Is Bio-Oss® an Osteoconductive Material When Used as an Onlay Graft in Combination with a Resorbable Membrane? A Prospective Experiment in a Rabbit Model.

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Dr. Abdulaziz Abbas, D.D.S.

Supervisor:
Dr. Timothy Head, D.D.S., M.Sc.

School of Graduate and Postdoctoral Studies
Faculty of Dentistry
McGill University
Montreal, Quebec, Canada
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ABSTRACT

PURPOSE: The present investigation evaluated the osteoconductive potential of bovine bone (Bio-Oss®) as an onlay grafting material in conjunction with a bioresorbable trimethylene carbonate, L-lactide and polyglycolide barrier, in a rabbit model. **MATERIAL AND METHODS:** In twelve White New Zealand rabbits, the lateral aspect of the mandible was exposed and four monocortical holes were drilled, under copious irrigation, to expose the marrow space. A bioresorbable trimethylene carbonate, L-lactide and polyglycolide chamber filled with Bio-Oss® and autogenous blood was adapted and fixed over the drilled holes, using four bioresorbable tacks. A similar procedure was performed on the opposite side but without using Bio-Oss®, to serve as a control. After three months of healing, the rabbits were euthanized and the chambers were retrieved. The specimens then underwent a micro-CT scan and non-demineralized histological and histomorphometrical analyses. **RESULTS:** Radiographically, new bone regenerated more significantly in the Bio-Oss® chambers than in the control, at rates of 10.8% ± 2.07% and 0.29% ± 0.33%, respectively ($p < 0.0001$). Also, the height of calcified tissue regenerated was greater in the Bio-Oss® chambers, measuring at 2.25 mm (75% of the total height under the chamber) ± 0.38 mm (12.67%) versus 1.35 mm (45%) ± 0.46 mm (15.33%) regenerated in the control chambers ($p = 0.0002$). Histomorphometrically, new bone volume percentage was significantly greater in the Bio-Oss® chambers compared to the control chambers, which measured at 18.28% ± 3.69% and 3.28% ± 2.0%, respectively ($p < 0.0001$). Partial degradation of the bioresorbable chambers was observed with collapse and distortion occurring in 16.67% of the Bio-Oss® filled chambers compared to 75% of the control chambers. **CONCLUSION:** Bio-Oss® was proven to be a good osteoconductive bone substitute when used under trimethylene carbonate, L-lactide and a polyglycolide bioresorbable barrier in a guided bone regeneration model in rabbits. However, further investigations in higher animal and human models are needed to confirm our results, prior to recommending this technique for clinical practice.

Résumé

OBJET: La présente enquête évalue le pouvoir ostéoconducteur de l'os bovin (Bio-Oss®) comme matériau de greffe par apposition en association avec un carbonate de triméthylène biorésorbable, du L-lactide et une barrière polyglycolide, sur le modèle du lapin. **MATÉRIEL ET MÉTHODES:** Sur douze lapins albinos New Zealand, l'aspect latéral du maxillaire inférieur a été exposé et quatre orifices monocorticaux ont été percés sous irrigation abondante pour exposer l'espace médullaire. Un carbonate de triméthylène biorésorbable, du L-lactide, une chambre polyglycolide remplie de Bio-Oss® et du sang autogène ont été adaptés et fixés sur les orifices percés en utilisant quatre tacks biorésorbables. Une procédure similaire a été effectuée du côté opposé mais sans utiliser Bio-Oss®, à fins de contrôle. Après trois mois de guérison, les lapins ont été euthanasiés et les chambres ont été prélevées. Les spécimens ont ensuite subi un scanner X haute résolution (micro-CT scan) et des analyses histologiques et histomorphométriques non-déminéralisées. **RÉSULTATS:** Radiographiquement, l'os s'est régénéré plus significativement dans les chambres avec Bio-Oss® que dans les chambres de contrôle, à des taux de 10,8% ± 2,07% et 0,29% ± 0,33%, respectivement ($p < 0,0001$). Également, la hauteur du tissu calcifié régénéré a été plus grande dans les chambres avec Bio-Oss®, mesurant 2,25 mm (75% de la hauteur totale sous la chambre) ± 0,38 mm (12,67%) contre 1,35 mm (45%) ± 0,46 mm (15,33%) régénérés dans les chambres de contrôle ($p = 0,0002$). Histomorphométriquement, le pourcentage en volume d'os neuf a été significativement plus grand dans les chambres avec Bio-Oss® que dans les chambres de contrôle, avec 18,28% ± 3,69% et 3,28% ± 2,0%, respectivement ($p < 0,0001$). Une dégradation partielle des chambres biorésorbables a été observée avec affaissement et distorsion chez 16,67% des chambres remplies avec Bio-Oss® comparés à 75% des chambres de contrôle. **CONCLUSION:** Il a été prouvé que Bio-Oss® est un bon substitut osseux ostéoconducteur quand il est utilisé sous carbonate de triméthylène, L-lactide et une barrière polyglycolide biorésorbable dans un modèle de régénération osseuse guidée chez le lapin. Toutefois, d'autres investigations sur des modèles animaux supérieurs et humains sont nécessaires pour confirmer nos résultats avant de recommander cette technique pour la pratique clinique.

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CHAPTER ONE
INTRODUCTION AND BACKGROUND

1.1.0 General Introduction

Due to significant advancements in biomaterials, design, surgical techniques and loading conditions, dental implant surgery has become a very predictable modality for restoring the function and aesthetics of edentulous dental arches (Albrektsson et al., 1987) (Adell et al., 1981). The success of implant surgery relies mainly on the concept of osseointegration that was initially described by Branemark in 1969. The concept describes the interface between a stable implant and bone, as a "direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant" (Branemark, 1985). Such direct, structural and functional connection between bone and implant has to occur at 50% or more of the total intraosseous implant surface and 90% or more of its cortical passage, in order to produce resistance to shear and tensile forces (Albrektsson, et al., 1987).

In order for an implant to successfully osseointegrate, its primary stability has to be achieved during placement and in the initial healing phase. Mobility of the implant after placement and in the initial healing phase is the main reason for soft tissue encapsulation and failure of implant anchorage (Uhthoff, 1973) (Cameron et al., 1973). Therefore, adequate bone quality and quantity is essential to ensure sufficient primary stability and thus successful osseointegration. Clinical correlation between poor anatomic structure, with regard to bone quality and quantity, and increased implant failure rate exists and is proven valid through well-designed clinical trials (Ekfeldt et al., 2001). In many clinical situations, placement of osseointegrated dental implants is complicated by suboptimal bone quantity and quality because of trauma, periodontal disease, traumatic dental extractions and defects created following removal of pathological lesions (Barber et al., 1993; Clokie et al., 2002) (Gbara et al., 2007) (Lacerda et al., 2009). To overcome this problem, different bone grafting techniques and materials have

been developed and used to augment the alveolar ridge, in an attempt to restore its quality and quantity.

Autogenous bone is considered the material of choice for grafting bone defects in the oral and maxillofacial region (Barone et al., 2007; Haas et al., 1998). Autogenous bone is osteogenic, biocompatible, does not elicit an immune response and is not associated with the risk of disease transmission (Keating et al., 2001). However, autogenous bone has its shortcomings, whether it is harvested from an intraoral or extraoral site. Harvesting bone from an intraoral site is associated with increased morbidity such as pain, increased risk of infection and neurosensory deficit. Also, intraoral sites do not provide sufficient quantities of bone for a grafting medium on large alveolar defects (Nkenke et al., 2002). When a larger quantity of bone is needed, an extraoral site such as the anterior or posterior ilium is the donor site of choice. Harvesting bone from extraoral sites is also not without risks and possible complications such as; extended anesthetic and surgical time, prolonged pain, bleeding, gait disturbance and neurosensory deficit (Cricchio et al., 2003) (Hallman et al., 2001; Norton et al., 2003). In addition, endochondral bone grafts such as those harvested from the ilium and ribs are known to have a high resorption rate during the course of healing (Borstlap et al., 1990; Zins et al., 1983).

To avoid the drawbacks of autogenous bone grafts, various grafting techniques and materials were developed and investigated. These techniques and materials yielded variable levels of effectiveness and predictability, when compared to autogenous bone. Therefore, to be able to better evaluate these materials, a thorough understanding of the characteristics and mechanisms by which autogenous bone grafts heal is essential.

1.2.0 Principles of Autogenous Bone Graft Healing

The incorporation of bone grafts into the recipient bone bed depends on three main factors: graft revascularization, host-graft union and new bone regeneration (Stevenson et al., 1996).

The presence of an independent vascular supply (pedicle) with the bone graft optimizes the quality and speed of revascularization, which results in faster incorporation and reliable healing of the graft (Stevenson, et al., 1996). In nonvascularized bone grafts, revascularization occurs mainly through the ingrowth of blood vessels from the recipient bone into the graft. This ingrowth of blood vessels occurs at a slow rate and thus prolongs the healing time of the graft compared to its vascularized counterpart. However, due to the technique sensitivity and associated morbidity with harvesting such grafts (flaps), using a vascularized bone graft (flap) is not usually recommended when small to medium size alveolar augmentation is all that is required, for the placement of dental implants. Host-graft union is aided by stabilization and fixation of the bone graft to the recipient bone. This is important for maximizing graft-host bone contact and ensuring successful ingrowth of perforating vessels into the graft.

As a consequence of successful vascularization and stabilization of the graft, new bone regeneration takes place. In an autogenous bone graft, new bone generation relies on the presence of three distinct characteristics -osteogensis, osteoinduction and osteoconduction- which are more or less present when different grafting materials are used. (Soost et al., 2001).

Osteogensis is defined as the generation of new bone from osteoprogenitor cells that either exist in the host bone or come from the grafted bone. Osteogenic grafts are very effective and predictable materials and have the advantage of being osteoinductive and osteoconductive as well. Autogenous bone graft is considered the only graft that is osteogenic in nature. (Abubaker et al., 2007; Albrektsson et al., 2001).

Osteoinduction is the process by which undifferentiated pluripotent cells are stimulated to differentiate into bone-forming cells by means of inducing compounds (Albrektsson, et al., 2001). Pluripotent cells are present in bone and are essential for bone healing. These cells require the correct stimulus to be recruited and induced to differentiate into pre-osteoblasts and subsequently to bone forming osteoblasts. Bone morphogenic proteins (BMPs) are the most commonly known osteoinductive agents. They are glycoproteins that belong to

the transforming growth factors β (TGF-β) super-family. There are many BMPs which possess variable degrees of osteoinductive activities. BMP-2, BMP-6, BMP-7, and BMP-9 are considered the most effective ones. In the presence of these proteins, bone formation occurs by means of endochondral ossification (Keating, et al., 2001).

Osteoconduction is a characteristic of a graft that permits migration of cells and ingrowth of blood vessels into and onto its surfaces. The graft is bioinert and serves only as a structural scaffold for vascular ingrowth and bone formation. A graft which is only osteoconductive cannot initiate or induce bone formation (Cornell et al., 1998) (Abubaker, et al., 2007; Albrektsson, et al., 2001).

1.3.0 Bone Graft Substitutes

1.3.1 Allogenic Bone Substitutes

Due to the limited supply and morbidity associated with autogenous bone harvesting, allografts have been researched and used clinically as an alternative grafting material (Eppley et al., 2005). An allogenic bone graft is a graft harvested from an individual of the same species. It is supplied in various forms such as cortical, cortico-cancellous, or cancellous with different configurations such as powder, cortical chips, cancellous cubes and cortical struts (Stevenson, 1999). It also can be processed as mineralized or demineralized, fresh-frozen or freeze-dried forms. The processing procedure is the principle factor in determining the biological and physical properties of the material.

Fresh-frozen allogeneic bone is derived from patients undergoing total hip replacement or from a fresh cadaver source (Perrott et al., 1992). It is minimally processed and its cellular and organic content is preserved. It is thought that fresh-frozen bone leads to better graft vascularization, incorporation and bone formation (Perrott, et al., 1992). Several studies found that fresh-frozen bone is effective and reliable as an inlay and onlay grafting material in restoring atrophic alveolar bone in humans (C. Contar et al., 2010; C. M. Contar et al., 2009; Franco et al., 2009). The material is also found to have a predictable pattern of resorption that mainly occurs throughout the first year of healing (Franco, et al., 2009).

However, the risk for disease transmission, although small, is still the most feared factor in considering this type of allogeneic graft, followed by the risk of immunocompatibility problems (Buck et al., 1989; Buck et al., 1990).

The freeze-dried allogeneic bone graft is another commonly used form that is easy to handle and convenient to store at room temperature (Stevenson, 1999). Freeze-drying creates a safer graft in terms of reducing the risk of host immunologic reactions and transmitting diseases (Bauer et al., 2000; Eppley, et al., 2005). However, it kills the osteopotent cells and denatures proteins in the graft, rendering it only osteoconductive, without any osteoinductive potential (Eppley, et al., 2005). Compared to autogenous bone grafts, freeze-dryied bone takes longer to become vascularized and thus incorporated (Perrott, et al., 1992). Freeze-drying also reduces the mechanical strength of the graft.

Allogeneic bone can also be processed so that its mineral content is removed. Demineralized bone matrix (DBM) is a term used when the demineralization process reduces the calcium content of bone to 8% or less. Reported advantages of this type of allogeneic graft are: diminished immune response and disease transmission, as a consequence of the demineralization treatment with hydrochloric acid increased osteoinductive potential due to the increased exposure of the graft's bone morphogenic proteins and enhanced osteoconductive potential through the abundant collagen matrix (Eppley, et al., 2005; Urist, 1965). DBM was found to effectively promote new bone formation when used as an inlay graft and in maxillary sinus floor augmentation. Clokie et al. found that, in rabbits, DBM resulted in 87.1% bone fill of iatrogenically created calvarial defects by six weeks and almost complete bone fill after twelve weeks (Clokie, et al., 2002). Moghadam et al. conducted a similar study and found that DBM, with or without calcium hydroxide treatment, was effective in grafting critical size defects created in rabbit calvaria compared to calcium-sulfate materials (Moghadam et al., 2004). Froum et al. also found that demineralized allograft is superior to anorganic xenograft (Bio-Oss®) in the amount of new bone generation when used to augment the maxillary sinus floor (Froum et al., 2006). However, the main drawbacks of DBM grafts are; their lack of mechanical

strength, difficulty in handling and manipulation and risk of disease transmission (although less than other allogenic types) (Eppley, et al., 2005).

Despite the studies that proved the efficacy of allografts in promoting bone generation, evidence of the ineffectiveness of some types of allografts also exists in the literature. Becker and Urist found that demineralized freeze-dried bone failed to promote new bone formation in human extraction sockets and bony defects in mice (Becker et al., 1994; Becker et al., 1995). Others have found that allogenic grafts are no more than weak osteoconductors (Jensen et al., 1998).

The use of allogenic bone as an onlay grafting material is not extensively reported in the literature. A few observational studies, such as case reports and clinical series, have described the use of allogenic bone blocks as an onlay grafting material in the alveolar bone (Barone et al., 2009; Waasdorp et al., 2010). However, due to the use of different treatment approaches, different types of allogenic grafts, short clinical follow-up periods and lack of appropriate study design, the data presented in the literature does not give clear evidence about the effectiveness and predictability of allogenic bone as an onlay grafting material. (Waasdorp, et al., 2010).

For these reasons along with the known risks of disease transmission and host immune reactions, other materials with a potential for more desirable properties have been developed and investigated.

1.3.2 Alloplastic Bone Substitutes

Alloplastic bone substitutes are purely osteoconductive, synthetic, bone grafting materials (Eppley, et al., 2005). Besides being used as the sole grafting material, they are also used either in combination with autogenous bone grafts to gain more graft volume, or as a carrier for osteoinductive preparations (Keating, et al., 2001; Ohgushi et al., 1989). Common types of alloplastic bone substitutes are the derivatives of calcium phosphate and calcium sulfate compounds. When either compound is processed under high temperature, in a process called sintering, crystalline structures of variable mechanical and degradation properties are produced and are termed ceramics (Eppley, et al., 2005; Keating, et al., 2001).

The commonly investigated and used alloplastic ceramics are synthetic hydroxylapatite crystals and β-tricalcium phosphates (Keating, et al., 2001; Miloro, 2004).

