AUTOGENOUS BONE AND BOVINE BONE MINERAL AS GRAFTING MATERIALS IN MAXILLOFACIAL SURGERY

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AUTOGENOUS BONE AND BOVINE BONE MINERAL AS GRAFTING MATERIALS IN MAXILLOFACIAL SURGERY

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VOOR LIESBETH
EMILIE, COEN EN JOB

CONTENTS

List of Abbreviations

ABG	Autogenous Bone Graft
BBM	Bovine Bone Mineral
(rh)BMP	(Recombinant Human) Bone Morphogenetic Protein
DFDBA	Demineralized Freeze Dried Bone Allograft
FDA	Food and Drug Administration
HA	Hydroxyapatite
OMF	Oral and Maxillo-Facial
OP	Osteogenic Protein
PDGF	Platelet Derived Growth Factor
PRP	Platelet Rich Plasma
TCP	Tri-Calcium Phosphate
TGF	Transforming Growth Factor

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

The history of autogenous bone grafting goes back to the nineteenth century. In 1821 VON WALTER was probably one of the first surgeons who used autogenous corticocancellous bone grafts to reconstruct lost parts of the maxillofacial skeleton (cited in HABAL and REDDI 1987, DE BOER 1988). Until the present day the use of autogenous bone grafts is still considered as the standard in reconstructive oral and maxillofacial (OMF) surgery (BURCHARDT 1983, CUTTING et al. 1984, HABAL 1992a). These grafts can be used to augment the severely atrophic edentulous alveolar ridges, to reconstruct alveolar defects in cleft palate patients, or to bridge defects that are the result of ablative surgery or trauma. Autogenous bone grafts, when adequately fixed, usually adapt to the recipient site and incorporate well into the defects. A layer of connective tissue will mostly cover the grafts while from the interface between the recipient bone and the graft revascularisation takes place ultimately leading to the formation of new bone (URIST 1980). Fresh autografts contain surviving cells and inductive proteins, which can stimulate osteogenesis. From a biological point of view, it is the best material available since it is non-immunogenic and it partially retains its viability immediately after transplantation. Alloplastic bone substitutes on the other hand, which are also commonly used as graft material, provide at best a scaffold for bone formation (ISAKSSON 1992).

The limited availability, the sometimes unpredictable resorption, the need for a second operation site, the time consuming procedure, donor site morbidity, the quantity of bone available and the increased costs of hospitalisation, has led to a search for alternatives. It is, therefore, the aim of the current study to gain insight into the healing capacity of three types of grafts with the idea to ultimately produce a graft that would alleviate or minimize the need for autogenous bone grafts.

1.2 MECHANISMS OF BONE GRAFT HEALING

A prerequisite for a bone graft to serve its purpose is healing. Many factors influence this healing process, which may also be considered as a kind of bone regeneration (GLOWACKI et al. 1981, MUTHUKUMARAN and REDDI 1985, SALIBIAN et al. 1987, CONNOLLY et al. 1989, WEILAND et al. 1990, HOLLINGER 1993). The healing process is complicated and involves factors like bone metabolism, changes in hormonal balance and external influences that are present for a prolonged time (HABAL and REDDI 1987). Revascularisation is mandatory for graft healing as newly formed blood vessels enable nutrients and humoral substances to be brought into the graft (LOZANO et al. 1976, LEE and LANGER 1983). If revascularisation is successful new bone is formed around the transformed osteocytes at the margins of

Table 1.1 *Mechanisms of bone healing (GLOWACKI 1992)*

Type	Physiologic principle	Examples of materials
Osteogenesis	Transplantation of viable osteoblasts and preosteoblasts	Cancellous bone, marrow, periosteum and vascularized grafts
Osteoconduction	Ingrowth of bone from the margins of defect with gradual resorption of implant	Cortical segments; banked allogenic or xenogenic bone; resorbable materials
Osteoinduction	Phenotypic conversion of mesenchymal cells into bone cells	Demineralized bone and dentin

the graft and the old bone is slowly replaced. This process is called 'creeping substitution'. Cancellous bone allows the easy penetration whereas cortical bone is more difficult to penetrate for capillaries (BURCHARDT and ENNEKING 1978, EITEL et al. 1980, KUSIAK et al. 1985, ARDEN and BURGIO 1992, HABAL 1992a). On the other hand, the volumetric maintenance of cortical bone grafts is superior to that of cancellous bone (SMITH and ABRAMSON 1974, ZINS and WHITAKER 1983, WILKES et al. 1985, DADO and IZQUIERDO 1989, PHILIPS and RAHN 1990). Corticocancellous bone usually produces the best results because it has characteristics of both. It enables adequate revascularisation and thus enhances incorporation of the graft into the surrounding structures and it also possesses mechanical strength. Differences in healing between several cortico-cancellous donor sites are related to the three dimensional osseous architecture (the cortical/cancellous ratio) rather than to the embryonic origin per se (membranous versus endochondral bone) (HARDESTY and MARSH 1990).

Osseous healing is achieved by direct osteogenesis, via osteoinduction or via osteoconduction (GLOWACKI 1992) (table 1.1). These mechanisms are not separate and distinct, but overlapping (MISCH and DIETSH 1993). Understanding these processes enables the surgeon to make the most appropriate selection for a particular application. Osteoinductive materials stimulate the differentiation of osteogenic cells from mesenchymal cells. Osteoconductive materials provide a permanent or resorbable scaffold for the ingrowth of bone from the margins of the defect.

It is possible to determine which healing mechanism(s) is (are) stimulated by a material by examining its effects in osseous and ectopic, non-osseous, sites (GLOWACKI 1992) (table 1.2). If implanted subcutaneously, osteoinductive materials elicit enchondral osteogenesis, whereas osteoconductive materials appear

Table 1.2 *Differences between osteoinductive and osteoconductive materials (GLOWACKI 1992)*

	Osteoinductive	Osteoconductive
In ectopic implants,		
Is bone formed?	Yes	No
In intraosseous implants,		
What is geometry of healing?	Field	From margins
Is healing enchondral?	Yes	No
In onlay implants,		
What is geometry of healing?	Field	From underlying bone
Is healing enchondral?	Yes	No
Examples	Demineralized bone	Porous particulate ceramics, collagen matrix

to be inert. The latter only provoke mild inflammatory reactions. Osteoconductive grafts allow ossification from the margins of the defect combined with the resorption of the non-vital matrix. This process is similar to the formation of periosteal collarbone in fracture repair.

1.3 TYPES OF GRAFT MATERIAL

Per definition there are four types of grafts, i.e. autografts, allografts, alloplasts and xenografts. The term autograft refers to tissue transplanted from one site to another within the same individual. Allografts are obtained from cadavers or living individuals from the same species. In human medicine they can be obtained from tissue banks (KÜBLER 1997). Alloplasts are synthetic materials consisting of biological inert substances. Xenografts are composed of tissue taken from another species (i.e. from an animal source, usually bovine). In case the organic material is removed from xenogenic bone, it may be considered as an alloplast (GARG 1999). The term 'composite graft' refers to grafts that are composed of materials from different origins, usually autogenous bone mixed with other materials (HABAL 1991).

1.3.1 Autogenous bone grafts

Autogenous bone grafts may consist of cortical or cancellous bone but usually a combination of both is used, called cortico-cancellous grafts. This type of graft is probably the graft most often used in reconstructive OMF surgery. The easier the

penetration of blood vessels into the graft to revascularise it, the less mechanical stress the graft can take.

1.3.1.1 Cortical bone grafts

Pure cortical grafts have limited application. They may be used as onlay grafts in the maxillofacial skeleton. In order to serve their purpose the graft needs to be completely remodelled by creeping substitution for which adequate revascularisation is required. This in turn demands rigid fixation of the graft to the recipient bone because any micromovement may jeopardise this process. Typical applications include onlay grafts to the maxilla to improve infra-orbital or paranasal contour (HABAL 1992a). The most often used donor site is the cranial vault (especially in older patients) first described by TESSIER (1982). There is hardly any morbidity associated with this donor site even if the dura is visualised or exposed (HABAL 1992b).

1.3.1.2 Cancellous bone grafts

Pure cancellous bone is commonly used in OMF surgery to fill bony defects such as alveolar clefts, for non healing fractures, around dental implant defects and other small osseous defects. Cancellous bone lacks the mechanical strength, desired for reconstruction of larger defects except when rigid appliances are used. Cancellous bone is used because of the ease of its application and its ability to improve healing. The porous structure of these grafts allows for rapid revascularisation, with cellular ingrowth and differentiation and subsequent remodelling and substitution. The most common donor sites for autogenous cancellous grafts are the anterior or posterior iliac crest or the tibial plateau.

1.3.1.3 Corticocancellous bone grafts

Cortico-cancellous bone grafts have a wide application in reconstructive OMF surgery. The cortical layer strengthens the graft, which implies that the graft can be contoured to fit the defect that needs to be filled or bridged. As a free graft it can be used to bridge a defect up to 5 cm provided adequate blood supply is assured from the enveloping soft tissues. Much larger cortico-cancellous bone grafts can be used when microvascular anastomoses guarantee the primary survival of the graft. In most cases these grafts have also to be fixed rigidly to the surrounding bone to allow proper healing, while the patient can still function with a more or less intact jaw. Typical donor sites are rib, iliac crest, fibula or radius, whereas oral sites include chin, retromolar area and mandibular body.

1.3.2 Allografts

The primary forms of allografts are frozen, freeze-dried (lyophilised), demineralized freeze-dried and irradiated bone. Because allografts are not osteogenic, bone formation takes longer and results in less volume compared to autogenous grafts (MISCH and DIETSH 1993). They revascularize quickly but the risk of an antigen transmission, especially in a high vascular area as the facial skeleton, increases the risk of rejection of the graft. In the OMF area demineralized bone grafts can be used after irradiation.

The most commonly used allograft is demineralized and freeze-dried bone. The latter is used for minimising the antigenicity (BLOCK and POSER 1995), resulting in a demineralized bone matrix (DFDBA). In 1965, URIST (1965) reported that demineralized bone and dentin provide postembryonic osteoinductive stimuli and that this material is capable of inducing ectopic bone formation when implanted subcutaneously or into muscle pouches in rodents. REDDI and HUGGINS (1972) found that implantation of particles of demineralized bone resulted in the induction of enchondral bone. This enchondral osteogenesis is similar to the process occurring in embryonic skeletogenesis, growth of long bones and healing of fractures (REDDI and HUGGINS 1972, URIST 1973, REDDI et al. 1987, HAMMONDS et al. 1991, SASANO et al. 1993). Successful experiments in animal studies led to the clinical application of DFDBA, with satisfactory results particularly in small defects (GLOWACKI et al. 1981, MULLIKEN et al. 1981, KABAN et al. 1982, SONIS et al. 1983, GLOWACKI and MULLIKEN 1985, MAXSON et al. 1990). In larger defects the results are reproducible in children, but not in adults (HABAL 1992b). In human sinus floor elevation procedures, allogenic DFDBA increases mineralised tissue volume mainly by osteoconduction, supported by remineralisation but not osteoinduction (GROENEVELD et al. 1999a).

1.3.3 Alloplasts and xenoplasts

A major category of grafting material consists of an anorganic matrix either obtained by sintering calcium phosphates such as tricalcium phosphate (TCP) or hydroxyapatite (HA), or consisting of deproteinized bovine bone mineral (BBM) or derived from the natural exoskeleton of reef-building sea coral. Anorganic alloplastic bone minerals, if used alone, are osteoconductive and consequently do not have the capacity to bridge critical size maxillofacial defects (BOYNE 1991). Nevertheless, they have been used for a variety of indications such as periodontal defects (BOWEN et al. 1989), alveolar clefts (EL DEEB et al. 1989), facial contour corrections (SALYER and HALL 1989, ROSEN and MCFARLAND 1990) and alveolar

ridge augmentations (HOLMES and HAGLER 1988, MERCIER et al. 1992). Numerous problems have been reported such as fracture, infection and exfoliation of the implanted material (HUPP and KENNA 1988, ROONEY et al. 1988, PIECUCH et al. 1990, SCHLIEPHAKE and NEUKAM 1991). These complications have been attributed to the fact that the grafts are devoid of living tissue and that they can only be used in uncompromised recipient beds (SIEBERT et al. 1986). It has been shown that the rate of bone ingrowth can be increased by using a resorbable membrane to allow for guided bone formation inside subperiosteally implanted HA blocks (SCHLIEPHAKE et al. 1994).

1.3.3.1 Tricalciumphosphate and Hydroxyapatite

Sintered calcium phosphate ceramics, particularly α-TCP and HA, are the anorganic graft materials most often used in OMF surgery. The rationale for their use is found in their biochemical and structural similarity to the mineral phase of bone. Both materials are available as solid or porous block, chips, or granules. Each has markedly different physical and degradative properties, strongly dependent on the structure (i.e. solid versus porous), its microstructure and the presence of minor impurities. In general, α-TCP is degraded within days or weeks after implantation, whereas high-density crystalline HA is irresorbable or its degradation may take years (HOLMES et al. 1984, SHIMAZAKI and MOONEY 1985, BUCHOLZ et al. 1989, NAGAHARA et al. 1992).

TCP is sintered into polycrystalline porous ceramics. Histological studies of α-TCP ceramic implanted in human osseous defects showed excellent tissue compatibility but no new bone formation (BALDOCK et al. 1985). However, a histological study of biopsies of soft tissues removed from defects filled with α-TCP revealed osteoid forming alongside and within many of the ceramic particles (FRANK et al. 1985). Viable mature bone fragments were found separate from the ceramic material (BOWERS et al. 1977, NAAMAN-BOU-ABBOUD et al. 1994). One of the drawbacks of α-TCP, however, is that it resorbs faster than it can be replaced by newly formed bone (REIDA et al. 1977, NERY et al. 1978). Probably this is the reason why only studies with α-TCP in small defects have been published, such as periodontal lesions (HASHIMOTO et al. 1995). Changes in the chemical structure of the material (β-TCP form) that are recently introduced, reduce the resorption rate and intend to provide a physical matrix suitable for deposition of new bone (MISCH and DIETSH 1993).

HA is, similar to α-TCP, synthesised by a procedure that results in a crystalline spatial structure close to that of cortical bone matrix (DESJARDIN 1985).

Its degradation does not start before 9 months after implantation (KENT et al. 1983, BUCHOLZ et al. 1987, BRAYE et al. 1996). Various authors studied the use of HA. They considered its slow resorption relative to the characteristic time span for bone regeneration, to be either an asset (HERR et al. 1993) or a liability (DOLL et al. 1990). In biopsies taken one year after mandibular ridge augmentation with a composite of HA particles in a matrix of purified fibrillar collagen, the HA particles appeared to be surrounded by dense fibrous connective tissue and trabeculae of woven and lamellar bone (MEHLISCH et al. 1990). This combination was found to be biocompatible with human tissue and receptive of direct bone apposition on the HA particles. An argument against HA is that as long as it maintains its mechanical strength, the bone that is formed within the porous network of the HA scaffold is stress shielded and, therefore, does not undergo mechanical loading that acts as a trigger for remodelling. Long-term persistence of remodelling bone would be more likely to occur if the initial scaffold would gradually degrade allowing the bone to be loaded. An argument in favour of HA is that its structure resembles natural hydroxyapatite more closely than TCP does and, therefore, it might represent a better scaffold for bone ingrowth (HAERS et al. 1991, YASZEMSKI et al. 1996).

1.3.3.2 Bovine Bone Mineral (BBM)

BBM is derived from bovine bone. The structure consists of a wide interconnective pore system that can easily be invaded by blood vessels resulting in osteoblastic migration. BBM contains pores of different sizes: macropores (300-1500 µm), micropores (Haversian and vascular marrow canals) and intracrystalline spaces (3-26 nm) (PEETZ 1997). This results in an overall porosity of 70% to 75%, reaching a surface area of almost 100 m^2/g. No other current commercially available synthetic material shows this high porosity (HÄMMERLE et al. 1997): e.g. synthetic HA reaches a surface area of approximately 1 – 10 m^2/g and natural coralline 3.1 m^2/g (WHITE and SHORS 1986, PEETZ 1997).

BBM is thermally and chemically treated in order to extract organic constituents and thereby eliminating its antigenicity and potential inflammatory response by the recipient (HISLOP et al. 1993, COHEN et al. 1994). Some authors, however, reported that BBM still contains collagen type I fibres, which might cause antigenic reactions (LINSEMAYER 1981). Antibodies against bovine collagen are rare and, therefore, are unlikely to be responsible for graft failure (DE LUSTRO et al. 1990). On the other hand, collagen I might stimulate the primary attachment of osteoblasts by an interaction with the β-1 integrin subunit of the pre-osteoblast resulting in a faster bone deposition as compared to HA (BASLE et al. 1998).

BBM is prone to resorption by osteoclasts or multinucleated giant cells (PINHOLT et al. 1991, DERSOT et al. 1995) although other studies refute this (MANDELKOW et al. 1990, STASSEN et al. 1994, SKOGLUND et al. 1997, YOUNG et al. 1999). Contrary to HA, which is not replaced by bone, because it does not resorb, the resorption of BBM finally leads to substitution and remodelling.

1.3.3.3 Coralline

Another hydroxyapatite structure is derived from coral (Porites spec.). The calcium carbonate skeleton is converted for 90% into HA, 3% remain calcium carbonate and the remainder consists almost entirely of TCP (HOLMES et al. 1987, MARTIN et al. 1989). It has fully interconnected pores of 100 – 500 µm in diameter and a surface to volume ratio of 3.1 m^2/g providing an good matrix for bone ingrowth by osteoconduction (WHITE and SHORS 1986). It has been used in bone regeneration studies with varied success (HOLMES et al. 1987, DOLL et al. 1990). The resorbability of this material is questionable (MARTIN et al. 1993, PAPACHARALAMBOUS and ANASTASOFF 1993, POLLICK et al. 1995, BRAYE et al. 1996).

BUCHOLZ et al. (1997) demonstrated bone healing in 46 cases of nonhealing fractures of metaphyseal and diaphyseal bones treated with coralline. One of the major disadvantages of coralline as an implant material is its initial weakness (55% of cancellous bone strength) (BUCHOLZ et al. 1987, MURPHY et al. 1992) and the difficulty of handeling it (ASHMAN 1992). This excludes its use in cases in which the initial mechanical load has to be borne by the graft alone (SARTORIS et al. 1987). After bone ingrowth, the mechanical properties of coralline implants are dramatically improved. Six months after implantation its strength is 1,5 – 3 times that of a cancellous autograft (BUCHOLZ et al. 1987).

1.3.3.4 Active glass ceramics

Bioactive glass ceramics (Bioglass, Perioglass, Biogran) are composed of calcium salts and phosphates in similar proportions as found in bone and teeth, as well as sodium salts and silicates which are essential for bone mineralisation. PerioGlas is the particulate form of Bioglass. Biogran is a soluble, hydrophilic and slightly hemostatic powder made of bioactive glass granules. The ceramic is available as an amorphous material that shows more favourable properties than the crystalline form, which is believed to degrade faster under the influence of tissue fluids by physical-chemical reactions. Because it is not porous, release of silicates and ingrowth of blood vessels and other tissue components into the material is not

possible. WILSON and NOLETTI (1990) mentioned two properties contributing to its usefulness: (1) the relatively fast host cells reaction and (2) the ability to bind to connective tissue collagen. The bioactivity of Bioglass may stimulate the reparative process (FETNER et al. 1994) which give rise to a relative short time needed for full bony ingrowth as compared to HA (OONISHI et al. 1997).

These materials are mainly applied in periodontal defects (ZAMET et al. 1997, LOVELACE et al. 1998, FROUM et al. 1998), pre-implant mandibular osseous defects (SCHEPERS et al. 1998), and peri-implant defects (HALL et al. 1999). There is only one study comparing bioactive glass particles with autogenous bone grafts in patients with skeletal deformities of the face after trauma or tumour surgery. Clinical and radiographical (without histological) examination showed no 'relapse' of the aesthetic result in both groups after one year follow-up (SUOMINEN and KINNUNEN 1996).

1.3.4 Composite grafts with autogenous bone

Per defenition a composite graft is composed of materials from different origin. Several attempts have been made to accelerate their healing process, by adding autogenous cancellous bone (COBB et al. 1990) or using electrical stimulation (LEW and MARINO 1991) but also by adding growth factors (BMP) (KAWAMURA et al. 1987, RIPAMONTI 1991) or platelet rich plasma (PRP) (MARX et al. 1998). LANG and MERTENS (1990) even tried to implant cultured homologous osteoblast-like cells in alloplastic scaffolds with success. All these attempts were ment to reduce the volume of autogenous bone needed and to accelerate the bone healing. It also may improve the efficiency and may even prevent resorption, possibly resulting in a dense new bone formation (MISCH and DIETSH 1993, SATOW et al. 1997).

Osteoconductive and osteoinductive materials may also be used in combination with autogenous bone. In case of an alloplastic material this is ment to be osteoconductive as to allow migration of capillaries and cells into the pores resulting in deposition of new bone (HAERS et al. 1991, SATOW et al. 1997). HA particles in conjunction with autogenous cancellous bone appeared to be excellent grafting material because it enhances the osteoconduction and fastens the solidification (VANASSCHE et al. 1988) while HA alone does not (WANGERIN et al. 1985). HABAL (1991) used composites of HA, autogenous corticocancellous bone, temporalis fascia slivers, fibrillar collagen, blood and antibiotics in cleft alveolus surgery in children between the ages of 6-21 years. His results showed no changes or interferences with growth of the facial skeleton of these children. The results,

however, were somewhat compromised by the simultaneous use of orthodontic appliances during the healing phase.

At present, no studies have been reported on composite grafts consisting of autogenous bone and BBM.

Recombinant human bone morphogenetic proteins (rhBMP), osteogenic proteins (OP), or autogenous platelet derived growth factors (PDGF) can be added to composite grafts. It might be a reasonable alternative to autogenous bone grafting in osseous reconstructive procedures, circumventing the limitations of donor bone supply and the risk of complications. Many osteoinductive factors have been added to composite grafts in several experiments. The results indicate that the combination of any composite graft with a BMP has the greatest potential for regeneration of experimental bony defects (DAMIEN et al. 1990, DOLL et al. 1990, RIPAMONTI et al. 1996). At present, the American Food and Drug Administration (FDA) has not registered rhBMP and, therefore, the experience is still in a phase II stadium. The results appear to be somewhat unpredictable (GROENEVELD et al. 1999b). Information concerning the efficacy and safety of rhBMP or OP, however, is essential for the design of future human applications. The use of demineralized allogenic or xenogenous bone may contain the risk of host pathogen transmission. The construction of collagenous matrices in which osteoinductive proteins are embedded would result in an inconsistent performance, a limited supply and tenuous inductive ability (GROENEVELD et al. 1999a). The use of autogenous platelet-rich plasma (PRP), which contains PDGF and transforming growth factor (TGF-β1 and -β2) seems more promising (MARX et al. 1998).

1.4 MODES OF APPLICATION.

Grafts can be used in large pieces called 'blocks' or may be manufactured or cut into small particles. Both forms can be obtained in a dense and a porous form referring to the microstructure of the graft. All types of graft material can be delivered in blocks. **Blocks** are usually shaped before application to precisely fit the defect and to bear limited mechanical stress. They are commonly used to restore continuity for example in mandibular reconstructive surgery. When autogenous grafts are used for this purpose the supply is somewhat limited. **Particulate grafts** are composed of small irregular chips easily obtained by cutting block grafts into chips by milling (ISAKSSON 1992). This type of graft is usually applied in areas where little mechanical strength is needed. It can be used to fill small defects, to correct minimal contour irregularities and for packing around larger grafts. The chips are usually of different shape and size and may facilitate and promote bone

regeneration (HABAL 1992a). Since the introduction of improved rigid fixation techniques, chips can also be used for larger defects. Trays offer an excellent means of holding particulate, autogenous grafts in the desired contour (BOYNE 1983, ALBERT et al. 1986, LEAKE et al. 1992, CHEUNG et al. 1994, TIDEMAN et al. 1998). As a consequence, the patient can have near-normal function during the healing phase, which may take as long as 2 years.

