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A Histologic and Radiologic Analysis of Bone Formation Under the Elevated Maxillary Sinus using Venous Coagulum as the Sole Filling Material

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ABSTRACT

Insufficient bone height is a common obstacle to placing dental implants in the posterior maxilla. Sinus lift procedures using various grafting materials have been shown to be a highly predictable way to increase bone height in the posterior maxilla (Jensen 1998). Given the wide range of materials that have proven successful in augmenting the sinus, the argument could be made that the presence of graft is not critical. Instead, the creation and maintenance of space, provided by two implants, along with the osteoinductive properties of the membrane, the periosteum, and growth factors provided by a blood clot are the only requirements for bone formation in the maxillary sinus.

The purpose of this "proof of principle" study is to demonstrate that elevation of the sinus, using the patient's venous coagulum alone, results in clinical, radiologic and histologic evidence of vital bone formation. A total of 5 sinus elevations with simultaneous placement of 2 dental implants were performed with venous blood coagulum as the sole filling biomaterial. After 8-9 months of healing, a postoperative cone-beam computed tomography(CBCT) was taken and the implants

were uncovered. During the uncovery procedures, core bone sample were taken from the lateral wall of the maxilla. The cores underwent micro-computed tomography(CT), histologic and histomorphometric analyses. Comparisons of pre-operative and post-operative alveolar crest height were made using cone-beam CT to determine the gain of bone height. Criteria for inclusion were: edentulism in the posterior maxilla, less than 10mm alveolar height beneath the maxillary sinus, greater than 18 years of age and systemically healthy. Gain in height was observed in all 5 sites ranging from 4.37mm to 10.01mm. Histological evaluation showed new bone formation in 4 of the 4 biopsies obtained. Based on the results of this study, it appears that bone graft materials in the sub-sinus cavity are not required for bone formation. Instead, stabilization of a blood clot under the sinus membrane appears to be the fundamental healing mechanism, allowing for bone formation after sinus elevation procedures.

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LIST OF ABBREVIATIONS

AO	Academy of Osseointegration
ASA	American Society of Anesthesiologists
BMP	Bone Morphogenetic Proteins
BBM	Bovine Bone Matrix
CBCT	Cone-Beam Computed Tomography
CT	Computed Tomography
DFDBA	Demineralized Freeze Dried Bone Allograft
DPBB	Deproteinized Bovine Bone
FGF	Fibroblast Growth Factor
HA	Hydroxyapatite
IGF	Insulin-Like Growth Factor
IRB	Institutional Review Board
PCNA	Proliferating Cell Nuclear Antigen
PDGF	Platelet-Derived Growth Factor
PRF	Platelet Rich Fibrin
PRP	Platelet Rich Plasma
TGF	Transforming Growth Factor
UAB	University of Alabama at Birmingham
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

Insufficient bone height, along with the financial and temporal investments of sinus lifts, are often roadblocks in the replacement of teeth with dental implants in the posterior maxilla. Grafting beneath the floor of the maxillary sinus has become the most common surgical modality to increase alveolar height (Wallace & Froum, 2003).

Sinus elevation procedures using various grafting materials have been shown to be a highly predictable way to increase bone height in the posterior maxilla (Jensen 1998). The goal of the sinus elevation is to lift the schneiderian membrane that lines the maxillary sinus from the floor of the sinus. By raising the membrane up into the sinus cavity, a new, more superiorly located sinus floor is constructed. The newly created space can then be filled with bone, or a suitable bone substitute material, to increase the total vertical height of bone in the posterior maxilla, allowing for placement of dental implants (Klokkevold 2006).

Augmentation of the sinus has been described using a variety of grafting materials that include autogenous particulate bone graft (Lundgren et al 1996, Froum et al. 1998, Wood & Moore 1988), demineralized freeze dried bone allograft (DFDBA) particulate (Chanavaz 1990, Chanavaz 1996), anorganic bovine bone particulate(Froum et al. 1998, Hurzeler et al. 1996, Valentini & Abensur 1997), non-resorbable hydroxyapatite (HA) (Small et al. 1993), autogenous block grafts (Wannfors 2000), bone morphogenic protein (BMP-2) (Boyne et al. 1997), platelet

rich plasma (PRP) (Mazor et al. 2009), venous blood (Hatano et al. 2007), and with no graft material at all (Lundgren et al. 2008, Thor et al. 2007, Palma et al. 2006).

Given the wide range of materials that have proven successful in augmenting the sinus, the argument could be made that the presence of graft is not critical. Instead, the creation and maintenance of space, provided by two implants, along with the osteoinductive properties of the membrane, the periosteum, and growth factors provided by a blood clot are the only requirements for bone formation in the maxillary sinus.

Determining that the stabilization of a fibrin clot, without the use of grafting material, results in bone formation will decrease cost and time from sinus lift to restoration. Utilizations of one's own blood as filling material removes any objections to grafting including religious, ethical or fear of disease transmission. Venous blood coagulum is a simple and inexpensive biomaterial, and its systematic use during a sinus lift may be a relevant option, ultimately leading to increased access to implant treatment options for patients.

We hypothesize that bone grafting materials in the subsinus cavity are not required for successful placement of implants. Stabilization of a blood clot under the sinus membrane may be the fundamental healing mechanism allowing for bone formation after sinus elevation procedures.

LITERATURE REVIEW

Dental Implants

Missing teeth lead to functional and cosmetic deficits and have traditionally been replaced with dentures or tooth-supported bridges. The use of dental implants has proved to be a predictable alternative when they are inserted into the jawbones. The success of dental implants relies on osseointegration, defined by P. I. Branemark as the maintenance of a direct structural and functional connection between living bone and the implant surface (Branemark et al. 1977). Dental implants require adequate bone height and width to achieve success (Klokkevold 2006).

Development of Insufficient Bone

Minimal bone height inferior to the sinus floor in the posterior maxilla often creates a unique challenge when treatment planning for dental implants. The inadequate bone volume often encountered results from a combination of post-extraction bone atrophy and ongoing maxillary sinus pneumatization associated with aging. Pneumatization refers to the expansion of the maxilla sinus floor toward the alveolar crest, often resulting in minimal or insufficient bone height (McAllister & Haghighat 2007). The maxillary sinus maintains its overall size while the posterior teeth remain in function. It is well known, however, that the sinus expands with age, especially when posterior teeth are lost. The average volume of a fully developed sinus is about 15mL but may range from 4.5-35.2mL. The sinus cavity expands both inferiorly and laterally, potentially invading the canine region.

This phenomenon may be due to atrophy caused by reduced strain from occlusal function (Lang & Lindhe 2008). Residual ridge height in the edentulous posterior maxilla was measured and 43% of the proposed implant sites had ≤4mm of bone crestal to the sinus (Lundgren et al. 1996). Alveolar bone resorption also occurs following tooth loss. A reduction of approximately 50% in both horizontal and vertical directions has been observed over 12 months. The rate and pattern of bone resorption may be increased if pathologic or traumatic processes have damaged one or more walls of the socket (Chen et al. 2004).

Sinus Elevation and Augmentation

The most common technique for augmenting the maxillary sinus is the lateral approach using the Caldwell-Luc osteotomy, first presented in 1976 by Tatum and later published by Boyne & James and modified by Wood & Moore (Tatum 1986, Boyne & James 1980, Wood & Moore 1998). Access is obtained by drilling a window in the lateral bony wall of the sinus. The sinus membrane is carefully elevated and mobilized together with the attached bony window and rotated medially. Careful elevation of the Schneiderian membrane creates a defined space between itself and the sinus floor to receive the bone-grafting material of choice. The creation of space, in the presence of osteoinductive cells with the exclusion of connective tissue and epithelial cells, encourages the formation of bone. This formation of bone increases the vertical dimension of bone, allowing for placement and osseointegration of dental implants. In humans, several techniques were reported for successful sinus augmentation, with average implant success rates of 92% (Wallace & Froum 2003).

An alternative technique was described by Summers to increase the available bone volume in the posterior maxilla. The floor of the sinus is accessed through the alveolar ridge using various instruments to form and shape the socket. Elevation of the sinus floor is performed by inward collapse of the residual crestal floor with specially designed osteotomes; this eliminates the need for a trap door access. The membrane is elevated and a bone graft material can be introduced through the prepared osteotomy, if needed, with or without simultaneous implant placement (Summers 1994). The amount of augmentation achieved by the osteotome technique ranged from 3 to 5mm. Dependent on the proposed length of implant, a minimum preoperative ridge height of 5mm is desired to achieve adequate elevation of the sinus floor without undue risk of perforation of the schneiderian membrane (Rosen et al. 1999).

When considering which approach to use, anatomical factors, such as pre-operative alveolar bone height and width dimensions and access, as well as the extent of the desired augmentation, must be considered. If sufficient bone volume and quality for achieving primary implant stability is present at the time of augmentation, a single staged approach may be used where implant placement is performed simultaneously (Jensen 1998). Survival of implants placed at the time of sinus augmentation using lateral window approach is increased with crestal ridge heights greater than 3mm (Fugazzotto & Vlassis 1998).

The placement of bioabsorbable or non-resorbable barrier membranes over the lateral sinus window and graft material has been shown to aid in graft containment, prevent soft tissue encleftation, and enhance the implant success rate

(Avera et al. 1997, Wallace et al. 2005). Histologic evaluations of regenerated bone following sinus augmentation has shown considerable variation in bone quality and quantity. Histomorphometric analysis of sinus graft biopsies revealed a large variation, typically 5% to 60% in vital bone area (Moy et al. 1993, Lundgren et al. 1996, Froum et al. 1998, Tarnow et al. 2000, Wheeler et al. 1996, Schenk et al. 1994, Schlegel et al. 2003).

