Analysis of Bone Volume Changes Following Lateral Guided Bone Regeneration

A Thesis

Presented to the Faculty of Tufts University School of Dental Medicine

in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Dental Research

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ABSTRACT

INTRODUCTION. Despite numerous reports in the literature on guided bone regeneration (GBR), there are no human randomized trials providing data on lateral ridge augmentation on pristine sites comparing absorbable and non-absorbable membranes. The current literature does not provide evidence-based criteria to guide a clinician select one technique for lateral GBR over the other. Due to lack of data, it was complex to design randomized clinical trial in order to get scientific outcomes. The aims of the present study were a quantitative and qualitative comparison of bone changes after lateral GBR procedures compared to baseline level between absorbable and non-absorbable membranes. The quantitative changes in bone volume were detected by a 3D digital software. The qualitative comparison derived from a histological analysis of bone core samples collected from the site of augmentation.

MATERIALS & METHODS. The study population was 20 subjects, 10 for the bio-absorbable membrane (Group 1) and 10 for the non-absorbable one (Group 2). The study received IRB approval. The inclusion criteria were systemically healthy subjects (>18 years old), non-smokers, presenting with at least one mandibular Kennedy Class III defects with 1 or 2 posterior adjacent missing teeth. A preoperative polyvinyl siloxane (PVS) impression of the edentulous mandible was poured into a stone cast that was subsequently scanned and digitalized. The crestal soft tissue thickness was measured by a periodontal probe and guided by a customized stent. After full thickness flap reflection, grafting material (FDBA) was placed in the defect and covered with one of the two membranes in randomized order. Three and six months postoperative stone casts were obtained and scanned. At the end of six months, the three models were compared using a

dedicated 3D software in terms of volumes gained after GBR. After six months, dental implants were placed in the areas of augmentation. During the procedure a core sampling was collected for analysis by histomorphometry to compare the two groups.

RESULTS. Analysis of volumetric changes between baseline (0 months) and time of implant placement (Visit 4 at 6-8 months) detected a mean changes of 434,78 mm^3 (SD ± of 136,67 mm^3) and 284,21 mm^3 (SD ± 96,31 mm^3) for Group 1 and 2, respectively. Bone changes in Group 1 between baseline and 3 months were 500.34 mm^3 (SD ± 163,398 mm^3) while they were -65,55 mm^3 (SD± 44,25 mm^3) between 3 and 6 months. In Group 2 (Cytoplast membranes) bone changes between baseline-3 months and 3- 6 months were 310,68 mm^3 (SD ± 145,42 mm^3) and -26,46 mm^3 (SD ±53,37 mm^3), respectively. The soft tissue had a mean thickness of 1,26 mm (SD ± 0,44 mm), which decreased, by 0,02 mm (SD ± 0,38 mm) at 6 months. Lateral bone gain had a mean of 2,82 mm (SD ± 1,02 mm). Histomorphometric analysis showed a mean of 44,39% of new bone, old bone and residual graft compared to the total area of the specimens. Group 1 specimens had a mean of 41,27% while for Group 2 was 48,59%.

CONCLUSIONS. From the present pilot study, it could be concluded that bio-absorbable and non-absorbable membranes provided sufficient bone for implant placement as planned prosthetically. No further grafting was needed after the initial augmentation. The results showed higher incidence of complications but lower graft resorption from 3 to 6 months for non-absorbable membrane Group. Specimens harvested from the non-absorbable group showed more mineralized tissue (bone and residual graft) compared to the bio-absorbable Group.

“It always seems impossible until it’s done” N.M.

DEDICATION

I dedicate this important achievement to my parents, who have always supported me during my education.

I would like to thank the committee members Dr. Hur, Dr. Hawley, Dr. Steffensen, Dr. Weber, Dr. Ogata and Dr. Finkelman for their precious suggestions and advices throughout this project.

ACKNOWLEDGMENTS

My first thank goes to Dr. Karacosta. Her constant dedication and support helped immensely the development of this research.

I truly appreciated the help of Mr. Kudara and Mr. Jones from the Prosthodontic department that assisted me in the volume calculation; Dr. Zoukhri and MS Kubin that helped me during the specimen preparation and shipping; Dr. Cavani that passed most of his Christmas holidays with me in the lab cutting, staining and analyzing histological slides; Miss Ferretti and Mrs. Sutton that let me use materials from Pros department; the UG students, Perio residents and faculty that referred me patients; Dr. Wang for taking over the research; the Perio Staff and Dispensary; Dr. Magnusson, Mr. Silberstein and Miss O'Connell for their help in preparing, submitting the protocol to IRB and get it approved;
In conclusion I thank all the patients enrolled in the study that cooperated with me during my research.

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LIST OF ABBREVIATIONS

3D = Three Dimensional

AIDS = Acquired Immune Deficiency Syndrome

ASA = American Society of Anesthesiologists

CAD-CAM = Computer Aided Design – Computer Aided Machinery

CBCT = Computer Beam Computer Tomography

ePTFE = Expanded Polytetrafluoroethylene

ESEM = Environmental Scanning Electron Microscopy

FDBA = Freeze Dried Bone Allograft

GBR = Guided Bone Regeneration

GTR = Guided Tissue Regeneration

HIV = Human Immunodeficiency Virus

ICF = Informed Consent Form

IRB = Institutional Review Board

MM = Milliliters

MM^2 = Squared Milliliters

MM^3 = Cubical Milliliters

PBS = Phosphate-buffered Solution

PLA = Polylactic Acid

PGA = Polyglycolic Acids

PTFE = Polytetrafluoroethylene

PVS = Polyvinyl Siloxane

SD = Standard Deviation

T1 = Thickness Time 1

T2 = Thickness Time 2

TID = Three in a day

TUSDM = Tufts University School of Dental Medicine

LIST OF SYMBOLS

® = Registered trademark

© = Copyright

TM = Unregistered Trademark

$\boldsymbol{\mu}$ = micron

Analysis Of Bone Volume Changes Following Lateral Guided Bone Regeneration

Introduction

1. Background

With the increase in the use of dental implants for restoration of partial or complete edentulism, more emphasis is being placed on restoration of the alveolar ridge to ensure the optimal implant placement and prosthetic treatment outcomes. Different teeth have different root diameters and, ideally, they should be replaced with an implant similar in size. However, after tooth extraction the shrinkage of horizontal dimension of jawbone is often a problem and results in deficiencies that complicate the ideal implant placement[1, 2].

The modern concept of implant therapy is based on the final position of the prosthesis. In the past, surgeons would place the implant in anatomically favorable area regardless of the relation of the future prosthesis. A prerequisite for the successful placement of implants in the ideal, prosthetic driven position is a minimum amount of bone volume (width and height) of the edentulous area that will provide a functional and esthetic implant restoration; this means that there is more emphasis on preservation of the alveolar ridge to ensure optimal implant placement and prosthetic treatment outcome. In order to make both prosthesis and implant successful, hard and soft tissues need to be present in adequate volumes, quality and in the correct position[3]. Lack of bone in the ideal position requires techniques that predictably regenerate bone for optimal prosthetic-driven implant therapy. These may include augmentation of the horizontal dimension of the ridge[4]. This procedure is called Guided Bone Regeneration (GBR).

Typically, augmentation of bone volume is accomplished through various methods including use of particulate and block bone grafting materials, split ridge technique, distraction osteogenesis, growth and differentiation factors, and GBR techniques. Horizontal ridge augmentation is employed to recreate the missing bone. Horizontal or lateral ridge augmentation is utilized by a variety of different techniques and materials for proper function, esthetics or prosthetic restoration of the edentulous sites with deficiencies[5-7] .

GBR is a surgical procedure that utilizes barrier membranes to direct the growth of new bone at sites with insufficient volume or dimension of bone by creating and maintaining a space during healing[8]. GBR was introduced as a therapeutic modality aiming to achieve bone regeneration, via the use of barrier membranes (Dahlin et al. 1988)[9]. Bassett in 1956[10] and Ashley (1959) [11] introduced the concept of obtaining a secluded anatomic environment with the goal to promote healing by using cellulose acetate filters for the regeneration of nerves and tendons. Murray in 1957[12] reported new bone formation beneath plastic cages adapted over decorticated defects in dogs. Further studies on animals reported enhanced osseous healing of bone defects via the use of cellulose acetate and Millipore filters (Hurley et al. 1959, Ruedi & Bassett 1967[13-15]).

The use of barrier membranes in the dental field was inspired by the periodontal regeneration techniques called guided tissue regeneration (GTR). GTR was developed in the early 80's by Nyman et al[16, 17, 17, 18]. The demonstration of the successful outcomes of GTR procedures led extensive research activity in the mid to late 80s. The research initially focused on expanded

polytetrafluoroethylene (ePTFE) which soon became the standard membrane for GTR and GBR during the development phase of both procedures.

The secluded space created by these membranes prevented the invasion of epithelial and fibroblast cells into the area where angiogenic and osteogenic cells were expected to proliferate in order to produce bone structure. These biological processes were described in a milestone paper by Schenk and Buser[19].

The utilization of PTFE membranes for GBR techniques in on patients was initiated in the late 80's[9, 20, 21].

The main use was to regenerate the peri-implant bone defects in implant sites with local bone deficiencies. The GBR technique has been used in two clinical scenarios: simultaneous or staged approach. GBR was mainly used for immediate post extractive implant placement to regenerate peri-implant bone defect[22, 23] or for implants with bone dehiscence defect[24]. The staged approach was based on a first stage of crestal bone augmentation followed by implant placement after 6 to 9 months of healing[25]. Soon, bone fillers such as autograft or allograft were recommended in order to prevent membrane collapse and subsequent failure of bone regeneration but also to enhance new bone formation through the osteogenic potential of autogenous bone grafts . The combination of membranes and bone graft provided improved clinical outcomes with both staged and simultaneous approaches[26-28].

In the mid-90's, several expert agreed that the GBR technique needed to be further improved due to multiple weaknesses: significant rates of membrane exposure (soft tissue dehiscence) leading to graft and membrane infection and a compromised regeneration outcome[29-32] difficult handling of the membrane requiring stabilization of the membrane[31, 33] and the need

of a second surgical access to remove the non-absorbable membrane. All these reasoning led to the development and subsequent use of bio-absorbable membranes.

Wang et al described the current standards to obtain a successful outcome with GBR procedures in 2006[33]. The surgical procedure to augment bone in the edentulous and resorbed area is accomplished by different steps. After exposing the edentulous ridge with the elevation of a surgical flap, bone-grafting material is placed on top of the denudated ridge. This material, as well as the adjacent bone, is covered by barrier membranes (either bio-absorbable or non-absorbable) in order to form a space for new bone growth while preventing the migration of other undesired cells[34] such as epithelial cells and gingival fibroblasts coming from the flap that is sutured on top of them. The secluded space favors the ingress of regenerative cells from the host bone (marrow) in order to replace the bone grafting with natural bone.

