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Maxillary Sinus Augmentation Using Growth Factors and Collagen Membranes

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DEPARTMENT OF HISTOLOGY, GROUP OF TISSUE ENGINEERING

Maxillary Sinus Augmentation Using Growth Factors and Collagen Membranes

Dissertation presented by Ismael El Khouly Castilla to obtain a PhD Degree at The University of Granada

This Thesis has been conducted at:

Bluestone Center for Clinical Research. New York University College of Dentistry , New York, NY, USA

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REGENERACIÓN ÓSEA DEL SENO MAXILAR. ESTUDIO SOBRE LA UTILIZACIÓN DE FACTORES DE CRECIMIENTO Y MEMBRANAS BIOLÓGICAS

Tesis realizada por Ismael El Khouly Castilla para obtener el titulo de Doctorado por la Universidad de Granada

La Tesis se ha realizado en:

Bluestone Center for Clinical Research. New York University College of Dentistry , New York, NY, USA

Departmento de Histología (Grupo de Ingeniería Tisular). Universidad de Granada, Granada, Spain

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A mi familia…

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ABBREVIATIONS

ABBM: Anorganic Bovine Bone Mineral

ACS: Absorbable Collagen Sponge

CT: Computed Tomography

DBM: Demineralized Bone Matrix

DFDBA : Demineralized Freeze-Dried Bone Allograft

EA : Extraosseous Anastomosis

HA: HydroxyApatite

IA: Intraosseous Anastomosis

lOA: Intraorbital Artery

MA: Maxillary Artery

MCBA: Mineralized Cortical Bone Allograft

OPT: Orthopantomography

PSAA: PosteriorSuperior Alveolar Artery

PTFE: Polytetrafluoroethylene

rhBMP: Recombinant Human Bone Morphogenetic Protein

rhPDGF: Recombinant Human Platelet-derived Growth Factor

TCP: TriCalcium Phosphate

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I. SUMMARY

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Traditional management of patients with missing teeth formerly involved the use of a variety of fixed or removable prostheses designed to utilize selected teeth (Bryan & Zarb 1998). However, many patients with removable prostheses experience difficulty achieving comfortable and efficient function. Fortunately, maladaptive complete denture patients respond very well to implant prostheses (Zarb & Schmitl 1989). The use of implants to prosthetically restore function and esthetics following the loss of teeth has become a common treatment alternative to conventional tooth-supported fixed or removable recontructions, mainly due to the benefit of avoiding the sacrifice of intact structure of adjacent teeth. However, a common problem of encountered in implant dentistry is insufficient bone quantity to allow implant placement according to standard protocol. Various clinical techniques have been developed to address these bone deficiency problems (Tonetti & Hämmerle 2008).

The maxillary sinus is the essential anatomical structure often involved in many oral and maxillofacial surgical procedures in the posterior maxilla. During augmentation procedures and/or dental implant placement, trauma to the maxillary sinus in situations where the residual ridge height is reduced. The loss of posterior maxillary teeth is often accompanaid by resorption of the remaining alveolar bone, which is frequently thin and qualitatively poor. With advancing age, pneumatization of the maxillary sinus increases its size at the expense of the remaining alveolar bone (Small et al. 1993). However, where necessary augmentation of the posterior alveolar bone is important, placement of dental implants in the atrophic posterior maxilla is a challenging procedure. Several techniques have been proposed to address this challenge and to obtain adequate bone dimensions for implant insertion. Furthermore, improvements in surgical techniques and advances in biomaterial research have resulted excellent outcomes. These advances have been reported in recent years for implant-supported rehabilitations, even in cases involving severe alveolar bone atrophy (Del Fabbro et al. 2008; Aghaloo & Moy 2007; Wallace & Froum 2003).

First presented by Tatum in 1977 and published by Boyne and James in 1980, maxillary sinus elevation became part of pre-prosthetic surgical site development (Boyne & James 1980; Tatum 1986). Since the introduction of this procedure, researchers have been

evaluating bone graft materials to determinate those best suited for endosseous implant placement. Initially, autogenous bone graft material was the material used and was harvested from the same patient using an oral or extra-oral donor site (Wood & Moore 1988; Boyne & James 1980). However, to minimize patient discomfort, increase patient acceptance, and decrease morbidity associated with donor sites harvesting, the focus of research shifted toward to use of other materials. A multitude of graft materials have been utilized and studied. The ideal graft material should provide a high percentage of vital bone after a reasonable healing period. Literature reviews of different graft materials reported a range of results (Van den Bergh et al. 2000; Tarnow et al. 2000; Piatelli et al. 1999). However, despite 36 years of clinical research, surgical experience, and advances in techniques and technology, no consensus regarding the best grafting material or surgical technique leading to best clinical outcomes has been identified. Arriving at a consensus has been made even more difficult with the introduction of new graft materials and biomimetic enhancement factors, as well as the continuous introduction of new surgical techniques.

Growth factors (GFs) have been suggested as having the potential to speed the healing process. Growth factors have been used in clinical scenarios to improve tissue regeneration. These natural biological mediators regulate key cellular events that are part of the process of tissue repair and regeneration. Binding of GFs to specific cell membrane receptors of target cells induces intracellular signaling pathways, which activate genes to ultimately change cellular activity and phenotype. This process is accompained by a complex system of feedback loops that induce other GFs, enzymes, and binding proteins. Recent advances in cellular and molecular biology provide a clearer understanding of GFs' functions and their participation in the different phases of wound healing. In vitro and in vivo studies have revealed that GFs can enhance a tissues' capacity to regenerate by controlling cell chemoattraction, differentiation, and proliferation. In sinus augmentation procedures, the most extensively studied GFs are platelet-derived growth factor (PDGF) (Nevins et al. 2003) and bone morphologic protein (BMP) (Tarnow et al. 2010). These GFs have demostrated broad wound healing properties for both hard (bone) and soft (skin or gingival) tissue (Nevins et al. 2003, 2009; Triplett 2008).

The purpose of this PhD Thesis is to analyze the use of tissue engineering, including growth factors which include bone morphologic protein, platelet-derived growth factor and

collagen membranes in sinus augmentation procedures. A histologic and histomorphometric analysis was performed following maxillary sinus floor augmentation in 65 human patients with a total of 120 maxillary sinuses. In the first part of this study, the role of recombinant human platelet derived growth factor (*Study I*) and recombinant human bone morphologic protein (*Study II*) as bone graft materials in sinus augmentation were compared with the use of xenografts and allografts respectively. In the second part of the study, the use of acellular collagen membranes for sinus augmentation in combination with rhBMP (*Study II*) and following sinus membrane perforation (*Study III*) was evaluated.

I. RESUMEN

El tratamiento tradicional de pacientes con pérdida de piezas dentarias incluye la utilización de prótesis fijas o removibles, que están diseñadas para reponer los dientes deseados (Bryan & Zarb 1998). Sin embargo, muchos pacientes con prótesis removibles experimentan dificultades para lograr una función cómoda y eficiente. Afortunadamente, los pacientes con prótesis completas mal adaptadas responden muy bien a tratamientos con implante dentales (Zarb & Schmitl 1989). El uso de implantes para restaurar la función y estética de la pieza dental perdida se ha convertido en una alternativa de tratamiento común, principalmente debido a la ventaja de evitar preparación dental de los dientes intactos adyacentes. Un problema común con implantes es una cantidad de hueso insuficiente para la colocación del implante de acuerdo con procedimientos estándar. Diversas técnicas quirúrgicas de aumento óseo se han desarrollado para hacer frente a estos problemas de deficiencia ósea (Tonetti & Hämmerle 2008).

Durante los procedimientos quirúrgicos de aumento óseo en la zona posterior del maxilar y colocación de implantes dentales, existe el riesgo de producir un trauma en el seno maxilar, debido a una altura ósea del reborde residual insufiente. La perdida de los dientos en la zona posterior del maxilar puede provocar una mayor reabsorción del hueso alveolar, que normalmente es fino y de poca calidad. Con edades avanzadas el seno maxilar neumatiza, aumentando de tamaño a espensas del hueso alveolar remanente (Small et al. 1993). Es por ello que la colocación de los implantes dentales en el maxilar posterior atrófico es un procedimiento difícil, especialmente con una altura del hueso maxilar reducida. Varias técnicas quirúrgicas se han propuesto para hacer frente a este reto y para obtener la dimensión del hueso adecuada para la colocación del implante en el seno maxilar. Además, las mejoras en las técnicas quirúrgicas y los adelantos en la investigación de biomateriales han creado excelentes resultados (Del Fabbro et al. 2008; Aghaloo & Moy 2007; Wallace & Froum 2003).

Presentado por primera vez por Tatum en 1977 y publicada por Boyne y James, en 1980, la elevación del seno maxilar se ha convertido en un procedimiento quirúrgico pre-protésico importante (Boyne & James 1980; Tatum 1986). Desde la introducción de este procedimiento, los investigadores han estado evaluando materiales de injerto óseo más

adecuados para la colocación de implantes endoóseos. Inicialmente, el material de injerto óseo por excelencia se extraía del mismo paciente (autoinjerto) utilizando una zona donante oral o extraoral (Wood & Moore 1988; Boyne & James 1980). Sin embargo, para minimizar el malestar del paciente, aumentar la aceptación del tratamiento por parte del paciente y disminuir la morbilidad asociada a las zonas donantes, la investigación se ha desplazado hacia otros materiales. Una multitud de materiales de injerto se han utilizado y estudiado en profundidad. El material ideal de injerto debe proporcionar un alto porcentaje de hueso vital después de un período de cicatrización razonable. Varias revisiones literarias sobre diferentes materiales de injerto han demostrado una serie de resultados (Van den Bergh et al. 2000; Tarnow et al. 2000; Piatelli et al. 1999). Sin embargo, a pesar de 36 años de investigación clínica y experiencia quirúrgica, los avances en las técnicas y la tecnología, no hay consenso sobre cuál es el mejor material de injerto o la técnica quirúrgica que resulte en mejores resultados clínicos. Conseguir un consenso es una tarea difícil debido a la introducción de nuevos materiales de injerto y de los factores de crecimiento, así como la continua reinvención de las técnicas quirúrgicas.

Desde hace tiempo se cree que los factores de crecimiento (FGs) tienen potencial para acelerar el proceso de cicatrización. Los FGs se han utilizado para la mejora en la regeneración de tejidos. Estos mediadores biológicos naturales regulan los procesos celulares que son parte del proceso de reparación de tejidos y la regeneración. La unión de los FGs a receptores específicos en la membrana celular induce una señalización intracelular en las células diana, en la cual se activan los genes involucrados en la actividad celular y en el fenotipo. Sin embargo, este proceso se rige por un complejo sistema de circuitos de retroalimentación, tales como otros FGs, enzimas, y proteínas de unión. Los recientes avances en biología celular y molecular proporcionan un entendimiento más claro de las funciones y la participación en las diferentes fases de la cicatrización. Estudios en in vitro e in vivo han revelado que los FGs pueden mejorar la capacidad de los tejidos para regenerarse mediante el control de quimioatracción, la diferenciación y la proliferación celular. Entre todos los procedimientos de elevación de senos, los FGs que más ampliamente se han estudiado son el factor de crecimiento derivado de plaquetas (PDGF) (Nevins et al. 2003) y la proteína morfogenética ósea (BMP) (Tarnow et al. 2010). Estos factores de crecimiento han demostrado poseer grandes propiedades curativas, tanto en

tejidos duros (hueso) y como en blandos (piel o encía) (Nevins et al. 2003, 2009; Triplett 2008).

El propósito de esta tesis doctoral es valorar el uso de la ingeniería de tejidos, incluyendo los factores de crecimiento, como proteína morfogenética ósea, factor de crecimiento derivado de plaqueta y membranas de colágeno en los procedimientos de aumento óseo de senos. Para ello, se ha analizado de forma histológica e histomorfométricamente las elevaciones de seno maxilar de 65 pacientes humanos con un total de 120 senos maxilares. En la primera parte de este trabajo se estudia el papel del factor de crecimiento recombinante humano derivado de las plaquetas (rhPDGF) (*Estudio I*) y la proteína morfogenética ósea humana recombinante (rhBMP) (*Estudio II*) como material de injerto óseo en la elevación de seno en comparación con el uso de aloinjerto y xenoinjerto, respectivamente. En la segunda parte, se evalúa el uso de la membrana de colágeno acelular durante la elevación de seno en combinación con rhBMP (*Estudio II*) o durante la perforación de la membrana sinusal (*Estudio III*).

II. INTRODUCTION

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1. ANATOMY OF THE MAXILLARY SINUS

The maxillary sinus starts to develop between the second and third month of pregnancy, with an invagination of the mucosa of nasal passage's lateral wall. The sinus size is about 0.1 to 0.2 cm^3 at birth and retains its similar size until the eruption of the permanent teeth (Van den Bergh et al. 2000). The development, in regard to pneumatization (increasing volume of air contained in it), is achieved by adolescence, although its volume may increase further after tooth loss. The maxillary sinus is the largest of the paranasal sinuses including the ethmoid, sphenoid, and frontal sinus, and occupies most of the jawbone. It is a cavity with a quadrangular pyramidal shape with several walls namely the medial wall lying closest to the nasal cavity, the rear wall near the tuberosity, the mesiobuccal wall along the canine fossa, the cranial or superior wall which is the orbit floor, and the wall of the sinus floor (Fig. 1) adjacent to the alveolar processes (McGowan et al. 1993).

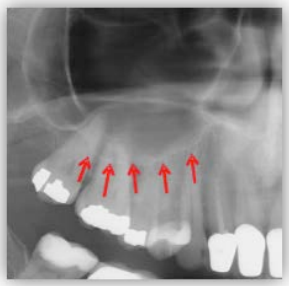


Fig.1 Floor of the maxillary sinus

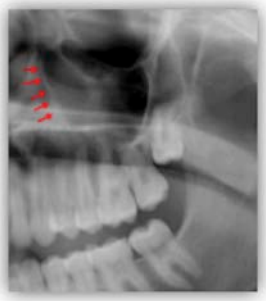


Fig.2 Medial Wall of the maxillary sinus

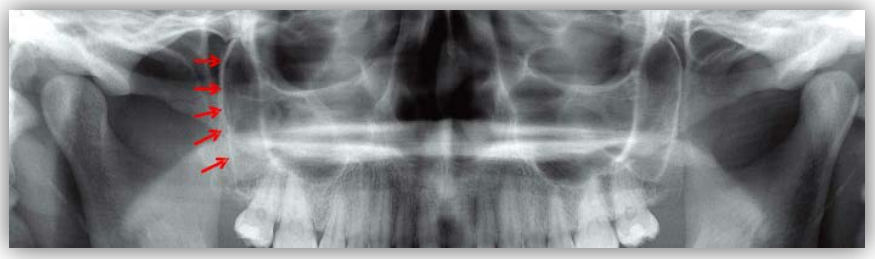


Fig.3 Posterior wall of the maxillary sinus

The maxillary sinus communicates with the nasal cavity through the ostium above the medial wall, which drains into the middle meatus. (May et al. 1990). All sinuses also

communicate with the nasal cavity. Functions of the sinus include air humidification and heating, contribute to weight reduction in the cranial bones, protection of the skull base against trauma, thermal isolation of some of the superior nerves, and influence in phonation (Ritter & Lee 1978; Blanton & Biggs 1969).

The two bony walls, the mesiobuccal and medial walls (Fig.2), are most often involved in sinus surgeries. The mesiobuccal wall is comprised of thin cortical bone containing a complex neurovascular system: the arterial anastomosis between the upper branch of the posterior artery, and the infraorbital nerve innervating the infraorbital region, anterior teeth, and periodontal component. In some cases, the wall's thickness can reach 2 mm, especially in brachyfacial patients (Testori 2009). This thickness cannot be determinated using panoramic radiographs, but only through CT-Scan analysis. The posterior teeth are innervated by complex neurovasculature from the maxillary tuberosity (de Mol Van Otterloo 1994). This anatomical aspect is vital due to limited space for sinus surgery. A surgery performed in the apical region of a vital tooth may increase the risk of devitalization of the tooth (Van den Bergh et al. 2000). The medial wall on the other hand is rectangular in shape and is the bone that separates the maxillary sinus from the nasal cavity. The inferior meatus of the nasal cavity corresponds to the lower part of this wall (Chanavaz 1990).

The detection of an accessory ostium in the medial wall may occur during surgery; hence, the membrane should not be elevated to a height of blocking the ostium (McGowan et al. 1993).

In adults with complete dentation, the sinus floor is the thickest of all the walls that make up the sinus. Usually it has some depressions near the premolars and molars. The sinus floor tends to resorb and form perforations around the roots with age, so that only the Schneiderian membrane separates the roots from the sinus cavity (Testori 2009).

1.1 Sinus Dimension

The average size of an adult maxillary sinus is approximately 12 to 15 cm^3 (with a large range from 3.5 to 35.2cm^3) with a height of 36 to 45 mm, length of 38 to 45mm, and width between 15 to 35 mm (Chang et al. 2012; Van den Bergh et al. 2000; Uchida et al.

1998a and 1998b; Eckert-Mobiu 1954). The sinus may further increase in size with aging and tooth loss due to continuous absorption of the upper, middle, and inferior walls. This form of pneumatization may vary from person to person and even between the two sinuses in the same person (Chan et al. 2012).

1.2 Septum

Nasal septum is found on the floor of the maxillary sinus. The average prevalence of one or more partitions is between 10-44% (Schwartz- Arad et al. 2004; Jensen 2003; Cho et al. 2001; Misch 1993). This is usually common in areas between the second premolar and first molar. Adults lacking teeth have a higher prevalence of maxillary sinus septa (Lindhe 2008).

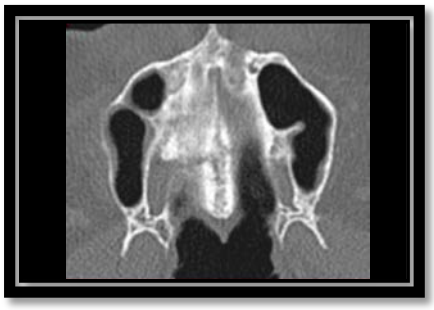


Fig.4 Horizontal view of septum in CT.

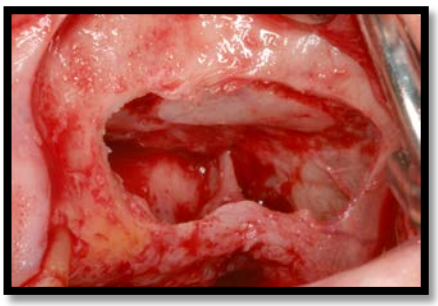


Fig.5 Intraoperative view of the septum.

1.3 Schneiderian Membrane

The maxillary sinus walls are lined with a mucous membrane, known as the Schneiderian Membrane which consists of the following (Testori 2009):

a) pseudostratified cylindrical epithelium with goblet cells and,

b) corium, or lamina propria, with a junction of blood vessels and glands

This membrane is an extension of the nasal respiratory epithelium. Normal membrane thickness ranges from 0.3-0.8mm (Morgensen & Cough 1977). The membrane can, however, suffer from injury causing increase in its thickness due to an inflammatory

reaction, known as sinusitis. In cases in which the thickness is greater than 3-4 mm, it is advisable for the patient see an Ear-Nose-Throat specialist. The sinus membrane includes a richly vascularized lamina propria (Srouji et al. 2009) consisting of two layers, a surface layer of connective tissue beneath the epithelium, and the deep compact layer below the vascular layer merging with the periosteum to form the mucoperiosteum (Watelet 2002). The innermost layer is similar to a periosteum-like structure (Srouji et al. 2008). Under normal conditions, the epithelium remains continuously moistened by fluid secretion from glands contained in Schneiderian membrane. This mucosal epithelium directs fluid towards the ostium that terminates in the nasal cavity (Stammberger 1986). This process is achieved by the 100-150 cilia present in every cuboidal cell epithelium, which vibrate at a frequency of 1000 strokes per minute. Because of its direct contact with air, this membrane has an immune defense capability, although less significant than the nasal mucosa.

The sinus membrane cells are capable of differentiating into osteoblasts, thus making osteogenesis in this region possible (Srouji et al. 2010; Kim et al. 2009; Srouji et at. 2008).

1.4 Vascularization

The maxillary vascular complex is particularly large, hence adequate blood is assured. This blood flow within the maxillary sinus is mediated through three branches of the maxillary artery: the infraorbital artery; the posterior lateral nasal artery (irrigates the medial wall); and the posterior superior alveolar artery (internal maxillary artery branch) (Flanagan 2005; de Mol Van Otterloo 1994; McGowan et al. 1993; Chanavaz 1990). The latter vessel creates an intraosseous anastomosis with the infraorbital artery, starting in the inner side of the maxillary sinus lateral wall at approximately 19mm from the base of the sinus (Elian et al. 2005). When this branch extends intraosseously, computed tomography can detect and visualized it (Elian et al. 2005; Solar et al. 1999).

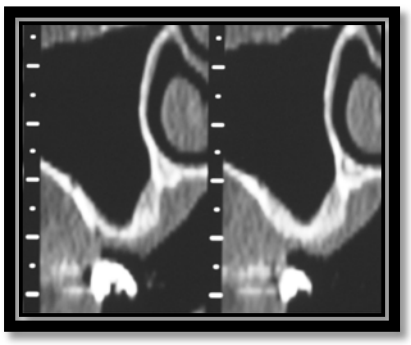


Fig.6 Preoperative CT scan view of the intraosseous artery through the lateral wall of the maxillary sinus.

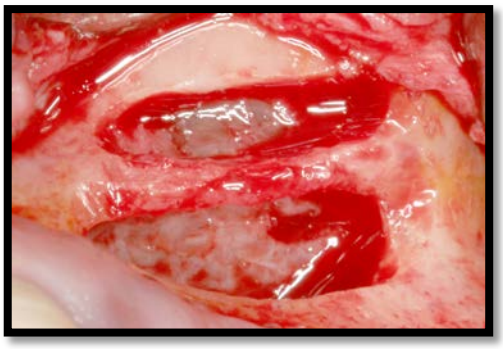


Fig.7 Intraoperative photograph of the bony window prepared in the lateral sinus wall during a sinus floor augmentation. Note a discernible intraosseous anastomosis.

During sinus floor elevation, the vascularization of graft material occurs through the three following branches (Solar et al. 1999):

• Extraosseous Anastomosis (EA): terminal branch of the posteriorsuperior alveolar artery (PSAA) branch from the maxillary artery (MA) with an extraosseous terminal branch of the intraorbital artery (lOA), another branch of the MA. It has a mean height of 23 to 26 mm from the alveolar margin. An extraosseous vestibular vascular anastomosis was detected in 44% of cases. These vessels may result to hemorrhage during flap preparation and periosteum releasing incisions.

• Intraosseous anastomosis (IA) or alveoloantral artery: second branch of PSAA (dental branch) with the lOA. It is located at a distance of 18.9 to 19.6 mm from the alveolar margin.

• Branches of these vessels (PSAA, lOA and IA) in the sinus membrane.

The middle part of Schneiderian Membrane is supplied by the pterygopalatine artery, the terminal branch of the MA. The existence of this anastomosis should be identified prior to surgery to prevent bleeding during surgery which occurs if this branch of artery is punctured during antrostomy.

Severe hemorrhages during maxillary sinus grafts are rather rare as main arteries do not run inside the surgical area. Small vessels may be punctured. If these are located in the

exposed Schneiderian membrane, hemostasis may occur naturally, possibly through applying slight pressure with gauze (Testori 2009). These vessels supply both sinus membrane and periosteal tissues as the PSAA often has an extraosseous course. The majority of blood vessels in the maxillary sinus (70 - 100%) come from the periosteum (Testori 2009; Chanavaz 1990). Healing and remodeling of the graft depends mainly on the vascularization from the sinus walls where new blood vessels are formed between the graft particles. It is also important to preserve blood flow to other structures involved in the surgical procedure, such as the Schneiderian membrane and the mucoperiosteal buccal flap.

The maxillary sinus venous return occurs toward the pterygomaxillary plexus, along two paths: the facial and the maxillary vein to the internal jugular vein, or through the ophthalmic vein into the cavernous sinus (Testori 2009; Solar et al 1999).

Marked reduction in vascularization of the bone is due to loss of maxillary teeth and aging. An interrelationship among the development of micro-vascular defects, bone atrophy and advancing age is observed (Testori 2009). In elderly, the stenotic processes reduce blood flow to the bone marrow, preventing osteoblast activity and causing mineralization delay.

Lymphatic drainage is achieved from the posterior region of the nasal cavity and nasopharynx to the retropharyngeal nodes and submaxillary glands. The healthy maxillary sinus requires postural drainage and action of the ciliated epithelial mucosa, which moves bacteria to the ostium. It also produces mucus-containing lysozyme and immunoglobulins. Vascularization of the sinus membrane maintains the body's defenses by providing access to lymphocytes and immunoglobulin from both the membrane and the sinus cavity (Lindhe 2008).

Having the communication from the nasal cavity to the maxillary sinus not located in the inferior part of the sinus (where graft is placed) is important in providing an anatomical foundation for sinus floor elevation. This allows grafting while avoiding the normal function of the sinus. A sinus lift may even enhance symptoms of sinusitis and congestion since the lifted floor is relocated closer to the drain port (Lindhe 2008).