Synthetic hydroxyapatite is a slowly reabsorbing highly crystalline structure that mimics the mineral phase of bone. The osteoconductivity of synthetic hydroxyapatite is controversial. While some authors found promising results regarding bone conduction of these materials (Johnson et al., 1996), others have found the opposite. In a Masters Thesis project conducted by Al-Masri, synthetic hydroxyapatite plus tricalcium phosphate (Straumann Bone Ceramic®) proved to be a good osteoconductive material both histologically and radiographically, when used as an onlay graft under a polylactic/polyglycolic acid bioresorbable barrier in rabbit mandibles (Al-Masri, 2009). On the other hand, Cancian et al. found that synthetic hydroxyapatite (Calcitite®), compared to bioactive glass, did not result in bone generation, when used as an inlay graft in the mandibles of four monkeys (Cancian et al., 1999). Clokie et al. reached a similar conclusion with synthetic hydroxyapatite bone paste (Bone Source®) and calcium phosphate bone cement (Norian CRS®). He found that these materials acted as physical barriers to new bone generation and were difficult to handle (Clokie, et al., 2002). In addition, this material has not been studied for different grafting techniques. For example, most of the literature available on synthetic hydroxyapatites consists of investigation of the material as an inlay graft and not enough data is available to give consensus on the use of the material as an onlay graft (Eppley, et al., 2005).

β-tricalcium phosphate is another available ceramic material that has been recently added to the armamentarium used in grafting bony defects in the oral and maxillofacial region. Some authors found that β-tricalcium phosphate is a good osteoconductive material (Zerbo et al., 2004). However, others found that this material is unreliable due to its early reabsorption during the course of healing, independent of new bone formation. Consequently, this leads to insufficient bone generation due to the early loss of the graft scaffold structure (Hirota et al., 2009). To overcome this problem, a mixture of β-tricalcium phosphate and another bone

substitute such as allogenic bone or synthetic hydroxyapatite is used (Hirota, et al., 2009).

Bioactive glass is an easy to handle alloplastic material composed of silicon dioxide, sodium oxide, calcium oxide, and phosphorous pentoxide (Wheeler et al., 2000). Bioactive glass has the ability to bind to hard and soft tissue, which enhances bone-graft contact and reduces the amount of soft tissue incorporation in the graft (Finkemeier, 2002; Wheeler, et al., 2000). In the literature, bioactive glass has resulted in variable amounts of new bone generation (Stavropoulos et al., 2003). Some authors found that this material is osteoconductive(Cancian, et al., 1999; Tadjoedin et al., 2002), while others found that it obstructs long-term bone formation (Stavropoulos, et al., 2003; Stavropoulos et al., 2004).

Calcium sulfate (also known as plaster of Paris) is considered the oldest alloplastic material used in bone grafting procedures. It has a simple chemical structure, calcium sulfate dihydrate. However, it is not very reliable clinically. When used, the setting chemical reaction leads to variable crystalline structures and thus inconsistent mineral properties. Subsequently, this leads to rapid resorption, which exceeds the capacity of the host tissue to generate bone (Keating, et al., 2001).

Advances in recombinant technology enabled scientists and researchers to recruit large quantities of a single or multiple bone morphogenic proteins (BMPs) for use in bone augmenting procedures. In augmentation of defects and atrophic ridges in the oral and maxillofacial region, recombinant BMP-2 carried in absorbable collagen sponge was found to be effective. Triplett et al. used recombinant BMP-2 carried in collagen to augment the posterior maxilla using the sinus lift technique. He found that a significant amount of new bone, that is suitable for the placement of dental implants, was generated, when compared to an autogenous bone graft. (Triplett et al., 2009). Despite his promising result, research on the use of these proteins and their techniques in the maxillofacial region is still in its infancy.

In summary, the majority of alloplasts have been used largely because of their osteoconductive potential and unlimited supply. However, some studies found that some alloplasts inhibited new bone formation (Clokie, et al., 2002; Moghadam, et al., 2004; Stavropoulos, et al., 2003). Despite the large variety of alloplastic materials available for clinical use, the literature still lacks data on their effectiveness and predictability in living tissue. Besides, some alloplastic materials are found to be technique sensitive and produce byproducts that elicit host immune response (Kurashina et al., 1998; Moghadam, et al., 2004). Osteoinductive materials such as recombinant bone morphogenic proteins delivered in carriers as bone substitutes are promising materials, yet they are new and require more investigation.

1.3.3 Xenogenic Bone Grafts

Another alternative to autogenous bone is a xenograft. Xenograft is a tissue harvested from one species and transplanted into an unlike species (Abubaker, et al., 2007). The common available xenografts are derived from coralline, porcine and bovine sources (Zhang, 2006).

Coralline hydroxyapatite is porous osteoconductive mineral, derived from sea coral, that resembles human cancellous bone. This xenograft is supplied in two forms "natural or synthetic," depending on the processing method of sea coral. The natural form is coral mineral that is cleaned and sterilized only, whereas the synthetic form is processed so that the carbonate component of the mineral is substituted with phosphates. Available synthetic forms are Interpore™ and Pro-Osteon™ (manufactured by Interpore International, Inc, Irvine, CA) (LeGeros, 2002).

Porcine hydroxyapatite is another source of xenogeneic hydroxyapatite. Pig bone mineral has shown to be osteoconductive, biocompatible and slowly resorbable. A common available form is Gen-Os™ (by *OsteoBiol*, Tecnoss, Italy). Gen-Os™ also contains collagenous material that is thought to facilitate clotting, subsequent repairing and bone generation (Wlodarski et al., 2005).

Bovine hydroxyapatite is by far the most commonly used and researched xenogeneic bone graft. As is the case for the other xenogenic materials, it has been shown to exhibit good osteoconductive potential in several studies and reports (Norton, et al., 2003). The commercially available forms of bovine mineral are: (1) unsintered (non-heat treated) with organic matrix, (2) unsintered without organic matrix (Bio-Oss®, Geistlich Biomaterials, Geistlich, Switzerland) and (3) sintered without organic matrix (Osteograf-N™, CeraMed Co, Denver, CO & Endobon™ Merck Co, Darmstadt, Germany) (LeGeros, 2002).

The unsintered form without organic matrix is by far the most clinically used bovine mineral. Being unsintered means that the mineral is not heat processed. Therefore, it has less crystalline structure and small particles than its sintered counterparts. Unsintered minerals are better reabsorbed and incorporated into the host bone. The lack of organic matrix adds the advantage of being more biocompatible with diminished potential for foreign body reaction and inflammation in humans (Cohen et al., 1994; Yildirim et al., 2000).

1.3.4 Bio-Oss® - Bovine Hydroxyapatite

Bio-Oss® (Geistlich, Wolhusen, Switzerland) is a commercial unsintered, inorganic, bovine, mineral matrix of calcium-deficient, carbonate apatite (Kasabah et al., 2002). It is supplied in block or granular forms of different particle sizes. It is highly porous and exhibits a large inner surface area to facilitate vascular ingrowth, osteoblastic proliferation and thus new bone generation (Peetz, 1997). The resemblance between the morphological and physical structure of Bio-Oss® and human cancellous bone facilitates harmonious bone apposition and particle resorption when used to augment bony defects in humans (Fukuta et al., 1992; Peetz, 1997; Yildirim, et al., 2000).

The biocompatibility and osteoconductivity of Bio-Oss® are well documented in the literature. Klinge et al. compared Bio-Oss® to synthetic forms of hydroxyapatite in grafting of intrabony defects created in rabbit calvaria. Histologically, he found that the Bio-Oss® was osteoconductive and resulted in

better quality and quantity, of newly generated bone, than the synthetic hydroxyapatites (Klinge et al., 1992).

Thaller et al. augmented intrabony defects in rabbit calvaria with Bio-Oss® versus Bio-Oss® with collagen. He found significant new bone formation in both Bio-Oss® groups versus control at 2, 4, 6, 8, 12, and 16 weeks postoperatively (Thaller et al., 1994).

Terheyden et al. used Bio-Oss® in maxillary sinus floor grafting in pigs. He compared Bio-Oss® plus osteogenic protein-1 (BMP-7) and Bio-Oss® alone. He found that significant amounts of new bone were generated in both groups with the exception that the Bio-Oss®+BMP-7 group resulted in faster apposition and better quality of bone. The only downside of this study is its lack of an appropriate control (Terheyden et al., 1999).

Cordaro et al. compared Struamann Bone Ceramic® and Bio-Oss® in grafting the maxillary sinus floor. He found that both materials were biocompatible, resulting in significant new bone formation that is suitable for dental implant placement. However, the Bio-Oss® group exhibited more bone-particle contact, less connective tissue incorporation and more residual particles after 180-240 days (Cordaro et al., 2008).

A similar conclusion was reached by Valentinit et al. who found that sinus floor grafting with bovine HA resulted in 21.08% and 27.55% of newly generated bone after six and twelve months of healing, respectively. Fifty-seven delayed implants were inserted with a four-year success rate of 98.2% (Valentini et al., 2000).

Although the literature lacks adequate data on the use of Bio-Oss® as an onlay graft, there are few published studies that found Bio-Oss® to be effective when used as an onlay graft. In six patients, Zitzmann et al. augmented maxillary alveolar deficiencies with onlay Bio-Oss® mixed with powder tetracycline and covered with collagen membrane. Re-entry biopsy and subsequent dental implant placements were performed after 6-7 months of healing. Histologically, significant amounts of new bone regenerated in the grafted sites with an average bone-particle contact of 36.7% (Zitzmann et al., 2001).

In fifteen patients, Norton et al. used Bio-Oss® as an onlay graft in augmenting atrophic alveolar ridges and as a filler graft in fresh extraction sockets. All grafted sites were covered with collagen membranes. After 4-10 months of healing, the osteoconductive behavior of Bio-Oss® was confirmed in 80% of the patients. Although the amount of new bone formed varied between grafted sites (average = 26.9%), the augmented ridges resulted in good bone quantity and quality sufficient for the placement of dental implants (Norton, et al., 2003).

In a Masters thesis written by Al-Harkan, the use of Bio-Oss® as an onlay graft under a titanium chamber was tested on the mandibles of eight rabbits. Histologically, he found that Bio-Oss® resulted in significant amounts of new bone formation compared to the control (empty chambers). He concluded that Bio-Oss® is a good osteoconductive material when used as an onlay graft in a guided bone regeneration model (Al-Harkan, 2008). Although many studies have proved the osteoconductivity and predictability of Bio-Oss®, some published studies and case reports found that Bio-Oss® inhibited new bone formation or interfered with long-term bone generation. Stavropoulos et al. used Bio-Oss® as an onlay graft under Teflon capsules in a guided bone regeneration model in rats. Histologically, he found that Bio-Oss® interfered with new bone generation and that empty capsules (control) resulted in more new bone generation at two and four months (Stavropoulos et al., 2001).

In 2003, the same author conducted another study with a similar design in order to test the osteoconductivity of Bio-Oss® and bioactive glass (Biogran®) in a guided bone regeneration model. After one year of healing, he found that bone generation in the Bio-Oss® and bioactive glass groups was inferior to the control group with values of new bone of 23%, 12.6% and 88.2%, respectively (Stavropoulos, et al., 2003).

In a study by Pinholt et al., sintered and unsintered bovine minerals were used as onlay grafts in rats' maxilla in order to test for potential osteoconduction. The minerals were also heterotopically grafted in the abdominal muscles to test for their osteoinductive potential. At four weeks, histological evaluation revealed

that neither osteoconduction nor osteoinduction of the minerals was observed. Instead, foreign body reaction was evident around the mineral particles. Partial resorption of the mineral particles was also observed (Pinholt et al., 1991).

Carmagnola et al. conducted a study investigating the effect of a protein sealant (Tisseel®) when mixed with Bio-Oss® in grafting mandibular defects in dogs. She found that Tisseel® had retarded new bone formation. However, the interesting finding was that the amounts of new bone formed in both the Bio-Oss® and the Bio-Oss® + Tisseel® groups were inferior to the amounts of bone formed in the control group, which filled up almost completely with new bone in three months (Carmagnola et al., 2002). In 2003, the same author conducted another study using Bio-Oss® to preserve fresh extraction sockets in 21 patients. The sockets were covered with collagen membranes alone, filled with Bio-Oss® and covered with a membrane, or left to heal spontaneously (control). She found that the collagen membrane alone group resulted in almost complete bone fill whereas the Bio-Oss® + membrane group filled with connective tissue surrounding the Bio-Oss® particles with minute amounts of new bone formation after seven months of healing (Carmagnola et al., 2003).

The resorbability of Bio-Oss® is another controversial issue. In general, the ideal grafting material is preferred to have a slow resorption rate. Fast resorption might result in loss of the graft prior to adequate bone conduction, and non-resorption might result in shielding of the newly formed bone from physiological stresses necessary for further remodeling and maturation. Different authors reached different conclusions regarding the resorbability of Bio-Oss®. In Klinge's study (1992) which was presented earlier, Bio-Oss® showed evidence of osteoclastic resorption 4 to 14 weeks after being grafted to rabbit calvaria (Klinge, et al., 1992). Zitzmann et al. (2001) also found evidence of osteoclastic resorption of Bio-Oss® with appearent Howship's lacunae 6 to 7 months after being grafted in the maxillary sinus floor of six patients. In a case report by Sartori, Bio-Oss® was grafted in the maxillary sinus of a 60-year-old man, with simultaneous placement of implants. Biopsies taken, at eight months, two years and ten years postoperatively, suggested a slow resorption process with an initial rate of 56.8%

in the first two-years where Bio-Oss® was replaced by lamellar bone (Sartori et al., 2003). However, in the Stavropoulos study (2001), no histologic evidence of Bio-Oss® resorption was found after one, two and four months of being grafted in rat mandibles (Stavropoulos, et al., 2001). Schlegel et al. used Bio-Oss® to graft jaw defects in seventy-one patients. On radiographic and histologic investigation, he found that Bio-Oss® particles persisted in the grafted area and did not resorb for six years and longer (Schlegel et al., 1998). In a case report by Valentini et al., a biopsy taken twelve months after maxillary sinus grafting with Bio-Oss® revealed the persistence of particles with no evidence of resorption (Valentini et al., 1998).

The safety of Bio-Oss® is another issue of concern to clinicians and patients. Altered bovine proteins (prions) are known to cause bovine spongiform encephalopathy and Creutzfeldt-Jakob disease (CJD). In a case report, Will et al. reported that a variant of CJD has been diagnosed in patients who ingested infectious bovine food (Will et al., 1996). Therefore, Wenz et al. conducted a study to investigate the effectiveness of prion inactivation in bovine mineral processing. He concluded that the processed bovine mineral (Bio-Oss®) is safe and poses no risk of disease transmission (Wenz et al., 2001). In another study by Benke et al., Bio-Oss® was subjected to detailed biochemical, histochemical and biophysical analysis and was confirmed to be safe and to contain no detectable amount of bovine proteins (Benke et al., 2001).

Bio-Oss® is a readily available bone grafting substitute that has been studied by many. Despite the few reports that raised some uncertainty regarding the osteoconductivity of Bio-Oss®, by and large, the biocompatibility and osteoconductivity observed in many other published animal and human studies seem to indicate the reliability and predictability of the material. However, the literature still lacks data and is inconsistent regarding Bio-Oss® use as an onlay grafting material. For this reason, we chose to investigate this material as an onlay graft in a guided bone regeneration model.

1.4.0 Guided Bone Regeneration (GBR)

1.4.1 Inlay and Onlay Bone Graft

Inlay bone graft is a term used when a graft is used to fill a bone defect within the confines of the anatomical skeleton. For example, the graft needed to fill a three-wall periodontal bony defect is an inlay graft. By contrast, when a graft is used to augment atrophic bone outside of the boundaries of the anatomical skeleton, the term onlay graft is more appropriate. An example of an onlay graft is the graft needed to increase the atrophic alveolar bone width of a future implant site. This three-dimensional positioning of the graft has an influence on the course of healing and thus the graft's success. Onlay grafts undergo a bit more complicated healing course than inlay grafts, which are confined within the existing anatomical boundaries and surrounded with host bone. Onlay grafts have a higher resorption rate in comparison to inlay grafts (Cordaro et al., 2002; Jensen, 2006; Stellingsma et al., 1998). The increased resorption seen in onlay grafts is due to two main reasons: (1) onlay bone grafts are less exposed to the recipient bone vasculature, which results in less bone apposition and remodeling; (2) onlay bone grafts are exposed to more biological forces from the surrounding soft tissue leading to more osteoclastic resorption in the areas exposed to these forces (Araujo et al., 2002; Hodges et al., 2006). Beside exerting more biological forces on onlay grafts, the fast proliferating soft tissue can compete with the slowly proliferating osteoprogenitor cells in occupying the grafted space, especially when a particulate onlay graft is used (Dahlin et al., 1989). To overcome this problem, a guided bone regeneration technique was developed.

1.4.2 Guided Bone Regeneration Technique

Guided bone regeneration (GBR) technique relies on the mechanical hindering of cellular components with fast proliferation rates, such as fibroblasts, from entering the grafted area in an attempt to give the time and space needed for the slowly proliferating osteoproginitor cells to deposit bone tissue (Dahlin, et al., 1989). When a particulate bone graft is used as an onlay graft, the guided bone regeneration model is assumed successful if it can: (1) impede fibroblasts migration into the grafted area, (2) provide stability for the graft material, and (3)

maintain the grafted space during the healing phase. To satisfy these criteria, a biocompatible barrier with desirable mechanical and structural properties is used (Becker et al., 1990; Buser et al., 1990). Different types of resorbable and nonresorbable barriers have been developed and will be discussed in the following sections.

