitative scoring of the 3D reconstructions showed similar results for bridging of the defects and slightly better for the CPC material in filling of the defect.

The histology and morphometric evaluation revealed that less bone was formed in the CPC sections than in AB after 12 weeks. Bloemers et al. presented similar results where calcium phosphate materials were compared with autologous bone in long bone defects. [159] Interestingly, the morphometric results showed nonetheless 47.7% bone in the CPC sections in this study. Another observation of this defect model is the ulnar thickening and union with ulna, described earlier i.e. by Bodde et al. and Zhao et al. and further confirmed in this study, especially in the AB sections. [119, 160] The ulnar union at the middle of the defect was noticed to be less prevailing in the CPC group. As mentioned earlier, the new bone formation followed the implant well and was found in sites throughout the whole CPC graft, even if it was primarily seen in contact to the fracture ends.

The difference in this study compared to our earlier study with ceramic compound, Trbakovic et al., [161] was that we used bovine deproteinized bone mineral as control, and AB in the present study. The bovine bone mineral was easy to recognize, and differentiate from newly formed bone, whereas a differentiation between old and new bone was not possible in the present study. This could have been achieved by using e.g. calcein green injections as suggested by Zhao et al.[160] Autologous bone chips of 0.5-2.0 mm^2 size were observed to induce larger initial bone volume than smaller or larger AB particles in a rat calvaria model and even if reduced amount of graft material remained after 4 weeks, it could still be found in the defects. [162] The AB chip size in our study was between 1-3 mm^2. Even if we can expect reduction of the graft volume, as was seen in the previously mentioned study by Pallesen et al., we still cannot conclude that the AB samples were graft- free after 12 weeks. [162]

In contrast to the previous conducted study in which we evaluated the same calcium phosphate material, we found in these CPC sections inflammatory cells including monocytes, macrophages, and multinucleated cells in these CPC section. The macrophages and multinucleated cells were often

found often in contact to the material, especially the small-detached material pieces. The inflammatory cells have been seen in contact to calcium phosphate materials in other studies [163] [164] and a recent review by Miron et al., presented the biomarkers to differentiate between the inflammatory cells found around calcium phosphates. They also argued that the roles of different inflammatory cells role is still not clear, as macrophages have been found to differentiate into M2-type, which can instead of having a pro-inflammatory role, acquire theoretically a bone-healing role. [165]

Mononucleotide cells were also seen in the AB samples, but here they were generally located in the periosteum-like layer where probably the collagen membrane was localized and were now no longer possible to differentiate from the soft tissue. Von Arx et al. studied this and saw similar degradation, but no inflammatory cell reaction, in the presented collagen membranes of their study.[166] The collagen membranes stabilizing effect on the bone chips that we desired to obtain in this study seems to not have been reached. A more rigid membrane, e.g. a titanium reinforced polytetrafluoroethylene (PTFE) membrane, could perhaps have held the formation better.[56, 167]

Even if we observed plenty of internal fractures in the implant walls, the new bone may have reinforced the implant to a degree, because only one dislocated implant fracture was seen in this otherwise brittle material. It would be of interest to evaluate the CPC implants mechanical stability and degradation after a longer healing period. As presented by Habraken et al., studies of calcium phosphates have shown the materials positive abilities regarding bone healing, but emphasizes also the importance of design of the scaffolds for this purpose.[84] The presented implant design in this study showed us that the bone formation benefitted from the hollow central canal but that bone formation was mostly seen in the walls. A design with several smaller canals that enable an increased blood flow through the implant would perhaps enhance bone formation. It would maybe also keep the necessary rigidity and stability until the bone formation is complete, but theoretically, also give rise to additional higher level of formation.

The long bone models in rabbits are useful for testing biomaterials and especially this model, were no fixation is needed. [168] The care needs to be taken to use adult rabbits with a defect >15 mm and to remove the radius periosteum, preferably 5 mm further from the radius fracture ends, as this otherwise may result in a non-critical defect. [119, 160, 169, 170] In the present study only adult rabbits were used and the defect of 20 mm with removed periosteum was created, which should have resulted in the desired critical defects.

Considerations and clinical implications of the suggested bone substitute materials

As presented in this thesis and in earlier studies, [96, 98] the materials tested here are biocompatible and non-toxic to the surrounding tissue, and they display varying amount of the desired new bone formation, as seen in the presented sinus elevation model in rabbits.

The bisphosphonate hyaluronan hydrogel was tested earlier in an in vitro model together with BMP-2, where it showed positive in vitro effects on differentiation of mesenchymal stem cells. The most important finding was that the hydrogel seemed to reduce the release of BMP-2.[98] The question was if the hydrogel would induce bone formation even without added growth factors? We saw that some bone formation was formed in the periphery of the material. We are not sure if this is due to the material itself or a result of the sinus membrane elevation procedure. The hydrogel is intended to be injected, even if it could be applied by other means. As it does not seem to be loadbearing and a bit delicate to apply, the material in the existing form is questionable as bone substitute material for maxillofacial use with or without growth factors.

The calcium phosphate material has the advantage of being mouldable and adjustable for different purposes.[84] The solid form of the presented specific CPC in these studies seems to be brittle, although the mechanical properties are not evaluated yet (for the granular and segmental implant). An accepted study by Linder et al., with similar CPC material composition, showed that a titanium-CaP scaffold (made of a titanium mesh incorporated into ceramic tiles) could absorb almost twice the load energy in a mechanical test compared to the titanium mesh alone.[171] The same study presented an explanted graft after 24 months and showed both woven and lamellar bone around and in-between the calcium phosphate tiles in histological evaluation. Interestingly, bone growth was observed at tiles without any other contact with original bone or other tiles. This notation is similar to the observations made of the CPC implant in our study, where new bone formation was seen in the middle of the implant, which had no contact with bone ends or the periosteum.

We believe that the granular form of the material also should be able to work as bone void filler in the maxillofacial area. It could perhaps be an alternative to bovine bone mineral, as it showed similar bone formation and well-integrated granules in the sinus augmentation model. For optimal bone gain, the material resorption rate and thereby the optimal granular size should be evaluated in future studies. For now, we are in the process of testing the CPC material in granular form in a human clinical sinus augmentation study with direct implant installation. As mentioned before, the design of the implant is important for the outcome and should carefully be considered for the different purposes. A design like the segment implant we presented attained, in all sections, internal fractures in a model that should have

been relatively stable. Again, in the study by Linder et al., it took a load of 550 N to break a ceramic tile.[171] This means that future solutions with individually custom-made solid implants, as seen in our radius model and the orthopaedic scull defects,[94] may soon be relevant for the maxillofacial area. In loadbearing areas of the face, a supplementary reinforcement could be necessary. This may be attained with different titanium solutions as in the skull defects. Further studies with granules and membranes for alveolar crest reconstruction, will also be of immediate interest for the immediate future.