1.5 Innervation

Innervation of the maxillary sinus occurs through the maxillary nerve, the second branch of the fifth cranial nerve (nervus trigeminal). It innervates the sinus floor in the posterior area with its posterior middle and superior alveolar branches, as well the molar and premolar teeth. The anterior superior alveolar branch, a branch of the infraorbital nerve from the infraorbital foramen, extends to the anterior wall of the sinus plexus and the maxillary teeth that are located below the sinus membrane. B

Before leaving the infraorbital foramen, some branches of the infraorbital nerve trunk innervate the medial wall of the maxillary sinus. Other branches involving the sinus mucosa are branches of the pterygopalatine ganglion and the sphenopalatine ganglion, with the long and short sphenopalatine nerve (Testori 2009).

1.6 Sinus Microbiota

Hemolytic and alpha-hemolytic Streptococcus spp. and Neisseria are normal commensal microbial flora of the maxillary sinus. Staphylococci, diphtheroids, Haemophilus spp., Pneumococcus, Mycoplasma spp., and Bacteroides spp. are also present in varying quantities (Timmenga et al. 2003).

2. BONE

According to Rho et al. 1998, bones are organized in a complex hierarchical structure. The general classification of mammalian bones including those of human beings at the macro level is cortical or cancellous (trabecular) bones. The cortical class of bones is located mainly in the shaft of long bones and the outside shell surrounding trabecular bone at the proximal and distal ends of bones and the vertebrae. On the other hand, trabecular bone is found inside cortical tissues, within the medullary cavities found at the ends of long bones and inside short bones such as spinal vertebrae (Wang et al. 2010). At a sub-microscopic level, the lamellae make up the osteons and trabeculae.

2.1 Cortical Bone

Cortical bone is both a primary and secondary bone and it accounts for approximately 80% of the human skeleton. Primary bone refers to tissue deposited on the existing bone surfaces at the developmental stage. This may also be made of circumferential lamellae, woven tissue or plexiform tissue. Circumferential lamellar bone is made up of lamellae, which is parallel to the surface of the bones. Inside the circumferential lamellar are primary osteons, which form when the blood vessels on the surface of the bone become part of the periosteal bone. Plexiform and woven bones exist in large animals and/or those developing fast and can develop after a fracture. Other components of the cortical bone are Haversian canals, resorption spaces and Volkmann's canals which occupy void spaces (Wang et al. 2010).

2.2 Trabecular Bone

Trabecular bone is located in the metaphysis, epiphyses, and medullary cavity of long bones, within flat bones, and within vertebral bodies. It consists of a three-dimensional structure of interconnected plates and rods known as trabeculae, each of which is approximately 200µm thick (Martin et al. 1998). Trabecular bone contains between 75%-95% porosity. The pores in trabecular bone are joined and filled with bone marrow.

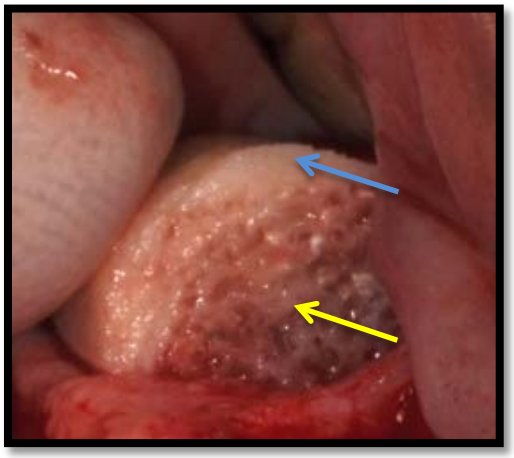


Fig.8 View of a bone block. Note the trabecular bone (yellow arrow) and the cortical bone (blue arrow).

2.3 Structure of Bone

Comparable for both cortical and trabecular bone, organic matrix (mostly type I collagen), apatite mineral (similar to hydroxyapatite crystals) and water together compose the ultrastructure of bone, characterized as a composite of mineral crystals and collagen fibrils. The collagen fibrils are placed in an organized manner and form a lamella upon mineralization (Figure 2.5). Bone material can therefore be simplified as a two-phase composite (Bonfield & Li 1967; Currey et al. 1962). Bone can be recognized as two-phase mixture in two different ways. First, bone tissue should be considered as a combined mineralized collagen fibers and organic matrix from the structural point view. Second is to consider it as a composite of mineral and collagen from the compositional point view.

2.4 Bone Components

Among the bone components, the mineral phase occupies up to 60% of the mass or 40% of the bone volume. This mineral composition is mainly calcium (Ca^{2+}) and phosphate (PO_3^{-4}) with a small fraction of carbonates (CO_2^{-3}). The organic matrix in addition occupies about 40% of the bone volume (Elliott et al. 1957). It comprises of more than 90% of type I collagen and non-collagenous proteins (e.g., osteocalcin, osteonectin, osteopontin, etc.), which are small in amount yet essential in bone structure and bone metabolism. Lastly, the water phase occupies up to 25% of the bone volume (Wang et al. 2010).

3. DENTAL IMPLANTS

Many types of implants have been used for teeth replacement, including subperiosteal and endosteal implants with fibrous encapsulation, and endosseous implants with direct bone contact (osseointegration). Originally proposed by Branemark et al. 1969, osseointegration was defined by Albrektsson et al. 1981 as a "direct structural and functional connection between vital bone and the surface of a load-bearing implant." Another clinical definition was suggested by Zarb & Albrektsson 1991 that it is the "process whereby a clinically asymptomatic rigid attachment of alloplastic materials is achieved and

maintained with the bone during functional loading." Schroeder et al. (1995, 1981, 1976) connotated the term "functional Ankylosis" to describe rigid fixation of the implant to the jawbone, where the "new bone is provided in direct contact on the surface of the implant, provided that rules are followed for the atraumatic implant placement (rotating cutting tool below 800 rpm, with cooling sterile physiological saline solution) and the primary implant stability features."

Therefore, the implant should demonstrate proper initial fixation (stability) after the installation at the receiving site to achieve osseointegration (or functional ankylosis). This initial stability is the outcome of the relationship of contact, or friction, established during implant insertion between the mineralized bone (often the cortical bone) in the receiver and the metal implant (Lindhe 2008).

3.1 Materials and Implant Surfaces

Implant materials have changed greatly over the past forty years. Commercially pure titanium has demostrated excellent biocompatibility and mechanical properties. When exposed to air, titanium forms a thick oxide layer from 2 to 10 nm directly on its surface (Sykaras et al. 2000). This layer is bioinert. However, strength problems of pure titanium have led manufacturers to utilize titanium alloy to increase implant strength. Most implants are titanium alloy. Using alloy significantly increases the force that an implant can withstand while decreasing fractures when small connections and internal diameter implants are used. Titanium alloy (Ti-6Al-4V) has been discussed as the ideal metal for endosseous dental implants. Several attempts to modify the surface characteristics of titanium implants have been made to improve implant anchorage in bone. A thin coating of hydroxyapatite (HA) has been plasma-sprayed onto a roughened and prepared titanium surface to enhance the bone implant connection. HA coatings that usually range from 50 to 70 µm, are applied to the implant surface with plasma-spray technology (Sykaras et al. 2000). Pressurized hydrothermal plasma-spray increases the crystalline HA content from 77% to 96% with an amorphous content of 4%. This coating demonstrated to improve bone adhesion as seen in several studies (Buser et al. 1991, Thomas et al. 1987). The achievements in orthopedics

with roughened titanium surfaces for endosteal appliances influenced dental implant manufacturers to modify the titanium surface by either adding titanium to the surface through plasma-spray technology or reduction procedures involving etching and blasting the surface. The titanium plasma-sprayed surface (TPS) was the first rough titanium surface introduced to implant dentistry. The TPS process is characterized by high-velocity molten drops of metal sprayed onto the implant body to a 10 to 40 µm thickness (Brunski et al. 2000). The original purpose was to cover a greater surface area for bone union. TPS implants have proven to provide long-term success for complete and partially edentulous patients. Techniques such as sand-blasting, titanium oxide blasting, acid etching, or the combination of the three can also be used to create rough titanium surfaces. The average values of bone-implant contact in five weeks are: 72.4% for an acid-etched surface, 56.8% for TPS, 54.8% for sand-blasted surfaces, and 48.6 % for machined surface implants (Cordioli et al. 2000). This may lessen the treatment time from implantation to implant loading resulting in faster healing and improved bone quality (Cochran et al. 2002). Despite positive outcomes with machined titanium implants, all manufacturers and clinicians have changed to rough surface implants. With few exceptions, most endosseous implants today have rough surface textures (Miloro et al. 2004).

3.2 Osseointegration Time

The healing period of a non-loaded machined surface dental implant in the mandible is usually 4-6 months and 6 months for the maxilla (Adell et al. 1985). These healing time periods were recommended to prevent fibrous encapsulation of the implant when prematurely loaded. These initial recommendations were based on clinical findings and not necessarily on biological reasons of implant integration. Due to advances in surfaces and implant designs, the original Brånemark protocol has been greatly modified. In recent years, histological studies reported specific topographic surfaces of micro-implants show earlier and increased bone-implant contact than healing times obtained with machined surface implants (Bornstein et al. 2010). Histological and clinical studies, investigating early loading and immediate-implant placement, revealed that these procedures could be performed earlier than previously recommended. In a study using Osseotite dental implants the effect

of early loading on implant performance and survival was documented with findings that loading can occur in less than 2 months post implant placement (Lazzara et al. 1998). Another study using Osseotite implants placed in the maxillary posterior for a clinical course of two months with a three-year follow-up period (Testori et al. 2001) showed overall implant survival rate after functional loading in the mandible and maxilla of 97.7% and 97.7%, respectively. The early loaded implants in clinical function had a survival rate of 96.2%, with single implants loaded after 3-week implant placement (Cooper et al. 2001). Most tapered-threaded implants were placed in type 3 bones with an 11 mm minimum length. The mean change in marginal bone level was 0.4 mm with an average gain of 0.61 mm papilla length at 12 months. Future changes in implant surfaces aim to greatly reduce integration time.

4. TREATMENT OPTIONS IN THE ATROPHIC POSTERIOR MAXILLA

The atrophic posterior maxilla serves as a challenge for implant placement not only by nature of the bone quality but also because of sinus pneumatization (Sogo et al. 2012). To treat insufficient bone quantity problems, several treatment options have been utilized in the posterior maxilla (Aghaloo & Moy 2007; Del Fabbro et al. 2008; Wallace & Froum 2003). The most conservative of these is the use of short implants to prevent implant placement into the sinus cavity. For short implant placements, at least 6 mm residual bone height is still required (Taschieri et al. 2013). Another way to avoid sinus augmentation is to place tilted implants in a position medial or distal to the sinus cavity when adequate bone support exist (Malo et al. 2011). Another option is the use of zygomatic implants which can be positioned lateral to the zygomatic bone (Aparicio et al. 2012).

On the other hand, in patients with residual bone height greater than 7.5 mm, the sinus membrane elevation can also be attained with the maxillary sinus transcrestal approach known as the osteotome technique or BAOSFE (Bone-Added Osteotomy Sinus Floor Elevation) (Ferrigno et al 2006; Rosen et al 1999; Summers 1994). This transcrestal approach is considered "minimally invasive" due to minimal flap elevation and lower postoperative morbidity (Engelke et al. 2003; Summers 1994), an inadequate bone height

problem can be resolved using a maxillary sinus floor elevation osteotome technique to provide sufficient bone for dental implant placement. However, limitations of this technique are decreased accessibility, limited visibility to the sinus membrane elevation, failed diagnosis and treatment of membrane perforations and potential paroxysmal positional vertigo (Peñarrocha-Diago et al. 2008; Di Girolamo et al. 2005).

Lateral sinus floor elevation (LSFE), is one of the procedures for sinus augmentation whereby an osteotomy "window" is made for access in the side wall of the sinus (Del Fabbro et al. 2004; Wallace & Froum 2003). If residual bone height is less than 4-5 mm, one or two stage sinus lift procedure with lateral access is recommended. The advantage of the lateral window technique is that it allows direct sinus cavity view, direct access for lifting the Schneiderian membrane and graft material addition. However, disadvantages are of time, additional cost, and increased morbidity have been documented (Barone & Santini 2006).

A shortened dental arch concept must also be understood. A study stated that patients sustained adequate capacity (50-80%) to chew using premolar occlusion. However, as the occlusion is restored to the first molar, this ability to chew increases to 90% (Kayser 1981).

4.1 Lateral Sinus Floor Elevation

The sinus floor graft procedure was introduced by Tatum in 1976, modified by Boyne and James in 1980, and further modified by Tatum in 1986. The surgical technique as reported by Tatum in 1986 is generally used at present. Based on this technique, access to the maxillary sinus is reached by a window osteotomy in the lateral maxillary sinus wall.

4.2 Pre-Surgical Evaluation

A full examination, which includes a medical and dental history, should be obtained prior to planning complex surgical procedures such as the sinus floor elevation. Using clinical and radiological examination methods, the dental and periodontal status is assessed. Vitality

of adjacent teeth are also checked. Upper facial, infraorbital, lateral nasal and labial areas must be inspected for tenderness, swelling, or asymmetry. The patient's medical and dental history along with the findings of the clinical examination are reviewed to obtain sufficient information for diagnosing acute, allergic and chronic sinusitis (Lindhe 2008).

Examples of a pre-operative assessment of potential pathological state in the maxillary sinus radiographic examination include orthopantomography (OPT), tomography or computed tomography (CT).

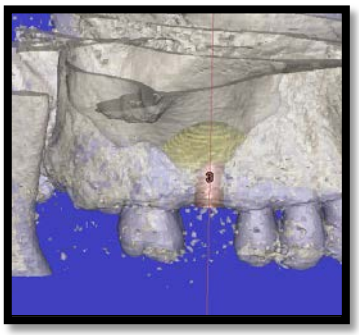


Fig.9 Maxillary CT-Scan: 3D view of implant planning.

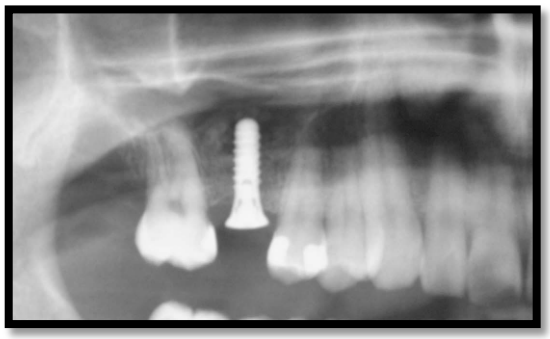


Fig.10 Panoramix view after implant placement.

All patients with sinusitis, polyps and tumors should complete medical evaluation and the necessary surgical treatment prior to the sinus lift procedure.

4.3 Indications

The maxillary sinus floor elevation using a lateral approach is mainly indicated in case of reduced residual bone height, when standard implants or implant placement using the osteotomy technique is not possible. In cases of reduced bone height due to the alveolar bone resorption and air pockets in the sinus cavity from a lateral approach, with or without horizontal bone augmentation is indicated (Lindhe 2008).

The following are some of the indications for the use of sinus bone grafting (Cohen 2007; Jensen 1998):

1. Insufficient vertical bone height (<5 mm) due to implant placement.

• Sinus Pneumatization

• Alveolar ridge resorption

• Combination of the above

2. Oroantral fistula repair

3. Alveolar cleft reconstruction

4. Le Fort I with graft interposition

5. Cancer with reconstruction of craniofacial prostheses

Guidelines to sinus grafting may also include the following:

1. Residual alveolar bone height (<10 mm)

2. At least 4 mm width of residual bone

3. No history of sinus pathology

4. No significant history of sinus disease

5. No anatomical limitations due to anatomical structures or scars after previous surgery

4.4 Contraindications

Contraindications for maxillary sinus augmentation include (Cohen 2007; Jensen 1998):

• General Medical contraindications:

1. Radiation treatment to the jaw region

2. Septicemia

3. Serious medical fragility

4. Uncontrolled systemic disease

5. Excessive smoking

6. Excessive alcohol or substance abuse

7. Psychophobias

• Local factors that may contraindicate subantral augmentation include:

1. Sinus infections

2. Chronic sinusitis

3. Alveolar ablation due to scar (a previous surgical procedure)

4. Odontogenic infections

5. Inflammatory lesions or pathological

6. Severe allergic rhinitis

4.5 Surgical Technique

The window or lateral approach, the original technique of Caldwell-Luc, described an osteotomy in a superior position just above the zygomatic. The two other positions that are described include the medial position in the jaw between the ridge and the zygomatic attachment area and the inferior and anterior position near the level of the existing ridge (Zitzmann and Scharer 1998; Lazzara 1996). The surgical technique is performed as follows (Lindhe 2008):

• Local anesthesia is induced on the buccal and palatal surgical sites.

• The initial incision (midcrestal) is extended well beyond the planned expansion of the osteotomy. The incision is performed to a position beyond the leading edge of the maxillary sinus and is made above and extended into the vestibule to facilitate mucoperiosteal flap elevation (Fig. 11).

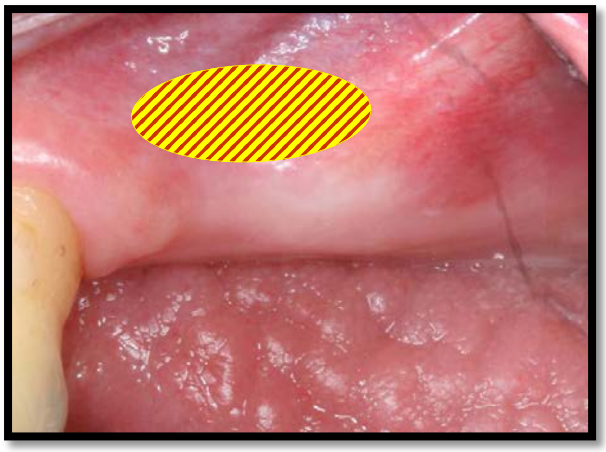


Fig.11 Pre-operative lateral view prior to sinus augmentation. Note the flap design (yellow line), osteotomy design (yellow circle), sinus floor location (white line) and location of implants (grey circles).

• The flap is lifted slightly to the expected height of the side window (Fig. 12).

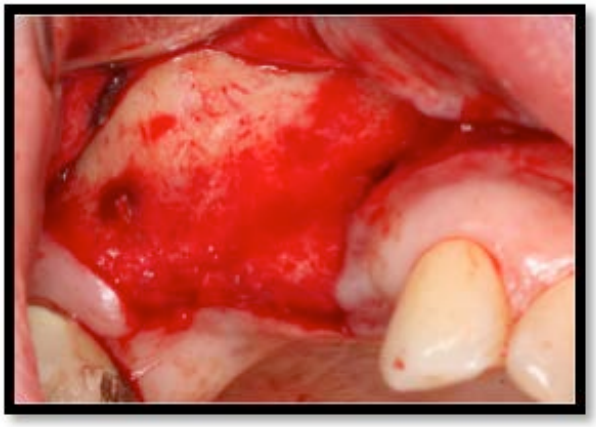


Fig.12 Full thickness flap performed to exposure the lateral wall.

• After exposing the sinus side wall, a round diamond or carbide bur high speed in a straight hand piece is used with copious irrigationto mark the outline of the osteotomy (Fig. 13). The preparation is continued with piezotomo after the bone has been reduced to a thin bone plate, until a bluish hue of the sinus membrane is observed.

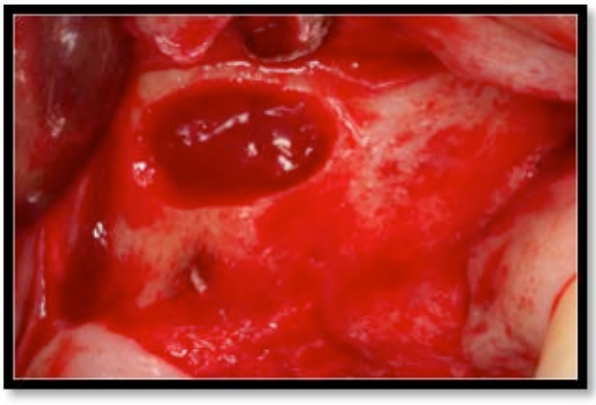


Fig.13 Osteotomy was created to access inside of the maxillary sinus.

Three methods for the vestibular cortical bone window manipulation have been discribed (Wallace et al. 2012). The most common of the three is the thinning of oral bone to a paper thin sheet with a round bur bone and then separating and removing it prior to the sinus membrane elevation. The second method involves the cortical bone infracture and using it as the upper edge of the sinus chamber, leaving it attached to the underlying mucosa. Since the cortical bone plate is resistant to bone resorption, it can protect and contain the graft. The last method is removing the cortical bone during the sinus floor elevation and then replacing it back over the lateral window to cover the graft material at the end of the procedure (Boyne 1993).

• The next step depends on the technique used. If the buccal wall is removed, the sinus membrane is elevated directly with blunt instruments (Fig. 14). The membrane elevation provides adequate space for the graft material placement. The sinus membrane should be carefully and completely elevated to avoid perforations. Depending on the clinical condition and the surgeon's preference, the clinician can use a delayed technique (in second subsequent stage with the implants are placed), or simultaneous placement of the implant.

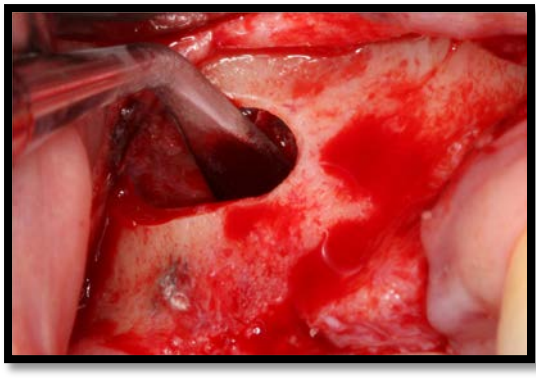


Fig.14 A blunt instrument was used to elevate the sinus membrane and create the space needed for the graft material.

I. Sinus lift in two stages (delayed implant placement following graft healing):

o The graft material is placed in the compartment apical to the elevated sinus membrane (Fig. 15). It should not be too thighly condensed, to avoid reducing the space required for new bone growth and formation. Moreover, pressure of the graft on the sinus membrane may result in perforations and migration of graft material (Wallace et al. 2012; Lindhe 2008).

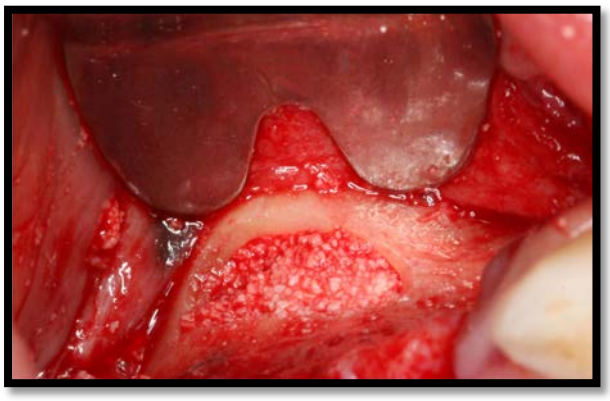


Fig.15 The sinus compartiment was filled using graft material (ABBM + MCBA)

o After filling the compartment with the graft material, the window is then covered with a resorbable or non- resorbable barrier (Fig. 16). The flap is then sutured without tension. In some cases, periosteal releasing incisions are made in order to advance the flad without tension.

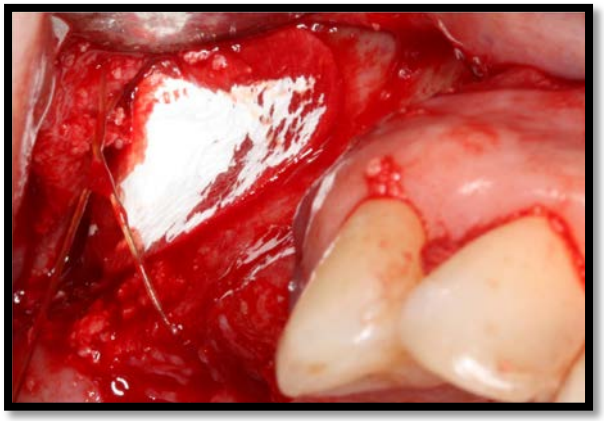


Fig.16 A resorbable collagen membrane placed over the lateral wall.

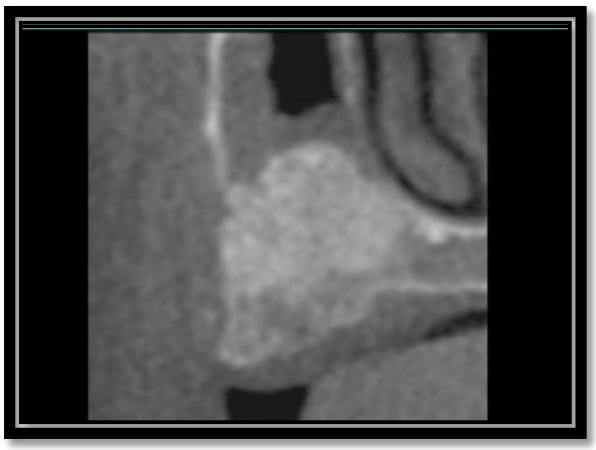


Fig.17 CT-Scan taken after 7 months of healing. Note the new bone formed.

II. Sinus floor elevation(or one stage) with simultaneous implant placement:

- Following the sinus membrane elevation, implant osteotomies are made using copious irrigation. If rotary instruments are used, the periosteal elevator is used to protect the sinus membrane. Osteotomes of different diameters can also be used, with care not to perforate the mebrane.
- The graft material is then inserted into the medial and anterior compartment of the sinus before implant placement. After placement, the lateral compartment and remaining areas are filled with graft material.
- Barrier placement and flap suturing follow the same technique as those described for the two-staged procedure.

