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Barrier Membranes For Ridge Augmentation - Is There An Optimal Pore Size?

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ABSTRACT

Background: - Alveolar bone loss is often a sequelae of edentulism. However, several reconstruction procedures with bone grafts and barrier membranes are used to restore the lost bony architecture. The value of titanium mesh barriers has been shown to be reliable both in vertical and horizontal ridge augmentation procedures. However, there is a paucity of literature supporting the role of pore size of barrier membranes in preventing soft tissue ingrowth during ridge augmentation procedures. There are multiple reports describing a layer of soft tissue with varying thickness beneath the mesh and adhering to the newly regenerated bone.

The objective of this study was 1) to identity the presence of an optimal pore size that facilitates qualitative bone regeneration, 2) to identify the critical pore size that excludes soft tissue ingrowth into regenerative sites, 3) to determine if cortical perforations have any effect on bone regeneration, and 4) to reiterate that bone graft containment is an important parameter for successful regeneration.

Methods: - The study involved 4 adult hound dogs that were randomly divided into 3 groups. Groups 1 and 2 consisted of one animal each, sacrificed at one month and two months respectively. Group 3 consisted of two animals, sacrificed at the end of four months after the surgical procedure. All the animals received corticocancellous tibial bone grafting to the bilateral mandibular body/ramus areas. The left mandible received

cortical perforations in all animals. The experiment analyzed three different pore sized meshes compared to the controls without mesh. Two different pore sized titanium meshes (1.2mm and 600 μm) and a resorbable mesh (1mm) were pre-formed into the shape to a cube with one face open. Each side of the cube measured approximately 10 millimeters in size. The cubes were open face on the surface facing the bone. A total of 31 sites were included in the study. Prior to sacrifice, all the animals received 2 doses of tetracycline as a marker for new bone formation.

Data Analysis: Histomorphometry was performed by using Bioquant image analysis software. Areas of new bone and soft tissue were measured. The rate of mineral apposition was also calculated. All the values obtained with histomorphometry were statistically analyzed with a student's t-test procedure.

Results: The amount of new bone growth into the macroporous titanium mesh was significantly higher than the other groups. The mean area of new bone formation in large and small titanium meshes was 66.26 mm^2 and 52.82 mm^2 respectively. In the resorbable mesh group, the mean area of new bone formed is 46.76 mm^2. The amount of new bone formed in the control group was 29.80 mm^2. There was no significant difference in the amount of bone formation between the left and right sides ($p=0.3172$). Resorbable meshes had significant soft tissue ingrowth (23.47 mm^2) compared to macroporous titanium mesh (16.96 mm^2), and microporous titanium mesh (22.29 mm^2). The controls had least amount of soft tissue ingrowth (9.41 mm^2). Mineral apposition rate was found to be higher in the resorbable group (2.41μm/day) and the rate was least (1.09 μm/day) in the large pore titanium mesh group.

Conclusion: - The macroporous membranes facilitated greater bone regeneration compared to microporous and resorbable membranes. The macroporous mesh also prevented significant soft tissue ingrowth compared to other meshes. Containment of the bone graft is the most critical issue in successful bone regeneration. The presence of cortical perforations did not have any effect on the quality or the quantity of regenerated bone. Further research should be directed towards identifying a critical pore size and manufacturing a reliable mesh that would prevent excessive soft tissue ingrowth in ridge augmentation procedures.

DEDICATION

This work is dedicated to my
MOM
for all her sacrifices, unconditional love, and support.

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INTRODUCTION

There are approximately twenty million teeth extracted each year in the United States alone. Tooth extraction in the United States results in more than 40% of all people over 60 years of age being edentulous in one or both the jaws [1]. Alveolar bone loss with edentulism is a more common finding [2]. This loss of alveolar bone is accentuated over a period of time and conventional techniques of denture restoration could hasten the bone loss [3]. Endosseous implants can slow down or prevent bone loss [4-6]. The long-term evaluations of osseointegrated implant-borne dental restorations have shown this to be a predictable treatment method with good long-term prognosis for replacement of missing dentition in both completely and partially edentulous patients [7-9].