2. Barrier Membranes

Types of membranes

A wide range of membrane materials has been examined in animal and clinical studies to achieve successful GBR. Both with absorbable and non-absorbable membranes can be used for GBR. Critical criteria for guided tissue regeneration membrane include biocompatibility, cell occlusiveness, integration by the host tissues, clinical manageability, and the space making function[35]. Lateral GBR is a predictable procedure even if some factors may affect its success rate. These factors include the ideal size of membrane perforations, membrane

stability, duration of barrier function, enhanced access of bone and bone-marrow-derived cells to the area for regeneration, blood fill of the space, and prevention of soft tissue dehiscence.

- *Non-absorbable membranes*

Non-absorbable membranes were early considered the gold standard in bone augmentation procedures[36]. These barriers are available as ePTFE, titanium reinforced ePTFE, or high-density PTFE[37-40]. The membranes are plain, with high stability in biological systems. Non-absorbable membranes have the advantage of resisting to the breakdown processes by host tissues and by microbes and the ability of maintaining adequate defect space. The stability of the non-absorbable materials as well as their ability to maintain a space for undisturbed bone matrix deposition, usually lead to more favorable clinical outcomes compared to bio-absorbable matrices[41-44]. These results are different in case of membrane exposure, which represents the most frequent post-operative complication. In this case, the underlying graft may get infected and defeat its own purpose. Another important disadvantage of non-absorbable membranes is the need of a surgical re-entry to remove them from the regenerated site. This procedure adds patient discomfort and soft tissue involvement. Several studies reckon that cases with no ePTFE membrane exposures, resulted in more favorable amount of bone formation[41-44].

- *Bio-absorbable membranes*

Bio-absorbable materials consist of natural or synthetic polymers. Natural membranes originate from various types of animal collagens whereas synthetic membranes are made of aliphatic polyesters. Among the last type we can find polylactic (PLA) and polyglycolic acids (PGA) mainly. The difference between these two types of membrane is their way of resorption; collagen products undergo enzymatic degradation[45-47], while synthetic membranes biodegrade to carbone dioxide and water via the Krebs cycle [48-50].

When inserted in an aqueous environment, such as a biological system, the biodegradable polymers undergo four stages of degradation, namely hydration, strength loss, loss of mass integrity and solubilisation via phagocytosis.

The time length of each stage and the overall degradation rate depend on the nature of the polymer, the pH, the temperature, the polymer crystallisation degree and the membrane volume [4, 51, 52].

Studies of bio-absorbable membranes aim to avoid the drawbacks of a non-absorbable membrane regarding subject morbidity, risk of tissue damage, and technique sensitivity. The first two outcomes are linked to the phase of membrane removal. The most important advantage of bio-absorbable membranes is that they do not need this step thanks to their natural breakdown. Patient and tissue morbidity can be drastically reduced.

Studies describe bio-absorbable membranes as being able to offer improved soft tissue healing, reduced risk of bacterial contamination and infection compared to non-absorbable membranes[53-55]. Nevertheless some authors have reported opposite findings regarding this matter[56]. Like the non-absorbable membranes, bio-absorbable membranes can experience

premature soft tissue dehiscence and exposure. The advantage of the bio-absorbable membrane is that the oral cavity accelerates its resorption rate, and that contamination of the underlying bone grafting drops. One of most important roles of a membrane is their space maintenance ability. On the other hand, the major disadvantages of bio-absorbable membranes are the insufficient space-making capacity, predictable duration of barrier function, and degradation process that varies according to biological structure and physical properties.

In conclusion, if a soft tissue dehiscence does not occur, non-absorbable membranes seem to support more bone regeneration than bio-absorbable membrane. The volume of regenerated bone generally is more substantial with non-absorbable membranes than with bio-absorbable membranes [42]. However, bio-absorbable membranes are easier to handle and associated with reduced morbidity. The choice of membrane depends largely on the requirements of the case, personal experience of the operator and scientific evidence at the present time[57, 58].
Literature does not provide clear data on when to prefer a bio-absorbable membrane over a non-absorbable and vice versa. This research aims to provide new data that will improve the decision-making process in lateral guided bone regeneration procedures.

It may be therefore concluded that the barrier function duration is not strictly controlled and that the resorption process may possibly interfere with the wound healing and bone regeneration process. Therefore, although the launch of bio-absorbable membranes eliminated the need for membrane removal surgery, thus simplifying the surgical protocols

and improving the cost-effectiveness, it has been suggested that the ePTFE membranes should serve as the gold standard for comparing the results obtained via the use of new materials[4, 51, 52]

3. CAD-CAM and Histomorphometry

Conventionally, the measurements of regenerated bone are usually extrapolated from a comparison of pre and post-operative CBCT images or stone casts. Based on the recent advancements in digital imaging, this study will utilize computer-aided design/computer-aided manufacturing (CAD-CAM) digital software to obtain volume measurements, and thereby to compare pre-operative bone defects with post-operative defect resolution. CAD-CAM technology is used to aid in the design, analysis, and manufacture of products. It is used in various fields like engineering and dentistry. This technique helps in obtaining precise measurements (micrometer), acquiring 3D digital figures and replicating identical copies of objects. In the dental field it is used to provide a range of dental restorations including: crowns, veneers, inlays and onlays, fixed partial bridges, dental implants abutments as well as orthodontic appliances. A recent paper utilized digital techniques to compare tissue changes after alveolar ridge preservation [59].

In this study, a pre-operative digital image of the stone model representing the defect was compared with the post-operative stone model representing the healing site after the bone augmentation procedure. The two merged images allowed for direct volume measurements with a dedicated software (Exocad, Lexington, MA, USA® and Minimagic – Materialise NV

© and Meshmixer Autodesk © 2011, Inc). We propose the use of CAD-CAM technology to measure volume changes as it eliminates the bias resulting from human measurements of volume changes.

CAD-CAM has not been utilized yet for this kind of measurement and we believe this may be used in the future as a standardized measuring technique. We have proposed a mathematical formula to calculate the expected bone gain. If we consider the mandible in a frontal section, the amount of bone gained is described as an area recognizable as a triangular shape with the base parallel to the alveolar crest and one side parallel to axis of the mandible. An expert opinion (HPW) proposed that 4 mm of horizontal bone ad 3.5 mm vertically (from the base of the defect to the crest) could be achieved with guided bone regeneration (7 mm^2). These measurements do not have strong scientific basis, therefore it has been decided to develop a study.

A considerable portion of the periodontal literature involves histological studies of sites that have been treated with materials, implants and biological substitutes. Most of the cases have involved animal-derived specimens while a minority has involved human samples. Histological (quantitative) analyses are central to the histological evaluations. This analysis can be broadly defined as the measurement of the shape or form of a tissue. Bone histomorphometry is a quantitative microscopic analysis of bone organization, structure and architecture. Unlike the dynamic version, static histomorphometry provides information on the amount of bone, bone phenotypes, cell count, quantity of cancellous bone and osteoid. It represents one of the analyses to define the success of regeneration procedures. Studies by

Lindhe at al. and Simion et al.[60, 61] have involved harvesting and processing of a core sample in order to be analyzed and compare the amount of newly formed bone over the remaining bone grafting particles. Bone cores for the histological analysis are usually collected by means of a trephine bur in the areas that will receive an implant. This rotary device sections a cylindrical portion of hard tissue that is subsequently analyzed under microscopy, after decalcification processes. The osteotomy sites are usually included in the preparation for implants, so that no unnecessary bone removal is performed. In this study, histomorphometry will be used to analyze differences in morphologic and histologic outcomes of the two procedures.

Significance of Research

Since the literature is scarce regarding expected volume change in GBR procedures[55] and there were no data providing specific volumes gained after the use of either bio-absorbable and non-absorbable membranes we propose a pilot study on the use of bio-absorbable (RCM6, ACE Surgical Supply; Brockton, MA, USA) and non-absorbable membranes (Cytoplast, Osteogenic Biomedicals; Lubbok, TX, USA) for lateral ridge augmentation. Digital impression technology was used to provide the quantitative analysis of volume changes. Histomorphometry was used to analyse differences in morphologic and histologic outcomes of the two procedures. This research provided new data to improve the decision-making process on the selection of either bio-absorbable or non-absorbable membranes in lateral guided bone regeneration procedures.

Aim and Hypothesis

1. The primary aim of this study was a quantitative comparison of bone volume changes using CAD-CAM technology after lateral guided tissue regeneration procedures compared to baseline level regardless of the type of membrane.
2. The secondary aim of this study was a comparison bone volume gain between the bio-absorbable and non-absorbable membranes group and to conduct histological analysis of core samples derived from the site of prosthetic-driven implant position.

This study has been made a pilot study as the measurement technique (using CAD-CAM to measure bone volume) is unique and novel, and literature does not exist to support a sample size calculation for a full randomized controlled trial. With the results from this exploratory study we hope to inform a future randomized clinical trial.

Research Design

The proposed study included patients requiring lateral ridge augmentation procedures based on the standard clinical treatment planning.

The primary aim of this Pilot Study is a quantitative comparison of bone volume changes after lateral guided tissue regeneration procedures compared to baseline level. Twenty subjects were divided in two groups: one treated with bio-absorbable (RCM6 ACE Surgical Supply; Brockton, MA, USA) and the other with non-absorbable (Cytoplast™ Osteogenic Biomedicals; Lubbok, TX, USA) membranes.

The inclusion criteria for this study required subjects to be systemically healthy with no conditions that could contraindicate periodontal surgery or could affect hard or soft tissue healing (e.g., uncontrolled hypertension and diabetes, severe Parkinson's disease, acute leukemias, agranulocytosis and lymphogranulomatosis), ASA (American Society of Anesthesiologists) Status I (healthy patient) or II (patient with mild systemic disease), be at least 18 years of age, be a non-smoker or former smoker (a former smoker is defined as someone who quit smoking at least 1 year before surgery), and presenting with at least one defective pristine site characterized by presence of horizontal ridge deformity on a partially edentulous mandible. The site had to present 1 or 2 posterior adjacent missing teeth with one tooth posterior to edentulous area (so defined Kennedy Class III), residual horizontal ridge width ≤ 6 mm (measured clinically with a caliper) and opposing occlusal surface (e.g., natural tooth, crown, or denture) with a minimum of 5 mm between occlusal surface and top of edentulous ridge;

The study excluded pregnant females, patients with medical contraindication to dental surgery (uncontrolled and/or poorly controlled diabetes (HbA1c>7),medical conditions affecting bone metabolism (e.g., severe osteoporosis, treatment with immunosuppressant drugs, chemotherapy, chronic corticosteroids,previous or current head and neck radiation therapy, long term steroid use denied as more than two weeks in the past two years, HIV/AIDS, and/or Hepatitis),a history of IV bisphosphonate therapy or >3 years of oral intake, a history of myocardial infarction, cerebrovascular accident, and/or a history of radiation therapy within the past 6 months. Subjects who did not comply with the study procedures, (e.g., did not return for follow up appointments, were unwilling to further participate in the study, had changes in medical/dental history as consistent with the previous

exclusion criteria) would have withdrawn from the study. If bone volume gained is not ideal for placement of an implant without having any surface exposed, the subject will be withdrawn and followed up with standard of care practices at the TUSDM periodontology clinic (e.g., redo GBR). Patient would have impressions and measurements made and then withdrawn from the study. The Principal Investigator determined whether subjects (either withdrawn subjects or subjects completing the study) were in need of additional treatment and/or follow-up observation as a result of participation in this trial.