The position and the technique used to prepare the side window, the lifting amount of the membrane sinus, graft used, and the choice of one stage or two-stage approaches are major differences between the methods used today and before.

5. COMPLICATIONS IN SINUS AUGMENTATION

Evidence-based studies support sinus augmentation as a highly predictable procedure for implant placement (Aghaloo & Moy 2007; Del Fabbro et al. 2008; Wallace SS, Froum 2003). However, these studies do not discuss the etiology and treatment of various complications or failure with the sinus augmentation procedure.

Situations may occur intra or postoperatively that require modification or abortion the surgical procedure in some cases. These complications or failures result in a delay in time of implant placement. The following are the most common complications observed in sinus augmentation procedures:

- Sinus membrane perforations (Testori et al. 2008; Fugazzotto & Vlassis 2003; Proussaefs & Lozada 2003; Pikos et al. 1999).
- Infection of the surgical site with an occasional resistance to antibiotics (Bandar et al. 2011; Barone et al. 2006; Peleg et al.1999).
- Inadequate bone volume or quality (Mardinger et al. 2010).

- Postoperative sinusitis related to compromised ostium patency, cilliary function or mucous production (Kim & Baik 2010; Zijderfeld et al. 2008; Raghoebar et al. 1999).
- Postoperative cyst formation (Garg et al. 2000; Lockhart et al. 2000)
- Wound dehiscence with subsequent graft loss graft (Misch 1992).
- Sequestration of graft material.
- Oroantral fistula (Anavi et al. 2008).
- Implant migration into the sinus (Kitamura & Zeredo 2010; Chappuis et al. 2009).

Even though complications requiring abortion of surgical procedure or re-entry surgery to treat postoperative problems are rare, clinicians performing the sinus elevation procedure should be familiar with the etiology and appropriate clinical management of such complications.

5.1 Sinus Membrane Perforation

The most common intraoperative complication related to maxillary sinus augmentation is membrane perforation (Zijderfeld et al. 2008). The main causes of perforations are improper rotary instrumentation and sinus membrane elevators usage. Literature reviews show that the percentages of reported perforations range from 11% to 44% and higher percentages with sinuses, with thin membranes, and with the presence of septa (Zijderfeld et al.2008; Schwartz-Arad et al. 2004). Sinus elevation surgery for single tooth replacements in the posterior maxilla can also cause perforations, due mainly to the difficulty in accessing the site (Kreinnmair et al. 2007). Inappropriate surgical access to a site with a previously attempted lateral window may also result in membrane perforation (Fig.18).

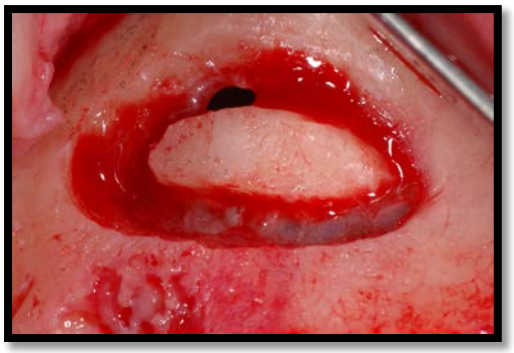


Fig.18 Sinus membrane perforation

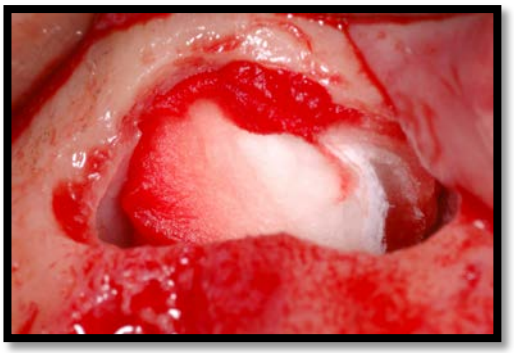


Fig.19 Placement of collagen membrane to repair sinus perforation

Based on literature studies, membrane perforations are associated with most postoperative complications such as acute or chronic sinus infection, bacterial invasion, swelling, bleeding, wound dehiscence, graft material loss, and disruption of normal sinus physiologic function (Proussaefs et al. 2004; van den Bergh et al. 2000; Chanavaz 1990). Lower implant survival rates have been reported with large perforations. Cho-Lee et al. 2010 reported that implant survival rate were lower (81%) in presence of surgical complications, membrane exposure or post-operative sinusitis compared to cases with no complications (97%). Several methods have been discribed to manage these perforations. Large perforation are usually managed by using a bio-absorbable membrane (Fig.18) (Proussaefs et al. 2004; Shlomi et al. 2004; van den Bergh et al. 2000; Vlassis et al.1999), collagen membranes stabilized with sutures and/or tacks (Schwartz-Arad et al. 2004; Vlassis & Fugazzotto1999), fibrin sealants (Chanavaz 1990), block grafts inserted with a cancellous graft (Vlassis & Fugazzotto1999) or by abandoning the procedure (Schwartz-Arad et al. 2004; Shlomi et al. 2004; van den Bergh et al. 2000; Chanavaz 1990). Testori et al. 2008 described two techniques for repair of large perforations with collagen membranes. Pikos et al. 1999 and Vlassis & Fugazzotto 1999 also described on collagen barrier membrane repairs for perforations of more than 10mm. Fugazzotto & Vlassis 2003 described a technique for repairing large sinus perforations with a pliable porcine membrane externally fixated and completely covering the internal bony walls of the sinus.

The “Loma Linda pouch” technique (Proussaefs & Lozada 2003) for membrane perfortion includes the usage of a slowly resorbing collagen membrane with external tack fixation that completely covers all internal bony walls including the sinus floor. This

technique results in delayed vascularization of the graft from the lateral sinus due to the membrane surrounding the enclosed graft (Froum 2010).

Several of the above mentioned techniques attempt to retain vestiges of the membrane to have enough fragments for suturing and stabilization to allow proper graft containment.

Perforations of more than 10 mm are more complicated since the repair may be non-stable. This may result in migration of graft particles into the sinus cavity resulting in blockage of the ostium, and leading to infection and sinusitis (Hernandez-Alfaro et al. 2008).

When membrane repair becomes very difficult, if not impossible, to accomplish during surgery, the procedure may be discontinued while the membrane is allowed to heal with a scheduled future re-entry and re-grafting procedure (Anavi et al. 2008; Ziccardi & Betts 1999). Studies showed that 6-12 months are required under normal conditions for new respiratory ciliated epithelium to regenerate before any further treatment is considered since normal sinus epithelium was removed during the Caldwell-Luc operation. If sinus epithelium is preserved during the perforation, a sinus re-entry can be performed 3 months postoperatively as a shortened healing period is sufficient enough for closure of the sinus membrane perforation (Watelet et al. 2002).

5.2 Sinus Perforation and Bone Grafting

The success rate of dental implants is improved when grafting materials are replaced or surrounded by newly formed bone, which originales from local host bone into the augmented area (Sihegel et al. 2003; Hass et al. 1998; Van de berg et al. 1998). Bone formation requires osteoblasts from progenitor cells of a mesenchymal linage (Bianco & Robey 2001; Ducy et al. 2000; Bruder & Fox 1994). Mesenchymal progenitor cells come from several sources including the bone marrow, cambium layer of periosteum, and pericytes surrounding blood capillaries (Bianco & Robey 2004; Doherty et al. 1998; Burder & Fox 1994). Whether the sinus mucosa, which covers approximately half of the augmentation material (Gruber et al. 2003), has osteogenic potential cells, continues to be a topic of

discussion. Hurzeler et al. 1997 and Haas et al. 2003 argued that the sinus membrane lacks osteogenic potential once elevated from the sinus. Both showed less bone formation close to membrane. Furthermore, some particles had penetrated the membrane in some cases, where more inflammatory cells at these sites were clearly found. However, Fuerst et al. 2004 reported that after sinus grafting with allogenic and xenogenic graft material prior to implant placement in mini pigs, bone formation was induced in the region adjacent to sinus membrane. In another study, Terheyden et al. 1999 discribed a continuous layer of bone adjacent to the Scheiderian membrane in the test sites (BMP-7 were used) and partially missing bone in control sites (ABBM were used). Mesenchymal progenitor cells differentiation into bone forming osteoblast is composed of multiple steps, which can be stimulated by local growth factors (Bianco & Robey 2001; Bruder & Fox 1994). BMP related proteins are likely to be involved in this process. The result reported by Terheyden proved the osteogenic potential of the maxillary sinus membrane.

Various methods are used to confirm the osteogenic potential of the cells, including alkaline phosphatase activity and mRNA expression of osteogenic markers (alkaline phosphatase, bone sialoprotein, osteocalcin and osteonectin). Gruber reported that cells derived from porcine sinus associated mucosa expressed STRO-1, a marker of osteogenitor cells; alkaline phosphatase (ALP); and that the amount of calcium accumulation within the extracellular matrix was increasing in response to BMP-6 and BMP-7. This result agrees with the Terheyden's study mentioned previously. Likewise, other studies confirmed that human maxillary sinus membrane cells could be made to express ALP, BMP-2, osteopontin, osteonectin, and osteocalcin, and to mineralize their extracellular matrix (Srouji et al. 2010; Kim et al. 2009; Srouji et al. 2008). Based on these studies, it is presumed that sinus membrane cells are capable of differentiation into osteoblasts, and osteogenesis is possible.

Sinus membrane perforations are followed by their own healing and repair. Healing of mucous membranes occur via cell migration from normal adjacent epithelium followed by multiplication and differentiation of progenitor cells (Forsgren et al. 1993; Bang et al. 1979). Epithelial regeneration begins within few hours at an estimated 4-20microm/hour velocity (Forsgren et al. 1993; Chopra et al. 1982). The healing of the mucosal lining is a systematic and well-coordinated process, which involves inflammation, cell proliferation,

matrix deposition and remodeling. It is also regulated by a range of growth factors and cytokines (Waletet et al. 2002). Injury causes bleeding to the highly vascularized membrane with numerous blood vessels in the tissue which lead to the formation of a fibrin network formation or scaffold. The combination of the activated progenitors and fibrin clot creates a natural cell-scaffold construct, which becomes an initiation center for new bone formation beneath the sinus membrane (Srouji et al. 2010). Platelets are vital components of this early response due to their concurrent release of numerous cytokines. PDGF, TGF-alfa, TGF-beta are released by damaged cells. Alfa granules within the aggregated platelets are also stimulated by fibrin to release PDGF, EGF, IGF-1, TGF-beta and FTF. All of these growth factors are capable of influencing bone healing and bone formation in the sinus after membrane perforation.

A rigid inflammatory reaction begins simultaneously together with the coagulation phase and remains over a period of several days. Macrophages of the lamina propia release a number of growth factor such as TGF-beta, FGF, EGF, TGF-alfa and PDGF. These stimulate proliferation of fibroblast and angiogenesis. After 4 days, a new stroma or granulation tissue can be observed. Tissue formation undergoes fibroplasia, angiogenesis and re-epithelization. This can be perceived in the fibroplasia process where the fibroblasts slowly mediate protein synthesis and growth factors. In the angiogenesis phase, angiogenic growth factors are released from injured nasal cells, platelets, which induce vascularization. During the re-epithelization phase, four different processes are functioning in regeneration including migration from adjacent epithelium, multiplication of undifferentiated cells, reorientation and differentiation (Whatelet et al. 2002; Norlander et al. 1992). Inayama et al. 1988 claimed that undifferentiated basal cells seem to be the main source of new progenitor cells in the sinus mucosa. This tissue remodeling may last up to 6 months (Watelet et al. 2002).

It has been reported that the regenerated mucosa showed significantly more vessels than the non-operated mucosa which contained rich microvasculation with local signs of angiogenesis (Forgren et al. 1999). Vascular cells may be one of or even the main contributor to the osteogenic cell population in the sinus membrane (Srouji et al. 2009). This increase of GF and angiogenesis around the perforated membrane increases the vessels

formed around the graft particles, affecting wound healing.

In an experiment by Forgreen et al. 1993, after the maxillary sinus membrane mucosa was removed in rabbits, new bone formation that lasted for 2moths was visualized with osteoid and palisades of osteoblasts. The sinus cavity showed a decrease in size. Hilding et al. 1963 stated that the new bone originated from the denuded bone itself as well as the adjacent mucoperosteum, and replaced connective scar tissue. Hilding et al. 1993 reported that removal of the mucoperiosteum of the sinus in dogs resulted in complete destruction of the cavity. Considering a study where a greater volume of bone was found, this may be explained by the injury of the membrane and its response. Unger et al. 1986 and Unlu et al. 1994 found that the most common postoperative change in the post Cadwell-Luc was due to fibro-osseous proliferation which resulted in antra wall thickening or total obliteration of lumen of the sinus. Likewise, a number of cases reported enhanced bone formation following cyst and tooth removal from the maxillary sinus in any bone material absence (Jung et al. 2007; Lundgren et al. 2003). Lundgren reported 3 months following intra-sinus mucosal cyst removal bone formation without the use of a bone graft, where they the membrane perforated. This bone formation was explained by the blood clot formation in the space between the sutured mucosa and the bone walls, and the sinus mucosa periosteum adding to this process.

6. BONE TISSUE ENGINEERING

Tissue engineering is an advanced scientific technology that uses a combination of protein, protein fragments, cells or scaffolds to repair, improve or replace damaged tissues restoring the organ to full function. Traditionally, a number of bone grafting therapies have been used as treatment options for damaged bones but these present a number of shortcomings. Tissue engineering applied to bone regeneration is seen as having the potential to overcome the limitations associated with traditional bone grafting therapies. Bone tissue engineering may involve the use of bioresorbable/biocompatible scaffold materials (Shikinami 2006; Boccaccini et al. 2005; Hutmacher 2000) in combination with cells obtained from a variety of sources [Kanczler & Oreffo 2008; Malicev et al. 2008; Xiao et

al. 2003) and growth factors (Tabata et al. 2006; Huang et al. 2005). This combination is considered optimal for sufficient and timely tissue regeneration.

Currently, three widely accepted methods of tissue engineering exist. The first method involves the use of three- dimensional, porous and degradable scaffolds to offer short-term mechanical support as the tissue regenerates. This tissue engineering approach relies on the body to induce the migration of host cells into the scaffold, where the cells differentiate into the appropriate tissue phenotype and replace the degrading scaffold. (Chen et al. 2007; Schantz et al. 2003; Tyler & McCobb 1980). In the second approach, the right cells are obtained from the patient, cultured in vitro and then transplanted back to the patient. (Kulakov et al. 2008; Peng & Huard 2004; Redlich et al. 1999]. The third approach involves culturing a patient's cells on a three- dimensional scaffold in vitro that is prepared in advance. The cultured cell-scaffold combination is then transplanted into the patient. (Howard et al. 2008; Tabata et al. 2006; Carstens et al. 2005).

Bone tissue engineering is a complex process whose advancement relies on multiple disciplines including science, biology, biomedicine and engineering. The rapid advancement success achieved in this field can be attributed to contribuitions by these disciplines. Advancements in cell and molecular biology have made it possible to isolate and manipulate cells, genes and growth factors (Park et al. 2009; Leonardi et al. 2008). Recent researchs in biomaterials have made it possible to produce new and innovative scaffold systems. Interactions between biology, material science and engineering have made it possible to deliver viable cells and grow tissue on compatible constructs.

While tissue engineering has been successful to date, there are still challenges to overcome especially in the repair and replacement of load-bearing tissues such as bone and dental hard tissues that are responsible for biomechanical function. The biggest challenge facing tissue engineering research is developing a conducive environment for the cells to proliferate and differentiate into functioning tissues (Wang et al. 2010).

6.1 Requirements for Bone Tissue Engineering

There are three essential elements for successful bone tissue engineering. These include the scaffold, the cells and the proper environment for culturing and conditioning the cell-scaffold constructs.

The design and function of the synthetic scaffolds should be optimized to preserve their structural integrity and ensure efficient control of the tissue regeneration process. It is possible to manipulate a variety of scaffold characteristics. Qualities that can be controlled include the shape and size of the pores that accommodate the cells, the mechanical characteristics of the scaffold, the type of coating used to encourage cell adhesion, and the combination of chemicals and growth factors required for successful tissue formation (Wang et al. 2010).

In addition to choosing the appropriate scaffold, the cells used must be sufficient and have osteogenic potential. Currently, there are four types of cells used in bone tissue engineering applications. These include bone marrow cells (Soltan et al. 2009; Connolly 1995), mesenchymal stem cells (Kulakov et al. 2008; Filho Cerruti et al. 2007), muscle cells (Bueno et al. 2009), and embryonic stem cells (Handschel et al. 2008; Tian et al. 2008). Cells can be obtained from there different sources. Cells extracted from the patient are known as autologous and are the best choice because they avoid immune rejection issues. However, they may not be the healthiest choice since the presence and multiplication of some factors in bone may damage bone structure and compromise the biological process of bone resorption. Cells donated from another human are known as allogeneic. Cells obtained from another species (xenogeneic) can also be used. Because of immune rejection and genetic incompatibility issues, both allogeneic and xenogeneic cells can only be used where it is possible to apply genetic engineering to overcome these limitations.

In addition to the scaffold and cells, there must be a proper environment for the culturing and conditioning of the cell-scaffold constructs to achieve successful control and optimization of tissue production. Currently, this has been accomplished in vitro using bioreactors whose development is still in the early stages. These bioreactors provide the cell-scaffold with essential nutrients and dissolvable gases, provide a favorable physical

environment that allows fluid flow, and provide essential growth factors and a controlled environment for the cells to proliferate and differentiate. Bioreactors are also responsible for eliminating cell wastes and providing an outlet for degraded materials. This process takes place in a porous three-dimensional scaffold that is controlled by the bioreactor. The type of bioreactor to use depends on the type of tissue and the type of scaffold being used (Wang et al. 2010).

6.2 Biological Mechanism of Bone Grafting

Bone tissue is very capable of regenerating, acquiring its original structure and completely resuming its functions. This does not happen when there are bone defects. In these cases, bone graft materials have to be used to promote healing (Lindhe 2008). The biological mechanism through which regeneration of bone graft consists of osteogenesis, osteoconduction and osteoinduction (Tonelli et al. 2011; Albrektsson T & Johansson 2001).

6.2.1 Osteogenesis

With Osteogenesis, viable osteoblasts from which osteoid are formed and then transferred together with the graft material to the bone defect to establish bone formation. At the developmental stage, osteoid formation is a natural process that occurs in the endosteum and periosteum of adjacent bone to promote growth. In medical osteogenesis, the remaining osteoblasts or stem cells such as autogenous grafts from iliac bone, mandibular bone and bone medulla transplants become the sources of new bone formation (Marx 2007).

6.2.2 Osteoconduction

With Osteoconduction, non-vital graft materials act as the matrix for in growth of osteoblast precursor into the defect. New bones form from the adjacent bone or periosteum facilitated by a matrix or scaffold that guides the bone formation. The matrix

must bind to molecules that enable cell adhesion to occur. These molecules include fibrin, fibronectin, vitronectin, and collagen (Marx 2007). Gradual resorption of the graft material then occurs. Examples of graft materials with osteoconductive characteristics include autogenous or allograft bone grafts and these as well as bone-derived substitutes or synthetics have similar osteoconductive qualities. The problem with this process is that degradation and viable bone replacement is poor in most of the cases especially if the implanted material is not reabsorbed, which occurs for example with more porous hydroxyapatite grafts. Bone incorporation is limited to the the surface of the material and substitution does not occur at the remodeling phase (Lindhe 2008).

6.2.3 Osteoinduction

With osteoinduction, new bone form from the stimulation and biochemical transformation of mesenchymal cells into bone producing cells (Marx 2007). Examples of graft materials in this category include platelet derived growth factor (PDGF), demineralized bone matrix (DMB), and bone morphogenetic proteins (BMPs) (Giannobile & Somerman 2003; Reynolds et al 2003; Opperman & Sykaras 2003).

6.3 Conditions for Successful Bone Regeneration

All the three basic mechanisms of bone formation described above are crucial in bone regeneration. Without them, bone regeneration may not occur because the cells from autologous cancellous bone grafts cannot survive the transplantation process. The graft materials serve a major function as a matrix for invasion of host cells. Although osteoblasts and osteocytes in the adjacent bone cannot migrate and divide the transplant is invaded by mesenchymal cells that later differentiate into osteoblasts.

It is therefore important to identify the three conditions necessary for successful bone regeneration (Lindhe 2008).

I. The cells must be capable of forming bone or differentiating into bone forming cells.

II. There must be osteoinductive stimuli to stimulate the differentiation of mesenchymal cells into osteoblasts.

III. There must be an osteoconductive matrix acting as the medium where invading tissue can proliferate and osteoprogenitor cells can differentiate into osteoblasts that will promote formation of bone.

6.4 Biocompatibility

Just like exogenous substances are foreign materials to the body, the body also reacts to endogenous tissue that has lost its functional connection with local tissue as foreign to the body (Donath1994). These can have different effects depending on the patient receiving the graft material and whether it is placed on hard or soft tissue (Jensen 2003; Donath 1991). How the tissues and specifically the bone tissues react to the foreign materials is the best indicator of biocompatibility of these materials.

Based on their biocompatibility, graft materials can be classified as biotolerated, bioinert, and bioactive materials (Heimke et al. 1981). Biotolerated materials trigger irritation of the nearby host tissue, the precursor cells then differentiate into osteoblasts and then distant osteogenesis forms a collagen-rich intermediate layer. Bioinert materials do not affect the surrounding tissue by causing a cellular response to the foreign body. Rather, they cause an enzymatic reaction where the implant is camouflaged against the host immune system and the contact osteogenesis remains possible. Bioactive materials cause collagen and hydroxyyapatite to deposit on the implant surface originated from the surrounding bone (osteogenesis bonding).

One limitation with this classification of graft materials is that it ignores the biological effect of the graft material itself in the classification method by Thielemann et al. (1983). These authors claim that osteoconductive grafts such as collagen preparations, macerated bone, porous ceramics, and spongy bone guide the formation of new bone from bone grafts and osteoinductive grafts such as demineralized bone matrix and its more

purified factors. These promote morphogenesis, cytodifferentiation, organogenesis of new bone formation, heterotopically inside the organism (Boyne 1999).

7. CLASSIFICATION OF BONE GRAFTS

The following table is a resume of the different types of bone graft materials and their sources:

Autograft	Intraoral	Mandibular Symphysis
		Mandibular Ramus
		Maxillary Tuberosity
	Extraoral	Calvarium
		Tibia
		Iliac crest
Xenograft	Bovine	ABBM (BioOss)
	Alga	fluorohydroxyapatite (FHA) - Algipore
Allograft	Mineralized	MFDB (mineralized freezed dried bone)
		MCB (mineralized cortical bone)
	Demineralized	DFDB (demineralized freezed dried bone)
Alloplastic	hydroxyapatite (HA)	Porous
		No porous
	Calcium Sulfate	Calcigen - Osteo/Graf
	Tricalcium Phosphate (TCP)	Osteo/Graf
	Bone Ceramic	HA + TCP
Growth Factors	Platelet rich plasma (PRP)	Autogenous bloof
	Bone morphogenetic protein (BMP)	rhBMP-2
	Platelet-derived growth factors (PDGF)	rhPDGF

7.1 Autogenous Bone or Autograft

One type of bone graft is autogenous bone. According to a number of studies, autogenous bone is the "gold standard" of graft material (Esposito et al. 2007; Esposito et al. 2006; Noton et al. 2002). Its characteristics include osteogenic, osteoinductive and osteoconductive properties (Giannoudis et al. 2005). Autogenous bone is harvested either intraorally or extraorally from donor areas from the same patient (Schlegel et al. 2006; Nkenke et al. 2004).

Most intraoral autogenous grafts are obtained from the mental (symphysis) and retromolar areas because there is easy access and no need to administer general anesthesia. In addition there is no visible scar following harversting. The retromolar donor area is considered the safest site (Nkenke et al. 2004) and there are few associated side effects. In contrast, there have been reports of increased sensory disturbances and loss of tooth vitality in the lower incisors and canines when a graft is obtained from the symphysis region (Marchetti et al. 2007; Nkenke et al. 2001). The limitation in both regions is that only limited amount of bone is available.

In cases where larger amounts of autogenous bone are needed, the iliac crest is the best autogenous donor region. However, obtaining grafts from this area often requires administration of anesthesia and is associated with an increased risk of complications such as contour defects, hernias, sacroiliac joint instability, pathological fracture, chronic pain, sensory loss, damage to the ureters, bruising and bleeding (Marchetti et al. 2007; Schlegel et al. 2006; Nkenke et al. 2004). The use of punches in the posterior iliac area is less traumatic and requires only local anesthesia but the amount of bone obtained is again limited.

Patients are often not amenable to use of these area because of a number of factors including postoperative morbidity issues, the surgery takes longer, and it is more costly (Froum et al. 2006). If the patient is not amenable, other types of bone replacement grafts can be offered.

A number of bone substitutes have been studied as alternatives to autogenous bone (Hallman et al. 2002). Because of the morbidity and more rapid autogenous bone

resorption, the results from a number of studies suggest that autogenous bone may not always be the best material (Esposito et al. 2006).