1.5 MICROSTRUCTURE OF THE GRAFT

Grafts, but particularly alloplasts, exhibiting large inner surface areas, are better recipient sites for bone ingrowth than materials with a comparatively small surface area (FRAME et al. 1987, HOLMES and ROSER 1987, KASPERK et al. 1988). The penetration of host bone into the inner part of the graft material is related to the porosity of the material (HOLMES and ROSER 1987, KASPERK et al. 1988, DACULSI and PASSUTI 1990, VAN EEDEN and RIPAMONTI 1994, HÄMMERLE et al. 1997).

Cortical bone is composed of a dense osteoid matrix and, therefore, difficult to penetrate by new blood vessels. Even years after application only the interface is the area that is revascularised, while the remainder of the cortical component may stay non-vital. In cancellous grafts, however, large open areas enable revascularisation very well, bringing cellular regeneration, resulting in a remodelling and substitution, with new bone forming as old bone is resorbed. The more dense the bone graft, the harder it is to be revascularised, to become incorporated, and to remain viable or to become replaced by viable bone by creeping substitution (HARDESTY and MARSH 1990, SULLIVAN and SZWAJKUN 1991, PHILIPS and RAHN 1992).

Ceramic calcium phosphate materials can be delivered in dense and porous forms of blocks and particles. A general disadvantage of porous ceramic blocks is that their strength decreases exponentially with the increase in porosity. Blocks have a high compressive strength but are brittle; thus they are not considered suitable for load bearing (GARG 1999). Hydroxyapatite (HA) particles, instead of blocks HA, are less brittle and circumvent this problem. Porous HA blocks have been used as an alternative to HA particles (FRAME 1987). BOYNE (1991) noted a decrease in the clinical use of non- resorbable, nonporous HA for alveolar ridge augmentation and an increase in the use of porous HA because the non-resorbable form tends to lack cohesive strength, whilst the particles migrate under stress during the healing process. The use of porous HA blocks, however, might result in mechanical fragile areas if used to fill defects in the cortex of long bones or thin mandibles (YAMAGUCHI et al. 1995).

1.6 EXPERIMENTAL SET-UP AND AIM OF THE STUDY

The experimental model chosen to test bone grafts or bone substitutes should preferably have similar physiologic properties as the clinical site where it will be used. Most of the tests on grafting materials are performed in animal calvaria. This has been shown to be a reproducible model (SIROLA 1960, GLOWACKI et al. 1981, REID et al. 1981, TAKAGI and URIST 1982, PROLO et al. 1982, ISAKSSON 1992, KLINGE et al. 1992). The calvarial model has many similarities to the maxillofacial region. Both the calvaria and the facial bones develop by membranous bone deposition and show morphological and embryological similarities (SCHMITZ and HOLLINGER 1986). In calvarial bone morphogenesis and healing, however, the dura plays a role because of its osteoinductive capacity making it an osteogenic site (HOBAR et al. 1993, GÜZEL et al. 1995, HOBAR et al. 1996, YU et al. 1997). In the facial skeleton respiratory epithelium is involved which is not osteoinductive and thus represents a non-osteogenic site. This respiratory epithelium in the growing individual acts as a resorptive endosteum, leading to an increase in overall size of the (para)nasal sinuses (ZINS et al. 1984). It is, therefore, questionable whether the calvaria is a suitable research model for OMF surgery, as bone grafts are often used to bridge gaps overlying a paranasal sinus with respiratory epithelium as a lining. In this thesis the frontal bone (over the frontal sinus) of young adult female goats as experimental site is chosen. This sinus has a large volume lying between the orbits. The mucosal lining is consists of a ciliar epithelium, similar to the maxillary sinus. Four full thickness standardized defects can be made under general anaesthesia with a trephine. In a pilot study defects with a diameter of 14 mm showed to be of critical size, which means they will not heal spontaneously. The selected site enables the study different grafting materials in one animal.

In the present experimental study BBM was chosen as an alternative for autogenous bone because this material has normal osseous porosity and undergoes progressive resorption when used as a bone substitute (BOYNE 1992). It also gives rise to the deposition of lamellated bone because of its osteoconductive capacity and it eventually will be resorbed. There are, however, no studies available on the behaviour of a composite graft consisting of autogenous bone and BBM. This study was designed to gain further insight in graft healing in general in the maxillofacial area with an emphasis on the mode of application (block graft versus particulate grafts). The primary aim was to study the healing of autogenous cancellous and cortical bone grafts alone and in composite grafts using BBM in a new, standardised animal model.

When considering the use of grafts in the maxillofacial area, alveolar cleft defects immediately come into mind. These sites require grafts that allow teeth to erupt (clefts). To study the eruption of teeth through bone grafts, an experimental model has to fulfil several requirements. The experimental animals must have preferable two generations of teeth, a deciduous and a permanent one. The development of the teeth has to start in the postnatal period and it has to be slow enough to allow accurate documentation by means of large series of radiographs. The animals have to be large enough to make standardized radiographs of the developing teeth and the mandibles possible without superposition of other structures. The experimental animals must have a known background, and the normal growth and development of the animals has to be well documented. Finally they must be easy to handle. Dogs, and especially beagles fulfil most of these requirements. The use of female dogs only is based on the data reported by MALTHA (1982). It is shown that sex differences in the pattern of emergence exist and that the variation in the age of emergence of different teeth in females is smaller than in males (KREMENAK 1967).

Another well known reason for bone grafting in the OMF area is 'sinus floor augmentation'. These sites form an adequate environment for endosteal implants, to create a fixation for a dental prosthesis. The final aim of the study was to review the current clinical literature with regard to various anorganic materials and alloplasts used for sinus floor grafting.

1.7 LITERATURE

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CHAPTER 2

INCORPORATION OF THREE TYPES OF BONE BLOCK GRAFTS IN THE FACIAL SKELETON

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ABSTRACT

The regenerative response on autogenous cancellous and cortical bone grafts, and on a commercially available xenogenous bovine bone mineral (BBM) (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland) was compared in standardized bony defects related to a paranasal sinus.

On 15 skeletally mature goats four critically size full thickness bone defects were trephined in the frontal bone. These defects were filled at random with a cortical bone plug, a cancellous bone plug, a plug of spongeous BBM cut into shape or left empty. Fluorochrome bone markers were injected subcutaneously 1 and 5 weeks after transplantation and one week before the animals were sacrificed. This took place 3, 6, 12 and 24 weeks after surgery.

Histological evaluation showed that autogenous bone grafts were all accepted and incorporated in a similar way as in calvarial defects. BBM was only osteoconductive. New bone was formed at the margins of the defects, and only little of the BBM was incorporated. Most of the BBM was gradually resorbed by multinucleated osteoclast-like cells.

2.1 INTRODUCTION

Several forms of autogenous bone transplants have been used in attempts to achieve rapid and proper bone healing in facial reconstruction (SALYER and TAYLOR 1987). Allografts and xenografts (e.g. deproteinized bone) or other bone substitutes (e.g. glass tubes, ceramics and plastics) which also are commonly used as graft material, only may provide a scaffold for bone formation (URIST 1980, ISAKSSON 1992).

In oral and maxillofacial surgery (OMS), gap bridging over a paranasal sinus with autogenous bone grafts is the 'accepted standard' (HABAL 1992). Two different types of autogenous bone grafts are most often used: cortical and cancellous. Each type has advantages and disadvantages based on differences in architecture of the graft itself (SMITH and ABRAMSON 1974, BURCHARDT and ENNEKING 1978, EITEL et al. 1980, SULLIVAN and SZWAJKUN 1982, ZINS and WHITHAKER 1983, KUSIAK et al. 1985, WILKES et al. 1985, DADO and IZQUIERDO 1989, HARDESTY and MARSH 1990, PHILIPS and RAHN 1990, 1992, ARDEN and BURGIO 1992).

Various allogenous and alloplastic materials have been developed to avoid the problems related to harvesting autogenous bone grafts. These materials are supposed to enhance bony ingrowth in non-weight-bearing gap and revision models, although they do not have bone inductive capacities.

Most of the tests on bone grafting materials are performed in animal calvaria. This has shown to be an accurate and reproducible mode (SIROLA 1960, GLOWACKI et al. 1981, REID et al. 1981, PROLO et al. 1982, TAKAGI and URIST 1982, ISAKSSON 1992, KLINGE et al. 1992). The calvarial model has many similarities to the maxillofacial region as acceptor site. Both the calvaria and the facial bones develop by membranous bone deposition and show morphological and embryological similarities (SCHMITZ and HOLLINGER 1986). In calvarial morphogenesis and bone healing the dura plays a central role (ISAKSSON 1992, HOBAR et al. 1993, GÜZEL et al. 1995, YU et al. 1997). However, in the morphogenesis of the facial skeleton not dura but respiratory epithelium is involved. Bone grafts in OMS are usually used to bridge gaps overlying a paranasal sinus with respiratory epithelium as underlining. This respiratory epithelium normally acts as a resorptive endosteum, leading to an increase in overall size of the (para)nasal sinuses (ZINS et al. 1984).

Therefore the purpose of this study was to evaluate bone healing in the facial skeleton overlying a paranasal sinus in response to autogenous cancellous and cortical bone grafts, and a commercially available xenogenous bovine bone mineral

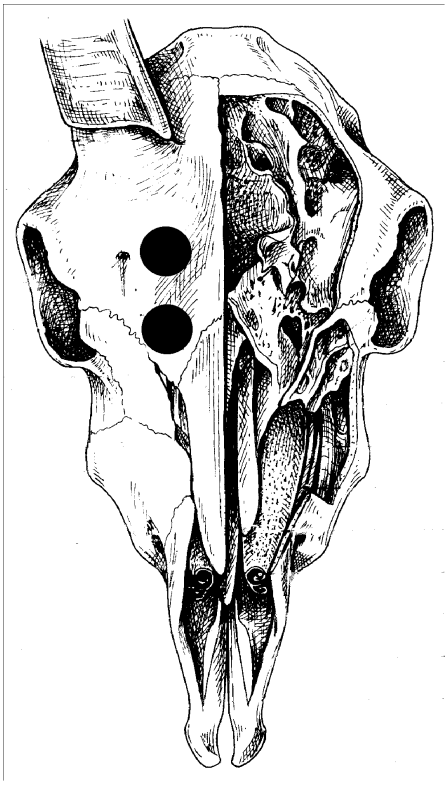

Fig 2.1 *Schematic drawing of the (prepared) skull of a goat with trephine holes over the frontal sinus (CHATELAIN 1987)*

(BBM) (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland).

2.2 MATERIAL AND METHODS

The study was performed on 15 skeletally mature female Dutch milk goats (*Capra Hircus Sana*) of about 2 years of age and weighing about 60 kg. Under general anaesthesia a cancellous bone plug was harvested from the anterior iliac crest with a trephine (inner ∅ 14 mm). In a similar way a cortical bone plug was removed from the caudal mandibular margin, without avulsing the mandibular nerve. Both bone plugs were stored in sterile 0.9% NaCl solution. The third type of material used in this research was Bio-Oss® (Geistlich-Pharma, Wolhusen, Switzerland), delivered as a spongiosa block measuring 1x1x2 cm. It was cut into a circular plug (∅ 14 mm, 0.5 cm thick) and stored in sterile 0.9% NaCl solution as well.

A longitudinal midsagittal incision was made over the snout. Four full thickness bone defects were made in the frontal bone with a trephine (outer ∅ 14 mm) overlying the frontal sinus leaving the mucosal lining and its fibrous attachment to the inner table around the defect intact (fig 2.1). In each animal the

Fig 2.2 *Radiograph of a frontal bone specimen, 3 weeks after transplantation, with tantalum implants around the defects. 1 = Control, 2 = Cancellous bone graft, 3 = BBM sponge, 4 = Cortical bone graft*

defects were filled at random with one of the implant materials or left empty. Prior to the implant procedure metal bone markers were inserted by the method of BJÖRK (1955), one adjacent to each defect to facilitate orientation at sectioning of histological specimens and to improve radiographic evaluation. The periosteum was repositioned and the incision closed with resorbable sutures.

For the histological study, fluorochrome bone markers were injected subcutaneously 1 week (Tetracycline 20 mg/kg), 5 weeks (Xylenol Orange 90 mg/kg) after transplantation, and one week before the animals were given up (Calceine 7 mg/kg).

Groups of 3 animals were sacrificed at 3, 6, or 12 weeks and 6 animals at 24 weeks after surgery with a lethal dose of pentobarbitone after normal anaesthetic induction procedures. Block resection of the frontal bone was performed. After stripping all soft tissue they were stored in 4% 0,1 M phosphate-buffered formaldehyde for fixation. Contact radiographs were obtained by placing the frontal bone specimen paranasal side down on a 5 x 7 cm intraoral film (Kodak Ektaspeed)

using a radiographic apparatus (Philips Oralix), set at 70 kV, and an exposure time of 0.7 seconds.

After fixation, the explants were divided into smaller specimens in such a way that each experimental site was split into two halves. One half was decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast® (Sherwood Medical, St. Louis, U.S.A.) (WIJDEVELD et al. 1991). Serial sections, 7 µm thick, were cut transversally in the midpart of the lesion and stained with haematoxylin and eosin. The other half of each defect was embedded in PMMA without decalcification and 30 µm sections were obtained using a Leitz 1600 rotating water-cooled diamond saw. These sections remained unstained. Two observers evaluated all sections.

2.3 RESULTS

2.3.1 Macroscopical and morphological assessment

Healing was uneventful in all instances without any signs of infection or wound dehiscences. Palpation of the defects in sacrificed animals revealed that after 3 weeks trephine rims of the empty defects were still marked, and after 6 weeks they were more rounded. After 12 and 24 weeks a tight sheet crossed the defect. The autogenous cortical and cancellous bone plugs were rigidly fixed in all animals. The BBM grafts also seemed to be fixed in the trephine defect at 3 and 6 weeks. After 12 and 24 weeks, however, hardly any BBM could be detected in the centre of the defect.

2.3.2 Radiographic analyses

The radiographs did not reveal any difference between the two types of autogenous grafts at any time. The original cortical or cancellous radiologic structure was still detectable after 3 weeks (fig 2.2). All implants seemed well amalgamated to the surrounding bony margins. The control defects showed no healing after 3 weeks but at the edges of some defects new bone mineralization was observed at later times. After 3 and 6 weeks the defects treated with BBM were nearly completely filled with mineralised tissue. Although BBM was less radiopaque after 6 weeks than after 3 weeks, the BBM structure was still identifiable. After 12 weeks tissue within the defects was not completely mineralised anymore and after 24 weeks the amount of mineralised tissue within the defect had decreased further, suggesting resorption of the implant.

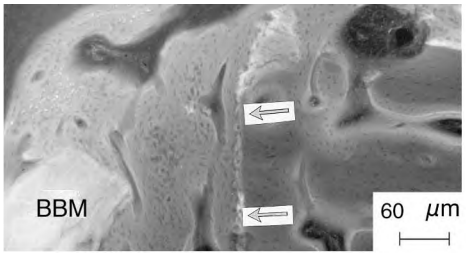

Fig 2.3a *BBM 6 weeks after implantation. Margin of a defect: osteoconduction in BBM sponge (x40). BBM = Incorporated particle of Bovine Bone Mineral, Arrows = Margin of the defect.*

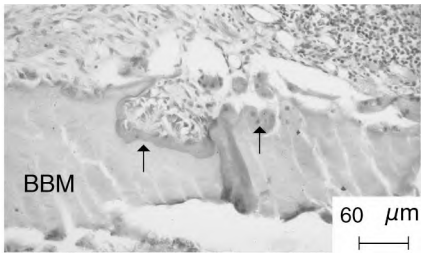

Fig 2.3b *BBM 6 weeks after implantation Centre of the same defect as 3a: osteoclasts around BBM (x40). BBM = Bovine Bone Mineral, Arrows = Active osteoclasts around non-incorporated BBM particle.*

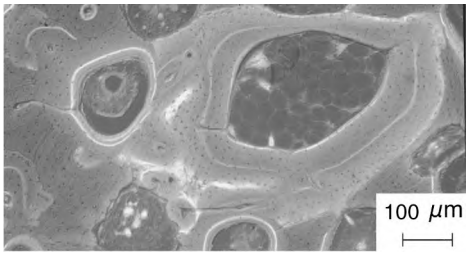

Fig 2.4 *Undecalcified section of cancellous bone graft 12 weeks after transplantation. Labelling reflecting a high remodelling activity. The original spongeous structure can be recognized (x25).*

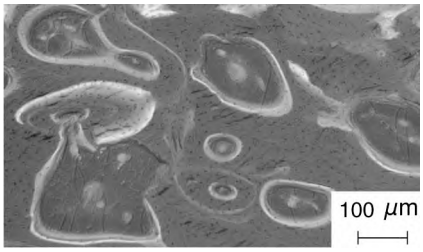

Fig 2.5 *Undecalcified section of cortical bone graft 12 weeks after transplantation. More regular labelling pattern with concentric circles over the whole graft reflecting a slow remodelling process. Mark the difference with fig. 2.4 (x25).*

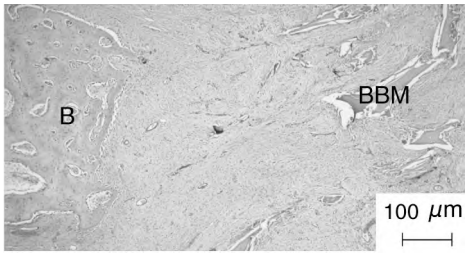

Fig 2.6 *BBM 12 weeks after implantation. Extensive osteoclastic activity around remnants of non-integrated, partly degraded BBM in the centre of the defect (x25). BBM = Bovine Bone Mineral, Arrows = Osteoclasts, B = Bone*

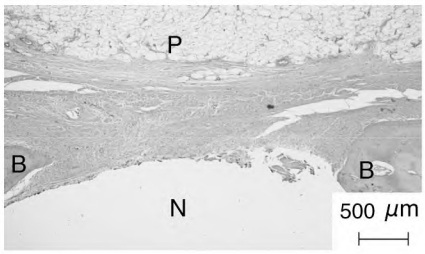

Fig 2.7 *Control defect 24 weeks after operation. The non-bridged defect showed variable amounts of bone deposition, extending to a considerable distance of the margins. Remaining osteoblasts or lining cells on the bone surface indicated a stable phase (x5). N = Nasal side, P = Periosteal side, B = Bony margin of the defect*

2.3.3 Histologic analyses

2.3.3.1 General features

In general no inflammatory activity, granulation tissue or encapsulation was observed in any of the specimens. The approximated periosteal flap and sinus epithelium seemed thicker over the defects. Pronounced subperiosteal bone deposition was observed close to the lesions. These phenomena could not be seen at the side of the sinus.

2.3.3.2 Three weeks after transplantation

Control: Bone formation was seen at the edge of the defect and many osteoblasts covered the bone, suggesting continuing bone deposition. Thick bands of tetracycline labelled bone indicated an active bone ingrowth in the first period. **Cancellous and cortical grafts**: For the first 3 weeks following transplantation, cancellous and cortical grafts underwent comparable repair processes and showed comparable revascularisation of the grafts. Necrotic tissue in the marrow spaces in the centre of the transplants seemed to be removed by macrophages. Local osteoclastic activity was seen. Tetracycline was deposited at the edge of the grafts, in smaller amounts in the cortical than in cancellous grafts. **BBM**: Woven bone was deposited at the edge of the defect growing centripetally into the porosity of the BBM. Spaces without ingrowing bone were infiltrated by loose immature fibrous connective tissue.

2.3.3.3 Six weeks after transplantation

Control: Centripetal bony ingrowth labelled with thick bands of tetracycline and thinner bands of calcein indicate continuing but slower bone deposition in the week prior to death. **Cancellous**: Membranous bone formation is found at the periosteal side of the bone graft while at the side of the sinus bone deposition and resorptive fields could be seen. Bone remodelling took place throughout the whole graft. No tetracycline labelling and a thick area of calcein labelling was seen. **Cortical**: subperiosteal and some submucosal bone formation over the donor-acceptor transition confirmed the acceptation of the bone graft. The graft itself appeared to be vital at the margins and remodelling took place by osteoclastic resorption of the bone and bone deposition within the central canals of osteons. There was no distinct tetracycline or calcein labelling in the centre of the graft. **BBM**: Fan-wise centripetal cancellous bone ingrowth in the BBM sponge emanated mostly from the margins of the defect (fig 2.3a) and in the centre of the defect an extensive activity of multinucleated osteoclast-like cells was seen around the BBM (fig 2.3b). A

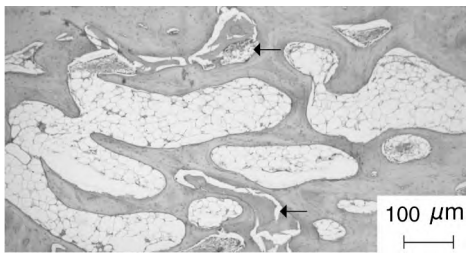

Fig 2.8 *BBM 24 weeks after implantation. Integrated BBM near the margin of a defect. No remodelling activity is seen around these particles. (x25). Arrows = Bovine Bone Mineral*

sheath of fibrous tissue separated the BBM from the new bone in most cases. A few BBM particles were incorporated in newly formed bone and had become part of the remodelling process.

2.3.3.4 Twelve weeks after transplantation

Control: Osteogenesis in the control defects was confined to their margins. The periosteum and the underlining paranasal mucosa was fused and included some isolated islands of young bone. The margins of the new bone were rounded and covered with fibrous tissue and some osteoblastic cells. The centripetal bone ingrowth was labelled with xylenol and calcein. The tetracycline labelled bone was already remodelled. **Cancellous**: High osteoblastic activity and some osteoclasts revealed activation of bone deposition on the graft. In fluorescence microscopy all 3 labels could be seen in an irregular pattern (fig 2.4). **Cortical**: Lamellar bone deposition was seen at the periosteal surface with a marked transition of donor/acceptor bone. The whole graft was involved in the remodelling process. Osteoblasts deposited osteoid within the central canals of osteons and areas of necrotic graft matrix were tunnelled by new osteons. All three labels were recognized over the whole graft, often in concentric circles (fig 2.5). **BBM**: In contrast to the control defects, the BBM prevented fusion of the periosteum and the paranasal mucosa. It created an area filled with loose connective tissue and remnants of BBM. Periosteum, paranasal mucosa and the margins of the defect delineated this area. At the margins, BBM was integrated in bone, which was lined with resting osteoblasts and some osteoclasts. All three labels were found in the bone. In the central area vascular connective tissue appeared more mature and an extensive bone resorption was found around remnants of non-integrated, partly degraded BBM (fig 2.6).

2.3.3.4 Twenty-four weeks after transplantation

Control: The non-bridged defects showed variable amounts of bone deposition, often extending to a considerable distance of the margins. Resting osteoblasts or lining cells on the bone surface indicated a stable phase in which only internal remodelling took place as was also indicated by the calcein labelling (fig 2.7). **Cancellous**: The cancellous grafts had a more cortical aspect than the original spongiosa but not the same as the acceptor bone. The grafts were completely integrated with normal remodelling of the cancellous bone and cortical bone deposition. No tetracycline, some remnants of xylenol and an equal distribution of calceine labelling could be detected over the whole graft. **Cortical**: Cortical grafts had returned to their original structure showing compact bone. No areas of tetracycline labelling could be detected. Xylenol and calcein labelling, however, were evident. **BBM**: Integrated BBM particles could only be detected near the original margins of the defect (fig 2.8). The majority of the particles in the centre appeared progressively to be resorbed and they were replaced by fibrous tissue. Only few remnants were still present. Thin xylenol and calcein bands and no tetracycline labelling were found in the newly formed bone around the BBM scaffold.

2.4 DISCUSSION

The purpose of this study was to investigate the feasibility of autogenous bone grafts and grafts of xenogenous anorganic resorbable bone mineral in osseous defects in the maxillofacial area overlying a (para)nasal mucosa. The rationale for this approach was that calvarial defects may show other responses than maxillofacial defects (MAYER et al. 1996).