The first aim of this research was characterized by a digital analysis of stone casts to calculate the change in volume before and after GBR techniques. A baseline (preoperative) PVS impression of the edentulous area was poured into a stone cast, scanned and digitalized with a 3D lab scanner (Activity 880 Smart optics ©, Germany).

The distance between a reference on a customized stent and the soft tissue surface on the edentulous area was measured. Every subject was randomly allocated to one of the two membrane groups, by a computer-generated randomization scheme. After the elevation of a full thickness flap, the GBR procedure was performed. Three and six months post-operative stone casts were obtained from PVS impressions and they were scanned and digitalized with the 3D lab scanner used for the baseline cast. At the end of 6th month there was a comparison between the volumes gained after lateral bone augmentation. The values were calculated from a comparison of the 0, 3 and 6 months digital files. The dedicated software (Exocad® and Minimagic – Materialise NV © and Meshmixer Autodesk © 2011, Inc) provided data on how much volume changed at 3 and 6 months. After the 6th month, core samples were harvested and in the same location dental implant were placed according to the

prosthetic plan. The secondary aim of the study was a qualitative analysis by a histomorphometric analysis of the core samples. The difference in bone versus graft was investigated after histologic preparation and microscopic analysis.

Materials and Methods

Sample Size

We proposed a sample size of 10 subjects per treatment group (for a total of 20 subjects). This would give an adequate representation of the patient population indicated for this procedure, 35 subjects were screened in order to find the qualifying 20 subjects.

Randomization

A randomization scheme was used to allocate subjects in the two treatment groups. The treated defects will be stratified in similar sizes after collection of all data (e.g., number of teeth missing in the study area – one or two). These groups will be then randomized in a 1:1 ratio using a balanced design based on a computer-generated randomization scheme using the website www.random.org. If the subject had two qualifying sites, the site used was selected randomly. The subject was assigned to one of the two groups at the time of Visit 2, before GBR procedure was started.

Blinding

Due to the nature of this study, neither the subject not the treating clinician was blinded. The laboratory investigator was blinded as to which group the specimens are from. The

investigators using the CAD-CAM software which measures volume on a computer program was blinded.

Products

All products were FDA approved as used in this study. They were used according to manufacturer's guidelines.

- Group 1:

  RCM6 (ACE Surgical Supply; Brockton, MA, USA) is a bio-absorbable, implantable collagen material that is intended for the use in periodontal and/or dental surgery procedures as a material for placement in the area of periodontal defects, dental implants, bone defects or ridge reconstruction to aid in wound healing post-surgery. This membrane is made of purified type I collagen fibers derived from bovine Achilles tendon. Its resorption eliminates the need to a second stage surgery to remove it from the site of implantation. Its structure delays invasion of epithelial cells into the wound site but at the same time it permits the exchange of essential nutrients for wound healing.

- Group 2:

  Cytoplast™ (Osteogenic Biomedicals; Lubbok, TX, USA) is a high-density Polytetrafluoroethylene non-absorbable membrane. This material is characterized by having porosity of less than 0.3$\boldsymbol{\mu}$ that creates impervious barrier to bacteria.

Being non-absorbable, this material cannot be processed and eliminated by the body in which is implanted. Therefore a second surgical procedure is often required to eliminate this membrane. In few cases, its exposure allows the clinician to remove it without elevating a flap.

- Groups 1 & 2:

  Mineross® is an allograft material prepared from tissue recovered from a cadaveric donor using aseptic surgical techniques and microbiologically tested during recovery. This material can be used in a variety of dental applications when appropriate. It can be used in bone grafting procedure in combination with autologous bone or other forms of allograft bone, or used by itself as an individual bone allograft. Mineross® is used routinely at Tufts University School of Dental Medicine (TUSDM) department of Periodontology in guided bone regeneration procedures. This material acts like a scaffold (space maintainer) in order for bone cells to invade the area and start bone deposition in the area to be augmented.

Study Procedures

The procedures used in this study were the current standard of care for Lateral Guided Bone Regeneration technique. The PVS impressions, stone models, the CAD-CAM measurements were utilized for research purposes; these procedures are not routinely followed for GBR procedures. The collection of cores was performed during the implant placement procedure. Osteotomies, the term referring to the hollow where the

implant will be placed, are usually performed by drilling out the bone and not by removing a solid core. It has to be noted that the final osteotomy can be made by using either of the two procedures without interfering with the implant success rate and the site health[62].

The study outline and visits are listed in *Table 1*.

The details on each visit were as following:

Visit 1 - Screening visit

The subject was given an informed consent form (ICF) to read, regarding the study proposed procedures. Subjects were given ample time to read the ICF and have any questions answered. If a subject decided to participate, he or she was instructed to sign the ICF. A copy of the ICF was given to the subject.

The subject was asked to complete demographic information and a medical history.

An oral exam, including evaluation of oral cavity, soft and hard tissues, was completed following standard of care procedures in US dentistry using a mouth mirror and dental explorer.

Inclusion/exclusion criteria were evaluated.

Residual horizontal ridge width (lingual to buccal) was measured with a calliper. The distance from the occlusal surface to the top of edentulous ridge was measured with a periodontal probe.

Two dental impressions of the mandibular arch were made of polyvinyl siloxane (PVS) following manufacturer's instructions. The double impression helped to determine the amount of difference between two independent measurements to provide stronger level of accuracy on the same subject and measured by CAD-CAM. A third dental impression of the mandibular arch was made using alginate following the manufacturer's instructions.

After Visit 1

After the visit, the silicone impression was poured up to obtain two stone casts (type IV stone) (these were considered the baseline casts), representative of the ridge topography prior to lateral augmenting procedures.

Additionally, the alginate impression was poured up to obtain a stone cast. This cast was used to fabricate a customized stent made of acrylic resin. This stent will have holes on the occlusal, lateral, and 45° angle which will allow a periodontal probe to fit through and standardize the placement of the measurements. The stent was used to measure the soft tissue and bone.

After the visit, two scans (by two blinded investigators) of each model using the optical 3D-scanner (Activity 880Smart optics ©, Germany) were done to obtain a virtual representation of the casts and, therefore, of the edentulous area prior bone augmentation procedures. The digital images were processed with the dedicated software (Exocad® and Minimagic – Materialise NV ©) to calculate volume of the area prior bone augmentation. This double scans helped in determining the amount of difference between two independent measurements to provide stronger level of accuracy on the same subject. Having two blinded investigators make the CAD-CAM allowed for the reporting of inter-observer variability.

Models were coded and stored together, and then all impressions were scanned at one time.

Visit 2 - Baseline/Surgery

Medical history was reviewed and any changes were noted.

Eligibility and subject withdrawal criteria were reviewed to ensure the subject still qualifies for the study.

An oral exam, including evaluation of oral cavity, soft and hard tissues, was completed following standard of care procedures in US dentistry using a mouth mirror and dental explorer *(Figure 1 a)*.

Subjects were randomized to one of two groups, either lateral ridge augmentation with RCM6 bio-absorbable membrane or lateral ridge augmentation with Cytoplast non-absorbable membrane group.

Surgery proceeded as follows, following standard of care procedures at TUSDM periodontology clinic. The only different step during surgery that is specific to the study was the use of the customized stent for measurements prior to and during surgery. Surgeries for both groups followed the same guidelines except for the type of membrane placed.

Local anesthesia was achieved following standard guidelines at TUSDM periodontology clinic.

Immediately before the surgery, the crestal soft tissue thickness was measured via a periodontal probe guided by a customized stent (made after Visit 1), under local anesthesia (bone sounding).

A crestal incision was made, followed by a full thickness flap reflection *(Figure 1 b)*. Bone measurements were taken using a customized stent (same as above) (specific to study). Then decortication of the alveolar ridge was done by means of a 0.14 mm surgical drill mounted on a hand-piece. Grafting materials (FDBA) was placed following manufacturer's instructions. Membrane was placed (RCM6) following manufacturer's instructions and primary closure was accomplished using conventional suturing procedures. The bone graft and the

membrane were completely covered by the flaps and will not communicate with the oral cavity [63].

If necessary, periosteal incisions were made in order to be able to provide complete primary closure.

Non-absorbable membranes were removed at implant placement unless exposure happens sooner. The bio-absorbable membrane underwent processes of resorption without the need of removal.

After surgery, the patients were provided with verbal and written post-operative instructions and prescriptions for post-operative care (Ibuprofen 800 mg TID for 3 days, then as needed, Amoxicillin 500 mg TID for 7 days, ice pack, chlorhexidine 0.12% mouth wash). Subjects were followed and seen for regular surgical follow up visits (including 1 week and 2 week postoperative visits).

Visit 3 – At 4 months (± 1 month) after Visit 2

Medical history was reviewed and any changes noted. Eligibility and subject withdrawal criteria were reviewed to ensure the subject still qualified for the study. Two dental impressions of the mandibular arch were made of polyvinyl siloxane (PVS) following manufacturer's instructions as at Visit 1.

After Visit 3

After the visit, the silicone impressions were poured up to obtain two stone casts (type IV stone) (this was considered the intermediate casts), representative of the ridge topography approximately 3 months after surgery.

After the visit, two scans (by two blinded investigators) of each model were done as at after Visit 1 and digital images were processed with the dedicated software to calculate volume changes compared to models prior bone augmentation. This double scansion helped in determining the amount of difference between two independent measurements to provide stronger level of accuracy on the same subject. Models were coded, stored together and scanned at one time.

Visit 4- At 6 months (± 1 month) after Visit 2

A medical history, control of eligibility criteria and an oral exam, including evaluation of oral cavity, soft and hard tissues, were completed following standard of care procedures in US dentistry using a mouth mirror and dental explorer.

Two dental impressions of the mandibular arch were made of polyvinyl siloxane (PVS) following manufacturer's instructions as at Visit 1.

After Visit 4

After the visit, the silicone impressions were poured up to obtain stone casts (final cast, type IV stone) representative of the ridge topography approximately 6 months

after surgery. Two scans (by two blinded investigators) of each model were done as at after Visit 3.

After the visit, two scans (by two blinded investigators) of each model were done as at after Visit 1 and digital images were processed with the dedicated software to calculate volume changes compared to models prior bone augmentation. This double scansion helped in determining the amount of difference between two independent measurements to provide stronger level of accuracy on the same subject. Models were coded, stored together and scanned at one time.