7.2 Allogeneic Bone or Allograft

Allogeneic grafts are obtained from donors of the same species and transferred to a recipient of the same species. Human allogeneic grafts are a viable alternative to autogenous bone because they eliminate the need for a second surgical site and as play osteoconductive characteristics. Human bone allografts have been successfully used for a variety of purposes such as periodontal regeneration (Wang et al. 2005; Becker et al. 1996), socket preservation (Minichetti et al. 2004; Wang et al. 2004; Block et al. 2002) and ridge augmentation around dental implants (Block & Degen 2004; Feuille et al. 2003). Examples of Allograft materials include mineralized cortical bone allograft (MCBA) and demineralized freeze-dried bone allograft (DFDBA). Sources include certified tissue banks (Reynolds et al. 2010). Sterilizing and processing of the different types of allograft materials is accomplished following different methods. Typical steps include cleaning, decontamination, microbiological treatment, freezing, lyophilization, packaging and sterilization to remove contaminants and prevent transmission of diseases and infections (Holtzclaw et al. 2008). Most grafts are composed of blocks or particles of cortical, cancellous, or cortico-cancellous bone and have been used to replace autogenous bone in sinus augmentaion procedures (Froum et al. 2005; Schöpf et al. 2005).

In essence, allograft bone has osteoconductive functions as a matrix and provides a structural framework on which the host cells can differentiate and mature resulting in formation of new bone. DFDBA has been reported to be osteoinductive and may have bone morphogenetic proteins as a component. Some authors do feel that DFDBA has limited osteogenic qualities depending on a variety of factors such as the age of the donor (Blokhuis et al. 2008). Tissue banks are often tested to verify the presence of bone morphogenetic protein in the grafts they provide.

7.3 Heterologous Bone or Xenograft

Characteristics of heterologous bone, most commonly from bovine sources, include carbonate apatite crystals components xenograft (ABBM) and absence of organic components which are removed following a delicate process of extraction. ABBM is a deproteinized anorganic bovine bone which has a 75% to 80% porosity and a crystal size of about 10 nm (Hurzeler et al. 1997). It is commonly presented as cortical, spongy cancellous and cortical blocks (Storgard-Jensen et al. 1996) and is normally available in different sizes (0.25 to 1.0 mm, 0.5 to 0.1 mm, and 1.0 to 2.0 mm). The chemical structure of anorganic bovine bone matrix is similar to the human bone (Berglundh & Lindhe 1997). The internal network of pores provides a favorable area for new bone to penetrate into the graft structure by providing a large surface area for the bone to colonize. The new bone is higher in density compared to other biomaterials and autologous bone (Boyne 1997). There have been reported no cases of local B or T cell inflammatory responses after the use of anorganic bone (McAllister et al. 1998; Hislop et al. 1998; Clergeau et al. 1996). It appears that ABBM provides a more effective hydroxyapatite for replacement by host bone when used for alveolar ridge reconstruction, and remodeling is characterized by the hostbone physiologically incorporated into the graft (Fig. 20-21) (Berglundh & Lindhe 1997; Storgard-Jensen et al. 1996). ABBM has also been found to be more biocompatible with oral hard tissues in both animals and humans while displaying osteoconductive qualities (Berglundh & Lindhe 1997; Wheeler et al. 1996; Denissen et al. 1980).

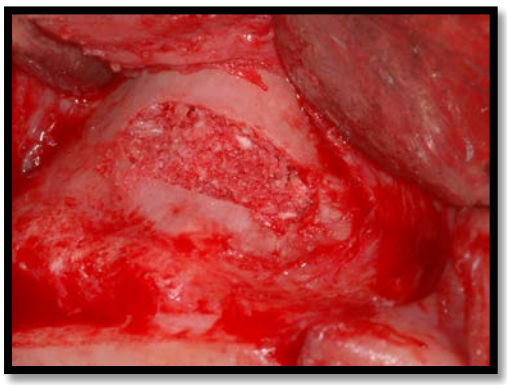


Fig.20 Intraoperative picture of a maxillary sinus filled with ABBM particles.

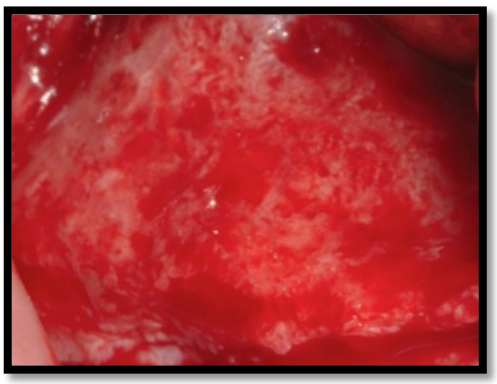


Fig.21 Six months post-operative picture of a sinus filled with ABBM particles.

There are different opinions on how ABBM degrades. Some histology show it being replaced rapidly by host bone and other cases are characterized by only a few resorption lacunae, which is a sign of slow (Berglundh & Lindhe 1997; Storgard-Jensen et al. 1996) or nonexistent resorptive activity (Valentini et al. 1998). This graft material is remodeled in three phases. The first phase involves the integration of the particles with the surrounding bone. In the second phase, resorption by osteoclasts occurs and new bone forms with osteoblasts replacing the particles. This dense lamellar bone forms in the final phase.

Xenografts as an osteoconductive biomaterial have been used in augmentation of bone defects, sinus lifts, and maxillary reconstruction (Simion et al. 1994).

7.4 Alloplastic

Alloplastic materials obtained from hydroxyapatite (HA) and tricalcium phosphate (TCP). These belong to a class of polycrystalline ceramics with a crystalline structure. These crystals are melted at a high temperature. The structure can be highly porous or dense depending on the manufacturing method.

Both HA and TCP are similar in chemical composition and structure to natural mineral bone (Szabó et al. 2001), but have different methods of resorption. Research has shown that after incorporation into dense bone, HA is minimally resorbed, but TCP is resorbed very quickly and resorption is complete in 8 weeks (Jensen et al. 2007). While porous HA is reabsorbed slowly, it is still considered a viable graft option because of its dense form (De Groot 1980; Jarcho 1981). Currently, HA displays in varying degrees of resorption and has different density depending on the diameter of the pores. If the pores are larger than 100 microns, bone ingrowth will occur (Simion et al. 1994; Davis & Martinoff 1984). Complete replacement of this biomaterial can take 6 to 12 months (Denissen et al. 1991; Cranin & Ronen 1980).

7.5 Growth Factors

Growth factors are chemicals produced by cells and are essential in regulating various cellular processes. They provide signals between the cells and trigger different

biological actions. For example, they can stimulate or inhibit cell adhesion, control proliferation, migration and differentiation by regulating protein synthesis, and regulate growth and resorption. Growth factors are essential for tissues to form. They also have a crucial role in tissue engineering. There is a high concentration of growth factors in bone. A number of these (BMPs, the TGF-b, the FGF, VEGF, IGF I and II and PDGF) are the most important in tissue engineering (Jadlowiec et al. 2003).

Advanced recombinant technology has made it possible to synthesize growth factors in a controlled environment making available commercial recombinant growth factors/matrices. Combining biomaterials is a recent trend in regenerative therapy and is becoming popular as a method of optimizing tissue regeneration. These products involve a combination of certain tissues or matrices containing a high level of bioactive proteins, to stimulate or produce progenitor cells to treat tissue deficiencies. Being able to combine highly concentrated signaling proteins with scaffolding has made it possible for scientists to develop improved clinical regenerative products that have the physical and chemical characteristics essential for specific binding, growth and differentiation of cells.

7.5.1 Bone Morphogenetic Protein (BMP)

Bone morphogenetic proteins (BMPs) refer to a group of osteoinductive proteins that have the ability to stimulate existing mesenchymal cells for new bone formation to occur. They are classified as Transforming Growth Facto beta (TGF-b) because their structure is similar. Marshall Urist discovered (Urist 1964) and named (Urist et al.1997) BMPs. This was followed by studies describing their purification and cloning (Wang et al. 1988.1990; Wozney et al. 1998). Currently, there are 15 to 20 known human BMPs and at least six of them (BMP-2, BMP-4, BMP-5, BMP-6, BMP-7 and BMP-9) are known to have osteoinductive qualities (Urist 1964).

Only a very small amount of BMP exists in bone matrix (approximately 1-2µg of total active protein per kg of cortical bone). The amount of BMP-2 varies in different individuals (Blum et al. 2004; Riley et al. 1996). It requires a large amount of bone to obtain a sufficient amount of BMP making it both difficult and expensive. With advanced techniques for

cloning BMP (recombinant human bone morphogenetic protein, rhBMP), production of BMP has become simpler making it available for clinical use. One challenge is that rhBMP has limited application in vivo due to its rapid degradation by proteases. This means that it takes large amounts of rhBMP to stimulate bone formation (Wang et al. 1990). For bone regeneration, rhBMP-2 concentrations of 0.5 to 2.5mg/ml are used.

Using animal models in various preclinal studies, researchers have reported that rhBMP-2 combined with other delivery systems can regenerate critical size cranial bone, long bone and mandibular defects (Smith et al. 1995; Gerhart et al.1991; Toriumi et al.1991). Findings from preclinical studies showed that rhBMP-2 is capable of increasing alveolar bone in dogs and the floor of the maxillary sinus in goats (Nevins et al. 1996; Sigurdsson et al.1995). The use of rhBMP-2 in clinical applications in oral surgery began in 2007. After it was approved by the U.S Food and Drug Administration (FDA), it became available for use in sinus augmentation and localized alveolar ridge deficiencies related to extraction sockets.

To determine the optimal concentration of rhBMP-2 for inducing adequate bone formation, a number of clinical tests were done in multiple centers in randomized controlled studies (Boyne et al. 2005). One study (Triplett 2008) involved 160 patients and demonstrated positive results with the use of rhBMP-2 for maxillary sinus augmentation. One thing that was clear from these studies is that a sufficient volume of material was necessary during grafting,and a high elevation of the sinus membrane is essential when placing implants with rhBMP-2. The findings also showed that the graft shrinks to a large extent and the bone obtained has low density. This is in addition to the fact that the material necessary is costly for sinus floor elevation.

Fig.22 Mix of rhBMP-2/ACS and ABBM.

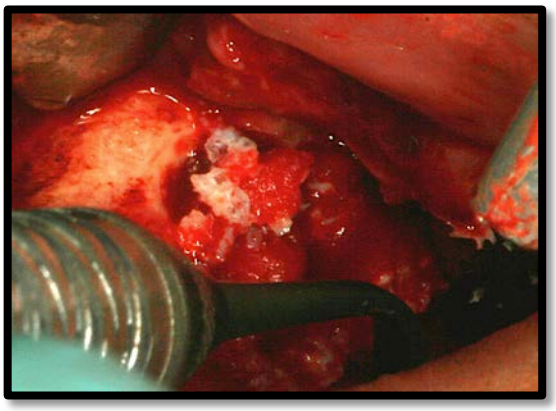


Fig.23 Sinus lift augmentation using rhBMP-2/ACS and ABBM.

Currently, the typical vehicle materials used to carry rhBMP-2 include collagen, tricalcium phosphate, demineralized bone matrix, hydrogels and synthetic polymers. However, these materials present a number of limitations including biodegradability issues and limitation in their ability to support a continuous release of BMP into the area of interest (Haidar et al. 2009a, b). The absorbable collagen sponge (ACS) lasts only three to six weeks and this has a negative impact on the release of BMP-2 at the time of degradation (Fig. 22-24). In the ACS carrier, rhBMP-2 is released in about eight days in in-vivo models. The molecule cannot be detected at the fourth week of implantation (Valdes et al. 2009). Following bone surgery, the initial step in the healing process is characterized by an inflammatory response similar to resorption and lasts for a period of three to four weeks.

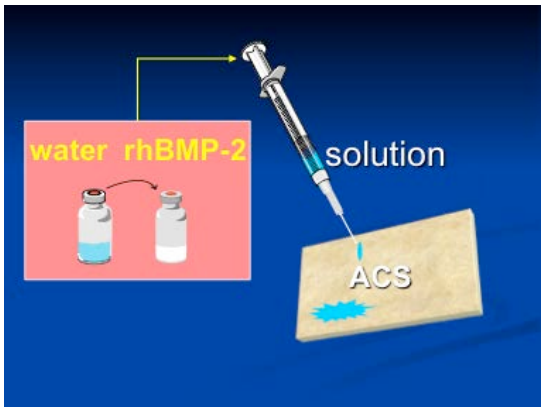


Fig.24 The protein solution is soaked into the sponge, which is designed to resorb (disappear) over time. The sponges keep the solution from migration away from the bone binding the BPM-2 at the site and acting as a scaffold for the formation of the new bone that the protein stimulates.

The pharmacokinetics of BMP-2 and the average retention time of BMP-2 vary depending on the process followed in obtaining the collagen sponge and the crosslinking method used (Geiger et al. 2003; Uludag et al. 2001). Only recently have the efforts to improve the design of the carrier and prolong the release of BMP been sucessfully (Haidar et al. 2009 a, b).

7.5.2 Platelet-Derived Growth Factor (PDGF)

Platelet-derived growth factor (PDGF) used as a molecular mediator promotes the regeneration of periodontal tissue such as bone, cementum and periodontal ligament (Lynch et al. 1989). This mediator was first discovered in the late 1980s by Lynch and colleagues in an animal study. More studies have been conducted and findings published in an attempt to offer a better understanding of the mechanisms involved and the therapeutic potential of this growth factor.

PDGF is a natural protein manufactured by platelets, monocytes, macrophages, endothelial cells and osteoblasts (Andrew et al. 1995) and its structure consists of four different polypeptide chains (A, B, C, D) combined together.

In case of an injury of hard or soft tissue, platelets naturally release PDGF in the blood clotting process. (Pierce et al. 1991). PDGF occurs in abundance in the bone matrix in at least three combinations: PDGF-AA, PDGF-AB and PDGF-BB (Alvarez et al. 2006). PDGF-BB is known as the most biologically potent because of its ability to bind due to a higher affinity with osteoblasts (Centrella et al 1991. Zhang et al 1991).

PDGF-BB appears to have two different mechanisms that affect bone regeneration. It may trigger a direct mitogenic effect on osteoblasts and osteoclasts or induce inflammatory cells such as macrophages to release growth factors that cause PDGF to bind to specific cell surface receptors, induce rapid migration (chemotaxis) and proliferation (mitogenesis) of cells in the injured area Ronnstrand & Heldin (2001) promoting healing. In both in vitro and in vivo processes, PDGF has proven to be a potent chemotactic and

mitogenic factor for the periodontal ligament and gingival fibroblasts, osteoblasts and cementoblasts (Lin et al. 2008; Lynch et al. 2006; Centrella et al. 1992; Lynch et al. 1989).

Although growth factor proteins have proven to be potent promoters of repair, the use of concentrated forms of these proteins only began in 1998 when Marx and colleagues suggested the use of autologous platelet concentrates (Marx et al. 1998). While platelet concentrates individually have good handling characteristic combined with other matrices, and this method still has major limitations as shown by recent research. Disadvantages include the need to draw blood from the patient and umpredictable response after treatment (Nikolidakis & Jansen 2008). The first recombinant protein to gain the approval of the FDA in the USA for use in treating ulcers in patients with chronic diabetes (Regranex, Ethicon, Inc., Somerville, NJ) was human platelet-derived growth factor (rh-PDGF) (Wieman et al. 1998; Steed et al. 1996).

The level of growth factor in the recombinant product could be as much as 3000 times of that of the whole blood. After extensive use of this application, the safety and efficacy of PDGF in tissue generation has been established (Margolis et al. 2005). In addition, the use of rhPDGF for bone regeneration has been tested and found to induce and control bone regeneration in humans (Margolis et al. 2005, Nash et al. 1994; Joyce et al. 1991).

The principles of tissue engineering have also made it possible to use enhanced growth factors made up of rhPDGF-BB combined with osteoconductive scaffolds (ie, autograft, allograft, xenograft or an array of synthetic materials, such as beta-TCP) to promote periodontal regeneration (Stephan et al. 2000). Recently, a commercial product containing PDGF-BB and b-TCP (GEM 21S ® Osteohealth, Luitpold Pharmaceutical Inc Shirley, NY, USA) was established as a safe and effective material when used clinically for periodontal regeneration (Nevins et al. 2005, 2003). All this was possible because PFGF induces angiogenesis, encourages the migration of cells into the bone defect margins of the surrounding tissue and has a positive effect on cell proliferation (Hollinger et al. 2008). Apart from acting as a delivery systeml for growth factors, the matrix also offers mechanical support essential for cells to migrate and plays a significant role in the formation of new bone, cementum and/or periodontal ligament.

Various human studies have been conducted on the use of rhPDGF for dental implant site development (i.e., sinus elevation) (Nevins et al. 2009), horizontal bone augmentation (Simion et al. 2007), and ridge preservation (Nevins et al. 2009). These clinical studies have mainly investigated the use of rhPDGF for periodontal and bone regeneration (McAllister et al. 2010). After three evidence based reviews failed to provide evidence to support the claimed improved outcomes in maxillary sinus elevation (Esposito et al. 2006; Boyapati & Wang 2006; Sanchez et al. 2003), the use of rhPDGF is currently considered an off-label use for sinus lift procedures.

8. CLASSIFICATIONS OF BIO-MEMBRANES

In theory, placing a membrane over the sinus graft osteotomy site to act as a barrier has both negative and positive effects. The positive aspect includes potential GBR effect resulting from excluding non-osteogenic flap connective tissue cells from wound healing; the particulate graft material remain contained; prevention of soft tissue encleftation; and a rise in bone formation leading to a higher rate of implant survival rate. On the other hand, the negative effects of membrane placement include potential reduction of vascular supply to the graft by excluding the buccal flap; more extensive flap reflection when placing and removing a membrane; and higher costs (Tarnow et al. 2000).

A variety of materials have been used in both experimental and clinical studies in GTR/GBR procedures to determine their effectiveness as tissue barrier materials. These materials include polytetrafluoroethylene (PTFE), expanded PTFE (e-PTFE), polyglactin 910, polylactic acid, polyglycolic acid, polyorthoester, polyurethane, polyhydroxybutyrate, calcium sulfate, freeze-dried fascia lata, freeze-dried dura mater allografts, native and/or synthetic collagen, micro titanium mesh, and titanium foils (Lungren et al. 1994; Gottlow 1993; Davarpanah et al. 1991; Fleisher et al. 1988).

There are two major classes of tissue barriers. The resorbable classes include (collagen membrane, polylactic acid, cargile membrane, polyglycolide, Vicryl, freeze-dried dura mate) (Gottlow 1993). The non-resorbable class includes (PTFE, e-PTFE, titanium mesh, d-PTFE) and collagen membrane are the most commonly used in sinus procedures (Wallace

et al. 2005; Froum et al. 2002; Tarnow et al. 2000; Froum et al. 1998).

8.1 Non-Resorbable Membranes

Non-resorbable barriers consist of thin sheets of materials, mainly polymers. Their characteristics include stability, non-degradability and biocompatibility. The earliest commercial and most popular non-resorbable membranes to be used were expanded polytetrafluoroethylene (ePTFE) membranes, which became a standard for bone regeneration shortly after GBR became an approved dental therapy (Simion et al.1994; Hämmerle et al. 1998).

8.1.1 Polytetrafluoroethylene (PTFE) Membranes

Expanded polytetrafluoroethylene membranes (e-PTFE) were considered a standard for bone regeneration barriers because of their early and successful application (Dahlin et al. 1991a, b; Davarpanah et al. 1991). PTFE refers to a polymer characterized by high stability in biologic systems in that it can resist breakdown by host issues and microbes and does not cause immunologic reactions. Using e-PTFE for bone regeneration produces very predictable results. However, this membrane requires a second surgery for removed and bacterial contamination can occur if the membrane is exposed. Due to inflammation of the surrounding tissues, it is important to remove the membrane early if there is exposure or contamination. Some studies have also investigated the use of biodegradable materials that can overcome these limitations (Simion et al. 1998; Machtei 2001).

8.2 Bioresorbable Membranes

Compared to non-resorbable materials, bioresorbable membranes are preferable and have many advantages. The main advantage is that there is no need for a second surgery to remove the membrane. Tissue healing is also better with these materials (Lekovic et al. 1998, 1997; Zitzmann et al. 1997). Furthermore, the membranes are incorporated by

the host issues and rapidly resorbed. Even when there is exposure, there are no microstructures to encourage bacterial contamination (Zitzmann et al. 1997). Bioresorbable materials used to make resorbable membranes are classified as natural or synthetic polymers. The most commonly used polymers in the medical field are aliphatic polyesters and collagen (from bovine or porcine). Currently membranes made of polyglycolide, polylactide or copolymers thereof or of collagen are used (Moses et al. 2005; Rothamel et al. 2005; Friedmann et al. 2002, von Arx et al. 2001; Hutmacher & Hürzeler 1995; Tal et al. 1996, 1991).

Several controlled studies which compared bioresorbable and nonresorbable membranes (Zitzmann et al. 1997, 2001; Christensen et al. 2003) reported no significant differences between these two membranes. However, bioresorbable membranes are considered better alternatives to non-resorbable e-PTFE membranes and are now the standard in most clinical situations. Studies and clinical applications have demonstrated that when bioresorbable membranes are used, the risk of complications is reduced (Wallace et al. 2005).

8.2.1 Collagen Membranes

Collagen belongs to a family of proteins characterized by sophisticated triple helical structure. Collagen has desirable qualities including biocompatibility, biodegradability and low immunogenicity. These make it a popular choice in pharmaceutical or biotechnological disciplines (Schlegel et al. 1997; Cooperman and Michaeli 1984). In several experimental and clinical studies aimed at investigating the suitability of different materials as regenerative tissue barriers, collagen was found to be an optimal choice and demonstrated the requirements of bioabsorbable materials. Of all these proteins, Collagen Type I is the most abundant. It constitutes about 25% of the body's proteins and accounts for about 80% of the connective tissue proteins. Collagen Type I polymerizes into aggregates of fibers and bundles. All collagens in the body undergo similar remodeling through degradation and synthesis. Type I collagen can only be degraded by collagenase, as it resists non-specific proteolytic degradation.

The building blocks of collagen membranes are porcine/bovine collagen fibers type I and III, which possess a double layer structure consists of a compact l and a porous layer. The compact layer with its smooth surface protects condensed connective tissue infiltration, while the porous layer provides the best passage for cellular invasion (Fig. 25). Applied in bone regeneration, the porous layer allows migration of osteogenic cells while the compact layer blocks connective tissue infiltration (Locci et al. 1997; Yaffe et al. 1984; Postlethwaite et al. 1978).

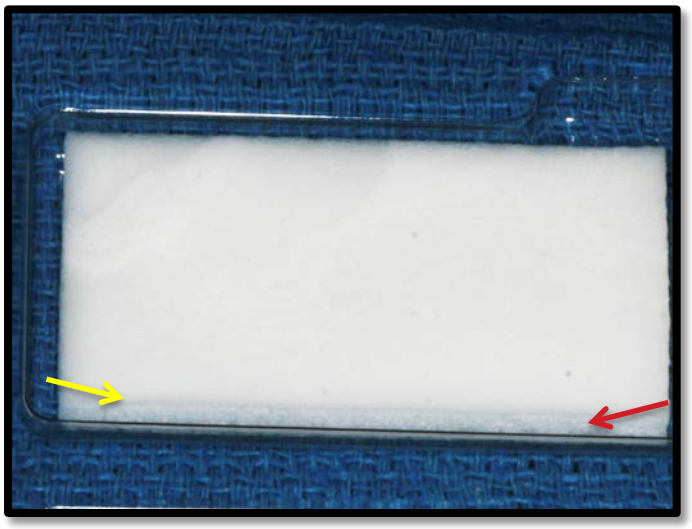


Fig.25 Collagen Membrane. Note double layer structure of a compact (yellow arrow) and a porous layer (red arrow).

Findings from animal studies show that mesenchymal cells can differentiate into osteogenic cells in the presence of certain conditions. Without the use of bone graft materials, collagen fibers applied in bone regeneration may act as a stimulus to osteogenic cells in bone defects while providing a barrier blocking the infiltration of connective tissue. Collagen fibers are the most extensive components of bone matrix. They can serve as a reservoir for many local factors in the cellular matrix of osteogenic cells (Gottlow et al. 1994).

Use of collagen membranes in treating intrabony defects produced similar results compared to those obtained with the use of e-PTFE membrane. Collagen reduced epithelial migration by up to 50% (Sandenberg et al. 1993).

9. SUCCESS OF BONE GRAFTS IN MAXILLARY SINUS AUGMENTATION

While early findings on long-term bone biomaterials are available at 9 (Traini et al. 2007) and 11 years (Mordenfeld et al. 2010), there is still no explanation on the effect of bone substituteS on vital bone formation. In a 2011 study by Chackartchi et al. (2011), there was a notable difference between small and large particle grafts. Testori et al. (2012) found a statistically significant difference. After healing for 6 months, vital bone formation was 26.8% when large particles were used and 18.8% when small particles were used.

9.1 Factors Affecting the Success of Bone Grafting in the Sinus

Regardless of whether bone or bone substitute biomaterials are used, the success of a sinus grafting procedures is determined by the following factors related to the host site (Boyne 1999):

1. The proliferative capacity the host site has for new bone formation. This could be low, high or nonexistent.
2. The degree of vitality and capacity for revascularizing of the grafting material.
3. Stability of the grafting materials when placed in the sinus.
4. The amount of bone morphogenetic proteins concentrated on the surface of the host bone.
5. The metabolic activity of the host organism.
6. The size and volume of the defect to be treated.

