Comparison of Post Tooth Extraction Healing Using different Xenograft Materials -A Pilot Study

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ABSTRACT

Aim & Hypothesis: Ridge preservation procedures may be used to overcome the challenges of dimensional changes of the alveolar ridge following tooth extraction. Various materials are used alone or in combination in extraction sockets. The choice of bone material used influences the bone quality of the edentulous ridge. Micro CT provides a good insight into the bone healing and the bone quality in three dimensions. The aim of this pilot study was to evaluate and compare the bone density using three different bone xenografts in ridge preservation procedures.

Materials & Methods: This randomized pilot study included ten patients requiring extraction of a non-restorable single rooted non-molar tooth. Comprehensive Periodontal and radiographic examination were performed prior to the extraction and before implant placement. Atraumatic tooth extraction was performed followed by ridge preservation surgery. Ten extraction sockets were randomly assigned to one of three groups: 1) Bio-Oss®, 2) Endobon® or 3) Equimatrix®. All grafted sockets were covered with a resorbable membrane (Mucograft) and partial primary closure was achieved. After 4 to 6 months, at the time of implant placement core biopsies were harvested using trephine burs. Samples were analyzed by Micro CT to compare bone density among the three different groups.

Results: A slight difference was evident among the different parametric values between the Bio-Oss®, Endobon® and Equimatrix® groups. Bio-Oss® and Equimatrix® groups showed similar bone density but all were higher than the Endobon® group. The mean density for the Bio-Oss®, Endobon® and Equimatrix® groups were 1275.42, 1217.62 and 1258.72 respectively.

The percentage of bone volume “BV/TV” was also higher in the Bio-Oss® and Equimatrix® groups when compared to the Endobon® group. Mean “BV/TV” was 0.2298 for the Bio-Oss®, 0.1287 for the Endobon® and 0.2556 for the Equimatrix®. However, significance testing was not performed due to the small sample size.

Conclusion: Results from this pilot study are inconclusive due to the small number of subjects. Preliminary data obtained from this pilot study suggested that Bio-Oss® and Equimatrix® had higher mean bone density and mean bone volume when bone cores were analyzed by Micro CT. These results would be valuable to design future clinical trials with a larger sample size based on sample size calculations. It sets the stage for future studies in which statistical significant differences between the groups may be determined.

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Introduction

Tooth loss can decrease quality of life by affecting mastication, speaking and socializing.(1) Implant therapy is highly accepted as the best option to restore lost dentition. (2) Adequate alveolar bone volume and ideal bone architecture are essential to achieve successful tooth reconstruction with implants.(3)

Success and failure of dental implants are influenced by many factors such as the quantity and quality of bone, patients' age, the dentist's experience, and oral hygiene control. (2)Implant stability is considered a primary factor affecting implant success.(4) Failure of implants is strongly related to inadequate amount of bone, active periodontal disease, uncontrolled systemic diseases, smoking, age and evidence of para-functional habits. (2) A failed implant is any lost or moving implant with bone loss more than 1.0 mm in the first year and more than 0.2 mm the second year. (5)

Normal Physiology of the Alveolar Bone

The alveolar bone is comprised of cortical bone, cancellous trabeculae and the alveolar bone proper. This alveolar bone proper contains compact bone, which forms the alveolus. Alveolar bone formation is accomplished by intramembranous ossification with the initial formation of woven bone. This less organized bone is replaced with the more organized lamellar bone. Osteons containing blood and nerves can be observed within the lamellar bone. It is important to understand the anatomy and normal physiology of the alveolar bone to better understand the changes and remodeling that follows tooth extraction.(3)

Post Extraction Healing Phases

Healing of extraction sockets involves phases including the formation of a coagulum that is replaced by a provisional connective tissue matrix, followed by woven bone. This woven bone is then transformed into lamellar bone and bone marrow.(6) The events of bone formation in healing extraction sockets were described in an experimental study in dogs.(6) Results showed that the first three days of healing evidence of blood clot was found in most of the extraction socket.(6)(Fig.1a,1b) After seven days the clot was partially replaced with a provisional matrix.(6)(Fig.1c) Fourteen days post extraction the tissue consisted of the provisional matrix and woven bone.(6)(Fig.2a) Mineralized bone was found to make 88% of the socket volume one month following extraction.(6) (Fig.2b) The portion filled with bone marrow was about 75% at two months and increased to 85% on day 180.(6)(Fig.2c, 3a, 3b, and 3c) *Ohta* studied healing of extraction sockets in monkeys. He divided the process into five stages: granulation stage, initial angiogenic stage, new bone formation stage, bone growth stage and bone reorganization stage.(3)

Molecular, Cellular and Tissue Changes Following Tooth Extraction

The healing process at the extraction sites occurs at molecular, cellular and tissue levels.(3) Thorough knowledge of the alterations in these levels is a key to understand the healing pattern and remodeling of the extraction sockets.(3) After extraction blood fills the socket containing proteins and damaged cells. Blood platelets are essential components to the formation of the fibrin clot that occupies the socket within the first hours.(3) Inflammatory cells are governed by growth factors in the coagulum. Macrophages and neutrophils play an important role in phagocytosis of bacteria and tissue debris(3). Synthesis and migration of mesenchymal cells are induced by the specific growth factors and cytokines released.(3)

Epithelialization and formation of the immature connective tissue takes place on the fourth day.(3) Granulation tissue replaces the clot within seven days while osteoid formation starts at the base of the extraction site.(3) Two thirds of the site is filled with immature bone at day 28.(3)

Dimensional and Volumetric Alveolar Ridge Alterations

The absence of a tooth initiates irreversible biological events causing alveolar ridge atrophy. The ridge volume reduction shows an impact on the outcome of implant therapy as stated by *Siebert and Salama.*(1) The progression of bone resorption after extraction renders the placement of implants difficult or complicates the manufacturing prosthesis in the future and the management of plaque control around these implants. (2) Resorption of the alveolar ridge occurs primarily during the initial period after tooth extraction. Most of this bone resorption occurs during the first three months. (2) This bone loss is more distinct in the horizontal dimension particularly the facial aspect of the ridge. Similarly loss of vertical ridge height can be observed mostly buccally(7) Many studies showed that the horizontal bone resorption was as great as 63% and vertical bone resorption was 22%.(8) Soft tissue is additionally affected by the volumetric dimensions of the alveolar ridge. *Covani et al.* found that considering the soft tissue with the hard tissue resulted in more vertical and horizontal resorption.(8)

Previous clinical and histologic investigations in studies performed on animals and humans have clearly proved that the buccal bone wall is often composed entirely of bundle bone.(9) This is attributed to the fact that the buccal bone is thin cortical bone and unsupported by medullary bone.(3) This results in greater resorption when compared with the lingual aspect of the alveolar bone, which is true for both maxillary and mandibular

arches(9). Socket remodeling is faster in the maxilla due to the abundant blood supply leasing to a faster resoprtion pattern.(3) The end product of this resorptive pattern is alteration of the diminished ridge and shift to a more palatal or lingual position.(7) This shift together with the reduced ridge vertical and horizontal dimensions may have a negative influence on optimum implant placement and restorative esthetics.(7)

Ungrafted extraction sockets may lose up to 50% of their ridge width in the first year post extraction.(10) (Fig.4) *Schropp et al.* studied alveolar ridge changes following tooth extraction in humans. Results showed that the width of the alveolar ridge was reduced up to 50% 12 months post extraction with most of the reduction occurring in the first 3 months.(3)*Chen et al.* reported that between six to twelve months following tooth extraction, 0.34 to 7.7 mm horizontal and 0.2 to 3.25 mm vertical bone loss occurs. The systematic review by *Lang et al.* found that the alveolar ridge undergoes 3.8 mm horizontal reduction and 1.24 mm of vertical reduction resulting in dimensional changes in the ridge width and height within 6 months after tooth extraction.(11) *Iasella et al.* found a 29% decrease in ridge width at nongrafted sites in the premolar and anterior areas.(9) *Botticelli et al.* also reported a mean 56% width reduction at untreated extraction sockets with immediate implant placement.(9) Additionally, in 2008, *Barone et al.* evaluated the healing of anterior and premolar extraction sockets at 7 to 9 months and reported a mean ridge width reduction of 4.5 mm, resulting in 42% horizontal loss.(9) The hard and soft tissues of the alveolar ridge undergo physiological changes after tooth extraction. This subsequently affects the restoration of lost dentition causing limited amount of bone volume for implant placement or unpredictable esthetics of the prosthetic replacement.(8) This explains the reason fresh

extraction sockets without bone grafting in the alveolar ridge are considered to be a major challenge in everyday clinical practice.(11)

Ridge Preservation Surgery

Substantial efforts and diverse methods have been conducted to preserve the residual alveolar ridge and prevent further bone resorption after teeth were extracted. Ridge preservation is a procedure that has proven to decrease further loss of ridge width during healing.(10) It is defined as preserving the ridge volume within the envelope existing at the time of extraction.(11) It is important to make a distinction between ridge preservation and ridge augmentation even though they share the same principles. Ridge augmentation has been defined as increasing the ridge volume beyond the skeletal envelope existing at the time of extraction.(11)

Thus, ridge preservation procedures offer predictable results to overcome the challenges of dimensional changes of the alveolar ridge following tooth extraction. Socket grafting can help maintain ridge height and width and reduce residual ridge resorption. This technique can increase the success rate of implant placement provide more pleasing esthetics and optimum functional stability. (12-14) *Tarnow et al.* related the mesial and distal osseous height to the presence or absence of an interdental papilla at the future implant site.(7) Based on the systematic review by *Vignoletti et al.* the reasons for ridge preservation are the maintenance of the existing soft and hard tissue envelope, preservation of a stable ridge volume for optimizing functional and esthetic outcomes and simplification of treatment procedures subsequent to the ridge preservation.(11) The major benefit is generation of adequate soft and hard tissue volume at the time of implant placement.(11) Ridge preservation improved the esthetic results as well as reducing the likelihood of need for

additional bone augmentation at the time of implant placement.(8) Careful patient and technique selection is critical to achieve a successful outcome with ridge preservation. (15) The principle of bone augmentation relies on using natural or synthetic materials to initiate physiological regeneration depending on the process of osteogenesis, osteoinduction and osteoconduction. (16)