In order to fully ascertain the efficacy of the sinus floor augmentation bone graft procedure, the Academy of Osseointegration (AO) organized a conference in November of 1996. Retrospective data from sinus floor augmentation bone grafts were collected from 38 surgeons for 1007 sinus grafts that involved the placement of 2997 implants over a 10-year period. The majority of implants were followed for 3 or more years post-restoration. Various grafting modalities and root-form implants were used. The various materials, including autographs, allografts, alloplasts, and combinations thereof, all seem to perform acceptably. In combination, all materials were 90% successful in the 3-to 5- year window, which is better than reported when implants are placed in native maxillary bone when no graft is used. Autografts and alloplasts performed better either alone or in combination. Autografts with alloplasts performed better than autografts with allografts. Allografts alone performed less well and when used in combination with other materials were not as successful as when the other materials were used alone (Jensen 1998).

A retrospective quantitative radiographic analysis was performed on a subset of available patients from the AO Consensus Conference. The purpose was to

determine the effect of graft material and smoking status on the maintenance of graft height over 3 years. Results showed that a reduction of mean graft height occurs for all graft materials studied (intraoral autograft and alloplast, alloplast, intraoral autograft, allograft & alloplast, hip autograft, and allograft). Maintenance of bone height was significantly greater in intraoral autogenous grafts versus allografts, which had the least favorable results, followed by hip autografts. The effect of smoking on implant loss revealed a significant difference in implant survival. Autogenous bone generally resulted in a more favorable outcome over a 3-year period. Smoking adversely impacted implant survival in sinus grafts (Geurs et al. 2001).

Definitive conclusions from the AO conference were difficult to draw because the database was so multivariate and multifactorial. Instead, the consensus conference developed and voted on multiple consensus statements derived by committee review for bone graft materials, type of implants, timing for implant placement, failure analysis, radiographic analysis, indications/contraindications, prosthetics, and nomenclature. Several consensus statements were obtained including that the sinus graft should be considered a highly predictable and effective therapeutic modality (Jensen 1998).

Sinus Lift Complications

There is a low incidence of significant complications following sinus augmentation, however the following have been reported: infection, bleeding, cyst formation, graft slumping, membrane tears, ridge resorption, soft tissue

invagination, sinusitis, wound dehiscence, and loss of implants (Avera et al. 1997, Wheeler 1997, Geurs et al. 2001). As long as the sinus graft does not extend high enough to interfere with ostium function, grafting can be considered a generally benign procedure (Drettner & Aust 1977). Evidence of acute sinusitis, chronic sinusitis, or other sinus pathology suggests the need to refer to the otolaryngologist for treatment prior to initiation of the sinus augmentation procedure (Misch 1999). Pre-operative sinusitis was a positive predictive factor for the development of post-operative acute sinusitis (Tidwell et al. 1992). There is an increase in the incidence of membrane tears in cases with smaller internal sinus angles (Froum et al. 1998). If a perforation, or tear, in the membrane occurs, a bioabsorbable collagen membrane can be used to assist in graft containment. One study of 91 patients requiring sinus augmentation with simultaneous placement of 259 implants evaluated the implant success with regard to the effects of sinus membrane perforations. Perforations were detected in 12 sinus sites. After proper treatment of perforations, 26 implants were placed into perforated sinus areas. Results showed that there was no statistically significant difference regarding peri-implant bone resorption and soft tissue conditions for implants placed into perforated-augmented sinus areas and augmented sinus areas. The authors concluded that perforation of the sinus membrane did not compromise the osseointegration process or the success of dental implants placed in the augmented maxillary sinus (Karabuda et al. 2006).

Bony Wound Healing Principles

Despite the mineral nature of bone, in which calcium and phosphate participate as functional pillars, it is a vital and dynamic tissue. The histogenesis of bone is directly from mesenchymal connective tissue (intramembranous bone formation) or from pre-existing cartilage (endochondral bone formation). Intramembranous bones are found in the mandibulo-craniofacial complex, ilium, clavicle, and scapula (Brighton et al. 1994). The intramembranous bone formation pathway is used when intraoral bone augmentations are utilized by the surgeon (Serletti et al. 1992).

The principles of osteogenesis, osteoconduction, and osteoinduction can be used to optimize therapeutic approaches to bone regeneration (Hollinger et al. 1996). Osteogenesis involves the direct transfer of vital cells to the area that will regenerate new bone. Osteoconduction encompasses the principles of providing the space and a substratum for the cellular and biochemical events progressing to bone formation (McAllister & Haghighat 2007). The space maintenance requirement for many intraoral procedures allows the correct cells to populate the regenerate zone (Aukhil et al. 1986). Osteoinduction embodies the principle of converting pluripotential, mesenchymal-derived cells along an osteoblast pathway with the subsequent formation of bone. This concept was established in 1965, with heterotopic ossicle formation induced by the glycoprotein family of morphogens known as the bone morphogenetic proteins (BMPs) (Urist 1965). All therapeutic bone reconstruction approaches use some or all of these principles in an attempt to maximize the clinical results of bone augmentation(McAllister & Haghighat 2007).

Bone formation during augmentation procedures requires ample blood supply and mechanical support. In the case of sinus augmentation, protection of a blood clot under the sinus, exclusion of gingival connective tissue and provision of a secluded space into which osteogenic cells from bone can migrate are essential for a successful outcome. Organization of the blood clot is followed by ingrowth of vascular tissue and deposition of woven bone. Reinforcement of this disorganized bone structure is accomplished by lamellar bone formation, which in turn, is remodeled soon after as is evident by the presence of secondary osteons (McAllister & Haghighat 2007).

Blood supply and angiogenesis play an important role in guided bone formation (Boeck-Neto et al. 2009, Degidi et al. 2006). The blood clot contains many growth factors involved in regulating the repair of bone. These factors include fibroblast growth factor (FGF), transforming growth factor(TGF), bone morphogenetic proteins(BMP), insulin-like growth factor(IGF), platelet-derived growth factor(PDGF), and vascular endothelial growth factor (VEGF), all of which are expressed during skeletal development and induced in response to injury (Bayliss et al. 2006). FGF, TGF, and VEGF are also involved in the development of new blood vessels, termed angiogenesis (Dai & Rabie 2007). It has also been demonstrated that cells derived from explants of schneiderian membrane can express markers of osteoprogenitor cells (Srouji et al. 2009). Further, titanium on the implant surface in contact with whole blood produces thrombin. In addition to cleaving fibrinogen, thrombin contributes to activation of osteoblasts via proteinase-activated receptors (Thor et al. 2007).

Sinus Graft Healing

The role of the sinus lining as an angioblast-osteoblast source, or as endoperiosteum is still being investigated but it's role is likely secondary in importance to the sinus floor(Summers 1995). The effect of BMP-2 on bone formation from the elevated sinus lining, where pluripotent cells and vascular capacity are present, has been observed (Carlsson et al. 1994). The BMP-2 sinus graft mineralization begins at the periphery and continues toward the central part of the graft. Bone begins to form from the floor and extends circumferentially around the cavity to join bone formation occurring, more minimally, along the sinus membrane. The remodeling of various sinus grafting materials and their process of angiogenesis, osteogenesis, consolidation, and osseointegration, requires further study in order to determine their exact mechanism of healing and establish optimal grafting material (Triplett & Lilly 1998, Tarnow et al. 1997).

The maxillary sinus can be considered a sterile environment and is maintained by a lining bathed in mucin, lactoferrin, and secretory antibodies which inhibit epithelial colonization of microorganisms along with ciliary action (Watzek et al. 1998, Brandtzaey et al. 1996). The rapid reparative capacity of the sinus lining allows the sinus to return to a sterile state soon after sinus graft wound healing (Drettner & Aust 1977, Jensen & Sennerby 1998).

Sinus Graft Materials

Three primary bone graft classifications exist including autograft, allograft, and alloplast. An autograft consists of bone harvested from one site of a patient's body and transplanted to another containing organic, autologous material that has the potential to possess osteogenic, osteoinductive, and osteoconductive properties. Allografts are bone grafts harvested from cadaveric specimens of the same species. Generally these are either cortical or trabecular processed bone grafts that possess osteoconductive properties and may or may not be osteoinductive. Alloplasts are natural or synthetic materials containing non-human calcium and phosphate materials that typically are osteoconductive (Klokkevold 2006).

One of the major focuses of the consensus conference was what constitutes a successful and/or superior bone grafting material or technique. Several surgeons have reported on biopsies of sinus grafts. A review of these findings demonstrates that bone forms endosteally from the sinus floor with every material reported. Results showed that when little or no grafting material is used, such as when the osteotome technique was used or when only a blood clot is present under the sinus floor, tented with implants, bone still forms as long as a space is maintained beneath an intact sinus lining to form a closed wound environment (Lazzara 1996, Summers 1995, Jensen 1998). The space maintenance, created by addition of osteoconductive alloplast materials have been associated with bone formation that ascends from the floor of the sinus several millimeters up into the graft (Fuerst et al. 2004). Osteoinductive materials have shown endosteal formation of new bone from the floor of the sinus as expected, but may additionally form new bone de novo within

the graft depending on it's osteoinductive capacity (Marx 1995, Frost 1998, Nevins et al. 1996, Sigurdson et al 1995). Autografts have been shown to be highly osteoinductive, in general, and therefore may be less dependent on sinus floor endosteal bone migration (Jensen & Sennerby 1998, Sennerby & Lungren 1998, Schenk et al. 1994). This inherent advantage allows autografts to be considered the material of choice, though definitive support for this conclusion is lacking (Marx 1995).

Autografts incorporate to a greater extent than allografts, but when loading occurs early in the healing period, the process of early bone-graft remodeling can undermine or alter fragile osseointegration (Roberts et al. 1989, Jensen et al. 1995). The less contact between the bone and the implant, the higher the chance of implant failure (Sennerby et al. 1992). One study using particulate autografts showed direct bone-implant contact to be minimal, from 10 to 15% after 6 months. Most of the osseointegration occurred from the sinus floor bone migration up the sides of the implant despite complete incorporation of the graft (Jensen & Sennerby 1998). Only a few point contacts within the graft were osseointegrated, suggesting that bone contact "spot welds" within the graft above the sinus floor are of secondary importance (Sennerby & Lundgren 1998).