Conclusions

Paper I
We found very few clinical problems connected to the reduced tooth-implant distance in this study, but an extra focus should perhaps be set on the buccal bone and the soft tissue aesthetics in these compromised sites. Thus, bone augmentation should be considered prior the NDI treatment in the aesthetic zone.

Paper II
Immediate loading protocol can be satisfactory for selected patients but for patients with severe risk factors, a more conventional implant protocol such as bone augmentation should be considered, and close follow-ups are recommended as late implant failures may still occur.

Paper III.
Scores for the FAU categories (eye tightening and ear position) were increased after surgery, but carprofen was not shown to reduce the pain or to affect the bone formation in sinus augmented rabbits.

Paper IV
From this in vivo study, we observed that the two test materials had different handling properties and the bone formation was larger in the CPC samples than HABP·CaP hydrogel but equivalent to bovine bone mineral.

Paper V
The tested CPC implant was integrated in the radius long bone and exhibited new bone formation in places along the whole scaffold. Further follow-ups to evaluate the long-term bone formation, material degradation and mechanical properties are required before human studies are performed.

Summary

The clinical studies in this thesis highlight a problem area that is still present, 60 years after the introduction of dental implantology. For most patients, a simple implant treatment without any additional actions is sufficient, but questions remain how we can help patients with limited alveolar bone volume, to gain long-term function and aesthetics with a fixed implant prosthesis? Many treatment options are offered for this problem, but they often include drawbacks as longer healing time, risk of donor site morbidity, higher costs, or not entirely optimal bone substitute materials.

The clinical studies we present here, evaluate if treatments with a faster implant protocol in compromised sites are sufficient to achieve good long-term implant function.

The first study consisted of a heterogenic age group of patients with limited bone in the aesthetic front area. An indication of long-term survival of narrow diameter implants placed in these challenging sites was achieved. Further, we found that even with closer distance to the neighboring teeth than generally recommended, the placement of these implants did not induce any clinical pathology at the neighbouring teeth. Interestingly a side issue was raised regarding the buccal bone and adjacent soft tissue. It seemed to, in some cases, have been negatively affected by the treatment, a complication that may have been avoided by augmentation.

In the second clinical study, patient satisfactory scores were high after receiving an implant treatment with immediate loading. Most of the patients had an uneventful healing process, except in three subjects, who showed early cluster implant failures. Most subjects experienced good survival and function of their implants until the last 8-11-year visit, but at this time-point we found additional four patients with failed implants. A lower implant survival and success rate, 81.9 % and 74.7 % respectively, was stated for this study compared to other similar implant studies with immediate loaded implants in the maxillae. As well as in the first study, we could conclude that bone augmentation, not stating which type of augmentation, together with implant treatment, would probably have been a more successful combination for these failed cases.

For augmentations, the goal lingers on to find a solution that may be more *biocompatible* than the patient's own bone and as mentioned before, donor site morbidity, graft resorption, longer healing time and costs motivates the research for other alternatives.

For the experimental part of this thesis, we tested two synthetic materials that have shown promising in vitro and in vivo results. We also evaluated if additional NSAIDs would reduces pain and bone formation in the sinus lift model. Here, we have started to explore the two test materials abilities to function as bone substitute materials in the maxillofacial area. This was performed in experiments with the materials in two different animal models in the rabbit; a sinus augmentation model with direct implant placement and a long bone defect model. One of these materials was a bisphosphate hyaluronan acid hydrogel and the other a calcium phosphate material in granular form. We observed larger bone formation and implant integration of the calcium phosphate material than the bisphosphate hyaluronan acid hydrogel. The CPC material had similar bone forming capabilities as a frequently used bovine bone mineral. The NSAID did not seem to affect the bone formation or reduce the pain scores. We proceeded with testing this material in a more challenging long bone model that bears resemblance with the compact mandibular bone. The 3D printed calcium phosphate implant, a replica of a radius segment, was inserted into critical radius defects. It displayed bone integration not only at the ends, were it was expected, but also generated new bone in places along the entire implant. Both these materials possess downsides. The calcium compound is brittle and the hydrogels have poor load bearing abilities, but by evaluating and adjusting these materials in vivo models that resemble the clinical situations, they can slowly be improved and in the future, may come to even replace autologous bone as graft material or be an alternative to bovine bone mineral.

Sammanfattning

De kliniska studierna i denna avhandling belyser ett problemområde som fortfarande existerar, 60 år efter det att implantat introducerats som behandlingsmetod. För majoriteten av patienter är en enkel implantatbehandling utan ytterligare åtgärder tillräcklig, men för patienter med begränsad benvolym och kvalitet kan det behövas ytterligare åtgärder. Många behandlingsalternativ finns även för denna grupp men innefattar ofta längre läkningstid, risk för komplikationer, exempelvis där man tagit bentransplantatet, högre kostnader eller material som inte är optimala för ändamålet (försvinner snabbt, animaliskt ursprung som kan innehålla proteinrester). De kliniska studierna som vi presenterar, utvärderar om behandlingar med kortare implantatprotokoll i områden med otillräcklig benvolym bibehåller funktionen under lång tid.

Den första artikeln behandlar en spridd åldersgrupp av patienter med begränsad benvolym i estetiska zonen. Den gav oss en indikation på hur väl dessa smala implantat fungerar samt att, trots den snäva placeringen till de närliggande tänderna än vad som generellt rekommenderas, vi såg väldigt få kliniska tecken som kunde härledas till det reducerade avståndet. En intressant observation var dock att buckala benet och närliggande mjukvävnad blev negativt påverkad av behandlingen, vilket troligtvis hade kunnat undvikas med benaugmentation innan, eller samtidigt med implantatinstallationen.

I den andra artikeln framgår det att de flesta patienter hade en problemfri läkningsprocess, förutom tre stycken som drabbats av tidiga implantatförluster. Utöver de tidigt påträffade fallen hade de flesta av patienternas implantat god funktion vid 1-, 3- och 5-årskontrollen samt även vid den sista 8 till 11-årskontrollen, utöver de ytterligare fyra patienter som bedömdes ha implantatförluster. En lägre överlevnadsgrad (81,9 %) och lyckandesgrad (74,7 %) fastställdes i denna artikel, i jämförelse med andra liknande studier med direktbelastade överkäksimplantat. Såsom i den första artikeln kom vi även här fram till att benaugmentation troligtvis hade varit en mer framgångsrik metod i de fall där implantaten fallerat. Det egna benet är det mest kompatibla alternativet för transplantation, men som tidigare påpekats så är komplikationsrisken, risken för okontrollerad transplantatnedbrytning, längre läkningstid och högre kostnader motiv för forskning efter andra alternativ.