Visit 5 – 6-8 months after Visit 2 (can be same day as Visit 4 if both visits are within the appropriate windows of time)

Implant placement occurred in this visit. The technique for the bone sample collection took place according to the following procedures. Removal of bone is standard during implant placement in order to create space for the fixture. For bone sampling purposes, a hollow, rather than solid, bur was used. Cores were taken at the same location as the ‘‘implant-to-be-placed’’ based on the prosthetic recommendation. The device collecting the bone was smaller in diameter compared to the final implant. The amount of bone removed was not greater than the implant diameter and the technique used did not affect the implant placement [64].

Implant and bone core specimen collection:

After local anesthesia was achieved, the crestal soft tissue thickness was measured via a periodontal probe guided by a customized stent, (bone sounding) (as at Visit 2). Muco-periosteal incision was made and a full thickness flap was reflected *(Figure 1 c)*. Non-absorbable membranes were removed in this stage, unless an unexpected exposure occurred before 6 months. Bone measurements were taken using the same customized stent (as at Visit 2) as a distance between a point of reference and the bone surface.

A trephine bur was used in a hand-piece to obtain bone core specimens for histomorphometric analysis. This device had a cutting length of 15 mm and a diameter of approximately 4 mm. It presented a circular cutting end that faced the area of harvesting. The hollow center in its entire length (8 mm) allowed sectioning a cylindrical specimen of bone of mm in diameter. Once the specimens were harvested and labelled with the subject ID number, they were fixed in sodium phosphate-buffered (PBS) 4 % paraformaldehyde pH 7.4 [65] and stored at 4°C for 24 hours or 12 hours at room temperature. They were then dehydrated in graded ethanol 70%. Two blinded collaborators (QT and DZ) supervised the preparation and handling of the specimens.

In the same site of bone core specimen collection, an implant bone osteotomy was developed and the fixture was inserted according to the previously determined treatment plan and following the conventional protocol *(Figure 1 d)*. Healing abutment or cover screws were applied to the implant and the flap was

sutured in order to provide primary closure of the wound *(Figure 1 e)*. Radiographic images were taken in order to confirm the correct connection between implant and healing abutment *(Figure 1 f)*.

After Visit 5

The digital files representing scansions of stone casts at baseline, 3 and 6 months were processed and volumes were calculated. The bone core samples were shipped following all applicable regulations to the Laboratory at Modena University (Italy) for the histomorphometric analysis; FC was responsible for this process.
These two procedures were completed as follow:

Bone Volume Calculation

All the stone casts deriving from the previous visits were scanned with a 3D desk scanner (Activity 880Smart optics ©, Germany). The result of these scansions was a series of STL files, a digital representation of the stone casts. Every subject had 6 related scansions (two per Visits 1, 3, and 4). These STL files were aligned and superimposed with Exocad® (Minimagic – Materialise NV ©) and subsequently trimmed with Meshmixer (Autodesk © 2011, Inc) after being turned into watertight images *(Figure 2)*. A dedicated tool calculated the Volume of every digital representing baseline, 3 and 6 months *(Figure 3)*.

Histologic Preparation

Core biopsies were embedded in blocks of methacrylate and were serially sectioned parallel to their longitudinal axis up to the center of the bone core with a diamond saw microtome (1600, Leica, Germany) then the remaining block was placed in a Reichert Jung Autocut 1150 microtome (Nussloch, Germany) to obtain a series of 5μ thick sections. A minimum of nine sections were sliced for each specimen. All the sections were examined at 2,5X and 4X magnifications to determine which represented the best sample for analysis. A single core section per individual was selected for histologic evaluation in its entirety from its most apical end to its most coronal end. The innermost aspect of the original core biopsy was selected as priority. In case of alteration of the innermost section, the nearest section was examined.

The sections were stained with *Solochrome Cyanaine* staining solution which differentiates between old and new bone. The sections were photographed under transmitted and polarized light using a Nikon Eclipse 90i microscope (Tokyo, Japan) equipped with a DS-Fi1 Nikon digital camera and driven by the Nikon ACT-2U software; Histomorphometric analysis was completed by one examiner (FC) who was blinded to the treatment groups during preparation and examination of all cores. Each section was examined at a 2,5X, 10X and 20X magnifications with a light microscope in order to identify the three main component layers (vital bone, residual graft, and connective tissue). Images were taken of each section in order to facilitate this process *(Figure 4 a and b)*.

For further analysis, sections were analysed under Environmental Scanning Electron Microscopy (ESEM QUANTA 200 Fei Company, Oxford Instruments) in order to highlight mineralized tissue in respect to non-mineralized one. Therefore, the remaining half blocks were gently polished on the sectioned surfaces using alumina. After polishing, the surfaces were analysed by ESEM under low vacuum using backscattered electron detection only in order to highlight the different mineral densities of new and old bone *(Figure 5)*.

Histomorphometric evaluations were performed using the ImageJ software (NHS, Bethesda, USA). These images obtained from the ESME analysis appeared as grey-scale images. These scansions were converted by the software into binary images (black and white), which allowed for analysis of the total area of each layer based on the number of pixels in each image. With dedicated tools, the core area was outlined and considered as the total field of measurements. By changing the contrast of the image, hard tissue was highlighted in red and its area was measured *(Figure 6)*. The results was a percentage of hard tissue compared to the total core area.

Statistical Analysis

Data deriving from six patients were expressed as counts and percentages for categorical data and mean and standard deviations were reported for continuous data. Once the study will be completed, all data deriving from the 23 subjects will be analysed with a t-test that will compare the amount of bone volume gain in pre- and post-operative bone ridges as well as the new bone deposition in the bone specimens within groups and between groups. Interclass correlation coefficient and corresponding standard error of measurement were calculated to quantify inter-observer variability. Visual presentations will include a Bland-Altman plot.

Results

Thirty subjects were screened for eligibility criteria. Twenty-three were enrolled in the study and randomly distributed in the two membrane groups as soon as they reached the day of guided bone regeneration on Visit 2 (*Table 2*).

The subjects not enrolled in the research did not meet the criteria due to previous grafting in the edentulous area, thickness of the ridge > 6 mm, not acceptance of human derived bone graft and financial limitations.

The demographic analysis resulted in a mean age of 55,6 with a male to female ratio of 6:17. The ethnical groups corresponded to a total of 11 Caucasians, 3 Blacks, 3 Asians and 6 Hispanics (*Table 3*).

Among the 23 subjects enrolled in the study, 12 underwent successfully lateral bone augmentation performed during Visit 2. Seven of them reached Visit 3 (Intermediate Cast) but only 6 completed Visit 4 where they received dental implants in the augmented edentulous areas *(Table 4*). A total of 10 implants were successfully placed in the area of 10 missing teeth (two premolars and eight molars) *(Table 5*).

Soft tissue thickness and bone lateral augmentation were first measured with the acrylic customized stent. The mean soft tissue thickness at baseline was 1,26 mm with SD 0,44 mm (Table 6). The change in soft tissue thickness calculated at the time of implant placement (Visit 4) was -0,02 mm with SD 0,38 mm *(Table 7).* With the same stent, linear bone changes were calculated as well. Considering the 45° and 90° measurements, the

mean value was 2.82 mm with a SD of 1.02 mm (*Table 8*). *Table 9* shows the linear values of every study subjects.

Among the subjects treated with GBR, two experienced post-operative complications that occurred between Visit 3 and 4 (LD005 and CG009) (*Figure 7*). In both cases the membranes used (non-absorbable) were removed between Visit 3 and 4 due to infection. Despite these problems, patients were not withdrawn from the study and one of them successfully received two fixtures. Up to this date, the second subject is still completing healing process between Visit 3 and 4.

Bone Volume Calculations and Data

The bone volume changes for every subject, before and after augmentation, are reported in *Table 10.* Analysis of volumetric changes between baseline (0 months) and time of implant placement (Visit 4 at 6-8 months) resulted in a mean of 434,78 mm^3 (SD ± of 136,67 mm^3) and 284,21 mm^3 (SD ± 96,31 mm^3) for Group 1 and 2 respectively. Bone changes for Group 1 between baseline and 3 months were 500,34 mm^3 (SD ± 163,3 mm^3) while between 3 and 6 months were -65,55 mm^3 (SD± 44,25 mm^3). As far as Group 2 (Cytoplast membranes) bone changes between baseline-3 months and 3-6 months were 310.68 mm^3 (SD ± 145,42 mm^3) and -26,46 mm^3 (SD ± 53,37 mm^3) respectively *(Table 11).* As stated before, no additional grafting was needed at time of implant placement. Every subject received the planned implant/s in the correct prosthetic position.

Histological analysis

Light microscopic evaluation at 2,5X, 10X and 20 X magnifications was used to identify vital bone, residual graft, and connective tissue (CT)/non-mineralized tissues.

Residual graft particles were defined by regions of lamellar bone with the absence of osteocytes in lacunae while vital bone was identified by the presence of osteocytes in lacunae. It was common to observe vital bone in direct contact with residual graft material in both groups.

All the specimens had some areas of well-defined organized lamellar structures with or without osteocytic lacunae, a presentation consistent with vital bone and residual allograft particles, respectively. The staining used for the histologic analysis could not clearly outline the boundaries between the residual graft particles and the vital bone so that they appeared to be the same structure.

Some lamellar structures containing osteocytic nuclei surrounded mineralized tissue generally lacking organized lamellar structure and canaliculi presenting a different orientation (*Figure 8)*. This difference was even more clear when a polarized filter was activated on the light microscope.

At higher magnification (light microscope 20X) it was possible to identify resorption bays with multinucleated giant cells consistent with osteoclasts. Scattered osteocytes and blood vessels were consistently observed throughout the vital bone.

The third category observed was CT/non-mineralized tissues (i.e. connective tissue, vessels, and cells of different origin). All specimens presented with variable amounts of fibrous stroma filled with spindle-shaped mesenchymal cells, fibroblasts, adipocytes and monocytes. Inflammatory cells were noted too especially on LD 005 sample, the subject that experienced

early non-absorbable membranes exposure and removal.

Numerous vascular structures were observed interspersed in the connective tissue matrix as well as in the center of osteons. It was noted that some vascular organizations occupied Haversian canals of residual graft particles *(Figure 9)*.

Images deriving from the ESEM analysis *(Figure 5)* were processed with the software ImageJ* *(Figure 6)* in order to identify all the mineralized tissue in the specimens. The images in greyscale were turned into a binary black and white configuration. The mean mineralized tissues extension in all the specimens was 44,39 % when compared to the total area of the specimens *(Table 12)*. Group 1 specimens had a mean of 41,25 % while for Group 2 was 48,59 % (*Table 13*).

Discussion

This document reports the final results of 6 subjects that successfully reached the stage of implant placement and bone core harvesting (Visit 4). The data deriving from the remaining subjects enrolled in the study will be analyzed in a future paper, as they complete Visit 4. The study will be concluded when all the enrolled patients will undergo implant placement and bone core harvesting.

The principal aims of this study were a quantitative and qualitative analysis of bone changes in terms of Volume gain and graft/new bone ratio in the area of lateral ridge augmentation procedures.