Bone Graft Materials

A bone graft material is determined superior when it is biocompatible, nontoxic, radiopaque, sterile, inexpensive and retains excellent mechanical properties.(17) The mechanical properties of bone materials are linked to porosity, trabecular dimension and thickness that have an influence on the rate of bone formation and osteointegration.(17) It has been presumed that pore morphology, size and connectivity are significant elements.(17) They have direct effects on the rate of bone regeneration and indirectly on the primary and secondary stabilization of implants. (17) Bone substitution materials such as autografts, allografts, xenografts and alloplasts used alone or in combination were reported to reduce the rate of ridge resorption. (2)

Autogenous bone grafts and allografts were considered to be the "gold standard" bone substitutes. The main drawbacks using these materials are the significant disadvantages of limited availability, second surgical site, undesirable post operative pain and morbidity.(18) To evaluate its efficacy in ridge preservation, *Araujo & Lindhe* conducted a dog study in 2011.(19) Extraction sockets in the mandibles of dogs were filled with either anorganic bovine bone or autogenous bone chips. After 3 months of healing, a histometric analysis was performed.(19) In the apical and middle portions of the sockets, no resorption was observed.(19) The coronal portions of the autogenous bone graft group underwent resorption

(25%) while there was a positive change (3.6%) in the xenograft group.(19) It seemed that autologous bone did not preserve the alveolar ridge. (19)

These various regenerative bone materials were evaluated in preserving the dimensions of the alveolar ridge following tooth extraction. These studies showed no evidence that any of these biomaterials could completely impede the physiological bone remodeling in extraction sockets. However, some of the bone grafts reduced the extent of dimensional changes of the alveolar bone. Several studies showed clear difference in the amount of alveolar ridge changes after bone grafting. This could be influenced by the nature of bone material and residual ridge thickness. *Horvath et al.* suggested that the bone quality of grafted sockets are affected by the choice of grafting material used.(8)

Xenograft Bone Material

Xenografts obtained from other animal species exhibit ample osteoconductive properties. They are found to be a valuable alternative considering their biocompatible properties. Several studies revealed the effectiveness of these bone substitutes and showed their ability to serve as a scaffold inducing bone growth. This is proven by their capability to encourage osteoblasts' differentiation and activation, an essential key factor in bone regeneration. (18) *Fiorellini et al., Nevins et al and Misch* showed significant reduction in ridge dimensions when extraction sockets were grafted with xenografts in comparison with spontaneous healing.

The majority of the xenografts used in bone regeneration procedures are either of bovine or porcine origin. (20) Bovine-derived bone mineral xenografts have been extensively studied and are commonly used in clinical circumstances. (21) The efficacy of bovine

mineral materials have been examined histologically and histomorphometrically to evaluate the quality and quantity of newly formed bone in healing sockets. (22)

Healing patterns of the newly formed tissues in relation to the amount of residual graft material can be determined histologically by collecting cross sections along tissue cores from treated extraction sockets.(22) These specimens are prepared to be examined histomorphometrically to facilitate the evaluation and determine the influence of the socket depth.(22) A study conducted by *Artzi et al.* showed that the generated tissues, bone and connective tissue in post healed sockets occupied approximately 70% of all the examined specimens.(22) Based on histomorphometric evaluation at 9 months, they concluded that resorption of the graft particles was not noticeable. These findings were similar to previous studies reaching the same conclusion of the healing patterns anticipated.(22)

It is of special interest to understand the mechanism and rate of resorption of xenografts. It is assumed that the ideal bone substitute should induce or conduct osteogenesis and eventually be replaced by new bone as it completely resorbs. Although many studies have demonstrated new bone formation following ridge preservation procedures, the resorption capability of the different grafted materials is not clear yet. Presence of bovine bone mineral at 6 months and up to 42 months has been recognized.(22) *Skoglund et al*. showed that the rate of resorption of bone mineral particles is extremely slow and may be caused by the direct new bone generation onto the grafted particles impeding their resorption.(22)

Bio-Oss® Xenograft

Bio-Oss®, a low temperature-processed bovine bone (LTB) mineral material, possesses great osteoconductive properties making it the most commonly recognized xenograft used in various bone regeneration procedures. (23) (Fig.5)It is a deproteinized sterile bovine bone containing 10 μm granules with 75% to 80% porosity. (23) Protein destruction is achieved by the use of chemical solvents and heat treatment to a temperature of 300°C. (17)

Endobon® Xenograft

BIOMET3i Endobon® is a relatively newly developed bovine xenograft incorporating porous ceramic hydroxyapatite particles.(Fig.6) It is a high temperature-treated bovine bone (HTB) containing a partially carbonate-substituted HA containing minor ionic impurities.(17) Endobon® undergoes a calcination process by its exposure at temperatures reaching a peak of 1,000°C to 1,200°C (17). This results in a crystalline-like structure composed mainly of HA with porosity between 30% to 80%.(17) It has been widely used in a variety of orthopedic and maxillofacial procedures taking advantage of its osteoconductive activity. (16)

Equimatrix® Xenograft

While some people believe there are safety concerns of bovine spongiform encephalopathy with the bovine derived materials, the literature reports this risk as essentially non-existent. Considering the safety of xenografts, equine-derived bone is proposed to be an alternative xenogenic bone substitute material. (21) Equimatrix® is a horse derived xenograft material. It is an equine bone utilizing a unique purification process that removes all organic matter. (Fig.7) The nano-crystalline surface of Equimatrix® seen under

high power scanning electron microscopy has been demonstrated to have a high affinity for osteoblast cell attachment. Cellular attachment to the bone matrix is a critical precursor to successful regeneration of new bone. This complex, porous network closely resembles natural human bone and results in the excellent osteoconductive properties of the material. There are few studies on equine-derived bone xenografts, which showed its ability to induce osteoblastic differentiation of human bone marrow mesenchymal stem cells or to induce ectopic bone formation in a rat model. (20)

Previous studies on socket preservation using Bio-Oss® have shown favorable results as it could limit the amount of horizontal and vertical alveolar ridge resorption. (24,25) *Cardaropoli et al.* conducted a randomized clinical trial in 2012, confirming that ridge preservation using the bovine bone mineral Bio-Oss® limits the amount of horizontal and vertical bone resorption when compared with tooth extraction alone.(24) Their histomorphometric evaluation revealed that the xenograft material ensures a large mineralized fraction with the formation of new bone.(24) It also has demonstrated favorable results in alveolar bone augmentation procedures especially with sinus floor elevation. (23) In 2013, sockets grafted with Bio-Oss® and compared to other xenografts in rats using micocomputed tomography at 14 days of extraction.(26) Bio-Oss® exhibited significantly higher trabecular bone numbers and significantly smaller trabecular separation values than others.(26) Bio-Oss® appeared to be a more potent graft material to stimulate new bone formation in the tooth extraction sockets in rats. (26)

Few studies on Endobon® have shown it can be used as bone substitute in extraction sockets to preserve alveolar ridge dimensions. (18) A case report revealed excellent properties by Endobon® that could be related to its porous microstructure and high

percentage of interconnected micropores promoting osteogenesis making the graft integration easier and faster. (17) Its similarity with cancellous bone structure promotes the complete infiltration of bone, bone marrow, and blood vessels.(27) Therefore, Endobon® acts as a scaffold demonstrating osteoconductive properties.(27) In 2010 an animal study showed evidence that Endobon® is biocompatible, osteoconductive and non-resorbable and can be considered a bone substitute that does not interfere with normal reparative bone processes. (16)

In 2013, *Barone et al.* conducted a prospective, randomized, controlled, multicenter study to evaluate and compare histologically extraction sockets grafted with Endobon® and Bio-Oss®.(28) Outcomes between the two xenografts were similar, with de novo bone for the Endobon® at 28.5% and for the Bio-Oss® group 31.4%. (28)This investigation provides support for the efficacy of bovine bone xenograft for socket preservation when subsequent implant placement is planned. (28)

One study presenting clinical case series showed optimal healing and positive results when Equimatrix® was used in socket preservation, sinus augmentation and bone grafting techniques. (29) Equine particulate bone showed significant differences in amount of new cementum, newly formed bone area, and bone volume fraction values when compared to the negative control and collagen membrane alone groups.(21) Another case series evaluating an equine spongy bone in alveolar ridge augmentation procedures indicated its biocompatibility and association with new vessel ingrowth during healing.(20) Their findings showed that the equine bone material can be safely and successfully used to perform mandibular ridge augmentations.(20) In 2014, a recent case series study was conducted to determine the efficacy of equine particulate bone Equimatrix® and its ability to preserve the volume of

bone at extraction sites.(30) Clinical and histologic evidence supported the suitability of equine particulate bone for extraction site augmentation that facilitated dental implant placement.(30) Considering the safety of xenograft material, equine-derived bone mineral is suggested to be an alternative xenogenic bone substitute material, which is rarely mentioned in the literature.(21)

Micro CT Analysis

Micro-CT is a well-documented method to study bone microstructures because it provides accurate 3D images and is time efficient. (31) Micro-CT images are the result of differences in x-ray attenuation properties of bone, marrow spaces, and soft tissues.(31) It can determine 3D bone structures at micrometer to submicrometer resolution and allows for quantification of architectural metric parameters.(31) Micro CT provides a good insight into the bone healing and the bone quality in three dimensions, compared to conventional histopathology. Several previous studies showed that micro-CT data were comparable and significantly correlated to histomorphometric data. (32,33) However, micro-CT is not suitable to sufficiently assess cells of bone tissue such as osteoid, osteoblasts, and osteoclasts. Despite these differences, previous studies revealed that micro-CT was still comparable to stereologic histomorphometry for determining bone tissue quantity.(33)

The majority of micromorphometric human bone studies examining larger series of samples have been carried out in extraoral locations, and there are a limited number of reports in the literature that analyze micro-CT parameters in human maxillary bones.(31) Despite the different locations of the biopsies reported in previous studies, they might be useful for comparing the microstructure of pristine bone versus the newly formed bone in grafted areas. (31)

Bio-Oss® has been supported by plenty of studies in animals and humans. It has been widely used by clinicians in the daily practice. On the contrary, Endobon® and Equimatrix® are recently introduced and the scientific data available for the use of both materials are inadequate. However, the limited studies related to two materials are mainly from non-human, case reports, and uncontrolled clinical trials. A major drawback of most of the reports available in literature to date is that the biopsies taken for analysis are not derived from the actual implant site. (31) This does not provide exact information on the quality of bone at the actual implant site. (31) Moreover, there are no prospective studies comparing the effectiveness of these three materials, Bio-Oss®, Endobon® and Equimatrix®, when placed in extraction sockets. Therefore, the purpose of the present pilot study was to conduct an interventional prospective clinical trial to evaluate the efficacy of these three different xenografts for ridge preservation by comparing the quality of newly formed bone using micro CT analysis.