I denna avhandling presenterar vi två syntetiska material som har visat sig ge lovande resultat i in vitro och in vivo studier. Vi undersöker dess förmåga att fungera som benersättningsmaterial inom det maxillofaciala området

genom att testa dem i två olika djurmodeller; en augmentationsmodell i bihålan med samtidig implantatplacering samt en rörbensmodell. Ett av de aktuella materialen var en hydroxyapatit hyaluronsyra-hydrogel med kopplad bisfosfonatgrupp och det andra en blandning av kalciumfosfatmaterial i granulform. Bentillväxt och implantatintegrering observerades i större grad i kalciumfosfatsnitten än i hydrogelen. Därför testades detta material i en mer utmanande rörbensmodell som har likheter med mandibeln. Implantatet av kalciumfosfat som installerades i den kritiska radiusdefekten påvisade benintegrering inte bara i ändarna som förväntat, utan även i områden längs med hela implantatet. Båda dessa material uppvisar nackdelar, kalciumfosfaten är skör och hydrogelen har dålig belastningsförmåga, men genom ytterligare utvärdering och justering av dessa material med hjälp av in vivo-modeller som liknar kliniska patientfall, kan de så småningom förbättras och i framtiden möjligtvis ersätta autologa bentransplantat.

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References

1. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. *Clin Implant Dent Relat Res* 2012; **14**: 783-791.
2. Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Factors Influencing Early Dental Implant Failures. *J Dent Res* 2016; **95**: 995-1002.
3. Erkapers M, Segerstrom S, Ekstrand K, Baer RA, Toljanic JA, Thor A. The influence of immediately loaded implant treatment in the atrophic edentulous maxilla on oral health related quality of life of edentulous patients: 3-year results of a prospective study. *Head Face Med* 2017; **13**: 21.
4. Marra R, Acocella A, Alessandra R, Ganz SD, Blasi A. Rehabilitation of Full-Mouth Edentulism: Immediate Loading of Implants Inserted With Computer-Guided Flapless Surgery Versus Conventional Dentures: A 5-Year Multicenter Retrospective Analysis and OHIP Questionnaire. *Implant Dent* 2017; **26**: 54-58.
5. Lekholm U ZG. In: Patient selection and preparation. Tissue integrated prostheses: osseointegration in clinical dentistry. Chicago
6. Cawood JI, Howell RA. A classification of the edentulous jaws. *Int J Oral Maxillofac Surg* 1988; **17**: 232-236.
7. Nystrom E, Nilson H, Gunne J, Lundgren S. Reconstruction of the atrophic maxilla with interpositional bone grafting/Le Fort I osteotomy and endosteal implants: a 11-16 year follow-up. *Int J Oral Maxillofac Surg* 2009; **38**: 1-6.
8. Dasmah A, Thor A, Ekestubbe A, Sennerby L, Rasmusson L. Marginal bone-level alterations at implants installed in block versus particulate onlay bone grafts mixed with platelet-rich plasma in atrophic maxilla. a prospective 5-year follow-up study of 15 patients. *Clin Implant Dent Relat Res* 2013; **15**: 7-14.
9. Dahlin C, Johansson A. Iliac crest autogenous bone graft versus alloplastic graft and guided bone regeneration in the reconstruction of atrophic maxillae: a 5-year retrospective study on cost-effectiveness and clinical outcome. *Clin Implant Dent Relat Res* 2011; **13**: 305-310.
10. Hallman M, Mordenfeld A, Strandkvist T. A retrospective 5-year follow-up study of two different titanium implant surfaces used after interpositional bone grafting for reconstruction of the atrophic edentulous maxilla. *Clin Implant Dent Relat Res* 2005; **7**: 121-126.
11. Barone A, Ricci M, Mangano F, Covani U. Morbidity associated with iliac crest harvesting in the treatment of maxillary and mandibular atrophies: a 10-year analysis. *J Oral Maxillofac Surg* 2011; **69**: 2298-2304.
12. Mackiewicz Z, Niklinska WE, Kowalewska J, Chyczewski L. Bone as a source of organism vitality and regeneration. *Folia Histochem Cytobiol* 2011; **49**: 558-569.
13. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003; **423**: 337-342.

14. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem* 2010; **285**: 25103-25108.
15. Marsell R, Einhorn TA. The biology of fracture healing. *Injury* 2011; **42**: 551-555.
16. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest* 2002; **109**: 1405-1415.
17. Goodman SB, Ma T, Mitsunaga L, Miyanishi K, Genovese MC, Smith RL. Temporal effects of a COX-2-selective NSAID on bone ingrowth. *J Biomed Mater Res A* 2005; **72**: 279-287.
18. Endo K, Sairyo K, Komatsubara S, Sasa T, Egawa H, Ogawa T, Yonekura D, Murakami R, Yasui N. Cyclooxygenase-2 inhibitor delays fracture healing in rats. *Acta Orthop* 2005; **76**: 470-474.
19. Oh N, Kim S, Hosoya K, Okumura M. Compensatory cellular reactions to nonsteroidal anti-inflammatory drugs on osteogenic differentiation in canine bone marrow-derived mesenchymal stem cells. *J Vet Med Sci* 2014; **76**: 629-636.
20. Giannoudis PV, MacDonald DA, Matthews SJ, Smith RM, Furlong AJ, De Boer P. Nonunion of the femoral diaphysis. The influence of reaming and non-steroidal anti-inflammatory drugs. *J Bone Joint Surg Br* 2000; **82**: 655-658.
21. Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V. BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. *Nat Genet* 2006; **38**: 1424-1429.
22. Neovius E, Lemberger M, Docherty Skogh AC, Hilborn J, Engstrand T. Alveolar bone healing accompanied by severe swelling in cleft children treated with bone morphogenetic protein-2 delivered by hydrogel. *J Plast Reconstr Aesthet Surg* 2013; **66**: 37-42.
23. Cattalini JP, Boccaccini AR, Lucangioli S, Mouriño V. Bisphosphonate-based strategies for bone tissue engineering and orthopedic implants. *Tissue Eng Part B Rev* 2012; **18**: 323-340.
24. Larsson S, Fazzalari NL. Anti-osteoporosis therapy and fracture healing. *Arch Orthop Trauma Surg* 2014; **134**: 291-297.
25. Kates SL, Ackert-Bicknell CL. How do bisphosphonates affect fracture healing? *Injury* 2016; **47 Suppl 1**: S65-68.
26. Abtahi J, Agholme F, Sandberg O, Aspenberg P. Effect of local vs. systemic bisphosphonate delivery on dental implant fixation in a model of osteonecrosis of the jaw. *J Dent Res* 2013; **92**: 279-283.
27. Abtahi J, Tengvall P, Aspenberg P. A bisphosphonate-coating improves the fixation of metal implants in human bone. A randomized trial of dental implants. *Bone* 2012; **50**: 1148-1151.
28. Cardemil C, Omar OM, Norlindh B, Wexell CL, Thomsen P. The effects of a systemic single dose of zoledronic acid on post-implantation bone remodelling and inflammation in an ovariectomised rat model. *Biomaterials* 2013; **34**: 1546-1561.
29. Lindhe J, Bressan E, Cecchinato D, Corra E, Toia M, Liljenberg B. Bone tissue in different parts of the edentulous maxilla and mandible. *Clin Oral Implants Res* 2013; **24**: 372-377.

30. Branemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg* 1969; **3**: 81-100.
31. Galindo-Moreno P, Nilsson P, King P, Worsaae N, Schramm A, Padial-Molina M, Maiorana C. Clinical and radiographic evaluation of early loaded narrow-diameter implants: 5-year follow-up of a multicenter prospective clinical study. *Clin Oral Implants Res* 2017; **28**: 1584-1591.
32. Pieri F, Forlivesi C, Caselli E, Corinaldesi G. Narrow- (3.0 mm) Versus Standard-Diameter (4.0 and 4.5 mm) Implants for Splinted Partial Fixed Restoration of Posterior Mandibular and Maxillary Jaws: A 5-Year Retrospective Cohort Study. *J Periodontol* 2017; **88**: 338-347.
33. Wennerberg A, Albrektsson T, Johansson C, Andersson B. Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. *Biomaterials* 1996; **17**: 15-22.
34. Abrahamsson I, Albouy JP, Berglundh T. Healing at fluoride-modified implants placed in wide marginal defects: an experimental study in dogs. *Clin Oral Implants Res* 2008; **19**: 153-159.
35. Rocci M, Rocci A, Martignoni M, Albrektsson T, Barlattani A, Gargari M. Comparing the TiOblast and Osseospeed surfaces. Histomorphometric and histological analysis in humans. *Oral Implantol (Rome)* 2008; **1**: 34-42.
36. Testori T, Galli F, Fumagalli L, Capelli M, Zuffetti F, Deflorian M, Parenti A, Del Fabbro M. Assessment of Long-Term Survival of Immediately Loaded Tilted Implants Supporting a Maxillary Full-Arch Fixed Prosthesis. *Int J Oral Maxillofac Implants* 2017; **32**: 904-911.
37. Toljanic JA, Ekstrand K, Baer RA, Thor A. Immediate Loading of Tilted and Axial Posterior Implants in the Edentulous Maxillary Arch: A Retrospective Comparison of 5-Year Outcomes. *Int J Oral Maxillofac Implants* 2018; **33**: 433-438.
38. Chrcanovic BR, Albrektsson T, Wennerberg A. Survival and Complications of Zygomatic Implants: An Updated Systematic Review. *J Oral Maxillofac Surg* 2016; **74**: 1949-1964.
39. Moraschini V, Velloso G, Luz D, Barboza EP. Implant survival rates, marginal bone level changes, and complications in full-mouth rehabilitation with flapless computer-guided surgery: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 2015; **44**: 892-901.
40. Hutton JE, Heath MR, Chai JY, Harnett J, Jemt T, Johns RB, McKenna S, McNamara DC, van Steenberghe D, Taylor R, et al. Factors related to success and failure rates at 3-year follow-up in a multicenter study of overdentures supported by Branemark implants. *Int J Oral Maxillofac Implants* 1995; **10**: 33-42.
41. Belser UM, W.; Jung, R.; Hämmerle, C., Schmid, B., Morten, D., Buser, D. ITI Tretament Guide, Implant therapy in the esthetic Zone Single tooth replacements. Quintessenve Publishing Co, Ltd
42. Galindo-Moreno P, Padial-Molina M, Nilsson P, King P, Worsaae N, Schramm A, Maiorana C. The influence of the distance between narrow implants and the adjacent teeth on marginal bone levels. *Clin Oral Implants Res* 2016.
43. Klein MO, Schiegnitz E, Al-Nawas B. Systematic review on success of narrow-diameter dental implants. *Int J Oral Maxillofac Implants* 2014; **29 Suppl**: 43-54.

44. Lee DW, Choi YS, Park KH, Kim CS, Moon IS. Effect of microthread on the maintenance of marginal bone level: a 3-year prospective study. *Clin Oral Implants Res* 2007; **18**: 465-470.
45. Chowdhary R, Halldin A, Jimbo R, Wennerberg A. Influence of Micro Threads Alteration on Osseointegration and Primary Stability of Implants: An FEA and In Vivo Analysis in Rabbits. *Clin Implant Dent Relat Res* 2015; **17**: 562-569.
46. Ellingsen JE, Johansson CB, Wennerberg A, Holmen A. Improved retention and bone-tolmplant contact with fluoride-modified titanium implants. *Int J Oral Maxillofac Implants* 2004; **19**: 659-666.
47. Wennerberg A, Hallgren C, Johansson C, Danelli S. A histomorphometric evaluation of screw-shaped implants each prepared with two surface roughnesses. *Clin Oral Implants Res* 1998; **9**: 11-19.
48. Albrektsson T, Gottlow J, Meirelles L, Ostman PO, Rocci A, Sennerby L. Survival of NobelDirect implants: an analysis of 550 consecutively placed implants at 18 different clinical centers. *Clin Implant Dent Relat Res* 2007; **9**: 65-70.
49. Polizzi G, Gualini F, Friberg B. A two-center retrospective analysis of long-term clinical and radiologic data of TiUnite and turned implants placed in the same mouth. *Int J Prosthodont* 2013; **26**: 350-358.
50. Thor A, Ekstrand K, Baer RA, Toljanic JA. Three-year follow-up of immediately loaded implants in the edentulous atrophic maxilla: a study in patients with poor bone quantity and quality. *Int J Oral Maxillofac Implants* 2014; **29**: 642-649.
51. Malchiodi L, Moro T, Cattina DP, Cucchi A, Ghensi P, Nocini PF. Implant rehabilitation of the edentulous jaws: Does tilting of posterior implants at an angle greater than 45 degrees affect bone resorption and implant success?: A retrospective study. *Clin Implant Dent Relat Res* 2018.
52. Cassetta M. Immediate loading of implants inserted in edentulous arches using multiple mucosa-supported stereolithographic surgical templates: a 10-year prospective cohort study. *Int J Oral Maxillofac Surg* 2016; **45**: 526-534.
53. Papaspyridakos P, Chen CJ, Chuang SK, Weber HP. Implant loading protocols for edentulous patients with fixed prostheses: a systematic review and meta-analysis. *Int J Oral Maxillofac Implants* 2014; **29 Suppl**: 256-270.
54. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. *Eur Spine J* 2001; **10 Suppl 2**: S96-101.
55. LeGeros RZ. Calcium phosphate-based osteoinductive materials. *Chem Rev* 2008; **108**: 4742-4753.
56. Dahlin C, Sennerby L, Lekholm U, Linde A, Nyman S. Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. *Int J Oral Maxillofac Implants* 1989; **4**: 19-25.
57. Nyman S, Lang NP, Buser D, Bragger U. Bone regeneration adjacent to titanium dental implants using guided tissue regeneration: a report of two cases. *Int J Oral Maxillofac Implants* 1990; **5**: 9-14.
58. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP. Observations on a new collagen barrier membrane in 16 consecutively treated patients. Clinical and histological findings. *J Periodontol* 2001; **72**: 1616-1623.
59. Buser D, Dula K, Hirt HP, Schenk RK. Lateral ridge augmentation using autografts and barrier membranes: a clinical study with 40 partially edentulous patients. *J Oral Maxillofac Surg* 1996; **54**: 420-432; discussion 432-423.