Osteoinductivity is a pharmacokinetic principle thought to be directly proportional to the concentration of bone morphogenetic protein (BMP) present in grafted material. Allografts, autografts, BMP-2 and BMP-7 are all materials that have been utilized in sinus grafts containing BMP (Wozney et al. 1988). BMP-2, used with a collagen carrier, has been shown to rapidly form bone de novo throughout

the sinus graft site (Margolin et al. 1997, Nevins et al. 1996). The osteoinductive capacity of allografts has recently been questioned, regardless of their preparation. Sinus allografts resulted in more late loading failures, more infections, and more stage 2 uncovering failures when compared to other materials (Block 1998, Jensen & Greer 1992). The poor performance of allografts may be due to their tendency to delay both bone formation and osseointegration, leading to incomplete annealing and poor implant fixation in the modeling phase (Aspenberg et al. 1988). Allografts have been shown to undergo incomplete replacement by creeping substitution, during remodeling, resulting in a mixture of non-vital and vital bone. Though the mechanical significance of this is unknown, it likely makes fatigue failure more likely (Frost 1998, Burkhardt & Enneking 1978).

Bovine material can be considered an alloplastic material and has been advocated because it acts as a slowly resorbing space maintainer. Dental implants installed in bovine bone matrix (BBM) grafted sinuses reported a bone-implant contact of 63%(Schlegel et al. 2003), 27%(Wetzel et al. 1995), and 38% (Terheyden et al. 1999), after a 6 month observation period. One study reported that in the BBM-only group, 23% newly formed bone was recorded at 12 weeks (Fuerst et al. 2004). Taken together, BBM is very slowly resorbed and appears to behave as a semipermanent grafting material. In a study in beagles, a volume reduction of 16% in the BBM group was reported at 180 days (Schlegel et al. 2003). Histologically, elevations with BBM correspond to an ongoing chronic inflammation in the marginal bone zone. Histomorphometic evaluation showed that in all groups the

average percentage of newly formed bone was found to be maximal (34%) at the 7.5 month time point (Sennerby et al. 1998).

Some controversy exists in the literature as to whether deproteinized bovine bone (DPBB) is resorbable or not. Some studies have reported signs of resorption such as the presence of osteoclasts on the particle surface, resorption lacunas, or a decrease over time of the fraction of DPBB compared with bone and soft tissues in the graft (Klinge et al. 1992, Berlundh & Lindhe 1997). Other authors claim that DPBB is not resorbable (Hallman et al. 2001, Schlegel et al. 2003). One study recently published by Mordenfeld and colleagues reported histological and histomorphometric analyses of DPBB biopsies harvested 11 years after sinus grafting. Core samples from 9 patients were taken at 6 months and 11 years following sinus grafting procedures. Biopsies harvested after 11 years showed that DPBB particles were easily identified in the regions of interest and consisted of 44.7% lamellar bone, 38% marrow space and 17.3%DPBB. Particles were often well integrated and surrounded by lamellar bone with no signs of resorption. The authors also noted that sometimes the bone tissue in close contact to the particles seemed to be less mature with no lamellar structures observed and presents of more irregular woven bone. Large multinucleated giant cells were noted in close connection to particles. The area fraction of the remaining DPBB particles (17.3%) and the particle area (0.063mm^2) after 11 years were in accordance with previous results from specimens retrieved at 6 months (14.5% and 0.066mm^2 , respectively) (Hallman et al 2001). The authors concluded that the absence of significant

decrease in particle size after 11 years may indicate that, if present at all, DPBB resorption is very minor (Mordenfeld et al. 2010).

When the implant is biomechanically reliant on graft, the vitality of the graft is probably important for implant longevity. In order to maintain osseointegration, the graft must be vital enough to respond to microdamage. The minimal requirement of vital bone within the graft is thought to be somewhere between 25 and 35% by volume for osseointegration to be maintained. This corresponds to the approximate value for normal maxillary cancellous bone (Lazzara 1996, Tarnow et al. 1997).

Implant Placement Timing and Preoperative Bone Levels

In grafted bone, rough-surface implants, including HA-coated and titanium plasma-sprayed implants have shown a greater capacity to osseointegrate and resulted in statistically better results (Carlsson et al. 1994). Implant placement, whether simultaneous or delayed, remains an unresolved topic. Advocates for delayed placement argue that more desirable position can be obtained and simplification of initial grafting surgery are advantages (Triplett & Schow 1996, Cawood et al. 1994). Only one study has shown an improvement in positioning and angulation when a delayed approach was used (Blomquist et al. 1997). Delayed placement may serve as an important fallback, however, when immediate implant placement is not possible due to perforation of the membrane or poor bone quality. Simultaneous placement proponents argue that single stage surgery is less invasive, more cost-effective, and more time-efficient. Simultaneous and delayed placement

results from the 1996 Consensus Conference were statistically equal but overall success rate is still not conclusive (Jensen 1998).

Amount of pre-operative bone present is a factor when treatment planning for sinus augmentation. Minimal pre-operative bone has been reported to be an important factor in failure to establish or maintain osseointegration, however, only a few reports suggest that the lack of preoperative bone is a factor in implant loss. There appeared to be a statistical difference in implant loss between sites with 4mm or less compared to 5mm or greater, but the limited number of cases made these findings inconclusive. (Jensen & Greer 1992, Jensen 1994). An accurate classification would require the implant osteotomy site to be measured directly at the time of bone grafting or implant placement. At the time of the conference, the only conclusion agreed upon was that implant loss occurs to a greater extent when less available bone is initially present. It was also recommended that sinus grafting take place when there is less than 8mm of bone available for implant placement (Jensen 1998).

Sinus Elevation without the use of Graft

A novel approach was developed based on the concept of guided bone regeneration. Lundgren and colleagues developed a surgical technique for maxillary floor augmentation which did not include bone grafting. The authors performed 10 sinus lifts in patients who received a total of 19 implants. The procedure included elevating the maxillary sinus and insertion of implants in the residual bone. The implants served as “tenting poles” and allowed new bone to fill

the created compartment in the antral sinus. Post-operative radiographs showed bone formation in all 10 patients, and 19 implants were stable after 12 months of loading (Lundgren et al. 2008). Two other publications confirmed Lundgren's findings that mere elevation of the schneiderian membrane with simultaneous placement of implants resulted in bone formation. In one study, 14 implants were placed in 6 patients using venous blood as the sole filling material. The authors found that the average height of newly formed bone around the implants was 10mm (Hatano et al. 2007). Thor and colleagues studied 20 patients with 44 implants placed at the time of the sinus membrane elevation. With an average follow-up period of 28 months, only 1 implant failed to integrate. The average new bone formation was 7mm. The authors concluded that the greater the length of the implants, the more new bone that was formed (Thor et al. 2007).

Mazor recently reported 41 implants placed in 25 sinuses augmented with platelet-rich fibrin (PRF) alone. No implant was lost and results showed that final bone gain was between 7 and 13 mm. Radiologic analyses revealed the final position of the sinus floor was always in the continuation of the end of the implant. All biopsies showed well-organized and vital bone. PRF requires a centrifuge, leading to considerable increases in cost and time. The study was performed without a control group and the authors concede that similar results could be reached with the physiologic blood clot as the sole filling material (Mazor et al. 2009).

A randomized controlled trial consisting of 15 patients in a split mouth design compared the use of autogenous graft (control) verses no graft (test) with

simultaneous implant placement. Results showed that simultaneous sinus membrane elevation, with or without bone graft, reach a comparable bone gain and implant survival at 6-month follow-up (Borges et al. 2011).

Bone formation underneath the elevated sinus is still not fully understood. Palma and colleagues compared the histological outcome of sinus membrane elevation in primates with and without grafts. Four tufted capuchin primates were given bilateral sinus augmentations. Two different implants were placed in each sinus (one machined and one oxidized). One sinus was filled with coagulum alone and the opposite sinus was filled with autogenous bone harvested from the tibia. After six months, the animals were sacrificed. The maxilla was retrieved en bloc. Histologic examination revealed that floor of the sinus provided approximately 2.2mm of cortical bone for primary stability. In most cases, the sinus membrane appeared intact morphologically and in contact with the apical surface of the implant. Sites under the elevated membrane showed most bone growth at the periphery, occasionally extending inward. Results of the study showed no difference in the amount of augmented bone in the maxillary sinus regardless of the addition of autogenous bone. The results showed no differences regarding implant stability, bone-implant contact, or bone area within and outside the implant threads between the two techniques. The authors noted that new bone was often deposited in contact with the membrane in coagulum alone sites, indicating the osteoinductive potential of the schneiderian membrane (Palma et al. 2006).

In a human study involving 10 patients, Sohn and colleagues were the first to demonstrate histologic evidence of new bone formation in the maxillary sinus with

membrane elevation only and simultaneous implant placement beyond the original sinus floor. As the methods of sealing the lateral access window of the sinus, patients were divided into 2 groups. A non-resorbable membrane was used to seal the lateral access window of the maxillary sinus after implant placement in 5 cases (group A). A replaceable bony window was used to seal the lateral wall of the sinus in another 5 cases (group B). Computed tomograms (CT) were taken immediately before and after surgery, at the uncovering of the implant, and after a 6 month healing period. A CT taken immediately after surgery revealed that the sinus was filled with blood clots under the sinus membrane. After a mean healing period of 6 months, bone biopsies were taken on the previous bony window to evaluate new bone formation. All 21 implants remained stable during the study period. New bone formation and new sinus floors were found in radiographic and histologic evaluations. The authors emphasized the importance of superior elevation of the sinus membrane to expose the medial wall of the sinus cavity, allowing mesenchymal stem cells to migrate from the exposed sinus wall. The authors' findings suggest there is great potential for new bone formation in the maxillary sinus without the use of additional bone grafts. There were no differences in clinical outcomes according to the sealing methods of the lateral access window of the sinus (Sohn et al. 2008).