Aim and Hypothesis

Primary aim:

Compared the bone density between Equimatrix®, Bio-Oss® and Endobon®. This was determined by Micro CT analysis.

We hypothesized that the bone density from Equimatrix® will be higher than Bio-Oss® and/or Endobon®.

Secondary aim:

Compare the percentage of newly formed bone between Equimatrix®, Bio-Oss® and Endobon®. histomorphometric analysis.

We hypothesized that the mean percentage of newly formed bone from Equimatrix® will be higher than Bio-Oss® and/or Endobon®.

Research Design

This was a randomized pilot prospective clinical trial in which Micro CT analysis was utilized to compare between Endobon®, Equimatrix®, and Bio-Oss®. Subjects enrolled in the study were patients recruited from TUSDM clinics from 2014-2015. These patients required extraction of single rooted non-molar tooth and were treatment planned to receive dental implants in the future. Inclusion and exclusion criteria were established for selecting eligible subjects to be recruited.

Inclusion criteria:

1. Patients aged 18 years and over.
2. Non-restorable single rooted tooth in anterior or premolar region: if a patient had two adjacent teeth meeting the inclusion criteria the patient was excluded from the study. If a patient had more than one tooth in different quadrants meeting the inclusion criteria only one tooth was included in the study. The day of the extraction and ridge preservation surgery involved only the tooth included in the study. (Routinely one quadrant of extraction and ridge preservation is done for each appointment as standard of care).
3. Patients previously treatment planned for implant procedure and implant restoration.
4. $\geq$ 10 mm from maxillary sinus or inferior alveolar canal (IAC) (as measured on radiograph from within the past 6 months or taken at Visit 1 according to standard of care).
5. Intact buccal bone (only minor dehiscence or fenestrations (approximately < 50% of socket depth) was accepted (as measured on radiograph from within the past 6 months or taken at Visit 1 according to standard of care).

6. Non-smokers.
7. Patients treatment planned for extraction and ridge preservation at Tufts University School of Dental Medicine.

Exclusion criteria:

1. Poor oral hygiene (plaque index>30%).
2. Systemic diseases that affect bone metabolism (self-report):
   i. Osteoporosis
   ii. Osteomalacia
   iii. Hyperthyroidism
   iv. Hyperparathyroidism
   v. Paget's disease
3. Inflammatory and autoimmune diseases of the oral cavity (severe bone loss) (self-report):
   i. Severe chronic periodontitis
   ii. Aggressive periodontitis
   iii. Necrotizing ulcerative periodontitis
   iv. Crohn's disease
   v. Multiple sclerosis
   vi. Rheumatoid arthritis
   vii. Systemic lupus erythematosus
4. History of radiation to the head and neck, and /or chemotherapy.
5. Current corticosteroid therapy.
6. History of IV Bisphosphonates therapy or >3 years of oral intake.

7. Infectious diseases such as HIV, tuberculosis, Hepatitis (self-report).
8. Known allergy to research related materials.
9. Self-reported pregnancy or lactation (as this is considered an elective surgery). (Elective periodontal procedures are postponed during pregnancy as standard of care in the TUSDM department of periodontology. Additionally, bone density has shown to be affected temporarily by pregnancy.)

Withdrawal termination criteria:

1. Non-compliance (e.g., subject fails to come to scheduled research appointments)
2. Unwillingness to further participate
3. Subjects who participated in other medical or dental studies during the course of this study were terminated.
4. If a subject became pregnant in the ongoing study, subject was withdrawn.
5. If subjects experienced infection they were withdrawn from the study and followed up with standard of care in the TUSDM periodontology clinic.

Experimental Procedures:

Patients received primary standard dental care prior to the surgical phase including comprehensive periodontal examination, radiographs, and dental photography at Visit 1. Eligible subjects were committed to a total of 3 visits including screening visit, extraction, ridge preservation, postoperative follow-ups, harvesting core biopsies and implants placement. (Fig.8) All patients who are disqualified received standard of care of TUSDM.

Groups and Randomization:

Randomization occurred at Visit 2. The statistical software package R (Version 2.11.1) was used to determine which tooth to be included in the study if more than one

qualified. The statistical software package R (Version 2.11.1) was used to conduct the randomization of study subjects to be assigned to one of the three groups:

A. Bio-Oss® + Mucograft

B. Endobon® + Mucograft

C. Equimatrix® + Mucograft

A printout of the randomization scheme was given to the investigators. The surgeon and the subject were not blinded. All products were FDA approved as used in this study and were used according to manufacturer instructions. The product was stored at room temperature (DHS1256).

Materials and Methods

Clinical and Surgical Methods:

Patient Recruitment and Pre-surgical Preparation:

The present study was approved by Institutional Review Board at Tufts Medical Center and Tufts University Health Sciences Campus. Subjects enrolled in the study were expected to participate for six to eight months. Potential subjects had a screening visit to obtain signatures for the informed consents, collect demographic data and medical history. Inclusion and exclusion criteria were evaluated to determine if a subject would be qualified or not. Clinical periodontal examination, according to standard of care at TUSDM, was conducted using a hand mirror and probe, including:

1. Full mouth Plaque Index: calculated as a percentage (6 sites/tooth)
2. Gingival Index: Loe and Silness criteria was used (Table 1) (4 sites/tooth)
3. Full mouth Bleeding on Probing (BOP): calculated as a percentage (6 sites/tooth)
4. Probing Depth (PD): from the base of the pocket to the gingival margin (mm) of all teeth (6 sites/tooth)
5. Recession: from the CEJ to the gingival margin (mm) of all teeth
6. Clinical attachment loss (CAL) (mm) of all teeth (6 sites/tooth)
7. Amount of keratinized gingiva KG (mm) on the buccal aspect of all teeth
8. Periapical radiographs (if none in the dental record from within the past 6 months as part of standard of care).

Surgical Procedures, Postoperative Care and Follow-up Visits:

Atraumatic extraction and ridge preservation was performed on the eligible subjects in the second visit after reviewing the medical history, inclusion and exclusion criteria. Randomization was done prior to the surgery to determine which xenograft material will be used. Pre surgical intra oral photographs were taken prior to the surgery. Topical and local anesthesia was used to numb the area where the tooth was going to be extracted. The tooth was extracted in a minimally invasive atraumatic manner using periotomes and minimal forceps rotation or sectioning of the tooth if needed. (Fig.9) Surgery was done with minimal flap reflection not extending more than approximately 2 mm beyond the alveolar crest. Complete curettage of the extraction socket, elimination of the granulation tissue and irrigation with saline was followed. Then the socket was thoroughly evaluated for any defects such as dehiscence and fenestrations. The grafting material selected was hydrated with saline and filled in the socket to or coronal to the crest of bone. Then Mucograft membrane was

placed over the graft according to manufacturer's instructions and stabilized with non-resorbable 5-0 Vicryl sutures. (Fig.10) Standard of care written post-operative instructions was given to the patient and reviewed verbally. Standard of care at TUSDM medications were prescribed including antibiotic therapy and post-operative pain management. For the antibiotic therapy Amoxicillin 500 mg three times a day for 10 days was prescribed. If a patient was allergic to Penicillin Clindamycin 300 mg three times a day for 10 days was given. Ibuprofen 800 mg three times a day for 3 days was given as an anti-inflammatory and pain medication. Prescription of Peridex 0.12% mouth rinse was also given to the patient. All subjects were seen for standard of care follow up appointments, which were not considered part of this study. (Fig.11, 12, 13)

Surgical Reentry and Implant Placement:

The third visit took place 4-6 months after extraction and ridge preservation to collect bone core samples before the implant placement. Medical history, inclusion and exclusion criteria were reviewed. Pre surgical periapical radiographs and intra oral photographs were taken. Following anesthesia, the bone core biopsy was harvested from the area planned for implant placement. Using a trephine drill with a 2 mm internal diameter about 2 x 8 mm core biopsy was harvested from the central part of the socket and then placed in 10% buffered formalin. (Fig.14) This method was slightly different from the standard clinical method of drilling. Removal of bone was standard for implant placement surgery and no more than the standard amount of bone was removed for this study. Intraoral photographs were taken before and after bone biopsy. (Fig.15, 16, 17) The implant was then placed following standard of care clinic guidelines. The implant placement was not considered part of the study. Post surgical periapical radiographs were exposed as standard of care. (Fig.18, 19, 20)

Laboratory and Micro CT Methods:

A total of ten bone core samples were retrieved from ten healthy individuals after ridge preservation procedures. The group evaluated received xenograft (Bio-Oss®, Endobon®, Equimatrix®) and Mucograft resorbable collagen membrane upon extraction. The bone core samples were collected immediately prior to implant placement and placed in 10% formalin. Each sample was labeled with the subject's ID and date of harvesting. (Fig.21) After at least 48 hours of fixation in formalin, the samples were washed with distilled water to ensure complete removal of the formalin solution and placed in 70% Ethanol for storage and prevent any bacterial contamination. Bone core samples were submitted to the Laboratory of Dr. Kawai in Forsyth Institute for Micro CT analysis to evaluate bone density. A PhD student (A.A) who was a masked blinded examiner in Forsyth Institute assisted and helped to prepare, submit and analyze the samples to Forsyth micro-CT core (director, Dr. Sasaki). Each bone core sample was assigned a number prior to the scanning process (Fig. 22).

The bone cores were analyzed with a tabletop micro CT system (mCT40, Scanco Medical, Bassersdorf, Switzerland) at energy of 70 kVp and a current of 140 mA to evaluate bone density. A standard-medium resolution mode (1024 pixels, 500 projections) with an integration time of 300 ms was chosen, resulting in an isotropic voxel of 37 mm and an in-plane dimension of 1024 X 1024 pixels. The scanning of the bone samples was completed overnight. After scanning, the 3D data of micro CT were reconstructed and analyzed using computer software where all of the measurements were performed (Fig. 24,25,26).