60. Lang NP, Hammerle CH, Bragger U, Lehmann B, Nyman SR. Guided tissue regeneration in jawbone defects prior to implant placement. *Clin Oral Implants Res* 1994; **5**: 92-97.
61. Bozkurt A, Apel C, Sellhaus B, van Neerven S, Wessing B, Hilgers RD, Pallua N. Differences in degradation behavior of two non-cross-linked collagen barrier membranes: an in vitro and in vivo study. *Clin Oral Implants Res* 2014; **25**: 1403-1411.
62. Omar O, Dahlin A, Gasser A, Dahlin C. Tissue dynamics and regenerative outcome in two resorbable non-cross-linked collagen membranes for guided bone regeneration: A preclinical molecular and histological study in vivo. *Clin Oral Implants Res* 2018; **29**: 7-19.
63. Gultekin BA, Cansiz E, Borahan MO. Clinical and 3-Dimensional Radiographic Evaluation of Autogenous Iliac Block Bone Grafting and Guided Bone Regeneration in Patients With Atrophic Maxilla. *J Oral Maxillofac Surg* 2017; **75**: 709-722.
64. Hollister SJ, Murphy WL. Scaffold translation: barriers between concept and clinic. *Tissue Eng Part B Rev* 2011; **17**: 459-474.
65. Moradi Haghgoo J, Arabi SR, Hosseinipanah SM, Solgi G, Rastegarfard N, Farhadian M. Comparison of the effect of three autogenous bone harvesting methods on cell viability in rabbits. *J Dent Res Dent Clin Dent Prospects* 2017; **11**: 73-77.
66. Dym H, Huang D, Stern A. Alveolar bone grafting and reconstruction procedures prior to implant placement. *Dent Clin North Am* 2012; **56**: 209-218, x.
67. Barone A, Ricci M, Calvo-Guirado JL, Covani U. Bone remodelling after regenerative procedures around implants placed in fresh extraction sockets: an experimental study in Beagle dogs. *Clin Oral Implants Res* 2011; **22**: 1131-1137.
68. Frohberg U, Mazock JB. A review of morbidity associated with bone harvest from the proximal tibial metaphysis. *Mund Kiefer Gesichtschir* 2005; **9**: 63-65.
69. Nkenke E, Neukam FW. Autogenous bone harvesting and grafting in advanced jaw resorption: morbidity, resorption and implant survival. *Eur J Oral Implantol* 2014; **7 Suppl 2**: S203-217.
70. Johansson B, Grepe A, Wannfors K, Hirsch JM. A clinical study of changes in the volume of bone grafts in the atrophic maxilla. *Dentomaxillofac Radiol* 2001; **30**: 157-161.
71. Dasmah A, Thor A, Ekestubbe A, Sennerby L, Rasmusson L. Particulate vs. block bone grafts: Three-dimensional changes in graft volume after reconstruction of the atrophic maxilla, a 2-year radiographic follow-up. *J Craniomaxillofac Surg* 2011.
72. Sbordone C, Toti P, Guidetti F, Califano L, Santoro A, Sbordone L. Volume changes of iliac crest autogenous bone grafts after vertical and horizontal alveolar ridge augmentation of atrophic maxillas and mandibles: a 6-year computerized tomographic follow-up. *J Oral Maxillofac Surg* 2012; **70**: 2559-2565.
73. Corinaldesi G, Pieri F, Sapigni L, Marchetti C. Evaluation of survival and success rates of dental implants placed at the time of or after alveolar ridge augmentation with an autogenous mandibular bone graft and titanium mesh: a 3- to 8-year retrospective study. *Int J Oral Maxillofac Implants* 2009; **24**: 1119-1128.

74. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; **85**: 638-646.
75. Thor A, Wannfors K, Sennerby L, Rasmusson L. Reconstruction of the severely resorbed maxilla with autogenous bone, platelet-rich plasma, and implants: 1-year results of a controlled prospective 5-year study. *Clin Implant Dent Relat Res* 2005; **7**: 209-220.
76. Hallman M, Lundgren S, Sennerby L. Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clin Implant Dent Relat Res* 2001; **3**: 87-96.
77. Cardaropoli D, Tamagnone L, Roffredo A, Gaveglio L, Cardaropoli G. Socket preservation using bovine bone mineral and collagen membrane: a randomized controlled clinical trial with histologic analysis. *Int J Periodontics Restorative Dent* 2012; **32**: 421-430.
78. Hallman M, Nordin T. Sinus floor augmentation with bovine hydroxyapatite mixed with fibrin glue and later placement of nonsubmerged implants: a retrospective study in 50 patients. *Int J Oral Maxillofac Implants* 2004; **19**: 222-227.
79. Mordenfeld A, Hallman M, Johansson CB, Albrektsson T. Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clin Oral Implants Res* 2010; **21**: 961-970.
80. Schmitt CM, Moest T, Lutz R, Neukam FW, Schlegel KA. Anorganic bovine bone (ABB) vs. autologous bone (AB) plus ABB in maxillary sinus grafting. A prospective non-randomized clinical and histomorphometrical trial. *Clin Oral Implants Res* 2015; **26**: 1043-1050.
81. Cordaro L, Bosshardt DD, Palattella P, Rao W, Serino G, Chiapasco M. Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenter clinical trial. *Clin Oral Implants Res* 2008; **19**: 796-803.
82. Grover LM, Wright AJ, Gbureck U, Bolarinwa A, Song J, Liu Y, Farrar DF, Howling G, Rose J, Barralet JE. The effect of amorphous pyrophosphate on calcium phosphate cement resorption and bone generation. *Biomaterials* 2013; **34**: 6631-6637.
83. Garcia-Gareta E, Coathup MJ, Blunn GW. Osteoinduction of bone grafting materials for bone repair and regeneration. *Bone* 2015; **81**: 112-121.
84. Habraken WH, P.; Epple, M. ; Bohner, M. Calcium phosphates in biomedical applications: materials for the future? *Materials Today* 2016; **19**: 69-87.
85. Kruppke B, Farack J, Wagner AS, Beckmann S, Heinemann C, Glenske K, Rossler S, Wiesmann HP, Wenisch S, Hanke T. Gelatine modified monetite as a bone substitute material: An in vitro assessment of bone biocompatibility. *Acta Biomater* 2016; **32**: 275-285.
86. Habibovic P, Yuan H, van der Valk CM, Meijer G, van Blitterswijk CA, de Groot K. 3D microenvironment as essential element for osteoinduction by biomaterials. *Biomaterials* 2005; **26**: 3565-3575.
87. Chan O, Coathup MJ, Nesbitt A, Ho CY, Hing KA, Buckland T, Campion C, Blunn GW. The effects of microporosity on osteoinduction of calcium phosphate bone graft substitute biomaterials. *Acta Biomater* 2012; **8**: 2788-2794.