Three weeks after implantation the only difference between cancellous and cortical grafts was an initial faster revascularisation and bone deposition in the former. After 6 weeks, the first deposited bone in cancellous grafts is already remodelled, while in cortical grafts the first deposited bone is still present. Also after 12 weeks remodelling in the cancellous grafts is more active than in the cortical grafts. After 24 weeks, the cortical grafts still showed slow remodelling while the cancellous grafts had a more stable aspect. These differences confirm the faster revascularisation and remodelling of the cancellous grafts (BURCHARDT and ENNEKING 1978, ARDEN and BURGIO 1992).

In case of a shortage of autogenous bone, it is interesting to know whether anorganic xenogenous BBM can be used as an alternative. This question has still to be answered. At the one hand, reports indicate structural similarity to cancellous

bone and successful and efficient applications in oral and maxillofacial surgery (BOYNE 1990, HISLOP et al. 1993, WETZEL et al. 1995), but at the other hand also failures are reported (HISLOP et al. 1993, STASSEN et al. 1994). Bio-Oss® is a BBM which closely parallels the structure of human bone enabling an early and effective bone apposition (SCHENK 1991, KLINGE et al. 1992) by osteoconduction (FUKUTA et al. 1992, KATTHAGEN 1993, JENSSEN et al. 1996). KLINGE et al. concluded from their comparison of Bio-Oss® with two synthetic dense non-resorbable hydroxyapatite granules in calvarial defects of rabbits, that no tendency of bone induction by the BBM was detectable, although the resorbable implant seemed to promote initial bone healing more extensively (KLINGE et al. 1992).

In this study centripetal ingrowth of new woven bone in the BBM was seen after 3 weeks and the situation was stabilised after 6 weeks. Slow resorption of integrated BBM is in accordance with the results of JENSEN et al. (1996). Fibroblasts which had migrated to the centre of BBM implanted defects may inhibit osteogenesis (NYMAN et al. 1982, GOTTLOW et al. 1984, DAHLIN et al. 1994, PETIT and RIPAMONTI 1994) by producing factors that hamper differentiation of osteoprogenitor cell populations (OGISO et al. 1989).

In a rabbit calvarial wound model THALLER et al. (1993) did not see osteoclastic activity 12 weeks after implantation of BBM but they found a continuing reactive bone formation. KLINGE et al. (1992), also working in rabbit calvaria, found osteoclasts in moderate amounts only close to the BBM implant tissue interface indicating an active remodelling but not around the non-integrated isolated BBM. This study shows active, ongoing resorption of non-integrated, non-functional BBM by multinucleated osteoclastlike cells. The same is reported by GLOWACKI et al. (1988), who consider this a normal physiological response. These results indicate that for answering questions on osseous wound repair in maxillofacial regions in relation to (para)nasal mucosa an experimental model as used in the present study is more favourable than a model using calvarial defects.

2.5 CONCLUSION

Osseous defects over (para)nasal sinuses filled with autogenous bone grafts, show acceptance and incorporation of the graft and heal equivalent to calvarial defects. Bio-Oss®, as a BBM, is only osteoconductive, and new bone is formed only at the margins of the defect like in non-filled control sites. The bone in the BBM defects is only partly in direct contact with the implant surface. Multinucleated osteoclastlike cells has gradually resorbed the non-functional, central part of the BBM.

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CHAPTER 3

INCORPORATION OF PARTICULATE BONE GRAFTS IN THE FACIAL SKELETON

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ABSTRACT

The purpose of this study was to compare the regenerative response of autogenous cortical and cancellous bone chips and a natural particulate bovine bone mineral (BBM) (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland) in standard bony defects related to a paranasal sinus together and to bone blocks.

On 13 skeletally mature female goats four standard critically size full thickness bone defects were made in the frontal bone overlying the frontal sinus. These defects were filled at random with cortical bone chips, cancellous bone chips, spongiosa granules of a BBM or left empty. Fluorochrome bone markers were injected subcutaneously 1 and 5 weeks after transplantation and one week before the animals were sacrificed. The animals were put down 3, 6, 12 and 24 weeks after surgery.

Particulate autogenous cancellous bone is the material of choice for bridging a bony defect in the maxillofacial area when there is no need for mechanical strength. It heals in the same way as a cancellous bone block will do. Particulate cortical bone is not reliable enough to be used as a solitary bone grafting material under these conditions. A cortical block as a solitary implant gives better results. Particulate BBM as a solitary implant in a critical size defect does not stimulate osteoconduction but gives rise to an extensive osteoclastic activity stimulated by the mutual loose relation. A solid block of BBM is, in a similar case, more reliable.

3.1 INTRODUCTION

The problem of reconstruction of bony defects in the maxillofacial region as a result of malformation, infection, trauma, or oncologic resection has not yet been satisfactory solved (REDONDO et al. 1995). The survival of bone grafts is determined partly by the degree and rapidity of the revascularisation (FONSECA et al. 1980). The revascularisation in turn is influenced by the architecture of the graft itself. Particulate bone grafts present fewer barriers to the diffusion of tissue fluids as well as to the subsequent ingrowth of vascular tissue of the host.

Bone chips or particulate bone are easily and conveniently obtained as surplus material when producing major grafts or performing osteotomies (ISAKSSON 1992). It can be used to fill small defects, to correct minimal contour irregularities and for packing around larger grafts. Particulate bone implants may facilitate and promote bony regeneration (HABAL 1992b).

When there is no surplus material of autogenous bone, a second surgical side has to be created and the operation time is increased. Therefore alloplastic materials have been developed and tested as possible substitutes (REDONDO et al. 1995). In this respect deproteinized bovine bone mineral (BBM) is supposed to offer excellent biocompatibility and physicochemical characteristics corresponding to the mineral component of the original bone (JARCHO 1981). Bio-Oss® (Geistlich-Pharma, Wolhusen, Switzerland) is such a natural material consisting of anorganic bovine bone containing carbonate apatite. Investigations have shown Bio-Oss® to have a natural morphologic structure, a chemical composition identical to that of bone, a large inner surface and porosity comparable to that of bone, a crystalline structure identical to that of bone tissue, and a composition that is purely anorganic (THALLER et al. 1993, PEETZ 1997).

A suitable bone substitute is of interest to reconstructive surgeons: it would obviate the need for harvesting autogenous bone with the concomitant risks and costs associated with a second operative site (DERSOT et al. 1995). Before such materials can be used clinically, they have to be tested in animal models. Many of these tests are performed in animal calvaria (GLOWACKI et al. 1981, REID et al. 1981, TAKAGI and URIST 1982, ISAKSSON 1992, KLINGE et al. 1992). Calvarial defect models, however, may show other responses to therapeutic interventions than maxillofacial surgical models (MAYER et al. 1996, MERKX et al. 1999). It is well known that the dura and periosteum have an osteogenic potential in young humans and animals (REID et al. 1981, GÜZEL et al. 1995). In osseous maxillofacial defects however the (para)nasal respiratory epithelium is involved which has an osteoclastic potential. In calvarial morphogenesis and bone healing the dura plays a central role (HOBAR et al.

1993, GÜZEL et al. 1995, HOBAR et al. 1996, YU et al. 1997). In the facial skeleton, however, not dura but respiratory epithelium is involved in the morphogenesis. In maxillofacial surgery bone grafts are usually used to bridge gaps overlying a paranasal sinus with respiratory epithelium as underlining. This respiratory epithelium normally acts as a resorptive endosteum, leading to an increase in overall size of the (para)nasal sinuses (ZINS et al. 1994).

We have previously evaluated the incorporation of three types of block implants (autogenic cancellous, autogenic cortical and a commercially available xenogenic BBM (Bio-Oss®)) in osseous maxillofacial defects overlying a paranasal sinus in a goat model (MERKX et al. 1999). That animal model appeared to be a valuable tool for answering questions on osseous wound repair in maxillofacial regions in relation to (para)nasal mucosa and bone block grafting.

The purpose of this study was to compare the healing response induced by particulate cortical and spongeous bone and BBM granules in this test model to one another and to bone blocks. From a clinical point of view it is important to know whether a graft is reliable in terms of stability and bone quality (ISAKSSON 1992).

3.2 MATERIAL AND METHODS

The study was performed on 13 skeletally mature female Dutch milk goats (*Capra Hircus Sana*) of about 2 years of age and weighing about 60 kg. Briefly, under general anaesthesia, with a trephine (inner ∅ 14 mm), a full thickness corticocancellous bone plug was harvested from the anterior iliac crest. In a similar way a cortical bone plug was removed without avulsing the mandibular nerve. Both bone plugs were cut into chips of 10-15 mm^3 by Tessier bone-mill (Leibinger, GmbH, Tuttlingen, Germany) and stored in sterile 0.9% NaCl solution. The third type of material used in this study was BBM (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland), delivered as spongiosa granules of 1 - 2 mm^3 stored in sterile 0.9% NaCl solution as well.

Four full thickness bone defects were made in the frontal bone with a trephine (outer ∅ 14 mm) overlying the frontal sinus, leaving the mucosal lining and its fibrous attachment to the inner table around the defect intact (fig 3.1). The defects were filled at random with one of the implant materials or left empty. Prior to the implant procedure metal bone markers were inserted by the method of BJÖRK (1955), one adjacent to each defect to facilitate orientation at sectioning of histological specimens and to improve radiographic evaluation.

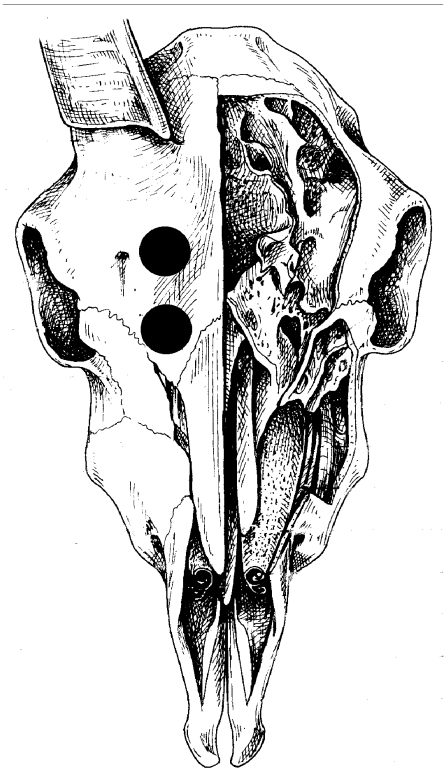

Fig 3.1 *Schematic drawing of the (prepared) skull of a goat with trephine holes over the frontal sinus (CHATELAIN 1987)*

For the histological study, fluorochrome bone markers were injected subcutaneously one week (Tetracycline 20 mg/kg), five weeks (Xylenol Orange 90 mg/kg) after implantation, and one week before the animals were put down (Calceine 7 mg/kg).

Groups of three animals were surrendered 3, 6, or 12 weeks and 4 animals 24 weeks after surgery. Block resection of the frontal bone was performed and all soft tissue was stripped. The blocks including the transplantation sites were stored in 4% 0,1 M phosphate-buffered formaldehyde for fixation. Contact radiographs were obtained by placing the frontal bone specimens paranasal side down on a 5 x 7 cm intraoral film (Kodak Ektaspeed).

After fixation, the explants were divided into smaller specimens in such a way that each experimental site was split into two halves. One half was decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast® (Sherwood Medical, St. Louis, U.S.A.) (WIJDEVELD 1991). From these specimens serial sections, 7 µm thick, were cut transversally in the midpart of the lesion and

stained with haematoxylin and eosin. Two observers evaluated all sections. The healing pattern was analyzed with particular reference to fibrous tissue involvement, new bone formation, graft incorporation, bone marrow development and bone maturation. The other half of each defect was embedded in PMMA without decalcification and 30 µm sections were obtained using a Leitz 1600 rotating water-cooled diamond saw. These sections remained unstained and were evaluated by fluorescence microscopy.

3.3 RESULTS

3.3.1 Macroscopical and morphological assessment

All goats tolerated the surgical procedure well, without anesthetic complications or sepsis. Healing was uneventful in all instances without any sign of local infection or wound dehiscences. Palpation of the defects in sacrificed animals revealed that at 3 weeks p.o. trephine rims of the empty defects were still marked, and at 6 weeks p.o. they were more rounded. At 12 and 24 weeks p.o. a tight sheet closed the trephine defect and the edges were hardly palpable. The defects filled with autogenous cancellous bone chips were difficult to detect after 24 weeks p.o. and bridged with a rigidly fixed layer of bone in all animals. The autogenous cortical chips inlays were still detectable after 24 weeks p.o. because they were pierced at some places by connective tissue strains. At 3 and 6 weeks the BBM grafted areas were bridged with a sheet of fibrous tissue. At the edges some new bone was formed and granules of BBM were centrally located. At 12 and 24 weeks p.o. however in the centre of the defect, BBM could hardly be detected in the fibrous tissue.

3.3.2 Radiographic analysis

The original cortical or cancellous radiological structure was still detectable at all moments. The control defects showed no healing after 3 weeks but at the edges of some defects new mineralized bone was observed at later instances. After 3 weeks (fig 3.2) the defects treated with BBM were more radiopaque than at 6 weeks. At 12 weeks tissue within the defects filled with BBM was not mineralized completely anymore (fig 3.3) and at 24 weeks the amount of mineralized tissue within the defect had decreased further, suggesting resorption of the implant.

3.3.3 Histologic analysis

3.3.3.1 General aspects

No generalized inflammatory activity, granular tissue or encapsulation was observed in any of the specimens. The approximated periosteal flap and sinusepithelium over

de control and BBM defects seemed thicker than in the areas grafted with autogenous bone. Pronounced subperiosteal bone deposition was observed close to the lesions. These phenomena could not be seen at the site of the sinus.

3.3.3.2 Three weeks after transplantation

Control: Bone formation was seen at the edge of the defect and superior to the acceptor cortex probably as an effect of lifting of the periosteum. Finger-shape ingrowth spicules were lined with active osteoblasts, suggesting a continuing bone deposition. Thick bands of tetracycline labelled bone near the edge of the defect indicated an active bony growth in the first period. **Cancellous**: At the acceptor as well as the implant site new bone was seen throughout the whole defect, produced by a layer of active osteoblasts around the grafted particles (fig 3.4). Multinucleated osteoclast-like cells were seen occasionally at the site of the sinus. Most of the lacunae in the particles were empty. The tetracycline labelling was vague and could hardly be recognized. The calceine labelling, however, could be seen over the whole grafted area in the new bone as well as in the former chips. **Cortical**: More osteoclast-like cells around the cortical particles were seen than around the cancellous particles, mainly located at the site of the sinus. The tetracycline and calcein labelling was evident at the acceptor site and around the chips but were less clear than in the cancellous grafted areas. The chips themselves were not stained. **BBM**: In some places at the edge of the defect new bone was deposited at the BBM particles by osteoblasts mostly independent of the implant material. In those areas the BBM particles seemed to be pushed forward as by a snowshovel. No signs of osteoconduction could be recognized. No labelling was found in or around the BBM granules but a clear calceine labelling was present in the newly formed bone originating from the edges of the defects.

3.3.3.3 Six weeks after transplantation

Control: Centripetal bony ingrowth labelled with thick bands of tetracycline and thinner bands of calcein indicated continuing but slower bone deposition in the last week. The structure of the new bone adapted to the original acceptor bone with a large central defect filled with fibrous tissue. **Cancellous**: The cancellous chips were still recognizable with fibrous connective tissue in between. Most lacunae in the chips were empty, but a few showed viable osteocytes. Osteoclastic but mainly osteoblastic activity around the chips caused a net bone deposition over the whole grafted area. In the undecalcified sections tetracycline and calceine labelled the acceptor site more extensively than the new bone in the grafted area. The chips themselves were vaguely

stained with calcein. **Cortical**: The cortical chips had a necrotic aspect with empty lacunae embedded in vital new bone coming from the acceptor bone as well as from new foci in the centre. This new bone had a cancellous aspect. In some places osteoclasts were seen on the paranasal side. One of the three defects was bridged with a small layer of new bone on the periosteal side. The other two defects were not bridged. A tetracycline and calcein labelling could only be detected at the margins of the defect while a solitary calcein labelling was seen in the new bone around the chips without staining the chips themselves. **BBM**: Centripetal deposition of new bone was found which was in contact with bone-BBM in some places. Osteoclastic activity was seen around the non-integrated BBM particles at the periosteal as well as paranasal side. The interstitial space between the implanted particles was infiltrated with immature fibrous connective tissue.

3.3.3.4 Twelve weeks after transplantation

Control: Osteogenesis in the control defects was confined to the margins of the defects. In the central area a fusion of the periosteum with the underlining paranasal mucosa was found with some isolated islands of young bone in between. The margins of the new bone were rounded and covered with fibrous tissue and some osteoblastic cells. The centripetal bone ingrowth was labeled with xylenol and calcein. The tetracycline labelled bone had already been remodelled. **Cancellous**: New bone was deposited from the edges of the defect as well as around the chips. At the periosteal side more bone formation was seen than at the paranasal side. The defects were totally filled with chips embedded in new bone. The structure of the chips was still recognizable in the irregular trabeculae of the new bone (fig 3.5). Only occasional osteoclasts and osteoblasts were present. Hardly a difference could be seen between the acceptor and donor tissue with no tetracycline, a vague xylenol and a clear calceine labelling in and around the chips which became part of the bony structure. **Cortical**: The irregular trabecular structures of the chips could be recognized with empty lacunae embedded in new woven bone arising from the acceptor and implant sites. The defects were not totally bridged but areas of new bone/chips alternated with islands of connective tissue. Xylenol and calcein labels were seen over the whole grafted area but not within the chips themselves. **BBM**: At the edge of the defect incorporated particles of BBM were involved in the physiologic remodelling process (fig 3.6). The space between periosteum and mucosa was filled with BBM particles embedded in a stroma of fibrous tissue. No bone deposition was found in that area. Abundant osteoclastic activity at the paranasal side around these non-integrated particles decreased the amount of implant material.

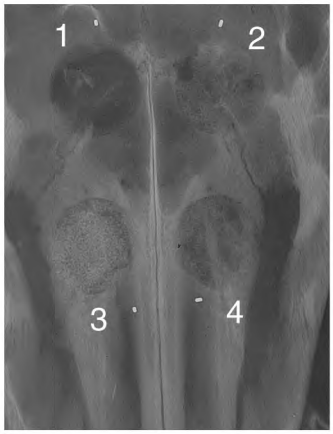

Fig 3.2 *Radiograph of a frontal bone specimen, 3 weeks after transplantation, with tantalum implants around the defects. 1 = Control, 2 = Cancellous bone graft, 3 = BBM sponge, 4 = Cortical bone graft*

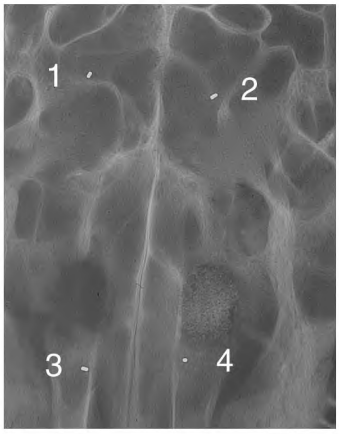

Fig 3.3 *Radiograph of a frontal bone specimen, 12 weeks after transplantation, with tantalum implants around the defects. 1 = Cortical bone graft, 2 = Cancellous bone graft, 3 = Control, 4 = BBM sponge*

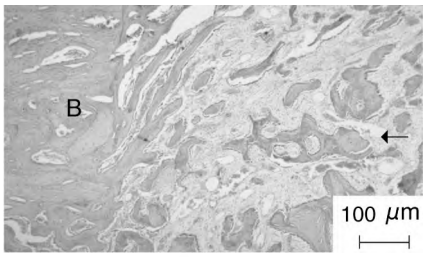

Fig 3.4 *Cancellous particulate bone, 3 weeks after implantation, embedded in young bone delineated by osteoblasts. (orig. magn. x25)*

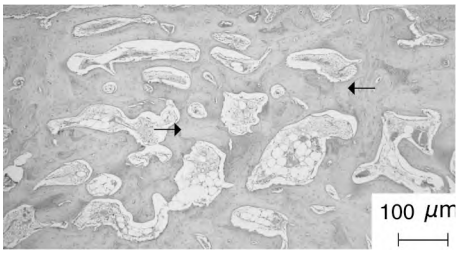

Fig 3.5 *Cancellous bone chips, 12 weeks after implantation. The structure of the chips is still recognizable in the irregular trabeculae of new bone (orig. magn. x25). Arrows = former chips with empty lacunae*

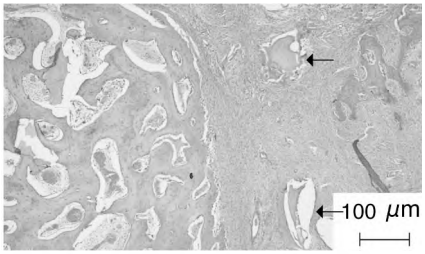

Fig 3.6 *BBM 12 weeks after implantation. Incorporated particles of BBM are involved in the physiologic remodelling process. (orig. Magn. x25)*

3.3.3.5 Twenty four weeks after transplantation

Control: The non-bridged defects showed variable amounts of bone deposition, often extending a considerable distance from the margins. Resting osteoblasts on the bonesurface indicated a stable phase in which only internal remodelling took place as indicated by calcein labelling. **Cancellous**: A uniform remodelling process over the whole defect could be seen. The former spongeous structure of the chips could hardly be recognized. In fluorescence microscopy more bone formation at the periosteal than at the paranasal side was seen. Some remnants of tetracycline were found at the edge of the original defect. In the centre mainly calceine was equally deposited. **Cortical**: Small non-union areas filled with connective tissue were still detectable without osteoblastic activity. Collagen fibres piarced the bone in a direction perpendicular to the bone surface, and the margins of these defects showed rounded edges. In the undecalcified slides tetracycline labelled bone surrounded the chip-like structure in the centre while xylenol and calcein labelling were found throughout the whole defect. **BBM**: Some isolated BBM particles in the centre of the defect were left and embedded in fibrous tissue with osteoclastic activity at the paranasal side. In comparison with 6 and 12 weeks the fibrous tissue was more mature and the amount and size of granules had decreased.

3.4 DISCUSSION

Particulate bone is composed of small chips and is applied in areas where no mechanical strength is required (DODD et al. 1988, HABAL 1992a). Clinically, chips are easier to put in a bony defect than block implants: the defect should be stented well without fitting exactly. Healing takes place as the bony chips become incorporated. Rapid revascularisation of the grafted tissue is followed by solidification which can withstand mechanical forces and weight bearing. In humans, this process may take more than a year to be completed (MURPHY et al. 1992, MERKX et al. 1997, PEETZ 1997, MERKX et al. 1999). Autogenous cortical bone particles of 'intermediate' size have the optimal potential for regeneration of bone. Small particle grafts (8mm^3) were quicker revascularized, showed more osteoclastic activity and therefore resorbed much quicker and more complete than the large particle sized grafts (50mm^3) (FONSECA et al. 1980). The average particle size of 10-15 mm^3 used in this study can be considered as 'intermediate'. FONSECA (1980) found initial edema and inflammation only in the large particulate grafts. This study shows similarities with the latter. On the other hand, 12 weeks postoperatively the structure of the cancellous particles was hardly distinguishable, which is in agreement with the small particulate areas (FONSECA et al. 1980).

In this research project the defects filled with autogenous cancellous bone chips healed uneventfully. After six weeks the defects were nearly bridged, but the trabecular structure of the chips could still be detected. In the defects filled with cortical chips, osteoblasts were trying to bridge the spaces between the particles. Fibroblasts between the cortical chips produced connective tissue strains perpendicular to the defects with an inhibitory effect on bone formation: they hampered the osteoblasts in bridging the remaining gaps. The chips themselves had a necrotic aspect and were not stained by the labels as abundantly as the cancellous chips. In cortical block grafts, at that time, a continuum of centripetal labelling of tetracycline and calcein was seen although not equally distributed over the whole graft (MERKX et al. 1999). So after 6 weeks particulate cortical bone seems not to be such a reliable grafting material as the cortical bone blocks are. The cancellous graft areas were bridged and accepted in the physiological remodelling process. This is similar to the results of bone-block implantation. The cortical graft areas, however, were pierced by bundles of collagen: they were not smoothly bridged. This indicates that a steady state had been reached: the defect will not heal any further by itself. This was also seen by HOLLINGER (1993) who compared autogenous cortical and cancellous particulate allergen antigen free bone.