Considering the above factors, the maxillary sinus is an ideal model for testing outcomes with various techniques and materials. Drowbacks of this model include the fact that bone and connective tissue portions of the antral mucosa (mucoperiosteum) surround the bone or bone substitute materials placed in the subantral space. New bone formation can also be limited by micro movements of the antral membrane during respirations, which prevent the formation of new bone causing sheathing of the connective tissue (pseudoarthrosis) (Boyne 1999).

Elevation of the Schneiderian membrane and formation of a lateral bone window causes surgical related trauma, which may jeopardize the blood supply to the local endosseous bone. This is worse in cases of severely atrophic maxillas and should not be underrated (Solar et al. 1999).

9.2 Factors for Bone Substitute Biomaterial

Histologically, the success of bone substitute biomaterials is linked to three factors (Wallace et al.2012):

I. Osteoconductive properties (about 25% of vital bone volume formed in 6-8 months).

II. Slow resorption (25% of new vital bone + 25% nonvital residual graft material).

III. The residual graft material does not come into direct contact with the implant surface so there is no effect on osseointegration.

9.3 Histological evaluation

Full-thickness bone core biopsies of the study area are usually harvested when testing for histologic and histomorphometric analysis. There are two different ways of collecting biopsies. One is to use a trephine of varying diameters drilling from the alveolar ridge toward the maxillary sinus in the intended dental implant position. The other method is to obtain the biopsy from the positioning the previous lateral wall osteotomy where the graft material was placed (Fig. 26-27) (Avila et al. 2010; Froum et al. 2008).

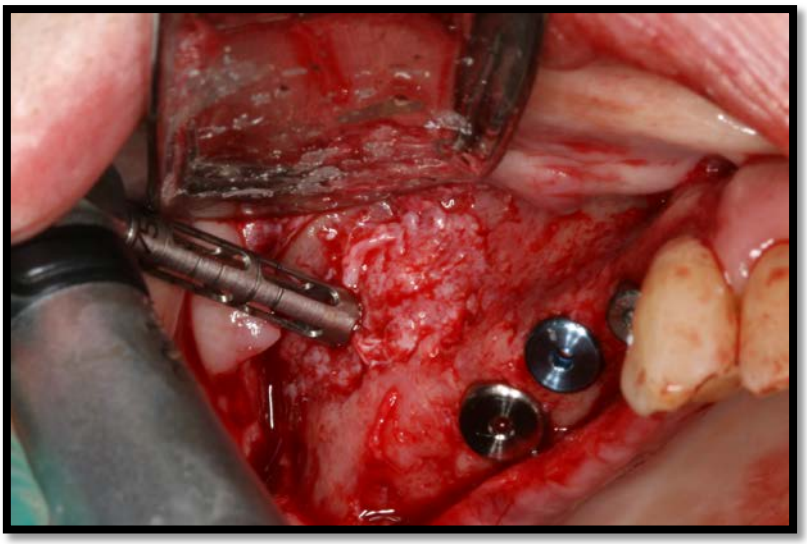


Fig.26 Trephine is place in the previous lateral wall osteotomy to obtain bone biopsy.

Fig.27 Full thickness bone core was obtained from the maxillary sinus augmentation.

Some of the stains that have been used include toluidine blue, hematoxylin eosin and blue, Gomori one-step trichromes´ stein (Froum et al. 1998), toluidine blue and pyronine (Yildirim et al. 2000), and Mayer's hematoxylin and eosin (Boyne et al. 2005). The specimens taken from sinuses augmented with various bone graft materials were used in histologic and histomorphometric studies. Each of the biopsy specimens were decalcified and evaluated for the presence of vital cortical and/or trabecular bone, thickness of the

osseous trabeculae, and the presence of lamellar bone, woven bone, and remaining bone substitutes. These were examined by an oral pathologist using computerized image analysis.

Traditionally, the healing time to obtain results using various graft materials applied in sinus augmentation procedures is 7-9 months Handschel et al. (2009).

9.3.1 Autogenous

The use of autogenous bone graft as a graft material can reduce the healing time as showed by several histological and clinical cases (Froum et al 1998; Valentini et al. 2000). A sinus augmentation procedure using autogenous bone, the original bone could not be differentiated from the grafted bone (Wiltfang 2003). A combination of autogenous bone with BMP, HA, or ABBM, produced more pronounced bone formation (Schlegel et al. 2003; Hürzeler et al. 1997; Haas et al. 1998a, b; Roldan et al. 2004). A 47% increase in bone formation was noted weeks after graft placement (Roldan et al. 2004). After 26 weeks healing implants placed into this grafted bone produced 30-36% greater bone-implant contact (BIC) compared to the control group (without grafting) where the BIC was 20-25% (Haas et al. 1998; Haas et al. 1998; Roldan et al. 2004). The rapid and unpredictable resorption of autogenous bone grafts may produce umpredictable outcomes in the long term, particularly in bone regeneration of considerable size (Davis et al. 1984). Bone resorption (up to 50%) was observed in sinus augmentation procedures performed in both beagle (Schlegel et al 2003) and human studies (Sbordone et al 2009; Browaeys et al 2007).

9.3.2 Allografts

Resorption of DBM graft materials and formation of new bones was demonstrated histologically, with a direct deposition on the surface of bone graft particles (Froum et al. 2005). New bone occupied the spaces between the particles of the graft and the majority was buried in new bone. Osteoid occurred in some of the cases, which is a sign of active bone formation within the graft material. The osteocytes and new connective tissue formed around bone marrow cavities. The bone marrow cavities had a high amount of new

connective tissue and blood vessels and osteoclasts were observed close to the graft material. There were no cases of inflammatory cell infiltration although invasion of irregular particles of new bone occured in some cases (Won et al. 2011).

The histological appearance observed with DFDB is similar to a chronic inflammatory process around the margins of the adjacent bone (Haas et al. 2002a). However, this inflammatory process does not affect the biomechanical implant stability that is comparable to the stability achieved in bone formed when autogenous iliac crest is used (Haas et al. 2002b).

9.3.3 Xenografts

Residual particles of xenograft when partly surrounded by new vital bone, present a histological pattern called "bone bridging" (Wallace et al. 2012) (Fig. 28-29). ABBM particles behave like osteoconductive resorbable material, as close contact was observed between the particles and the newly formed bone and whitout gaps at the interface (Storgard-Jensen et al 1996; Hürzeler et al 1997; Valentini et al.1998). The ABBM's porous grid, size and structure favor the growth of internal bone (Storgard-Jensen et al 1996; Clergeau et al. 1996). The possibility of osteoclasts to resorb ABBM particles was shown (Storgard-Jensen et al 1996; Hammerle et al. 1998; Klinge et al. 1992). However, other studies did not show any signs of resorption of the graft particles (Valentini et al. 1998; Clergeau et al. 1996). In one human study, no bovine inorganic matrix was observed after 20 months (Wallace et al. 1996).

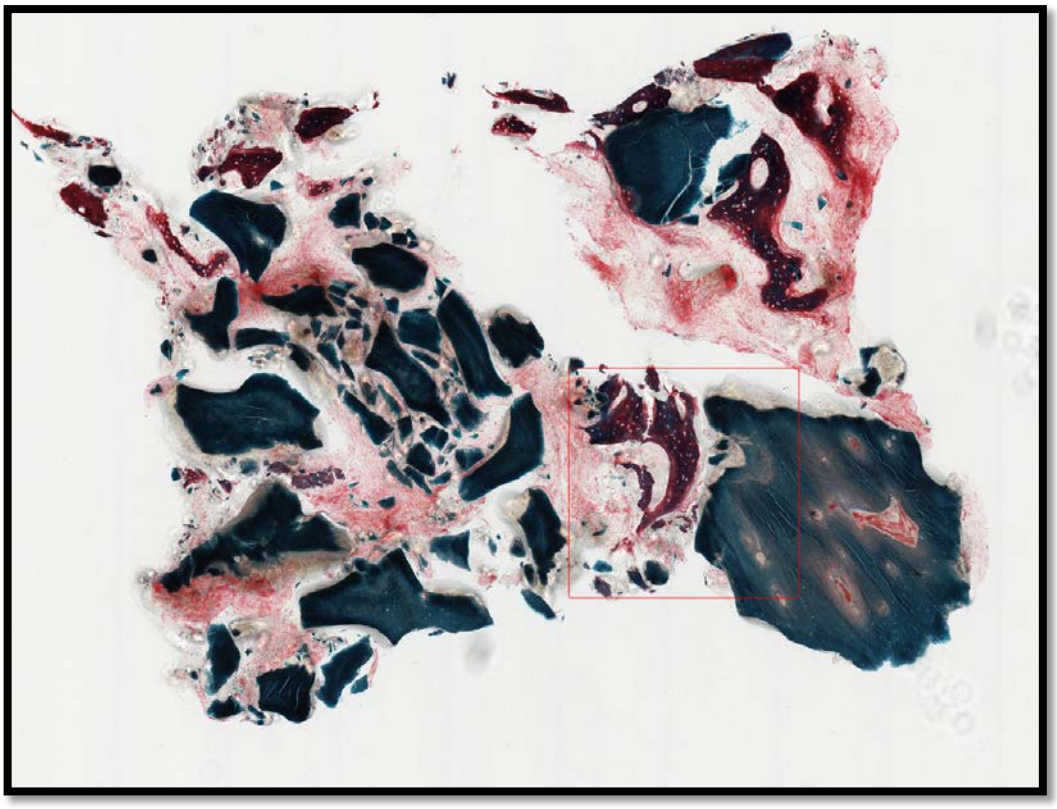


Fig.28 Cross section of a core sample from a site with ABBM alone showing bone formation of varying maturity with 15.78% vital bone. Gomori Trichrome Stain (20x0.15).

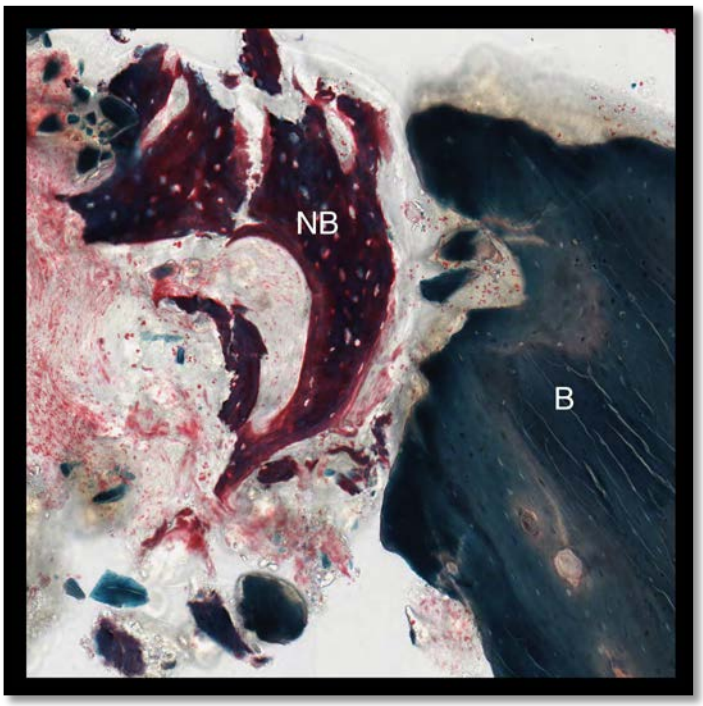


Fig.29 High-power imageof core in fig.28 showing immature newly formed bone (NB) around particles (B) of MCBA. Vital bone formation is apparent between the residual MCBA particles. Gomori Trichrome Stainx (x20).

One study demonstrated an average vital bone volume of 24% 6 to 9 months post surgery, both with and without autogenous bone, compared to 33% vital bone volume between 12 and 15 months (Froum et al. 1998). Another study of sinuses grafted with 100% ABBM resulted into a 21.08% vital bone at 6 moths and 27.55% at 12 months (Valentini et al. 2000). A recent study confirmed there is a relationship between the mean vital bone volume and healing duration (Lee et al. 2006).

9.3.4 Growth Factors

Over the last 10 years, biomimetic stem cell technology have been applied in clinical practice to produce better results or achieve results that resemble those achieved by autogenous bone grafts without having to use bone substitues. The evaluation of bone morphogenetic proteins (BMP) has been ongoing for approximately 40 years (Urist 1964, 1997; Boyne et al. 2005).

Efforts to overcome the limitations of graft shrinkage and low density by combining mineralized bone replacement grafts with collagen sponges have largely being unsuccessful, as studies demonstrate a rapid resorption of BMP-2 (Fig. 29-30) (Tarnow et al. 2010). In a recent study comparing bilateral elevations (Kao et al. 2012), the results showed less favorable results when rhBMP-2/ACS was added to xenografts compared to xenografts used alone.

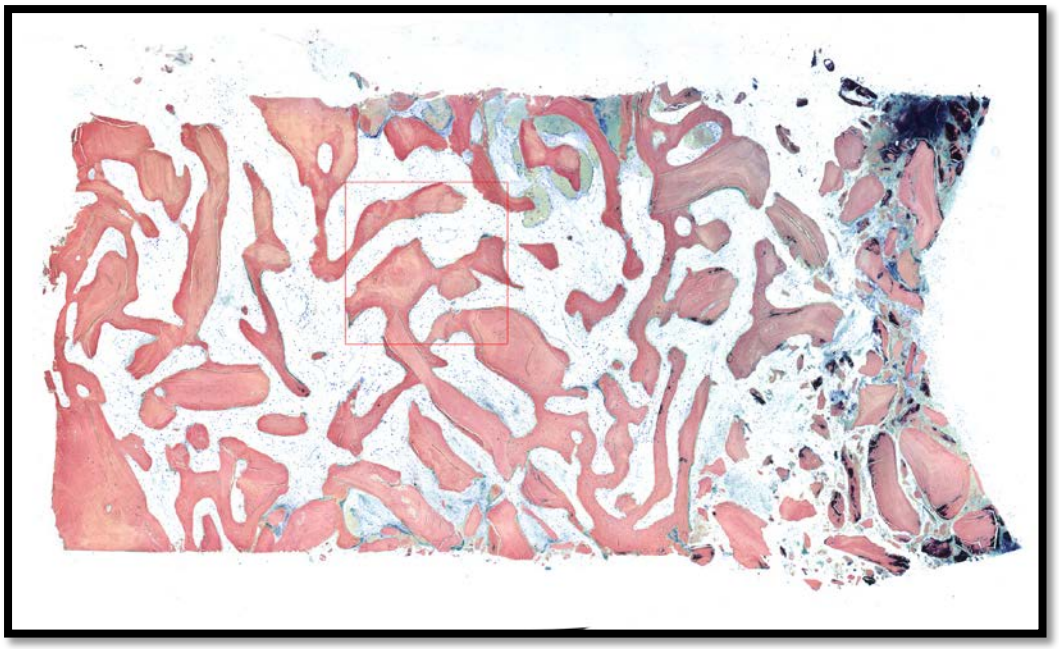


Fig.29 Histomorphometric analysis of MCBA + rhBMP-2/ACS core composed of 24.44% vital bone and 25.71% of residual bone at 7 months. Stevenel blue and van Gieson stain (20x0.15).

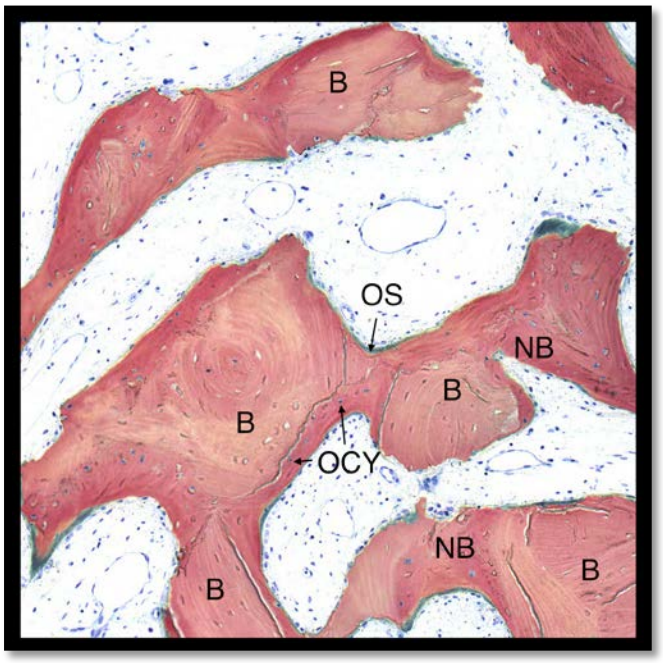


Fig.30 High-power image showing immature, newly formed bone (NB) around particles of MCBA (B), and osteoid (OS). Note the osteocytes (OCY) around the MCBA (B). Stevenel's blue, van Giesson's picro fuschin (x20).

Platelet rich plasma (PRP), formed by centrifuging freshly drawn venous blood from a patient, has shown to be an autologous source of multiple growth factors. PDGF-ββ is one of the main growth factors when using PRP. Results obtained from several studies demonstrated significant improvements in soft tissue healing and formation of more vital bone when PDGF was used as a graft material (Fig. 31-32). A human study showed vital

bone formation in maxillary sinuses using a combination of PDGF-ββ and xenograft (Nevins et al. 2009). One study involving fresh-frozen allograft (bone matrix multipotential cell) enhanced with stem cells (Nuvasive, San Diego, CA) used in sinus lift surgery (Gonshor et al. 2011) resulted into 32.5% vital bone within 3 to 4 months versus 18.3% when compared with a conventional allograft (DFDBA).

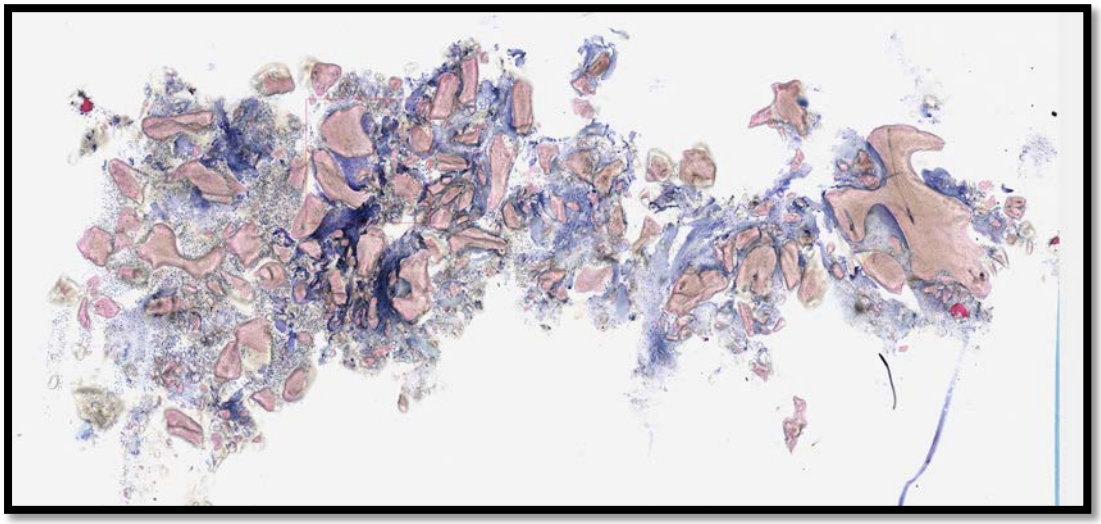


Fig.31 Histomorphometric analysis of core sample from a site with ABBM + rhPDFG- showing 16.43% vital bone and 34.67% of residual ABBM, in which the ABBM particles are generally incorporated into the newly formed bone (NB). Stevenel blue and van Gieson stain (20x0.15).

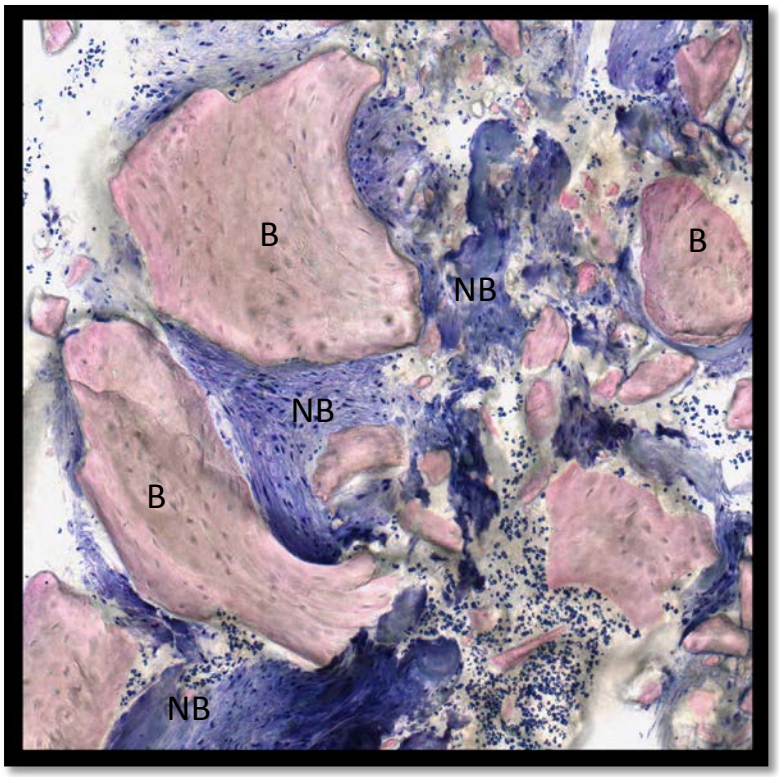


Fig.32 High power view of vital bone formation (NB) directly on the residual MCBA particles (B). Stevenel's blue, van Gieson's picro fuschin (x20).

9.4 The Survival Rate of Implants in Augmented Sinus

Wallace & Froum (2003) published a systematic review of the literature on the effect of maxillary sinus augmentation and dental implant survival in human studies with a minimum of 20 interventions and a minimum of one year of functional loading follow up. Here is a summary of the findings from this review:

1. For implants placed simustaneously in conjunction with the lateral approachsinus lift, the survival rate of implants ranged between 61.7% and 100% with an average survival rate of 91.8%.
2. Implants survival compared favorably with the survival rates of implants placed in the posterior native bone.
3. Rough surface implants demonstrated a higher rate than machined surface implants.
4. The survival rate of implants increased when barrier membranes were used to cover the lateral window.
5. Implants placed in sinus as graftedwith autograft particulate grafts demonstrated better survival rates than the ones placed in sinuses augmented with block grafts.
6. The survival of the implant increased when bone substitutes were used compared to autogenous bone grafts.

One shortcoming of this review is that it does not explain whether the residual native bone height apical to the sinus elevation influenced the success rate of the implants. A subsequent systematic review was conducted to study the survival rates of grafts and implants placed in sinus augmented sites, with an average height of residual bone of 6 mm or less, and the prevalence of surgical complications occurrance (Pjetursson et al. 2008). The main results of this study were:

1. At the implant level, there was an approximate annual failure rate of 3.5% and a 90.1% survival rate at three years based at the implant level. Based on subject level, the annual failure rate was 6.04% and there was loss of implants in 16.6% of the subjects over three years.
2. Machined surface implants showed a failure rate of 6.9% which was significantly higher ($p < 0.0001$) compared to a 1.2% rate with rough surface implants.

3. The failure rate was greater in the absence of the use of a membrane to cover the lateral window following grafting (4.0%) compared to 0.7% (p = 0.001) when a membrane was used.
4. With rough surface implants, the rate at 3 years was between 96.3% and 99.8% depending on the graft material used.
5. Rough surface implants demonstrated the lowest annual failure rate (0.1%) when particulate autologous bone graft was used.
6. Whether using bone substitutes or combinations of autograft bone and bone substitutes, the annual failure rate with rough surface implants was constant at 1.1%.

According to a review by Handschel et al. (2009), autogenous bone was more effective than bone substitutes in the short term but after 9 months both types of grafting material produced similar results. These findings were in agreement with those of Tong et al. (1998) who conducted a meta analysis for implants placed in grafted maxillary sinuses and found similar results whether autografts, allografts or alloplastic were used.

Esposito et al. (2010) published a review article that recommended that bone substitutes could be used as alternatives to autogenous bone for sinus grafting because bone substitutes such as Bio-Oss (ABBM) and Cerasorb (TCP) demonstrated a similar effectiveness to that of autogenous bone grafts used for augmenting atrophic maxillary sinuses. These studies did not find any evidence that the addition of platelet-rich plasma (PRP) to autogenous bone grafts or bone substitutes produced improved results in sinus lift procedures prior to implant placement.

Drilling of the sinus membrane was the most surgical common complication and occurred in 19.5% of the procedures. Graft infection following surgery occurred in 2.9% of the cases. In 1.9% of cases, graft loss prevented the placement of the implant.

III. OBJECTIVES

III. OBJECTIVES

The overall aim of the present thesis was to evaluate bone regeneration following sinus lift procedures using tissue engineered, such as platelet derived growth factor, bone morphogenetic protein and acellular collagen membranes.

Specific aims

• _To prospectively evaluate histological and histomorphometrically new bone following maxillary sinus augmentation in patients undergoing this procedure with the use of xenograft and recombinant human platelet derived growth factor (rhPDGF). (*Study I*).

• _To prospectively evaluate histological and histomorphometrically the new bone following maxillary sinus floor augmentation in patients undergoing this procedure with the use of allograft and two different doses of recombinant human bone morphogenetic protein type 2 (rhBMP-2). (*Study II*).

• _To retrospectively evaluate the amount of bone regeneration following repair of sinus membrane perforations using collagen membranes in patients undergoing maxillary sinus lift procedures. (*Study III*).