Micro CT Data Analysis:

Micro CT post analysis yielded valuable outcome variables for each sample specified as:

1. Bone density of the sample. The higher the number, the higher the density.
2. Bone volume/Tissue volume "BV/TV" .The higher the number, the higher bone volume.
3. Trabecular number "Tb.N". The lower the number, the better the bone.
4. Trabecular thickness "Tb.Th". The higher the number, the higher trabecular bone thickness.
5. Trabecular space "Tb.Sp". The higher the number, the higher trabecular bone space.

BV/TV: This parameter relates BV to total tissue volume. (31) The measurement of BV/ TV is of major importance in bone research because it is the principal determinant of bone strength. (31)TbTh, TbSp, and TbN: Measurements of the mean thickness, the separation, and density of the trabeculae within the specimen, respectively. (31) These measurements provide information on the amount of bone in the specimen and its organization. (31)

Statistical Analysis

As this was a pilot study, no formal sample size calculation was required. Our goal was to have a sample size of n=5 per group complete the study. Up to 40 subjects were screened to find qualifying subjects.

Descriptive statistics were calculated as means and standard deviations for all three groups. Inferential statistics (confidence intervals and p-values) were not computed due to the fact that the study was a pilot. All analyses were conducted using SPSS (Version 21).

Results

Study Subjects:

A total of ten healthy patients were included in the study and ten bone cores were obtained for Micro CT analysis. The study subjects included eight males and two females. The Bio-Oss® group had three subjects with a mean age of 57.33 and SD of 10.41(Table 2). The Endobon® group had four subjects with a mean age of 56.75 and SD of 6.29(Table 2). The Equimatrix® group had three subjects with a mean age of 54.67 and SD of 9.29 (Table 2).

Surgical Sites:

Teeth extracted were three maxillary anteriors and seven maxillary and mandibular premolar teeth (Table 2). Reasons for extraction included non-restorable teeth due to extensive caries, crown fracture, root fracture or endodontic reasons. None of these teeth were periodontal involved with mobility or severe bone loss. Dental implants were successfully placed in all patients with no complications and adequate primary stability was achieved in all grafted sockets (Fig. 15-20).

Clinical Data:

Mean probing depths (PD) and clinical attachment loss (CAL) means and SDs were calculated for each group (Table 3). Mean probing depths for the Bio-Oss®, Endobon® and Equimatrix® groups were 2.56, 3.71 and 3.05 respectively. Mean Clinical attachment loss was 2.89 for Bio-Oss®, 3.88 for Endobon® and 3.33 for Equimatrix®.

Mean $\pm$ SD for the percentages of the mesial and distal bone to the root length were calculated (Table 4). The mean percentages of mesial bone to root length for the Bio-Oss®, Endobon® and Equimatrix® groups were 76.99, 90.81 and 93.35 respectively. The mean

percentages of distal bone to root length were 92.71 for Bio-Oss®, 88.35 for Endobon® and 91.88 for Equimatrix®.

Micro CT Outcomes:

Table 5 lists the means, standard deviations of all the subjects in the study for every measured parameters, distinguishing between the three groups. A slight difference was evident among the different parametric values between the Bio-Oss®, Endobon® and Equimatrix® groups. Bio-Oss® and Equimatrix® groups showed similar bone density which was higher than the Endobon® group. The percentage of bone volume "BV/TV" was also higher in the Bio-Oss® and Equimatrix® groups when compared to the Endobon® group. The trabecular bone number "TbN" was lower in the Endobon® group than in the Bio-Oss® and Equimatrix® groups indicating better bone quality associated with the Endobon® group. The Trabecular bone thickness "Tb.Th" was higher in the Bio-Oss® and Equimatrix® groups. The Trabecular bone separation "Tb.Sp" was higher in the Endobon® group than in the Bio-Oss® and Equimatrix® group. However, significance testing was not conducted due to the small sample size.

Probing depths and Regenerated Bone:

The Bio-Oss® and Equimatrix® groups showed less bone density with deeper probing depth (Table 6). This findings did was not relevant with the Endobon® group where the deeper probing depths showed higher bone density than shallower probing depths. Bone volume showed varying results with deeper probing depths in all three groups. There seems to be a trend for less bone density in the newly formed bone with deeper probing depths.

Discussion

The aim of this randomized pilot study was to evaluate and compare the bone quality following grafting of extraction sockets with three different xenografts: 1) Bio-Oss®, 2) Endobon® or 3) Equimatrix®. To our knowledge no clinical studies were executed to compare and evaluate histologically these three xenograft materials when placed in extraction sockets. This pilot study would provide initial evidence of tangible difference between the three bone replacement materials when used in ridge preservation procedures. It would set the basis for estimating the difference in the efficacy of each bone material. The results would provide preliminary evidence to answer the question if Equimatrix® can be considered an alternative xenogenic bone material to the commonly used Bio-Oss® Furthermore, it would guide us for conducting future randomized controlled clinical trials based on the results and experience gained with these three bone graft materials.

The hard and soft tissues of the alveolar ridge undergo physiological changes after tooth extraction. Previous studies evaluated healing after tooth extraction and its effect on the dimension of the residual alveolar ridge. (3,9,10,11) Progressive alveolar ridge resorption following tooth extraction has been considered a negative factor for future implant placement. Hence, ridge preservation was justified to maintain the ridge volume and prevent any further loss of ridge width following extraction. Different materials and techniques have been used to preserve the ridge after tooth loss. In the premolar and anterior areas *Iasella et al.* and B*arone et al.* found a 29% to 42% decrease in ridge width at non-grafted sites.(9) This horizontal ridge reduction affected the placement and stability of dental implants. Therefore, ridge preservation procedures can help maintain ridge height and width and reduce residual ridge resorption. In the present study clinical observation of the edentulous

ridges at reentry showed favorable results. All grafted sockets showed sufficient ridge width clinically for predictable implant placement. All ten sockets received dental implants successfully and achieved primary stability. No post-operative complications were reported. These findings confirmed the results of the systematic review by *Vignoletti et al.* stating that ridge preservation can maintain the alveolar ridge volume for optimizing implant placement, increasing the success of the outcomes. (11) It also supported the recent study by *Barone et al.* indicating that ridge preservation improved the esthetic results and reduced the probability for additional bone augmentation at the time of implant placement.(8) Outcomes of our study support results from *Cardaropoli et al.* confirming that ridge preservation using bovine bone mineral graft material limits the amount of bone resorption when compared with tooth extraction alone.(24) Their histomorphometric evaluation revealed that the xenograft material ensured a large mineralized fraction with formation of new bone.(24)

Bone core biopsies were collected from all ten patients and the bone density was analyzed with Micro CT. It was suggested from previous studies that the bone quality of grafted sockets is majorly affected by the choice of bone material used and this subsequently affects the success of implants placed.(8) Observation of the grafted sockets showed residual remaining graft particles that did not completely amalgamate or incorporate. This issue was more evident with sockets grafted with Endobon® than with Bio-Oss® or Equimatrix®. This observation supports that Bio-Oss® is considered the gold standard bone graft material with its superior osteoconductive properties. Results from our study are in agreement with the results from the study by *Artzi et al.* of the healed sockets after grafting with Bio-Oss®.(22) Histomorphometric evaluation revealed that resorption of the graft particles was not noticeable.(22) The clinically detectable graft particles after 4 to 5 months in the present

study corresponds to previous studies in which the presence of bovine bone mineral at 6 months and up to 42 months has been recognized.(22)

Clinical performance revealed significant variation in the handling process of the three graft materials. Bio-Oss® was easy to manipulate and manage during the grafting procedure. Equimatrix® was reasonable to manage but less manageable than Bio-Oss®. Endobon® was clinically difficult to use as particles scattered even when hydrated.

A major weakness in the previous studies in literature was that the biopsies harvested for Micro Ct analysis were not collected from the actual implant site. (31) This did not accurately determine the bone quality at the actual implant site.(31) In the current study, the biopsies were taken directly from the grafted site prior to implant placement.

Bone quality was assessed from the Micro CT analysis of the core samples obtained prior to implant placement. Results from previous Micro CT demonstrated that Bio-Oss® exhibited higher trabecular bone numbers and significantly smaller trabecular separation values than others.(26) Our Micro CT analysis revealed similar results for Bio-Oss®. Significant finding was that Equimatrix® also had comparable high trabecular bone numbers and smaller trabecular bone separation values especially when Bio-Oss® and Equimatrix® were compared to Endobon.

Overall the outcome variables obtained from the Micro CT analysis were in favor of Bio-Oss® and Equimatrix®. They had similar mean bone densities, which were higher than those of Endobon®. Additionally they both had higher mean bone volume, trabecular bone number and trabecular bone thickness than Endobon®. On the contrary, Bio-Oss® and Equimatrix® had lower mean trabecular bone separation than Endobon®. The data from the Micro CT analysis favors the xenografts Bio-Oss® and Equimatrix® over Endobon® when

considering bone quality. However, since this was a pilot study no p-values were calculated to determine statistical significance. Careful interpretation of our results is necessary due to the small sample size.

The present pilot Micro CT investigation of three different xenografts - filled extraction sockets confirmed de novo bone formation after a healing period of 4-5 months. The bone density of newly formed bone associated with ridge preservation procedures utilizing Bio-Oss® and Equimatrix® was similar but higher than Endobon®. Nonetheless, all three xenografts tested provided implant with equal primary stability despite the differences in bone quality.

Within this pilot study limitations could be attributed to time, effort and cost needed to conduct such study design in a specific timely manner. Furthermore, the drop out of some study subjects further limits the scope of inferences from the results, especially with a small sample size. Moreover, absence of negative controls could be considered a weakness in the study.

Conclusion

Drawing a definitive absolute conclusion is incomprehensible due to nature of the study design and small number of subjects. Preliminary data obtained from this pilot study suggested that Bio-Oss® and Equimatrix® had higher mean bone density and mean bone volume when bone cores were analyzed by Micro CT.

However, this pilot study will be valuable to design future clinical trials with a larger sample size based on results and sample size calculations. This may permit developing evidence-based clinical protocols to serve as a key for clinicians in decision making when considering which bone graft material to choose for ridge preservation procedures.