1.4.3 Bone Graft and GBR

It has been observed in several published studies that guided bone regeneration models resulted in significant amounts of bone generation without using bone graft materials (refer back to the Bio-Oss® section for studies published by Stavropoulos 2001 & 2003; Pinholt 1991; Carmagnola 2002 & 2003). However, in order for a GBR model to be effective without the use of a bone graft or substitute, the space under the barrier must be maintained throughout the healing period, and this space should be of reasonable dimensions. However, in most clinical scenarios the use of bone graft material can be beneficial in two ways: (1) it acts as a physical support for the barrier preventing its collapse; (2) it can enhance the quality and quantity of new bone generated by means of osteogensis, osteoinduction and/or osteoconduction depending on the properties of material used (Simion, Baldoni, et al., 1994).

However, the benefit of different types of bone substitutes used as onlay grafts in guided bone regeneration models has not been established in the literature, due to the lack of publish data. This is also the case for bovine hydroxyapatite as we saw in the Bio-Oss® section of this paper where the few published studies reached different conclusions.

1.4.4 Guided Bone Regeneration Barriers

Non-resorbable Barriers

Different types of non-resorbable barriers have been investigated and described in the literature. Expanded polytetrafluroethylene (PTFE) with or without titanium support is one of the oldest non-resorbable barriers used in alveolar guided bone regeneration. It underwent extensive research and was

found to be an excellent GBR device in both animal and human studies (Buser et al., 1993; Buser et al., 1995). However, it has a few drawbacks that led researchers to continue the search for a more ideal barrier. The main drawbacks of expanded PTFE are: (1) barrier exposure during healing, (2) susceptibility for bacterial colonization and infection and (3) the need for a second surgical procedure for barrier removal (Lekovic et al., 1998; Lekovic et al., 1997). Simion et al. found that early barrier exposure hinders bone generation by 96.6%, when expanded PTFE was used around immediate dental implants placed in recent extraction sockets (Simion, Trisi, et al., 1994). To overcome this problem, a modified form of this barrier has been developed. The nonexpanded or dense PTFE is known for its simplicity and ease of surgical manipulation. This barrier can be left intentionally exposed without considerable risk of bacterial colonization. Thus, primary closure is not required, which eliminates the need for extensive flaps and minimizes the risk for surgical complications (Barber et al., 2007; Bartee, 1998, 2001). The decreased risk of bacterial colonization in the dense PTFE is attributed to the low porosity of the barrier material, which prevents bacteria from being incorporated into the barrier(Bartee, 2001). Despite the advantage this barrier has over the expanded PTFE, the need for a second meticulous removal surgery and the risk for exposure, although low, keeps the search for a more ideal material ongoing.

Titanium Mesh

Titanium mesh is a biocompatible and durable material used to support onlay autogenous bone grafts in reconstruction of atrophic alveolar bone. Boyne et al. investigated the use of titanium mesh to support onlay autogenous bone, in augmenting severely atrophic maxillary ridges in fifteen patients, followed for 3-10 years. The technique resulted in good amounts of new bone generation with graft resorption not exceeding 10-20%, in height. The titanium mesh provided only structural support without any occlusive property against the ingrowth of soft tissue. When the mesh was removed, a thick layer of connective tissue was

encountered on the surface of the newly generated bone, underneath the mesh (Boyne et al., 1985).

In two separate studies published by Von Arx et al., atrophic ridges were augmented with autogenous onlay graft supported by titanium mesh. In both studies, the technique proved successful in augmenting ridge height and width with minimal resorption. The minimal resorption was attributed to the shield effect, of the titanium mesh, against exterior trauma and non-functional forces (Von Arx et al., 1996; Von Arx et al., 1998).

Therefore, the titanium mesh is an excellent material for providing good structural support for autogenous bone grafts. However, the fact that it is not resorbed and does not prevent the ingrowth of soft tissue can be a disadvantage. This is especially true when it is used in combination with bone substitutes which lack osteogenic potential and require good isolation to induce or conduct bone generation.

Resorbable Barriers

The first line of resorbable barriers available is cross-linked type I & III collagen barriers derived from porcine or bovine sources (Bunyaratavej et al., 2001). Among the advantages of collagen barriers, they are known to be: (1) biocompatible, (2) capable of being integrated into host tissue, (3) occlusive against unwanted cells, (4) semi-permeable, thus facilitating nutrient transfer to the grafted area, and (5) easy to manipulate and adapt (Hardwick et al., 1994; Schwarz et al., 2006). In addition, collagen barriers are haemostatic in nature and are thought to aid in early wound stabilization (Postlethwaite et al., 1978). Schwarz et al. hypothesized that collagen barriers allow early angiogenesis and vascular perforation from the flap side, into the graft area. This could enhance *de novo* bone formation away from the host bone (Schmid, Wallkamm, et al., 1997; Schwarz, et al., 2006). However, the main drawbacks of collagen barriers are: (1) rapid biodegradation rate induced by enzymatic activity, and (2) lack of structural strength to maintain the secluded space and stabilize the bone graft (Rothamel et al., 2005; Sela et al., 2003).

To slow down the rate of resorption, more chemically sophisticated cross-linked collagen barriers were developed, to prolong their enzymatic degradation time. However, prolonging biodegradation comes at the expense of decreased vascularization and the exacerbation of foreign body reaction toward these materials (Kodama et al., 1989; Rothamel, et al., 2005).

The remaining major disadvantage is the lack of structural strength of collagen barriers. To address this, researchers started to investigate the use of polymer compounds that provide a stiffer, and stronger barrier material, that is still able to biodegrade and resorb.

Derivatives of the aliphatic polyester family of polymers have been extensively used in manufacturing resorbable surgical devices, for decades. Of this family of polymers, poly α-hydroxy acids such as polyglycolic and polylactic acids are the most commonly used, in making resorbable surgical sutures and meshes. In the GBR arena, the literature shows that these polymers are biocompatible, biodegradable and bioresorbable, and yet mechanically strong and durable materials for space maintaining during healing (Bostman et al., 1989; Manninen et al., 1992; Mellonig et al., 1998; Suuronen, 1991). In addition, these polymers are easy to handle and provide a good barrier, against the ingrowth of unwanted cells. They were also shown to integrate well in the recipient tissues (Mellonig, et al., 1998).

In contrast to collagen barriers that resorb by means of enzymatic activity, poly α-hydroxy acids first degrade by means of hydrolysis into monomer acids. These monomer acids are then resorbed by the body and converted to carbon dioxide and water through the Krebs cycle (Mellonig, et al., 1998; Spenlehauer et al., 1989). However, when the barrier is composed of only one polymer, the single degradation byproduct substantially increases in concentration, leading to severe local inflammatory reaction. For this reason, most barriers are now made of more than one polymer (copolymer) so that when the barrier degrades, it produces multiple monomer byproducts with lower concentrations and less local inflammation.

Many co-polymers with different compositions have been manufactured and used clinically. However, of particular interest to us is the co-polymer composed of trimethylene carbonate, poly L-lactide, and polyglycolide, which is used in making the bioresorbable barrier used in the present study (Inion GTR™, Inion Inc., Finland). The manufacturer claims that this material is biocompatible, blocks cell migration, integrates well with tissues, maintains space is easy to use, and acts as a barrier for 8-12 weeks (Inion™, 2004).

Although not enough data has been published on the use of poly α-hydroxy acids as GBR barriers, the existing data shows that these materials exhibit excellent potential for clinical use. Polyglycolic (PGA) and polylactic (PLA) acids were found to be biocompatible and osteoconductive polymers when used in rabbit models. Rimondini et al. and Imbronito et al. conducted two separate studies filling bony defects with PLA/PGA polymers. Both authors found that PLA/PGA polymers were osteoconductive, biocompatible and did not induce a foreign body reaction (Imbronito et al., 2005; Rimondini et al., 2005).

Von Arx et al. investigated the durability and barrier effect of a glycolide-lactide-trimethylene carbonate barrier. The author used a glycolide-lactide-trimethylene carbonate barrier along with six other types of resorbable collagen and synthetic barriers to cover iatrogenically created inlay defects in rabbit calvaria. Evidence of degradation and decreased strength of the barrier was not seen before 12 weeks postoperatively. However, foreign body reaction was seen around the barrier throughout the healing time, which the author thought was a consequence of the accumulation of glycolide and lactide byproducts (von Arx et al., 2005).

Amano et al. found that poly L-lactic acid (PLLA) barrier is a good GBR device for inlay bone defects in dog mandibles. After 36 weeks, the defects covered with PLLA barrier filled with significantly greater amounts of bone compared to the control defects ($P < 0.05$). The barrier did not start degrading before 36 weeks and there was no evidence of foreign body reaction (Amano et al., 2004). The success of PLLA barrier, seen in this study, confirms the results published by Schmid et al. (1997) that were presented earlier in this paper.

Mellonig et al. (1998) found that the degradation pattern of the PLA/PGA barrier resulted in premature barrier collapse and inferior bone regeneration, when compared to the nonresorbable expanded PTFE. He covered iatrogenic bone defects, around titanium implants placed in dogs, with expanded PTFE or PLA/PGA barriers. After 3.5 months, the ePTFE group yielded more bone-implant contact, thread coverage, and new bone height than the PLA/PGA group. He concluded that the poor results were possibly due to the early degradation and resorption of the PLA/PGA barrier, which led to loss of stiffness and then collapse. The author suggested that the use of a filler graft under the resorbable barrier could provide support and prevent the collapse of the barrier and subsequently maintain the secluded space. No foreign body reaction to the PLA/PGA barrier was seen.

Different combinations of polymers have been developed as bioreabsorbable barriers, for guided bone regeneration. However, despite some promising data in the literature, animal studies and clinical trials are still lacking in terms of the effectiveness and predictability of these barriers. For this reason, we chose to test the effectiveness of one of the commonly available copolymer barriers Inion GTR™, in combination with a Bio-Oss® bone substitute, in a rabbit, guided bone regeneration, model.

CHAPTER TWO
MATERIALS AND METHODS

2.1.0 Experimental Model:

The protocol of our experiment was reviewed and approved by the Animal Care Committee at McGill University. We chose adult New Zealand White rabbits as our experimental model. This specific species has been used extensively in research of bone grafting, for the oral and maxillofacial region (Rimondini, et al., 2005). The New Zealand White rabbit has a shorter bone remodeling cycle (sigma = 6 weeks) than humans. The human sigma value is 17 weeks and the ratio between the two bone remodeling sigmas has been used to extrapolate conclusions from rabbit models to human equivalents (Roberts et al., 1999).

We purchased our rabbits from Charles River Breeding Laboratories, Inc. All rabbits weighed 3.0 to 4.0 kg at purchase. During the first two weeks, after arrival,each animal was kept in quarantine, in the research facility at the Montreal General Hospital.

2.2.0 Treatment Groups

Our sample consisted of twelve rabbits (n=12) (Appendix I). The sample size was calculated using the "simple sample size calculator" software by Dr. Jose Correa. Each individual rabbit served as its own control. One side of the mandible was used as the experimental side and the other side served as the control. By a process of random assignment, eight rabbits received chambers filled with Bio-Oss® on the right side, and four rabbits received the Bio-Oss® chambers on the left side of the mandible. The rabbits were named and assigned a number, according to order of operation, where animal number 1 (Punch) was operated on first and animal number 12 (Horex) was operated on last (Appendix I).

2.3.0 Experimental Armamentarium

A dense porous copolymer barrier consisting of trimethylene carbonate, poly L-lactide and polyglycolide (Inion GTR™, Finland) of 0.2mm thickness was plasticized using N-Methyl-Pyrolidone plasticizer solution included in the barrier package (Figures 1&2). The barrier was then molded into a chamber by pressing it firmly against a cylindrical titanium mesh template (specifications and design are shown in Appendix II) with a diameter of 5.5 mm and height of 3 mm. The same titanium template was used to create all of the resorbable chambers used in both experimental and control sides. Each side received only one chamber. All chambers have a uniform volume of 71.3 mm^3. On one side, the chamber was filled with Bio-Oss® and autogenous blood from the rabbit ear vein, and on the other side it was filled only with autogenous blood (Figure 3). The chambers on both the experimental and the control sides were fixed using resorbable tacks made of the same bioresorbable material (Figures 1 & 2).

Figure 1: Inion biodegradable barrier (left) and tacks (right).

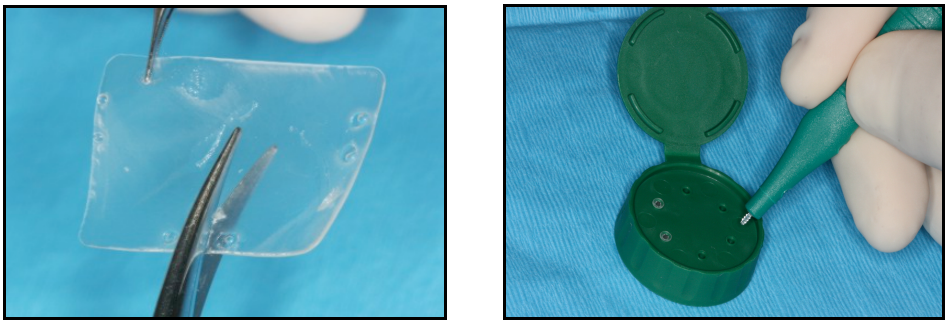


Figure 2: Inion biodegradable barrier after being plasticized (left), bioresorbable tacks open package (right).

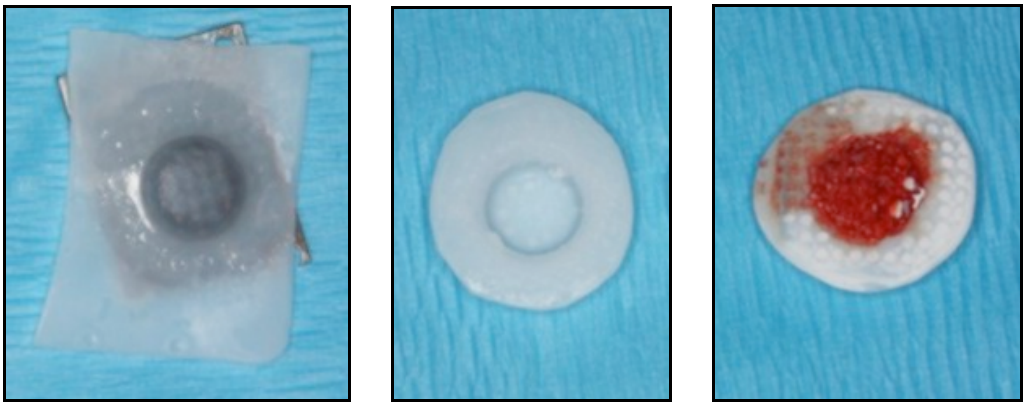


Figure 3: Titanium template used to shape the bioresorbable chambers (left), empty chamber (center) prior to filling with either autogenous blood only or Bio-Oss®, with autogenous blood (right).

2.4.0 Operative Technique

One surgeon performed all surgical procedures on all rabbits. The surgeon had successfully completed the advanced level of the theory training course on animal use for research and teaching as well as the rabbit methodology workshop, offered by McGill University (Appendices IV). Animal handling, manipulation, anesthesia and injections were performed by the Montreal General Hospital animal health technician. The technician and the surgeon shared the duty of delivering the postoperative analgesic injections.

2.4.1 Preparation and Anesthetic Technique

The rabbits were quarantined and acclimatized , for two weeks, following their arrival at the animal research facility. They were kept in individual cages and were given water and a normal diet for the entire period. None of the rabbits developed any sign of illness or lost appetite throughout the course of the study. Therefore, none of the animals were excluded from the experiment.

Food and water were withheld for 2-3 hours prior to surgery. On the day of surgery, each rabbits was sedated one hour preoperatively. Xylazine 1-3 mg/kg and Acepromazine 1 mg/kg were injected subcutaneously. Then after 10 minutes, a subcutaneous dose of Ketamine 35-50 mg/kg was also delivered. The rabbit was then transferred to the operating room. Intravenous access using a 22-gauge catheter through the marginal ear vein was established, through which the rabbit was hydrated with lactated ringer's solution. General anesthesia was induced by injecting 0.5 cc of intravenous Thiopental 2.5% solution and titrated to effect with subsequent doses of 0.25 cc. The rabbit was then intubated with a 2.5-3.5 mm non-cuffed pediatric endotracheal tube (ET). The ET tube was then connected to the ventilation set to 30-40 breaths/minute (4 L/min) and general anesthesia was maintained through diffusion of 2% Isoflurane gas. Anesthetic monitoring consisted of a pulse oximeter, placed on the lower extremity, and a rectal thermometer. All rabbits had a sound anesthetic course without complications.