IV. LIST OF PUBLISHED SCIENTIFIC ARTICLES

IV. LIST OF PUBLISHED SCIENTIFIC ARTICLES

This thesis is based on the following studies:

I. A HISTOMORPHOMETRIC COMPARISON OF BIO-OSS ALONE VERSUS BIO-OSS AND PLATELET-DERIVED GROWTH FACTOR FOR SINUS AUGMENTATION: A POSTSURGICAL ASSESSMENT.

Authors: Froum SJ, Wallace S, Cho SC, Rosenburg E, Froum S, Schoor R, Mascarenhas P, Tarnow DP, Corby P, Elian N, Fickl S, Ricci J, Hu B, Bromage T, Khouly I.

Journal: International Journal of Periodontics and Restorative Dentistry. 2013 May-Jun;33(3):269-79.

Impact Factor: 1.197

II. HISTOMORPHOMETRIC COMPARISON OF DIFFERENT CONCENTRATIONS OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN WITH ALLOGENIC BONE COMPARED TO THE USE OF 100% MINERALIZED CANCELLOUS BONE ALLOGRAFT IN MAXILLARY SINUS GRAFTING.

Authors: Froum SJ, Cho SC, Wallace S, Khouly I, Rosenberg E, Corby P, Froum S, Bromage T, Schoor R, Norman R, Tarnow D.

Journal: International Journal of Periodontics and Restorative Dentistry. (Accepted for publication in November 2013). 10.11607/prd.1736

Impact Factor: 1.197

III. EFFECT OF MAXILLARY SINUS MEMBRANE PERFORATION ON VITAL BONE FORMATION AND IMPLANT SURVIVAL: A RETROSPECTIVE STUDY.

Authors: Froum S, Khouly I, Favero G, Cho SC.

Journal: Journal of Periodontology. 2013 Aug;84(8):1094-9.

Impact Factor: 2,602

V. SCIENTIFIC ARTICLES

V. SCIENTIFIC ARTICLES

In this section a summary of the scientific articles mentioned in the previous section has been made.

1. A HISTOMORPHOMETRIC COMPARISON OF BIO-OSS ALONE VERSUS BIO-OSS AND PLATELET-DERIVED GROWTH FACTOR FOR SINUS AUGMENTATION: A POSTSURGICAL ASSESSMENT.

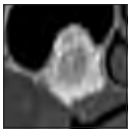
The purpose of this study was to assess vital bone formation at 4 to 5 months and 7 to 9 months following sinus augmentation with anorganic bovine bone matrix (ABBM) with and without recombinant human platelet-derived growth factor (rhPDGF).

METHODS:

Twenty-four subjects received bilateral sinus elevation surgery with ABBM on one side and ABBM and rhPDGF on the contralateral side. Twelve patients had core sampling at 4 to 5 months and 12 patients at 7 to 9 months postoperatively. In subjects with cores taken at 4 to 5 months, mean vital bone, connective tissue, and residual graft were 11.8%, 54.1%, and 33.6%, respectively, with ABBM alone. Cores of sinuses filled with ABBM and rhPDGF showed mean 21.1% vital bone, 51.4% connective tissue, and 24.8% residual graft. Paired t test showed a statistically significant difference in vital bone.

RESULTS:

In cores taken at 7 to 9 months, the values for ABBM alone and ABBM + rhPDGF were 21.4% vs 19.5% vital bone, 28.4% vs 44.2% connective tissue, and 40.3% residual graft vs 35.5%. There was no statistically significant difference in vital bone at 7 to 9 months after surgery. Test and control groups showed clinically acceptable levels of vital bone both at 4 to 5 months and 7 to 9 months postsurgery. However, vital bone formation was significantly greater in the 4- to 5-month sections of ABBM + rhPDGF vs the Bio-Oss alone. In the 7- to 9-month specimens, this difference disappeared.



time and implant survival rates that are equal to or better than those achieved with autogenous bone.

Anorganic bovine bone matrix (ABBM) is a bone substitute manufactured from bovine bone mineral that is processed and sterilized for use in intraoral grafting procedures. It is composed of only the mineral portion of extremity bone. This material, alone or in combination with autogenous bone, enjoys widespread use as the graft material of choice for many practitioners performing sinus augmentations procedures. In fact, 10 published evidence-based systematic reviews concluded that the results with xenografts are the most favorable, complete, and well-documented in the published literature.[1–10]

The safety standard of ABBM derives from the fact that sources include only extremity cow bone from Australia, where bovine spongiform encephalopathy (BSE) has not been detected, that has undergone chemical processing in strong alkaline solutions and subsequently subjected to heat. Examination of ABBM for protein residues using validated analyses with regard to BSE is performed on each batch. Proof of deorganification is obtained through Bio-Rad assay, SDS-Page testing, and SDS – Page + Western blotting.[20,21]

Platelet-derived growth factor (PDGF) is a wound-healing hormone that is naturally produced by the body at sites of soft tissue and bone injury. It is a well-characterized tissue growth factor long recognized for its broad wound healing effects in both soft and hard tissues. This growth factor, along with insulin-like growth factor-1, has been shown to be safe and effective in a series of well-controlled human clinical trials as well as in patient use for nearly 10 years.[22–30]

In periodontics, numerous studies in humans have demonstrated the effectiveness of PDGF in regenerating bone, ligament, and cementum.[22,23] Recombinant human platelet-derived growth factor BB (rhPDGF-BB) (Osteohealth, Luitpold Pharmaceuticals) was the first recombinant protein therapeutic approved for treatment of periodontal defects. It has been shown that the use of purified rhPDGF-BB mixed with bone allograft resulted in periodontal regeneration in both Class II furcations and interproximal intrabony defects.[22] Subsequently, it was shown that moderate to severe periodontal intrabony defects treated with 0.3 mg/mL rhPDGF-BB + β-tricalcium phosphate (β-TCP) had significantly greater clinical attachment level (CAL) gains and less gingival recession at 3 months and significantly greater radiographic linear bone growth and percent bone fill at 6 months compared to sites treated with the control (β-TCP + buffer).[23]

The recombinant PDGF-BB used in this study was of human origin. Tissue engineering allowed for the amplification of this human-derived protein.

At the time of the present study, PDGF-bb in sinus grafting was considered to be off-label use, as it had not been FDA-cleared. This study was approved by the New York University internal review board (IRB).

The purpose of this prospective, blinded, randomized controlled investigation was to compare the efficacy of ABBM with and without PDGF in producing vital bone at both 4 to 5 months and 7 to 9 months following sinus augmentation.

Method and materials

Twenty-four subjects were selected from those presenting to the Ashman Department of Implant Dentistry at New York University College of Dentistry, New York, New York, who desired maxillary posterior implants and did not have sufficient bone for the procedure. Each subject required bilateral subantral sinus grafting to be eligible for this study. Moreover, the subjects had to have no more than 4 to 5 mm of crestal bone below the sinus floor as determined on a computerized axial tomographic (CAT) scan (Fig 1). A panograph and a CAT scan were taken prior to patient selection and inclusion in the study as part of routine departmental diagnostic procedures. The patient was advised that a second CAT scan would be taken 1 to 2 weeks before implant placement and core sampling (Fig 2). The study exclusion criteria are listed in Table 1.

Informed consent was presented verbally and each subject who agreed to participate signed an informed consent form approved by the IRB. The use of this growth factor was off-label for sinus augmentation.

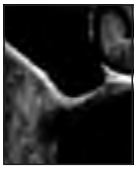


Fig 1 (left) *Presurgical measurements were obtained using a CAT scan.*

Fig 2 (right) *CAT scan of sinus in Fig 1 (test group) after a 5-month healing period and just prior to core removal.*

Table 1 Exclusion criteria

Patients requiring antibiotic prophylaxis for dental procedures
Any sinus pathology contraindicating the graft procedure
Patients who could not undergo standard oral surgery procedures for any reason
Patients who smoked more than 10 cigarettes per day
Patients with uncontrolled or poorly controlled diabetes or patients with other uncontrolled metabolic diseases
Patients with chronic or acute sinus problems
Women who were pregnant or who desired to become pregnant during the course of the study
Patients under 18 years of age

Surgical procedures

Investigators performed a standardized calibration session prior to the first surgery to ensure that the surgical technique for the sinus augmentation procedure had minimal variation between investigators. Each subject was required to take antibiotic prophylaxis using 2 g amoxicillin (Teva Pharmaceuticals) or 600 mg clindamycin (Watson Laboratories) 1 hour prior to surgery. Clinical photographs were taken prior to, during, and postsurgery. Procedures were performed with local anesthesia. The following anesthetic agents were used depending upon patient medical history and operator preference: Lidocaine HCL 2% with 1:100,000 epinephrine, Lidocaine HCL 2% with 1:50,000 epinephrine, Mepivacain/Carbocaine 3% without epinephrine, or Bupivacaine HCl 0.5% with 1:200,000 epinephrine (Abbott Laboratories).

Reflection of a full-thickness flap was performed exposing the lateral wall of the sinus.

Preparation of a hinge or complete osteotomy of the lateral sinus wall was performed using a rotary bur or piezoelectric surgery as the circumstances dictated and according to operator preference. The wall and sinus membrane were elevated. If the bony window was removed to facilitate elevation of the membrane, it was not added to the bone to be grafted.

ABBM (Bio-Oss, Osteohealth) alone was placed in one subantral compartment and ABBB + rh-PDGF was placed in the contralateral subantral compartment. The mixture material in the control sinus was composed of 2.5 g (50%) of 0.25- to 1.0-mm particle size and 2.5 g (50%) of 1.0- to 2.0-mm particle size (total, 5 g). The same ratio of small to large particles was used if any additional material was required to fill a larger sinus. A computer-generated randomized code was used to determine the test and control sites. Depending on the sinus anatomy, a total of 4 to 7 g of material were grafted in each sinus. Two units of PDGF (0.5 mL at concentration of 0.3 mg/mL) were mixed with 1 g of ABBM. The ABBM and rh-PDGF mixture was then thoroughly combined with an additional 4 g of ABBM, yielding a total volume of 5 g of graft material to be placed in the test sinus. The same ratio of rh-PDGF to ABBM was used if any additional material was required to fill a larger sinus. The sinuses were either grafted at the same visit or no more than 6 to 8 weeks apart.

A resorbable porcine collagen membrane (BioGide, Osteohealth) was hydrated in sterile saline prior to insertion and placed over the lateral window. The membrane extended at least 3 mm beyond

the limits of the prepared window and was adapted to the surrounding bone. Primary flap closure was achieved with silk, polyglactin 910, chromic gut (Ethicon), or expanded polytetrafluoroethylene (Goretex) sutures. A postoperative panoramic radiograph was taken to ensure that all the graft material was in place.

Temporary fixed or removable appliances positioned over the surgical sites were relieved prior to reinsertion.

Subjects were placed on 7 to 10 days of antibiotic coverage depending on their history of drug allergy and appropriate analgesics (Tylenol with Codeine #3 or #4, Ortho-McNeil-Jansen Pharmaceuticals) or Motrin 600 mg (Ortho-McNeil-Jansen Pharmaceuticals). Rinses with 0.12% chlorhexidine digluconate for 2 weeks were also prescribed. Subjects returned to the clinic 7 to 14 days postsurgery for suture removal, if required, and a postoperative site evaluation. A postoperative evaluation was also performed at 1 to 2 months following surgery. Core sampling was performed at 4 to 5 months for the first 12 patients enrolled in the study. The second patient cohort had core samplings performed at 7 to 9 months.

At stage-one surgery when implants were placed, a trephine core sample (10 mm in length and 2.7 to 4.0 mm in diameter) was retrieved from the distal and most superior area of the previous window site. If a patient chose not to continue with implant therapy, a core sample was still taken from the appropriate site. Uniformity of core harvest location ensured that the cores were taken through the previously drilled window and in the most apical area of the graft, which was considered to be the least mature due to the greater width of the sinus in this area.[31]

The cores, obtained from both test and control sites, were harvested by the listed investigators in a manner that did not compromise the implant receptor sites. The timing of core harvesting with the respective study maturation periods was strictly adhered to.

Data analysis and evaluation technique

Specimens were fixed in 10% buffered formalin, embedded undecalcified in polymethyl methacrylate (Polysciences), sectioned to 60-µm thickness along the full midline longitudinal length of the core with a Isomet low-speed saw (Buehler), and ground/polished using an automated 400 CS Grinding System (Exakt Technologies). Sections were stained with Stevenel blue and Van Gieson picro fuchsin. High-resolution image montages were acquired with a ScanScope Digital Scanner (Aperio) and analyzed using in-house algorithms developed for the Quantimet image analysis system (QWin version 3.0, Leica Microsystems). The percentages areas of new bone, grafting material, fibrous connective tissue, and marrow were calculated from each image montage. Volumetric and height measurements were made on all CAT scans for comparison. Data were separated into cores taken 4 to 5 months postsurgery and those taken 7 to 9 months postsurgery and then combined to determine the results from the entire study.

Statistical analysis

A repeated-measures analysis of covariance (ANCOVA) was used to determine whether there was a statistically significant difference in percent vital bone growth. There were two main effects factors: time (baseline, core sample) and material (ABBM only, ABBM + PDGF). The interaction effect time/material was also examined. The covariate in the analysis was the time elapsed from grafting (baseline) to core sampling.

A linear mixed effects model was fit for each outcome for all subjects to assess for differences in vital bone, residual graft, and connective tissue between the treatment (ABBM + rhPDGF) and control (ABBM only) groups and to assess the effect of time of core removal. Initially, the model included fixed effects of treatment group, core time, and their interaction with a random intercept for each subject. If the interaction term was not statistically significant then the model was refit without the interaction term. If the interaction term was statistically significant then tests of simple effect were performed.

Table 2 Group A subjects

	ABBM only				ABBM + PDGF			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Vital bone	11.8%	9.2%	2.1%	29.3%	21.1%	11.8%	2.0%	42.7%
Connective tissue	54.1%	17.5%	26.7%	82.0%	51.4%	10.1%	33.0%	67.0%
Residual graft	33.6%	12.0%	12.5%	49.3%	24.8%	11.4%	0.2%	48.2%

Table 3 Group B subjects

	ABBM only				ABBM + PDGF			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Vital bone	21.4%	8.6%	8.1%	36.1%	19.5%	10.7%	0.0%	39.9%
Connective tissue	38.4%	6.6%	24.7%	46.9%	44.2%	10.4%	27.3%	60.8%
Residual graft	40.3%	6.7%	30.2%	54.5%	35.5%	9.4%	20.1%	48.9%

Table 4 All 24 subjects

	ABBM only				ABBM + PDGF			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Vital bone	16.6%	10.0%	2.1%	36.1%	20.3%	11.0%	0.0%	42.7%
Connective tissue	46.3%	15.2%	24.7%	82.0%	47.8%	10.7%	27.3%	67.0%
Residual graft	36.8%	10.2%	12.5%	54.5%	30.2%	11.6%	0.2%	48.9%

Results

Twenty-four patients were enrolled and all completed the study. Each subject received two sinus augmentations, one with ABBM (control) and one with ABBM + rhPDGF (treatment). The 10 female and 14 male patients had an average age of 61.2 ± 7.7 years. There were two different groups of subjects based on the time between surgery and core removal. Group A (12 subjects) had cores taken between 4 and 5 months (mean ± standard deviation, 4.25 ± 0.34 months after surgery. Group B (12 subjects) had cores taken between 7 and 9 months (mean ± SD, 8.13 ± 0.53 months) after surgery.

Table 2 shows the summary data for the 12 subjects with cores taken at 4 to 5 months, Table 3 shows the same summary data for the subjects in the 7- to 9-month core group, and Table 4 shows the summary data for all subjects combined.

For vital bone, the interaction term of the model was nearly significant (P = .053); therefore, tests of simple effects of group and time were performed. These simple effects are the effect of treatment group tested separately for each core time and reciprocally the effect of core time tested separately within each treatment group. Paired t tests showed a statistically significant difference in vital bone between the ABBM (mean ± SD, 11.8% ± 9.2%) (Figs 3a and 3b)

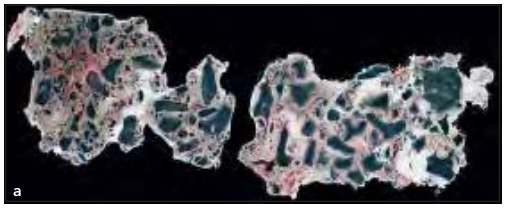


Fig 3a *Cross section of a core sample from a control site (4 months) showing bone formation of varying maturity with 10.1% vital bone* (NB). *Also evident are various-sized particles of Bio-Oss. The new bone formation is present between the Bio-Oss particles* (B), *bridging these particles and resulting in a cancellous bone pattern incorporating the Bio-Oss (Stevenel blue and van Gieson picro fuchsin; field width = 3.401 mm).*

Fig 3b *High-power image showing immature, newly formed bone* (NB) *around particles of Bio-Oss* (B). *In the center of the largest particle of B, a small area of new bone is evident (Stevenel blue, van Geisson picro fuchsin; field width = 1.244 mm).*

Fig 4a *Cross section of core sample from test site (4 months) showing 25.39% vital bone* (NB) *(Stevenel blue, van Gieson picro fuchsin; field width = 490 µm).*

Fig 4b *High-power image showing the new bone formation* (NB) *bridging the particles of Bio-Oss* (B), *along with active osteoid* (OS). *In the center of the largest particle of Bio-Oss in the upper right of the image, a small area of new bone is evident (Stevenel blue, van Gieson picro fuchsin; field width = 516 µm).*

and ABBM + rhPDGF (mean ± SD, 21.1% ± 11.8%) groups (Figs 4a and 4b) among group A subjects ($P = .043$). There was no statistically significant difference in vital bone in group B subjects between control and treatment (ABBM: 21.4% ± 8.6%; ABBM + rh-PDGF: 19.5% ± 10.7%; $P = .645$) (Figs 5 and 6). Independent sample *t* tests showed a significant difference in vital bone between group A (11.8% ± 9.2%) and group B (21.4% ± 8.6%) subjects in the ABBM group ($P = .015$), but no significant difference in the ABBM + rh-PDGF group (group A cores, 21.1% ± 11.8%; group B cores 19.5% ± 10.7%; $P = .723$).

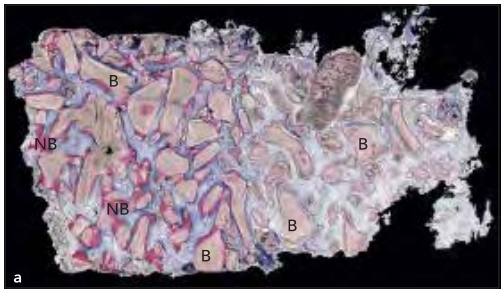


Fig 5a *A core specimen from control site at 7 months demonstrates Bio-Oss particles* (B) *surrounded by and interconnected with 20.85% newly regenerated bone* (NB) *(Stevenel blue, van Gieson picro fuchsin; field width = 5.320 mm).*

Fig 5b *High-power view showing new bone regenerated in a test site (7 months) around Bio-Oss particles (Stevenel blue, van Gieson picro fuchsin; field width = 2.262 mm).*

Fig 5c *High-power view showing osteoclasts* (OC) *around the residual graft material.*

For connective tissue, the interaction term of the initial model was not statistically significant ($P = .218$) and the model was therefore refit without the interaction. The second model showed no significant effect of ABBM treatment group ($P = .653$) but did show an effect of core timing ($P = .001$) on connective tissue. The amount of connective tissue in each treatment group was lower in group B subjects that in group A subjects.

For residual graft, the interaction term of the initial model was not statistically significant ($P = .477$) and the model was therefore refit without the interaction. The second model showed a significant effect of both treatment group ($P = .016$) and core timing ($P = .004$) on residual graft. Residual graft was higher in the ABBM group than in the ABBM + rhPDGF for both core times (see Tables 1 and 2). It was also higher in group B subjects than in group A subjects for both treatment groups.

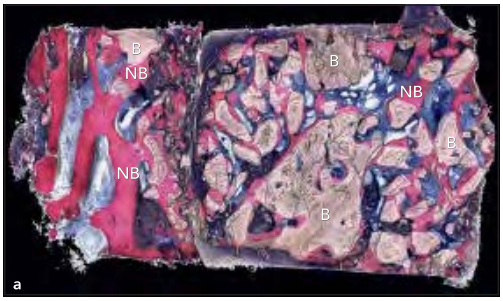


Fig 6a *Low-power view of a core showing trabeculae in which the Bio-Oss particles* (B) *are generally incorporated into the newly formed bone* (NB) *in a test site (7 months) with 19.95% of vital bone (Stevenel blue, van Gieson picro fuchsin; field width = 4.545 mm).*

Fig 6b *High-power view of vital bone formation* (NB) *directly on the residual Bio-Oss particles* (B) *in Fig 6a (Stevenel blue, van Gieson picro fuchsin; field width = 1.292 mm).*

Discussion

An important debate topic in implant dentistry is the choice of grafting material for sinus augmentation procedures.[1–10] These graft materials include autograft,[11,12,14,16] allografts,[15] xenografts,[18,19,32] alloplasts,[16,17] bioactive agents,[33] or a combination (composite) of grafts.[34] The literature shows a wide range of results with different grafting materials.[1–10] In the review by Del Fabbro et al,[2] sinuses grafted with 100% bone replacement graft had an implant survival rate of 96.2% compared with 87.7% for sinuses grafted with 100% autogenous bone. All the reviews demonstrated equal or better implant survival rates with xenografts than those achieved with autogenous bone. The inclusion of rhPDGF-BB in the sinus grafting protocol used along with Bio-Oss has been associated with positive clinical results and may provide opportunities to improve long-term clinical outcomes for this procedure.[33]

The use of growth or differentiation factors for bone regeneration has shown significant potential. Preclinical and clinical studies have demonstrated superior outcomes in terms of the amount and rate of new bone formation when these agents were compared with traditional bone grafting materials.[33,35] These factors are present at low concentrations in bone matrix and plasma and are essential mediators of tissue repair through their stimulatory effects on angiogenesis, cell proliferation, cell differentiation, and matrix synthesis. Among the myriad growth factors, rhPDGF has received the most attention. Recombinant human platelet-derived growth factor BB is a well-characterized tissue growth factor that has been used in various human[36] and animal studies.[37,38] Ross et al[39] and Westermark[40] published comprehensive reviews of the biology of rh-PDGF. Numerous references in the periodontal literature relate to the effectiveness and mode of action of rhPDGF in periodontal regeneration,[22,23,35] ridge augmentation procedures,[36,38,41,42] and maxillary sinus elevation.[33] Results of a preclinical canine study demonstrated that purified recombinant PDGF-BB, used in combination with a deproteinized bovine block and without placement of a barrier membrane, has the potential to regenerate significant amounts of new bone in severe mandibular ridge defects.[38] A case series in humans using various combinations of ABBM and rhPDGF-BB for maxillary sinus elevation reported successful histologic results.[33]

The most effective way to evaluate the effect of rhPDGF on bone formation in a sinus graft is to use the standard bilateral study model, with the addition of rhPDGF being the only controlled variable. This is the first randomized controlled clinical trial to report on a direct comparison of an ABBM alone to an ABBM with rhPDGF in sinus augmentation. However, even in this model, factors such as differences in the size and morphology of the sinuses, the amount of residual crestal bone, and operator differences remain as potential confounding variables.

The present study did not assess implant survival rates; rather, it examined the percent of vital bone present after grafting bilateral sinuses with ABBM or ABBM and rhPDGF at specific time intervals. The vital bone formation was significantly greater in group A in the ABBM + rhPDGF (21.1 ± 11.8%) group vs the ABBM (11.8 ± 9.2%) alone group. However, in group B, this difference disappeared. Overall, the longer healing period showed an improved percent vital bone in the ABBM alone group. Froum et al[34] used ABBM with and without autogenous bone in nine sinuses and reported 24% mean vital bone volume at 6 to 9 months, compared with 33% vital bone volume at 12 to 15 months. Valentini et al[32] examined sinuses grafted with 100% ABBM and showed a mean percentage of vital bone of 21.08% at 6 months and 27.55% at 12 months. A similar study by Lee et al[43] also showed a correlation between mean vital bone and healing time. In 14 sinuses grafted with 100% ABBM and covered with a collagen membrane, the mean percent of vital bone was 18.3% at 6 months and 26.6% at 12 months.

The shorter healing time group (group A) was chosen to highlight any earlier benefits likely to occur for the treatment options tested. The results of the present investigation support the potential of rh-PDGF to improve bone formation in the early stage of bone healing. At 4 to 5 months, almost twice the percentage of vital bone was observed in the ABBM + rhPDGF (21.1% ± 11.8%) sinuses compared with control (11.8% ± 9.2%). However, after 7 to 9 months of healing, the vital bone percentage was similar in test and control groups. One possible interpretation for these findings is that bone formation in the ABBM + rhPDGF group is accelerated or jump-started, but that the total amount generated at the 7- to 9-month endpoint is the same. Previous studies have also reported favorable results in terms of bone formation when rhPDGF was used.[44–46] The effects of rhPDGF-BB reported in the literature appear to be most significant during the early stages of bone healing.[44,45,47,48] Sarment et al[48] reported that the highest bone turnover rate was measured at 6 weeks in rh-PDGF-BB–treated intrabony defects of humans when compared with the 24-week observational period. Thus, when a longer healing time is used, any differences in bone formation resulting from rhPDGF treatment become less obvious.