88. Yamada S, Heymann D, Bouler JM, Daculsi G. Osteoclastic resorption of biphasic calcium phosphate ceramic in vitro. *J Biomed Mater Res* 1997; **37**: 346-352.
89. Yamada S, Heymann D, Bouler JM, Daculsi G. Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/beta-tricalcium phosphate ratios. *Biomaterials* 1997; **18**: 1037-1041.
90. Grover LM, Gbureck U, Wright AJ, Tremayne M, Barralet JE. Biologically mediated resorption of brushite cement in vitro. *Biomaterials* 2006; **27**: 2178-2185.
91. Sheikh Z, Zhang YL, Grover L, Merle GE, Tamimi F, Barralet J. In vitro degradation and in vivo resorption of dicalcium phosphate cement based grafts. *Acta Biomater* 2015; **26**: 338-346.
92. Orriss IR, Arnett TR, Russell RG. Pyrophosphate: a key inhibitor of mineralisation. *Curr Opin Pharmacol* 2016; **28**: 57-68.
93. Fleisch H, Bisaz S. Mechanism of calcification: inhibitory role of pyrophosphate. *Nature* 1962; **195**: 911.
94. Engstrand T, Kihlström L, Neovius E, Skogh AC, Lundgren TK, Jacobsson H, Bohlin J, Åberg J, Engqvist H. Development of a bioactive implant for repair and potential healing of cranial defects. *J Neurosurg* 2014; **120**: 273-277.
95. Engstrand T. Biomaterials and biologics in craniofacial reconstruction. *J Craniofac Surg* 2012; **23**: 239-242.
96. Engstrand T, Kihlström L, Lundgren K, Trobos M, Engqvist H, Thomsen P. Bioceramic Implant Induces Bone Healing of Cranial Defects. *Plast Reconstr Surg Glob Open* 2015; **3**: e491.
97. Nejadnik MR, Yang X, Bongio M, Alghamdi HS, van den Beucken JJ, Huysmans MC, Jansen JA, Hilborn J, Ossipov D, Leeuwenburgh SC. Self-healing hybrid nanocomposites consisting of bisphosphonated hyaluronan and calcium phosphate nanoparticles. *Biomaterials* 2014; **35**: 6918-6929.
98. Hulsart-Billström G, Yuen PK, Marsell R, Hilborn J, Larsson S, Ossipov D. Bisphosphonate-linked hyaluronic Acid hydrogel sequesters and enzymatically releases active bone morphogenetic protein-2 for induction of osteogenic differentiation. *Biomacromolecules* 2013; **14**: 3055-3063.
99. Hilborn J. In vivo injectable gels for tissue repair. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2011.
100. Stefanski S, Svensson B, Thor A. Implant survival following sinus membrane elevation without grafting and immediate implant installation with a one-stage technique: an up-to-40-month evaluation. *Clin Oral Implants Res* 2016.
101. Mazor Z, Peleg M, Gross M. Sinus augmentation for single-tooth replacement in the posterior maxilla: a 3-year follow-up clinical report. *Int J Oral Maxillofac Implants* 1999; **14**: 55-60.
102. Danesh-Sani SA, Loomer PM, Wallace SS. A comprehensive clinical review of maxillary sinus floor elevation: anatomy, techniques, biomaterials and complications. *Br J Oral Maxillofac Surg* 2016; **54**: 724-730.
103. Thor A, Sennerby L, Hirsch JM, Rasmusson L. Bone formation at the maxillary sinus floor following simultaneous elevation of the mucosal lining and implant installation without graft material: an evaluation of 20 patients treated with 44 Astra Tech implants. *J Oral Maxillofac Surg* 2007; **65**: 64-72.
104. Srouji S, Kizhner T, Ben David D, Riminucci M, Bianco P, Livne E. The Schneiderian membrane contains osteoprogenitor cells: in vivo and in vitro study. *Calcif Tissue Int* 2009; **84**: 138-145.

105. Sbordone C, Toti P, Guidetti F, Califano L, Bufo P, Sbordone L. Volume changes of autogenous bone after sinus lifting and grafting procedures: a 6-year computerized tomographic follow-up. *J Craniomaxillofac Surg* 2013; **41**: 235-241.
106. Lutz R, Berger-Fink S, Stockmann P, Neukam FW, Schlegel KA. Sinus floor augmentation with autogenous bone vs. a bovine-derived xenograft - a 5-year retrospective study. *Clin Oral Implants Res* 2015; **26**: 644-648.
107. Mordenfeld A, Lindgren C, Hallman M. Sinus Floor Augmentation Using Straumann(R) BoneCeramic and Bio-Oss(R) in a Split Mouth Design and Later Placement of Implants: A 5-Year Report from a Longitudinal Study. *Clin Implant Dent Relat Res* 2016; **18**: 926-936.
108. Kim YS, Kim SH, Kim KH, Jhin MJ, Kim WK, Lee YK, Seol YJ, Lee YM. Rabbit maxillary sinus augmentation model with simultaneous implant placement: differential responses to the graft materials. *J Periodontal Implant Sci* 2012; **42**: 204-211.
109. Sohn DS, Kim WS, An KM, Song KJ, Lee JM, Mun YS. Comparative histomorphometric analysis of maxillary sinus augmentation with and without bone grafting in rabbit. *Implant Dent* 2010; **19**: 259-270.
110. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater* 2007; **13**: 1-10.
111. Stübinger S, Dard M. The rabbit as experimental model for research in implant dentistry and related tissue regeneration. *J Invest Surg* 2013; **26**: 266-282.
112. Masoud I, Shapiro F, Kent R, Moses A. A longitudinal study of the growth of the New Zealand white rabbit: cumulative and biweekly incremental growth rates for body length, body weight, femoral length, and tibial length. *J Orthop Res* 1986; **4**: 221-231.
113. Wang X, Mabrey JD, Agrawal CM. An interspecies comparison of bone fracture properties. *Biomed Mater Eng* 1998; **8**: 1-9.
114. Valiense H, Barreto M, Resende RF, Alves AT, Rossi AM, Mavropoulos E, Granjeiro JM, Calasans-Maia MD. In vitro and in vivo evaluation of strontium-containing nanostructured carbonated hydroxyapatite/sodium alginate for sinus lift in rabbits. *J Biomed Mater Res B Appl Biomater* 2016; **104**: 274-282.
115. Keating SC, Thomas AA, Flecknell PA, Leach MC. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. *PLoS One* 2012; **7**: e44437.
116. Toljanic JA, Baer RA, Ekstrand K, Thor A. Implant rehabilitation of the atrophic edentulous maxilla including immediate fixed provisional restoration without the use of bone grafting: a review of 1-year outcome data from a long-term prospective clinical trial. *Int J Oral Maxillofac Implants* 2009; **24**: 518-526.
117. Toljanic JA, Ekstrand K, Baer RA, Thor A. Immediate Loading of Implants in the Edentulous Maxilla with a Fixed Provisional Restoration without Bone Augmentation: A Report on 5-Year Outcomes Data Obtained from a Prospective Clinical Trial. *Int J Oral Maxillofac Implants* 2016; **31**: 1164-1170.
118. Benke D, Olah A, Mohler H. Protein-chemical analysis of Bio-Oss bone substitute and evidence on its carbonate content. *Biomaterials* 2001; **22**: 1005-1012.