2.4.2 Operative Procedure

All rabbits received an intravenous, prophylactic dose of Cefazolin 25mg/kg (Novopharm Limited, Toronto, Canada) intraoperatively. The rabbits were first placed in the right decubitus position to start the operation. The right side was treated as either the experimental (Bio-Oss®) or the control side, depending on the random assignment. (Appendix I).

The skin over the mandibular body was shaved bilaterally. The skin was then prepped with povidine and then draped with a sterile sheet. Using a number 15 blade, a 3 cm submandibular incision was made from skin to bone over the mandibular body area. Subperiosteal dissection was performed to expose the

lateral aspect of the body of the mandible. Using a 701 bur, four 0.8 mm diameter, monocortical holes were carefully drilled through the buccal cortex exactly where the chamber would sit (Figure 4). A titanium template without the cylindrical chamber was used to determine the location where the holes would be drilled. The reason behind drilling these holes is to expose the secluded area under the chamber to the host bone marrow.

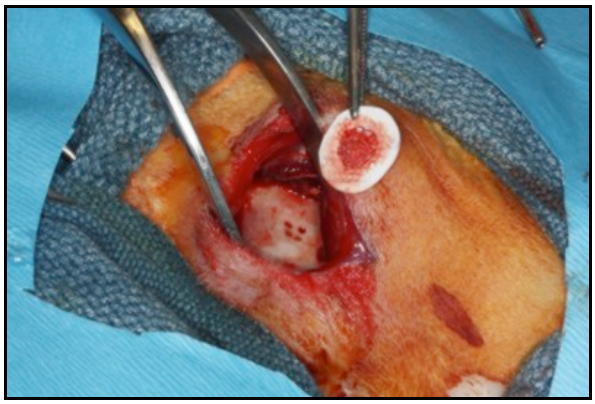


Figure 4: Subperiosteal exposure of the lateral aspect of the mandible with four monocortical holes dirlled and the chamber with Bio-Oss® soaked in blood is ready for fixation.

If the right side was assigned to be the experimental side, the pre-shaped bioresorbable chamber was filled with particulate Bio-Oss® (Geistlich Pharma AG Wolhusen, Switzerland) of particle size of 0.25-1.0 mm soaked in autogenous blood drawn from the ear vein (Figure 4). The chamber was then adapted and fixed to the lateral aspect of the mandibular body using four bioresorbable tacks (Figure 5). The incision was then closed in three layers. The periosteum and muscle layers were closed with 3-0 Vicryl suture in continuous fashion and the skin was closed with continuous 4-0 monocryl suture. The same procedure was performed on the left side with or without using Bio-Oss®, depending on the random assignment of the left side to either the experimental or the control group.

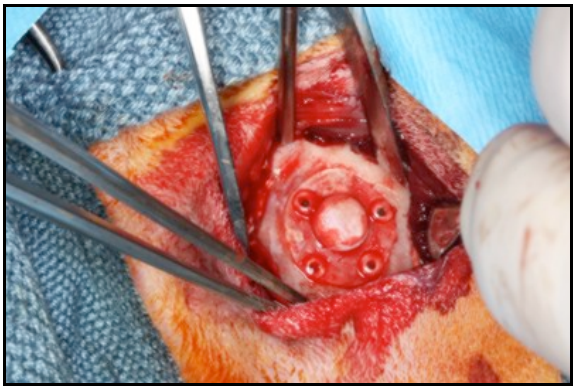


Figure 5: The chamber with the graft fixated to the mandible with bioresorbable tacks.

2.4.3 Post-operative Care

After completion of surgery, care of the rabbit was returned to the animal health technician for awaking and extubation. The isoflurane gas was stopped and mechanical ventilation with oxygen was continued until the rabbit started breathing spontaneously. Two minutes after spontaneous breathing, the rabbit was extubated and monitored closely for thirty minutes. The rabbit was then returned to its' cage. All rabbits underwent surgery and all recovered uneventfully.

Postoperative analgesia consisted of subcutaneous injections of Buprenorphine (Reckitt Benckiser plc, Slough, UK) 0.04 mg/kg every eight hours. Delivery of the analgesic was continued for two days. Periodic examination of the rabbits was done by either the animal health technician or the surgeon in order to assess the need for further analgesic injections. All rabbits received normal diet postoperatively. In the first postoperative week, the rabbits were examined twice daily by the animal health technician. An animal was weighed only if developed diarrhea, anorexia, or lethargy.

2.5.0 Post-mortem Assessment and Preparation

2.5.1 Animal Euthanasia

All rabbits were euthanized three months after surgery. Prior to euthanasia, the rabbits were sedated with subcutaneous injections of Torbugesic (butorphane) 1 mg/kg and Acepromazine 1 mg/kg. Fifteen minutes later,

intravenous access with a 22-gauge catheter was established through the marginal ear vein. The rabbits were then euthanized with intravenous injection of sodium pentobarbital (J. M. Loveridge p.l.c., Southamton, UK) at 100mg/kg. After cessation of cardiac pulsation was confirmed by the animal health technician, the rabbits were transferred to the operating room for retrieval of the specimens. Both sides of the mandible were sharply dissected, and gross enbloc resection of the portion of the mandible containing the chambers was performed, using a reciprocating saw. The bony specimens were trimmed to about 5 mm of bone surrounding the chambers. Attempts were made to preserve the remnants of the bioresorbable chamber through careful meticulous dissection. The prepared specimens were immediately placed in 4% formaldehyde containers. Each container was labeled with only the name, number and side of the jaw of the rabbit. No information regarding the group that the specimen belonged to was included in the label. All specimens were transferred to the bone laboratory on the same day, for processing and analysis.

2.5.2 Micro-CT Scan Analysis

A standard desktop micro-CT scanner (SkyScan™ - 1072, Aartselaar, Belgium) was used to scan all specimens. The scanner parameters were set to a power of 45 kV, 222 μA and a resolution of 9.38 μm per pixel. An attached 12-bit, cooled CCD camera (1024X1024 pixels) coupled with a fiber-optic taper to an x-ray scintillator was used to capture the scanned images. The rotation of the specimens was set at 0.9 degrees and 5000 ms exposure, each step for 180 degrees. Images were sectioned perpendicular to the native bone with a 21.88 μm distance between each cross-section. Each cross-section was reduced to half its size in order to facilitate the analysis, giving a voxel size of 21.88 X 21.88 X 21.88 μm^3. The NRecon (v1.6.1.3) and CT-Analyser (v1.8.1.2) software, provided with SkyScan™ scanner, were used to reconstruct the three-dimensional images of the specimens. The newly regenerated bone volume and height was measured on these images. Bone volume percentage was calculated by subtracting the volume of the dense Bio-Oss® particles from the total calcified

volume and dividing the remainder of the total volume of the chamber (Appendix III). Bone height was defined as the height from the basal bone to the top of the regenerated hard tissue, under the bioresorbable barrier.

2.5.3 Histological Slides Preparation

The specimens were prepared by immersion in the following solutions: 4% formaldehyde for fixation, 70% ethanol and for desiccation, 95% ethanol and water also for desiccation, ether/acetone (JT Baker Inc., Jackson, TN) 1:1 solution for degreasing and defatting, and finally anhydrous ethanol for final drying. For each of these solutions, magnetic stirring was facilitated to allow permeation through the contents of the specimens.

Prior to embedding, the specimens were pre-soaked in a solution of polymethylmethacrylate (PMMA) (Aldrich Chemicals, Oakville, ON) inhibited with 10 ppm of hydroquinone for a period of 48 hours. The PMMA solution was activated with the addition of 3.5g of bynzoyl peroxide (Aldrich Chemicals, Oakville, ON) per liter of PMMA monomer. The pre-soaked specimens were magnetically stirred and stored in a refrigerated environment at 4°C.

The liquid monomer used in the polymerization process was prepared as follows; inhibited PMMA monomer was activated with the addition of 3.5g of benzoyl peroxide per liter monomer. The activated monomer was then heated at 55°C in a hot water bath for about six hours. The monomer was stirred every half an hour while being heated. When the consistency of the partially polymerized solution was similar to thin syrup and became yellowish in color, the process of heating was stopped, and the solution was cooled down under tap water and stored at 1°C. The specimens were then placed in aluminum, prefabricated molds. The partially polymerized solution was added to the molds until it completely covered the specimens. The specimens were then placed in a vacuum of 70 mmHg for 12 hours. During the initial stages of vacuum treatment, the vacuum was interrupted regularly to force the polymer into the specimen and prevent the molds from overflowing. The molds containing the specimens were then kept in sealed Ziplock bags for a period of five days in order for the polymer to fully cure

and harden. To ensure complete coverage of the specimens, additional stock PMMA was added as needed to compensate for gaps around the specimens that had occurred due to evaporation and shrinkage. Once it was ensured that all soft areas and liquid inclusions around the specimens were eliminated, the specimens were placed in a heated chamber (35°C) for three days to ensure complete hardening. During the first twelve hours of this final heating step, the specimens were placed in an oven for one hour and then cooled down for another hour, with this process repeated six times. At this point, the specimens had become embedded in hard and transparent plastic blocks that are well-preserved and mechanically stable for further processing.

The blocks generated were then sectioned into 4-5 μm thick slices using a microtome. The sections were produced in a plane perpendicular to the outer cortex of the mandible. Unfortunately, the sliced were too thin to preserve the Bio-Oss® particles, which wre mostly lost from the specimens, during sectioning. One representative section from the center of each specimen was chosen and mounted on a glass slide. The slides were then deplastified using ethylene glycol, monoethyl ether and acetate for 30 minutes each.. The slides were then stained with Von Kossa and Toluidine blue stains. Then, they were incubated in 3% silver nitrate, exposed to UV light for 30 minutes and washed with running tap water. Next, they were incubated in Toludine working solution (1g sodium brate, 1g toluidine blue, and 100 ml distilled water) for 2-3 minutes then washed under running tap water. Finally, the slides were dried and mounted for microscopic analysis. Under the microscope, an image of each slide was captured at 2.5x magnification using a specialized connected digital camera.

2.5.4 Histomorphometric Analysis

The histological images already captured were histomorphometrically analyzed using Image-J software (version 1.37v). The software was calibrated so that 245.65 pixels equaled to 1 mm. The total calcified tissue was shaded and the particle analyzer feature in the software was used to calculate the total surface area of calcified tissue within each slide. Thereafter, the calcified tissue surface

area and distribution were evaluated and divided over the total surface area to yield the percentage of new bone in each slide.

2.6.0 Statistical Analysis

Since each rabbit served as its own control, paired samples t-test was selected for analysis to test for any significant difference between the experimental and control sides. The paired samples t-test examined the mean statistical difference between the experimental and control specimens in the following parameters: (1) radiographic bone height under the barrier, (2) radiographic percentage of new bone generated under the chamber barrier, and (3) histomorphometric percentage of new bone generated under the chamber barrier in a representative mid-section histological slide of each specimen. The statistical analysis of this experiment was performed by Dr. Jose Correa.

CHAPTER THREE
RESULTS

3.1.0 Clinical Course

All purchased rabbits were operated on and experienced no complications throughout the course of the study. Neither wound dehiscence nor infection was observed during the healing period. The rabbits tolerated the regular diet after surgery. The twelve rabbits were euthanized three months after the surgery, according to the previously described protocol. None had to be prematurely euthanized due to weight loss or excessive acquired morbidity.

3.2.0 Gross Description

Upon dissection of the rabbit mandibles the gross morphology and geometry of the grafted chambers appeared to be preserved in most of the samples. The bioreabsorbable chambers and their tacks were found to have lost their mechanical strength and were partially degraded. In some specimens, a very thin shell layer of bone-like tissue was found partially covering the outer aspect of the chamber. Also, a layer of dense connective tissue covering some areas of the chambers was seen and was carefully dissected off. No Bio-Oss® particles were found outside of the boundaries of the chambers. In some areas where the chamber was degraded, a uniform, hard, yellowish tissue was seen. Bio-Oss® shadow was also seen through these gaps in the experimental group specimens (Figure 6).

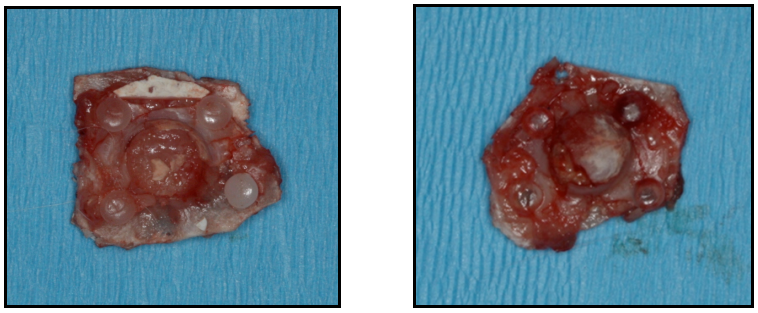


Figure 6: A postmortem experimental (left) and control (right) specimens with obvious partial degradation of the bioresorbable chambers and tacks.

3.3.0 Micro-CT Analysis

A total of 24 specimens (twelve in each group) were analyzed in order to determine the percentage of the total calcified tissue and new bone, under the bioresorbable chamber. Also, the height of the calcified tissue was measured from the basal bone toward the top of the chamber (Appendix III).

In the experimental group specimens, the Bio-Oss® particles were clearly identified on the micro-CT scan section slides. New bone was observed more in the lower half of the chambers. New bone was also seen bridging the particles in the central portion of the specimens. In most of the specimens, new bone formation took place in the inner and, for some, the outer aspects of the bioresorbable chambers in a linear semi-continuous fashion. The general morphology and dimensions of the chambers appeared to be preserved in most samples (Figure 7). In this group, the percentage of new bone generated in the secluded space ranged from 8.64% to 14.73% with a mean of 10.8% and a standard deviation of 2.07%. The height of the calcified tissue under the chambers ranged from 1.66 mm to 2.80 mm with a mean of 2.25 mm and a standard deviation of 0.38 mm.

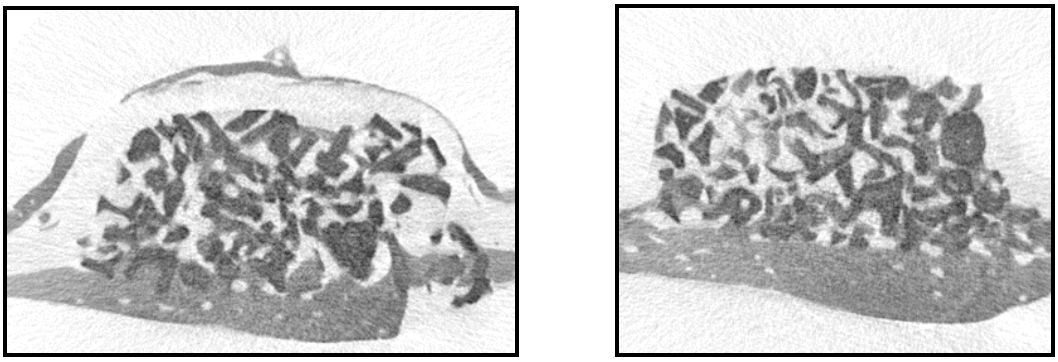


Figure 7: Midsection micro-CT images of two different experimental specimens. Note the difference between the particles opacity and the newly generated bone. Thin layer of new bone on the exterior of the chamber was seen in some slides (left). On the images you need to put arrows to indicate what is Bio Oss and what is bone

In the control group, more chambers appeared to have partially collapsed and exhibited a more distorted morphology than in the experimental group (Figure 8). The secluded space under the chambers contained less hard tissue with a noticeable radiopaque rim formed in the inner aspect of the bioresorbable mesh. A few specimens also showed bone formation on the outer aspect of the chamber. In this group, the percentage of new bone generated ranged from 0.005% to 1.23% with a mean of 0.29% and a standard deviation of 0.33%. The height of calcified tissue from the basal bone toward the roof of the chamber ranged from 0.65 mm to 1.96 mm with a mean of 1.35 mm and a standard deviation of 0.46 mm.