In case of a shortage of autogenous bone, anorganic xenogenous BBM might be a valuable alternative (BOYNE 1990, HISLOP et al. 1993, PEETZ 1997). However, some failures have been reported as well (STASSEN et al. 1994, WETZEL et al. 1995). Bio-Oss® is such a natural material consisting of anorganic bovine bone matrix containing carbonate apatite.

Histologically, the BBM-implanted sides underwent initial fibroblastic response, with formation of immature connective tissue around the granules. The centripetal ingrowth of new woven bone in BBM, seen in a previous study with bone block implants (MERKX et al. 1999), could not be seen in the particulate BBM 3 weeks after implantation. The new bone pushed the granules forward as by a 'snowshovel' and no signs of osteoconduction were seen. This 'snowshovel' phenomenon was also observed in BBM implanted sides overlying erupting premolars in beagle dogs (MERKX et al. 1997). The assumption that more time for incorporation for the BBM granules may improve the results, cannot be supported. To prevent this 'snowshovel' effect, it seems better to implant an exact fitting block instead of particulate BBM. It is noteworthy that the present results are in agreement with previous findings showing that granular hydroxyapatite failed to initiate bone differentiation in heterotopic and orthotopic sites (RIPAMONTI and REDDI 1992, VAN EEDEN and RIPAMONTI 1994).

The local mechanical environment has great influence on bone regeneration and healing. In areas with physiological mechanical loading, bone is remodelled very slowly (SALAMA 1983, KLEIN-NULEND et al. 1993), while in areas with more mechanical loading bone will resorb, bone grafts will not incorporate (e.g. over erupting teeth (MERKX et al. 1997)) or bone fractures will not heal resulting in a pseudoarthrosis (MURPHY et al. 1992). The multinucleated giant cells/osteoclasts observed around the granules 6 and 12 weeks p.o. is also reported in the block study but not that excessively (MERKX et al. 1999). The mutual loose relation of the granules may stimulate these cells more and in an earlier stage. The presence of multinucleated giant cells around the granules 6 weeks p.o. is also reported by other investigators who examined the tissue responses to non-resorbable hydroxyapatite (HA) granules (DERSOT et al. 1995). They stated that the giant cells generally flattened and assumed a non-foreign-body type of configuration. In this experiment, after 24 weeks, osteoclasts/multinuclear giant cells are still observed around the non-incorporated particles suggesting a continuing resorption. It is reported that macrophages with intracytoplasmic granular particles can be detected 1 year after an augmentation procedure of the alveolar ridge in which non-porous HA had been used (DONATH 1988).

3.5 CONCLUSION

In conclusion it may be stated that autogenous particulate cancellous bone is the choice material for bridging a bony defect in the maxillofacial area when mechanical strength is not needed. They heal in the same way as cancellous bone blocks do. Cortical bone chips are not reliable enough to be used as solitary bone grafting material under these conditions. A cortical block as a solitary implant gives better results. BBM granules as solitary implant in a critical size defect do not stimulate osteoconduction but give rise to an extensive osteoclastic activity, probably stimulated by the mutual loose relation or the continuous mechanical changes. A solid block of BBM is in a similar case is more reliable but not as good as a cortical block.

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CHAPTER 4

INCORPORATION OF COMPOSITE BONE GRAFTS IN THE FACIAL SKELETON

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ABSTRACT

The purpose of this study was to evaluate the capacity of composite grafts consisting of either particulate cancellous or particulate cortical bone and anorganic bovine bone mineral (BBM) (Bio-Oss®) to induce regeneration in standardized bony defects overlying the frontal sinus. Four full thickness bone defects of critical size were made in the frontal bone in each of 8 skeletally mature female goats. These defects were filled at random with composite cancellous bone/BBM grafts or composite cortical bone/BBM grafts. Control defects were not included but could be evaluated using data from a previous study in which the same experimental setting was used[19]. Fluorochrome bone markers were injected subcutaneously 1 and 5 weeks after implantation, and 1 week before the animals were sacrificed. Two animals were put down at 3, 6, 12 and 24 weeks after surgery respectively. The results were evaluated by histological means including fluorescence microscopy. In conclusion, composite grafts consisting of autogenous cancellous bone/BBM yielded good results, combining the advantages of each material alone and reducing the disadvantages of each when used separately. Critical size defects in the maxillofacial area, overlying a paranasal sinus, filled with this material heal uneventfully within 12 weeks. Composite grafts consisting of cortical bone and BBM show less favourable results. These grafts induce osteoclasts, probably by the presence of non-functional BBM, resulting in resorption of the cortical bone chips.

4.1 INTRODUCTION

Augmentation of the atrophied maxilla by sinuslifting or reconstruction of osseous defects in the maxillofacial skeleton in contact with a paranasal sinus is still a great challenge. The use of autogenous bone grafts has been the 'golden standard' so far (HABAL 1992). Disadvantages of this procedure are the long operation time, the creation of a donor site and the subsequent morbidity. Furthermore, there is often a shortage of donor bone. Some of these disadvantages can be overcome or decreased by the use of bone substitutes.

Bone substitutes may be divided into two major categories. One consists of an organic matrix made by either demineralizing xenogenous bone or by the in vitro construction of collagenous lattices in which osteoinductive proteins are embedded. Demineralized bone may have the risk of host pathogen transmission, inconsistent performance, limited supply and tenuous inductive ability. The use of recombinant human osteoinductive proteins embedded in collagenous matrices are promising but till now not registered by the American Food and Drug Administration.

The other major category consists of an inorganic matrix made by either sintering calcium phosphates such as hydroxyapatite (HA) or by deproteinizing xenogenous bone. Inorganic bone minerals, if used alone, do not show the capacity to bridge critical size maxillofacial defects (BOYNE 1991, MERKX et al. 1999a and 1999b). Composite bone grafts, consisting of HA and autogenous corticocancellous bone, however, seem to be more promising according to studies in cleft alveolus cases (HABAL 1991), in preprosthetic surgery (HAERS et al. 1991, CAWOOD et al. 1994, TOLMAN 1995) and in post-oncologic surgical reconstructions (COBB et al. 1990, BOYNE 1992). Composite grafts would combine the advantages of their components and minimise their disadvantages (HABAL 1991). The primary role of anorganic additives would be the enhancement of osteoconduction, during the second phase of bone healing (KENT et al. 1983).

Reports on composite grafts in which anorganic bovine bone mineral (BBM) such as Bio-Oss® (Geistlich-Pharma, Wolhusen, Switzerland) is combined with particulate autogenous cortical or cancellous bone are lacking up to now. The purpose of this study was to evaluate the possibilities of a BBM in combination with both kinds of autogenous particulate bone in bridging critical size osseous defects in an experimental setting.

4.2 MATERIAL AND METHODS

The study was performed on 8 skeletally mature female Dutch milk goats (*Capra Hircus Sana*) of about 2 years of age and weighing about 60 kg. The surgical

procedures have been described extensively elsewhere[19]. Briefly, under general anaesthesia a cancellous bone plug was harvested from the anterior iliac crest with a trephine. In a similar way a cortical bone plug was removed from the mandible without avulsing the mandibular nerve. Both bone plugs were cut into chips of approximately 10-15 mm^3 by Tessier bone-mill (Leibinger, Tuttlingen, Germany) (CHIAPASCO and RHONCHI 1994) and mixed (1:1) with a xenogenous anorganic bone mineral (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland), delivered as spongiosa granules of 1-2 mm^3.

Four full thickness bone defects were made in the frontal bone with a trephine (outer diameter 14 mm) overlying the frontal sinus, leaving the mucosal lining and its fibrous attachment to the inner table around the defect intact (fig 4.1). In each animal the defects were filled at random with one of the two implant materials. Prior to the implant procedure metal bone markers were inserted (BJ[illegible]RK 1955), one adjacent to each defect to facilitate orientation at sectioning of histological specimens and to improve radiographic evaluation. The periosteum was repositioned and the incision closed with resorbable sutures. Control defects were not included but data from previous studies with the same experimental setting were used (MERKX et al. 1999a and 1999b).

For the histological study, fluorochrome bone markers were injected subcutaneously 1 week (Tetracycline 20 mg/kg), 5 weeks (Xylenol Orange 90 mg/kg) after implantation, and 1 week before the animals were killed (Calceine 7 mg/kg).

Groups of 2 animals were put down 3, 6, 12 and 24 weeks after surgery. Normal anaesthetic induction procedures were performed after which a lethal dose of pentobarbitone was administered. Block resection of the frontal bone was performed and all soft tissue was stripped. The blocks including the transplantation sites were stored in 4% 0.1 M phosphate-buffered formaldehyde for fixation. Contact radiographs were obtained after placing the frontal bone specimen with the paranasal side down on a 5x7 cm intraoral film (Kodak Ektaspeed).

After fixation, the explants were divided into smaller specimens in such a way that each experimental site was split into two halves. One half was decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast® (Sherwood Medical, St Louis, MO, USA) (WIJDEVELD et al. 1991). From these specimens serial sections, 7 µm thick, were cut transversally in the midpart of the lesion and stained with haematoxylin and eosin. Two observers evaluated all sections. The healing pattern was analysed with particular reference to fibrous tissue involvement, new bone formation, graft incorporation, bone marrow development

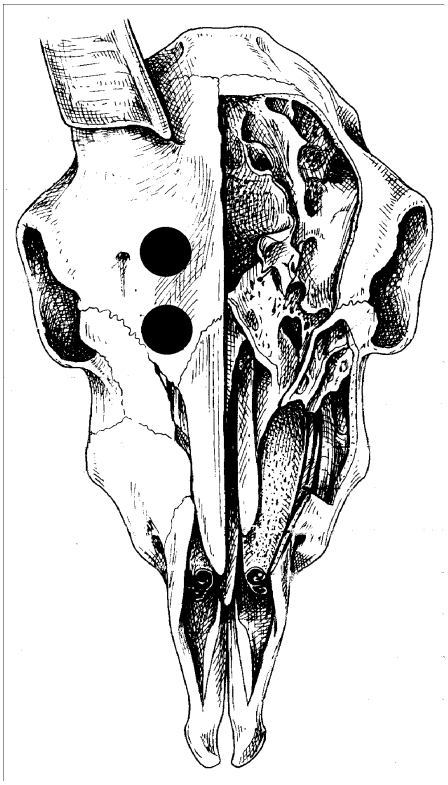

Fig 4.1 *Schematic drawing of the (prepared) skull of a goat with trephine holes over the frontal sinus (CHATELAIN 1987).*

and bone maturation. The other half of each specimen was embedded in PMMA without decalcification and 30 µm sections were obtained. These sections remained unstained and were evaluated by fluorescence microscopy.

4.3 RESULTS

4.3.1 Macroscopical and morphological assessment

All goats tolerated the surgical procedure well, without anaesthetic complications or sepsis. Healing was uneventful in all instances without any sign of local infection or wound dehiscences. The defects filled with composite cancellous bone/BBM grafts were difficult to detect clinically after 12 and 24 weeks postoperatively and they were bridged with a rigidly fixed layer of bone in all animals. At 24 weeks postoperatively the composite cortical bone/BBM grafts were still manually palpable because of a rough surface at some places interrupted by persisting perforations.

4.3.2 Radiographic analysis

The radiographs did not reveal major differences between the two types of composites after 3 weeks. The original chip structure of the cortical/BBM grafts, however, was better recognizable than in the cancellous/BBM grafts. After 6 weeks the solitary cortical chips could be recognized while the cancellous/BBM grafts were more equally amalgamated. After 12 and finally after 24 weeks the cancellous/BBM grafted areas could hardly be recognized anymore. The cortical/BBM grafted areas were not totally amalgamated but had some radiolucencies over the former defects.

4.3.3 Histologic analysis

General aspects. No generalised inflammatory activity, granular tissue or encapsulation was observed in any of the specimens during the experimental period. Pronounced subperiosteal bone deposition was observed close to the lesions. This phenomenon could not be seen at the paranasal side.

Three weeks after transplantation. **Cancellous/BBM grafts**: An active bone formation was observed at the edges of the defects and from individual cancellous bone chips (fig 4.2). New bone was deposited along and around a few solitary BBM particles. It is self-evident that this 'integrated/accepted' BBM itself had a non-vital necrotic aspect with empty lacunae. Most of the BBM particles showed no adjacent bone formation. Multinucleated giant cells were resorbing the BBM. Vital staining showed calceine labelling at the edges of the BBM granules suggesting some active calcium uptake in the BBM. The calceine labelling of the cancellous bone chips was more intense. **Cortical/BBM grafts**: Bone sprouted mainly from the edges of the defect. Osteoclastic activity was found around individual cortical and BBM chips (fig 4.3). In cortical chips most of the lacunae were empty. No fluorochrome markers were found in these chips whereas the new bone at the edges was labelled with calceine.

Six weeks after transplantation. **Cancellous/BBM grafts**: The defects were almost bridged with integrated BBM surrounded by 'multinucleated' cells and resorption lacunae throughout the whole defect (fig 4.4). Some BBM particles were integrated. They were mainly located under the periosteum or the paranasal mucosa, embedded in fibrous tissue and surrounded by multinucleated cells. In vital staining, the former cancellous bone chips could be recognized by a lower staining intensity in their centre surrounded by a zone of intense calceine labelling covering almost the whole defect. The BBM granules were equally vaguely stained by calceine. **Cortical/BBM grafts**: At the edges some integrated BBM granules and necrotic

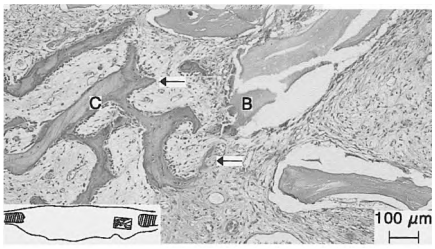

Fig 4.2 *Cancellous/BBM graft 3 weeks after implantation. An active bone formation sprouted from solitaire cancellous bone chips. The BBM particles are surrounded by multinucleated cells (orig. magn. x25), B = Bovine bone mineral; C = Cancellous bone chip surrounded by new bone; arrow = active bone formation.*

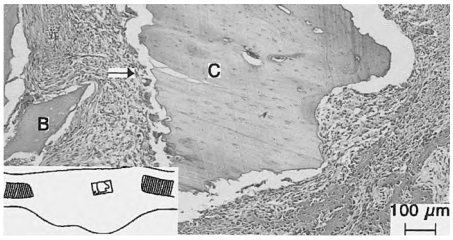

Fig 4.3 *Cortical/BBM graft 3 weeks after implantation. Osteoclastic activity was found around individual cortical and BBM chips (orig. magn. x25). No new bone formation was seen around the original cortical chips. B = Bovine bone mineral; C = cortical bone chip; arrow = multinucleated cells.*

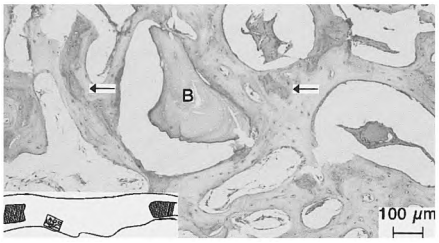

Fig 4.4 *Cancellous/BBM graft 6 weeks after implantation. Integrated BBM surrounded by new bone in which the former cancellous and the BBM particles could be recognized (orig. magn. x25). B = Bovine bone mineral; arrow = former cancellous bone chip.*

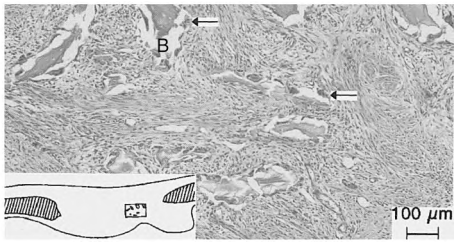

Fig 4.5 *Cortical/BBM graft 6 weeks after implantation. In the centre of the defects osteoclast-like cells surrounded the cortical chips and the BBM granules. No bone deposition was seen. (orig. magn. x25). B= Bovine bone mineral; arrow = multinucleated cells.*

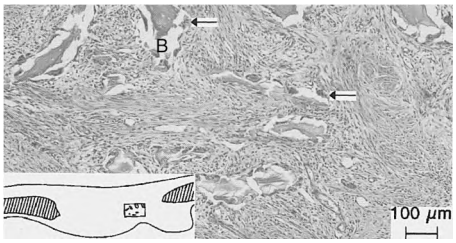

Fig 4.6 *Cancellous/BBM graft 12 weeks after implantation. The BBM particles are integrated in more compact bone than in Fig. 4. The original structure of the cancellous chips could hardly be recognized anymore (orig. Magn. x25). B = Bovine bone mineral.*

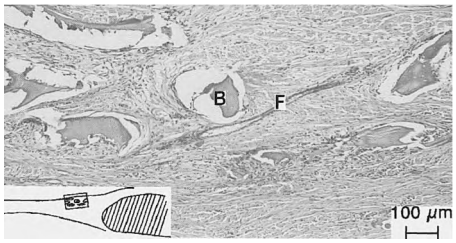

Fig 4.7 *Cortical/BBM graft 12 weeks after implantation. In all cases BBM granules in the central area were embedded in mature fibrous tissue surrounded by multinucleated cells (orig. magn. x25). B = Bovine bone mineral; F = fibrous tissue.*

cortical chips were seen in the newly formed bone without osteoclastic activity. Most of the BBM granules were not integrated but embedded in a very dense connective tissue stroma. In the centre of the defects osteoclast-like cells surrounded these granules (Fig 4.5). No tetracycline or calceine labelling of the BBM and the cortical chips was seen over the whole defect whereas at the edges the new bone was intensely labelled.

Twelve weeks after transplantation. **Cancellous/BBM grafts**: All defects were bridged with bone showing some osteoblastic and osteoclastic activity. The BBM particles were integrated in young bone with a physiological remodelling showing resorption lacunae (Fig 4.6) but the quantity of BBM was reduced compared to the 3 and 6 weeks defects. The vital staining showed a xylenol and calceine labelling throughout the whole defect. Hardly any tetracycline could be recognized. **Cortical/BBM grafts**: None of the defects was totally bridged. In all cases BBM granules and some cortical chips in the central area were embedded in mature fibrous tissue surrounded by multinucleated cells (Fig. 4.7). The new bone formed from the edges of the defects was stained with tetracycline, xylenol and calceine.

Twenty four weeks after transplantation. **Cancellous/BBM grafts**: The defects were filled with cancellous bone. The initial edges of the defects could hardly be recognized anymore. Some small pieces of BBM were integrated and bone remodelling was found throughout the former defect. In vital staining no tetracycline, a vague xylenol and normal calceine labelling was seen in which the original structure of cancellous chips had disappeared. **Cortical/BBM grafts**: None of the defects was bridged. In the centre of the defects some BBM particles and some cortical chips could be seen embedded in mature fibrous connective tissue without osteoclastic activity. In vital staining the newly formed bone at the edges of the defects showed less xylenol and calceine labelling in comparison to the cancellous/BBM grafts. At the edges BBM was faintly stained with xylenol and not with tetracycline or calceine.

4.4 DISCUSSION

Autogenous bone grafts from different locations are mainly used in augmentation procedures of the atrophied maxilla or in osseous reconstructions in the maxillofacial area. Problems with resorption of the autogenous grafts, shortage of donor site bone and morbidity of the donor site have led to the search for alternatives or additives. One of the alternatives to compensate for shortage of donor site bone is the use of alloplastic (synthetic) inorganic materials and

autogenous particulate corticocancellous or cancellous bone in composite graft-implant substrates (HABAL 1991, HAERS et al. 1991, CAWOOD et al. 1994, TOLMAN 1995). These grafts can lead to a better bone regeneration by enhancement of the osteoconduction. HA is used as a porous non-biodegradable ceramic resembling the anorganic bone matrix.

Another possibility is the use of a biological anorganic material such as Bio-Oss®, which has a bovine origin. This material has a normal osseous structure and is prone to normal osteoclastic resorption (PINHOLT et al. 1991, MERKX et al. 1999a and 1999b), although other studies refute this (MANDELKOW et al. 1990, STASSEN et al. 1994, SKOGLUND et al. 1997, YOUNG et al. 1999). It is osteoconductive and becomes involved in normal bone remodelling in which it is replaced by new lamellar bone (BOYNE 1992). It is brittle and lacks the basic elasticity of normal bone. This makes it less suitable for use on its own (MERKX et al. 1999a and 1999b). However, in combination with autogenous bone grafts, it can provide an additional stimulus for osteoconduction and as an expander (HABAL 1991). The advantages of this additive as supporting material for the particulate autogenous bone should be compared with the advantages of the use of this bone graft alone.

A bone/HA ratio varying from 1:1 to 1:2 is recommended, to avoid excessive fibrous encapsulation of the HA granules (COBB et al. 1990, TIDWELL 1992). Because there was no information about the ideal ratio for bone/BBM a 1:1 ratio is used in this study.

The maxillofacial defects used in the present study (∅ 14 mm) were of critical size (MERKX et al. 1999a), which means that they will not heal spontaneously (SCHMITZ and HOLLINGER 1986). This size may resemble the magnitude of an osseous defect, e.g. in preprosthetic surgery, in a cleft alveolus or an osteotomy gap. In the same experimental setting (MERKX et al. 1999a) the healing pattern of empty defects were described extensively and therefor not used in this experiment as a control.

Cancellous/BBM composite grafted areas in our study showed a stable situation after 12 weeks. All the defects were bridged and integrated BBM particles had to a large extent been replaced by newly formed bone due to physiological remodelling. After 24 weeks the same features were seen, although hardly any BBM particles could be seen anymore.

The involvement of implanted autogenous iliac crest bone in normal physiological remodelling has recently been reported by BLOMQVIST et al. in an osteometric and morphometric analysis of sinus lifting procedures[3]. They described

an active bone remodelling (deposition and resorption) instead of only bone deposition as soon as one month after transplantation. These findings may indicate that cancellous/BBM composite bone graft healing is accomplished within 12 weeks. Extension of the follow-up period with another 12 weeks does not seem to result in a better bone quality. When cancellous chips are implanted without an additive the same healing response is seen (MERKX et al. 1999b): after 6 weeks the defects are nearly bridged with the trabecular structure still detectable while after 12 weeks in a stable remodelling phase this structure had disappeared.

Cortical/BBM composite grafts showed a more extensive osteoclastic activity around non-vital cortical bone chips compared to grafts consisting of particulate cortical bone alone (MERKX et al. 1999b). This study showed that both at 12 and 24 weeks after implantation of cortical/BBM grafts none of the defects was bridged, while after implantation of cortical bone alone in the same setting only small fenestrations were found in the former defects. More osteoid tissue was seen when pure cortical chips as a bone grafting material was used (MERKX et al. 1999b). This difference can be clarified by the presence of more multinucleated cells around individual granules and the cortical chips. These cells, directly recruited by the presence of BBM, are able to resorb the non-vital cortical chips (GLOWACKI and COX 1986).

Another problem in bone grafting is the morbidity of the donor site. Reduction of the morbidity can be achieved by using a trephine for harvesting cancellous bone from the iliac crest (KALK et al. 1996, LUNDGREN et al. 1997) or the tibia plateau (VAN DAMME and MERKX 1996) instead of harvesting a corticocancellous bone block from the iliac crest (BANWART et al. 1995) due to leaving most of the muscular attachment uncompromised. The present study showed that the combination of BBM/cortical chips led to a more pronounced osteoclastic activity than the combination of BBM/cancellous bone. This means that the cortical components in particulate cortico-cancellous bone grafts may not have a positively additive influence and can be omitted, leading to a reduction of the donor site morbidity.

4.5 CONCLUSION

In conclusion, a composite graft consisting of autogenous cancellous bone and BBM is reliable, combining the advantages of each material alone and minimising the disadvantages of each when used separately. Critical size defects in the maxillofacial area, heal uneventfully within 12 weeks, when filled with this material. An additional 12 weeks may not yield a better bone quality. Cortical bone

in a composite graft at least has no additive value in bone healing, and probably an opposite effect is reached by the resorption of the cortical chips by osteoclasts induced by the presence of non-functional BBM.