The dose level of rhPDGF used in this study (0.3 mg/mL) was higher than that reported in previous studies (40 to 50 µg/mL).[44,45,47–49] The rationale for using a higher dose level was because rhPDGF has a high clearance rate in vivo[27] and the effects of rhPDGF-BB on mitogenesis and chemotaxis of osteoblasts appear to be proportional to the concentration administered.[50,51] Nevins et al[33] used the same 0.3 mg/mL concentration in a recent sinus study. Furthermore, the 0.3 mg/mL dose level of rh-PDGF-BB is the same as used in the product GEM 21S, which is FDA-cleared for clinical use in periodontal regeneration as it has been shown to be safe in humans even with dose levels of up to 1 mg/mL.[52]

The histologic observations revealed a visible difference in the rate of graft resorption when rhPDGF was used (Table 3). This difference was greater in the earlier healing group (group A) compared to the later group (group B). Recently, an animal study showed similar results.[51] The same phenomenon of accelerated replacement–resorption of bone substitute particles saturated with rhPDGF-BB was also found in human subjects.[33] It may be speculated that the use of the growth factor accelerated the biodegradation of the graft material, a finding confirmed in the present study.

Conclusion

Within the limitations of this study, it can be concluded that *(1)* both the test and control groups showed acceptable vital bone formation at both the 4- to 5-month and 7- to 9-month maturation times; *(2)* vital bone formation was significantly greater at 4 to 5 months in the ABBM + rhPDGF group vs the ABBM alone control; however, in the 7- to 9-month cores, this difference disappeared; *(3)* the longer healing period resulted in an increased percent of vital bone in the ABBM alone group; however, this was not true in the ABBM + rh-PDGF group where the percent of vital bone was similar in the 4- to 5-month cores and 7- to 9-month cores; and *(4)* the more rapid formation of vital bone may allow for earlier implant placement. Further clinical studies using rhPDGF-BB should be performed to validate the findings of this study and to evaluate the outcome of implant survival in both standard and early loading protocols.

Acknowledgment

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2. HISTOMORPHOMETRIC COMPARISON OF DIFFERENT CONCENTRATIONS OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN WITH ALLOGENIC BONE COMPARED TO THE USE OF 100% MINERALIZED CANCELLOUS BONE ALLOGRAFT IN MAXILLARY SINUS GRAFTING.

The posterior maxilla often requires bone augmentation prior to implant placement. The gold standard, autogenous bone graft, requires additional surgery with associated morbidity, while bone biomaterials may not support relevant bone formation. Recombinant human bone morphogenetic protein-2 (rhBMP-2) in an absorbable collagen sponge (ACS), however, induces significant, clinically relevant bone formation in several settings including the maxillary sinus floor.

The purpose of this study was to histomorphometrically evaluate the percentage of vital bone after grafting of maxillary sinuses using 2 different concentrations of Infuse (rhBMP-2/ACS) combined with mineralized cancellous bone allograft (MCBA) and to compare the results to a control sinus grafted with MCBA only.

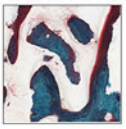
METHODS:

Thirty-six sinuses in 18 patients had 2 of 3 of the graft combinations including 1) Control MCBA only, 2) Test 1MCBA + 5.6 mL of rhBMP-2/ACS (containing 8.4 mg of rhBMP-2), 3) Test 2 MCBA +2.8 mL of rhBMP-2/ACS (containing 4.2 mg of rhBMP-2). Histological cores were taken 6-9 month following sinus augmentation.

RESULTS:

The results showed no statistically significant differences in vital bone between the higher dose of rhBMP-2 or lower dose group compared to the control sinus group treated with MCBA alone.

Histomorphometric Comparison of Different Concentrations of Recombinant Human Bone Morphogenetic Protein with Allogenic Bone Compared to the Use of 100% Mineralized Cancellous Bone Allograft in Maxillary Sinus Grafting



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The purpose of this study was to histomorphometrically evaluate the percentage of vital bone after grafting of maxillary sinuses using two different concentrations of Infuse (rhBMP-2/ACS) combined with mineralized cancellous bone allograft (MCBA) and to compare the results to a control sinus grafted with MCBA only. Thirty-six sinuses in 18 patients had two of three of the graft combinations including: (1) control MCBA only, (2) test one, MCBA + 5.6 mL of rhBMP-2/ACS (containing 8.4 mg of rhBMP-2), or (3) test two, MCBA + 2.8 mL of rhBMP-2/ACS (containing 4.2 mg of rhBMP-2). Histologic cores were taken 6 to 9 month following sinus augmentation. The results showed no statistically significant differences in vital bone between the two test groups compared to the control sinus group treated with MCBA alone. More cases and survival of implants placed in these augmented sinuses are needed to verify the results of this randomized prospective study. (Int J Periodontics Restorative Dent 2013;33:XX–XX. doi: 10.11607/prd.1736)

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The subantral augmentation (sinus elevation) procedure has been shown to be a predictable method for placing root form implants in areas of the posterior maxilla with deficient bone quantity and/or quality. The original protocol used autogenous bone from intraoral or extraoral sources.[1–4] The need for a second surgical site increased the length of the surgical intervention, the surgical risk, and postsurgical morbidity since healing was required in multiple sites.

Bone replacement graft materials have been used in the sinus elevation procedure to avoid the drawbacks inherent in the harvesting of autogenous bone. They have been shown to be effective and have demonstrated high implant success rates.[5–7] These graft materials include allografts,[8,9] xenografts,[10–13] and alloplasts.[14–20] Recently, a growth factor (bone morphogenic protein) has been successfully used to regenerate bone in extraction sockets and sinus augmentation procedures.[21–24]

The recombinant human bone morphogenetic protein (rhBMP-2/ACS) used in the present study (Infuse bone graft, Medtronic) has

been extensively reviewed for use as an augmentation material.[25–33]

The bone graft consists of two parts: a solution containing rhBMP-2 and the ACS (absorbable collagen sponge) from bovine type I collagen designed to resorb over time. The protein is a genetically engineered version of a natural protein normally found in small quantities in the body. During surgery, the protein solution is soaked into the ACS. The ACS binds to the BMP-2 at the site and acts as a scaffold for the formation for the new bone that the protein stimulates. The safety and effectiveness of the Infuse bone graft were previously evaluated in a clinical study,[34] and it has been approved by the United States Food and Drug Administration (USFDA) for use in sinus augmentation.

Mineralized cancellous bone allograft (MCBA) has been used for grafting in multiple intraoral applications to treat infrabony defects,[35] extraction sites,[36–40] and for ridge augmentation.[41,42] This material has been used as a bone replacement for sinus augmentation procedures.[9,43–45] The purpose of this study was to histomorphometrically evaluate the amount of vital bone formed 6 to 9 months after grafting in the maxillary sinus, using two different doses of rhBMP-2/ACS combined with MCBA (Community Tissue Services) and compare this to the control sinus grafted with MCBA only.

Method and materials

Twenty-one of 30 subjects were selected from patients presenting to the Implant Department and Bluestone Center for Clinical Research at New York University College of Dentistry who wished to receive maxillary posterior implants, but did not have sufficient bone for the dental implant placement. This number was chosen as necessary for power to determine a statistically significant different in results between the tests and control groups. Study inclusion and exclusion criteria were identical to those in a previous study.[46] Each of these patients required bilateral subantral sinus grafting to be eligible for this study.

Surgical procedure

Diagnosis of the need for implants and a sinus augmentation procedure was made with the aid of panorex and computer axial tomography scan radiographs. Presentation of the study to the subject both verbally and with an informed consent accepted by the New York University School of Medicine Institutional Review Board. Alternative treatments, including and excluding implants in the maxillary posterior area, were presented. If the subject agreed to participate in the study, the consent forms were signed, and a copy given to the patient.

Each subject was required to take antibiotic prophylaxis 1 hour prior to surgery. This included amoxicillin (Novopharm) 2,000 mg or clindamycin (Ranbaxy Pharmaceuticals) 600 mg, for patients allergic to penicillin. Administration of local anesthesia was performed. Depending on the patient's medical history, operator preference, and/or surgical circumstance, these included: lidocaine HCl 2% with 1:100,000 epinephrine, lidocaine HCl 2% with 1:50,000 epinephrine, mepivacain/carbocaine 3% without epinephrine, or bupivacaine HCl 0.5% with 1:200,000 epinephrine (Abbott Laboratories).

Reflection of a full-thickness flap was performed exposing the lateral wall of the sinus.

Preparation of hinge or a complete osteotomy of the lateral sinus wall was performed as the circumstances dictated. The sinus membrane and osseous wall, if retained, were elevated. If the bony window was removed to facilitate elevation of the membrane or as part of the piezoelectric osteotomy, it was not added to the grafted bone. If there was a perforation of the sinus membrane of > 10 mm, the perforation was repaired with a membrane barrier (BioGide, Osteohealth) or Biomend Extend (Zimmer) and the patient exited from the study. Any perforation ≤ 10 mm was repaired with the same collagen barriers and the augmentation procedure completed.

By block randomization, each of the patients had one of the three possible graft combinations placed in each sinus. The three groups were: control (MCBA only), test 1 (T1) (MCBA + 5.6 mL of rhBMP-2/ACS [four sponges], containing

8.4 mg of rhBMP-2), or test 2 (T2) (MCBA + 2.8 mL of rhBMP-2/ACS [two sponges], containing 4.2 mg of rhBMP-2).

In the control sinus, 100% MCBA was placed. The subantral compartment was filled using a total of 3 to 6 mg of MCBA depending on the size of the area to be augmented. The volume of MCBA was calculated using a volumetric syringe (ACE Dental Implant System). The mixture for the MCBA was composed of 50% of .25 to 1.0-mm particle size and 50% of 1.0 to 2.0-mm particle size.

In the T1 sinus, four pieces of collagen gauze were each saturated with 1.4 mL of rhBMP-2 for 15 minutes prior to placement. The concentration of rhBMP-2 was the same for each sponge (1.5mg/mL). The subantral compartment was then filled with 2 to 4 mg of MCBA depending on the size of the area to be augmented, mixed with cut up pieces of the four saturated collagen sponges (total dose: 8.4 mg rhBMP-2/ACS). In the other test sinus (T2), a similar total amount of graft material consisting of MCBA (2 to 4 mg) combined with cut up pieces from two of the saturated collagen sponges (total dose: 4.2 mg of rhBMP-2/ACS) were placed. Collagen membrane barriers were placed over the lateral window osteotomies in all surgeries as consistently done in previous studies at NYUCD.[9,10,13,43,46]

Six patients received control and T1 sinus augmentations. Six patients received control and T2 sinus augmentations. Eight patients received T1 and T2 sinus augmentations. Two patients were added following randomization to replace two patients who were exited from the study: one during surgery due to a sinus perforation > 10 mm and the other following suture removal who decided to withdraw from the study. Three patients were removed from the study because they were lost to follow-up and core removal was not performed.

Primary closure of the flap was performed with silk, polyglactin 910 (vicryl), or chromic gut. A panoramic radiograph was taken after sinus elevation surgery to ensure the materials were in the desired location.

Relief of the provisional fixed or removable prosthesis over the edentulous area was performed prior to insertion of the provisional. The patients were placed on 7 to 10 days of the same antibiotic coverage used preoperatively (amoxicillin or clindamycin), and analgesics (acetaminofen with codeine no. 3 or 4, or ibuprofen 600 mg every 6 hours). Rinses were prescribed with 0.12% chlorhexidine digluconate (on prescription) for 2 weeks.

All patients returned to the clinic 7 to 14 days postsurgery for suture removal (if required) and postsurgical examination.

At stage-one surgery when implants were placed (following a 6- to 9-month healing phase), a trephine core sample (10 mm in length and 2.7 to 4.0 mm diameter) was retrieved between the superior and inferior position of the lateral window osteotomies made during the sinus elevation surgery. If the patient decided not to have implants, a core sample was still taken of the healed bone 6 to 9 months post-sinus elevation surgery, which required a small incision and sutures to close the area. This technique ensured that the cores were taken through the previously drilled window and in the central area of the graft, which is considered to be the least mature and not to compromise implant placement. Five investigators performed the sinus augmentation. The cores were obtained from both the study and control sites by one of the investigators who examined all photographs and radiographs of the augmentation procedures to ensure the cores were taken from the center of the grafted sinus. The patients signed the consent form informing them that cores would be taken 6 to 9 months postsurgery as part of the study whether or not they decided to have implants placed at the time.

The same antibiotics and analgesics were prescribed following implant placement core retrieval surgery as prescribed for the sinus elevation surgery. Sutures were removed, if necessary, 7 to 14 days postsurgery.

Blinded histomorphometric analysis was performed on the bone core samples collected 6 to 9 months after sinus surgeries to determine the vital bone and residual graft material content. Native crestal bone was delineated and evaluated separately from the new bone created in the sinus.

Data analysis and evaluation technique

Methodology used for the histologic analysis was described in an earlier article[46]; briefly, the technique was as follows. Specimens were fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol, embedded undecalcified in poly-methylmethacrylate (Polysciences), sectioned to 60 µm thickness along the full midline longitudinal length of the core with a low speed saw (Isomet, Buehler), and ground/polished using an automated Exakt 400 CS grinding system (Exakt Technologies). Sections were stained with Stevenel blue and Van Gieson picro fuchsin. High resolution image montages were acquired with a ScanScope Digital Scanner (Aperio) and analyzed on computers using in-house algorithms developed for the Quantimet image analysis system QWin version 3.0 (Leica Microsystems) .

Prior to the start of the project and midway through, density-dependent backscattered electron microscopy in the scanning electron microscope (BSE-SEM) (Zeiss EVO 50 SEM) was employed on a selection of histologic thin section to reach a definitive evaluation of graft material in comparison to original bone. Knowledge gained from observations of the histologic thin sections by both BSE-SEM and ~~LM~~ [Au: Please define **LM**] was used to detect and measure areas within each section for two categories of vital bone (new bone and original bone), and allograft, fibrous connective tissue, and marrow were calculated from each image montage (total area was also recorded as a check against the summed measured areas). Relative measured areas (in %) were recorded. Images of thin sections by BSE-SEM unambiguously characterized new bone by its low mineralization density compared to original bone and allograft. This low mineral density fraction was assigned depending on the staining regimen. For specimens receiving Gomori trichrome stain, new bone was stained in hues of red, grading to purple with increasing age of the bone packet. For specimens stained with Stevenel blue and van Giesson picro fuschin, new bone absorbed more van Gieson picro fuschin stain than original bone and allograft. The discrimination of new bone with each staining method was also aided further by the presence of a cement line between new and original bone or allograft, and by differences in osteocyte lacunae organization and viability of cells, the latter determined by the presence of nuclei (original bone typically contains apoptotic cells and empty lacunae and allograft lacunae are always empty).

Statistical analysis

The percentage of bone, residual MCBA particles, and soft tissue in sinus cores taken from each of the test and control groups 6 to 9 months following sinus graft surgery were determined.

This was an average of the three sections from each core and expressed in % vital bone, % remaining MCBA particles, and % marrow and soft tissue.

The distribution of all variables were evaluated using measures of central tendency, variability, and higher moments as well as distribution plots and frequency histograms.

A linear mixed effects model with a between subject factor of treatment and a subject specific random intercept was used to identify differences in % vital bone between the treatment groups. Similar analysis was performed for % residual MCBA remaining and % soft tissue and marrow between the treatment groups. Differences between T1 and control and T2 and control were assessed for new bone and separately for residual graft with a linear mixed model using fixed effects of dummy coded group (T1 and T2, with the control group as a reference) and a random intercept for each subject. This was repeated with a fixed effect for group T2 with group T1 as the reference group. Simpler tests such as *t* tests are not appropriate due to the split mouth nature of this study with three different possible pairings of treatments/control.

Results

Thirty-two maxillary sinus augmentation sites were treated in this study. A total of 32 cores (11 cores with control, 10 cores with T1, and 11 cores with T2) were prepared,

Table 1 New bone and residual graft (%)

	Control (MCBA only)		T1 (8.4 mg rhBMP-2/ACS)		T2 (4.2 mg rhBMP-2/ACS)	
	New bone	Residual graft	New bone	Residual graft	New bone	Residual graft
Total	11	11	10	10	11	11
Mean	21.5	23.2	25.3	10.5	17.5	22.6
SD	11.6	12.9	15.3	12.8	10.9	7.0
Minimum	0	0.0	2.4	0.0	0.7	13.1
Maximum	35.0	40.2	52.2	35.8	32.2	37.1

thin-sectioned, imaged, and analyzed. Table 1 shows the summary statistics for the percentage of new bone and residual graft at the time of core removal in the control (MCBA only), T1 (MCBA + 5.6 mL of rhBMP-2/ACS), and T2 (MCBA + 2.8 mL of rhBMP-2/ACS) groups.

The analysis showed that there was no statistically significant difference in new bone between T1 (25.3%) and control (21.5%) (P = .252) or between T2 (17.5%) and control (P = .215). There was a statistically significant difference in new bone between T1 and T2 (P = .019). For residual graft, there was a statistically significant difference between T1 (10.5%) and control (23.2%) (P = .003) but not between T2 (22,6%) and control (P = .631). There was a statistically significant difference in residual graft between T1 and T2 (P = .011).

The perforation of the sinus membrane (≤ 10mm) was observed in 5 sinuses (15.6%). Four of these sinuses were in the T1 and one in T2. No perforations were detected in control group. Due to the small sample, it was not possible to find any statistically difference in new bone formation between perforation (18.04%) and nonperforation sinuses (21.9%). Sinuses with perforation in T1 (22.37%) showed slight higher percentage of vital bone when was compared to nonperforation sinuses (21.9%).

Discussion

The goal of this study was to evaluate whether mineralized cancellous bone allograft (MCBA), an osteoconductive material when added to two different concentration of recombinant bone morphogenetic protein-2 cellular collagen sponge (rhBMP-2/AC), an osteoinductive graft, would be effective in being able to reduce the volume of rhBMP-2/AC, while producing similar amounts of vital bone for implant placement in sinuses grafted with these materials. The randomized bilateral selection of material and blinded histomorphometric analysis was designed to reduce any bias or confounding variables in the study. The results demonstrated that there was a statistically significant (SS) higher % of vital bone in the test group (T1) with the higher concentration of rhBMP-2/ACS (8.4 mg)/MCBA compared to the lower dose rhBMP-2/ACS 4.2 mg/ MCBA) graft (T2). ~~These findings are consistent with those of Boyne et al,[25] although they pretend to difference the difference in bone difference [Aut: This sentence is confusing, please rephrase] rather than vital bone which the present study reported on.~~ Moreover in the Boyne study, the authors used much higher doses in the test group treated with 1.50 mg/mL rhBMP-2/ACS with a range 10.8 to 24.0 mg of rhBMP-2. In the present study, the dose was 8.4 mg of rhBMP-2 in T1 and 4.2 mg of rhBMP-2 in T2. These doses were significantly lower than in the Boyne study although the concentration was the same.

However, there was no statistically significant difference between T1 and the control, MCBA only, or T2 and the control group. The percentage of vital bone in the three groups varied from T1 25.3%, T2

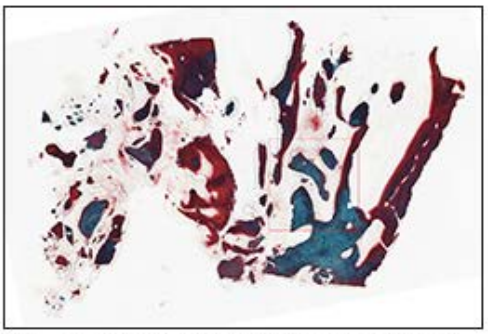

Fig 1 *Cross-section of a core sample from a control site showing bone formation of varying maturity with 21.02% vital bone (8 months) (Gomori trichrome stain; field width = 1.403 mm).*

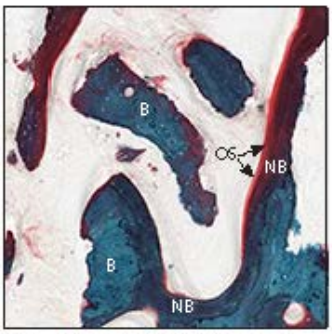

Fig 2 *High-power image showing immature, newly formed bone (NB) around particles of MCBA (B). Vital bone formation is apparent between the residual MCBA particles (Gomori trichrome stain; field width = 4.887 mm).*

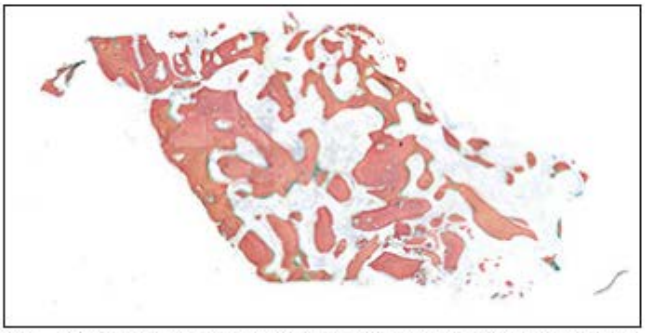

Fig 3 *Histomorphometric analysis of MCBA + 8.4 mg of rhBMP-2/ACS core composed of 24.57% vital bone at 7 months (Stevenel blue and van Gieson stain; field width = 1.403 mm).*

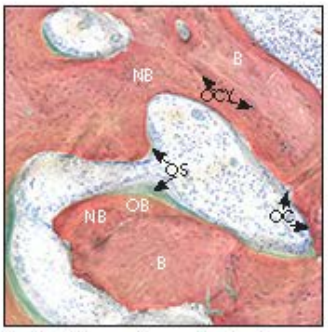

Fig 4 *High-power image showing immature, newly formed bone (NB) around particles of MCBA (B), along with osteoid (OS) and osteoblasts (OB). Note the osteocytes (OCY) and osteoclasts (OC) around the MCBA (B) (Stevenel blue, van Gieson picrofuschin; field width = 6.96 mm).*

17.5%, and control 21.5%. The mean vital bone seen in the MCBA group (control) in the present study was similar to that found in a previous study (28.25%) that compared anorganic bovine bone and MCBA as sole grafting materials in a bilateral sinus study.[9] Histology of all three materials revealed vital bone in close proximity to the residual graft with evidence of osteoid indicating ongoing new bone formation (Figs 1 to 6). The residual graft material showed a statistically significant difference between T1 and control and between T1 and T2 but not between T2 and control. It appears that there was either less volume of MCBA used in combination with T1 (because of the volume occupied by the sponges) or more

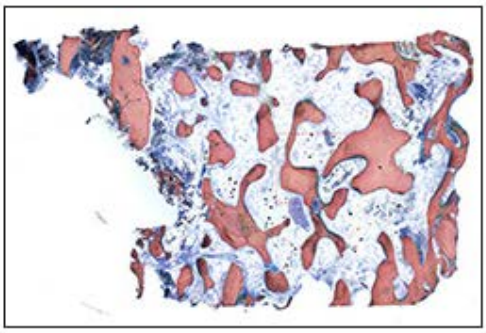

Fig 5 *Histomorphometric analysis of core sample from T2 site (MCBA + 4.2 mg of rhBMP-2/ACS) showing 24.57% vital bone (9 months), in which the MCBA particles (B) are generally incorporated into the newly formed bone (NB) (Stevenel blue and van Gieson stain; field width = 1.403 mm).*

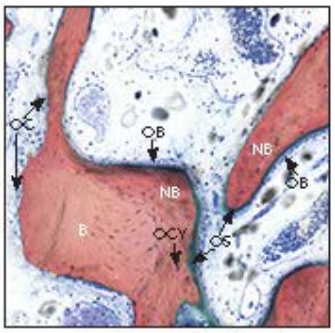

Fig 6 *High-power view of vital bone formation (NB) directly on the residual MCBA particles (B) (Stevenel blue and van Gieson picro fuschin; filed width = 6.800 mm).*

resorption of the MCBA materials. The former appears to be the more reasonable since there was no statistically significant difference in the remaining graft in T2 and the control, which if the rhBMP-2 resulted in increased graft resorption would be evident in this comparison. All cores as in previous studies were taken 6 to 9 months postsurgery.[9,10,13,43] This window was necessary to ensure a minimum drop rate for noncompliance with the timing required for core harvested. Moreover, although bone formation may be greater the 9 months postoperative time compared to the 6-month time period. The timing for the resorption and ultimate replacement of these graft materials with vital bone is not yet understood completely.[43]

The results of previous studies using rhBMP-2 as a sole graft material in maxillary sinus floor augmentation were encouraging with the de novo tissue growth produced adequate bone for successful placement of endosseous implants.[27,47] In the later prospective study, rhBMP-2 was used with an ACS, as in the present study, and compared to autogenous bone graft. A total of 160 patients were followed in a 21 center study in the United States. Although no histology was performed at 6 months postoperatively, a mean change in bone height was seen with the rhBMP-2/ACS patients of 7.83 ± 3.52 mm versus 9.46 ± 4.11 mm for the bone graft patients. The implant success rate 6 months after loading was 79% for the rhBMP-2/AC group.[47]

In an attempt to decrease the amount of rhBMP-2/ACS material required (and, thus, decrease the cost of the procedure), several attempts were made to decrease amounts of the protein with xenografts and allograft materials. One recently published study compared 11 patients treated with rhBMP-2/AC and bovine bone xenografts (Bio-Oss, Geistlich) to the use of bovine bone xenograft alone. Results indicated that the addition of rhBMP-2 to Bio-Oss had a negative effect on bone formation when compared with Bio-Oss alone. The combination graft rhBMP-2/ACS + Bio-Oss was used in an 80/20 ratio. The histologic results reported that percentages of new bone were 16.04% ± 7.45 with the combination vs 24.85 ± 5.82 with the Bio-Oss alone.[48]

In the present study, this negative effect was not seen when MCBA was combined with rhBMP-2. In fact, a case report using a combination of 8.4mg of rhBMP-2/2ACS combined with MCBA reported an improved quality, while "permitting more bone volume to be generated."[49]

Lastly, in a report of three cases that evaluated bilateral sinuses grafted with two concentrations of rhBMP-2/AC combined with either xenografts or allograft, vital bone was found to be similar with the two rhBMP-2/ACS concentrations with either composite.[50]

Sinus membrane perforation is the most prevalent complication of sinus floor elevation procedures (11% to 44%).[51,52] In the present study, sinus membrane perforation was observed in five sinuses, representing an incidence of 15.6%. The effect of sinus perforation on vital bone formation has not been widely studied. Proussaefs et al[53] showed less vital bone formation when sinuses were perforated larger than 2 mm. However, more recently, Froum et al[54] reported more vital bone formation with small perforation (< 10 mm). In the presented study, due to small sample size, the prevalence of sinus perforation was not found to be related to the vital bone formation. In fact, slightly less bone formation was found in sinuses with perforation (18.04%) when compared with nonperforation sinuses (21.9%). Quite interestingly, more vital bone formation was observed in perforated sinuses with higher doses of rhBMP-2/ACS (22.37%).