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APPENDICES

Appendix A: Tables

Appendix B: Figures

Appendix A: Tables

Table 1 Gingival Index*: Loe and Silness*

Table 1 Gingival Index*: Loe and Silness*	
0	Normal gingiva
1	Mild inflammation – slight change in color and slight edema but no bleeding on probing
2	Moderate inflammation – redness, edema and glazing, bleeding on probing
3	Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding

Table 2 Demographical & Clinical Data of Study Subjects

Table 2 Demographical and Clinical Data of Study Subjects divided by group					
Group	N	Age (y)	Gender (female/male)	Tooth (Anterior/premolar)	Arch (maxillary/mandibular)
A Bio-Oss®	3	Mean 57.33 SD 10.41	1/2	0/3	0/3
B Endobon®	4	Mean 56.75 SD 6.29	1/3	3/1	4/0
C Equimatrix®	3	Mean 54.67 SD 9.29	0/3	0/3	2/1

Table 3 Mean Clinical Attachment Loss and Probing Depth

Table 3 Mean Clinical Attachment Loss and Probing Depth			
Group	N	Mean PD (mm)	Mean CAL (mm)
A Bio-Oss®	3	3.17	3.67
		2.33	2.33
		2.17	2.67
	Mean	2.56	2.89
	SD	0.54	0.70
B Endobon®	4	2.67	2.67
		3.17	3.67
		3.00	3.17
		6.00	6.00
	Mean	3.71	3.88
	SD	1.54	1.47
C Equimatrix®	3	3.50	3.83
		2.83	3.33
		2.83	2.83
	Mean	3.05	3.33
	SD	0.39	0.50

Table 4 Percentages of Mesial and Distal Bone to Tooth Root Length

Table 4 Percentages of Mesial and Distal Bone to Tooth Root Length			
Group	N	Percentage of mesial bone to root length	Percentage of distal bone to root length
A Bio-Oss®	3	78.74	94.49
		54.29	85.71
		97.94	97.94
	Mean	76.99	92.71
	SD	21.88	6.31
B Endobon®	4	95.38	93.85
		94.62	88.46
		97.09	94.17
		76.15	76.92
	Mean	90.81	88.35
	SD	9.83	8.06
C Equimatrix®	3	93.33	94.67
		95.45	88.18
		91.27	92.78
	Mean	93.35	91.88
	SD	2.09	3.34

Table 5 Micro CT Data Analysis of Bone Core Samples

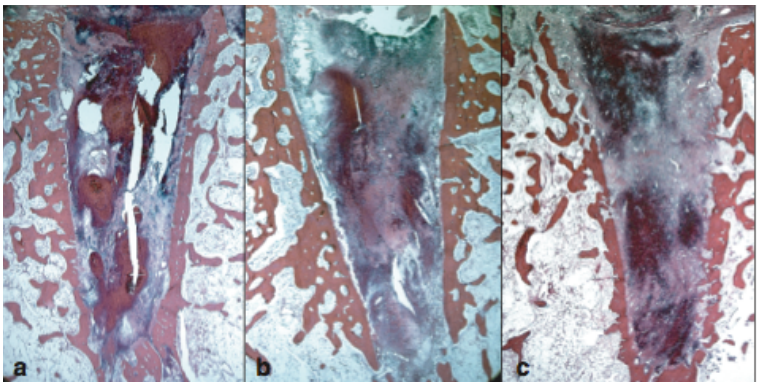
Table 5 Micro CT Data Analysis of Bone Core Samples						
Group	N	Bone density	Bone volume/tissue volume "BV/TV"	Trabecular number "Tb.N"	Trabecular thickness "Tb.Th"	Trabecular space "Tb.Sp"
A Bio-Oss®	3	1091.35	0.2048	2.62	0.2435	0.3988
		1283.96	0.3184	4.79	0.1581	0.1628
		1450.94	0.1662	3.80	0.1452	0.2440
	Mean	1275.42	0.2298	3.74	0.1823	0.2685
	SD	179.95	0.0791	1.09	0.0534	0.1199
B Endobon®	4	908.59	0.2308	2.17	0.2518	0.4501
		1690.80	0.2191	4.13	0.1584	0.2247
		1133.05	0.0294	2.39	0.0443	0.4306
		1138.02	0.0353	1.49	0.1086	0.6923
	Mean	1217.62	0.1287	2.55	0.1408	0.4494
	SD	333.11	0.1113	1.12	0.0875	0.1914
C Equimatrix®	3	970.56	0.4680	4.47	0.2667	0.1420
		1569.92	0.1183	2.04	0.1762	0.5120
		1235.67	0.1806	4.30	0.1401	0.2670
	Mean	1258.72	0.2556	3.60	0.1943	0.3070
	SD	300.34	0.1865	1.36	0.0652	0.1882

Table 6: Relationship between Probing Depths& Regenerated Bone

Table 6 Relationship between Probing Depths & Regenerated Bone				
Group	N	Mean PD	Bone Density	" BV/TV"
A Bio-Oss®	3	3.17	1091.35	0.2048
		2.33	1283.96	0.3184
		2.17	1450.94	0.1662
	Mean	2.56	1275.42	0.2298
	SD	0.54	179.95	0.0791
B Endobon®	4	2.67	908.59	0.2308
		3.17	1690.80	0.2191
		3.00	1133.05	0.0294
		6.00	1138.02	0.0353
	Mean	3.71	1217.62	0.1287
	SD	1.54	333.11	0.1113
C Equimatrix®	3	3.50	970.56	0.4680
		2.83	1569.92	0.1183
		2.83	1235.67	0.1806
	Mean	3.05	1258.72	0.2556
	SD	0.39	300.34	0.1865

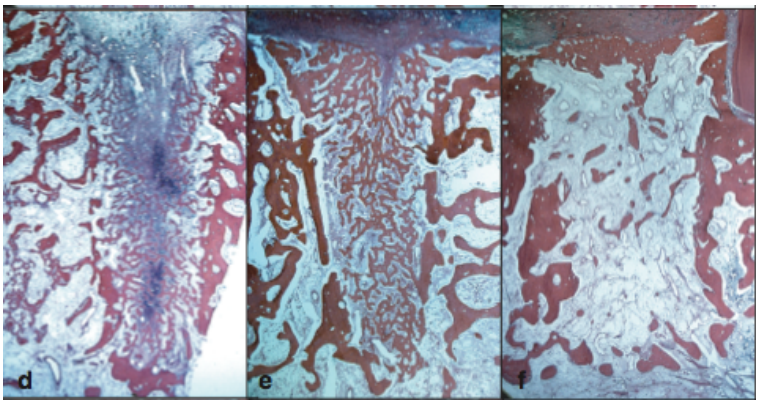
Appendix B: Figures

Figure 1: Healing of extraction socket: a) day 1 b) day 3: clot formation c) day 7: clot is replaced with provisional matrix.(6)



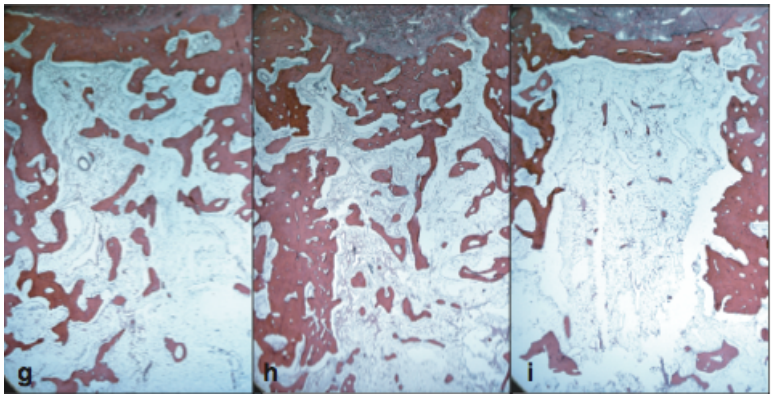
a b c

Figure 2: Healing of extraction socket: a) day 14: provisional matrix and woven bone. b) day 30: mineralized bone occupies most of the socket volume c) day 60: bone marrow formation.(6)



a b c

Figure 3: Healing of extraction socket: a) day 90 b) day 120 c) day180 involving bone maturation to lamellar bone and increased percentage of bone marrow.(6)



a b c

Figure 4

Difference in healing between Ungrafted Vs. grafted extraction socket.(1)

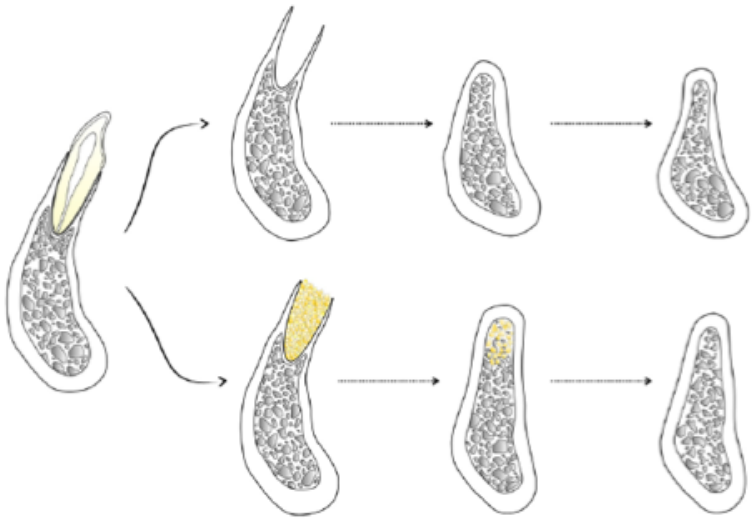


Figure 5

Bio-Oss® Bovine xenograft (34)

Figure 6

Endobon® Bovine xenograft (35)

Figure 7

Equimatrix® Equine xenograft (36)