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CHAPTER 5

TOOTH ERUPTION THROUGH AUTOGENOUS AND XENOGENOUS BONE TRANSPLANTS: A HISTOLOGIC AND RADIOGRAPHIC EVALUATION IN BEAGLE DOGS

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ABSTRACT

The effect of implanting autogenous and anorganic xenogenous bone transplants into metabolically active sites within Beagle dog mandibles during permanent premolar tooth eruption was examined. Ten beagles, 14 weeks old were used. Before the onset of the radiographic experiments, metal bone markers were placed in the caudal margin of the mandible at an age of 10 weeks. The deciduous first and third molar were extracted and their sockets over the second and fourth premolar were implanted with autogenous particulate enchondral iliac crest bone, autogenous particulate membranous mandibular body bone, xenogenous anorganic bovine bone mineral (BBM) spongiosa granules (1-2 mm^3) (Bio-Oss®, Geistlich Pharma, Switzerland) or left empty. The third premolar served as control side. Standardized oblique lateral radiographs were made once a week. A number of co-ordinates of defined points and structures were determined by means of a co-ordinate digitising system. The animals were put down 4, 10, and 16 weeks after bone transplantation for histologic examination of the transplantation sides.

All premolars showed no delay in eruption or disruption of crown and root development. In histology the BBM particles were not resorbed or integrated in the alveolar bone but pushed forward into the gingiva.

We have demonstrated that there is no difference in eruption curve of the permanent premolars in the 4 groups (ANOVA $p > 0.5$) and that bone transplantation has no inhibitory effect on eruption (ANOVA $p > 0.3$) and crown development of the underlying permanent premolar, but that Bio-Oss® has not the same resorbable or integrating capability as autogenous bone grafts.

5.1 INTRODUCTION

Secondary bone grafting of the residual alveolar cleft in patients with cleft lip and palate has become a well established procedure with well-accepted advantages (FREIHOFER et al. 1993, KALAAJI et al. 1996). One of these advantages is to promote the eruption of the permanent canine into the alveolar defect (WITSENBURG and REMMELINK 1993). Therefore the bone grafting has to be performed prior to permanent canine eruption, at the stage in which this tooth has formed about one half to two thirds of its root. Usually, free autogenous bone from the anterior iliac crest, the rib or the chin of the mandible is used (WITSENBURG and REMMELINK 1993). The age of the patient by that time varies between 8 and 12 years. The bone graft stabilises the dento-osseous segments, improves alveolar continuity, decreases tooth loss due to periodontal disease, increases alar cartilage support, and allows any unerupted teeth in the area to erupt through osseous tissue (WITSENBURG and REMMELINK 1993). These grafts may be autografts, allografts or xenografts. Different sources of autografts for closing palatal defects are described: chin, rib, calvarium and ileum. Chin bone is reported to give the best results (FREIHOFER et al. 1993). Advantages of the chin as donor site are reduction of operation time, less morbidity, decreased admission time in the hospital and an invisible scar (HOPPENREIJS et al. 1992). Also the way of healing is in favour of the use of chin bone: intramembranous bone i.e. ectomesenchymal bone maintains more of its volume (SMITH and ABRAMSON 1974) because of less post-operative resorption (ZINS and WHITHAKER 1983, KOOLE et al. 1991). This is probably due to earlier vascularization of the transplant (KUSIAK et al. 1985) in comparison with enchondral, i.e. mesenchymal, bone transplants.

Bone grafting also has disadvantages, such as the need for surgery at another site, extension of surgery time, increased possibility of infection, and other complications (HOPPENREIJS et al. 1992, SUGIMOTO et al. 1993). A special disadvantage of the chin as donor site is its small volume: if the cusps of the mandibular permanent canines are not yet erupted, the space to take a graft is very small (B[redacted]HR and COULON 1996). Damage of these teeth is an unacceptable complication. So, if there is a chance of damaging the toothbuds, autografts taken from rib, ilium or calvarium are indicated (BORSTLAP et al. 1990, WITSENBURG and FREIHOFER 1990, COHEN et al. 1991, KORTEBEIN et al. 1991, KOOLE et al. 1991).

Apart from autografts also xenografts taken from different species are advocated, but they do not have extensive clinical application in palatal surgery. The use of xenografts has the potential to reduce morbidity as harvest of autogenous bone is unnecessary. The antigenic potential of xenografts can be diminished or

eliminated by chemical treatments. Bio-Oss® (Geistlich-Pharma, Wolhusen, Switzerland) is a natural material consisting of anorganic bovine bone mineral (BBM) containing carbonate apatite. Electron microscopic examinations have shown that its macro- and microconfigurations closely parallel the structure of human bone. BBM, which is commercially available in either spongiosa or cortical blocks and particles, has been completely deproteinized by means of a proprietary extraction process that makes it free of antigenicity. This permits its implantation as a matrix that appears to stimulate all normal physiologic responses closely, mimicking the stages of bone repair (KLINGE et al. 1992). Previous clinical reports have demonstrated its efficiency in oral and maxillofacial surgery (BOYNE 1990, HISLOP et al. 1993, WETZEL et al. 1995).

Before using xenogenous bone transplants in alveolar clefts more has to be known about their effect on tooth eruption. Therefore, their effects on the eruption of permanent premolars were studied in beagle dogs after extraction of the deciduous predecessors. The extraction wounds were filled with different autogenous and xenogenous bone grafts and the subsequent tooth eruption was studied by radiographical and histological techniques.

5.2 MATERIAL AND METHODS

5.2.1 Surgical procedures

The experiment was performed in 10 female beagle dogs of 14 weeks old. They were housed in light- and temperature controlled rooms and access to food and water was allowed ad libitum. Four experimental treatments (mandibular bone graft, iliac bone graft, BBM, sham) were carried out in the extraction-sockets of the mandibular m1 and m3, the predecessors of the P2 and P4. Treatment modalities were in each dog determined by at random permutation. The eruption of the P3 served as control.

Prior to the surgical procedures, the animals were premedicated with 0.5 ml Thalamonal® (fentanyl, 0.05 mg/ml plus droperidol 2.5 mg/ml; Janssen Pharmaceutica, Beerse, Belgium) and 0.5 ml atropine (atropine sulphate 0.5 mg/ml). Subsequently, they were anaesthetised with an intravenous injection of 30 mg/kg Narcovet® (sodium pentobarbital 60 mg/ml; Apharmo, Arnhem, The Netherlands). After intubation, anaesthesia was maintained with Ethrane® (enflurane 15 mg/ml; Abott, Amstelveen, The Netherlands). The oral mucosa and the dentition were cleaned with chlorhexidinedigluconate 0.1% in water. In addition, approximately 6 ml Xylocaine® (lidocaine hydrochloride 0.4 mg/ml; Astra Chemicals, Rijswijk, The

Netherlands) was injected into the vestibular mucosa to avoid excessive bleeding during surgery.

After routine aseptic measures the anterior iliac crest was exposed by means of a 2-3 cm incision through the skin, subcutaneous tissue and periosteum. With a dental bur a 0,5-1 cm^3 bone graft was harvested. A similar procedure was used to harvest a bone graft from the angle of the mandible which was used as a substitute for chin bone. The bone grafts were placed in isotonic saline and finally cut into small chips up to about 10 mm^3 by a bone grinder.

The m1 and m3, the predecessors of the P2 and P4, of the right and left mandible were extracted. The extraction sockets were filled with either autogenous iliac crest bone chips, autogenous mandibular bone chips, BBM spongiosa granules (1-2 mm^3) (Bio-Oss®, Geistlich Pharma, Switzerland) or left empty. The mucosal defects were primary closed by a mucosal flap and sutured with a resorbable wire (Vicryl 3-0, Ethicon, Norderstedt, Germany). The m2 was left in place to evaluate the normal eruption pattern of its successor, the P3.

All animals were medicated prior to surgery with 1 ml of Albipen® 15% (ampicilline anhydrate 150 mg/ml; Mycopharm, de Bilt, The Netherlands) and maintenance doses of 1 ml Albipen® LA (ampicillin anhydrate 100 mg/ml; Mycopharm, de Bilt, The Netherlands) on days 2 and 4 postoperatively. All animals received a normal diet after surgery.

5.2.2 Radiographic procedures

Before the start of the study, under general anaesthesia, tantalum implants (Ole Dich, Hvidovre, Denmark), measuring 1.2 mm in length and 0.5 mm in width were inserted as bone marker in the margin of the mandible deep to the m2, the m3 and as far distal as possible (BJÖRK 1955). This was done 4 weeks before the extraction of the m1 and m3 at an age of 10 weeks (fig 5.1). During that period every week standardized oblique-lateral radiographs of the right and left halves of the mandible of each dog were made under general anaesthesia to follow the normal eruptive movements. To that end, the animals were fixed in an especially constructed cephalostat (MALTHA 1982) with two ear rods and with a pin in the midsagittal plane. For the radiographs Kodak® X-ray speed photo (Kodak Nederland BV, Driebergen-Rijsenburg, The Netherlands) was used. Radiographs were taken with a Philips Practic® X-ray Machine (Philips Nederland, The Hague, The Netherlands), set at 20 mA and 90 kV, the focus-film distance being 3.0 m, the object-film distance 7 cm became loose, a new one was inserted immediately, and the

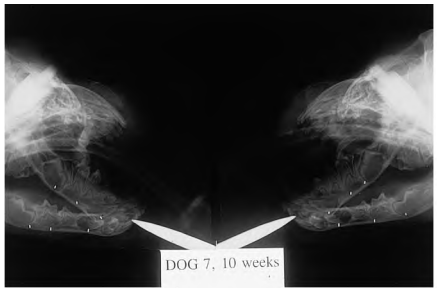

Fig 5.1 *Radiograph of the mandible of dog 7, at an age of 10 weeks after insertion of the tantalum bone markers.*

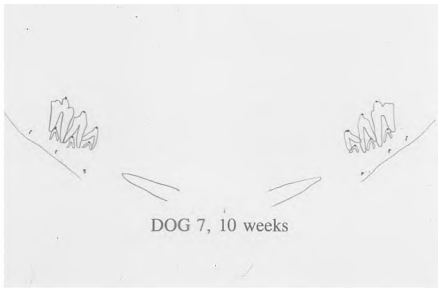

Fig 5.2 *Tracing of the mandible of dog 7, at an age of 10 weeks with points digitised with an electronic measuring table.*

radiographic procedure was repeated.

5.2.3 Radiographic analysis

The definition of tooth eruption used in this study has been restricted to the vertical movement because only small mesio-distal movements were observed. Therefore the changes in the X-co-ordinates of the tips of the teeth during the eruption were all omitted.

For each of the radiographs the points of the 3 metal bone markers and the tips of the deciduous m1, m2, m3, P2, P3 and P4 were digitised (fig 5.2). The co-ordinates of these points were digitised with an electronic measuring table (VAN DER LINDEN et al. 1972) with a accuracy of 0.01 mm. Due to the stable metal bone markers the X-axis was defined as a straight line through the most distant bone markers, and the Y-axis as a straight line perpendicular to the X-axis with the origin situated at a defined distance from the most proximal bone marker. The co-ordinate system was chosen in such way that all the co-ordinates in the mandible were situated in the first quadrant, so they had all positive values (MALTHA 1982). To describe the eruption of the premolars, the Y-co-ordinates of each measurement were plotted against the age of the animals.

5.2.4 Statistical analysis

The developmental stages for the P3 and the P4 are attained almost simultaneously (MALTHA 1982). Therefore the data of the P3 could function as a control for the

eruption of the P4. The eruption of the P2 had no controlled data. The total vertical tooth displacement in the experimental, sham and control groups in the period from 18-23 weeks is compared, using a one-way analysis of variance (ANOVA).

All recordings were performed twice by the first author resulting in a measurement error of 0.007 mm for the bone markers and 0.11 mm for the tips of the teeth. Finally the mean of both recordings was used reducing the measurement error with $\sqrt{2}$.

5.2.5 Histological evaluation

The animals were sacrificed for the histological study. One animal 4 weeks, 2 animals 10 weeks, and 6 animals 16 weeks after surgery. One animal had died during the surgical intervention at the beginning of the experiment. The animalswere brought under general anaesthesia using 30 mg/kg Narcovet®, after which 0.5 mg/kg heparin (Thromboliquine®; Organon Tetnika, Boxtel, The Netherlands) was administered. After making the final radiographs a lethal dose of Narcovet® was injected intravenously. The thorax of the animals was opened and the vascular system was perfused via the arch of the aorta with physiological saline, followed by 4% neutral formaldehyde as a fixative. After perfusion the mandibles were dissected and immersed in 4% neutral formaldehyde, decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast® (Sherwood Medical, St. Louis, U.S.A.) (WIJDEVELD et al. 1991). Serial mesiodistal sections were cut at 7 µm and stained with haematoxylin and eosin.

5.3 RESULTS

5.3.1 Radiology

The eruption curves of the P2, P3 and P4 in all experimental groups are comparable. In first instance the premolar moves slowly in an occlusal direction. At a certain age the rate of the movements increases, finally reaching a stable plateau after an abrupt slowing down of the vertical movement.

Test sites: Two weeks after transplantation (age 16 weeks), the permanent P3 and P4 started to erupt through the bone grafts and they were seen intra-orally at 21 weeks of age without dysplasia or resorption of the crown (SUGIMOTO et al. 1993) and without difference between P3 and P4 (ANOVA, $p > 0.3$). The mean vertical eruption from 18-23 of age showed no difference between the four experimental groups (ANOVA, $p > 0.3$) (tab 1). The average vertical eruption in the sham group seemed to start 1 week earlier than in the other groups without any statistical value (fig 5.3).

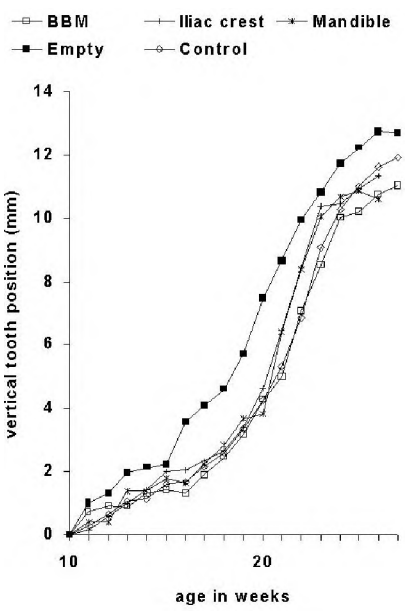

Fig 5.3 *Mean relative vertical tooth displacement of P4 (experimental and sham groups) and P3 (control group)*

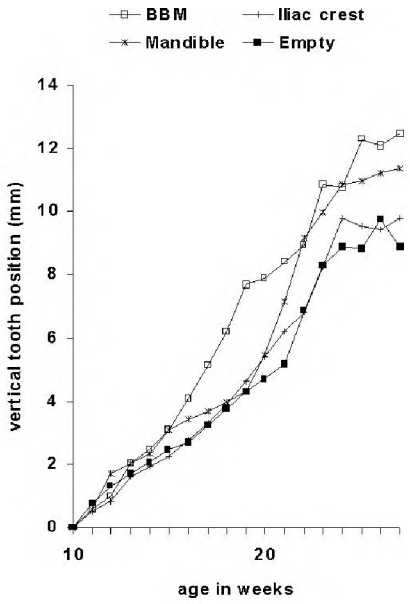

Fig 5.4 *Mean relative vertical tooth displacement of P2 (experimental and sham groups)*

Table 5.1 *Mean vertical displacement (in mm) ± SD from 18 to 23 weeks of age.*

	P2	P3	P4
Control		6.59 ± 1.47	
Sham	5.78 ± 1.38		6.90 ± 1.23
BBM	4.40 ± 0.65		6.36 ± 1.49
Mandible	5.93 ± 1.44		7.40 ± 0.56
Iliac Crest	4.35 ± 0.84		7.50 ± 0.92

LEGEND
P2: No difference between experimental and sham groups (ANOVA, $p > 0.3$)
P4: No difference between experimental, sham and control groups (ANOVA, $p > 0.3$)
P2 < P4 for experimental and sham groups (ANOVA, $p < 0.001$)

The eruption curve of the experimental P2 is not as uniform as the one of the P4, probably due to mesio-distal and linguo-buccal movements (fig 5.4). Although the vertical movement of the P2 in the period 18-23 weeks is slower than the P3 - P4 (ANOVA, $p < 0.001$), the rate of eruption shows no significant differences between the four experimental groups (ANOVA, $p > 0.3$).

Control sites: In these sites resorption of the m2 tooth roots and the eruption of the successive permanent P3 started 4 weeks after the beginning of the experiment. At an age of 21 weeks, the P3 erupted in the oral cavity and its roots were almost completely formed. The eruption lasts to a mean age of 23.1 ± 1.0 weeks. From that point the tip of the P3 remains stable in nearly all animals.

5.3.1 Histology

Mandibular bone chips: In eighteen-week old beagle dogs (four weeks after transplantation) the cortical bone chips had a non-vital aspect with only some osteocytes in the lacunae and no signs of inflammation (fig 5.5). The surfaces of the grafts occlusal to the erupting tooth were integrated in the normal physiological process of resorption. The periodontal ligament was intact with a normal structure and orientation of the fibres. At an age of 24 weeks the teeth showed no delay of eruption or disruption with an attachment of the functional epithelium on the enamel-cement border. The implanted bone grafts were incorporated in the developing alveolar bone. After 30 weeks all premolars were erupted to their functional level with normal alveolar bone height, intact periodontal ligament, normal crown and roots of a normal length. No remnants of bone grafts were seen anymore.

Iliac crest bone chips: At an age of 18 weeks (four weeks after transplantation) the cancellous iliac crest bone chips were fully incorporated with osteons among the whole transplant, without signs of inflammation (fig 5.6). The structure and orientation of the parodontal ligament of the erupting tooth was normal. Some cartilage remnants, probably from the iliac crest, were seen. The bone grafts were integrated in the physiological resorption process occlusal to the erupting tooth. At an age of 24 weeks no abnormalities were seen. After 30 weeks the premolars had emerged with intact periodontal ligament, crown and roots. Remnants of the bone chips were integrated in the alveolar bone.

BBM: The number of BBM granules was reduced at 18 weeks (four weeks after transplantation) because connective tissue was lying between particles and they were not compressed anymore (fig 5.7 and 5.8). The granules were not yet incorporated, lying in the empty alveolus as well as occlusal to the alveolar bone

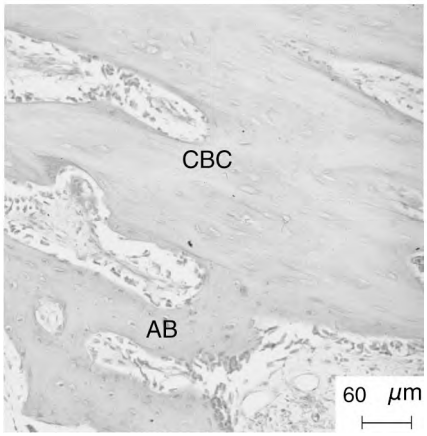

Fig 5.5 *Photomicrograph of cortical bone chips four weeks after transplantation at an age of 18 weeks showing the non-vital aspect of the bone chips with only some osteocytes in the lacunae. (Haematoxylin and Eosin; x40). CBC = cortical bone chips, AB = alveolar bone*

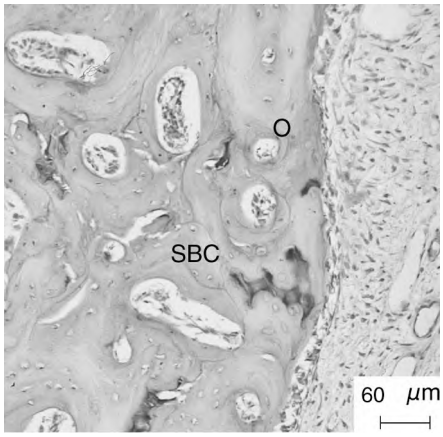

Fig 5.6 *Photomicrograph of spongious bone chips four weeks after transplantation at an age of 18 weeks after transplantation showing the incorporated bone chips with osteons among them. Arrow indicates remnants of cartilage (Haematoxylin and Eosin; x40). SBC = spongious bone chips, O = new osteon*

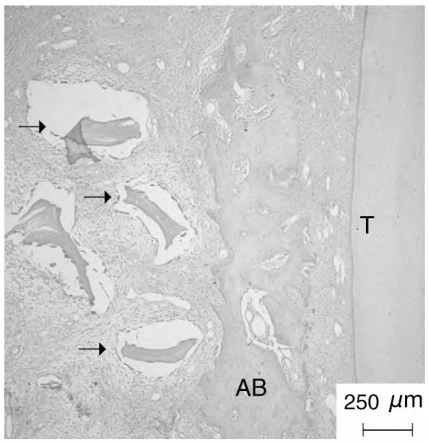

Fig 5.7 *Photomicrograph of BBM particles four weeks after transplantation at an age of 18 weeks showing the connective tissue layer around them without signs of inflammation. Arrow indicates BBM particle (Haematoxylin and Eosin; x10). T = tooth, AB = alveolar bone*

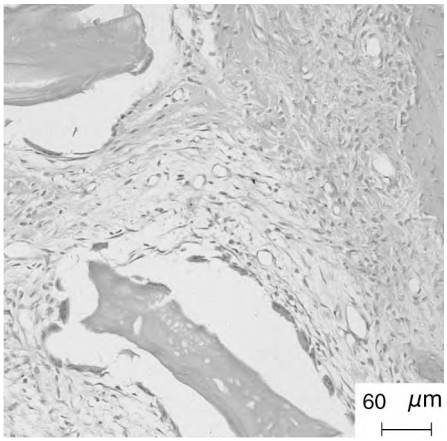

Fig 5.8 *Detail of fig 5.7. Osteoclast-like cells surrounding the particles (Haematoxylin and Eosin; x40)*

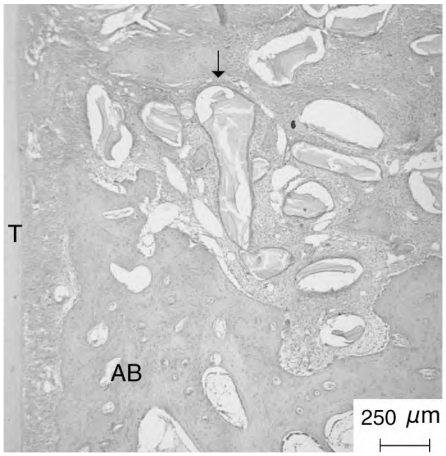

Fig 5.9 *Photomicrograph of BBM particles 16 weeks after transplantation at an age of 30 weeks. Arrow indicates BBM particle (Haematoxylin and Eosin; x10). T = tooth, AB = alveolar bone*

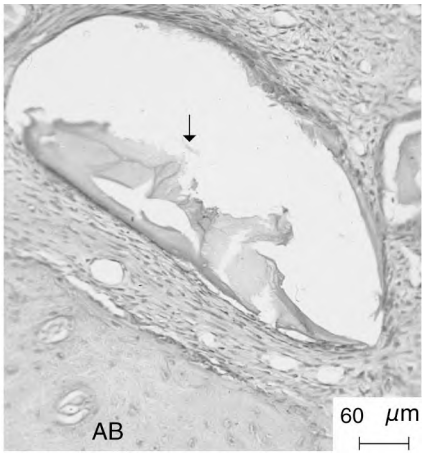

Fig 5.10 *Photomicrograph of one BBM particle 16 weeks after transplantation at an age of 30 weeks showing the thin fibrous sheet around the particle lying in connective tissue, not integrated in the alveolar bone. Arrow indicates BBM particle. (Haematoxylin and Eosin; x40)*

within connective tissue. Macrophages and osteoclast-like cells surrounded them. The attachment of the fibres of the periodontal ligament of the erupting tooth had a normal height at the enamel-cement border. At 24 weeks foreign body giant cells were seen in the vicinity of the particles without further signs of inflammation. The presence of the granules was restricted to the connective tissue occlusal to the enamel-cement border.