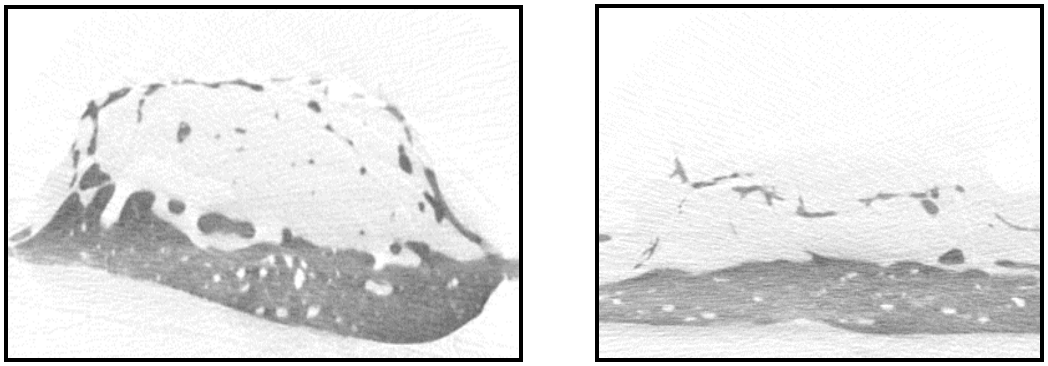
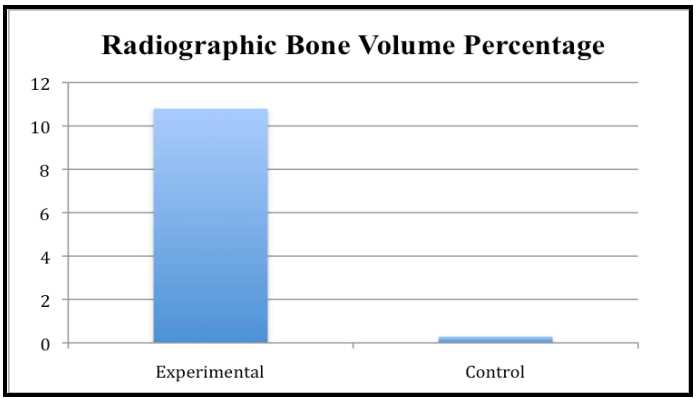


Figure 8: Midsection micro-CT images of two control specimens. Linear bone formation is seen in close proximity to the bioresorbable barrier (left). More collapse of the secluded space is seen in the control group (right).

Using the paired samples t-test, we found a significant difference between the percentage of new bone in the experimental group (mean= 10.8%, SD= 2.07%) and the control group (mean= 0.29%, SD= 0.33%). These results show that more bone was generated in the experimental group than the control group with the p-value being less than 0.0001 (table 1 and graph 1).

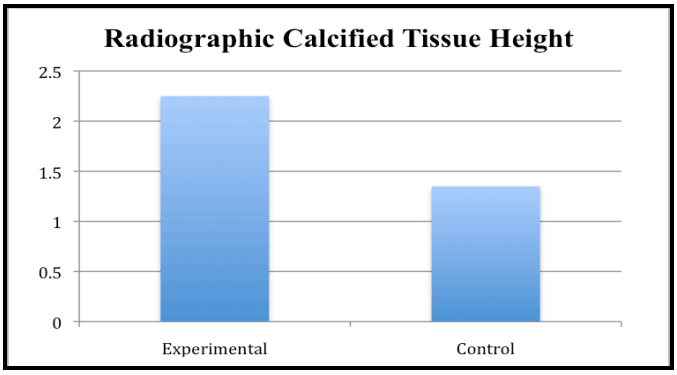
Group	Mean volume percentage of New bone (%)	Mean height of calcified tissue (mm)
Experimental	**10.8**	**2.25**
Control	**0.29**	**1.35**

Table 1: Comparison between experimental and control calcified tissue height and bone volume means.



Graph 1: Comparison between experimental and control mean radiographic bone volume.

We also found a significant difference in the height of calcified tissue between the experimental group (mean= 2.25 mm (75% of total height under the chamber), SD= 0.38 mm (12.67%)) and the control group (mean= 1.35 mm (45%), SD= 0.46 mm (15.33%)). These results show that more calcified tissue height was achieved in the experimental group than the control group with p-value equal to 0.0002 (table 1 and graph 2).



Graph 2: Comparison between experimental and control mean calcified tissue height.

3.4.0 Histological Description

In the experimental group, new bone was generated more in the area close to the basal bone than in the upper half of the chambers. New bone was also seen bridging the areas between particles and on the inner aspect of the bioresorbable chambers (Figure 9). The secluded space around the new bone was filled with soft tissue with sporadic fatty-like tissue (Figure 9). The Bio-Oss® particles were lost during microtome sectioning of the specimens' blocks. In the center of the specimens, new bone was mainly found around the empty spaces that were previously occupied by Bio-Oss® particles (Ps). The bone-particle interface appeared to be rough with projections of bone appearing to have grown into the particle pores and concavities, from which few have shed due to the shearing forces during particles dislodgement (Figure 10). No intervening soft tissue was found in the bone-particle interface in any of the specimens giving the impression that possibly an intimate contact existed between new bone and the particles. Also, Since Bio-Oss® particles were lost, no conclusion could be drawn regarding Bio-Oss® resorption after three months of healing.

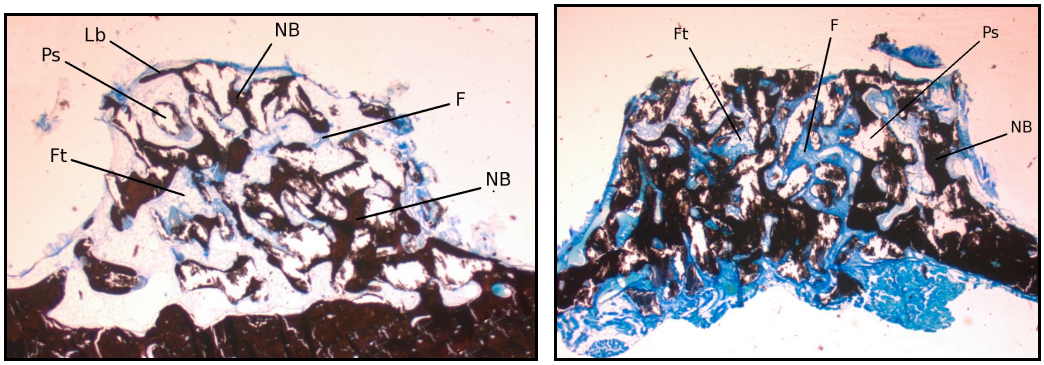


Figure 9: Histological slides from the experimental group stained with Von Kossa & Toluidine Blue (2.5X magnification). New bone (NB) is formed in greater quantity close to the basal bone. Also, new bone formed bridging the area between particles (particles' spaces, Ps). Fibrous (F) and fatty (Ft) connective tissue can be seen around the calcified tissue formed. Linear pattern (Lb) of bone also formed in close proximity to the barrier.

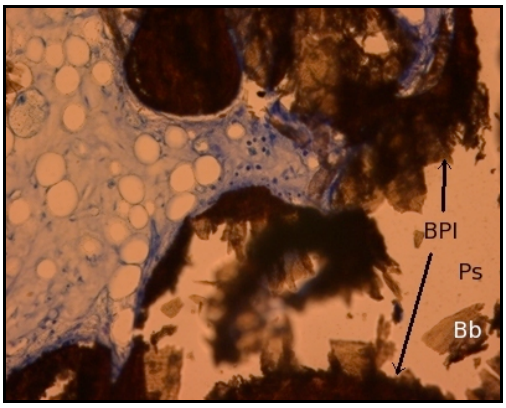


Figure 10: The rough bone-particles interface (BPI) with no intervening soft tissue. The Bio-Oss® particles are lost during sectioning of the specimens' blocks. Note the broken bone (Bb) processes into the particles' space (Ps).

In the control group, nine of the twelve chambers have partially collapsed and presented with distorted geometry on microscopic examination. The area under the chamber was mostly filled with soft fibrous and fatty tissues. New bone had formed in a linear pattern on the inner aspect of the barrier chamber or as sparsely distributed islands (Figure 11).

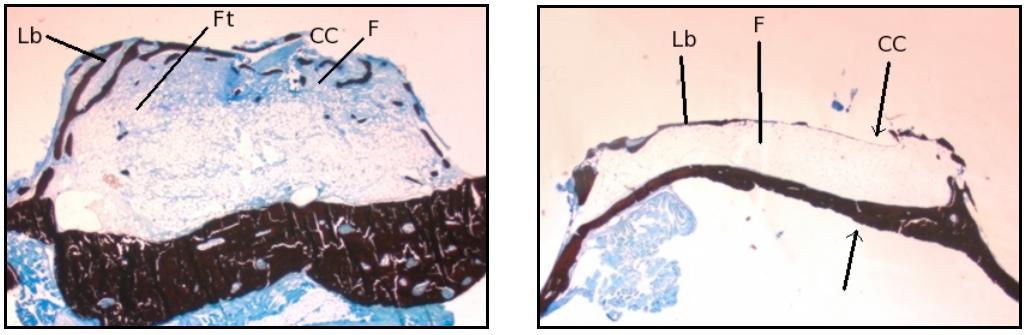


Figure 11: Histological slides from the control group stained with Von Kossa & Toluidine Blue (2.5X magnification). Both slides show variable amounts of chamber collapse (CC). Note the linear pattern of bone (Lb) formation in the inner aspect of the barrier chamber and the amount of fibrous (F) and fatty (Ft) soft tissue.

By comparing the two groups, we could clearly see that the vertical and horizontal dimensions were more preserved in the experimental (Bio-Oss®) group as compared to the control. Also, due to grafting there was more new bone formation, with more even distribution in the experimental than in the control group, after three months.

3.4.1 Histomorphometric Analysis

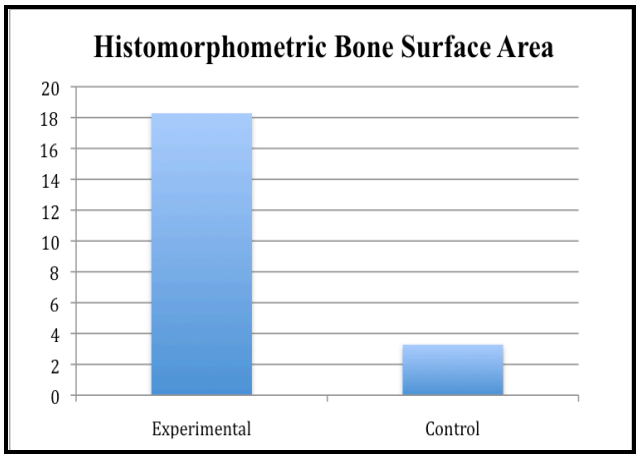
The bone surface area of a midsection representative histological slide per each sample was calculated using ImageJ software (version 1.37v). Detailed measurements of each sample are included in Appendix IV.

In the experimental group, the bone surface area ranged from 12.56% to 23.00% with a mean of 18.28% and a standard deviation of 3.69%. Most of the new bone was seen to occupy the lower half of the chamber space.

In the same way, bone surface area was calculated for the control group samples. The measurements ranged between 0.57% and 7.6%, with a mean of 3.28%, and a standard deviation of 2.02%. Bone in the control group has mostly formed either near the basal bone or in close proximity to the inner aspect on the barrier chamber.

A paired samples t-test was used to compare the two groups after confirming the test's robustness and that the data points' distribution resembles that of normal distribution. There was a statistically significant difference in the

bone surface area between the two groups in favor of the experimental group with the p-value being less than 0.0001 (graph 3).



Graph 3: Histomorphometric comparison of experimental and control bone surface area.

The geometry of the chambers underwent changes after three months of healing in both the experimental and control groups. Histologically, two of the twelve experimental (Bio-Oss®) samples underwent moderate distortion and collapse (16.67%). However, nine of twelve samples in the control group underwent distortion and collapse (75%), two of which were severe. By comparison, the secluded space was maintained significantly more in the experimental group than the control group (Appendix IV).

CHAPTER FOUR
DISCUSSION AND CONCLUSION

4.1.0 Experimental Model

The rabbit model is the most commonly used animal model in medical research, specifically in bone research. Approximately 35% of musculoskeletal research investigations are carried out using rabbits (Pearce et al., 2007). The rabbit model is unique for its availability, appropriate size, ease of handling, and short bone remodeling cycle (sigma = 6). As it is the case for all animals used in research, the macrostructure and microstructure of rabbit bone differs from that of a human. However, despite the obvious gross anatomical differences, there exists only a minor difference in bone composition (Pearce, et al., 2007). Indeed, bone mineral density in rabbits is found to be similar to that of humans. Since the human bone remodeling cycle is about three times longer than it is in rabbits (signa = 17 weeks) and since Bio-Oss® requires about 6-9 months of healing time in human, we have designated three months as the healing time in our rabbits according to the sigma ratio between human and rabbit bone.

4.2.0 The Experimental Group

Bio-Oss® is one of the most researched bone substitutes available for augmentation of small to medium size bony defects. Except for a few reported studies, Bio-Oss® demonstrates good osteoconductive potential as an inlay and onlay bone graft in block and particulate forms. However, the effectiveness of particulate Bio-Oss® as an onlay graft in guided bone regeneration models is scarcely reported in the literature. In the present study, Bio-Oss® effectiveness as an onlay graft in combination with bioresorbable barrier using GBR technique was investigated in a rabbit model. Our results show that Bio-Oss® exhibits good osteoconductive potential, and are in agreement with animal studies and clinical trials published by Zitzmann el al (2001), Slotte et al. (2003), Norton et al. (2003), Al-Harkan (2007) and Cordaro et al. (2008). The calculated histomorphometric

percentage of new bone regenerated in the present study equals 18.28%. This percentage is comparable to the percentages of new bone reported in previous studies. Zitzmann et al. found 22.6% new bone regeneration after 6-7 months of onlay grafting of Bio-Oss®, in the maxilla of six partially edentulous patients (Zitzmann, et al., 2001). Slotte et al. found that 17.3% of new mineralized and non-mineralized bone (12.0% and 5.3%, respectively) regenerated when Bio-Oss® filled titanium chambers were fixed on rabbit parietal bones and left to heal for twelve weeks (Slotte et al., 2003). Norton et al. used Bio-Oss® in maxillary sinus lift and extraction sockets preservation procedures in fifteen patients and yielded 29.9% new bone after six months of healing (Norton, et al., 2003). Al-Harkan used particulate Bio-Oss® under a titanium chamber as an onlay graft using GBR technique in rabbit mandibles and yielded 18.4% new bone regeneration, after three months (Al-Harkan, 2008). In a clinical trial by Cordaro et al., 23 maxillary sinuses were augmented with an organic bovine mineral and yielded 19.8% new bone after 180-240 days (Cordaro, et al., 2008). However, a few other studies reached a different conclusion regarding the effectiveness of bovine bone as an osteoconductive material. Slotte et al (1999) investigated the osteoconductive potential of Bio-Oss® under silicone domes fixed on rat calvaria. After eight weeks of healing, he found that the domes containing Bio-Oss® resulted in inferior quantities of mineralized bone when compared to the control. He concluded that Bio-Oss® is only biocompatible, and not an osteoconductive material (Slotte et al., 1999). He found that the total unmineralized tissue was greater in the Bio-Oss® group and that new bone distribution was more even throughout the space in the Bio-Oss® group compared to control, where bone formed closer to the basal bone. The author mentioned that the origin of the unmineralized tissue, seen more in the Bio-Oss® group, could not be identified despite the sporadic areas of osteoid deposits encountered on histological examination. Since the evaluation was only done at one time point (eight weeks), conclusions regarding how much of this tissue might eventually mineralize is unknown. The author also mentioned that the sagittal skull suture could not be avoided and that soft tissue from the suture might have grown into the secluded

space. The author also reported that the bovine particles collapsed to 69% of their original volume due to "dense packing" after eight weeks. The cause of this "dense packing" was not well explained. It is not known whether this change of volume was due to micromovement of the dome and subsequently the graft under it, which might have affected graft stability needed for new bone formation.

Stavropoulos et al. found that Bio-Oss® inhibited new bone generation. In his two studies conducted in 2001 and 2003, new bone generation under the empty control Teflon capsules yielded more bone than the Bio-Oss® filled capsules (Stavropoulos, et al., 2001; Stavropoulos, et al., 2003). The discrepancy between the results of these studies and the present study may be attributed to the method of barrier chamber fixation used in each study. In both of the Stavropoulos studies, the Teflon capsules were not rigidly fixed to the surface of the mandible, but rather sutured using silk sutures, which might have caused movement and an incomplete sealing of the capsule against soft tissue ingrowth. The effect of proper fixation of the GBR barrier device was demonstrated in the study by Amano et al. (2005). He placed the GBR barriers over defects in dog mandibles. He found that non-fixed polylactic acid barriers resulted in inferior amounts of bone fill (53.2%) when compared to appropriately fixed barriers (62.2%) (Amano, et al., 2004).

Pinholt et al. also found that Bio-Oss® in both sintered and unsintered forms was neither osteoinductive nor osteoconductive when implanted in rat maxillas and abdominal muscles and left to heal for four weeks (Pinholt, et al., 1991). The author found some evidence of osteoblast-like cells in close proximity to the Bio-Oss® particles, but without evidence of formal osteoconduction. The difference in these results compared to the present study could be attributed to the fact that guided bone regeneration principle was not appreciated in Pinholt's study. The Bio-Oss® particles implanted in the surface of the maxilla were neither isolated from the surrounding soft tissue nor were they stabilized in place. Also, the healing time in his study was seemingly short.