119. Bodde EW, Spauwen PH, Mikos AG, Jansen JA. Closing capacity of segmental radius defects in rabbits. *J Biomed Mater Res A* 2008; **85**: 206-217.
120. Donath K. The diagnostic value of the new method for the study of undecalcified bones and teeth with attached soft tissue (Säge-Schliff (sawing and grinding) technique). *Pathol Res Pract* 1985; **179**: 631-633.
121. Johansson CB, Morberg P. Importance of ground section thickness for reliable histomorphometrical results. *Biomaterials* 1995; **16**: 91-95.
122. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. *J Oral Pathol* 1982; **11**: 318-326.
123. Johansson CBR, K.; Jimbo, R.; Hammarström Johansson, P.; Larsson, L; Östberg, A.K. 3.16 Histological Analysis. *Comprehensive Biomaterials II* 2017; **3**: 335-363
124. Sohrabi K, Mushantat A, Esfandiari S, Feine J. How successful are small-diameter implants? A literature review. *Clin Oral Implants Res* 2012; **23**: 515-525.
125. Maiorana C, King P, Quaas S, Sondell K, Worsaae N, Galindo-Moreno P. Clinical and radiographic evaluation of early loaded narrow-diameter implants: 3 years follow-up. *Clin Oral Implants Res* 2015; **26**: 77-82.
126. Galindo-Moreno P, Padial-Molina M, Nilsson P, King P, Worsaae N, Schramm A, Maiorana C. The influence of the distance between narrow implants and the adjacent teeth on marginal bone levels. *Clin Oral Implants Res* 2017; **28**: 704-712.
127. Esposito M, Ekestubbe A, Gröndahl K. Radiological evaluation of marginal bone loss at tooth surfaces facing single Brånemark implants. *Clin Oral Implants Res* 1993; **4**: 151-157.
128. Tarnow DP, Cho SC, Wallace SS. The effect of inter-implant distance on the height of inter-implant bone crest. *Journal of Periodontology* 2000; **71**: 546-549.
129. Grunder U, Gracis S, Capelli M. Influence of the 3-D bone-to-implant relationship on esthetics. *Int J Periodontics Restorative Dent* 2005; **25**: 113-119.
130. Buser D, Martin W, Belser UC. Optimizing esthetics for implant restorations in the anterior maxilla: anatomic and surgical considerations. *Int J Oral Maxillofac Implants* 2004; **19 Suppl**: 43-61.
131. Staubli N, Walter C, Schmidt JC, Weiger R, Zitzmann NU. Excess cement and the risk of peri-implant disease - a systematic review. *Clin Oral Implants Res* 2016.
132. Allum SR, Tomlinson RA, Joshi R. The impact of loads on standard diameter, small diameter and mini implants: a comparative laboratory study. *Clin Oral Implants Res* 2008; **19**: 553-559.
133. Weltman B, Vig KW, Fields HW, Shanker S, Kaizar EE. Root resorption associated with orthodontic tooth movement: a systematic review. *Am J Orthod Dentofacial Orthop* 2010; **137**: 462-476; discussion 412A.
134. Cardaropoli G, Lekholm U, Wennstrom JL. Tissue alterations at implant-supported single-tooth replacements: a 1-year prospective clinical study. *Clin Oral Implants Res* 2006; **17**: 165-171.

135. Misch CE, Perel ML, Wang HL, Sammartino G, Galindo-Moreno P, Trisi P, Steigmann M, Rebaudi A, Palti A, Pikos MA, Schwartz-Arad D, Choukroun J, Gutierrez-Perez JL, Marenzi G, Valavanis DK. Implant success, survival, and failure: the International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference. *Implant Dent* 2008; **17**: 5-15.
136. Vervaeke S, Collaert B, De Bruyn H. The effect of implant surface modifications on survival and bone loss of immediately loaded implants in the edentulous mandible. *Int J Oral Maxillofac Implants* 2013; **28**: 1352-1357.
137. Collaert B, Wijnen L, De Bruyn H. A 2-year prospective study on immediate loading with fluoride-modified implants in the edentulous mandible. *Clin Oral Implants Res* 2011; **22**: 1111-1116.
138. Windael S, Vervaeke S, Wijnen L, Jacquet W, De Bruyn H, Collaert B. Ten-year follow-up of dental implants used for immediate loading in the edentulous mandible: A prospective clinical study. *Clin Implant Dent Relat Res* 2018.
139. Rasmusson L, Roos J, Bystedt H. A 10-year follow-up study of titanium dioxide-blasted implants. *Clin Implant Dent Relat Res* 2005; **7**: 36-42.
140. Mertens C, Steveling HG. Implant-supported fixed prostheses in the edentulous maxilla: 8-year prospective results. *Clin Oral Implants Res* 2011; **22**: 464-472.
141. Jemt T, Johansson J. Implant treatment in the edentulous maxillae: a 15-year follow-up study on 76 consecutive patients provided with fixed prostheses. *Clin Implant Dent Relat Res* 2006; **8**: 61-69.
142. Testori T, Taschieri S, Scutella F, Del Fabbro M. Immediate Versus Delayed Loading of Postextraction Implants: A Long-Term Retrospective Cohort Study. *Implant Dent* 2017.
143. Chrcanovic BR, Albrektsson T, Wennerberg A. Bone Quality and Quantity and Dental Implant Failure: A Systematic Review and Meta-analysis. *Int J Prosthodont* 2017; **30**: 219-237.
144. Ekfeldt A, Christiansson U, Eriksson T, Linden U, Lundqvist S, Rundcrantz T, Johansson LA, Nilner K, Billstrom C. A retrospective analysis of factors associated with multiple implant failures in maxillae. *Clin Oral Implants Res* 2001; **12**: 462-467.
145. Schwartz-Arad D, Laviv A, Levin L. Failure causes, timing, and cluster behavior: an 8-year study of dental implants. *Implant Dent* 2008; **17**: 200-207.
146. Chrcanovic BR, Kisch J, Albrektsson T, Wennerberg A. Analysis of risk factors for cluster behavior of dental implant failures. *Clin Implant Dent Relat Res* 2017.
147. Leach MC, Allweiler S, Richardson C, Roughan JV, Narbe R, Flecknell PA. Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. *Res Vet Sci* 2009; **87**: 336-347.
148. Flecknell PAL, J.H. Assessment of the analgesic action of opioid agonist-antagonists in the rabbit. *Journal of the Association of Veterinary Anaesthetists of Great Britain and Ireland* 1990; **Volume 17**: Pages 24-29.
149. Carpenter J. Exotic Animal Formulary. 3rd ed. . Elsevier St. Louis, USA.
150. Pal US, Sharma NK, Singh RK, Mahammad S, Mehrotra D, Singh N, Mandhyan D. Direct vs. indirect sinus lift procedure: A comparison. *Natl J Maxillofac Surg* 2012; **3**: 31-37.
151. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res* 2002; **17**: 963-976.