However to achieve a predictable long-term outcome for osseointegrated implants, a sufficient volume and quality of alveolar bone must be present at potential implant recipient sites. Several anatomic limitations of the residual alveolar bone preclude ideal implant placement thus resulting in compromised esthetics and function. The “site” of reconstruction of deficient alveolar ridges that lacks sufficient volume, contour, and height is often achieved with the use of biologically acceptable materials to permit locally found cells to permit bone formation.

Traditionally, alveolar ridge augmentation is achieved with various graft materials and barrier membranes to prevent soft tissue ingrowth. Although the value of titanium mesh barriers has been shown to be reliable in vertical ridge augmentation procedures, little literature supports the role of pore size of barrier membranes to allow adequate

vascular ingrowth and in preventing soft tissue ingrowth. Studies have shown a layer of connective tissue adhering to the local newly regenerated bone. For successful placement of implants the quality and quantity of regenerated bone is important.

The need therefore existed for literature and research, which leads to more effective and reliable means of alveolar ridge regeneration. This research will determine the optimal pore size to prevent soft tissue ingrowth into titanium mesh barriers used for alveolar ridge augmentation, and whether the grafting technique has role in the soft tissue ingrowth. The goal of this research is to develop a better understanding of bone regeneration procedures. This knowledge may lead to bioengineered materials which will modify and improve bone regenerative procedures in reconstructive defects.

BONE GRAFT MATERIALS

Bone has a remarkable intrinsic regenerative potential. Bone regeneration would greatly benefit from a more predictable enhancement of its natural repair process. Bone grafts would enhance this natural regeneration process. Bone grafts must be gradually absorbed and replaced with new living bone tissue. Cytokinal mobilization of endogenous factors such as bone morphogenetic protein, various platelet-derived, and insulin like growth factors are implicated in bone regeneration because they play a role in the healing of all wounds [10]. However, osteoprogenitor cells and osteogenic precursor cells play a significant role in the formation of bone in alveolar ridge regeneration procedures**.** There are different types of bone grafts used in reconstructive procedures. These bone grafts are generally classified based on the source and are divided into 1) autogenous, 2) allogenic, 3) alloplastic, & 4) xenogenic grafts.

Autogenous Bone Grafts

Autogenous bone grafts are often referred as the gold standard in grafting procedures. They are further divided based on the nature of the bone, into cortical blocks & particulate corticocancellous bone. Although autogenous bone is considered as the gold standard, it frequently is associated with donor site morbidity, and additional surgery. Autogenous cancellous bone has the highest osteogenic potential of any graft material.

Allogenic Bone Grafts

Allogenic bone is derived from a human donor not related to the recipient. However, this is associated with occasional reports of graft failure, and rare transmission of certain diseases. The rationale for using allografts is its osteoinductive potential [11]. It has also been reported that allografts often contain bone morphogenic proteins. However, the amount of bone morphogenetic proteins present in these materials has always been questioned [12].

Alloplastic Bone Grafts

Alloplastic materials are generally synthetic in nature. They act as a scaffold for bone formation. These materials aid in the formation of new bone through the process of osteo conduction. However, there is apparently no evidence that commercially available alloplasts would initiate the cascade of events that lead to significant amounts of bone formation [13].

Xeno Grafts

Xenogenic bone materials are obtained from a different species, usually bovine or porcine. Similar to alloplastic materials they only provide a scaffold for bone deposition (Osteoconduction) but do not affect bone formation (Osteoinduction).