In an animal study in 2011, Sohn evaluated new bone formation in maxillary sinuses with and without bone grafts through immunochemical analysis. Bilateral sinus augmentation procedures were performed in rabbits. In the first group the bony window was repositioned after elevation of the sinus mucosa without bone

grafting. A mini-screw was inserted into the bony window to support the elevated sinus membrane. In the second groups, Bio-Oss, a DPBB, was placed under the elevated sinus membrane and a collagen membrane was placed over the bone graft. The rabbits were sacrificed after 1, 2, 4, 6, and 8 weeks. Immunochemical analysis evaluated the augmented sinuses for proliferating cell nuclear antigen (PCNA), type I collagen, and osteocalcin content. According to the results, faster and greater new bone formation was observed in sites that received no grafting material. The authors suggested that the repositioned bony window may accelerate new bone formation earlier during healing versus the placement of a collagen membrane and grafting material in the sinus. The study showed that the repositioned bony window had more beneficial effects than the collagen barrier membrane on new bone formation in the sinus. The expression of PCNA, type I collagen, and osteocalcin was revealed along the floor of the repositioned bony window from the first week of healing to the eighth week in the graftless sites. The authors stated that the bony window not only acted as a barrier membrane, it also accelerated new bone formation as an osteogenic substrate (Sohn et al. 2011).

Cone-beam Computed Tomography and Micro-Computed Tomography

Cone-beam computed tomography (CBCT) is a relatively new imaging modality that offers significant advantages for the evaluation of implant patients. The xray source and the detector are diametrically positioned and make a 360-degree rotation around the patient's head within the gantry. The CBCT scanner generates a cone-shaped xray beam, which images a larger area. Images are

generated in 1-degree increments. At the end of one single complete rotation, 360 images of the area are generated. The computer can then use these images to generate a digital, three-dimensional map of the face. Once generated, multiplanar reconstructions as well as axial, coronal, sagittal, or oblique sections of various thickness can be reconstructed from the data (Tetradis et al. 2006).

Bone morphometric analysis has traditionally been assessed in two-dimensional (2D) histologic sections, with a third dimension added on the basis of stereology. In an attempt to better evaluate bone connectivity, other three-dimensional (3D) procedures have been proposed. Micro-computerized tomography (micro-CT) scanning is a nondestructive alternative approach to outline and quantify bone in three dimensions allowing higher-resolution 3D images and quantitative measurements of the trabecular bone structure. This technique uses X-ray images to create cross-sections of a 3D-object that can be used to recreate models without destroying the original sample (Chopra et al. 2009).

PURPOSE OF STUDY

The purpose of this study was to conduct a clinical, radiographic, histologic and histomorphometric investigation to assess the clinical healing of sinus lifts performed with simultaneous placement of dental implants using venous coagulum as sole filling material.

METHODS AND MATERIALS

Patients

The patient pool for this study consisted of 4 patients from The University of Alabama Birmingham (UAB) Graduate Periodontal Clinic requiring sinus augmentation and replacement of 2-3 maxillary teeth. One patient had bilateral sinus lifts performed. Orthopanoramic radiographs were used to screen patients. If the radiograph and clinical evaluation revealed that the patient needed sinus augmentation prior to implant placement, the first cone-beam computer tomography (CBCT)-scan was scheduled. This pre-operative CBCT-scan was used to quantify the amount of available bone under the maxillary sinus to decide whether the patient could be included in this study. Harvesting of tissue biopsies were reviewed and approved by the UAB Institutional Review Board (IRB).

A complete medial history and head and neck examination was performed. Preoperative intraoral examinations were performed to rule out any uncontrolled infection, disease, or local oral pathology. Impressions were obtained, models were constructed and mounted, and surgical guides were fabricated to determine the placement of the implants. Post-operative wound healing and oral hygiene were monitored.

The inclusion criteria for the study required that patients were adults (older than 18 years of age), missing 3 posterior maxillary teeth, had insufficient bone

height beneath the maxillary sinus and were class I or II as defined by the American Society of Anesthesiologists Physical Status Classification System (ASA). Only non- or light (less than 10 cigarettes/day) smokers were eligible for participation. Additional inclusion criteria included healthy maxillary sinus with no pathology of neighboring teeth and sufficient primary implant stability at the time of surgery.

Patients with absolute contraindications to this elective procedure including uncontrolled systemic disease, bleeding disorders, or excessive tobacco use (greater than 10 cigarettes/day) were excluded from this study. All patients were informed of the requirements for participating in the study including biopsy/bone core harvesting. Patients who fulfilled the inclusion criteria were screened and interviewed for possible participation in the study. Patients who agreed to the protocol signed a consent form, approved by the IRB (Protocol number X090803001) prior to entering the study.

Surgical Technique

All surgeries were performed using the following standardized protocol by the primary author in the Graduate Periodontal Clinic at UAB. This was performed under the direct supervision of the faculty mentor (MSR).

Prior to surgery the patients rinsed with Peridex mouth rinse (0.12% Chlorhexidine Gluconate, Procter & Gamble) for 1 minute. The surgeries were performed with local anesthesia and conscious sedation at patient's request. Prior to beginning the procedure, approximately 40cc of the patient's venous blood was obtained and allowed to clot for 50 minutes to a consistency that was easy to

handle. A mid-crestal incision was made in the posterior maxilla, a muco-periosteal flap was elevated and the lateral wall of the maxillary sinus exposed (Figure 1- and 3-B). A bone window was outlined using a diamond bur or piezoelectric bone surgery device under saline irrigation (Figure 1- and 3-C). The size of the window was dependent on the existing bone and the number of implants required for the treatment. The lateral window was designed to be large for two reasons. First, we wanted this window to be large enough to rest apically on the implants after placement. Second, we wanted the window to be large enough to allow for an area from which to harvest the bone biopsy after healing. Measurements of the location and dimension of the lateral window were taken at the time of surgery. After careful elevation of the membrane, the bone window was left attached to the membrane, rotated medially and served as the new sinus floor. Osteotomies were prepared for Straumann Bone Level implants (Straumann AG, Switzerland) (Figure 1- and 3-D). Immediately prior to implant placement, the venous coagulum was placed into the newly created compartment under the elevated sinus. 14-mm Straumann Bone Level implants were placed (Figure 1-E). The Venous coagulum (Figure 1-F) was then placed into the newly created compartment under the elevated sinus (Figure 1-G). A collagen membrane (OsseoGuard, Biomet 3i, Palm Beach, FL) was placed over the lateral window (Figure 3-E). Primary closure was obtained using vicryl sutures. Patients were instructed to not use any removable prostheses until sutures were removed 10-14 days post-operatively. Post-operative wound healing and oral hygiene were monitored.

Following a healing period of 8-9 months, a new cone-beam CT was taken and the implants were uncovered and healing abutments were placed. At the time of uncovery, a biopsy was taken through the lateral wall of the maxilla using a 2mm trephine bur (Figure 2-A&B, Figure 4-B). The biopsy was taken from the area where the lateral window was previously outlined based on measurements taken at the first surgery. A collagen membrane (OsseoGuard, Biomet 3i, Palm Beach, FL) was placed over the site of the biopsy.

For postoperative management, the following medications were prescribed: Chlorhexidine rinse 0.12% (Rinse for 30 seconds twice a day for fourteen days), Augmentin (500mg three tablets a day for seven days), Ibuprofen (600mg one tablet three times a day for one week) and pain medication as needed. Patients allergic to penicillin related drugs received Clindamycin 150mg three times a day for seven days.

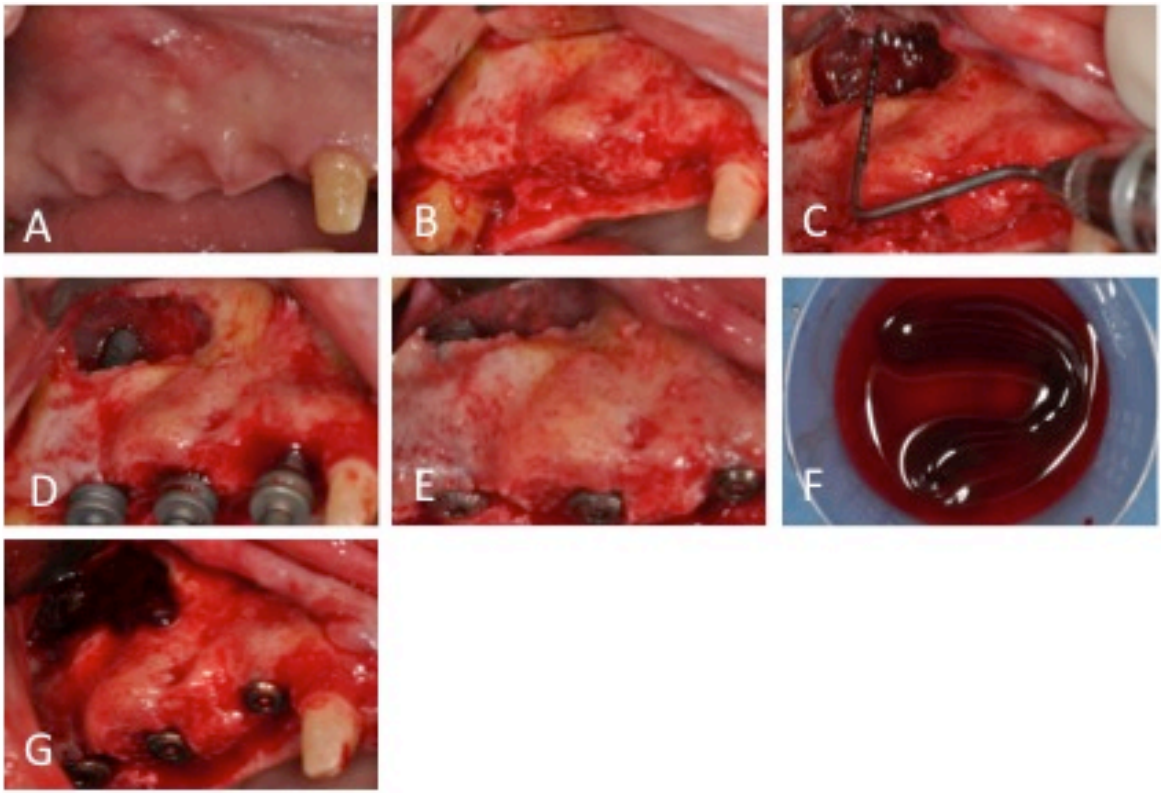

Figure 1. Surgical Procedure Site #1 with Implant Placement #3, 5 & 6. (A) Preoperative photograph of the right maxilla. (B) Flap elevation. (C) Lateral window subcrestally 7 to 15mm. (D) Guidepins in place. (E) Implants in place. (F) Venous coagulum after 40minutes clotting time. (G) Coagulum placed.