Figure 8

Study timeline

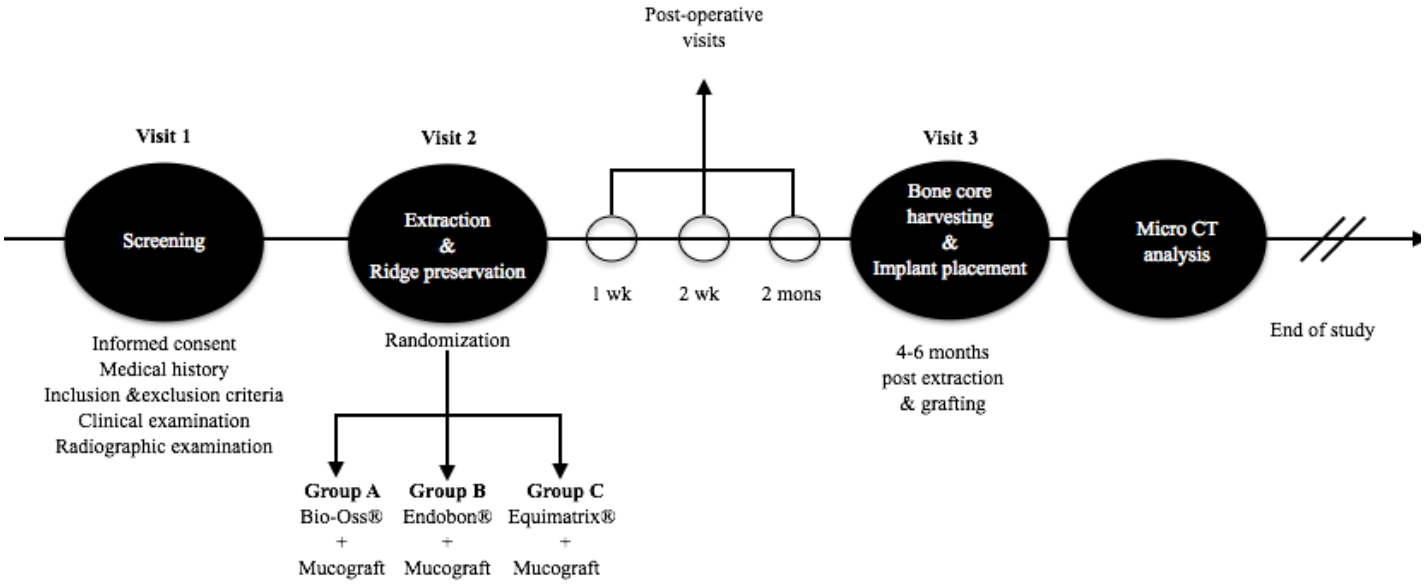


Figure 9

Extraction procedure.(37)
a. Using a periotome
b. Atraumatic extraction with forceps

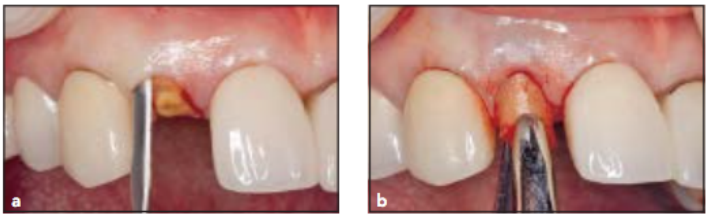
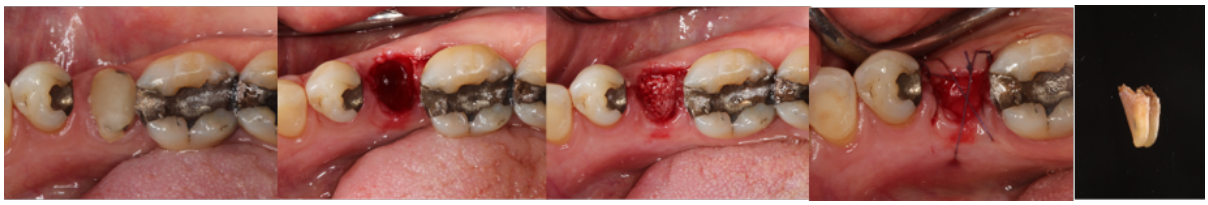


Figure 10

Mucograft collagen membrane(38)

Figure 11

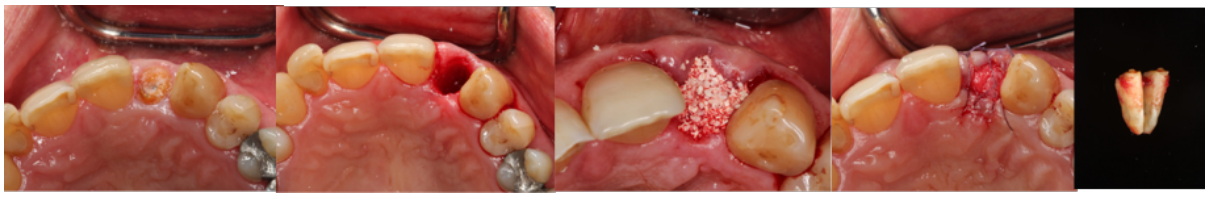
Group A Bio-Oss®: Extraction and ridge preservation surgery



a. Initial | b. Extraction | c. Socket grafting | d. Suturing | e. Extracted tooth

Figure 12

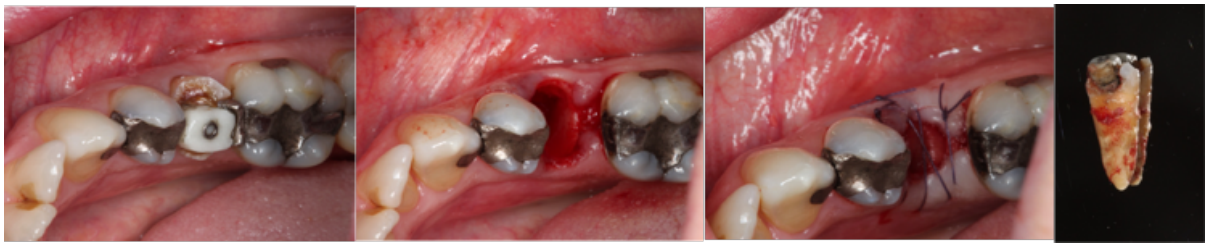
Group B Endobon®: Extraction and ridge preservation surgery



a. Initial | b. Extraction | c. Socket grafting | d. Suturing | e. Extracted tooth

Figure 13

Group C Equimatrix®: Extraction and ridge preservation surgery



a. Initial | b. Extraction | c. Suturing | d. Extracted tooth

Figure 14

Surgical reentry: Bone core biopsy harvesting using trephine bur

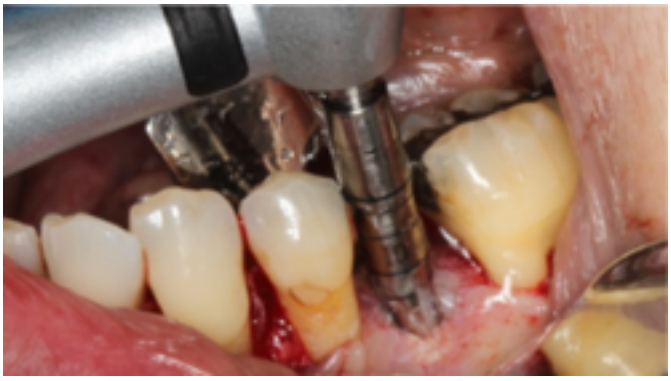
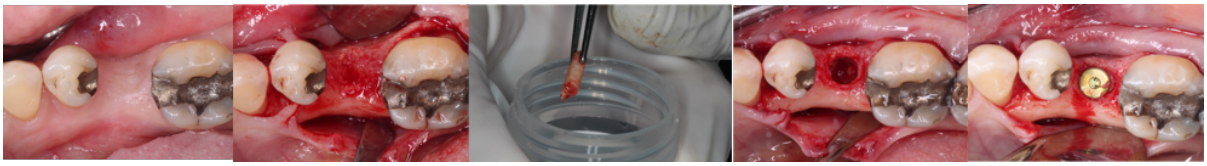


Figure 15

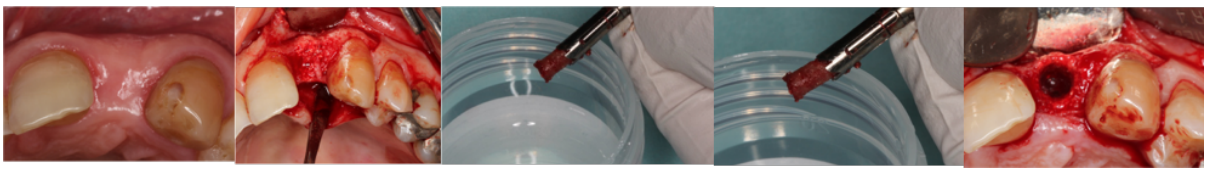
Group A Bio-Oss®: Core biopsy and implant placement



a. Initial | b. Reentry | c. Bone core biopsy | d. Ridge after harvesting core | e. Occlusal view after implant placement

Figure 16

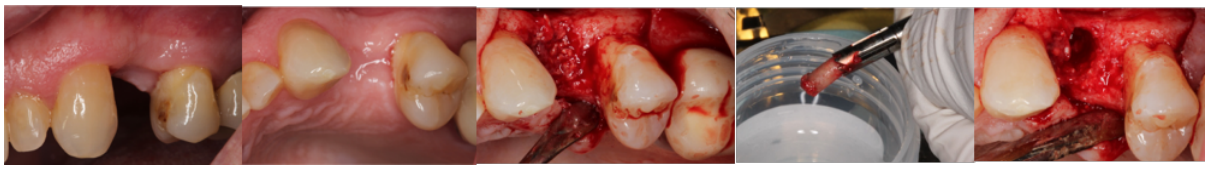
Group B Endobon®: Core biopsy and implant placement



a. Initial | b. Reentry | c. Bone core biopsy | d. Bone core biopsy | d. Ridge after harvesting core

Figure 17

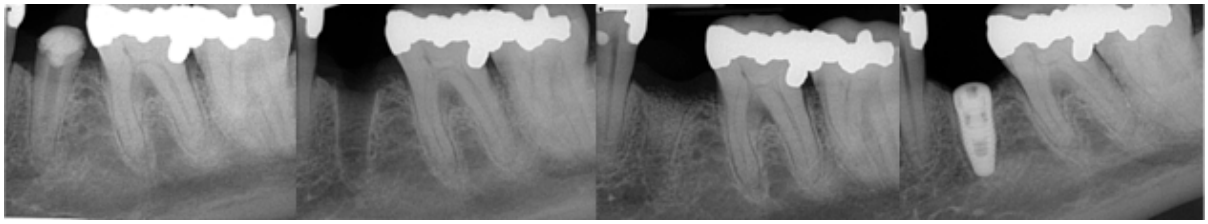
Group C Equimatrix®: Core biopsy and implant placement



a. Initial | b. Initial | b. Reentry | c. Bone core biopsy | d. Ridge after harvesting core