The cycle of creeping substitution, which includes revascularization, resorption, and formation of new bone can take up to 3-4 years in humans [14]. Completeness of this is only achieved with cancellous bone grafts [15]. Autogenous cancellous bone has the highest osteogenic potential of any graft material [16]. Furthermore, it exhibits perfect remodeling, thus contributing to the osseous integration of the endosseous implants. A cancellous bone graft heals by immediate and continuous bone formation that results in a larger and more rapidly consolidated graft. Autologous onlay block grafts may be resorbed rapidly and block grafts do take longer time to integrate than cancellous bone grafts [15]. The amount of vertical augmentation achieved with bone grafting is difficult to predict, with the resorption rate being high over a short period of time. Augmentation of the cranio-maxillofacial skeleton by onlay bone grafting is associated with variable degree of resorption [17].

GUIDED BONE REGENERATION

Treatment of patients with severely resorbed edentulous jaws using osseointegrated dental implants remains one of the most challenging goals of implant dentistry. Resorption patterns following tooth extractions greatly alter the width and height of the residual alveolar ridge; especially when tooth loss results from maxillofacial trauma, severe periodontal disease or traumatic extractions. Implants placed in situations where there is significant alveolar resorption without bone grafting can result in malposition/failure. Malpositioned implants may affect coronal form, emergence profile, establishment of physiologic bucco-lingual relationship, esthetics, and the function of the final implant-supported restoration [18].

There are several reconstructive procedures that are available to increase both height and width. The graft materials that are available include both particulate and block graft form. A particulate material cancellous bone (PMCB) graft heals by immediate and continuous bone formation that result in a larger and more rapidly consolidated graft. With exception to cranial bone, autogenous onlay block grafts may be resorbed rapidly and block grafts do take a longer time to integrate than PMCB grafts [10]. The amount of vertical augmentation achieved with this approach is difficult to achieve, with the resorption rate being high over a short period of time. Whatever grafting material is used, the quality of the reconstructive procedure depends on whether the grafted site can be protected from soft tissue ingrowth. To meet this requirement, physical barriers between soft tissue and consolidating graft is employed.

Compromised bone sites have been corrected with guided bone regenerating techniques using physical barriers to compartmentalize the wound-healing event. Guided bone regeneration has been defined as “the principle of physically sealing off an anatomic site for improved healing of a certain tissue type and directing regeneration by some type of mechanical barrier”. This concept has been used in experimental reconstructive surgery since the mid-1950’s. The concept of providing a defined space by tissue separation in which osteogenesis may take place was first applied by Berg [19] and the principle of guided bone regeneration (GBR) was first described by Hurley et al for treatment of experimental spinal fusion in 1959 [20].

Studies of the dog ilium as early as 1957 demonstrated that if a bony defect is secluded from the surrounding connective tissue, a proper bone fill occurs. In the 1960’s Boyne et al tested the healing of defects in long bones and jaws using microporous cellulose acetate laboratory filters [21-23]. Membranes have been used to isolate, create a

protected space, and to prevent space collapse due to pressure from the overlying soft tissue. The result is migration of bone-forming elements into this space and thus new bone formation [24, 25]. Murray et al described three things necessary for the new bone growth: (i) presence of blood clot; (ii) Preservation of osteoblastic cells; and (iii) Contact with living tissue [24]. Melcher and Dreyer studied the healing process of a defect in the rat femur; in which the blood clot was protected with either a plastic or an organic shield during healing [26]. They concluded that the role of the shield or barrier was to protect the graft material from invasion by the overlying soft tissue. The efficacy of barrier membranes in conjunction with bone healing and reconstruction is probably the result of several different mechanical, cellular, and molecular mechanisms.

The early barriers were used to establish a suitable environment for osteogenesis is by excluding fibrous connective tissue cells from bone defects [27]. These barriers lined metal cribs for autologous cancellous bone placement. Kahnberg found that Teflon prevented ingrowth of fibrous tissue and allowed bone regeneration to occur in the healing of mandibular defects in rabbits [28]. Epithelial cell and fibroblast growth must be regulated to allow time for bone cell migration. A secluded space created by a barrier membrane allows angiogenesis and vascular ingrowth [29]. In animal experiments in which bone augmentation was performed using a membrane to create a secluded space, it seemed that a connective tissue matrix always preceded the formation of mineralized bone [30].