Figure 2. Implant Uncovery and Biopsy Site #1. (A) Biopsy taken from mesial of implant #3 at approximately 9 to 11mm subcrestally. (B) Bone core biopsy in trephine. (C) Bone core embedded in block prior to microCT.

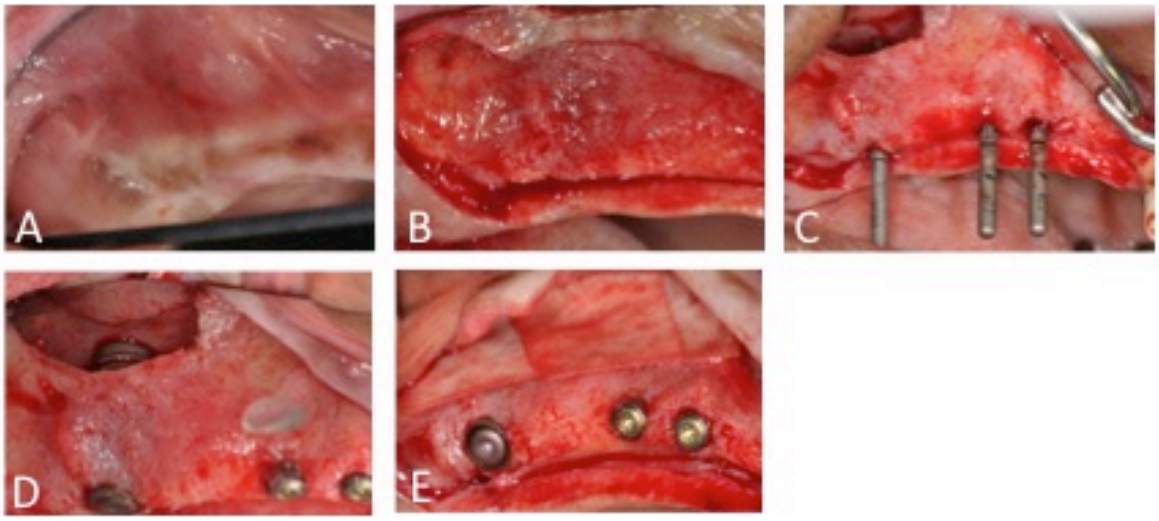

Figure 3. Surgical Procedure Site #5 with Implant Placement #3,5 & 6. (A) Pre-operative photograph of right maxilla. (B) Flap elevation. (C) Guide pins in place, lateral window located 8 to 15mm subcrestally. (D) Implants in sites #3,5,6. (E) Collagen membrane covering window.

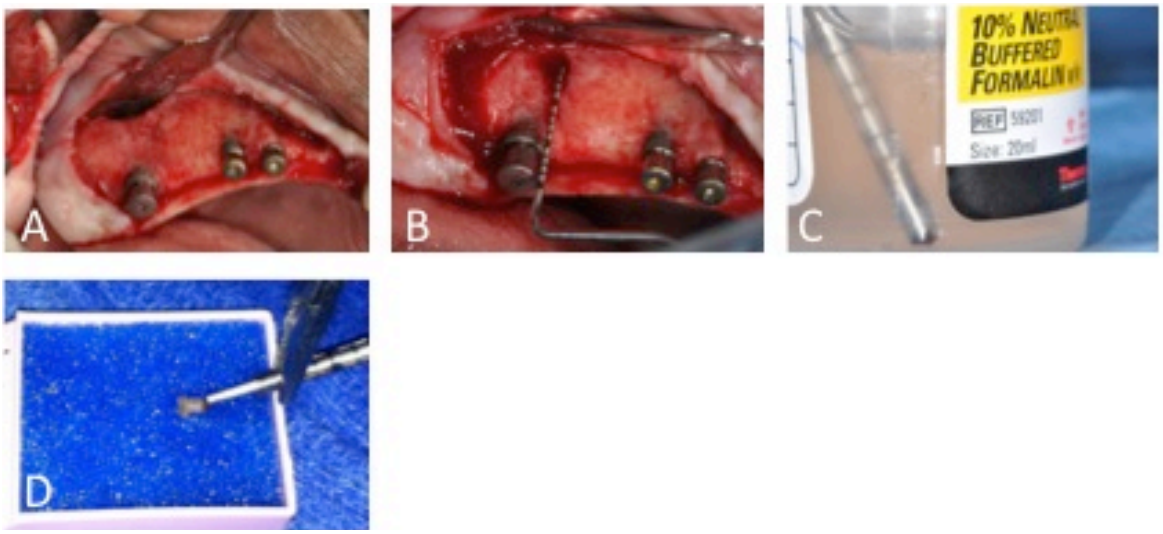

Figure 4. Implant Uncovery and Biopsy Site #5. (A) Flap elevation 8 months following surgery. (B) Biopsy site 9 to 11mm subcrestally. (C) Trephine in Formaline. (D) Bone core being pushed out of trephine.

Radiographic Evaluation

To evaluate any potential changes in radiographic bone height, measurements were made on the pre-operative CBCT-scan to evaluate the existing bone height from the crest of the alveolar ridge to the floor of the sinus. To improve measurement reliability, the average of 3 measurements, taken at 3 different times was calculated (Figure 5-and 6-A,C,E). After 8-9 months of healing, the post-operative CBCT-scan was made. Again, 3 measurements were made adjacent to the distal implant and their average was calculated to serve as the post-operative bone height (Figure 5- and 6-B,D,F). The gain in bone height was calculated as the difference between pre- and post-operative alveolar height.

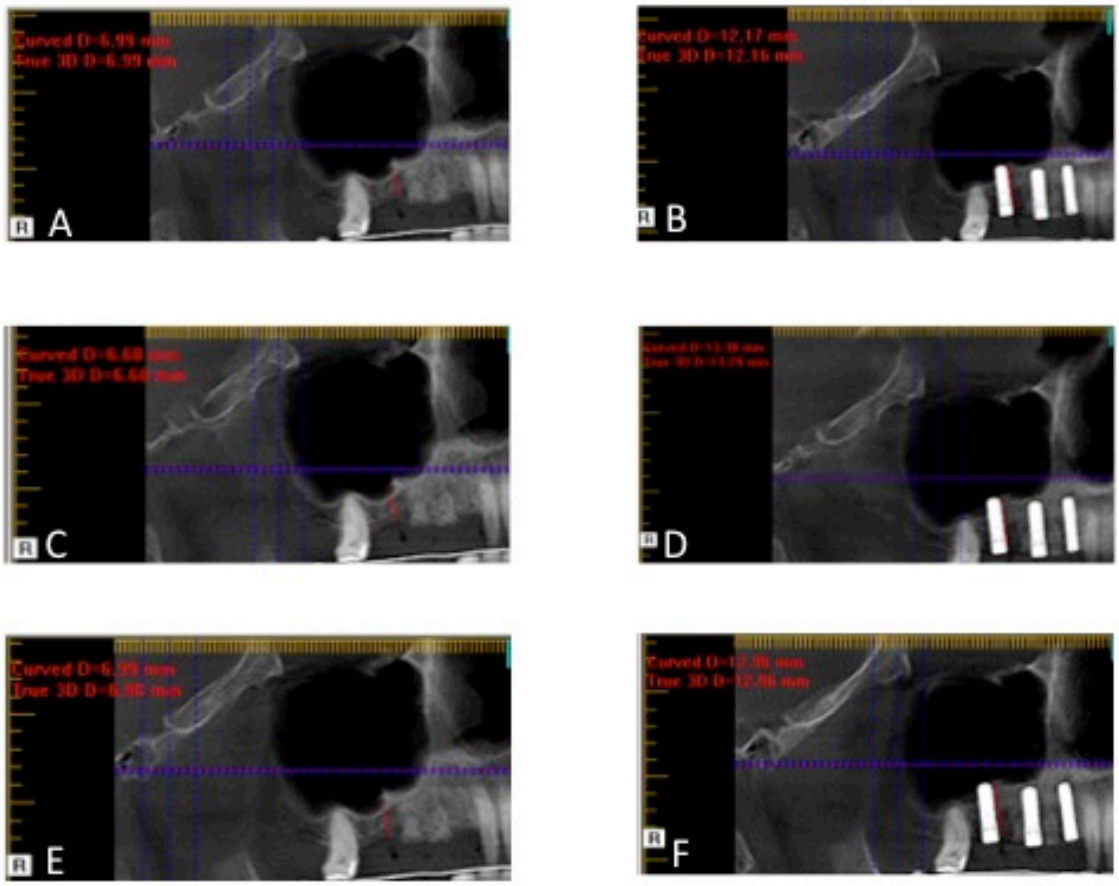

Figure 5. Pre- and Post-Operative CBCT-Scans Site #1. (A) Pre-op measurement 1=6.99mm. (B) Post-op measurement 1=13.30. (C) Pre-op measurement 2=6.60mm. (D) Post-op measurement 3=12.17mm. (E) Pre-op measurement 3=6.99mm. (F) Post-op measurement 3=12.96mm.