At an age of 30 weeks a thin fibrous sheath without an inflammatory response or osteoclastic activity surrounded most of the BBM particles (fig 5.9 and 5.10). Some of them were incorporated in the bone but most of them sat in connective tissue occlusal to the alveolar bone. The premolar eruption was undisturbed with a normal structure of the periodontal ligament, crown and roots.

Control: The eruption processes in the control group were not disturbed with a normal orientation and structure of the fibres of the periodontal ligament. In the period between extraction of the deciduous molar and emergence of its successor no alveolar bone was deposited in the empty alveolus.

5.4 DISCUSSION

There are still unanswered questions to the physiology of bone transplantation into the alveolar defect. One of these questions concerns the effect of the bone graft on the eruption of the permanent canine. In our institute chin bone, of ectomesenchymal origin, is the chosen grafting material (FREIHOFER et al. 1993). Membranous bone has several advantages over enchondral bone: it usually is removed with less donor site morbidity and less postoperative pain (TESSIER 1982, ROCHE and SCHWARTZ 1993, BÄHR and COULON 1996) and it has been shown that it is less subject to postoperative remodelling and resorption (SMITH and ABRAMSON 1974, ZINS and WHITHAKER 1983, FEINBERG and VITT 1988, BÄHR and COULON 1996). In this experiment there were no indications of a slower eruption through the mandibular bone graft. Only the eruption through the 'empty' alveolus of the P4 seemed to start earlier than through the grafted alveolus.

Studies of premolar eruption in dogs have shown that it is accomplished primarily by localised resorption of alveolar bone on the coronal side of the tooth (CAHILL 1969, MARKS and CAHILL 1986, MARKS et al. 1993), that the osteoclasts are activated on bone surfaces just prior to eruption (MARKS et al. 1983, WISE et al. 1985) and that eruption depends on the dental follicle proper, a thin collagenous investment of the crown of each developing tooth (CAHILL and MARKS 1980, MARKS and CAHILL 1984, MARKS et al. 1993). This cell-mediated process results in a local tuning of the erupting tooth and the overlying alveolar bone.

In our experiment the erupting premolars are able to resorb the cancellous iliac crest and cortical mandibular bone grafts without any difference to each other and to the control sites. The number of BBM granules is reduced, although we did not see resorption sites. The presence of osteoclasts on the surface of BBM granules at 4 and 10 weeks after surgery as observed by us and KLINGE et al. (1992) only suggest resorption in a more physiological manner. Most of the BBM granules are pushed forward into the soft tissues or probably discharged into the oral cavity during the eruption. Maybe more time for incorporation is needed in comparison with autologous bone transplants because of the lack of osteoinductive capacity (THALLER et al. 1994).

The results of our study are in accordance with a study which showed that the effect of four kinds of calcium phosphate ceramic particle insertion (hydroxyapatite (HA) dense and porous, and tricalciumphosphate (TCP) dense and porous) on tooth eruption in 20 3-month old dogs gave no delay of tooth eruption, no dysplasia and no resorption of dental hard tissue (SUGIMOTO et al. 1993). Studies on the effect of calciumphosphate on tooth eruption in forty 3- to 4-month old

kittens indicated that the use of a nonresorbable HA for grafting, however, resulted in impediment of eruption and distortion of crown development. This was in contrast to the TCP, which had a minimal effect on tooth eruption and development (FEINBERG and VITT 1988, FEINBERG et al. 1989). So the uniformity of eruption curves in our experimental (and control) groups indicates that BBM may be considered as a resorbable calcium phosphate.

5.5 CONCLUSION

The results of this study indicate that the use of cortical mandible bone or spongeous iliac crest bone in residual alveolar clefts has no negative influence on the eruption of permanent teeth.

The number of BBM particles decreased during eruption. Probably the time interval between insertion of the particles and the start of the eruption is too short for incorporation into the bone because of the lack of osteoinductive capacity. BBM particles separated from new bone are pushed into peripheral soft tissue or discharged into the oral cavity at the time of eruption. Iliac crest bone, mandibular bone and BBM, when implanted into extraction sockets of deciduous teeth, have no inhibitory effect on eruption and crown development of the underlying permanent mandibular premolars. Before using this xenogene bone mineral in alveolar clefts, more has to be known of the healing process in osseous defects, resembling the volume of alveolar clefts.

5.6 LITERATURE

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CHAPTER 6

ANORGANIC BONE MINERALS IN ORAL AND MAXILLOFACIAL SURGERY: A LITERATURE REVIEW

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Submitted

ABSTRACT

A variety of anorganic alloplastic grafting materials are used in the oral- and maxillofacial (OMF) area to bridge osseous defects or to create adequate bone-volume for placement of endosseous implants. Because of the variation in literature data about the effectiveness of these materials in reconstructive procedures of the maxilla in comparison with autogenous bone grafts, it is difficult to make protocols and select proper indications. A literature search in 'Medline' from 1989-1999 was performed to get a better insight into the pros and cons of the different modalities. Studies in which different graft materials were used in contact with the maxillary sinus with histomorphometric data were selected. Eight studies met the inclusion criteria: in 6 studies bone grafts were used in sinus floor elevation procedures and in 2 studies osteotomy gaps were bridged. It is concluded that autogenous bone gives the highest amount of osteoid volume, independent from the healing interval (1-24 months), application form (particulate versus block) and donor site (iliac crest versus chin). The results of inorganic calcium phosphate materials (bovine bone mineral or hydroxyapatite), solitary or as additive in composite grafts with autogenous particulate bone or demineralized freeze dried bone, shows a large range of values, but in all cases the results are inferior to those of autogenous bone grafts. The data on anorganic calcium phosphate materials cannot be compared mutually, because of differences in healing interval, mixture ratios and histological procedures, but they do indicate that the use of composite grafts is to be preferred over the use of solitary anorganic bone mineral.

6.1 INTRODUCTION

Bone grafting in oral- and maxillofacial (OMF) surgery is a common procedure. Well-known indications for osseous reconstructive surgery are the augmentation of the floor of the maxillary sinus in the severely atrophic posterior maxilla, the reconstruction of the alveolus in cleft palate patients, bridging osteotomy gaps or reconstruction of (post) traumatic osseous defects. Various therapeutic protocols, surgical techniques, and materials are used. The debate still continues about the best graft material and the proper indications and protocols (KENT and BLOCK 1989, CHANAVAZ 1990, PETTIS et al. 1990, REICHE and GARK 1991, WAGNER 1991, CRANIN et al. 1993, MOY et al. 1993, VLASSIS et al. 1993, HÜRZELER et al. 1997). Interpretation of literature is hampered by personal preferences of the authors which hinders direct comparison of studies concerning human bone grafting in the OMF field. Nevertheless such a comparison may be worthwhile for the development of a consistent protocol to optimise the success of surgery in the long term. The three-dimensional form of the defect, the vitality and quality of the (newly formed) bone and the subsequent success of implantology, orthodontics, and stability and bone continuity dictates the protocol of choice after orthognathic and trauma surgery.

In the past, autografts were the only material of choice for bridging an OMF osseous defect (HABAL 1992). Bone substitutes nowadays are commonly used to minimise donor site morbidity and to diminish the length of hospitalisation. The amount of osteoid in the grafted area is used as an indication of success of the procedure. This can be measured by histomorphometric analyses of core biopsies. Such evaluation is not possible in orthognathic surgery, trauma cases or bridged cleft alveolus, as in these cases a re-entry into the acceptor site has to be avoided and thus standardized biopsies are not taken. In preprosthetic surgery, however, histologic data can be obtained during implant- or abutment placement after several months of graft healing and so information about bone quality can be provided. This could be extrapolated to an indication for the success of bone grafting in orthognathic-, trauma- and cleft palate surgery, e.g. for the stabilisation of fractures and the ability to withstand the stresses induced through mastication.

The majority of the reports on the augmentation of the bony floor of the maxillary sinus in humans, have been in the form of clinical reports on implant survival over a given period with autogenous bone graft (ABG) from the iliac crest (KENT and BLOCK 1989, LOZADA et al. 1993, NYSTROM et al. 1993, RAGHOUBAR et al. 1993, CAWOOD et al. 1994, KELLER et al. 1994, MISCH and DIETSH 1994, GUNNE et al. 1995, BLOMQVIST et al. 1996, 1998a, CHAN et al. 1996, BLOCK and KENT 1997, DAELEMANS et al. 1997, LUNDGREN et al. 1997, 1999, SCHLIEPHAKE et

al. 1997, JENSEN and SENNERBY 1998), bone substitutes such as allografts (SMILER et al. 1992, FUGAZZOTTO 1994, VALENTINI and ABENSUR 1997, JENSEN and SENNERBY 1998), hydroxy-apatite (HA) (WAGNER 1991, SMILER et al. 1992, SMALL et al. 1993, FUGAZZOTTO 1994, HÜRZELER et al. 1996, WHEELER 1996, WHEELER et al. 1997) and bovine bone mineral (BBM) (SMILER et al. 1992, HÜRZELER et al. 1996, VALENTINI and ABENSUR 1997) or combinations of different kinds of materials (TIDWELL et al. 1992, MOY et al. 1993, SMALL et al. 1993, HÜRZELER et al. 1996, KAPTEIN et al. 1998).

Evaluations of survival rates only are not indicative for the quality of the bone graft (WHEELER et al. 1996, BLOMQVIST et al. 1998b, TONG et al. 1998). For the evaluation of bone quality histomorphometric analyses of core biopsies are indicated as they enable quantification of bone healing and remodelling. The purpose of this paper is to review the literature of histomorphometric data on different anorganic grafting materials used in the OMF sinus area in humans in comparison to autogenous bone. These data will be compared with data derived from animal experiments because these models are in principle accurate and reproducible although they will not always completely imitate the human situation. A meta-analysis of the literature would be preferable as it offers a structured method to systematically identify, review and analyse published reports. However, due to variations in experimental set-ups and data reporting, this appeared to be impossible.

6.2 LITERATURE SEARCH

Two reviewers (MM, JM) searched Medline from 1989-1999 for literature on histological evaluation of bone grafts in sinus, facial, orofacial and maxillofacial surgery in human studies in which anorganic matrices such as HA, coral or bovine bone mineral (BBM) were used, in the English literature. The reviewers used the following criteria: Reports about clinical histomorphometric analysed bone cores after sinus floor bone grafting or bridging osteotomy gaps were accepted, despite differences in histological techniques. In some instances older literature was included in order to clarify the background of choices and decisions reported.

'One area grafted' case-reports and papers only concerning the osseointegration of dental implants, the quality of the sinus epithelium, or the treatment of periodontal defects were excluded. Evaluations of bone grafts or substitutes in combination with membranes for guided bone regeneration were also excluded. Furthermore clinical studies dealing with the function of osteoinductive proteins (BMP's) in graft healing were not evaluated because they are not admitted

for clinical use by the American Food and Drug Administration (FDA) and the research is (therefore) still in a phase II stadium with unpredictable results (GROENEVELD et al. 1999b).

6.3 CORE PUBLICATIONS

Eight clinical papers reporting on anorganic or autogenous grafting materials in contact with the maxillary sinus (6 sinus floor grafts and 2 osteotomy gaps), were found in which biopsies, taken before implant or abutment placement, were evaluated histomorphometrically (table 6.2) (MOY et al. 1993, NUNES et al. 1996, WHEELER et al. 1996, AYERS et al. 1998, BLOMQVIST et al. 1998b, LORENZETTI et al. 1998, FROUM et al. 1998, HANISCH et al. 1999). AYERS et al. (1998) and NUNES et al. (1996), from the same institute, determined the long-term bone ingrowth into and -apposition along HA porous implants placed to bridge osteotomy gaps. BLOMQVIST et al. (1998b) tried to determine the importance of bone graft quality for implant integration after maxillary sinus reconstruction with iliac ABG. FROUM et al. (1998) reported the data of 34 sinus floor elevations that used BBM alone (n=5) or in combination with ABG (n=7) or demineralized freeze-dried bone allograft (DFDBA) (n=8) or a combination of these three materials (n=14) as a graft material. The results of their 79 sinus floor elevations which membranes were used for guided bone regeneration are not discussed. HANISCH et al. (1999) evaluated mineralization stages and processes during bone healing 6, 8, 10 or 12 months after allogenic-xenogenic bone grafting sinus augmentation procedures in 20 patients. LORENZETTI (1998) discussed in a 'preliminary' report the bone augmentation of the inferior floor of the maxillary sinus with ABG (chin block (n=3), chin particulate (n=3), iliac crest (n=8)) or a composite (HA/ABG chin) (n=3) bone graft. MOY et al. (1993) used histomorphometric analysis to quantitate the bone composition of a corticocancellous chin bone block (n=1), HA (n=1), a composite of these two materials (1:1) (n=4) and HA mixed with demineralized bone powder (n=1) in maxillary sinus augmentation. The single cases will not be discussed. WHEELER et al. (1996) evaluated the results of 36 sinusfloor augmentations with HA (n=16), HA/oral ABG (n=12), HA/iliac crest ABG (n=6), BBM (n=1) and iliac crest ABG (n=1). Because case reports were excluded the last two materials are not discussed. Several aspects of these papers will be discussed in relation to other literature.

Table 6.1 *Histometric evaluated healing intervals in months of grafts implanted in human maxillary sinus.*

Literature	AYERS et al. 1998	BLOMQVIST et al. 1998	FROUM et al. 1998	HANISCH et al. 1999	LORENZETTI et al. 1998	MOY et al. 1993	NUNES et al. 1997	WHEELER et al. 1996
Grafting material								
ABG chin block					10			
ABG chin particulate					4			
ABG iliac bone		1, 2, 4, 6, 12			6			
BBM			6					
BBM/ABG			6					
BBM/ABG/DFDBA			6					
BBM/DFDBA			6	6, 8, 10, 12				
HA	4 - 36 [19]						14 - 30 [19]	6
HA/ABG chin (1:1)					12	6, 10		
HA/ABG iliac crest (1:4)								6
HA/ABG oral (4:1)								6

LEGEND
ABG = Autogenous Bone Graft
BBM = Bovine Bone Mineral
DFDBA = Demineralized Freeze-Dried Bone Allograft
HA = Hydroxyapatite
DBP = Demineralized Bone Powder
n = Months

Table 6.3 *Osteoid volume in % of total field after sinus floor elevation procedures.*

Grafting material Versus time	Standard	1 mns	2 mns	4 mns	6 mns	8 mns	10 mns	12-24 mns	n	Author
ABG chin block							69.3		3	LORENZETTI et al. 1998
ABG chin particulate				62.6					3	LORENZETTI et al. 1998
ABG iliac bone					53.0				8	LORENZETTI et al. 1998
ABG iliac bone		41	23	38	33			26	4, 5, 6, 4, 2	BLOMQVIST et al. 1998
Alveolar ridge residual	32.6								17	HANISCH et al. 1999
BBM					8.5				5	FROUM et al. 1998
BBM/ABG					18.5				7	FROUM et al. 1998
BBM/ABG/DFDBA					19				14	FROUM et al. 1998
BBM/DFDBA					14				8	FROUM et al. 1998
BBM/DFDBA					8.1	9.0	11.8	20.7	5, 5, 5, 5	HANISCH et al. 1999
HA					16.4				16	WHEELER et al. 1996
HA								27	8	NUNES et al. 1997
HA								23	17	AYERS et al. 1998
HA/ABG chin (1:1)								43.6	3	LORENZETTI et al. 1998
HA/ABG chin (1:1)						44.4			4	MOY et al. 1993
HA/ABG iliac crest (1:4)					19.3				6	WHEELER et al. 1996
HA/ABG oral (4:1)					11.3				12	WHEELER et al. 1996

LEGEND
ABG = Autogenous Bone Graft
BBM = Bovine Bone Mineral
DFDBA = Demineralized Freeze-Dried Bone Allograft
HA = Hydroxyapatite
DBP = Demineralized Bone Powder
n = number of grafted sinuses in each group

6.3.1 Histology

Evaluation of the core literature is hampered by the variation in histological techniques used. Histomorphometrical evaluation of bone is performed in most cases by some kind of grid method. In this method a grid is placed over the microscopical image. By counting the number of points or measuring the length of the lines that overlie the item of interest, the volume of that item can be estimated. The value of bone histomorphometry is dependent on impeccable tissue preservation and proper section thickness. The effect of tissue thickness refers to an increase in the visualisation of tissue compartments as section thickness increases (HOLMES and ROSER 1987). In general, as the specimen thickness is increased, the accuracy of the histomorphometric assessment decreases. The optimal thickness of specimen is 4.5 – 5 µm (JOWSEY 1977). Another method of quantitative analysis used the backscattering scanning electron microscopy. This technique does not depend on sectioning of the tissue (HOLMES and ROSER 1987). Both techniques are used in the core articles (table 6.2). In 3 papers 30-45 µm histologic sections were used (MOY et al. 1993, WHEELER et al. 1996, FROUM et al. 1998), while in the other studies 10-20 µm (HANISCH et al. 1999), 7 µm (LORENZETTI et al. 1998) or 4 µm (BLOMQVIST et al. 1998b) section thickness was used. In the studies in which HA bone grafts were placed to bridge osteotomy gaps, 400 µm sections were ground (NUNES et al. 1997, AYERS et al. 1998). In spite of these differences and because of the absence of other, more uniform literature, we put these data together knowing the results are only indicative without secure value.

The amount of osteoid from the residual alveolar process, which has been undisturbed during the grafting procedure as measured by Hanisch et al. (1999), was 32.6%. This datum was used as an internal standard. The values in table 6.3 can be related to this standard as far as measurements with the backscatter method are concerned (table 6.2). This can give an indication of the vitality of different grafting materials used in clinical sinus floor elevation procedures. Because of the difference in patient numbers and time intervals between implantation and biopsy, no definite conclusion can be drawn. Most data are available at a six months healing time interval. Histomorphometric analyses of ABG in the core publications gave the highest amount of bone content in the grafted area (23.0% - 69.3%) independent of the interval used for healing. No clear difference could be detected between different donor sites and application forms (chin versus iliac crest or block versus particulate). The histomorphometric values of composite grafts consisting of ABG and an anorganic mineral (HA or BBM) showed no difference in the amount of bone in the grafted area (11.3% - 19%), whereas HA alone (16.4% - 20.3%)

differed slightly from BBM alone (8.5%) (table 6.3). All composite grafts have a lower osteoid content (8.1% – 19.3%) than the residual alveolar ridge (32.6%). The histomorphometric data in the core studies on composites also have a lower osteoid content than ABG. It seems that ABG's are in favour to composites as they show at least temporarily a higher osteoid content than the residual alveolar ridge.

In animal studies in which grafts consisting of cancellous ABG/BBM (1:1) were used. Good results were obtained, combining the advantages of each material alone and reducing the disadvantages of each when used separately (MERKX et al. 2000).

Table 6.2 *Techniques used for histomorphometric analyses in core papers.*

Author	Year	Slide Thickness (µm)	Histomorphometric Technique
AYERS et al.	1998	400 - grinding	Histomorphometry
BLOMQVIST et al.	1998	4	Histomorphometry
FROUM et al.	1998	35-45	Histomorphometry (computerized)
HANISCH et al.	1999	10-20	Backscatter electron microscopy
LORENZETTI et al.	1998	7	Histomorphometry
MOY et al.	1993	30-40	Backscatter electron microscopy
NUNES et al.	1997	400 - grinding	Histomorphometry
WHEELER et al.	1996	30-40	Backscatter electron microscopy

6.3.2 Healing period

Another problem in the evaluation of the core publications is the difference of time interval between bone grafting and biopsy taken between the studies and sometimes even during a research project. For example, LORENZETTI et al. (1998) evaluated the bone quality after sinus elevation procedures of four different grafting materials (iliac crest bone blocks, chin bone blocks, particulate chin bone and composite chin/HA (1:1)). The 'iliac crest bone' group was biopted after a 6 months' healing period "to limit the graft volume resorption that has been reported with iliac crest bone grafts" (SMITH and ABRAMSON 1974, BURCHARDT 1987, NYSTROM et al. 1995). The 'chin bone block' group was biopted after 8-12 months "to achieve sufficient revascularization and viability of this type of graft, essentially consisting of compact bone" (URIST 1980, BURCHARDT 1987). The 'particulate chin bone' group was biopted after 4 months, "since the original compact bone structure had been removed by the particulation process and graft volume could be presumed to occur during the first phase of healing" (SHIROTA et al. 1996). The healing for the

fourth group (chin ABG/HA (1:1)) was prolonged to 12 months to "allow adequate new bone formation, as indicated for this type of graft" (MOY et al. 1993).

Only two prospective studies evaluated the mineralization stage with the same grafting material at various time intervals (BLOMQVIST et al. 1998b, HANISCH et al. 1999). HANISCH et al. (1999) used BBM/DFDBA and evaluated it after 6, 8, 10 and 12 months. In their study no statistically significant difference was seen between the amount of newly formed bone in the grafted areas during the different intervals was seen. They concluded that the mineralization process of this composite is already complete after six months, but new bone formation continues at least up to 12 months resulting in a higher osteoid content (table 6.3). At that time, however, the amount of newly formed bone was still lower than the volume of residual bone. BLOMQVIST et al. (1998b) reported a highly increased bone remodelling (deposition and resorption) activity already 2 months after grafting and concluded that an extension of the healing period by an additional 2 or 4 months would not yield any advantage but may rather induce progressive bone resorption.

In other human and animal studies different measures were used for healing capacity and also time scales differed (LUNDGREN et al. 1999). In some human studies it appears that a period of six or even twelve months is too short to allow complete incorporation of the bone graft (NYSTROM et al. 1993; AYERS et al. 1998). When HA is implanted solitarily, the amount of bone present within the implant increases with implantation time and appears to reach a plateau around the 20 months time frame (AYERS et al. 1998). In course of time the soft tissue gave way to primarily woven and lamellar bone. If cancellous chips, with or without an anorganic additive, are implanted in a frontal sinus wall defect of goats, bone graft ingrowth is accomplished within 12 weeks (MERKX et al. 1999b, 2000). Extension of the follow-up period with another 12 weeks did not seem to result in a better bone quality. This kind of studies revealed that new bone formation in grafts in contact with the maxillary sinus varies considerably depending on bone graft type and healing time.

6.3.3 Materials

Twelve different (combinations of) materials are used in the core publications and evaluated at different time intervals (1-24 months) (table 6.1). The only anorganic bone substitutes used in the core studies are BBM and HA. Their action is based on osteoconduction, which means that new bone formation takes place along their surface. In one study also DFDBA is used in composites. DFDBA may result in both osteoinduction and osteoconduction (MISCH and DIETSH 1993), although the

inductive capacity is disputable (GROENEVELD et al. 1999). It is generally believed that BMP's and other non-collagenous proteins in the matrix are responsible for the osteoinduction of this material (ZHANG et al. 1997).

Particulate bone material is easily and conveniently obtained as a surplus material when harvesting major grafts or during osteotomies. It may be used to fill small defects, to correct minimal contour irregularities and for packing around larger grafts. Also particulate bone can easily be mixed with anorganic bone additives. LORENZETTI et al. (1998) evaluated the differences in behaviour of blocks versus particulate materials. They suggest that graft architecture, conformation, and composition may influence the mean bone content at the end of the healing. In an animal study it was stated that autogenous cancellous bone chips are the material of choice for bridging a bony defect in the maxillofacial area when there is no need for mechanical strength. They heal in the same way as cancellous bone blocks do and are easily and conveniently obtained as surplus material when producing major grafts or performing osteotomies (MERKX et al. 1999a, b).

The combination of an anorganic bone mineral with autogenous bone grafts, can provide an additional stimulus for osteoconduction and as an expander (HABAL 1991, LINDHOLM et al. 1994). The ideal composite ratio is still unknown, as no research has pointed out this parameter in detail. However, the different ratios of composite mixtures may be of influence on the results. An ABG/HA ratio varying from 1:1 to 1:2 is recommended to avoid excessive fibrous encapsulation of the hydroxyapatite granules (COBB et al. 1990; TIDWELL et al. 1992). In one of the core papers the mixture rates differed: WHEELER et al. (1996) said there "usually was a mixture of 4:1 ratio of HA to autogenous intraoral bone whereas the reverse was true when using bone harvested from the iliac crest". An argument for this difference is not given. When the ratio of anorganic bone mineral versus ABG increased, the radiological evaluation showed that resorption of the bone additive decrease exponentially (HORCH and STEEGMANN 1985).