Carmagnola et al. (2002) investigated the effect of adding fibrin glue (Tissel®) on Bio-Oss® in grafting mandibular inlay bone defects, covered by a

collagen membrane, in Labrador dogs. The Bio-Oss® + Tissel® group showed inferior new bone regeneration compared to the Bio-Oss® alone group, and both were inferior to the control group (defect covered by collagen membrane only). Although more new bone filled the control defects after 1 and 3 months, the quality of bone at the crest of the ridge differed significantly between the Bio-Oss® and the control groups. In the Bio-Oss® groups, comparatively larger amounts of woven and lamellar bone formed and underwent significant remodeling at the crest of the ridge and lateral walls of the defects after three months of healing, whereas in the control group, only a thin layer of saddle-shape mineralized bone covered the entrance of the defect with underlying large marrow spaces. The denser quality of crestal bone observed with the use of Bio-Oss® might add an advantage of achieving greater stability for dental implants at the crest of the ridge.. The author also mentioned that highly cellular and vascular connective tissue was observed around the Bio-Oss® particles in the center of the experimental specimens when compared to the marrow-filled central region of the control specimens. Schenk et al. and Hammerle et al. showed that highly vascular and cellular connective tissue proliferation always precedes bone neo-formation in such situations (Hammerle et al., 1995; Schenk et al., 1994), and that mineralization of such connective tissue can only be observed if longer healing time was allowed. Despite the superiority of bone regeneration in the small inlay defects of the control group in this study, larger inlay or onlay defects may not show the same degree of bone regeneration. In those cases, a filler graft might add the benefit of providing support for the barrier and promoting new bone regeneration.

Schmid et al. used particulate bovine bone (OsteoGraf/N-300®) as an onlay graft in combination with a bioresorbable polylactic acid chamber barrier on rabbi calvaria. The author concluded that particulate bovine bone only accelerated bone neogensis over the first month of healing. However, after two months of healing, he found that the control group surpassed the OsteoGraf/N-300® group in the amount and height of newly regenerated bone (Schmid, Hammerle, et al., 1997). The osteoconductive characteristic of bovine bone

observed by Schmid et al. was confirmed in the present study. However, the pattern of new bone regeneration observed by Schmid, over time, could not be observed in the present study. Schmid compared the pattern of new bone regeneration from two postoperative follow-up periods, whereas in the present study, we only observed the pattern of new bone formation at one time point,. Interestingly, Okazaki et al. conducted a similar study on rabbits using rabbit deproteinized particulate bone as a grafting material and a titanium reinforced, expanded, PTFE barrier. Okazaki observed the pattern of new bone regeneration after 2, 4, 8 and 12 weeks. He found that bone regeneration was significant in both the experimental and control groups after four weeks. However, he found more new bone regenerated in the experimental group, when compared to the control group, after eight weeks. This contradicts the results observed by Schmid et al. Therefore, more studies are needed to confirm the results regarding the use of natural bone substitutes as onlay grafts in guided bone regeneration.

In the present experiment, we observed that most of the new bone regenerated was close to the host bone. Experiments carried out by Al-Harkan (2008), Slotte (2003), and others (Al-Jandan, 2007) reached a similar conclusion. However, while most published studies agree on the fact that new bone mostly regenerates close to the host bone, the reported gain in height from the surface of the host bone varies among these studies. The reported bone height gain, in guided bone regeneration models, ranges from about 1.5 mm to 5 mm. This variation in height gain was seen by some as a result of incomplete vertical bone regeneration due to insufficient healing time allowed before observation and analysis (Caplanis et al., 1997). The healing time needed for guided bone regeneration to result is still unknown (Hermann et al., 1996). In the present study, the height of the generated bone was significantly higher in the Bio-Oss® group with a mean of 2.25 mm (75% of the total height under the chamber) versus 1.35 mm (45%) in the control. Since the height of the regenerated calcified tissue in both the experimental and control groups are less variable with small standard deviations of 0.38 (12.67%) and 0.46 (15.33%) respectively, we concluded that the difference in the means in both groups is significant and is more strongly

attributed to the use of Bio-Oss®. However, looking at the actual difference in millimeters, we found that Bio-Oss® resulted in only 0.9 mm of hard tissue height gain compared to GBR without Bio-Oss®. Thus, our results practically mean that the use of the described technique in this study is effective when placement of a dental implant in an atrophic ridge requires 2.25 mm of bone augmentation in order for the future implant to be surrounded with enough bone in all directions. However, the grafting of larger defects, which require more than a 3 mm of additional bone width, need to be investigated further.

The resorbtion of Bio-Oss® has been an issue of controversy. While histological evidence of Bio-Oss® resorption was reported in animal studies (Carmagnola, et al., 2002; Klinge, et al., 1992) and in human studies (Sartori, et al., 2003; Zitzmann, et al., 2001), some published studies and reports did not observe any resorbtion of Bio-Oss® particles, after healing periods ranging from four months up to six years or longer (Schlegel, et al., 1998; Stavropoulos, et al., 2001; Valentini, et al., 1998). In the present study, we encountered technical difficulty in sectioning of non-demineralized Bio-Oss® particles resulting in shedding of the particles and their absence in the final histological and histomorphometrical analysis. Therefore, we did not reach a conclusion regarding Bio-Oss® resorbtion.

Despite the loss of Bio-Oss® particles from the histology slides and the fact that we could not measure the exact histomorphometrical percentage of bone-particle contact, we noticed that the particle-new bone interface was rough with bony microprocesses and adjacent sequestrated bone chips. In all of the analyzed slides, we did not see any trace of soft tissue in the new "bone-lost particle" interface. From this histological presentation, we speculated that new bone was in direct contact with the Bio-Oss® particles confirming its osteoconductive potential. However, due to resemblance between the slides presentation in our study and other previously conducted studies, we assume that the amount of bone-particle contact is comparable to those reported in previous studies. Slotte et al. (2003) found that mean bone-Bio-Oss® particle contact is 46.4% ± 13.3% in a rabbit model after 12 weeks. In the clinical trial of Zitzmann et al. (2001) bone-

particle contact was 36.7% ± 26.6% after 6-7 months. Carmagnola et al (2002) found that bone-particle contact in the Bio-Oss® alone group (without Tissel®) was 40.4% ± 31.6%, after three months of healing in Labrador dogs.

To date, the literature lacks data on the use of poly α-hydroxy acids polymers as barriers in guided bone regeneration. However, the available published studies indicate that these bioresorbable polymers are biocompatible and occlusive against the ingrowth of soft tissue. In the Schmid et al. study, the polylactic acid chamber proved to be an adequate occlusive and structurally supportive barrier in onlay, bovine bone, grafts in rabbits. In a Masters project by Al-Jandan, the use of Inion GTR™ copolymer as a GBR barrier with autogenous bone filler graft was compared to titanium mesh in rabbits' mandibles. He found that the copolymer barrier is an excellent substitute for titanium mesh in terms of maintaining the secluded space and supporting the particulate onlay autogenous graft. In another Masters project, Al-Masri found that Inion GTR™ barrier is an adequate GBR occlusive barrier when used to support Straumann Bone Ceramic® as an onlay graft in rabbits' mandibles. The present study adds more data to the literature regarding the adequacy of trimethylene carbonate, L-lactide and a polyglycolide bioresorbable barrier (Inion GTR ™) as GBR occlusive barrier that structurally maintains the secluded space and stabilizes the particulate onlay graft.

The manufacturer of Inion GTR™ claims that this material provides a barrier effect for 8-12 weeks. Al-Jandan found that this barrier lost its barrier effect and partially degraded after eight weeks of healing (Al-Jandan, 2007). Al-Masri also reached a similar conclusion that it degraded after twelve weeks of healing (Al-Masri, 2009). However, the polylactic acid barrier used by Schmid et al. (1997) was structurally intact after one and two months of healing confirming the structural durability of these copolymers at two months. In our study, partial degradation and loss of physical strength was obvious in the majority of the specimens after three months of healing both radiographically and histologically. Thus, our results come in accordance with those claimed by the manufacturer and reported in previously conducted studies. However, whether or not 8 to 12 weeks

of barrier effect (claimed by the manufacturer of Inion GTR™) is sufficient for bone graft healing in humans needs to be further investigated.

Foreign body reaction and inflammation were found to be associated with the *in vivo* use of some of the poly α-hydroxy acids polymers in previous investigations (von Arx, et al., 2005). However, no clinical signs of inflammation were observed during the healing course or during dissection of the specimens in our study. Although no soft tissue histological slides were obtained, careful analysis of the hard tissue histological slides did not raise any suspicion regarding the existence of inflammation or foreign body reaction in any of the slides. The lack of foreign body reaction and inflammation observed by other studies support our findings (Al-Jandan, 2007; Al-Masri, 2009; Amano, et al., 2004; Mellonig, et al., 1998; Schmid, et al., 1997).

4.3.0 The Control Group

The amount of newly regenerated bone using a guided bone regeneration technique without a bone graft or substitute varies greatly in the literature. Stavropoulos et al. (2001) found that 38.7% of the volume of Teflon capsules (control) fixed on rat mandibles filled up with new bone after four months of healing. A similar study by the same author (2003), found that the space under empty Teflon capsules filled up with 88.2% more new bone after one year. Schmid et al. (1997) found that 20% of the space under the control polylactic acid chambers had filled up with new bone two months after from surgery. In Al-Harkan's experiment, the new bone fill under titanium mesh in the control group constituted only 5% of the space after three months of healing. In recent work by Al-Masri (2009), new bone fill under Inion GTR™ chamber barrier was 3.1%. In the present study, the average new bone regenerated in the control group was 3.28%, which is comparable to the amounts reported by Al-Harkan and Al-Masri. One explanation for this similarity is that the study design and surgical technique used in Al-Harkan's and Al-Masri's experiments were similar to those used in the present study. On the other hand, dissimilarity between our results and those of others can be explained by the same reason. Variations in the study design and

surgical techniques such as the use of a different animal model, not filling the secluded space with autogenous blood and not communicating host marrow space with the secluded space via monocortical perforations can all be possible explanations for variations in the results reported in GBR studies (Al-Harkan, 2008). However, there is no clear correlation in the literature between these factors and the pattern and magnitude of new bone regeneration.

It is obvious in Al-Masri's experiment that the use of a bone substitute preserved the geometric stability of the bioresorbable chamber. After twelve weeks of healing, he found that the presurgical geometry was preserved in 90.9% of the grafted chambers, and 36.36% of the empty chambers. The results of the present study confirm those of Al-Masri's. We found that when Bio-Oss® was used under the bioresorbable chamber, 83.33% of the chamber geometry was preserved, whereas 25% of the chambers' geometry was preserved when the chambers were empty. In the Schmid et al. study (1997), all of the chambers preserved their original geometry and none of them underwent distortion after two months. A possible explanation for this is that the healing time of Schmid's study was only two months and most of the available poly α-hydroxy acids polymer barriers are claimed to stay durable and intact for at least two months.

Rimondini et al. used polylactide/polyglycolide copolymer (PLA/PGA) as a filler material in grafting inlay defects created in rabbits' femoral condyles. He found that the material is osteoconductive and resulted in 75.98% to 95.34% of new bone regeneration after three months as compared to the control (Rimondini, et al., 2005). A similar conclusion was reached by Imbronito et al. when he used PLA/PGA as a grafting material in defects created in rabbit tibias and left to heal for 30 days (Imbronito, et al., 2005). In the present study, we observed variable amounts of new bone, which was generated in a linear fashion in close proximity to the bioresorbable barrier after three months of healing. This was noticed more clearly in the control group specimens where new bone was concentrated mainly in two areas; close to the basal bone and adjacent to the partially resorbed barrier.

4.4.0 Conclusion and Recommendations

The results of this study confirm that Bio-Oss® is an osteoconductive material when used as a bone substitute under trimethylene carbonate, L-lactide and a polyglycolide bioresorbable barrier. We suggest that our technique be applied clinically to horizontally augment atrophic alveolar ridges prior to implant surgery. To consolidate these findings, similar investigations on higher animals and human models are needed to assess the predictability and effectiveness of this technique.

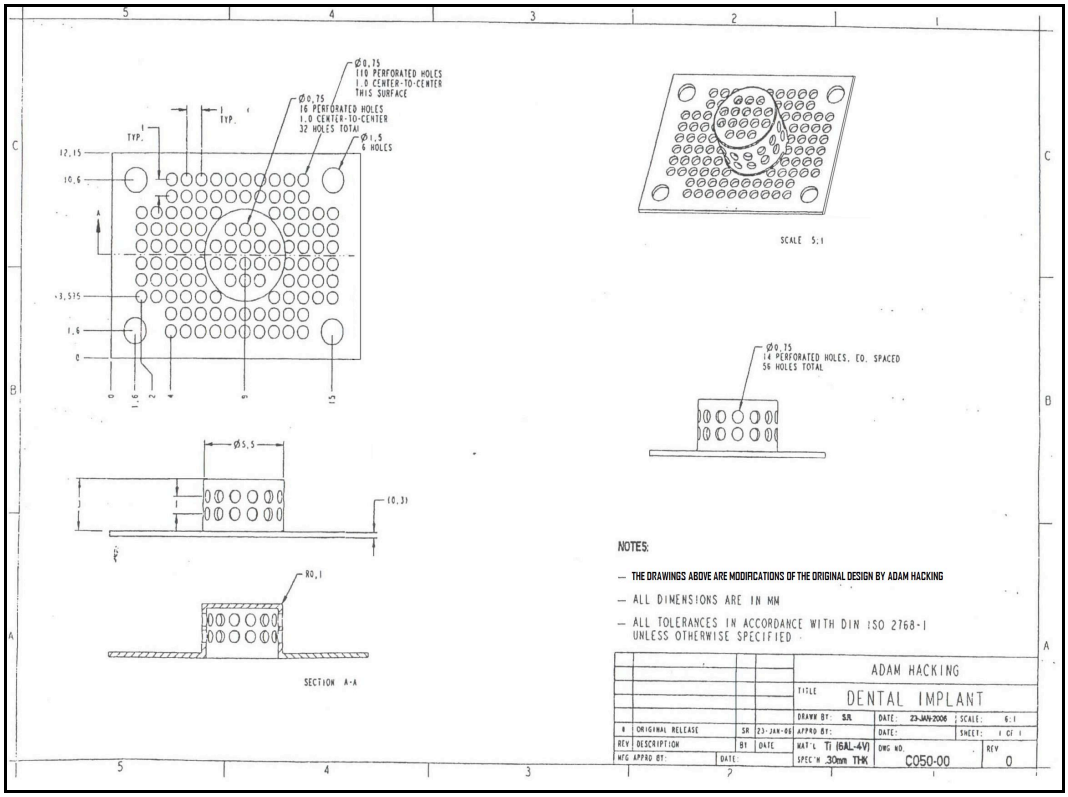
Appendix I

Order of Operation, Surgery and Euthanasia Dates, and Randomization of Rabbits

Animal number	Animal name	Surgery date	Euthanasia date	Experimental (Bio-Oss®) side
1	**Punch**	**Oct 6, 2009**	**Jan 7, 2010**	Left
2	**Gitane**	**Oct 7, 2009**	**Jan 7, 2010**	Right
3	**Amazonas**	**Oct 7, 2009**	**Jan 7, 2010**	Right
4	**Ardie**	**Oct 7, 2009**	**Jan 7, 2010**	Right
5	**Militaire**	**Oct 7, 2009**	**Jan 7, 2010**	Right
6	**Derbie**	**Oct 7, 2009**	**Jan 7, 2010**	Right
7	**Buell**	**Oct 7, 2009**	**Jan 7, 2010**	Left
8	**Liberia**	**Oct 7, 2009**	**Jan 7, 2010**	Left
9	**Pope**	**Oct 8, 2009**	**Jan 7, 2010**	Right
10	**Griffon**	**Oct 8, 2009**	**Jan 7, 2010**	Right
11	**Jawa**	**Oct 8, 2009**	**Jan 7, 2010**	Left
12	**Horex**	**Oct 8, 2009**	**Jan 7, 2010**	Right

Appendix II

The Design of the Titanium Template Used to Mold the Bioresorbable Chambers



(This design is a modification of the titanium mesh designed by Adam Hacking)

Appendix III

Micro-CT Scan Analysis Results

Experimental Group (Bio-Oss®)