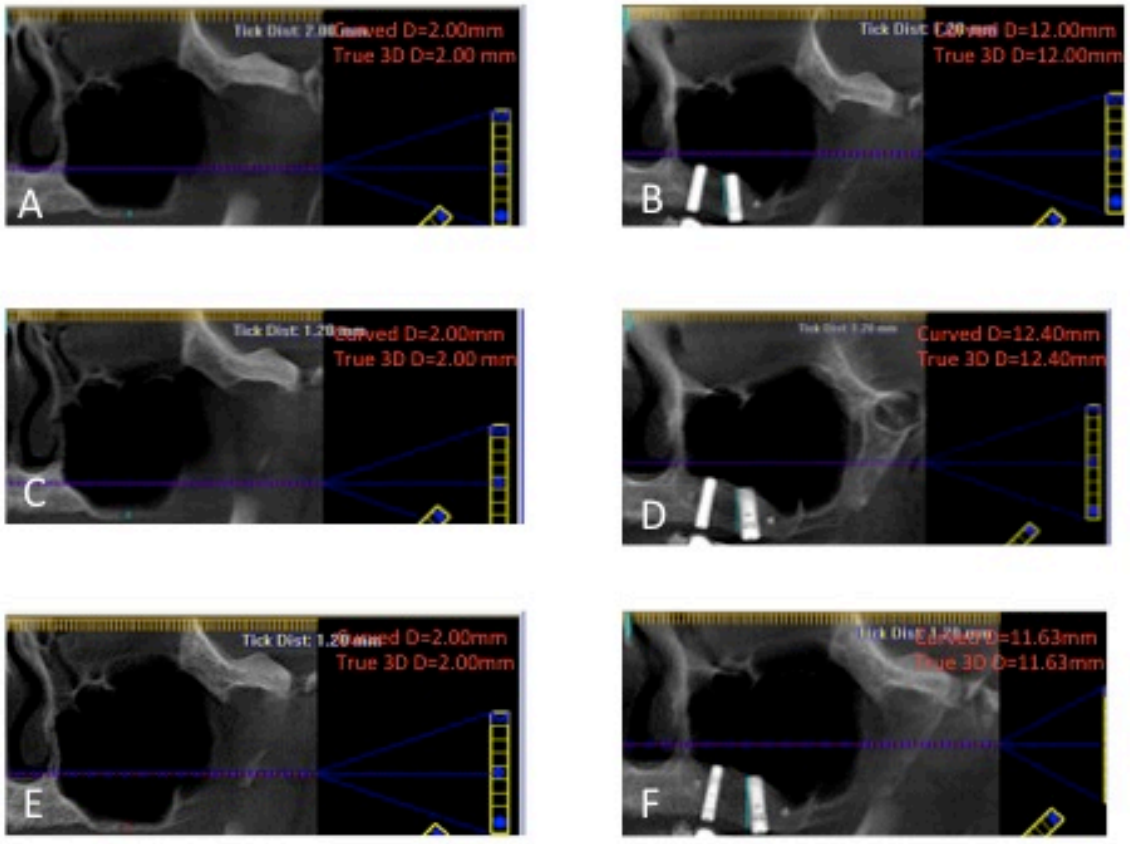

Figure 6. Pre- and Post-Operative CBCT Scans Site #4. (A) Pre-op measurement 1=2.00mm. (B) Post-op measurement 1=12.00. (C) Pre-op measurement 2=2.00mm. (D) Post-op measurement 3=12.40mm. (E) Pre-op measurement 3=2.00mm. (F) Post-op measurement 3=11.63mm.

MicroCT

After 8-9 months of healing, at the time of implant uncovery, biopsies were obtained adjacent to the implants, in the area of the bony window, perpendicular to the longitudinal orientation of the implants. This was performed with a 2.0mm trephine bur (ACE Surgical Supply Co, Brockton, MA). Cores were immediately placed in 10% Neutral Buffered Formalin. The samples were removed from the trephine by mechanical "push out" technique. The biopsies measured 2.5 to 4.5mm. The samples were longitudinally oriented in a sample holder and fixed in 10% Neutral Buffered Formalin for at least 24 hours. Core bone biopsies were sent for processing to the UAB Center for Metabolic Bone Disease. All the samples were dehydrated through graded ethanols (80% ETOH X 1, 95% ETOH X 2, and 100% ETOH X 4) to three changes of xylene prior to the infiltration solution (95% Methyl Methacrylate, (MMA), and 5% Dibutyl phthalate, (DBP). Infiltration solutions for all the samples were refreshed every 3 days, for a total of 4 changes. After infiltration, all the samples were embedded in the embedding solution which was composed by 95% MMA and 5% DBP with 0.25% perkodox as the initiator. The samples were then exposed to UV light for polymerization. Once the sample blocks were fully polymerized (plasticized), they were trimmed. The embedded samples were scanned using the Scanco microCT40 desktop cone-beam micro-CT scanner (Scanco Medical AG, Brüttisellen, Switzerland). The samples were placed vertically in 12mm diameter scanning holders and a scout scan was performed to locate the bone core within the embedding material and the entire core was scanned at the following settings: 6mm resolution, 70kVp, 114μA with an integration time of 200ms.

Histology

Following microCT, the fully polymerized sample blocks were trimmed and cut to obtain the 5μm thin sections through the longitudinal axis of the bone core. Paragon stain was then performed for histomorphometry.

The UAB Center for Metabolic Bone Disease lab, in conjunction with the lead author, evaluated the histological and histomorphometric specimens of the core bone samples. All samples were analyzed for new bone formation and soft tissue invasion. These parameters were recorded and photographed.

Histomorphometry

Paragon stained sections of bone cores were analyzed using Bioquant Image Analysis software (R&M Biometrics, Nashville, TN®) under the light microscope. The Area measurements were made of the Total Tissue Area (including Bone, Soft Tissue and Marrow Voids), Bone Area and Soft Tissue Area. A thresholding tool was used to collect the Area Data by selecting the desired pixels based on the intensity of the stain. Percentages of Bone Area, Soft Tissue Area and Marrow Void Area per Total Tissue Area were calculated using the formula: (Bone Area/Total Tissue Area ×100); (Soft Tissue Area/Total Tissue Area ×100); and ([1-(Bone Ar./Tt.Tissue Ar.) – (Soft Tissue Ar./Tt.Tissue Ar.)] ×100). All the measurements were taken at MAG 10X.

RESULTS

Patients

Patients presented for follow-up 7-14 days post-operatively to monitor healing and remove sutures. An orthopanoramic radiograph was taken at a 6 week follow-up appointment to monitor bone formation and implant stability. After 8-9 months, a second CBCT-scan was obtained. A total of 13 implants were placed under 5 elevated sinuses in 4 patients (3 unilateral, 1 bilateral). All patients who participated were female with ages ranging from 46-57 years. 1 patient, site #1, was a light smoker<10cigarettes/day. No post-operative infections occurred following the sinus elevation or implant uncovery surgeries. 2 of the 5 sinuses showed greater than 5mm bone height beneath the sinus prior to the sinus elevation while the remaining 3 patients had less than 3mm crestal bone. In one patient, site #5, IV access could not be obtained and therefore no venous coagulum was placed beneath the elevated sinus.

Radiographic Results

The analyses of the elevated sinuses included radiographic and histologic images including qualitative and quantitative evaluation. Height gain was observed in all 5 elevated sinuses. The post-operative Conebeam CT showed qualitative differences from traditional sinus lifts using allograft and/or xenograft materials. The bone beneath the sinuses was more radiolucent than is observed when using

grafting material. Direct measurements on CBCT scans revealed a significant gain in bone height in all 5 sites, ranging from 4.37 to 10.01mm. The average increase in bone height following 8-9months healing was 7.176mm. The radiographic results are summarized in Tables 1, 2, and 3.

The amount of bone surrounding the distal implant was calculated as a percentage of the implant length (Average Post-operative bone height)/Implant length. The amount of bone ranged from 62.36% to 92.5% with a mean of 79.33% (Table 3).

Table 1. Pre-Operative Height from Radiographic CBCT-Scan

Site	Pre-op Height 1 mm	Pre-op Height 2 mm	Pre-op Height 3 mm	Pre-op Height Avg mm
1	6.99	6.60	6.99	6.86
2	1.60	2.04	1.60	1.75
3	2.00	1.20	1.60	1.60
4	2.00	2.00	2.00	2.00
5	6.33	8.99	7.44	7.44

Table 2. Post-Operative Height from Radiographic CBCT-Scan

Site	Post-op Height 1 mm	Post-op Height 2 mm	Post-op Height 3 mm	Post-op Height Avg mm
1	13.29	12.96	12.60	12.95
2	9.29	10.80	10.00	10.03
3	8.48	10.47	7.25	8.73
4	12.00	12.40	11.63	12.01
5	11.39	10.88	13.16	11.81

Table 3. Change in Height (mm and %) from Radiographic CBCT-Scan

Site	Pre-op Height Avg mm	Post-op Height Avg mm	Change in Height mm	Change in Height %	Peri-implant bone height/implant length %
1	6.86	12.95	6.09	88.78	92.5
2	1.75	10.03	8.28	473.14	71.64
3	1.60	8.73	7.13	445.63	62.36
4	2.00	12.01	10.01	500.50	85.79
5	7.44	11.81	4.37	58.74	84.36
Mean			7.176	313.36	79.33

Clinical results

Out of the 5 procedures, 2 perforations of the Schneidarian membrane occurred (sites #3 and 4 both on the same patient). They were repaired with a collagen membrane (OsseoGuard, Biomet 3i, Palm Beach, FL) prior to placement of venous coagulum. Interestingly, at the time of uncovery, it was these two sites that had incomplete closure of the lateral window accompanied with soft tissue invagination. In one site, site #3, the window was filled approximately two-thirds with fibrotic tissue. The fibrous tissue was partially removed but the location prevented the harvesting of a bone core. Therefore, radiographic results are reported for 5 sinuses, while microCT and histology are reported for only 4 cores.

Bone density evaluated by hand drilling appeared mostly medium/normal (D2 to D3) as estimated by the surgeon. All implants had osseointegrated at the time of uncovery. However, at uncovery, 1 implant (site #2) was explanted and repositioned palatally due to prosthetic concerns and buccal dehiscence.