The first outcome to be noteworthy is that all implants placed in the augmented defects demonstrated uneventful healing after a minimum of two months post-operative. None of the patient treated with dental implants in the augmented sites needed additional grafting to cover bony dehiscence and exposed threads. This result shows a comparable outcome of the two membranes that provided enough bone volume for implant therapy.

The null hypotheses of this research were that non-absorbable membranes would be equal to the bio-absorbable in terms of total volume gain and quality of bone regeneration. Literature review showed that if soft tissue dehiscence did not occur, non-absorbable membranes supported more bone regeneration than bio-absorbable membrane. The volume of regenerated bone generally is more substantial with non-absorbable membranes than with bio-absorbable membranes [42]. Yet, a growing volume of publications on bio-absorbable membranes used for guided bone regeneration seems to support comparable results with non-absorbable ones [66].

The results obtained from the present preliminary investigation, showed comparable values between the two membranes with bio-absorbable outperforming non-absorbable on volume gain but yielding lower values in terms of percentage of mineralized tissue at bone core analysis. It is important to understand that statistical analysis on a Pilot Study does not include statistical significance and that the number of subjects treated at this stage is limited. The results deriving from the entire pool of subjects enrolled in this study may differ from the present.

Bone Volume change

The first aim of this study was to calculate changes in volume before and after lateral guided bone regeneration in localized mandibular defects. The preliminary data on volume gain shows contrasting results compared to literature. It would be expected that non-absorbable membrane would provide more volume but Group 1 (bio-absorbable membrane) yielded 34,7% higher volume gain if compared to Group 2 (434,78 mm^3 vs 284,21 mm^3) at 6 months after ridge augmentation. Despite the limited amount of data available at this stage, it can be asserted that bio-absorbable membranes are not only comparable to non-absorbable, but apparently superior in terms of volume gain. Yet, the discrepancy between residual ridge thickness and mesio-distal extension of the edentulous ridges treated in this study, suggests caution on the conclusions on which membrane performed better. One patient who experienced early membrane removal due to infection, showed a decrease in the mean volume gain at 3 months compared to 6. Another patient presented a site that was 6 mm thick

and the planned implant did not require an extensive grafting; the result was an inferior volume gain compared to the mean from other subjects.

Furthermore, it has to be noted that these volume values include soft and hard tissues and not only bone. One of the drawbacks of this protocol is the impossibility to separate soft and hard tissue volumes and calculate them separately. A CBCT analysis, before and after treatment, associated with the present protocol would have provided those information. IRB processes would have not approved this double radiographic investigation so that the protocol assumed this design. To counteract this limitation, linear average measurements were taken to show that the majority of the volumes calculated are represented by bone and not by a thickening of soft tissues. The average mucosal thickness represented 30,8% of the linear tissue gain, while the rest is occupied by mineralized tissue (bone). This concept connects to another limitation represented by risk of "fake" bone volumes beneath non-absorbable membranes. In fact, the titanium frameworks of Cytoplast™ were shaped at the time of grafting in order to replicate an ideal crestal topography that got lost during post-extractive ridge resorption. The tenting effect of the Ti-reinforced membrane could hide a possible absence of hard tissues in case of altered graft maturation; yet, the external aspect of the ridge would still give the idea of underlying bone structure. The soft tissue measurements with the stent may deceive the clinician on the real extent of bone underneath the membrane. This is why the mean soft and hard tissue linear values were measured as a confirmation of underlying bone presence.

The values deriving from the bone volumes calculations at 6 months, show lower values if compared to values at 3/4 months of healing. It is well know how ridges resorb after tooth extraction with no grafting procedure [1, 2]. In particular Tan in 2012 [2] demonstrated horizontal dimensional change of 32% at 3 months, and 29–63% at 6–7 months. Soft tissue changes

demonstrated 0,4–0,5 mm gain of thickness at 6 months on the buccal and lingual aspects. Horizontal dimensional changes of hard and soft tissue (loss of 0.1–6.1 mm) was more substantial than vertical change (loss 0,9 mm to gain 0,4 mm) during observation periods of up to 12 months. Casts were utilized as a means of documenting the changes.

Even though the volume changes recorded in the present study cannot really be compared to the abovementioned study, they still experienced a decrease in value. A paper that seems to confirm our results is the one from Simon in 2000[67] who showed a loss in width of grafted bone after 4 months of healing ranged from 52,1% to 58,0% 3 mm from the crest, 47,6% to 67,4% 5 mm from the crest, and 39,1% to 46,7% 10 mm from the crest. Also the method used to calculate bone changes was not three-dimensional but linear, with a customized stent. In the present study, the lower values showed by the non-absorbable membrane may be justified by the tenting activity of the titanium framework.

No references were found regarding grafted bone changes between 4 and 6 months after GBR procedures.

Another limitation of this study is the lack of standardization on the graft amount used for regeneration. No PVS impression was taken right after the augmentation procedure, to record the grafted volume before the healing started. The need of primary flap closure may decrease the vestibule of the treated area due to tissue advancement. The PVS impression would not capture the real extension of the graft but also the "tented" mucosa with an altered volume representation .

Our results can be read as a difference of grafted sites resorption between 3 and 6 months, using either bio-absorbable or non-absorbable membranes. There are no studies reporting

comparison of bone volume changes after lateral guided bone regeneration using bio-absorbable and non-absorbable membrane in pristine sites. This data can add knowledge to the rising evidence that bio-absorbable membranes can be compared, if not outperform, to the gold standard non-absorbable membranes.

When measuring ridge volumes from a stone cast, many problems may arise. One was discussed above and it concerns soft tissue thickening and the tenting effect of titanium frameworks. In addition, reduction of vestibular depth after grafting may occur because of the coronal advancement of the mucogingival junction. The coronally-shifted mucosa can mask and hide part of the ridge that with the folding of the vestibule is not captured by the impression tray.

Histology

The secondary aim of this study was to histologically evaluate the quality of the regenerated tissue. The original goal was to calculate the ratio between newly formed bone and residual graft in order to identify which of the two membranes allowed higher new bone deposition.
All sites examined in this study displayed evidence of new bone formation since the areas of residual graft particles, containing empty osteocytic lacunae, were circled and embedded by mineralized lamellar tissue containing osteocytic lacunae with evidence of nuclei. As described in the results, the staining used for the histology did not outline visually the graft particles form the vital bone. In this study the expected role of *solochrome cyanaine* staining

solution was to differentiate between old and new bone. It appears that it defeated its purposes. If from one hand it was not possible to calculate new bone and residual grafted particles, from the other it was noted an intimate connection between the majority FDBA and the autogenous bone with no immature tissue interposition. This leads to think that the bone graft integration was successful.

Histomorphometric analysis deriving from other publications revealed a mean new bone of 47.6% ± 14.2% with a mean residual graft particles of 52.4% ± 15.1% [68] and 57% bone (36% graft material and 21% new bone[69]. Urban et al[66, 70] reported 31% autogenous bone and 25% xenograft presence in bone core harvested after mandibular lateral augmentation with collagen membranes. The same author treated posterior mandible defects with PTFE membranes obtaining 36.6% autogenous and 16.6% of ABBM after bone core analysis[70]. Iasella et al. [71] reported an average of 54% vital new bone formation 6 months after extraction and socket grafting with mineralized freeze-dried bone, and most of the new bone was woven bone, with less lamellar bone formation.

It is important to stress that those studies presented different characteristics compared to the present one. First of all, vertical ridge augmentation differs very much in terms of technique, bone healing patterns and complications. FDBA is described to have more potential in bone induction compared to ABBM[70]. Also, ridge preservation represents a scenario where grafting material heals in a faster process compared to lateral bone augmentation [72].

It appears that total mineralized tissue found in ridge preservation specimens varies from ∽40% (Fotek et al. [73]) to ∽60% (Beck et al. [74]). These results can be compared to the values obtained by Trombelli et al. [75] in an undisturbed socket, where they found only 32.36% woven bone formation 6 months after tooth extraction.

Another consideration of this study is the location of the bone core harvesting and the discrepancy between patients as far as the residual crest thickness. For example, a 5,7 mm-thick ridge that requires a narrow diameter implant to replace a premolar is a very different scenario than a 2 mm-thick ridge that is planned for a wide diameter implant to replace a molar. In the first case, only part of the supplemented area would be included in the core with the majority represented by pristine autologous bone; in the second scenario the core would be almost entirely composed by grafted bone. What the authors thought to be more relevant was the total amount of mineralized tissue (bone) in the area of implant placement, rather than the residual graft versus vital bone. The ESEM analysis provided this data. At the completion of the investigation the total percentage of mineralized tissue in the bone cores may vary, due to an increased number of samples.

Another important consideration regards soft tissue thickness and quality after regeneration therapies. Data deriving from this investigation showed a loss of thickness at 6 months of healing. The assumption was that the membrane or graft might have interfered with the soft tissue vascularity during healing. The lowest value for Group 2 was -1 mm decrease while for Group 1 was -0,33 mm; despite no statistical significance of these values, Cytoplast^{TM} might have interfered more compared to RCM6.

GBR Complications

The previous paragraph leads to another important discussion about membranes complications. As reported in the literature, the benefit of spontaneous resorption and the structure of the absorbable membrane help to reduce the risk of post-operative complications. Despite the limited number of subjects and data available at this time, it appeared that the rate of post-operative complications (soft tissue dehiscence) was higher for the non-absorbable group compared to the bio-absorbable, confirming the previous literature reports.

Subject LD005 after 4 months from Visit 2, reported pain and discharge deriving from the area of bone augmentation. This subject was included in the non-absorbable (Cytoplast) membrane group. Upon clinical evaluation, it was noted a distal displacement of the membrane that caused a communication with the periodontal sulcus of tooth #31. Membrane was infected based on the clinical signs of pus discharge and pain upon palpation of edentulous area. Patient was prescribed with Clindamycin 300 mg TID for 10 days. After infection reduction the membrane was removed along with one tack used to stabilize it. Patient successfully underwent implant placement #29, 30 and bone core sampling according to prosthetic plan. The other complication occurred to subject GC009. Patient presented with an extremely resorbed edentulous ridge in the region of missing #29, 30. The area presented a buccal undercut with ridge thickness of 3 mm. GC009 was randomly assigned to the non-absorbable membrane group (Cytoplast). After 3 weeks post-operative, patient reported discomfort on the lingual side of the edentulous ridge, treated with lateral GBR. Upon clinical observation we noted a 2x2 mm exposure of Cytoplast membrane in proximity of the

lingual side of the ridge. Minimal extent of exposure, reduced plaque accumulation and slight erythema were considered not sufficient to remove the membrane at this stage. Patient was dismissed with instructions on how to maintain the exposure free of plaque and potential infection. After 3 months of the procedure patient was contacted for another follow up. Clinical case presented with increased area of exposure, increased plaque accumulation and discomfort. After local anesthesia the membrane was exposed and removed along with the metal tacks used to stabilize it. Epithelial growth under the membrane was noticed and some residual bone graft apparently non-integrated was carefully removed. The area was flushed with sterile saline water in order to remove any residual grafting material. The thickness of the buccal flap was increased if compared to the one elevated during GBR procedure 3 months prior. The titanium structure that was removed resulted in a collapse of the buccal volume once the flaps were sutured to obtain primary closure.