Animal Name	Bovine Bone + New Bone (mm^3)	Bovine Bone Only (mm^3)	Bone Only (mm^3)	Template $\pi*(2.75)^2*3$ (mm^3)	BV/TV (%)	Bone Height (mm)
Amazonas	28.30	19.40	8.90	71.30	12.48	2.44
Ardie	27.69	18.56	9.13	71.30	12.81	2.22
Buell	18.51	12.02	6.49	71.30	9.11	1.75
Derbie	11.26	4.83	6.43	71.30	9.02	2.58
Gitane	22.37	15.06	7.31	71.30	10.25	1.95
Griffon	31.35	20.84	10.51	71.30	14.73	2.8
Horex	22.76	14.15	8.60	71.30	12.07	2.5
Jawa	20.31	14.13	6.17	71.30	8.66	2.2
Liberia	26.30	20.13	6.16	71.30	8.64	2.66
Militaire	18.33	11.10	7.23	71.30	10.15	1.86
Pope	30.59	21.51	9.08	71.30	12.73	2.42
Punch	19.49	13.09	6.40	71.30	8.98	1.66

Control Group

Animal Name	Bovine Bone + New Bone (mm^3)	Bovine Bone Only (mm^3)	Bone Only (mm^3)	Template $\pi*(2.75)^2*3$ (mm^3)	BV/TV (%)	Bone Height (mm)
Amazonas	0.09	0.01	0.08	71.30	0.11	1.07
Ardie	0.12	0.04	0.07	71.30	0.10	0.74
Buell	0.29	0.02	0.27	71.30	0.38	1.52
Derbie	0.29	0.07	0.22	71.30	0.31	1.05
Gitane	0.96	0.08	0.88	71.30	1.23	1.78
Griffon	0.42	0.10	0.32	71.30	0.45	1.85
Horex	0.09	0.01	0.09	71.30	0.12	1.75
Jawa	0.07	0.01	0.06	71.30	0.09	1.96
Liberia	0.16	0.03	0.13	71.30	0.18	1.55
Militaire	0.12	0.03	0.10	71.30	0.13	0.82
Pope	0.36	0.10	0.25	71.30	0.36	0.65
Punch	0.00	0.00	0.00	71.30	0.00	1.48

Appendix IV

Histomorphometric Analysis Results

Experimental Group (Bio-Oss®)

Animal Name	Upper or lower compartment area (μm^2)	Lower compartment		Upper compartment		Average percentage of new bone of both compartments (%)	Chamber geometry
		New bone area (μm^2)	Percentage of new bone (%)	New bone area (μm^2)	Percentage of new bone (%)		
Amazonas	8,250,000	1791988.45	21.72	670463.36	8.13	14.92395036	intact
Ardie	8,250,000	2275822.83	27.59	1375283.8	16.67	22.12791897	intact
Buell	8,250,000	3190288.15	38.67	561804.93	6.81	22.73995806	intact
Derbie	8,250,000	2712958.27	32.88	618010.44	7.49	20.18768915	intact
Gitane	8,250,000	1690751.82	20.49	381013.32	4.62	12.55615236	intact
Griffon	8,250,000	2234711.08	27.09	630685.87	7.64	17.36604212	intact
Horex	8,250,000	2676933.37	32.45	1117939.28	13.55	22.99922818	moderate collapse
Jawa	8,250,000	2059590.06	24.96	1136702.25	13.78	19.37146855	intact
Liberia	8,250,000	2202855.73	26.70	806640.81	9.78	18.23937297	intact
Militaire	8,250,000	2015726.41	24.43	202389.87	2.45	13.44312897	intact
Pope	8,250,000	2224037.04	26.96	1208835.43	14.65	20.8052877	intact
Punch	8,250,000	1915573.86	23.22	494841.99	6.00	14.60858091	moderate collapse

Control Group

Animal Name	Upper or lower compartment area (μm^2)	Lower compartment		Upper compartment		Average percentage of new bone of both compartments (%)	Chamber geometry
		New bone area (μm^2)	Percentage of new bone (%)	New bone area (μm^2)	Percentage of new bone (%)		
Amazonas	8,250,000	546127.43	6.62	290367.34	3.52	5.069665273	moderate collapse
Ardie	8,250,000	252924.8	3.07	0	0.00	1.532877576	severe collapse
Buell	8,250,000	497593.89	6.03	0	0.00	3.015720545	moderate collapse
Derbie	8,250,000	890865.68	10.80	0	0.00	5.399185939	moderate collapse
Gitane	8,250,000	1220760.34	14.80	32939.43	0.40	7.598180424	moderate collapse
Griffon	8,250,000	549129.51	6.66	81889.93	0.99	3.824360242	intact
Horex	8,250,000	371590.14	4.50	0	0.00	2.252061455	intact
Jawa	8,250,000	371423.36	4.50	268852.47	3.26	3.880459576	intact
Liberia	8,250,000	345905.72	4.19	167115.49	2.03	3.109219455	moderate collapse
Militaire	8,250,000	303960.07	3.68	0	0.00	1.842182242	moderate collapse
Pope	8,250,000	207560.11	2.52	0	0.00	1.257940061	severe collapse
Punch	8,250,000	93314.49	1.13	0	0.00	0.565542364	moderate collapse

References

Abubaker, A. O., & Benson, K. J. (2007). *Oral and Maxillofacial Surgery Secrets* (Second ed.). Missouri: Mosby Elsevier.

Adell, R., Lekholm, U., Rockler, B., & Branemark, P. I. (1981). A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg, 10*(6), 387-416.

Al-Harkan, A. (2008). *Is Bio-Oss® an osteoconductive material when used as an onlay bone substitute? An experimental study in the mandible of the rabbit.* Unpublished Masters', McGill University, Montreal.

Al-Jandan, B. (2007). *The efficacy of biodegradable mesh as a fixation device for support of autogenous onlay bone grafts : a radiographic and histomorphometric analysis.* Unpublished Masters', McGill University, Montreal.

Al-Masri, M. (2009). *Is Bone Ceramic® an osteoconductive material when used as an onlay graft? A prospective experiment on rabbit mandibles.* Unpublished Master's, Mcgill University, Montreal.

Albrektsson, T., & Albrektsson, B. (1987). Osseointegration of bone implants. A review of an alternative mode of fixation. *Acta Orthop Scand, 58*(5), 567-577.

Albrektsson, T., & Johansson, C. (2001). Osteoinduction, osteoconduction and osseointegration. *Eur Spine J, 10 Suppl 2*, S96-101.

Amano, Y., Ota, M., Sekiguchi, K., Shibukawa, Y., & Yamada, S. (2004). Evaluation of a poly-l-lactic acid membrane and membrane fixing pin for

guided tissue regeneration on bone defects in dogs. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 97*(2), 155-163.

Araujo, M. G., Sonohara, M., Hayacibara, R., Cardaropoli, G., & Lindhe, J. (2002). Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol, 29*(12), 1122-1131.

Barber, H. D., & Betts, N. J. (1993). Rehabilitation of maxillofacial trauma patients with dental implants. *Implant Dent, 2*(3), 191-193.

Barber, H. D., Lignelli, J., Smith, B. M., & Bartee, B. K. (2007). Using a dense PTFE membrane without primary closure to achieve bone and tissue regeneration. *J Oral Maxillofac Surg, 65*(4), 748-752.

Barone, A., & Covani, U. (2007). Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. *J Oral Maxillofac Surg, 65*(10), 2039-2046.

Barone, A., Varanini, P., Orlando, B., Tonelli, P., & Covani, U. (2009). Deep-frozen allogeneic onlay bone grafts for reconstruction of atrophic maxillary alveolar ridges: a preliminary study. *J Oral Maxillofac Surg, 67*(6), 1300-1306.

Bartee, B. K. (1998). Evaluation of a new polytetrafluoroethylene guided tissue regeneration membrane in healing extraction sites. *Compend Contin Educ Dent, 19*(12), 1256-1258, 1260, 1262-1254.

Bartee, B. K. (2001). Extraction site reconstruction for alveolar ridge preservation. Part 1: rationale and materials selection. *J Oral Implantol, 27*(4), 187-193.

Bauer, T. W., & Muschler, G. F. (2000). Bone graft materials. An overview of the basic science. *Clin Orthop Relat Res*(371), 10-27.

Becker, W., & Becker, B. E. (1990). Guided tissue regeneration for implants placed into extraction sockets and for implant dehiscences: surgical techniques and case report. *Int J Periodontics Restorative Dent, 10*(5), 376-391.

Becker, W., Becker, B. E., & Caffesse, R. (1994). A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets. *J Periodontol, 65*(12), 1128-1133.

Becker, W., Urist, M. R., Tucker, L. M., Becker, B. E., & Ochsenbein, C. (1995). Human demineralized freeze-dried bone: inadequate induced bone formation in athymic mice. A preliminary report. *J Periodontol, 66*(9), 822-828.

Benke, D., Olah, A., & Mohler, H. (2001). Protein-chemical analysis of Bio-Oss bone substitute and evidence on its carbonate content. *Biomaterials, 22*(9), 1005-1012.

Borstlap, W. A., Heidbuchel, K. L., Freihofer, H. P., & Kuijpers-Jagtman, A. M. (1990). Early secondary bone grafting of alveolar cleft defects. A comparison between chin and rib grafts. *J Craniomaxillofac Surg, 18*(5), 201-205.

Bostman, O., Makela, E. A., Tormala, P., & Rokkanen, P. (1989). Transphyseal fracture fixation using biodegradable pins. *J Bone Joint Surg Br, 71-B*(4), 706-707.

Boyne, P. J., Cole, M. D., Stringer, D., & Shafqat, J. P. (1985). A technique for osseous restoration of deficient edentulous maxillary ridges. *J Oral Maxillofac Surg, 43*(2), 87-91.

Branemark, P. I. (1985). *Introduction to Osseointegration. In: Tissue integrated Prostheses. Osseointegration in Clinical Dentistry*. Berlin, Chicago, Tokyo: Quintessence.

Buck, B. E., Malinin, T. I., & Brown, M. D. (1989). Bone transplantation and human immunodeficiency virus. An estimate of risk of acquired immunodeficiency syndrome (AIDS). *Clin Orthop Relat Res*(240), 129-136.

Buck, B. E., Resnick, L., Shah, S. M., & Malinin, T. I. (1990). Human immunodeficiency virus cultured from bone. Implications for transplantation. *Clin Orthop Relat Res*(251), 249-253.

Bunyaratavej, P., & Wang, H. L. (2001). Collagen membranes: a review. *J Periodontol, 72*(2), 215-229.

Buser, D., Bragger, U., Lang, N. P., & Nyman, S. (1990). Regeneration and enlargement of jaw bone using guided tissue regeneration. *Clin Oral Implants Res, 1*(1), 22-32.

Buser, D., Dula, K., Belser, U., Hirt, H. P., & Berthold, H. (1993). Localized ridge augmentation using guided bone regeneration. 1. Surgical procedure in the maxilla. *Int J Periodontics Restorative Dent, 13*(1), 29-45.

Buser, D., Dula, K., Belser, U. C., Hirt, H. P., & Berthold, H. (1995). Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. *Int J Periodontics Restorative Dent, 15*(1), 10-29.

Cameron, H. U., Pilliar, R. M., & MacNab, I. (1973). The effect of movement on the bonding of porous metal to bone. *J Biomed Mater Res, 7*(4), 301-311.

Cancian, D. C., Hochuli-Vieira, E., Marcantonio, R. A., & Marcantonio, E., Jr. (1999). Use of BioGran and Calcitite in bone defects: histologic study in monkeys (Cebus apella). [Comparative Study]. *The International journal of oral & maxillofacial implants, 14*(6), 859-864.

Caplanis, N., Sigurdsson, T. J., Rohrer, M. D., & Wikesjo, U. M. (1997). Effect of allogeneic, freeze-dried, demineralized bone matrix on guided bone regeneration in supra-alveolar peri-implant defects in dogs. [Research Support, Non-U.S. Gov't]. *The International journal of oral & maxillofacial implants, 12*(5), 634-642.

Carmagnola, D., Adriaens, P., & Berglundh, T. (2003). Healing of human extraction sockets filled with Bio-Oss. *Clin Oral Implants Res, 14*(2), 137-143.

Carmagnola, D., Berglundh, T., & Lindhe, J. (2002). The effect of a fibrin glue on the integration of Bio-Oss with bone tissue. A experimental study in labrador dogs. *J Clin Periodontol, 29*(5), 377-383.

Clokie, C. M., Moghadam, H., Jackson, M. T., & Sandor, G. K. (2002). Closure of critical sized defects with allogenic and alloplastic bone substitutes. *J Craniofac Surg, 13*(1), 111-121; discussion 122-113.

Cohen, R. E., Mullarky, R. H., Noble, B., Comeau, R. L., & Neiders, M. E. (1994). Phenotypic characterization of mononuclear cells following anorganic bovine bone implantation in rats. *J Periodontol, 65*(11), 1008-1015.

Contar, C., Sarot, J., da Costa, M., Bordini Jr, J., Lima, A. A., Azevedo-Alanis, L., et al. (2010). Fresh-Frozen Bone Allografts in Maxillary Ridge Augmentation: Histological Analysis. *J Oral Implantol*.

Contar, C. M., Sarot, J. R., Bordini, J., Jr., Galvao, G. H., Nicolau, G. V., & Machado, M. A. (2009). Maxillary ridge augmentation with fresh-frozen bone allografts. *J Oral Maxillofac Surg, 67*(6), 1280-1285.

Cordaro, L., Amade, D. S., & Cordaro, M. (2002). Clinical results of alveolar ridge augmentation with mandibular block bone grafts in partially edentulous patients prior to implant placement. *Clin Oral Implants Res, 13*(1), 103-111.

Cordaro, L., Bosshardt, D. D., Palattella, P., Rao, W., Serino, G., & Chiapasco, M. (2008). Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenter clinical trial. *Clin Oral Implants Res, 19*(8), 796-803.

Cornell, C. N., & Lane, J. M. (1998). Current understanding of osteoconduction in bone regeneration. *Clin Orthop Relat Res*(355 Suppl), S267-273.

Cricchio, G., & Lundgren, S. (2003). Donor site morbidity in two different approaches to anterior iliac crest bone harvesting. *Clin Implant Dent Relat Res, 5*(3), 161-169.

Dahlin, C., Sennerby, L., Lekholm, U., Linde, A., & Nyman, S. (1989). Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. *Int J Oral Maxillofac Implants, 4*(1), 19-25.

Ekfeldt, A., Christiansson, U., Eriksson, T., Linden, U., Lundqvist, S., Rundcrantz, T., et al. (2001). A retrospective analysis of factors associated with multiple implant failures in maxillae. *Clin Oral Implants Res, 12*(5), 462-467.

Eppley, B. L., Pietrzak, W. S., & Blanton, M. W. (2005). Allograft and alloplastic bone substitutes: a review of science and technology for the craniomaxillofacial surgeon. *J Craniofac Surg, 16*(6), 981-989.

Finkemeier, C. G. (2002). Bone-grafting and bone-graft substitutes. *J Bone Joint Surg Am, 84-A*(3), 454-464.

Franco, M., Viscioni, A., Rigo, L., Guidi, R., Brunelli, G., & Carinci, F. (2009). Iliac crest fresh frozen homografts used in pre-prosthetic surgery: a retrospective study. *Cell Tissue Bank, 10*(3), 227-233.

Froum, S. J., Wallace, S. S., Elian, N., Cho, S. C., & Tarnow, D. P. (2006). Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: histomorphometry at 26 to 32 weeks after grafting. *Int J Periodontics Restorative Dent, 26*(6), 543-551.

Fukuta, K., Har-Shai, Y., Collares, M. V., Lichten, J. B., & Jackson, I. T. (1992). Comparison of inorganic bovine bone mineral particles with porous hydroxyapatite granules and cranial bone dust in the reconstruction of full-thickness skull defect. *J Craniofac Surg, 3*(1), 25-29.

Gbara, A., Darwich, K., Li, L., Schmelzle, R., & Blake, F. (2007). Long-term results of jaw reconstruction with microsurgical fibula grafts and dental implants. *J Oral Maxillofac Surg, 65*(5), 1005-1009.

Haas, R., Donath, K., Fodinger, M., & Watzek, G. (1998). Bovine hydroxyapatite for maxillary sinus grafting: comparative histomorphometric findings in sheep. *Clin Oral Implants Res, 9*(2), 107-116.

Hallman, M., Lundgren, S., & Sennerby, L. (2001). Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clin Implant Dent Relat Res, 3*(2), 87-96.

Hammerle, C. H., Schmid, J., Lang, N. P., & Olah, A. J. (1995). Temporal dynamics of healing in rabbit cranial defects using guided bone regeneration. [Research Support, Non-U.S. Gov't]. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons, 53*(2), 167-174.

Hardwick, R., Scantlebury, T. V., Sanchez, R., Whitley, N., & Ambruster, J. (1994). Guided Bone Regeneration in Implant Dentistry. Berlin: Quintessence Publishing Co.