Micro CT

Scans were automatically reconstructed into 2-D slices, and all the slices with visible bone were analyzed using the microCT Evaluation Program (v5.0A, Scanco Medical). The region of interest (ROI) was drawn on each of the slices to include all the bone. Bone was separated from less dense tissue and embedding material by thresholding at 240. The 3-D reconstruction was performed using all the outlined slices. Data was obtained on bone density. Images of two of the cores are shown in Figures 5 and 6.

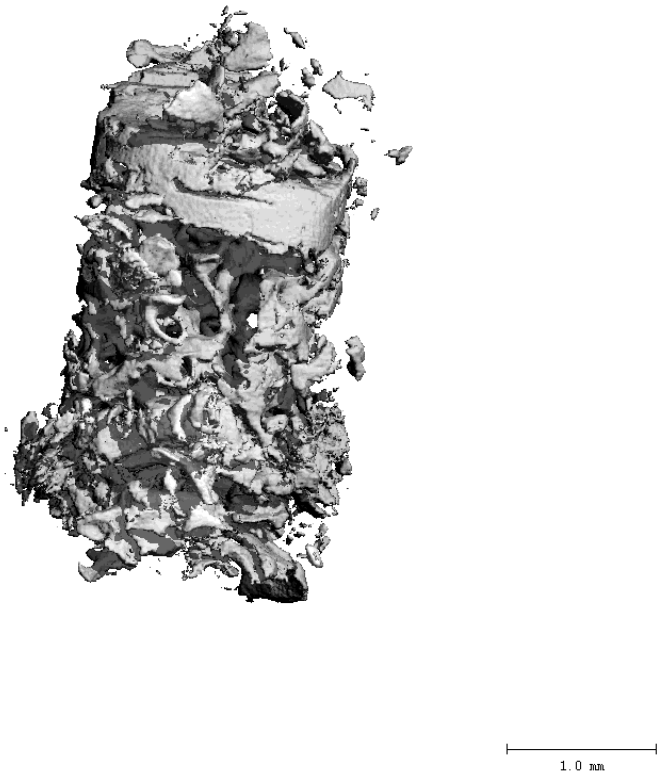

Figure 7. Micro CT from Site #2. Three-dimensional micro-CT reconstruction of a cylindric biopsy retrieved from a sinus grafted with venous coagulum after 9 months healing. This sample shows a representation of the well organized trabecular bone.

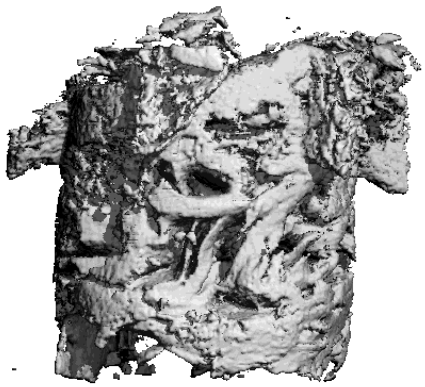

Figure 8. Micro CT from Site #4. Three-dimensional micro-CT reconstruction of a cylindric biopsy retrieved from a sinus grafted with venous coagulum after 9 months healing. This sample shows a representation of the bone trabeculae.

Histology and Histomorphometry

All biopsies showed evidence of new bone formation. Areas of new bone formation were identified and photographed (Figures 7 and 8). Polarized light microscopy was performed to show the orientation of the collagen fibers. Note that detailed information is provided in the figure captions.

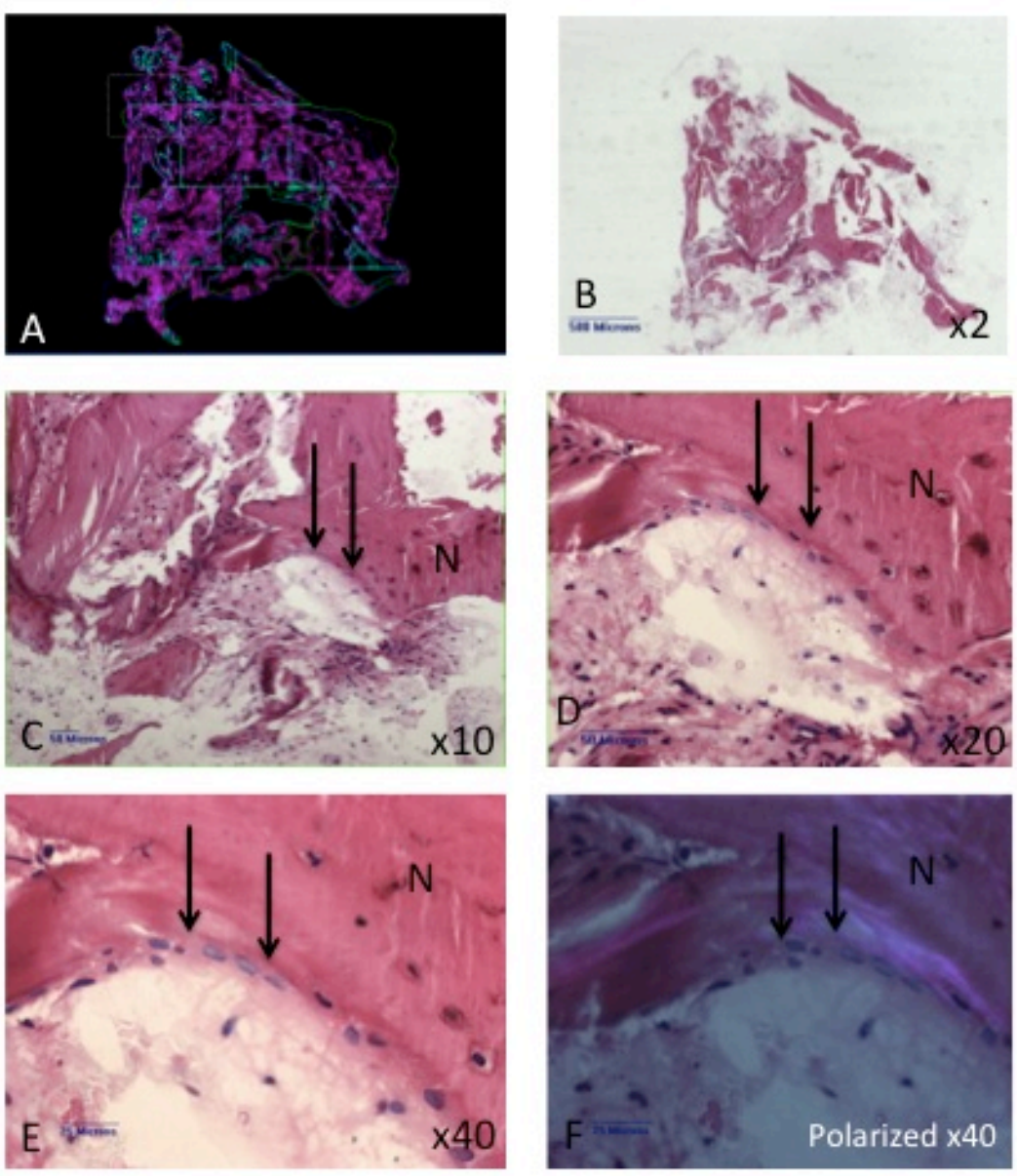

Figure 9. Histology from Site #1. (A) A photomicrograph of the paragon stained tissue demonstrating the tracings implicated during the process of measurements of total tissue area (green tracing) and light blue (bone area) and pink (non-bone area including soft tissue) utilizing the Bioquant computerized system (original magnificationX2). (B)Light microscopy(Paragon, original magnification x 2).(C-F) Light microscopy (paragon) showing new bone formation. An organizing cellular front of osteoblasts (arrowheads) observed. Osteocytes in round lacuna observed throughout new bone (N). Residual red blood cells present.