These two cases clearly illustrate the effects of membrane contamination on the surrounding soft tissue. It appears then once bacteria colonize the surface of the membrane (i.e. through incision lines, teeth sulci) and the patient cannot control it, the soft tissues tend to migrate away from this source of irritation, fenestrations and dehiscence.

This scenario describes one of the limitations of this protocol in calculating bone volume based on dental impressions. The volume calculated from the PVS impression may not represent the real amount of bone present under the non-absorbable membrane. The main reason is the tenting activity determined by the membrane titanium framework that is shaped at the time of GBR. The dental impression may show a total volume gain that does not correspond to the actual bone structure underneath with the framework compensating a potential void between the membrane and the pristine bone.

The importance of this study stands in it potential role as a reference for future and more complex randomized clinical trials on the same topic. At the conclusion this study will provide data deriving from 20 subjects treated with bio-absorbable and non-absorbable membranes. As stated in the introduction, no previous studies had been completed with the same structure as the present.

Conclusion

Based on the preliminary results of this investigation it can be concluded that:

1. Bio-absorbable and non-absorbable membranes provided a sufficient amount of bone that allowed implant placement as planned;
2. The bio-absorbable membrane group demonstrated 35 % more volume gain compared to the non-absorbable one at 6 months;
3. The bio-absorbable group demonstrated more volume resorption from 3 to 6 months compared to the non-absorbable group;
4. Histological sections of the non-absorbable membrane group cores, demonstrated a higher amount of bone/residual graft.

Despite the limited amount of subjects treated at this stage, it was shown that bio-absorbable membrane performed as proficiently as non-absorbable one. The latter demonstrated a higher amount of bone but an increase incidence of complications such as infection and membrane removal.

These conclusions need to be taken with caution due to the design of the study and due to the preliminary nature of the results. A higher number of subjects may over these results or confirm them. At the end of this study it will be possible to provide valuable data to calculate a sample size for a future Randomized Clinical Trial.

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APPENDICES

Appendix A: Tables

Appendix B: Figures

Appendix C: Post Operative Instructions

Appendix A: Tables

Table 1. Study outline and visits.

STUDY OUTLINE and VISITS					
Appointment / Procedures	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Informed Consent	X				
Demographics	X				
Medical History Review	X	X	X	X	X
Inclusion/Exclusion Criteria	X	X	X	X	X
Oral Mucosal Tissue Examination	X	X	X	X	X
Bone Measurements with Stent		X			X
Impressions	X			X	X
Assess Eligibility & Withdrawal Criteria	X	X	X	X	X
Soft Tissue Measurements with Stent		X			X
GBR Surgery		X			
Membrane Removal for Non-absorbable Group					X
Bone Core Specimen Collection					X
Adverse Event Assessment		X	X	X	X

Table 2. Randomization scheme. Every enrolled subject was assigned to Group 1 or 2 at the time of Visit 2 (GBR procedure). To be noted that the blank lines correspond to subjects that will be assigned to one of the groups once ready for GBR.

RANDOMIZATION SCHEME	GROUP	SUBJECT
1	1	RMA001
2	2	LD007
3	2	FK006
4	2	HHH004
5	1	GH003
6	2	KL007
7	1	SA010
8	1	GBC009
9	2	TTT017
10	1	RS015
11	2	TL016
12	1	RM018
13	2	
14	1	
15	2	
16	2	
17	2	
18	1	
19	1	
20	1	

Table 3. Study Subjects' demographics. Sex, mean age and race are listed in the table

STUDY SUBJECTS' DEMOGRAPHICS

	SUBJECT	MALE	FEMALE	AGE	RACE
1	RMA001		X	67	C
2	GH003	X		54	H
3	HHH004		X	66	C
4	LD005		X	61	B
5	FK006		X	67	A
6	KL007	X		66	C
7	TB008		X	38	H
8	GC009		X	56	B
9	SA010		X	44	H
10	SR011		X	48	H
11	BH012	X		68	C
12	VM013	X		32	H
13	CK014		X	47	C
14	RS015		X	53	H
15	TL016	X		38	A
16	HR017			54	C
17	TTT018		X	39	A
18	FJ019		X	69	C
19	LMC020		X	50	C
20	MR021	X		66	C
21	DM022		X	58	B
22	HW023		X	65	C
23	CMF024		X	73	C
	TOT	**6**	**17**	**55,6**	

Table 4. Subject Timeline. Distribution of Groups 1 and 2 (bio-absorbable and non-absorbable membrane) regarding stage of treatment, complications and dropouts.

SUBJECTS TIMELINE

	Subject	GROUP 1	GROUP 2	VISIT 1	VISIT 2	VISIT 3	VISIT 4	COMPLICATIONS	DROPOUTS
1	RMA001	X		X	X	X	X		
2	LD005		X	X	X	X	X	1	
3	GH003	X		X	X	X	X		
4	HHH00		X	X	X	X	X		
5	FK004		X	X	X	X	X		
6	KL007	X		X	X	X			
7	SA010	X		X	X	X	X		
8	GC009		X	X	X	X		1	
9	TTT018	X		X	X				
10	RS015	X		X	X				
11	TL016		X	X	X				
12	MR021	X		X	X				
	TOT	**7**	**5**	**12**	**12**	**7**	**6**	**2**	

Table 5. Subjects with corresponding teeth missing and implants placed.

		Teeth Missing	Implants	Premolars	Molars
1	RMA001	2	2		2
2	GH003	1	1		1
3	HHH004	2	2	1	1
4	LD005	2	2		2
5	FK006	1	1		1
6	SA010	1	1	1	
	TOT	**10**	**10**	**2**	8

Table 6. Soft Tissue Thickness. The mean soft tissue thickness measured with custom stent at baseline (Visit 2) when GBR procedure was performed.

Soft Tissue Thickness	Mean	SD
T1	1,26mm	± 0,44 mm

Table 7. Soft Tissue Thickness Change. Soft tissue difference between Visit 2 and Visit 4 (at the time of implant placement). Measurements collected with customized stent.

Soft Tissue Thickness Change	Mean	SD
T2-T1	-0,02 mm	±0,38 mm

Table 8. Bone Linear Changes calculated at Visit 2 (before augmentation) and at Visit 4 (at time of implant placement) considering the 45° and 90° angles of entrance of the probe on the customized stent.

Bone Linear Changes	Mean	SD
	2,82 mm	± 1,02 mm

Table 9. Soft Tissue And Bone Linear Changes Soft tissue thickness, bone linear measurements and SDs for every subject in the two groups, measured with the customized stent.

SOFT TISSUE AND BONE LINEAR CHANGES

Linear Changes*		T1		Diff T (1-2)		Diff B (1-2)	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
RCM6§	RMA001	**1,55**	*± 0,52*	**0**	*± 0,70*	**3,16**	*± 0,75*
	GH003	**1**	*± 0*	**0**	*± 0*	**2,5**	*± 0,54*
	SA010	**1,66**	*± 0,51*	**-0,33**	*± 0,51*	**2,5**	*± 0,57*
Cytoplast+	LD005	**1**	*± 0*	**0**	*± 0*	**2,5**	*± 0,54*
	FK006	**1,33**	*± 0,5*	**0**	*± 0*	**3,66**	*± 0,81*
	HHH004	**1,11**	*± 0,33*	**-1**	*± 0,33*	**3**	*± 0,89*

*in mm.
§RCM6 (ACE Surgical Supply; Brockton, MA, USA);
+Cytoplast (Osteogenic Biomedicals; Lubbok, TX, USA).

Table 10. Volume Changes. Bone volume changes, before and after augmentation. Values after 3 months and 6 months of healing and the difference between 3 and 6 months. The last column shows the number of teeth missing per patient in the area of augmentation.

Volume Changes*					
	Subject	***0-3 months***	***0-6 months***	***3-6 months***	***Teeth Missing***
RCM6[§]	RMA001	665,29	586,94	-78,35	2
	GH003	338,73	322,42	-16,6	1
	SA010	169,46	221,56	52,1	1
Cytoplast[+]	HHH004	188,28	189,6	1,32	1
	KL007	304,36	304	-0,36	1
	FK006	233,38	233,07	-0,31	1
	LD005	526,72	410,19	-106,52	2

[*]in mm^3
[§]RCM6 (ACE Surgical Supply; Brockton, MA, USA);
[+]Cytoplast (Osteogenic Biomedicals; Lubbok, TX, USA).

Table 11. Mean Volume Changes. Mean volume changes between baseline and 3 months post GBR, baseline and 6 months post GBR and difference between 3 and 6 months.

Volume Changes*	**RCM6[§]**		**Cytoplast[+]**	
0-6 months	**Mean**	SD	**Mean**	SD
	434,78	± 136,67	284,21	± 96,31
0-3 months	**Mean**	SD	**Mean**	SD
	500,34	± 163,3	310,68	± 145,42
3-6 months	**Mean**	SD	**Mean**	SD
	-65,55	± 44,25	-26,46	± 53,37

*in mm^3
§RCM6 (ACE Surgical Supply; Brockton, MA, USA);
+Cytoplast (Osteogenic Biomedicals; Lubbok, TX, USA).

Table 12. Bone/graft Core Content. Percentage of Bone and Graft content in the core samples for every subject. Area of the core slices occupied by mineralized tissue (bone and residual graft particles). ImageJ software was used for calculations after obtaining images with ESEM images.

BONE/GRAFT CORE CONTENT				
Subject	**Specimen**	**Area**	**Perimeter**	**Area %**
RMA001	*30*	*22*	*22*	43,36%
	31	*28,56*	*23,26*	45,74%
GH003		*27,71*	*24,32*	33,10%
HHH004	*20*	*23,33*	*19,91*	48,78%
LD005		*22,62*	*20,34*	56,18%
FK006		*9,11*	*11,98*	40,80%
SA010		*23,58*	*21,49*	42,78%
TOT				**44,39%**

Table 13. Mean Bone/graft Core Content. Mean Percentage of Bone and Graft content in the core samples for Group 1 and 2. Area of the core slice occupied by mineralized tissue (bone and residual graft particles).

MEAN BONE/GRAFT CORE CONTENT

Subject		GROUP 1	Subject		GROUP 2
RMA001	#30	43,36%	HHH004	#20	48,78%
	#31	45,74%			
SA010		42,78%	LD005		56,18%
GH003		33,10%	FK006		40,80%
Mean		**41,25%**	**Mean**		**48,59%**

Appendix B: Figures

Figure 1 (a/f). Clinical sequence of the study surgical procedures; images a and b show the ridge defect before and after full thickness flap elevation. Images c and d show the ridge after GBR and implant placement. Images e and f show implant placement and after suturing the flap and radiographic image of the fixture.