A barrier membrane can be used either in a 2-stage technique or as a single stage technique. An ideal barrier membrane would satisfy important design criteria such as: 1. biocompatibility, 2. cell occlusivity, 3. tissue integration, 4. clinical manageability, 5. space maintenance, 6. adequate stiffness, and 7. predictability. Currently, there are very

few barrier membranes that would satisfy the ideal requirements. A membrane should also direct mechanical stresses away from the graft site. A stress-free environment is very critical for adequate bone regeneration. Even 10µm to 20µm of movement during the early stages of wound healing is enough to direct mesenchymal cell differentiation into fibroblasts instead of osteoblasts [31]. Animal experiments demonstrated that micromotions would interfere with guided bone augmentation [32]. Regardless of the bone grafting techniques, the limiting factor has been maintenance of the graft itself and the competition with healing fast growing soft tissues in and around an osseous defect [33]. With guided bone regeneration, smaller defects (<70 mm^3) regenerate almost completely, while larger defects (>90 mm^3) regenerate between 90% and 93% and premature membrane removal resulted in incomplete regeneration [34]. In one study it was reported that membrane placement was associated with a reduction of peri-implant bone, caused by inflammatory soft tissue reactions [35]. This finding is in contrast with the results of many other studies, which have reported a positive effect of barrier membranes on bone regeneration in peri-implant bone defects [36, 37].

Although guided bone regeneration using barrier membranes has become an established treatment modality in implant dentistry, many problems remain and must be resolved to increase the predictability: Collapse of the barrier membrane, local infection, membrane exposure, soft tissue ingrowth, an incomplete bone formation within the space provided by the membrane [30, 38]. These problems increase when grafting defects with fewer residual bony walls (e.g. vertical ridge augmentation). Barrier membranes used for the process of guided bone regeneration are divided into 2 types, non-resorbable and resorbable. It has been shown many times that the regenerated bone is maximal when the membrane remains in place during the entire healing period [39].

Non-resorbable Membranes

Early investigations used various materials for guided bone regeneration. These materials ranged from silicone sheets, cellulose acetate laboratory filters, e-PTFE filters, titanium reinforced e-PTFE, and titanium meshes [21, 27]. However, some issues like brittleness, and inability to integrate with surrounding tissue and the need to remove them due to infection were noted [40, 41]. Currently, the popular nonresorbable membrane barriers used for guided bone regeneration are e-PTFE, nonexpanded PTFE, titanium mesh or titanium foil [33, 42-46]. Whereas resorbable materials do not require second surgery for removal, the non-resorbable membranes requires additional surgery to remove the membrane and facilitate implant placement. However, nonresorbable membranes are expected to be more reliable in terms of space maintenance [47, 48].

e-PTFE has a long history of effective use as an implantable medical material [49]. Due to the tendency to collapse during regenerative healing, investigators have explored the potential for reinforced, pre-formed, or moldable e-PTFE membranes in large defects that are screw retained. But even with screws, there is a possibility of collapse of non-rigid membrane [50]. A review of literature indicates that several studies have demonstrated that mesh exposure can occur with associated detrimental effects on the graft material [16, 34, 51-56]. It often requires extensive flap reflection to remove the barrier after premature exposure and infection that could lead to resorption of the underlying bone. If an exposed e-PTFE membrane is not removed once exposed, a wound infection usually occurs [57, 58].

Titanium mesh has been used in oral and maxillofacial surgery for the reconstruction of large and small defects [23, 59, 60]. Boyne inaugurated the use of a

titanium mesh in oral and maxillofacial surgery in 1969 for the reconstruction of large continuity defects. This method was then adapted for the reconstruction of severely atrophied maxillary ridges semi-rigid fixation of fractures and osteotomies. The rigidity of titanium mesh prevents contour collapse, its elasticity prevents mucosal compression, and its stability prevents graft displacement. The mechanical qualities of titanium mesh also allows for a predictable vertical or horizontal ridge augmentation. The mesh can be shaped and trimmed individually, making it easy to adapt to the alveolar ridge.