Figure 18

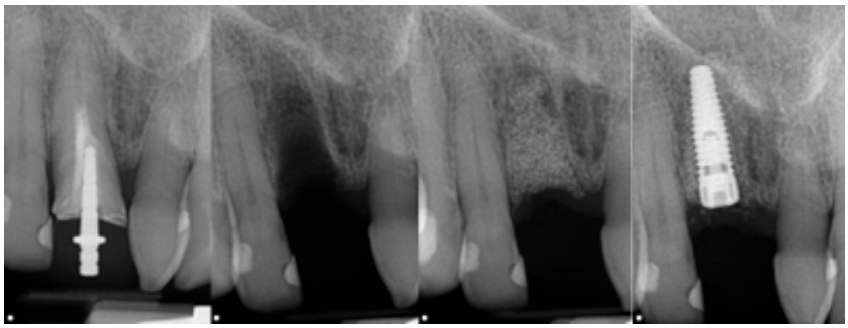
Group A Bio-Oss®: Periapical radiographs series



a. Initial | b. Post extraction | c. 4 months after grafting | d. Implant placement

Figure 19

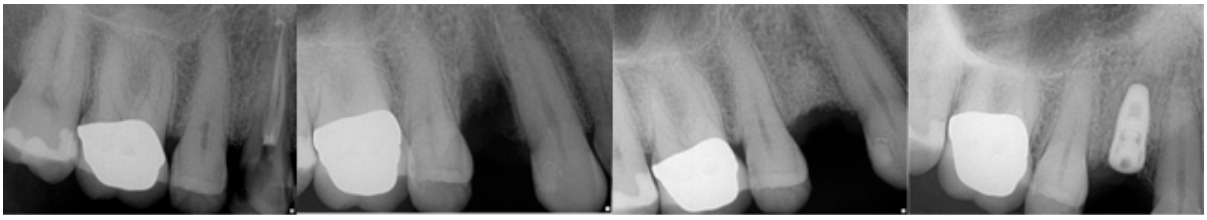
Group B Endobon®: Periapical radiographs series



a. Initial | b. Post extraction | c. 4 months after grafting | d. Implant placement

Figure 20

Group C Equimatrix®: Periapical radiographs series



a. Initial | b. Post extraction | c. 4 months after grafting | d. Implant placement

Figure 21

Labeled bone core samples placed in 70% Ethanol and stored at room temperature

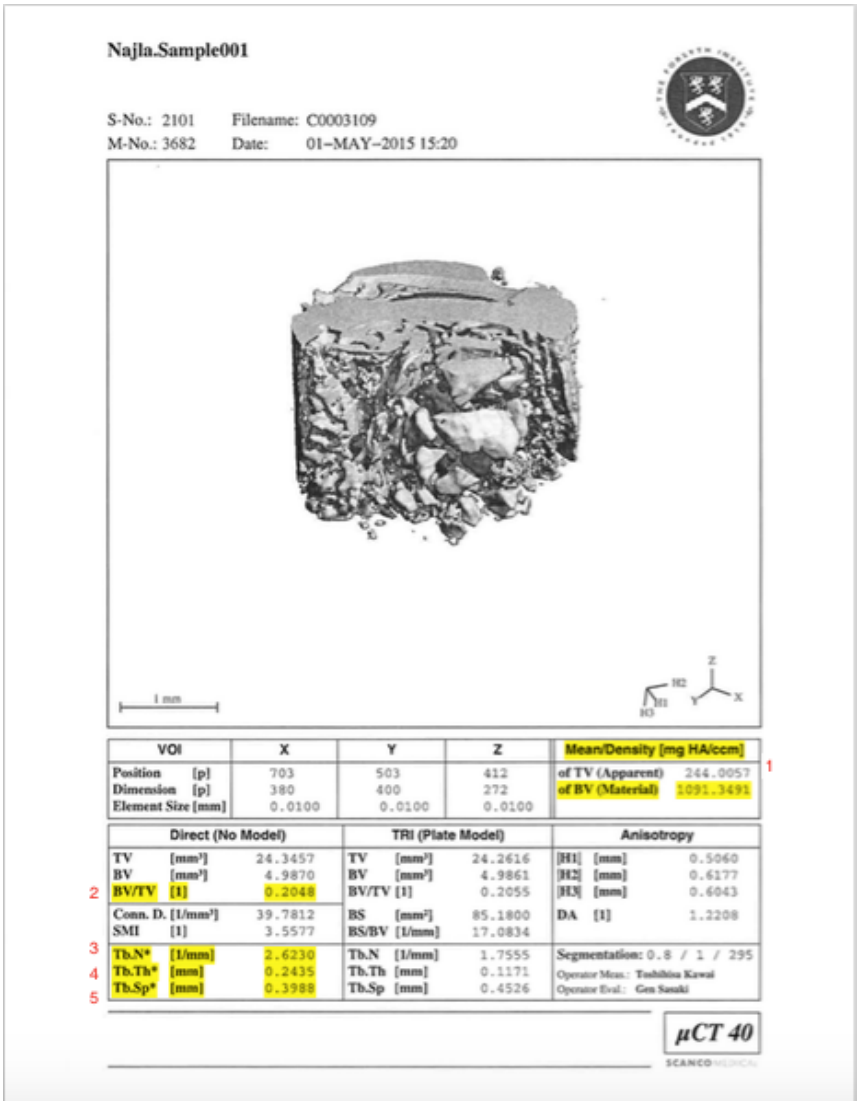
Figure 22

μ CT Index for the retrieved bone core samples

Sample	μCT reference
001	2101
003	2102
004	2103
005	2104
006	2105
010	2106
011	2107
012	2108
014	2109
017	2110

Figure 23

Detailed example of the Micro CT data analysis and outcome variables obtained for one core sample



Najla.Sample001

S-No.: 2101 Filename: C0003109
M-No.: 3682 Date: 01-MAY-2015 15:20

1 mm

VOI		X	Y	Z	Mean/Density [mg HA/ccm]		
Position	[p]	703	503	412	of TV (Apparent)	244.0057	1
Dimension	[p]	380	400	272	of BV (Material)	1091.3491	
Element Size	[mm]	0.0100	0.0100	0.0100			

	Direct (No Model)			TRI (Plate Model)			Anisotropy		
	TV	[mm³]	24.3457	TV	[mm³]	24.2616	[H1] [mm]	0.5060	
	BV	[mm³]	4.9870	BV	[mm³]	4.9861	[H2] [mm]	0.6177	
2	BV/TV	[1]	0.2048	BV/TV	[1]	0.2055	[H3] [mm]	0.6043	
	Conn. D.	[1/mm³]	39.7812	BS	[mm²]	85.1800	DA [1]	1.2208	
	SMI	[1]	3.5577	BS/BV	[1/mm]	17.0834			
3	Tb.N*	[1/mm]	2.6230	Tb.N	[1/mm]	1.7555	Segmentation: 0.8 / 1 / 295		
4	Tb.Th*	[mm]	0.2435	Tb.Th	[mm]	0.1171	Operator Meas.: Toshihisa Kawai		
5	Tb.Sp*	[mm]	0.3988	Tb.Sp	[mm]	0.4526	Operator Eval.: Gen Sasaki		