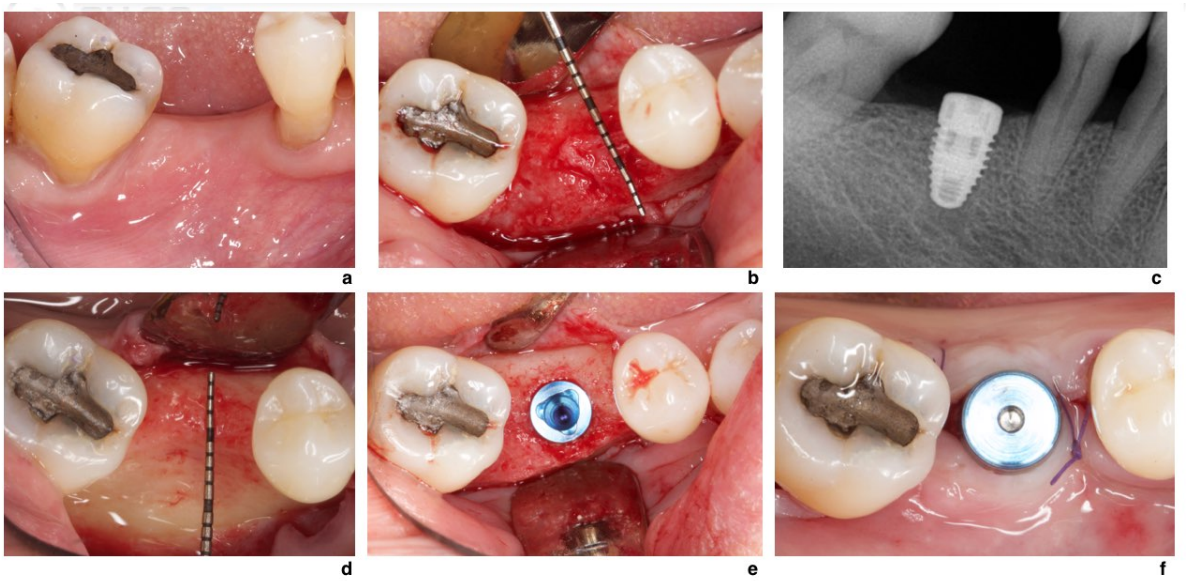


Figure 2. Snapshot of STL files processing with Meshmixer (Autodesk © 2011, Inc) software. Files are aligned and sectioned with a cutting tool.

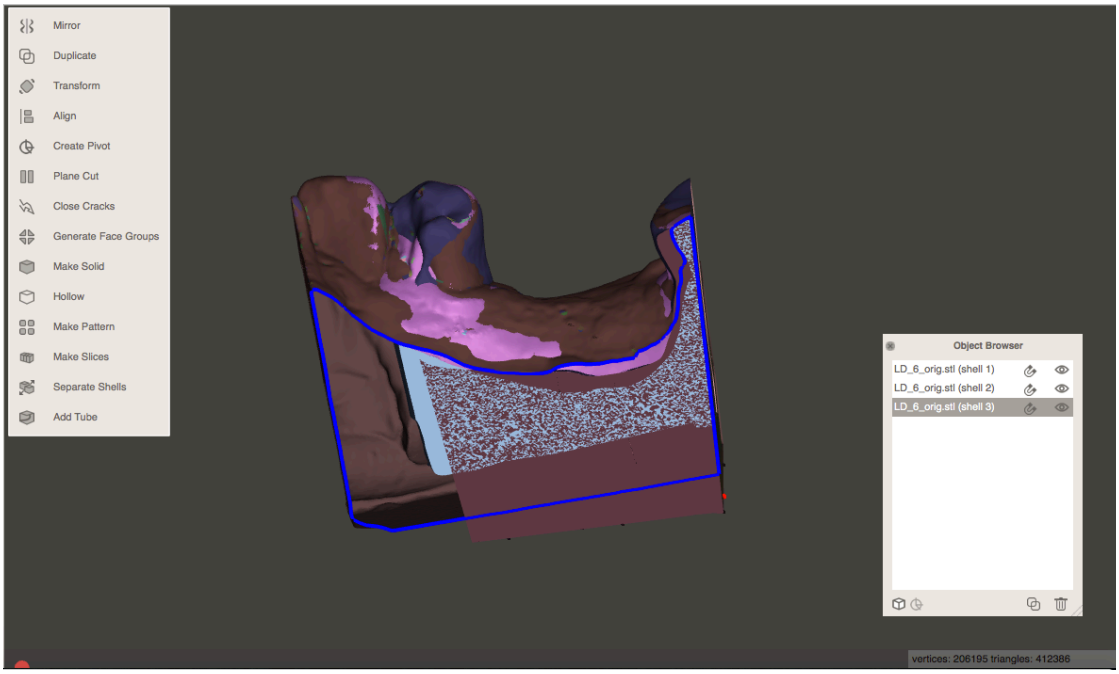


Figure 3. Comparison of 3 STL files representing 0, 3 and 6 months after GBR procedure. Their volume is measured with a dedicated tool of Meshmixer (Autodesk © 2011, Inc).

Figure 4 (a and b). Histologic image of a sample analysed under light microscopy (Nikon Eclipse 90i microscope - Tokyo, Japan equipped with a DS-Fi1 Nikon digital camera and driven by the Nikon ACT-2U software). Figure 4a shows a magnification at 2,5X while Figure 4b a magnification of 4X.

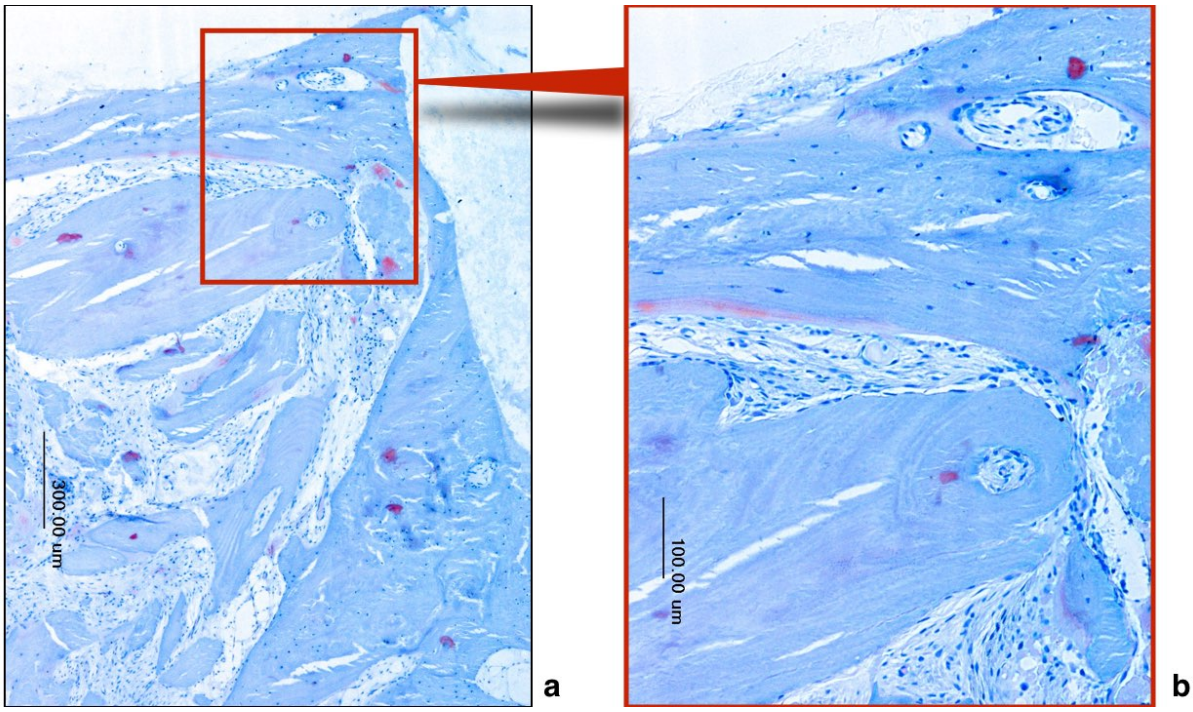


Figure 5. The image represents the bone core structure after ESEM analysis. The black areas represent non-mineralized tissue while grey-scale shades represent different grade of mineralized tissues.

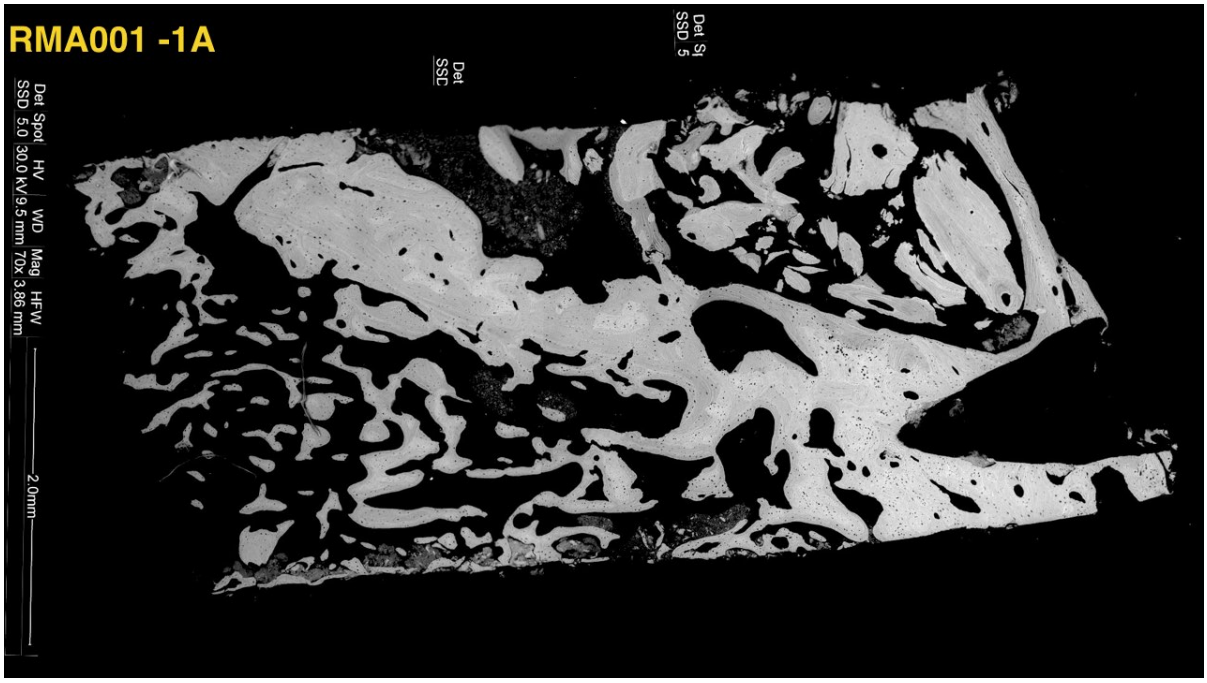


Figure 6. ESEM image processed with ImageJ software (NHS, Bethesda, USA) in order to calculate the area occupied by mineralized tissue (highlighted in red). First the core is outlined, mineralized tissue is highlighted, outliers are removed (artifacts of contrasting) and final area measurements are calculated.