152. Armstrong S, Lees P. Effects of carprofen (R and S enantiomers and racemate) on the production of IL-1, IL-6 and TNF-alpha by equine chondrocytes and synoviocytes. *J Vet Pharmacol Ther* 2002; **25**: 145-153.
153. Cara-Fuentes M, Machuca-Ariza J, Ruiz-Martos A, Ramos-Robles MC, Martinez-Lara I. Long-term outcome of dental implants after maxillary augmentation with and without bone grafting. *Med Oral Patol Oral Cir Bucal* 2016; **21**: e229-235.
154. Martínez-Sanz E, Ossipov DA, Hilborn J, Larsson S, Jonsson KB, Varghese OP. Bone reservoir: Injectable hyaluronic acid hydrogel for minimal invasive bone augmentation. *J Control Release* 2011; **152**: 232-240.
155. Bergman K, Engstrand T, Hilborn J, Ossipov D, Piskounova S, Bowden T. Injectable cell-free template for bone-tissue formation. *J Biomed Mater Res A* 2009; **91**: 1111-1118.
156. Martínez-Sanz E, Varghese OP, Kisiel M, Engstrand T, Reich KM, Bohner M, Jonsson KB, Kohler T, Müller R, Ossipov DA, Hilborn J. Minimally invasive mandibular bone augmentation using injectable hydrogels. *J Tissue Eng Regen Med* 2012; **6 Suppl 3**: s15-23.
157. Shi L, Zhang Y, Ossipov D. Enzymatic degradation of hyaluronan hydrogels with different capacity for in situ bio-mineralization. *Biopolymers* 2018; **109**.
158. Watanabe K, Niimi A, Ueda M. Autogenous bone grafts in the rabbit maxillary sinus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **88**: 26-32.
159. Bloemers FW, Blokhuis TJ, Patka P, Bakker FC, Wippermann BW, Haarman HJ. Autologous bone versus calcium-phosphate ceramics in treatment of experimental bone defects. *J Biomed Mater Res B Appl Biomater* 2003; **66**: 526-531.
160. Zhao MD, Huang JS, Zhang XC, Gui KK, Xiong M, Yin WP, Yuan FL, Cai GP. Construction of Radial Defect Models in Rabbits to Determine the Critical Size Defects. *PLoS One* 2016; **11**: e0146301.
161. Trbakovic A, Hedenqvist P, Mellgren T, Ley C, Hilborn J, Ossipov D, Ekman S, Johansson CB, Jensen-Waern M, Thor A. A new synthetic granular calcium phosphate compound induces new bone in a sinus lift rabbit model. *J Dent* 2017.
162. Pallesen L, Schou S, Aaboe M, Hjorting-Hansen E, Nattestad A, Melsen F. Influence of particle size of autogenous bone grafts on the early stages of bone regeneration: a histologic and stereologic study in rabbit calvarium. *Int J Oral Maxillofac Implants* 2002; **17**: 498-506.
163. Barrere F, van Blitterswijk CA, de Groot K. Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics. *Int J Nanomedicine* 2006; **1**: 317-332.
164. Basle MF, Chappard D, Grizon F, Filmon R, Delecrin J, Daculsi G, Rebel A. Osteoclastic resorption of Ca-P biomaterials implanted in rabbit bone. *Calcif Tissue Int* 1993; **53**: 348-356.
165. Miron RJ, Zohdi H, Fujioka-Kobayashi M, Bosshardt DD. Giant cells around bone biomaterials: Osteoclasts or multi-nucleated giant cells? *Acta Biomater* 2016; **46**: 15-28.
166. von Arx T, Broggini N, Jensen SS, Bornstein MM, Schenk RK, Buser D. Membrane durability and tissue response of different bioresorbable barrier membranes: a histologic study in the rabbit calvarium. *Int J Oral Maxillofac Implants* 2005; **20**: 843-853.

167. Caballe-Serrano J, Munar-Frau A, Ortiz-Puigpelat O, Soto-Penaloza D, Penarrocha M, Hernandez-Alfaro F. On the search of the ideal barrier membrane for guided bone regeneration. *J Clin Exp Dent* 2018; **10**: e477-e483.
168. Bigham-Sadegh A, Oryan A. Selection of animal models for pre-clinical strategies in evaluating the fracture healing, bone graft substitutes and bone tissue regeneration and engineering. *Connect Tissue Res* 2015; **56**: 175-194.
169. Kasten P, Vogel J, Geiger F, Niemeyer P, Luginbuhl R, Szalay K. The effect of platelet-rich plasma on healing in critical-size long-bone defects. *Biomaterials* 2008; **29**: 3983-3992.
170. Niemeyer P, Szalay K, Luginbuhl R, Sudkamp NP, Kasten P. Transplantation of human mesenchymal stem cells in a non-autogenous setting for bone regeneration in a rabbit critical-size defect model. *Acta Biomater* 2010; **6**: 900-908.
171. Burenstam Linder LK, Birgersson U, Lundgren K, Illies C, Engstrand T. Patient-specific titanium reinforced calcium phosphate implant for the repair and healing of complex cranial defects. *World Neurosurg* 2018.

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