μCT 40
SCANCO MEDICAL

Figure 24

Group A Bio-Oss®: 3 D image obtained from Micro CT analysis

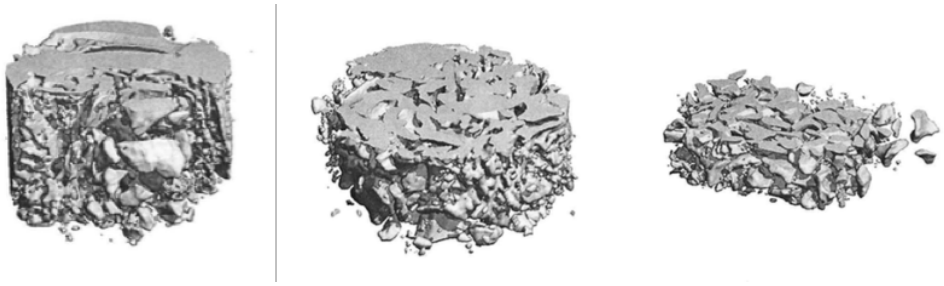


Figure 25

Group B Endobon®: 3 D image obtained from Micro CT analysis

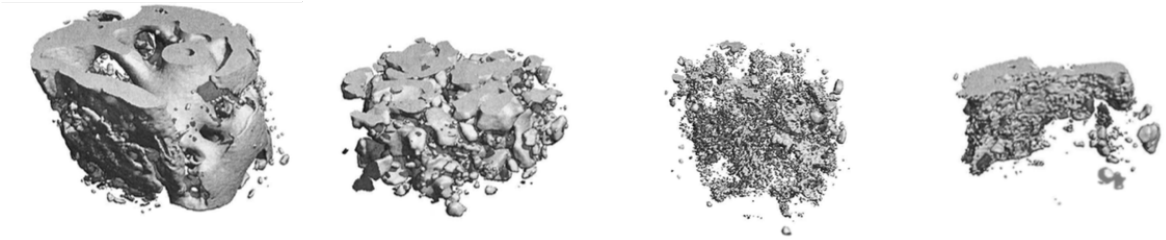


Figure 26

Group C Equimatrix®: 3 D image obtained from Micro CT analysis

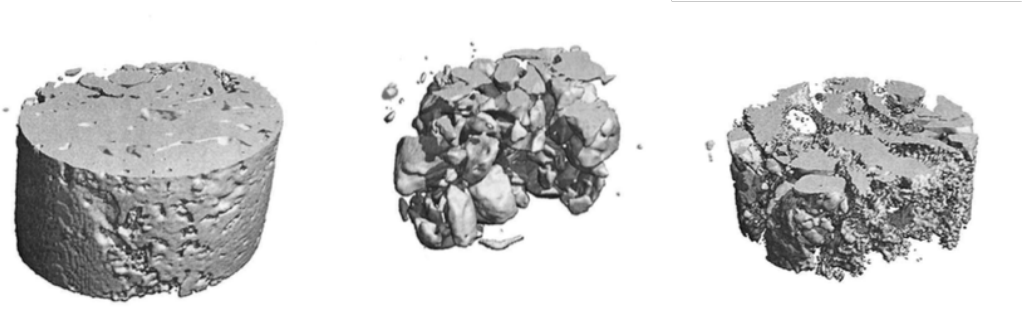


Figure 27

Micro CT Data Analysis of Bone Density

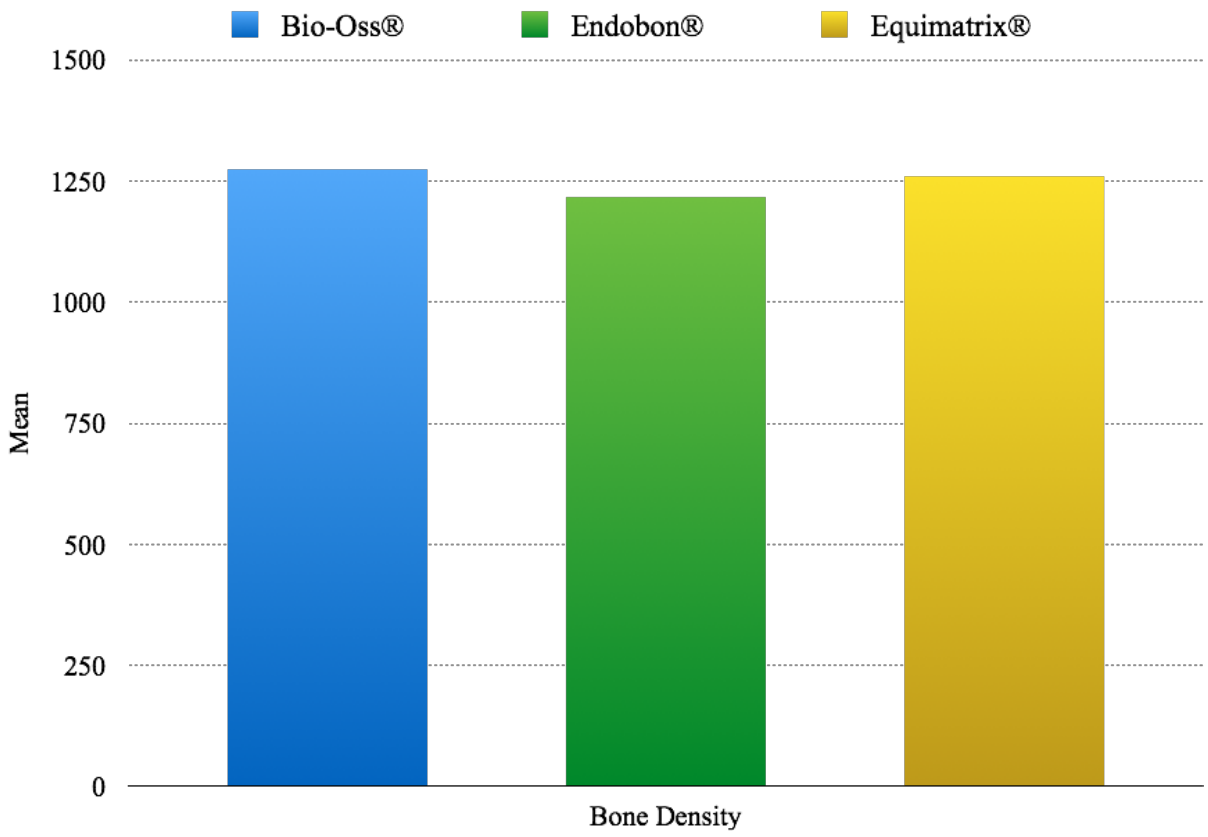
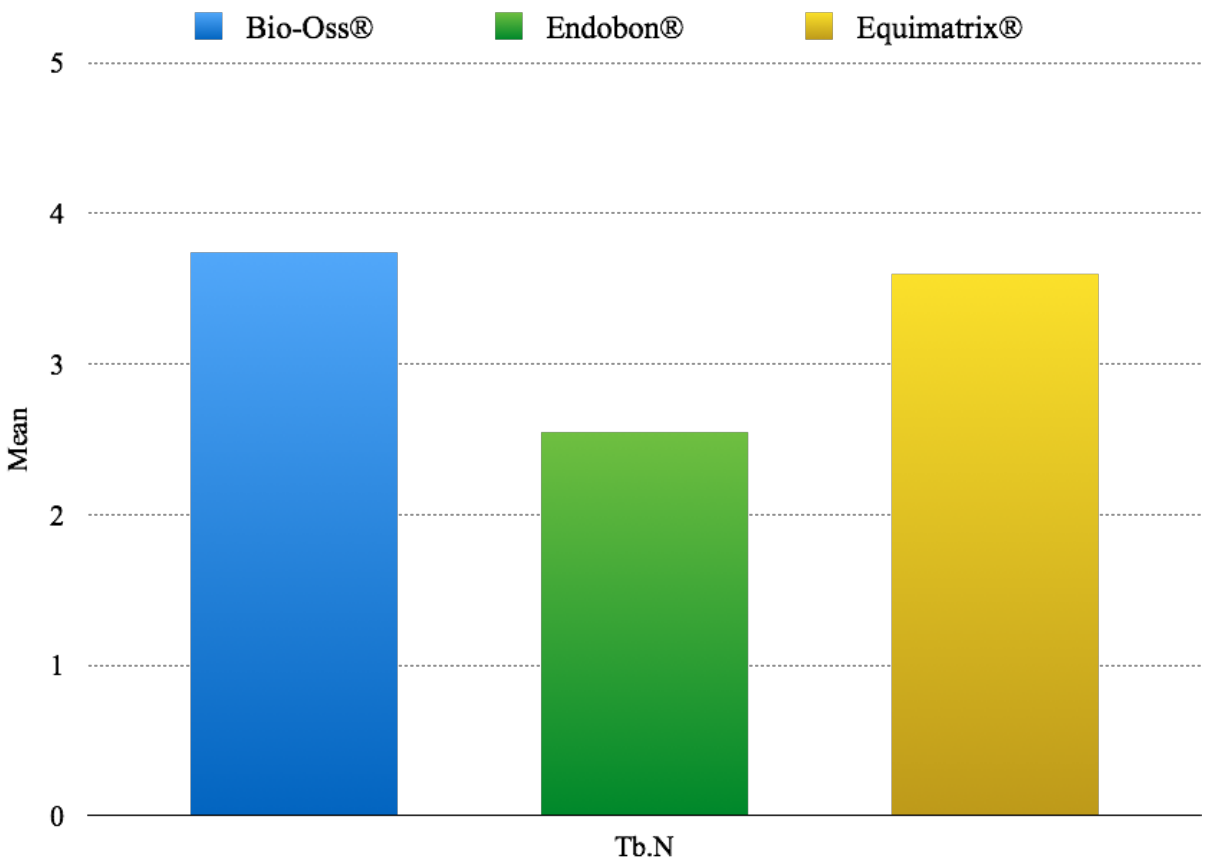


Figure 28

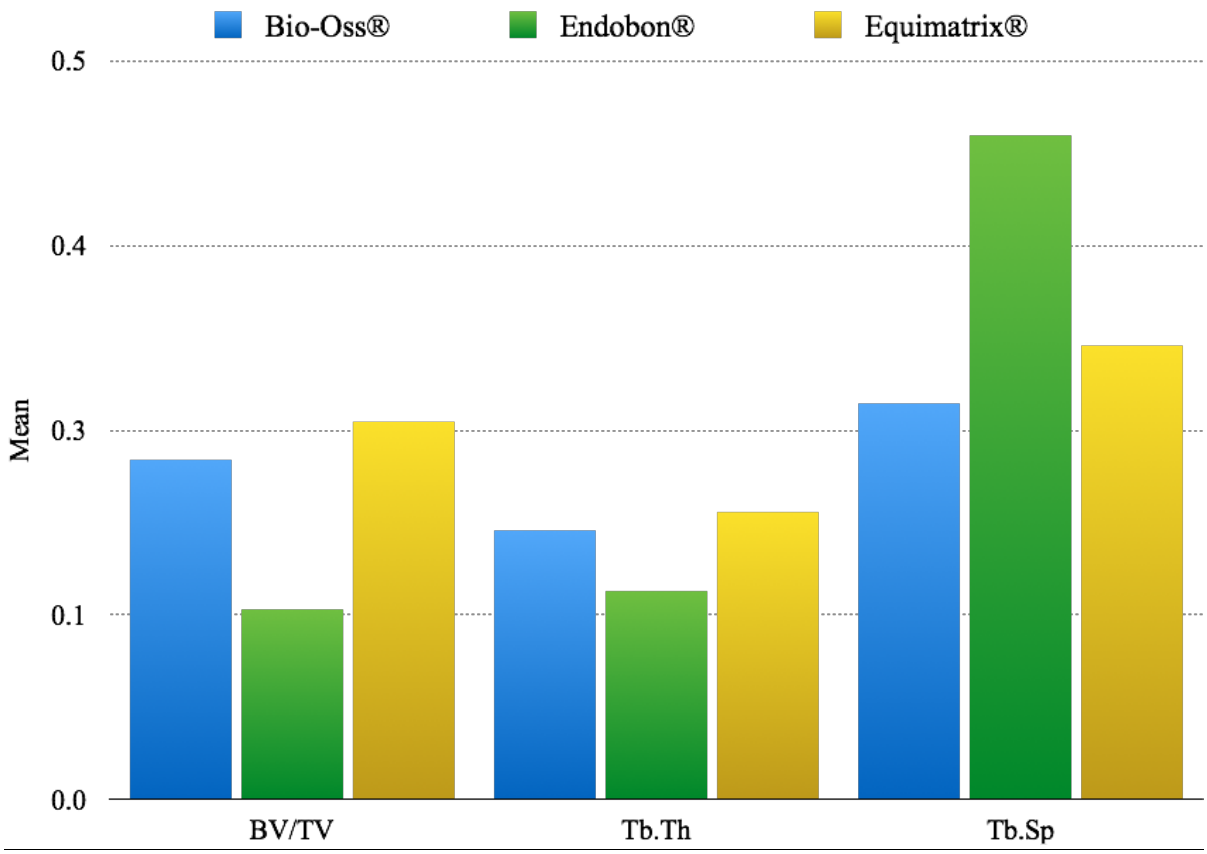
Micro CT Data Analysis of Trabecular Bone number



Tb.N: Trabecular bone number

Figure 29

Micro CT Data Analysis of Bone Volume, Trabecular Bone Thickness and Trabecular Bone Separation



BV/TV: Bone volume/ tissue volume

Tb.Th: Trabecular bone thickness

Tb.Sp: Trabecular bone separation