The findings reported in this study should be verified with additional cases with the success of implants placed in these sinuses reported and followed.

Conclusion

Analyses of the vital bone 6 to 9 months post-graft surgery showed that the group with higher dose of rhBMP-2 combined with MCBA (T1) had more new bone formed when compared with the group with the lower dose combined with MCBA (T2) and to control sinus treated with MCBA, but there was no statistically significant differences between either rhBMP-2 group combined with MCBA in either dose compared to the control sinus treated with MCBA alone. Results from this study also showed that the T1 group had less residual graft material after 6 to 9 months of receiving treatment compared to T2 and control. The residual graft material showed a statistically significant difference between T1 and control and between T1 and T2 but not between T2 and control. More cases are needed to confirm these trends.

Acknowledgment

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3. EFFECT OF MAXILLARY SINUS MEMBRANE PERFORATION ON VITAL BONE FORMATION AND IMPLANT SURVIVAL: A RETROSPECTIVE STUDY.

The maxillary sinus augmentation procedure (SAP) using the lateral window technique has been documented to be a highly predictable procedure. However, the most common intraoperative complication has been reported to be membrane perforation. The present study evaluated the percentage of vital bone and implant survival in sinuses that had perforations repaired during surgery vs. a non-perforated sinus group.

METHODS:

Data was obtained retrospectively from an IRBA approved anonymous database at New York University, Kreiser Dental Center, Department of Periodontology and Implant Dentistry from 23 patients who had undergone SAP with a total of 40 treated sinuses. Sinuses were grafted with mineralized cancellous bone allograft, anorganic bovine bone matrix, or biphasic calcium phosphate. Perforation complications occurred in 15 sinuses with 25 non-perforated sinuses. All perforations were repaired during surgery with absorbable collagen membrane barriers. Histological cores were taken from all treated sinuses 26-32 weeks post-surgery. The implant success rate of 79 placed implants were recorded.

RESULTS:

The average percentage of vital bone was 26.3± 6.3% in the perforated/ (repaired) sinuses vs. 19.1 ± 6.3% in the non-perforated sinuses. The differences were statistically significant (SS). The implant success rate was 100% (0/35) compared to 95.5% (2/45) in the non-perforated sinuses. There was no SS difference in implant failure rates.

Effect of Maxillary Sinus Membrane Perforation on Vital Bone Formation and Implant Survival: A Retrospective Study

Stuart J. Froum,* Ismael Khouly,† Giovanni Favero,†§ and Sang-Choon Cho*

Background: The maxillary sinus augmentation procedure (SAP) using the lateral window technique has been documented to be a highly predictable procedure. However, the most common intraoperative complication has been reported to be maxillary sinus membrane perforation (MSMP). The present study evaluates the percentage of vital bone and implant survival in sinuses that had perforations repaired during surgery versus a non-perforated sinus group.

Methods: Data were obtained retrospectively from an Institutional Review Board–approved anonymous database at New York University, Kriser Dental Center, Department of Periodontology and Implant Dentistry, New York, New York, from 23 patients who had undergone SAP with a total of 40 treated sinuses. Sinuses were grafted with mineralized cancellous bone allograft, anorganic bovine bone matrix, or biphasic calcium phospate. Perforation complications occurred in 15 sinuses with 25 non-perforated sinuses. All perforations were repaired during surgery with absorbable collagen membrane barriers. Histologic cores were taken from all treated sinuses 26 to 32 weeks after surgery. The implant success rate of 79 placed implants was recorded.

Results: The average percentage of vital bone was 26.3% ± 6.3% in the perforated (repaired) sinuses versus 19.1% ± 6.3% in the non-perforated sinuses. The differences were statistically significant (SS). The implant success rate was 100% (35 of 35) compared to 95.5% (43 of 45) in the perforated/repaired vs. non-perforated sinuses, respectively. There was no SS difference in implant failure rates.

Conclusions: The augmented sinuses in this study that exhibited MSMPs that occurred during the SAP (which were treated during surgery) show SS greater vital bone percentages compared with the non-perforated sinus group. There were no SS differences in implant survival in the perforated versus non-perforated groups. In this study, sinus MSMPs, when properly repaired during surgery, do not appear to be an adverse complication in terms of vital bone production or implant survival. *J Periodontol 2013;84:1094-1099.*

KEY WORDS

Bone grafting; dental implant; histology; sinus floor augmentation.

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Maxillary sinus augmentation surgery using the lateral window technique has been shown to be a highly predictable method of regenerating bone for implant placement in the atrophic posterior maxilla. The implants placed in these augmented sinuses have been reported to have high survival rates of ≈92%.[1-3] The sinus augmentation procedure (SAP) is generally considered to be a safe treatment modality with a low incidence of complications. However, complications can and do occur during and after SAP and may have an adverse effect on postoperative outcomes.[4-7] Maxillary sinus membrane perforation (MSMP) is one of the most frequent intraoperative complications that can occur with the SAP. The incidence of MSMP has been reported to vary considerably from 10% to 44% in various studies.[8-11]

Traditionally, a successful SAP is oftentimes determined by the amount of vital bone formation after graft maturation, as well as the subsequent long-term survival rates of implants placed in that bone. It is therefore of clinical interest to know the effect of the MSMP on vital bone formation and implant survival.

There is limited information in the literature related to histologic evidence and implant survival of the SAP with MSMP. The purpose of this study is to evaluate the effect of the MSMP on the percentage of vital bone and implant survival obtained after the SAP.

MATERIALS AND METHODS

Clinical data in this study are obtained from the Implant Database (ID) at New York University College of Dentistry (NYUCD). This dataset was extracted as de-identified information from the routine treatment of patients at the Ashman Department of Periodontology and Implant Dentistry at NYUCD Kriser Dental Center.

The study was granted exempt status by the NYUCD Review Committee based on the fact that the research involved the Department of Periodontology and Implant Dentistry De-Identified ID. The ID was certified by the Office of Quality Assurance at NYUCD and met all Institutional Review Board (IRB) and Health Insurance Portability and Accountability Act requirements. The dataset for this retrospective study consists of a pool of patients who had participated in studies from 1998 to 2008 in which they received bilateral sinus augmentation using the lateral wall technique (Figs. 1 through 3). In each of these patients, <5 mm of crestal bone had to be present below the sinus floor for the patient to be considered for inclusion in this study. All patients signed an IRB-approved consent form before participating in the sinus augmentation

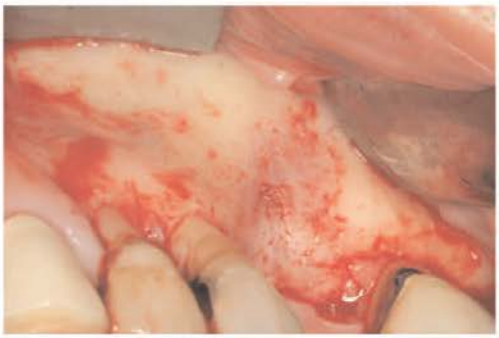

Figure 1.
Exposure of the lateral sinus wall after flap reflection.

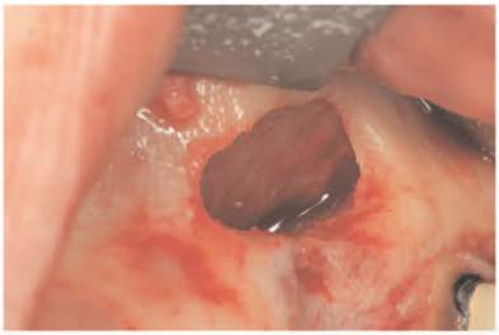

Figure 2.
Lateral window osteotomy and Schneiderian membrane exposure and elevation.

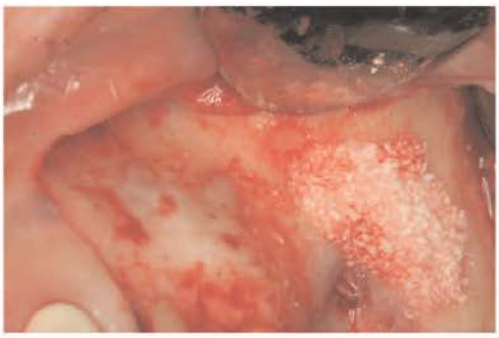

Figure 3.
Graft material placed after membrane elevation.

study. A total of 23 patients (40 sinuses; 10 males and 13 females) were selected. Six sinuses were excluded because the core for histologic evaluation was not obtained in the study time period (26 to 32 weeks) required by the protocol. The age range of patients included was 46 to 75 years (mean: 59 years).

Exclusion criteria for this study included: 1) patients who could not undergo standard oral surgery procedures for any reason; 2) patients who smoked >10 cigarettes per day; and 3) females who were pregnant or nursing a child. Each patient selected had to have taken a computerized axial tomographic scan before the SAP to determine that the amount of crestal bone present was <5 mm as required by the protocol. All patients selected for inclusion received an antibiotic regimen of 2.0 g amoxicillin given 1 hour before surgery and 500 mg

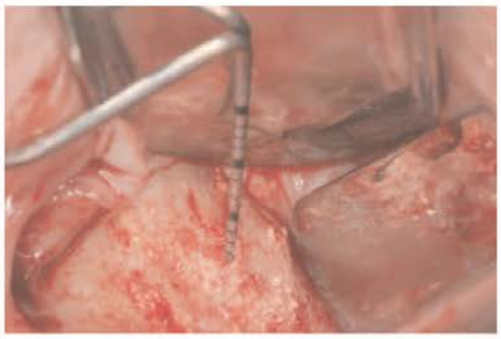

Figure 4.
Six months after healing: reflection of flap and exposure of healed lateral wall.

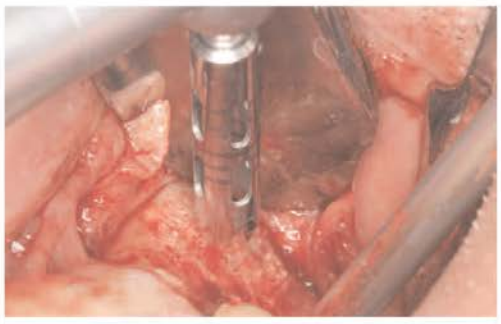

Figure 5.
Biopsy core removed with trephine in location of previous lateral wall osteotomy.

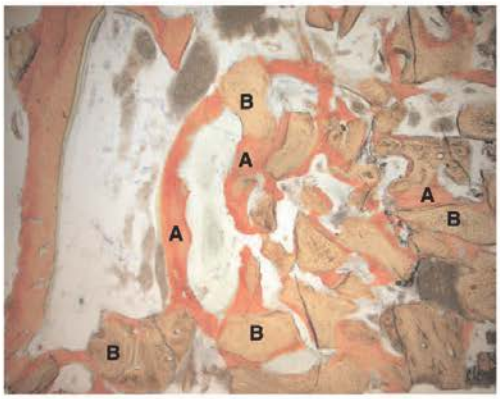

Figure 6.
Histomorphometric analysis of ABBM core composed of 12.44% vital bone. Vital bone formation (A) is apparent between the residual ABBM particles (B). (Stevenel's blue and Van Gieson's stain; original magnification ×40.)

three times daily thereafter for 7 to 10 days. The exception included patients who were allergic to penicillin, who received 600 mg clindamycin given 1 hour before surgery and 150 mg twice daily for 7 to 10 days thereafter. All patients were instructed to rinse with 0.12% chlorhexidine gluconate twice daily for 2 weeks after the surgery. The SAP and implant placement surgeries were done at the Ashman Department of Periodontology and Implant Dentistry at the NYUCD by postgraduate periodontal and implant residents under direct supervision of experienced faculty.[12,13]

Each patient selected was part of a previous study that required histomorphometric analysis of the healed sinus graft material. The sinuses were grafted with three different materials: 1) anorganic bovine bone matrix (ABBM);‖ 2) biphasic calcium phosphate (BCP);¶ or 3) mineralized cancellous bone allograft (MCBA).# In all patients at the stage 1 surgery visit, when implants were being placed (after a 26- to 32-week healing phase), a trephine core sample (10 mm in length and 3 mm in diameter) was retrieved near the superior position of the original lateral window osteotomy (Figs. 4 and 5). Masked histomorphometric analysis was performed on the bone core samples by independent examiners at the Hard Tissue Research Laboratory at the University of Minnesota, Plymouth, Minnesota, to determine the vital bone,

‖ Bio-Oss, Geistlich, Princeton, NJ.
¶ BoneCeramic, Straumann, Andover, MA.
# Puros, Zimmer Dental, Carlsbad, CA.

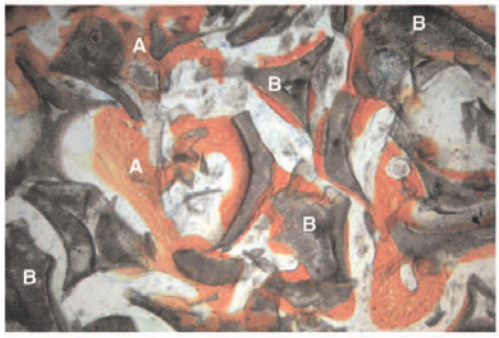

Figure 7.
Histomorphometric analysis of BCP core composed of 30.6% vital bone. Vital bone formation (A) is apparent between the residual BCP particles (B). (Stevenel's blue and Van Gieson's stain; original magnification ×40.)

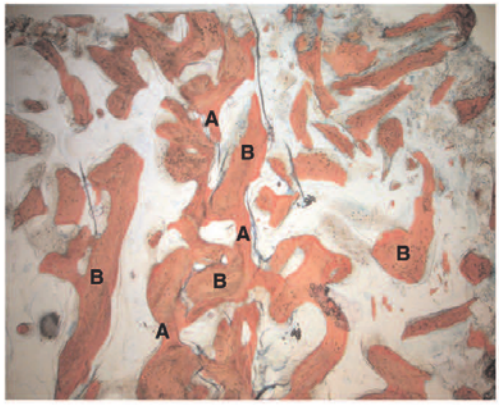

Figure 8.
Histomorphometric analysis of MCBA core composed of 28.25% vital bone. Vital bone formation (A) is apparent between the residual MCBA particles (B). (Stevenel's blue and Van Gieson's stain; original magnification ×40.)

connective tissue, and residual graft material content. These were reported for each SAP performed as part of the study protocol.

In the present study, implant survival and vital bone data are collected from the ID data. All intraoperative and postoperative complications were recorded. All of the perforations reported were <10 mm in diameter. In areas in which the sinus membrane was perforated, a resorbable collagen membrane, either bovine** or porcine,†† was trimmed and placed to cover the perforation before insertion of the graft material. The observations of implant survival after placement were reported from 6 to 32 months.

RESULTS

Twenty-three patients were included who had undergone SAPs, for a total of 40 treated sinuses. Eighty rough-surface implants from four different implant companies were placed, and two were lost (97.5% survival rate). Perforation complications were reported in 15 sinuses (37%). All the perforations were classified as small-to-medium size, <10 mm in diameter.[14] The average percentage of vital bone was 28.25% for MCBA, 12.44% for ABBM, and 30.6% for BCP (Figs. 6 through 8). The implant success rate in perforated sinuses was 100% (35 of 35) compared with the non-perforated sinuses with 95.5% (43 of 45). There was no statistical significance in implant failure between non-perforated sinuses and implant failure in the perforated group ($x^2 = 0.326$). The linear mixed-effect model showed that there was a statistically significant difference in the vital bone percentage ($z = 2.19$, $P = 0.028$) between the non-perforated (19.1% ± 13.7%) and perforated (26.3% ± 6.3%) sinuses, after adjusting for any treatment effect and any individual subject effect on vital bone. There was no statistically significant effect for treatment. The intraclass correlation indicating the contribution of the subject effects to vital bone was 0.093. There was insufficient data to investigate whether there was a treatment–interaction effect on vital bone, so this was not included in the model. (Table 1)

DISCUSSION

A total of 40 SAPs were performed in 23 patients in the present study. The sinuses were grafted with three different materials: 1) MCBA; 2) ABBM; and 3) BCP. The average percentage of vital bone was 28.25% for MCBA, 12.44% for ABBM, and 30.6% for BCP. Eighty implants were placed in the augmented sinus, with only two implant failures. These implant failures occurred in non-perforated sinuses. The results for implant survival in the present study are similar to those reported in evidence-based reviews.[1-3] The reviews analyzed and compared various grafting materials and implants. Also, the different implant surfaces have been compared, and it was reported that the survival rate of rough-surface implants was 95.98% compared with a survival rate of 85.64% with smooth-surface implants.[1,2] All implants in the present study are rough-surface implants.

** CollaTape, Zimmer Dental.
†† Bio-Gide, Geistlich.

Table 1.

Summary Statistics for Percentage of Vital Bone in Perforated/Non-Perforated Sinus Membrane Groups

		Vital Bone			
Group	Sinuses Observed (n)	Mean (%)	Statistical Deviation (%)	Minimum (%)	Maximum (%)
Perforated	24	19.1	13.7	7	55
Non-perforated	16	26.3	6.3	13	37

There is limited information pertaining to implant survival after perforation and repair of the sinus membrane. Studies by Jensen et al,[15] Proussaefs et al,[16] and Khoury[17] reported that implant survival was negatively affected by sinus MSMP. However, the studies by Jensen et al.[15] and Khoury[17] did not clearly report the implant survival rate and the perforation size. Hernández-Alfaro et al.[14] reported that the implant survival rate is inversely proportional to the size of the MSMP. They found significantly higher implant survival rates when perforations were <10 mm compared with perforations >10 mm: 95% to 97% and 74%, respectively. Other authors presented data showing that survival rates were not affected by perforations.[4,5,18-22] The high implant survival rate (100%) in the present study of implants placed in the sinuses in which perforations were reported appears to indicate that MSMPs do not appear to significantly influence implant survival. However, in the present report perforations are <10 mm in diameter and are repaired under the supervision of experienced faculty. Therefore, it is not known whether in cases of larger (>10 mm) untreated or unknown perforations the survival rate would be affected.

In the present study, sinus MSMP is observed in 15 sinuses, representing an incidence of 37.5%. According to the literature, the incidence of MSMP has been reported to vary considerably, from 10%,[9] 20%,[8] 35%,[10] to 56%.[11] The presence of antral septa may increase the potential for MSMP. However, in a study by Schwartz-Arad et al.,[4] the presence of a septum did not significantly increase the incidence of MSMP.

The effect of perforation of the sinus membrane on the vital bone has not been widely reported. Proussaefs et al.[16] compared results in 12 bilateral cases in which only one side had a perforation. They showed that a sinus MSMP >2 mm reduced the percentage of bone regeneration from 33.58% ± 7.45% for non-perforated to 14.15% ± 7.06% when the sinus membrane was perforated.[16] They also reported significant inflammation of the sinus membrane in most cases of MSMP after surgery. During the repair procedure in their study, the clinician was not able to stabilize the bioabsorbable collagen membrane over the perforation. Moreover, in that same study, a barrier membrane was not placed over the lateral window. The repair technique performed on all patients included in the present study involves the placement of two separate stabilized biosorbable membranes over the perforation and lateral window. There were no reports of symptoms of sinus inflammation after any surgery. The current study includes 15 perforations, all <10 mm in diameter, whereas Proussaefs et al.[16] included 12 with MSMPs >2 mm. In the present study, a higher percentage of vital bone (26%) is found in the sinuses with perforated membranes compared with those with no perforations (19.5%). Similar results were found by Testori et al.,[20] who reported 22% to 26% of the vital bone in sinuses with perforations using a bioabsorbable collagen membrane that completely contained the particle material.

The reasons for the increased vital bone seen in the perforated and repaired sinuses compared with the non-perforated group may be related to the membrane over the perforation creating and acting as a second barrier (one was placed over the lateral window), thus providing an additional barrier function that prevented soft tissue migration. Another explanation may be that the membrane acted to better contain and immobilize the graft particles during the bone healing phase. Additional research is needed to elucidate the role of this barrier used to treat and cover Schneiderian MSMPs.

CONCLUSIONS

In the present study, treated sinuses that exhibited MSMPs that occurred during the SAP (which were treated during surgery) showed statistically significant greater vital bone percentages compared with the non-perforated sinus group. However, there were no statistically significant differences in implant survival in the non-perforated versus perforated sinus groups. Additional studies should assess the consequences of successful and unsuccessful MSMP repairs on outcomes of implant success and

document the prevalence of possible future complications.

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VI. CONCLUSIONS

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From the results obtained in the present PhD Thesis, the following can be concluded:

i. Following the results presented in this PhD Thesis and according to evaluated histological patterns, the use of rhPDFG and rhBMP-2 demostrate that vital bone can be formed in the human maxillary sinus which will allow the placement of dental implants.

ii. The addition of rhPDGF to xenograft bone particles results in a more rapid formation of vital bone. The histological analysis of rhPDGF plus xenograft bone particles showed that the amount of new vital bone formed at four to five months after sinus augmentation is suitable for implant placement. The amount of vital bone formed at that time is comparable to the amount formed at seven to nine months without the addition of rhPDGF. These findings suggest that the addition of rhPDGF allows for earlier implant placement after sinus augmentation, reducing treatment time to between three to five months.

iii. According to the results presented in this PhD Thesis, the use of rhBMP-2 demonstrated a direct relationship between the dose of rhBMP-2 and the amount of bone formation. However there is no statistically significant difference in vital bone formation between the groups with different doses compared to the control group with agraft material only of bone allograft particles. It was demonstrated that there is an indirect relationship between the dose of rhBMP-2 and the amount of residual graft material and that higher dose of rhBMP-2 resulted in less residual graft material.

iv. The results presented in this PhD Thesis demonstrated that sinus membrane perforations, when properly repaired with collagen membranes during surgery, did not adversely effect formation of vital bone or implant survival. In fact, the augmented sinuses, with repaired membrane perforations, which occurred during the procedure, showed statistically significant greater vital bone percentages compared with the non-perforated sinus group. In this study included in the present PhD thesis, reported that there was no statistically significant difference in implant survival rates between the perforated group when compared to the non-perforated group.

IV. CONCLUSIONES

De los resultados obtenidos en la presente Tesis Doctoral, se pueden inferir las siguientes conclusiones:

i. La utilización de los factores de crecimiento rhPDFG y rhBMP-2, según el protocolo establecido en la presente tesis doctoral, pone de relieve que la generación de hueso vital en los senos maxilares humanos de acuerdo con los patrones histológicos evaluados, hace posible la colocación de implantes dentales a nivel de dichos senos.

ii. La adición de rhPDGF a las partículas de injerto óseo permite una formación más rápida de hueso vital en los senos maxilares. El análisis histológico llevado a cabo entre los cuatro y cinco meses después de la cirugía pone de relieve un incremento de hueso vital adecuado para la colocación de implantes que resultó ser similar al hueso vital que existe entre los siete y nueve meses. De ello se infiere que dicho procedimiento permite una colocación más temprana del implante reduciendo el tratamiento entre tres y cinco meses

iii. La utilización de rhBMP-2 según el protocolo descrito en la presente tesis doctoral pone de relieve una relación directa entre la dosis de rhBMP-2 y el grado de incremento de hueso neoformado sin que existan sin embargo diferencias significativas entre las dosis analizadas y el grupo control formado solo por partículas de injerto óseo. Existe asimismo una relación indirecta entre dosis de rhBMP-2 y el material de injerto residual, de tal forma que a mayor dosis menor nivel de residuo.

iv. El estudio realizado pone de relieve que Las perforaciones de la membrana del seno maxilar, cuando se reparan correctamente con membranas de colágeno durante la cirugía, no constituyen una complicación adversa en relación con la producción de hueso vital o de la supervivencia de los implantes. Los senos aumentados con perforaciones de la membrana producidas durante los procedimientos de aumento de senos mostraron un porcentaje mayor estadísticamente significativo de hueso vital en comparación con el grupo sinusal con membrana no perforada. En el estudio llevado a cabo en esta tesis doctoral no se han demostrado diferencias estadísticamente significativas, en lo que a la

supervivencia de los implantes se refiere, entre los casos con membrana perforada en comparación con los casos de membrana no perforada.

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