In an animal histological study it is shown that cancellous ABG/BBM (1:1) composite grafted in frontal sinus wall defects showed a stable situation after 12 weeks and that the BBM particles had become replaced to a large extent by newly formed bone due to physiological remodelling. After 24 weeks the same features were seen, but BBM particles could hardly be detected anymore (MERKX et al. 2000).

DFDBA showed lower osteoid contents after 6 months than the autogenous grafts (FROUM et al. 1998, HANISCH et al. 1999). It was observed that formation of

mature lamellar bone in these kinds of grafts took at least twice as much time as in autogenous bone grafts (JENSEN and SENNERBY 1998).

Two differences between HA and BBM may be important for the clinician. First, the three-dimensional structure, including the porosity of the grafting material, which has an important effect on bone healing (HOLMES 1987, KASPERK et al. 1988, DACULSI and PASSUTI 1990, VAN EEDEN and RIPAMONTI 1994, HÄMMERLE et al. 1997). Materials exhibiting large surface areas showed better bone-graft contact than materials with comparatively small surface area (HOLMES and ROSER 1987, KASPERK et al. 1988). The surface area of each graft particle of BBM is considerably larger than that of porous bioceramics (e.g. HA) (HÄMMERLE et al. 1997). Therefore it might be a better grafting material. The second reason is that sintered HA particles, used as a space filler, are not substituted with normal bone because of their irresorbability. The use of porous HA blocks might result in mechanical fragile areas if used to fill defects in the cortex of long bones or thin mandibles (YAMAGUCHI et al. 1995). BBM is prone to osteoclastic resorption (PINHOLT et al. 1991, MERKX et al. 1999a, b) although other studies refute this (MANDELKOW et al. 1990, STASSEN et al. 1994, SKOGLUND et al. 1997, YOUNG et al. 1999). The resorption of BBM finally leads to a physiological substitution and remodelling which could be an advantage in favour for BBM. However studies about this specific subject are lacking so far.

6.4 CONCLUSION

Allogenic (ceramic) bone replacing materials as reported in literature, which all consist of calcium phosphate complexes, differ in origin (xenogenic, ceramic or coral), structure (pore size, absolute surface area and interconnections), chemical composition and finally (therefore) in resorbability. All these materials are supposed to provide a scaffold for bone ingrowth by means of osteoconduction which is enabled by the establishment of an environment allowing early migration of osteoblasts or osteoprogenitor cells into the area in which bone regeneration is intended (HÄMMERLE et al. 1997).

The successful regeneration of bone requires cells that will produce osteoid, an appropriate scaffold upon which the new bone is deposited and bioactive molecules to modulate the process. The host site should, of course, provide a vascular supply and nutrients. The transplant has to meet some mechanical requirements in order to approximate the mechanical properties of the bone that is replaced in the reconstructed area. The anatomy, functional loading, and chemical circumstances of a particular reconstruction dictate the exact mechanical

requirements. Technology is now able to apply each of the required bone regeneration components separately (HOLLINGER 1993, YAZEMSKI et al. 1996).

Anorganic bone substitutes as solitary graft material, do not contribute to an early bone ingrowth and do not fulfil the requirements of an early functional loading. If it is necessary to use anorganic bone additives, the material that re-establishes the integrity of the resorbed or injured maxillofacial skeleton should be a temporary material and be mixed with autogenous bone of cancellous origin. This is necessary because only then, the bone that is formed is soon able to bear functional load e.g. of dental implants, chewing or orthodontic forces. Consideration should be given to the intended clinical use of the materials. Prospective clinical studies should start to compare the healing response of different (anorganic) grafting materials at different acceptor sites in the maxillofacial area. This can provide information about the ideal graft composition, the optimal healing time, the value of anorganic additives, the effect of functional loading and, in the future, the impact of the use of osteoinductive proteins.

6.5 LITERATURE

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

7.1 GENERAL DISCUSSION

The primary aim of this study is to assess the healing of critically size bone defects using various types of autogenous bone grafts and composites of bovine bone mineral (BBM) and autogenous bone. A research model was developed over the frontal sinus, which purposely avoided the calvaria as experimental site because of the postulated osteogenic capacity of the dura. This modified model is likely to mimic the clinical situation better because bone grafts in oral- and maxillofacial (OMF) reconstructive surgery are often used in an area where the paranasal sinuses are exposed and nasal epithelium is lining the defect.

A prerequisite for successful grafting material is the presence of an appropriate scaffold allowing the ingrowth of bone to achieve the desired three-dimensional structure. This could be the structure of the graft itself (block grafts) or trays made up from alloplastic materials. It also requires the presence of vital bone cells that produce osteoid and bioactive substances to modulate the process. The host site should, of course, provide an adequate vascular supply and nutrients. The graft usually has to fulfil some specific mechanical requirements as well, approximating the mechanical properties of the bone that is replaced in the reconstructed area. The anatomy and the functional loading of a particular reconstruction site dictate these mechanical requirements. At present, the technology is available to deal with each of the required bone healing components separately (HOLLINGER 1993, YASZEMSKI et al. 1996).

The size and form of the bony defect and the function of the location in question should determine the use of autogenous bone or alloplasts, alone or in combination (MISCH and DIETSH 1993). The choice and composition of the graft material should depend on the site and dimensions of the defect (FROUM et al. 1998). In general, alloplasts can be used for small, non-critically size defects and three- to five-wall defects. Autogenous bone has to be added to the graft for one-, two-, or three-wall defects or relatively large, critical size defects. In general, the larger the defects, the greater the amount of autogenous bone required. Alveolar osseous defects in cleft palate patients or sinus floors to be augmented to receive implants, resemble a two- or three-wall defect.

The healing of cortical and cancellous bone block grafts in the present model, confirmed the observations made by other authors who used other recipient sites (BURCHARDT and ENNEKING 1978). Cancellous bone grafts revascularise and remodel faster than cortical grafts. Acceptance and incorporation of the bone grafts, however, were in all cases comparable to studies in calvarial defects (SCHMITZ and HOLLINGER 1986, ARDEN and BURGIO 1992).

For bridging or filling an osseous defect in the maxillofacial area particulate autogenous cancellous bone is as good as a cancellous block graft. It heals in the same way and in both cases there was hardly any difference to be seen between acceptor and donor bone tissue after 12 weeks. When there is no need for initial mechanical strength in two- or three-wall osseous defects it is probably easier to use particulate bone rather than have a bone block cut exactly to fit the defect. Particulate bone is conveniently obtained as surplus material when harvesting major grafts. Cancellous bone can be harvested by dissection of a part of the iliac crest, but it can also be obtained from the iliac crest or tibial plateau with a trephine. This reduces donor site morbidity and shortens operation time. Autogenous cancellous bone is still the material of choice when fast and reliable healing is wanted. The graft, however, lacks intrinsic mechanical strength and thus it is of limited use in case larger defects have to be bridged. This problem may be solved when three-dimensional scaffolds are developed that contain the graft and at the same time provide rigidity for the time needed for healing. Probably these scaffolds will come on the market in the near future.

Our study indicates that cortical bone chips are questionable as a solitary bone graft in the OMF region. Even after 24 weeks small areas filled with connective tissue were detectable lacking any osteoblastic activity, while at that time cancellous particulate grafts had healed completely. Cortical block grafts were still involved in a remodelling process after 24 weeks, while the cancellous block had completely been remodelled. These findings point to the fact that purely cortical autogenous bone grafts have only limited use in clinical practice. If their use is inevitable one should allow for enough time for the bone to be remodelled before mechanical loading of the graft is considered.

Alloplastic bone replacing materials consisting of calcium phosphate complexes, differ in origin (xenogenic, ceramic or coral), structure (pore size, absolute surface area and interconnections), chemical composition and, therefore, in resorbability. All these materials are supposed to provide a scaffold for bone ingrowth by means of osteoconduction, which requires an environment allowing early migration of osteoblasts or osteoprogenitor cells into the area in which bone regeneration is intended (HÄMMERLE et al. 1997). In the present study the use of a block of BBM resulted in centripetal ingrowth of newly woven bone at 3 weeks and the situation was stabilised after 6 weeks with an active, ongoing resorption of non-integrated, non-functional BBM by multinucleated osteoclast-like cells. These findings are contrary to the results of THALLER et al. (1993) who did not see osteoclastic activity within 12 weeks after implantation of BBM in rabbit calvarial

wounds, but found continuing reactive bone formation instead. KLINGE et al. (1992), also using rabbit calvaria, found osteoclasts in moderate amounts, but only close to the BBM implant-tissue interface indicating an active remodelling but not around the non-integrated isolated BBM. Although species differences between rabbits and goats cannot be excluded, the most plausible explanation for the conflicting results is that the model used in this study mimics the clinical situation more closely because the defects were lined by sinus epithelium instead of dura mater.

BBM granules as solitary implant in a critically size defect in contact with respiratory epithelium did hardly show osteoconduction but gave rise to extensive osteoclastic activity in the centre of the defect. This is probably due to the fact that the solitary particles tend to be mobile under the influence of outside forces. Jaw movements and respiration generate these forces. At the edges new bone pushed the granules forward as a 'snowshovel'. A block of BBM in similar conditions, however, gives better results because in the centre of the defect a BBM block prevents fusion of the periosteum and the paranasal mucosa. Initially it gives rise to an area filled with loose connective tissue and remnants of BBM. Periosteum, paranasal mucosa and the margins of the defect delineate this area. Finally also the BBM in the centre will resorb and be replaced by fibrous tissue, but during that space maintaining period osteoid will be deposited by osteoconduction.

Thus, the current findings seem to indicate that BBM alone is not the graft material to be recommended for general use in OMF reconstructive surgery.

A composite graft, consisting of autogenous cancellous bone and BBM particles, however, shows more promising results, combining the advantages of each material alone and minimising their disadvantages. These composite grafts heal uneventfully within 12 weeks. All defects were bridged and integrated BBM particles were replaced to a large extent by newly formed bone due to physiological remodelling. At 24 weeks hardly any BBM particles were seen anymore. This mixed graft material certainly has something to offer. In selected cases it may be used to fill defects, minimising the need for a large volume of autogenous bone.

Cortical bone in a composite graft does not have a beneficial effect on healing and probably even has an opposite effect, as resorption of the cortical chips by osteoclasts seems to be induced by the presence of non-functional BBM.

A six months time interval is generally accepted for implants to be placed after sinus floor elevations and grafting. A rationale for the choice of this interval is the maturation of the grafted bone and thus, the expected better integration of the

implants. The results of the present study and analysis of recent literature seem to indicate that this period may safely be shortened to three months.

The study on the eruption of premolars through particulate autogenous cortical bone, cancellous iliac crest bone or BBM, shows normal progress. These results, however, should be regarded with caution. The fact that BBM did not disturb eruption does not imply that other alloplastic materials would behave the same way. It is still recommended to use autogenous grafts for alveolo-palatal clefts in children in whom canines are supposed to erupt through the graft. BBM, however, may be mixed with these grafts if necessary. It will probably not hamper eruption of the canines.

The present study, as well as recent literature, shows that solitary anorganic bone grafts do not contribute to early bone ingrowth and do not fulfil the requirements for early functional loading (WHEELER et al. 1996, FROUM et al. 1998). When selecting an alloplastic anorganic component for a possible composite grafts it has to be mixed with particulate autogenous bone.

7.2 CONCLUSIONS

- A critical size osseous defect over a frontal sinus in a young adult female goat, as used in the present study, can be used to assess graft healing.
- Healing and incorporation of an autogenous cancellous bone graft proceed irrespective of the way it is applied i.e. as block or particulate graft.
- Particulate cortical autogenous bone grafts used as implant material show less favourable results than particulate cancellous grafts.
- Particulate BBM shows no osteoconductive capacity but is prone to resorption. A BBM block graft, however, appears to be osteoconductive allowing bone ingrowth.
- BBM alone should not be used as a bone substitute in two- or three wall osseous critical size defects, but it may be added to particulate autogenous cancellous bone to form a composite graft.
- A composite graft consisting of a mixture of particulate BBM and autogenous cancellous bone combines the advantages of each material separately and minimises the disadvantages when used separately. In the experimental model used in this study these grafts heal uneventfully within 12 weeks, giving rise to a solid vital mixture of bone/BBM. As time proceeds, the BBM will be completely replaced by new bone.
- A composite graft consisting of particulate BBM and autogenous cortical bone chips does not seem to be a good choice. Resorption of the cortical bone appears to be enhanced by the presence of BBM particles.

- Autogenous cancellous bone, cortical bone and BBM, when implanted into extraction sockets of deciduous teeth, have no inhibitory effect on eruption of the underlying mandibular premolars.

7.3 SUGGESTIONS FOR FUTURE RESEARCH

There is a need for prospective clinical and experimental studies to assess the efficacy of various composite grafts. Special attention has to be paid to the reduction of donor site morbidity because of harvest of autogenous bone. This implies a search for better resorbable scaffolds and the testing of growth factors such as BMP and autogenous growth factors in platelet rich plasma such as platelet derived growth factors (PDGF). The various indications for bone grafting in the OMF area require tailored solutions. This implies that research must also be focussed on experimental models mimicking the various clinical situations.

7.4 LITERATURE

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CHAPTER 8

SUMMARY
SAMENVATTING

SUMMARY

Bone grafting in the oral and maxillofacial region with autogenous bone is a common procedure to reconstruct the extremely resorbed edentulous alveolar ridge, the cleft alveolus or osseous defects after ablative tumour surgery or trauma. The limited availability, the unpredictable resorption, the donor site morbidity, the need for a second operation area, the waste of bone material, the time consuming procedure and the increasing costs of hospitalisation, led to investigations for other, possibly effective alternatives.

The general purpose of this study was to gain insight into several aspects of bone grafting and graft healing in the maxillofacial area with emphasis on the application form, graft origin and the role of a commercially available bovine bone mineral (BBM) (Bio-Oss®, Geistlich-Pharma, Wolhusen, Switzerland) in oral and maxillofacial surgery. Most of the test models on bone grafting are performed in animal calvaria in which the dura mater plays a central role. In OMF surgery, however, the respiratory epithelium is involved, and therefore a standardized animal research model was developed in the maxillofacial skeleton in contact with a paranasal sinus. In a pilot study, trephined defects (∅ 14 mm) in the frontal bone overlaying a large frontal sinus on skeletally mature female goats, appeared to be of a critical size, which means that they will not heal spontaneously. This experiment model is used in chapter 2, 3 and 4 for answering questions concerning the regenerative response on autogenous cancellous and cortical bone grafts, and on a commercially available BBM. The materials were mutually compared in different application forms (block versus particulate) and composites (cancellous/BBM versus cortical/BBM graft).

A literature review (**Chapter 1**) exposed the mechanisms of bone-graft healing and the types of graft material (application forms, types and origins). In the first study (**Chapter 2**) the defects, in the newly developed model, were filled at random with a cortical bone block, a cancellous bone block, a spongeous BBM block cut into shape, or left empty. Histological evaluation showed that autogenous bone grafts were all accepted and incorporated in a similar way as in calvarial defects. Defects filled with BBM showed new bone formation at the margins of the defects and only little of the implant material was incorporated. Multinucleated osteoclast-like cells gradually resorbed most of the BBM. These results were in contrast to other studies. One of the reasons for early and continuous resorption of the non-incorporated BBM and the subsequent formation of fibrous tissue in the centre of the defect might be the continuous change in mechanical loading of the paranasal mucosa by breathing.

The purpose of the second study (**Chapter 3**) was to compare the regenerative response of autogenous particulate cortical and cancellous bone and particulate BBM in the same setting as used in chapter 2. Autogenous particulate cancellous bone is the material of choice for bridging a bony defect in the maxillofacial area if mechanical strength is not needed. Particulate cancellous bone healed in the same way as cancellous bone blocks but has the advantage of an easier applicability. Particulate cortical bone is not reliable enough to be used as a solitary bone grafting material under these conditions. After 24 weeks only small fenestrations remained in the former defect. A cortical block as a solitary implant gave better results. Particulate BBM as solitary implant material in a critical size defect did not stimulate osteoconduction but gave rise to an extensive osteoclastic activity, probably stimulated by the mutual loose relation. A solid block of BBM is more reliable in a similar case. The centripetal ingrowth of newly woven bone in BBM, seen in the 'block-study' 3 weeks after implantation, could not be observed around particulate BBM. The new bone pushed the granules forward as by a 'snowshovel' and no signs of osteoconduction were seen. To prevent this 'snowshovel' effect, it seems better to implant a securely fitting block instead of particulate BBM.

In **Chapter 4** the capacity of composite grafts consisting of either particulate cancellous or particulate cortical bone or BBM was evaluated. In conclusion, composite grafts consisting of autogenous cancellous bone/BBM yield good results, combining the advantages of each material in itself and reducing the disadvantages of each when used separately. Critical size defects in the maxillofacial area, overlaying a paranasal sinus, which are filled with this material heal uneventfully within 12 weeks. This is the same response as if particulate cancellous bone was implanted without an additive. Composite grafts consisting of cortical bone and BBM show less favourable results. These grafts induce osteoclasts, probably by the presence of non-functional BBM, resulting in resorption of the particulate cortical bone. More osteoid tissue was seen when pure cortical chips were used as a bone grafting material.

In **Chapter 5** the effect of the use of different autogenous and BBM transplants on mandibular permanent premolar tooth eruption in beagle dogs was examined by radiology and histology. The deciduous first and third molar were extracted and their sockets over the second and fourth premolar were filled with autogenous particulate spongeous iliac crest bone, autogenous particulate cortical mandibular body bone, BBM granules (1-2 mm^3), or left empty. The third premolar served as a control site. Standardized oblique lateral radiographs were made once a

week. A number of co-ordinates of defined points and structures were determined by means of a co-ordinate digitising system. None of the premolars showed delay in eruption or disruption of crown and root development. Histological evaluation showed that particulate BBM was not resorbed or integrated in the alveolar bone, but pushed forward into the gingiva. The eruption curves of the permanent premolars in all groups were similar and bone transplantation had no inhibitory effect on their eruption.

A variety of anorganic alloplastic grafting materials are used in the OMF area to bridge an osseous defect or to create adequate bone volume for placement of endosseous implants. Because of the variation in literature data about the effectiveness of these materials in reconstructive procedures of the maxilla, in comparison with autogenous bone grafts, it is difficult to make protocols and select proper indications. A literature search in 'Medline' from 1989-1999 reported in **Chapter 6** was performed to get a better insight in the pros and cons of the different modalities. Studies in which different graft materials were used in contact with the maxillary sinus and with histomorphometrical data were selected. Eight studies met the inclusion criteria: in 6 studies bone grafts were used in sinus floor elevation procedures and in 2 studies osteotomy gaps were bridged. It is concluded that autogenous bone gives the highest amount of osteoid volume, independent from the healing interval (1-24 months), application form (particulate versus block) and donor site (iliac crest versus chin). The results of inorganic calcium phosphate materials (bovine bone mineral or hydroxyapatite), solitary or as additive in composite grafts with autogenous particulate bone or demineralized freeze dried bone, show a large range of values, but in all cases the results are inferior to those of autogenous bone grafts. The data on anorganic calcium phosphate materials cannot be compared mutually, because of differences in healing interval, mixture ratios and histological procedures, but they do indicate that the use of composite grafts is to be preferred over the use of solitary anorganic bone mineral.

In the General Discussion (**Chapter 7**) the conclusions are listed as reported in the separate chapters. The future has to be focussed on prospective clinical and experimental studies to assess the efficacy of various composite grafts. Attention has to be paid to the role of different growth factors in bone healing in the OMF area.

SAMENVATTING

Bottransplantatie in het Mond-, Kaak- en Aangezicht- (MKA) gebied met autoloog bot, is een standaardprocedure om bijvoorbeeld de extreem geresorbeerde edentate processus alveolaris, de aangeboren kaakspleet of botdefecten na oncologische chirurgie of ongevallen te reconstrueren. De beperkte hoeveelheid donorbot die geoogst kan worden, de onvoorspelbare resorptie van het transplantaat, de morbiditeit van de donorplaats, de noodzaak voor een tweede operatiegebied, de verkwisten van botmateriaal, de tijd nodig voor het winnen van het bot, en de toenemende kosten in de gezondheidszorg, zijn allemaal aanleidingen voor onderzoek naar alternatieven.

Het doel van deze studie was om inzicht te krijgen in de verschillende aspecten van bottransplantaties en botgenezing in het aangezichtsskelet met de nadruk op de toepassingsvorm, de oorsprong van het transplantaat en de rol van een commercieel verkrijgbaar runder botmineraal (BBM, Bio-Oss®, Geistlich-Pharma, Wolhusen, Zwitserland) in de MKA-chirurgie. De meeste dierexperimenten, uitgevoerd om materialen voor bottransplantatie te onderzoeken gebruiken het schedeldak als onderzoekslocatie, alwaar de dura mater een centrale rol speelt. In het MKA-gebied hebben we echter te maken met slijmvlies dat de luchtwegen bekleedt, het respiratoir trilhaarepitheel. Daarom hebben we een diermodel ontwikkeld waarbij het MKA-gebied wordt nagebootst door botdefecten te creëren in de voorwand van het os frontale over de sinus frontalis. In een pilotstudy, bleken botdefecten met een diameter van 14 mm of meer, gemaakt met een trepaanboor, bij jonge volwassen vrouwelijke geiten niet meer spontaan te genezen. Dergelijke defecten zijn dus van kritische grootte. Dit onderzoeksmodel werd gebruikt in hoofdstuk 2, 3 en 4 om vragen over de genezingscapaciteit van autoloog spongieus bot, autoloog corticaal bot en het commercieel verkrijgbare BBM te beantwoorden. Deze materialen werden onderling vergeleken met het accent op de applicatie vorm (botblok versus botmoes) en de mengvormen (spongieus/BBM versus corticaal/BBM).

In een literatuuroverzicht (**Hoofdstuk 1**) wordt een beschrijving gegeven van de transplantaatgenezing en de verschillende types van transplantaten (applicatie vorm, type transplantaat en origine). In de eerste studie (**Hoofdstuk 2**) werden de botdefecten, in het nieuw ontwikkelde diermodel, ‘at random’ gevuld met een autoloog corticaal botblok, een autoloog spongieus botblok, een spongieus BBM blok of leeg gelaten. Histologische evaluatie toonde aan dat de autologe bottransplantaten werden geaccepteerd en op dezelfde wijze vast groeiden als in schedeldakdefecten. Defecten gevuld met BBM lieten een botingroei zien aan de

randen van de defecten. Slechts een geringe hoeveelheid van het BBM werd geïncorporeerd. Multinucleaire osteoclast-achtige cellen resorbeerden het BBM langzaam. Een van de redenen voor deze vroege en continue resorptie van het niet geïncorporeerde BBM en de daaropvolgende bindweefselvorming in het centrum van het botdefect zou de continue mechanische belasting van de paranasale mucosa kunnen zijn, veroorzaakt door aan de ademhaling gerelateerde factoren.

Het doel van de tweede studie (**Hoofdstuk 3**) was het bestuderen van de regeneratieve capaciteit van autoloog spongieus, corticaal en BBM botmoes in hetzelfde onderzoeksmodel als gebruikt in hoofdstuk 2. Autoloog spongieus botmoes is het te verkiezen materiaal om een botdefect in het MKA-gebied te overbruggen wanneer er geen mechanische ondersteuning nodig is. Autoloog spongieus botmoes geneest op dezelfde wijze als autologe spongieuze botblokken maar heeft als voordeel dat het makkelijker aan te brengen is. Een corticaal botblok als solitair transplantaat onder dezelfde omstandigheden geeft een beter resultaat dan corticaal botmoes. Na 24 weken werd het defect niet egaal met nieuw bot overbrugd maar bleven er uiteindelijk perforaties zonder genezingstendens over.