Hermann, J. S., & Buser, D. (1996). Guided bone regeneration for dental implants. *Curr Opin Periodontol, 3*, 168-177.

Hirota, M., Matsui, Y., Mizuki, N., Kishi, T., Watanuki, K., Ozawa, T., et al. (2009). Combination with allogenic bone reduces early absorption of beta-tricalcium phosphate (beta-TCP) and enhances the role as a bone regeneration scaffold. Experimental animal study in rat mandibular bone defects. *Dent Mater J, 28*(2), 153-161.

Hodges, N. E., Perry, M., Mohamed, W., Hallmon, W. W., Rees, T., & Opperman, L. A. (2006). Distraction osteogenesis versus autogenous

onlay grafting. Part II: biology of regenerate and onlay bone. *Int J Oral Maxillofac Implants, 21*(2), 237-244.

Imbronito, A. V., Scarano, A., Orsini, G., Piattelli, A., & Arana-Chavez, V. E. (2005). Ultrastructure of bone healing in defects grafted with a copolymer of polylactic/polyglycolic acids. *J Biomed Mater Res A, 74*(2), 215-221.

Inion™. (2004). Inion GTR™ Biodegradable Membrane System. In A. C. O. Inion Inc. (Ed.) (pp. 8). Oklahoma City: Inion Intelligent Solution.

Jensen, O. T. (2006). Alveolar segmental "sandwich" osteotomies for posterior edentulous mandibular sites for dental implants. *J Oral Maxillofac Surg, 64*(3), 471-475.

Jensen, O. T., Shulman, L. B., Block, M. S., & Iacono, V. J. (1998). Report of the Sinus Consensus Conference of 1996. *Int J Oral Maxillofac Implants, 13 Suppl*, 11-45.

Johnson, K. D., Frierson, K. E., Keller, T. S., Cook, C., Scheinberg, R., Zerwekh, J., et al. (1996). Porous ceramics as bone graft substitutes in long bone defects: a biomechanical, histological, and radiographic analysis. *J Orthop Res, 14*(3), 351-369.

Kasabah, S., Simunek, A., Krug, J., & Lecaro, M. C. (2002). Maxillary sinus augmentation with deproteinized bovine bone (Bio-Oss) and Impladent dental implant system. Part II. Evaluation of deprotienized bovine bone (Bio-Oss) and implant surface. *Acta Medica (Hradec Kralove), 45*(4), 167-171.

Keating, J. F., & McQueen, M. M. (2001). Substitutes for autologous bone graft in orthopaedic trauma. *J Bone Joint Surg Br, 83*(1), 3-8.

Klinge, B., Alberius, P., Isaksson, S., & Jonsson, J. (1992). Osseous response to implanted natural bone mineral and synthetic hydroxylapatite ceramic in the repair of experimental skull bone defects. *J Oral Maxillofac Surg, 50*(3), 241-249.

Kodama, T., Minabe, M., Hori, T., & Watanabe, Y. (1989). The effect of various concentrations of collagen barrier on periodontal wound healing. *J Periodontol, 60*(4), 205-210.

Kurashina, K., Kurita, H., Kotani, A., Kobayashi, S., Kyoshima, K., & Hirano, M. (1998). Experimental cranioplasty and skeletal augmentation using an alpha-tricalcium phosphate/dicalcium phosphate dibasic/tetracalcium phosphate monoxide cement: a preliminary short-term experiment in rabbits. *Biomaterials, 19*(7-9), 701-706.

Lacerda, S. A., Lanzoni, J. F., Bombonato-Prado, K. F., Campos, A. A., Prata, C. A., & Brentegani, L. G. (2009). Osteogenic potential of autogenous bone associated with bone marrow osteoblastic cells in bony defects: a histomorphometric study. *Implant Dent, 18*(6), 521-529.

LeGeros, R. Z. (2002). Properties of osteoconductive biomaterials: calcium phosphates. *Clin Orthop Relat Res*(395), 81-98.

Lekovic, V., Camargo, P. M., Klokkevold, P. R., Weinlaender, M., Kenney, E. B., Dimitrijevic, B., et al. (1998). Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. *J Periodontol, 69*(9), 1044-1049.

Lekovic, V., Kenney, E. B., Weinlaender, M., Han, T., Klokkevold, P., Nedic, M., et al. (1997). A bone regenerative approach to alveolar ridge maintenance

following tooth extraction. Report of 10 cases. *J Periodontol, 68*(6), 563-570.

Manninen, M. J., Paivarinta, U., Taurio, R., Tormala, P., Suuronen, R., Raiha, J., et al. (1992). Polylactide screws in the fixation of olecranon osteotomies. A mechanical study in sheep. *Acta Orthop Scand, 63*(4), 437-442.

Mellonig, J. T., Nevins, M., & Sanchez, R. (1998). Evaluation of a bioabsorbable physical barrier for guided bone regeneration. Part I. Material alone. *Int J Periodontics Restorative Dent, 18*(2), 139-149.

Miloro, M. (2004). *Peterson's Principals of Oral and Maxillofacial Surgery* (second ed. Vol. 2). Hamilton, London: BC Decker Inc.

Moghadam, H. G., Sandor, G. K., Holmes, H. H., & Clokie, C. M. (2004). Histomorphometric evaluation of bone regeneration using allogeneic and alloplastic bone substitutes. *J Oral Maxillofac Surg, 62*(2), 202-213.

Nkenke, E., Radespiel-Troger, M., Wiltfang, J., Schultze-Mosgau, S., Winkler, G., & Neukam, F. W. (2002). Morbidity of harvesting of retromolar bone grafts: a prospective study. *Clin Oral Implants Res, 13*(5), 514-521.

Norton, M. R., Odell, E. W., Thompson, I. D., & Cook, R. J. (2003). Efficacy of bovine bone mineral for alveolar augmentation: a human histologic study. *Clin Oral Implants Res, 14*(6), 775-783.

Ohgushi, H., Goldberg, V. M., & Caplan, A. I. (1989). Repair of bone defects with marrow cells and porous ceramic. Experiments in rats. *Acta Orthop Scand, 60*(3), 334-339.

Pearce, A. I., Richards, R. G., Milz, S., Schneider, E., & Pearce, S. G. (2007). Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater, 13*, 1-10.

Peetz, M. (1997). Characterization of xenogenic bone material. In P. J. Boyne (Ed.), *Osseous reconstruction of the maxilla and the mandible – surgical techniques using titanium mesh and bone mineral* (pp. 87–100). Chicago, Berlin: Quintessence.

Perrott, D. H., Smith, R. A., & Kaban, L. B. (1992). The use of fresh frozen allogeneic bone for maxillary and mandibular reconstruction. *Int J Oral Maxillofac Surg, 21*(5), 260-265.

Pinholt, E. M., Bang, G., & Haanaes, H. R. (1991). Alveolar ridge augmentation in rats by Bio-Oss. *Scand J Dent Res, 99*(2), 154-161.

Postlethwaite, A. E., Seyer, J. M., & Kang, A. H. (1978). Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. *Proc Natl Acad Sci U S A, 75*(2), 871-875.

Rimondini, L., Nicoli-Aldini, N., Fini, M., Guzzardella, G., Tschon, M., & Giardino, R. (2005). In vivo experimental study on bone regeneration in critical bone defects using an injectable biodegradable PLA/PGA copolymer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 99*(2), 148-154.

Roberts, W. E., & Garetto, L. P. (1999). Bone physiology and metabolism. In C. E. Misch (Ed.), *Contemporary implant dentistry* (pp. 225). St Louis: Mosby.

Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A., & Becker, J. (2005). Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Implants Res, 16*(3), 369-378.

Sartori, S., Silvestri, M., Forni, F., Icaro Cornaglia, A., Tesei, P., & Cattaneo, V. (2003). Ten-year follow-up in a maxillary sinus augmentation using anorganic bovine bone (Bio-Oss). A case report with histomorphometric evaluation. *Clin Oral Implants Res, 14*(3), 369-372.

Schenk, R. K., Buser, D., Hardwick, W. R., & Dahlin, C. (1994). Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. *Int J Oral Maxillofac Implants, 9*(1), 13-29.

Schlegel, A. K., & Donath, K. (1998). BIO-OSS--a resorbable bone substitute? *J Long Term Eff Med Implants, 8*(3-4), 201-209.

Schmid, J., Hammerle, C. H., Fluckiger, L., Winkler, J. R., Olah, A. J., Gogolewski, S., et al. (1997). Blood-filled spaces with and without filler materials in guided bone regeneration. A comparative experimental study in the rabbit using bioresorbable membranes. *Clin Oral Implants Res, 8*(2), 75-81.

Schmid, J., Wallkamm, B., Hammerle, C. H., Gogolewski, S., & Lang, N. P. (1997). The significance of angiogenesis in guided bone regeneration. A case report of a rabbit experiment. *Clin Oral Implants Res, 8*(3), 244-248.

Schwarz, F., Rothamel, D., Herten, M., Sager, M., & Becker, J. (2006). Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clin Oral Implants Res, 17*(4), 403-409.

Sela, M. N., Kohavi, D., Krausz, E., Steinberg, D., & Rosen, G. (2003). Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria. *Clin Oral Implants Res, 14*(3), 263-268.

Simion, M., Baldoni, M., Rossi, P., & Zaffe, D. (1994). A comparative study of the effectiveness of e-PTFE membranes with and without early exposure during the healing period. *Int J Periodontics Restorative Dent, 14*(2), 166-180.

Simion, M., Trisi, P., Maglione, M., & Piattelli, A. (1994). A preliminary report on a method for studying the permeability of expanded polytetrafluoroethylene membrane to bacteria in vitro: a scanning electron microscopic and histological study. *J Periodontol, 65*(8), 755-761.

Slotte, C., & Lundgren, D. (1999). Augmentation of calvarial tissue using non-permeable silicone domes and bovine bone mineral. An experimental study in the rat. *Clin Oral Implants Res, 10*(6), 468-476.

Slotte, C., Lundgren, D., & Burgos, P. M. (2003). Placement of autogeneic bone chips or bovine bone mineral in guided bone augmentation: a rabbit skull study. [Comparative Study Research Support, Non-U.S. Gov't]. *The International journal of oral & maxillofacial implants, 18*(6), 795-806.

Soost, F., Koch, S., Stoll, C., Amthauer, H., Grosse-Siestrup, C., & Zorn, P. (2001). Validation of bone conversion in osteoconductive and osteoinductive bone substitutes. *Cell Tissue Bank, 2*(2), 77-86.

Spenlehauer, G., Spenlehauer-Bonthonneau, F., & Thies, C. (1989). Biodegradable microparticles for delivery of polypeptides and proteins. *Prog Clin Biol Res, 292*, 283-291.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R., & Karring, T. (2001). Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clin Implant Dent Relat Res, 3*(3), 156-165.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R., & Karring, T. (2003). Deproteinized bovine bone (Bio-Oss) and bioactive glass (Biogran) arrest bone formation when used as an adjunct to guided tissue regeneration (GTR): an experimental study in the rat. *J Clin Periodontol, 30*(7), 636-643.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R., & Karring, T. (2004). Fate of bone formed by guided tissue regeneration with or without grafting of Bio-Oss or Biogran. An experimental study in the rat. *J Clin Periodontol, 31*(1), 30-39.

Stellingsma, C., Raghoebar, G. M., Meijer, H. J., & Batenburg, R. H. (1998). Reconstruction of the extremely resorbed mandible with interposed bone grafts and placement of endosseous implants. A preliminary report on outcome of treatment and patients' satisfaction. *Br J Oral Maxillofac Surg, 36*(4), 290-295.

Stevenson, S. (1999). Biology of bone grafts. *Orthop Clin North Am, 30*(4), 543-552.

Stevenson, S., Emery, S. E., & Goldberg, V. M. (1996). Factors affecting bone graft incorporation. *Clin Orthop Relat Res*(324), 66-74.

Suuronen, R. (1991). Comparison of absorbable self-reinforced poly-L-lactide screws and metallic screws in the fixation of mandibular condyle

osteotomies: an experimental study in sheep. *J Oral Maxillofac Surg, 49*(9), 989-995.

Tadjoedin, E. S., de Lange, G. L., Lyaruu, D. M., Kuiper, L., & Burger, E. H. (2002). High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. [Comparative Study Research Support, Non-U.S. Gov't]. *Clinical Oral Implants Research, 13*(4), 428-436.

Terheyden, H., Jepsen, S., Moller, B., Tucker, M. M., & Rueger, D. C. (1999). Sinus floor augmentation with simultaneous placement of dental implants using a combination of deproteinized bone xenografts and recombinant human osteogenic protein-1. A histometric study in miniature pigs. *Clin Oral Implants Res, 10*(6), 510-521.

Thaller, S. R., Hoyt, J., Dart, A., Borjeson, K., & Tesluk, H. (1994). Repair of experimental calvarial defects with Bio-Oss particles and collagen sponges in a rabbit model. *J Craniofac Surg, 5*(4), 242-246.

Triplett, R. G., Nevins, M., Marx, R. E., Spagnoli, D. B., Oates, T. W., Moy, P. K., et al. (2009). Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg, 67*(9), 1947-1960.

Uhthoff, H. K. (1973). Mechanical factors influencing the holding power of screws in compact bone. *J Bone Joint Surg Br, 55*(3), 633-639.

Urist, M. R. (1965). Bone: Formation by Autoinduction. *Science, 150*(3698), 893-899.

Valentini, P., Abensur, D., Densari, D., Graziani, J. N., & Hammerle, C. (1998). Histological evaluation of Bio-Oss in a 2-stage sinus floor elevation and implantation procedure. A human case report. *Clin Oral Implants Res, 9*(1), 59-64.

Valentini, P., Abensur, D., Wenz, B., Peetz, M., & Schenk, R. (2000). Sinus grafting with porous bone mineral (Bio-Oss) for implant placement: a 5-year study on 15 patients. *Int J Periodontics Restorative Dent, 20*(3), 245-253.

von Arx, T., Broggini, N., Jensen, S. S., Bornstein, M. M., Schenk, R. K., & Buser, D. (2005). Membrane durability and tissue response of different bioresorbable barrier membranes: a histologic study in the rabbit calvarium. *Int J Oral Maxillofac Implants, 20*(6), 843-853.

Von Arx, T., Hardt, N., & Wallkamm, B. (1996). The TIME technique: a new method for localized alveolar ridge augmentation prior to placement of dental implants. *Implant Dentistry, 6*(1), 61.

Von Arx, T., Walkamm, B., & Hardt, N. (1998). Localized ridge augmentation using a micro titanium mesh: a report on 27 implants followed from 1 to 3 years after functional loading. *Clinical Oral Implants Research, 9*(2), 123-130.

Waasdorp, J., & Reynolds, M. A. (2010). Allogeneic bone onlay grafts for alveolar ridge augmentation: a systematic review. *Int J Oral Maxillofac Implants, 25*(3), 525-531.

Wenz, B., Oesch, B., & Horst, M. (2001). Analysis of the risk of transmitting bovine spongiform encephalopathy through bone grafts derived from bovine bone. *Biomaterials, 22*(12), 1599-1606.

Wheeler, D. L., Eschbach, E. J., Hoellrich, R. G., Montfort, M. J., & Chamberland, D. L. (2000). Assessment of resorbable bioactive material for grafting of critical-size cancellous defects. *J Orthop Res, 18*(1), 140-148.

Will, R. G., Ironside, J. W., Zeidler, M., Cousens, S. N., Estibeiro, K., Alperovitch, A., et al. (1996). A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet, 347*(9006), 921-925.

Wlodarski, P. K., Haberko, K., Haberko, M., Pyda, A., & Wlodarski, K. H. (2005). Implantation of natural hydroxyapatite from porcine bone into soft tissues in mice. *Folia Biol (Krakow), 53*(3-4), 183-187.

Yildirim, M., Spiekermann, H., Biesterfeld, S., & Edelhoff, D. (2000). Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood. A histologic and histomorphometric study in humans. *Clin Oral Implants Res, 11*(3), 217-229.

Zerbo, I. R., Zijderveld, S. A., de Boer, A., Bronckers, A. L., de Lange, G., ten Bruggenkate, C. M., et al. (2004). Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. *Clin Oral Implants Res, 15*(6), 724-732.

Zhang, G. (2006). Maxillofacial Bone Augmentation and Replacement *Polymers for Dental and Orthopedic Applications* (Vol. null): CRC Press.

Zins, J. E., & Whitaker, L. A. (1983). Membranous versus endochondral bone: implications for craniofacial reconstruction. *Plast Reconstr Surg, 72*(6), 778-785.

Zitzmann, N. U., Scharer, P., Marinello, C. P., Schupbach, P., & Berglundh, T. (2001). Alveolar ridge augmentation with Bio-Oss: a histologic study in humans. *Int J Periodontics Restorative Dent, 21*(3), 288-295.