Figure 7. Clinical image showing edentulous ridge of subject CG009 at baseline and after membrane removal at 2 months due to early exposure and infection. The last image shows suturing after membrane removal and site debridement with sterile saline water.

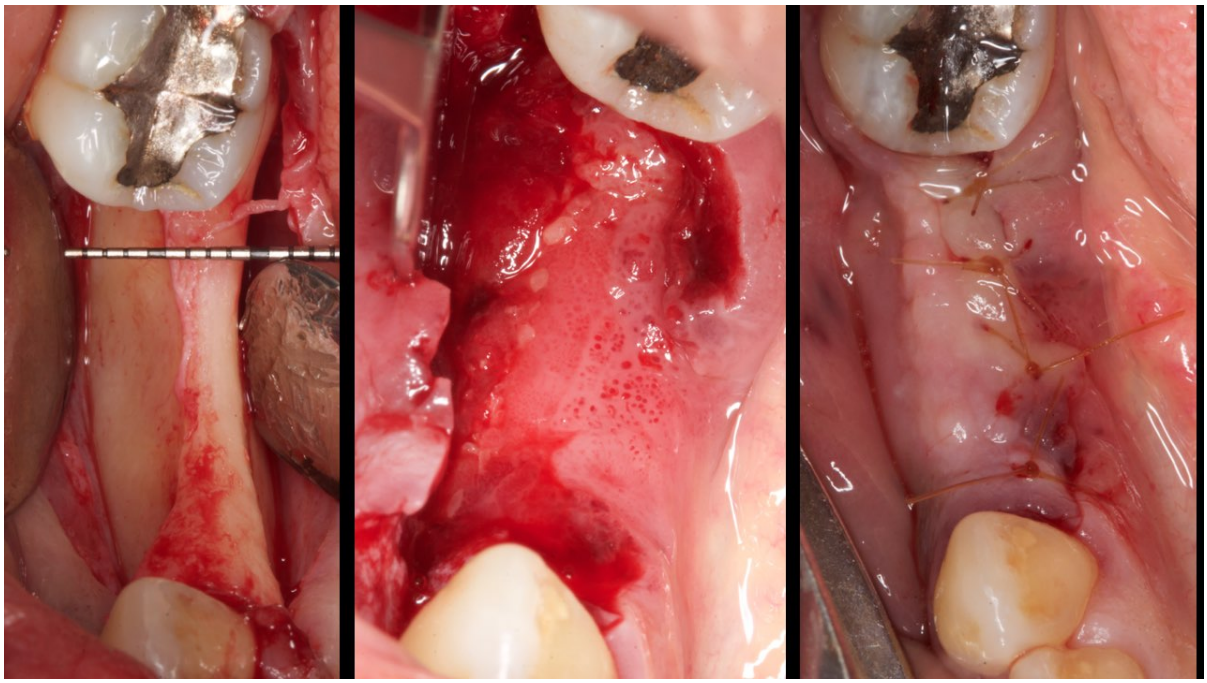


Figure 8. Histological analysis of one bone specimen. Some lamellar structures containin osteocytic nuclei surrounded mineralized tissue generally lacking organized lamellar structure and presenting empty osteocytic lacunae (Residual graft particles.)

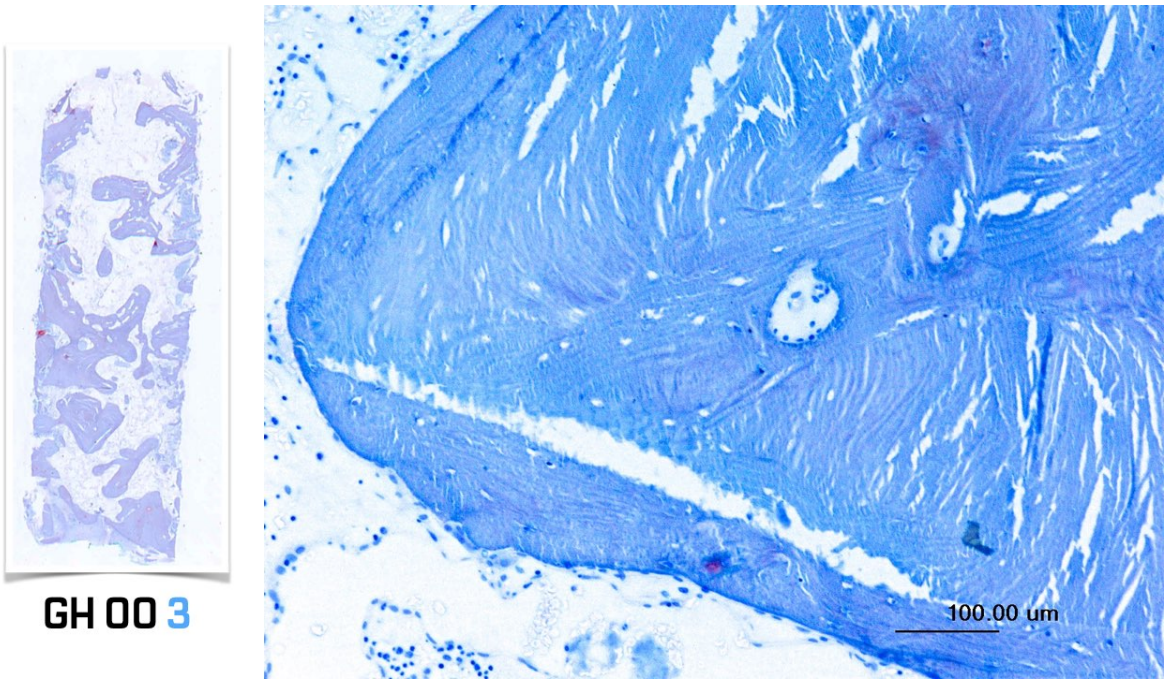
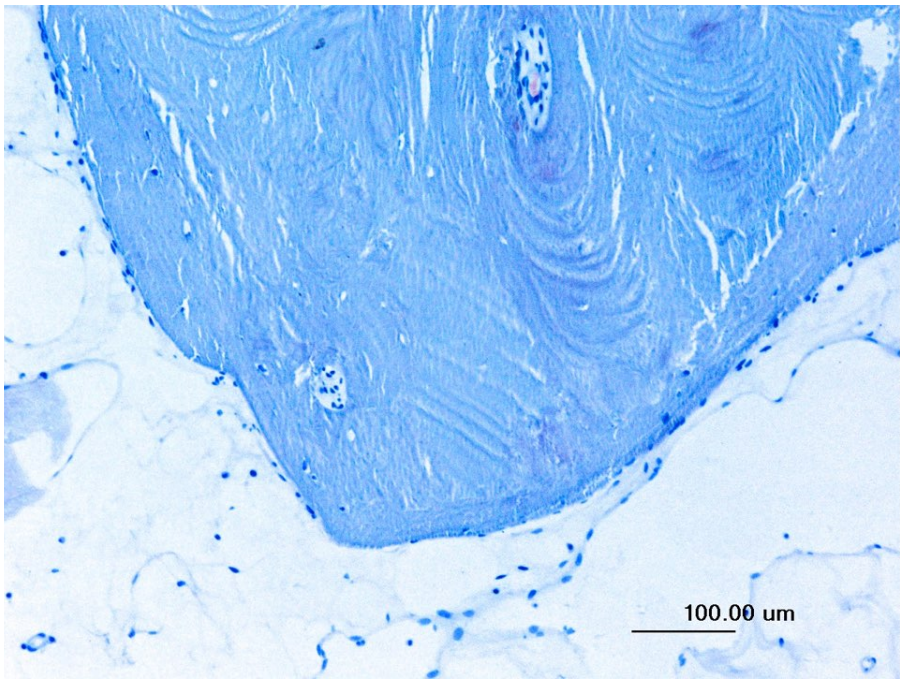


Figure 9. Histological image showing how a vascular organization occupied Haversian canals of residual graft particles.



Appendix C: Post-Operative Instructions

Tufts UNIVERSITY | School of Dental Medicine

DEPARTMENT of PERIODONTOLOGY

_____

POST OPERATIVE INSTRUCTIONS

Patient Name: _____

Date of Surgery: _____

Site: _____

DISCOMFORT

Discomfort is expected and it is usually controlled with pain medications (over the counter and/or prescribed).

The discomfort usually will disappear shortly after the sutures have been removed.

You may experience a feeling of slight weakness, chills or fever during the first 48 hours.

ACTIVITY

After leaving the clinic, relax for the remainder of the day. Keep your head elevated. Avoid strenuous activity for several days. Sunbathing should be avoided for two days to avoid swelling and fatigue.

BLEEDING

There should be minimal bleeding after the surgery is completed. If there is considerable amount of bleeding beyond this, wipe the area, locate the bleeding and apply gentle pressure to the inside of the mouth for 20 minutes. Use moist gauze to apply pressure over the area.

SWELLING

Some swelling will be present the day after surgery and usually peaks at 48 to 72 hrs. after surgery. This should disappear after 6 to 7 days.

Sip ice water or similar cold liquids to keep the surgical area cold for the remainder of the day. Do not use a straw, as suction can start bleeding.

Swelling can be minimized by placing a pack of ice on the outside of the face. Take the ice pack off every 20 minutes for about 5 minutes.

Keep your head elevated and sleep with 2-3 pillows the first day of the surgery.

DRUGS PRESCRIPTION

- ANTI-INFLAMMATORY / PAIN MEDICATION;

  - ☐ Ibuprofen 800mg TID for 3 days, then as needed
  - ☐ Acetaminophen 325mg TID for 3 days
  - ☐ Other prescribed: _____

    _____

- ANTIBIOTIC;

  - ☐ Amoxicillin 500mg TID for 7 days
  - ☐ Clindamycin 300mg TID for 7 days
  - ☐ Other prescribed: _____

    _____

- ICE PACK;

  - ☐ For 5 hours (intervals of 20 minutes on and 5 minutes off)

ORAL HYGIENE

Keep normal oral hygiene habits on the rest of the mouth.

On the surgical area:

- CHLOREXIDINE 0.12% MOUTH WASH;

  - ☐ After every meal for 14 days

Resume normal hygiene habits on the area after consultation with the resident that performed the surgery.

DIET

No hot drinks and foods for 48 hours.

Cold/room temperature drinks and/or soft and cold/room temperature foods advised for 2-3 days.

Chew on the opposite side of the surgical site. Avoid chewing on the side of the surgical site for 6 weeks.

PROBLEMS or CONCERNS

Please contact Dr. Lorenzo Mordini at 617-292-3433 or the front desk of the Periodontology Department at 617-636-6888.