BBM botpartikels als solitair implantatiemateriaal in een botdefect met een kritische grootte was geen stimulans voor botingroei maar liet een uitgebreide osteoclastische activiteit zien, mogelijk gestimuleerd door de onderlinge beweeglijkheid. Een BBM botblok is betrouwbaarder. De botingroei vanaf de randen, die gezien werd in de 'blokstudie' bij een BBM-blok (hoofdstuk 2), 3 weken na implantatie, werd niet gezien in de 'moesstudie' bij BBM partikels. Het nieuw gevormde bot duwt de BBM partikels opzij zoals een 'sneeuwschuiver', zonder tekenen van botingroei tussen de partikels. Om dit 'sneeuwschuiver' effect te voorkomen lijkt het beter een exact passend BBM blok te gebruiken dan BBM partikels.

In **Hoofdstuk 4** werd de genezingscapaciteit van gemengde transplantaten geëvalueerd (autoloog spongieus botmoes/BBM partikels versus autoloog corticaal botmoes/BBM partikels). Geconcludeerd kan worden dat een mengsel van autoloog spongieus bot en BBM goede resultaten geeft, waarbij de voordelen van beide materialen afzonderlijk worden gecombineerd en de afzonderlijke nadelen worden gereduceerd. Botdefecten met een kritische grootte in het MKA-gebied in contact met een paranasale sinus, gevuld met dit mengsel genezen zonder complicatie binnen 12 weken. Dit is even snel als de genezing van botdefecten wanneer autoloog spongieus zonder BBM wordt gebruikt. Een mengsel van autoloog corticaal bot met BBM geeft een minder resultaat. Deze mengsels stimuleren osteoclasten, mogelijk door het afunctionele BBM, wat resulteert in een

progressieve resorptie van het corticale botmoes. Meer osteoid weefsel werd gezien wanneer er puur corticaal botmoes als transplantatie materiaal werd gebruikt.

Het effect van het gebruik van de twee verschillende autologe materialen en BBM op de tanderuptie van blijvende premolaren in de mandibula van beagle honden werd onderzocht in **Hoofdstuk 5**. De eerste en derde melkkies werden op een leeftijd van 10 weken getrokken waarna de ontstane botdefecten over de blijvende tweede en vierde premolaar werden opgevuld met autoloog corticaal botmoes, autoloog spongieus botmoes, BBM granulaat of leeg werden gelaten. De derde blijvende premolaar diende als controle element. Wekelijks werden gestandaardiseerde röntgenfoto's genomen. Aan de hand van vaste referentiepunten werd middels een gedigitaliseerd systeem de verplaatsing tijdens de tanderuptie vastgelegd. Geen van de premolaren vertoonde een vertraging van de doorbraak of een verstoring van de kroon- of wortelontwikkeling. Histologische evaluatie liet zien dat de BBM partikels niet resorbeerden noch integreerden in het alveolaire bot, maar dat de partikels werden verplaatst naar de gingiva. De eruptiecurven van de blijvende premolaren in alle groepen vertoonden geen onderlinge verschillen.

In de MKA-chirurgie wordt een verscheidenheid aan anorganische en synthetische, biologisch inerte (alloplastische) transplantatie materialen gebruikt om botdefecten te overbruggen of om voldoende botvolume te creëren voor het plaatsen van tandwortel implantaten. Vanwege de variatie in de literatuur over de effectiviteit van deze materialen in vergelijking met autologe materialen, met name in reconstructieve procedures van de bovenkaak, is het soms moeilijk protocollen te ontwikkelen en de juiste indicaties te selecteren. In **Hoofdstuk 6** wordt een analyse gegeven van de literatuur, gerefereerd in 'Medline' van 1989-1999 met betrekking tot de voor- en nadelen van de verschillende modaliteiten. Studies waarin verschillende transplantatie materialen waren gebruikt in contact met de sinus maxillaris en waarvan histomorfometrische gegevens waren gerapporteerd werden geselecteerd. Acht studies werden in de analyse betrokken: in 6 studies werden bottransplantaten geplaatst voor sinusbodem-elevaties en in 2 studies ter overbrugging van osteotomie defecten. Over het algemeen kan worden geconcludeerd dat autoloog bot uiteindelijk de grootste hoeveelheid botvolume geeft, onafhankelijk van de genezingsperiode (1-24 maanden), wijze van aanbrengen (botmoes versus botblok) en donorlocatie (crista iliaca versus kin). De resultaten van anorganische calcium fosfaat materialen (BBM of hydroxyapatiet), solitair of in gemengde vorm met autoloog botmoes of gedemineraliseerd allogeen bot, vertoonden een grote variatie in de hoeveelheid osteoid dat werd afgezet. In alle gevallen waren deze waarden lager dan die bij de autologe bottransplantaten. De

data van de verschillende anorganische materialen kunnen niet eenvoudig met elkaar vergeleken worden vanwege de verscheidenheid van genezingsinterval, de mengverhoudingen en de verschillende histologische procedures. Zij geven wel aan dat het gebruik van mengsels van autoloog bot met anorganische materialen de voorkeur verdient boven het gebruik van solitaire anorganische bot mineralen.

In een afsluitende discussie (**Hoofdstuk 7**) worden conclusies getrokken uit het experimentele werk. De toekomst zal gericht moeten zijn op prospectieve klinische en experimentele onderzoeken om de effectiviteit van de verschillende samengestelde transplantaten vast te stellen. Daarbij zal ook aandacht moeten worden besteed aan de rol van verschillende groeifactoren bij botgenezing in het MKA-gebied.

TEN SLOTTE

Het werk, zoals beschreven in deze dissertatie, is een leerproces geweest dat verschillende stappen en fasen heeft gekend. Gelukkig heb ik deze meestal niet alleen gezet of doorgemaakt. Velen hebben hun bijdrage geleverd, ieder op hun eigen wijze. Zonder iemand tekort te willen doen, wil ik de belangrijksten noemen.

Hooggeleerde promotor Freihofer, beste Hans-Peter, dank voor je stimulerende impulsen bij de eerste stappen in het vakgebied en met name bij het opzetten en afronden van dit 'project'.

Zeergeleerde co-promotor Maltha, beste Jaap, de rode draad is van jouw hand. Als een coach heb jij een 'clinicus practicus' op het wetenschappelijke pad gehouden. De stappen gingen niet altijd vanzelf! Dank voor je nooit aflatende steun.

Staf en medewerkers van de afdeling Mond- en Kaakchirurgie van het UMC St. Radboud, jullie wil ik met name bedanken voor de ruimte en de tijd.

Medewerkers van het Centraal Dierenlaboratorium en de afdeling Orale Biologie, dank voor de hulp bij het uitvoeren van de experimenten en het bewerken van het verkregen materiaal.

Met mijn paranimfen Huib en Luc heb ik de eerste stappen gezet respectievelijk als student en assistent. Veel ervaringen hebben we gedeeld.

Mijn ouders wil ik bedanken voor hun warme belangstelling tijdens deze 'fase'. Dat ik met mijn vader een speciale band heb, moge duidelijk zijn: 'de appel is niet ver van de boom gevallen'!

Last but certainly not least het thuisfront, Liesbeth, Emilie, Coen en Job. Dit werkstuk is niet voor niets aan jullie opgedragen, ……..……..!

CURRICULUM VITAE

Thijs Merkx werd geboren op 30 mei 1959. In 1978 behaalde hij op de scholengemeenschap Canisius College/Mater Dei te Nijmegen het eindexamen VWO-β. Na te zijn uitgeloot in Nederland, heeft hij de eerste Kandidatuur Tandheelkunde aan het Rijks Universitair Centrum te Antwerpen gevolgd. In 1979 startte hij de studie Tandheelkunde (zes-jarige opleiding) aan de Rijks Universiteit te Groningen welke in maart 1985 met goed gevolg werd afgesloten. Aansluitend werd begonnen met de studie Geneeskunde. In juni 1987 werd het doctoraal examen gehaald. Na de co-assistentschappen te hebben doorlopen in Deventer (St. Deventer Ziekenhuizen), werd in april 1989 het artsexamen aan de RUG behaald. Van mei 1989 tot en met januari 1990 was hij arts-assistent (niet in opleiding) op de afdeling Algemene Heelkunde van het Canisius Wilhelmina Ziekenhuis te Nijmegen (opleider dr. H.J.M. Joosten). Op 1 februari 1990 startte hij de opleiding tot Mond- en Kaakchirurg te Nijmegen (opleider Prof.dr. H.P.M. Freihofer). Sinds het afronden van deze opleiding (1 februari 1994) is hij aan deze afdeling verbonden als staflid. In november 1994 ontving hij vanuit de Nederlandse Vereniging voor Mondziekten en Kaakchirurgie de 'Rhône-Poulenc Research Grant' voor de studieopzet, welke de start was van de voor U liggende dissertatie. Zijn speciale aandacht gaat uit naar de chirurgie en oncologie van het hoofd-hals gebied.

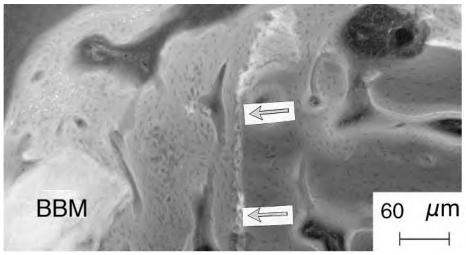


Fig 2.3a *BBM 6 weeks after implantation. Margin of a defect: osteoconduction in BBM sponge (x40). BBM = Incorporated particle of Bovine Bone Mineral, Arrows = Margin of the defect.*

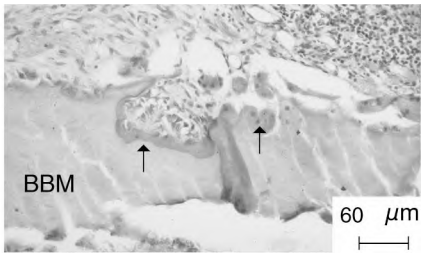


Fig 2.3b *BBM 6 weeks after implantation Centre of the same defect as 3a: osteoclasts around BBM (x40). BBM = Bovine Bone Mineral, Arrows = Active osteoclasts around non-incorporated BBM particle.*

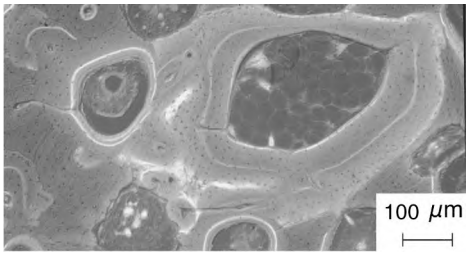


Fig 2.4 *Undecalcified section of cancellous bone graft 12 weeks after transplantation. Labelling reflecting a high remodelling activity. The original spongious structure can be recognized (x25).*

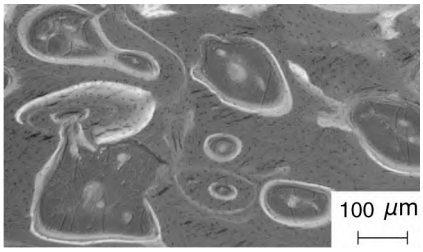


Fig 2.5 *Undecalcified section of cortical bone graft 12 weeks after transplantation. More regular labelling pattern with concentric circles over the whole graft reflecting a slow remodelling process. Mark the difference with fig. 2.4 (x25).*

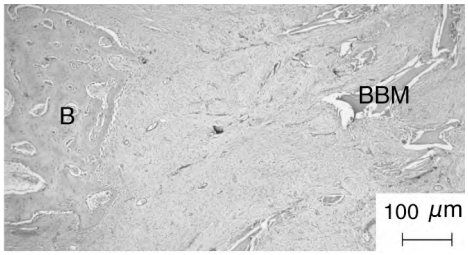


Fig 2.6 *BBM 12 weeks after implantation. Extensive osteoclastic activity around remnants of non-integrated, partly degraded BBM in the centre of the defect (x25). BBM = Bovine Bone Mineral, Arrows = Osteoclasts, B = Bone*

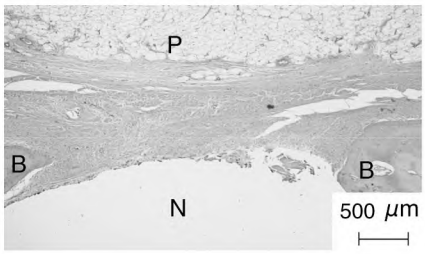


Fig 2.7 *Control defect 24 weeks after operation. The non-bridged defect showed variable amounts of bone deposition, extending to a considerable distance of the margins. Resting osteoblasts or lining cells on the bone surface indicated a stable phase (x5). N = Nasal side, P = Periosteal side, B = Bony margin of the defect*

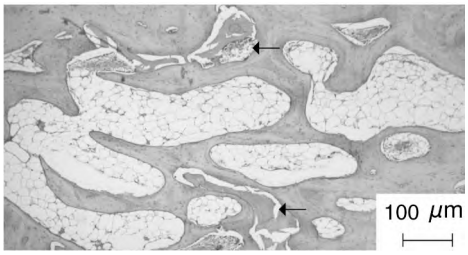


Fig 2.8 *BBM 24 weeks after implantation. Integrated BBM near the margin of a defect. No remodelling activity is seen around these particles. (x25). Arrows = Bovine Bone Mineral*

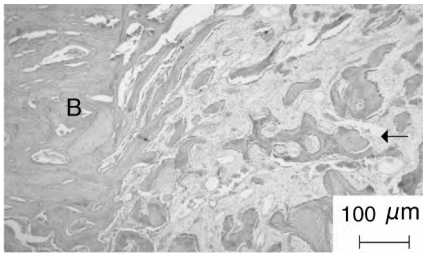


Fig 3.4 *Cancellous particulate bone, 3 weeks after implantation, embedded in young bone delineated by osteoblasts. (orig. magn. x25).*

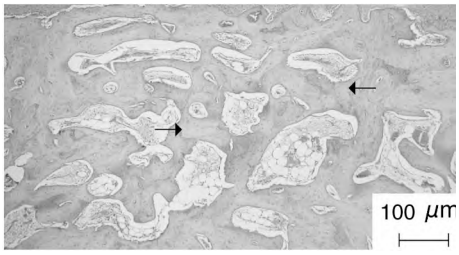


Fig 3.5 *Cancellous bone chips, 12 weeks after implantation. The structure of the chips is still recognizable in the irregular trabeculae of new bone (orig. magn. x25). Arrows = former chips with empty lacunae*

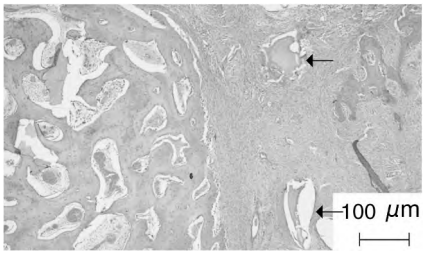


Fig 3.6 *BBM 12 weeks after implantation. Incorporated particles of BBM are involved in the physiologic remodelling process. (orig. Magn. x25)*

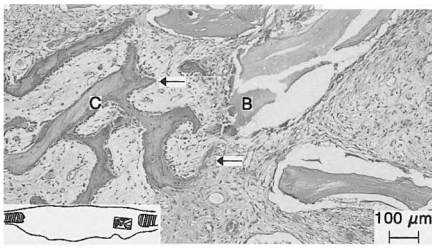


Fig 4.2 *Cancellous/BBM graft 3 weeks after implantation. An active bone formation sprouted from solitaire cancellous bone chips. The BBM particles are surrounded by multinucleated cells (orig. magn. x25), B = Bovine bone mineral; C = Cancellous bone chip surrounded by new bone; arrow = active bone formation.*

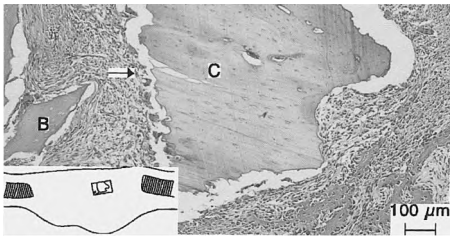


Fig 4.3 *Cortical/BBM graft 3 weeks after implantation. Osteoclastic activity was found around individual cortical and BBM chips (orig. magn. x25). No new bone formation was seen around the original cortical chips. B = Bovine bone mineral; C = cortical bone chip; arrow = multinucleated cells.*

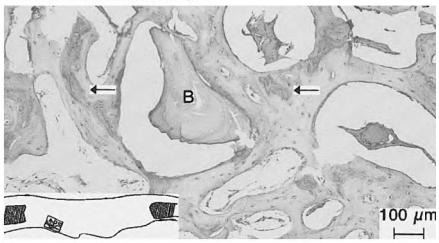


Fig 4.4 *Cancellous/BBM graft 6 weeks after implantation. Integrated BBM surrounded by new bone in which the former cancellous and the BBM particles could be recognized (orig. magn. x25). B = Bovine bone mineral; arrow = former cancellous bone chip.*

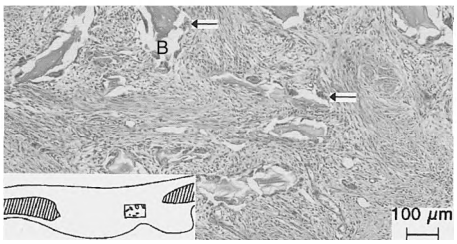


Fig 4.5 *Cortical/BBM graft 6 weeks after implantation. In the centre of the defects osteoclast-like cells surrounded the cortical chips and the BBM granules. No bone deposition was seen. (orig. magn. x25). B= Bovine bone mineral; arrow = multinucleated cells.*

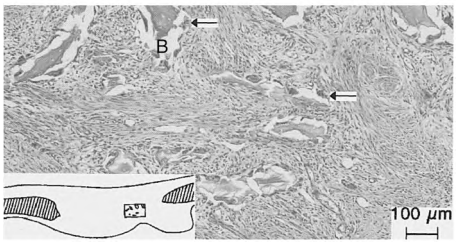


Fig 4.6 *Cancellous/BBM graft 12 weeks after implantation. The BBM particles are integrated in more compact bone than in Fig. 4.4. The original structure of the cancellous chips could hardly be recognized anymore (orig. Magn. x25). B = Bovine bone mineral.*

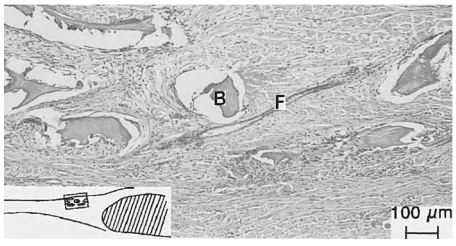


Fig 4.7 *Cortical/BBM graft 12 weeks after implantation. In all cases BBM granules in the central area were embedded in mature fibrous tissue surrounded by multinucleated cells (orig. magn. x25). B = Bovine bone mineral; F = fibrous tissue.*

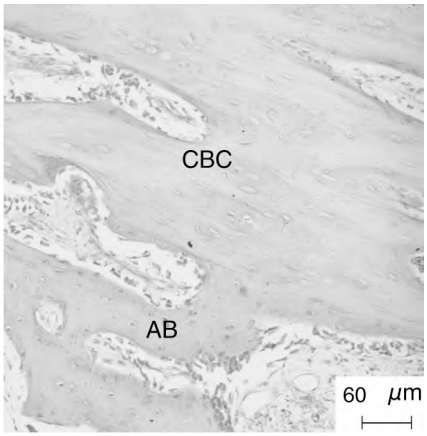


Fig 5.5 *Photomicrograph of cortical bone chips four weeks after transplantation at an age of 18 weeks showing the non-vital aspect of the bone chips with only some osteocytes in the lacunae. (Haematoxylin and Eosin; x40). CBC = cortical bone chips, AB = alveolar bone*

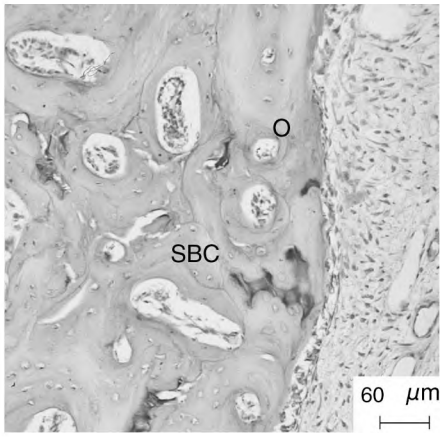


Fig 5.6 *Photomicrograph of spongious bone chips four weeks after transplantation at an age of 18 weeks after transplantation showing the incorporated bone chips with osteons among them. Arrow indicates remnants of cartilage (Haematoxylin and Eosin; x40). SBC = spongious bone chips, O = new osteon*

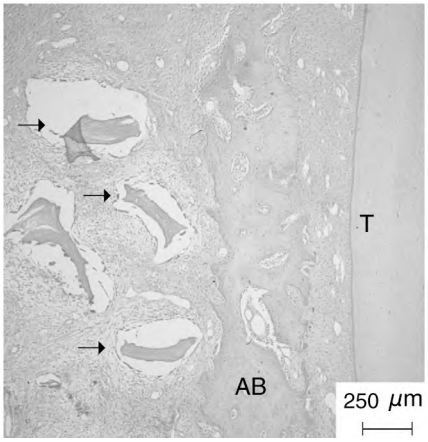


Fig 5.7 *Photomicrograph of BBM particles four weeks after transplantation at an age of 18 weeks showing the connective tissue layer around them without signs of inflammation. Arrow indicates BBM particle (Haematoxylin and Eosin; x10). T = tooth, AB = alveolar bone*

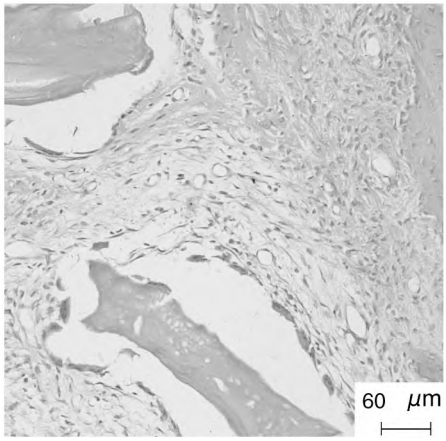
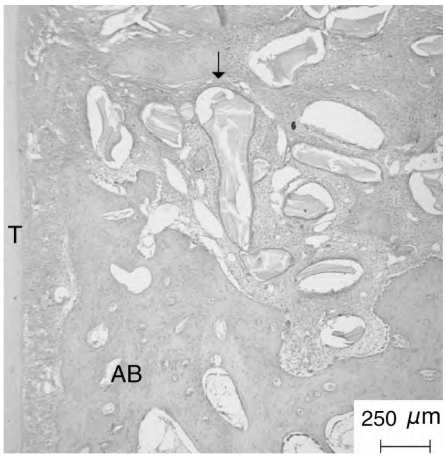


Fig 5.8 *Detail of fig 5.7. Osteoclast-like cells surrounding the particles (Haematoxylin and Eosin; x40)*



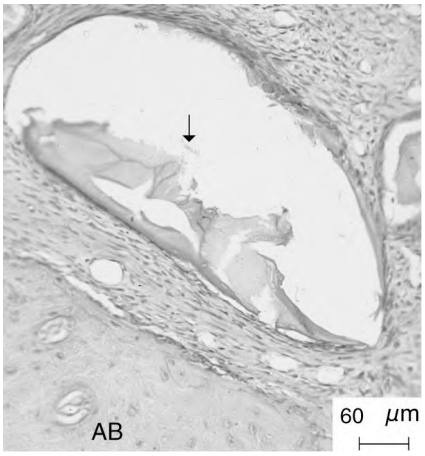


Fig 5.9 *Photomicrograph of BBM particles 16 weeks after transplantation at an age of 30 weeks. Arrow indicates BBM particle (Haematoxylin and Eosin; x10). T = tooth, AB = alveolar bone*

Fig 5.10 *Photomicrograph of one BBM particle 16 weeks after transplantation at an age of 30 weeks showing the thin fibrous sheet around the particle lying in connective tissue, not integrated in the alveolar bone. Arrow indicates BBM particle. (Haematoxylin and Eosin; x40)*