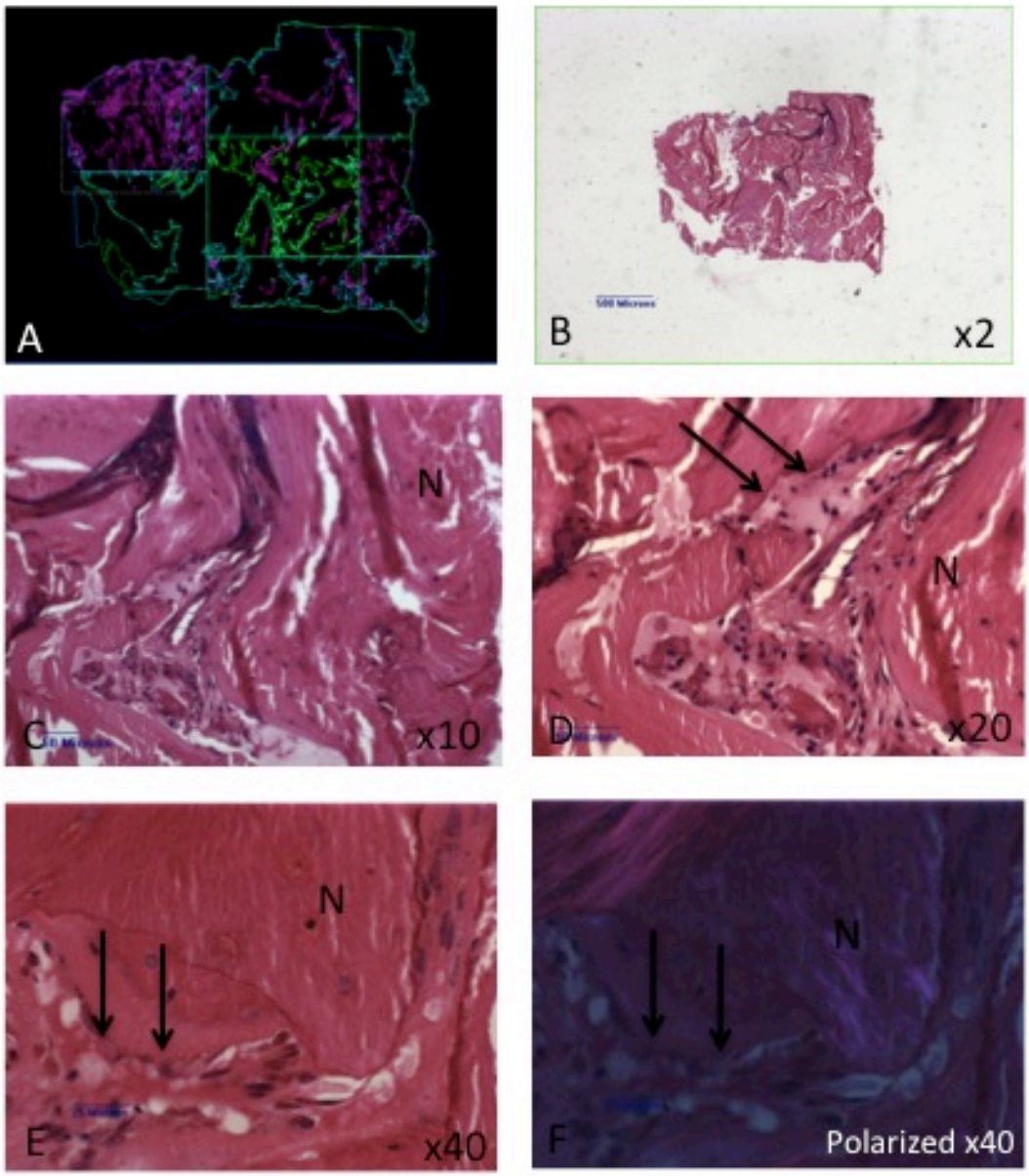

Figure 10. Histology from Site #2 (A) A photomicrograph of the paragon stained tissue demonstrating the tracings implicated during the process of measurements of total tissue area (green tracing) and light blue (bone area) and pink (non-bone area including soft tissue) utilizing the Bioquant computerized system (original magnificationX2). (B) Light microscopy(Paragon, original magnification x 2).(C-F) Light microscopy (paragon) showing new bone formation. An organizing cellular front of osteoblasts (arrowheads) observed. Osteocytes in round lacuna observed throughout new bone (N). Residual red blood cells present.

Histomorphometric analyses from histological sections of the bone cores are presented in Table 4. The percent of total bone area/total tissue area ranged from 37.406% to 74.285%. The summary of findings by site are presented in Table 5.

Table 4. Histomorphometry, Bone Core Data

	Site #1	Site #2	Site #4	Site #5	Mean
Total Tissue Area (mm^2)	3.388	3.192	2.567	3.903	3.263
Total Bone Area (mm^2)	1.267	1.561	1.907	2.893	1.907
Total Non-bone Area (mm^2)	1.126	0.402	0.156	0.737	0.605
% Bone Area/Total Tissue Area	37.406	48.896	74.285	74.106	58.673
% Non-Bone Area/Total Tissue Area	33.227	12.603	6.073	18.890	17.698
% Void Area/Total Tissue Area	29.367	38.501	19.642	7.004	23.629

Table 5. Summary of Findings by Site.

Site	Change in Height mm	Change in Height %	Bone Area/ Tissue Area %
1	6.09	88.78	37.406
2	8.28	473.14	48.896
3	7.13	445.63	NA
4	10.01	500.50	74.285
5	4.37	58.74	74.106
Mean	7.176	313.36	58.673

DISCUSSION

The use of CBCT-scans were invaluable in measuring pre- and post-operative alveolar bone heights. Gain in bone height, as assessed by measurements from CBCT, ranged from 4.37mm to 10.01mm with an average gain in bone height of 7.176mm (Table 3). The greatest gain in bone height occurred in sinuses with the least amount of native bone present. This indicates that the total bone regenerated may be a function of the implant length above the existing bone serving to support the sinus membrane analogous to a tent pole.

In this study, preoperative bone height beneath the maxillary sinus ranged from 1.6 to 7.4mm. Previous studies have shown that dental implants can be placed along with sinus augmentations in ridges with as little as 2mm native bone (Hatano et al. 2007, Thor et al. 2007). Results from this study support these findings. It appears that obtaining primary stability, rather than a minimum amount of native bone, it the critical factor in achieving osseointegration of implants placed simultaneously with sinus elevation.

The bone cores showed structural integrity even though some of the periphery was damaged, likely due to the trauma of "pushing out" from the trephine. This peripheral damage prevented estimations of bone volume from Micro-CT. The micro-CT did allow the analysis of 3D bone architecture. The core biopsies showed well-structured trabecular patterns. The biopsies were taken from the area where the lateral window was previously located, that is, where no bone was present. The

fact that bone cores were able to be harvested shows hard tissue was present where there previously was none.

The results of the histomorphometry report % bone area/total tissue area ranging from 37.4% to 74.3%. The mean %bone area/total tissue area was 58.7%. Other papers have reported on % bone area/total tissue area. In a study aimed to correlate clinical assessment of bone quality to the histologic structure quantified by histomorphometric evaluation of bone density, Trisi and Rao harvested small bone biopsies in 56 patients during oral implant surgery. The results of histomorphometric analysis were expressed as percentage of bony trabeculae over the total biopsy area. Samples of D1 showed a mean histomorphometric density of 76.54%±16.19. Samples from D2 showed a mean value of 66.78%±15.82. D3 specimens had a mean histomorphometric density scoring 59.61%±19.55. Finally, D4 samples had a mean value of 28.28%±12.02. In light of his findings, it appears on average the bone cores from the current study are similar to the D3 bone. Interestingly, densities from site 4 (74.285%) and 5 (74.106%) correlate to D1 type bone (Trisi & Rao 1999).

The incomplete closure of the osseous window seen in bilaterally in one patient, sites #3 and 4, may be a result of the large size of the windows. It may be also be that the blood clot and collagen membrane could not provide resistance to the pressure in the sinus. Previous studies have shown that a re-expansion process occurs, caused by intrasinusal positive pressure inside the sinus (Lambert et al. 2011, Hatano et al. 2004). A more rigid membrane may be a viable option, providing more strength than a collagen membrane.

No post-operative complications were reported or observed in the present study. Patients reported only mild discomfort following the procedures. One patient, site #1, had previously had a traditional sinus lift using a mixture of deproteinized bovine bone and mineralized bone allograft on the opposite site, performed by the main author. The patient reported less swelling and less use of pain medication following the sinus elevation using only venous coagulum. Lack of postoperative infections and patients reports of only "mild" discomfort may indicate that this procedure has less of an inflammatory response than those seen when using bone grafts.

SUMMARY AND CONCLUSIONS

Bone formation under the elevated sinus with simultaneous implant placement, using venous coagulum alone, was demonstrated in this study clinically, radiographically, and histologically. This limited study resulted in the following conclusions:

- Bone graft is not required for bone formation beneath the elevated sinus.
- Osseointegration can occur in the absence of bone graft.
- Successful osseointegration can occur when implants are placed in ridges with <2mm alveolar height.
- Use of venous coagulum under the elevated sinus did not result in any post-operative complications. Indeed, the lack of graft may contribute to the absence of post-operative infections and decreases in patient discomfort.

Based on the findings of this study, it can be concluded that bone grafting is not needed to augment atrophic maxillary sinuses, since it is sufficient to maintain the space with implants and venous coagulum alone.

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Project Revision/Amendment Form

Form version: October 28, 2010

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- **Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for Investigators for additional information.**
- **Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.**

1. Today's Date	August 10, 2011

2. Principal Investigator (PI)

Name (with degree)	Michael S. Reddy	**Blazer ID**	mreddy
Department	Periodontology	**Division (if applicable)**	
Office Address	412 SDB	**Office Phone**	4-4506
E-mail	mreddy@uab.edu	**Fax Number**	4-7901

Contact person who should receive copies of IRB correspondence (Optional)

Name	Sandra Haigh	**E-Mail**	shaigh@uab.edu
Phone	4-7513	**Fax Number**	4-7901
Office Address (if different from PI)			

3. UAB IRB Protocol Identification

3.a. Protocol Number	X090803001
3.b. Protocol Title	Tissue Procurement for Periodontal and Implant Research

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ **Study has not yet begun** — **No participants, data, or specimens have been entered.**
- ☒ **In progress, open to accrual** — **Number of participants, data, or specimens entered:** 6
- ☐ **Enrollment temporarily suspended by sponsor**
- ☐ **Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)**
  - **Date closed:**
  - **Number of participants receiving interventions:**
  - **Number of participants in long-term follow-up only:**
- ☐ **Closed to accrual, and only data analysis continues**
  - **Date closed:**
  - **Total number of participants entered:**

4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- ☐ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☒ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
  - ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
    In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OGCA tracking number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.
- ☐ **Add or remove performance sites**
  In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

▶

5. Description and Rationale

In Item 5.a. and 5.b, check Yes **or** No **and see instructions for** Yes **responses.**
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

☐Yes ☒No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐Yes ☒No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol. ✓ Added in SIRB. CRC

▶ We are adding Kathleen Beaudry, DMD as a sub-investigator. Dr. Beaudry is a Post-doctoral Resident, Department of Periodontology.

Dr. Beaudry will use tissue scraps collected under this protocol to study histologic healing of human subjects after periodontal surgery to develop a Master's thesis. The title of her thesis: "A Histologic and Radiologic Analysis of Bone Formation under the Elevated Maxillary Sinus using Venous Coagulum as Sole Filling Material"

Dr. Beaudry's project/analysis falls well within the original intent of this protocol; she has no conflicts interest to disclose.

5.d. Consent and Recruitment Changes: In the space below,
(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

▶

Signature of Principal Investigator _____ Date 8-10-11

FOR IRB USE ONLY

☐ Received & Noted ☑ ~~Approved~~ Expedited* ☐ To Convened IRB

Marilyn Dass — Signature (Chair, Vice-Chair, Designee)

8-16-11 — Date

DOLA 5/10/11

Change to Expedited Category **Y / N / NA**

*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111

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