Titanium is a metal with excellent biocompatibility and has been used in numerous surgical applications [61]. Titanium mesh has been shown to be rigid enough to prevent soft tissue collapse, thus maintaining a space for grafted bone [36, 62-64]. Titanium mesh with PMCB along or PMCB mixed with other bone minerals is commonly recommended for isolated vertical and combination of vertical-horizontal defects. Smooth surfaced titanium barriers are less susceptible to bacterial contamination than are resorbable membranes. Although exposure of titanium barriers is noted in the literature, an obvious cause for early exposure of titanium barriers is mechanical irritation of mucosal flaps [65]. Also sharp edges caused by cutting, trimming and kinking might be responsible for delayed exposure of titanium barriers [66]. Hence careful preparation of the flap and the barrier membrane would prevent early and delayed exposure during the critical healing period. Collapse and dislocation of membranes will not occur with titanium mesh barriers. Various studies have shown that titanium membranes maintain space more predictably and better resist collapsing than do e-PTFE membranes alone or resorbable membranes [67-70]. The presence of pore might facilitate nutritious and metabolic exchange through the microperforations but is not proved.

Currently in the market we have different types of titanium meshes with different pore sizes but we are interested in looking into which mesh best provides an adequate protection of the reconstructed site from invasion of soft tissue cells and thus formation of the healthy bone. One of the disadvantages of using titanium mesh is wound dehiscence with exposure. This is probably due to rigidity of the mesh, failure to recognize sharp edges, large vertical augmentations, and failure to release the mucosal flaps completely. During the use of titanium mesh, it is critical to have a smooth junction between the local bone and the membrane; otherwise sharp edges of the mesh may cut through the mucosa resulting in exposure. Also it is reported that the risk of membrane exposure is higher in more extensive reconstructions, which is probably the result of wound tension [55].

Nondegradable membranes give the clinician greater control over the length of time the membrane would remain in place during the regeneration period. This is certainly an advantageous factor as studies have indicated that healing times may vary between different types or sizes of defects. This is particularly true with bony defects of the alveolar ridge [25]. Non-resorbable membranes may thus, provide more predictable performance, less risk for long-term complications and ease of clinical management. The time for which the membrane has to stay in the site has still been under investigation. Buser et al suggested 9 months of healing when using bone grafts and membrane for alveolar ridge augmentation [44]. While others have reported that 6 months of healing is the optimal time for bone regeneration [71]. In one study the authors noted if the mesh was in place for at least 4-6 weeks, the grafted material was sufficiently stabilized by the newly formed bone [66]. Length of time needed for retention of the mesh and complete bone healing is obviously dependent on several factors and thus dictated by the clinical

situation. Although the problem of mesh exposure is evident, several studies have in general reported good results with the use of titanium mesh [59, 72-76].

Resorbable membranes

Although numerous, possible degradable biomaterials exist, most work has centered on, poly-lactide polymers, poly-glycolide polymers and collagen. Biologic membrane barriers are also available which include freeze-dried demineralized laminar bone sheets, freeze-dried dura, and fascia latta [77]. In most of the studies, other than general material composition, little information is available in the literature regarding the structural and mechanical characteristics of the membranes used in these studies.

Copolymers of lactides and glycolides have a long history of safety and biocompatibility in their use as a mesh or suture. Polymeric membrane materials like polylactic and polyglycolic acids vary in their structural and degradation characteristics. This is controlled by thickness and chemical composition. These materials are broken down to carbon dioxide and water. In general, membranes that are non-crystalline and are primarily composed of polyglycolic acids will undergo faster resorption. Membranes composed primarily of polylactic acids and are highly crystalline will undergo slower resorption. The incidence of significant inflammatory reactions such as granulomas or sterile abscess formation increases with highly crystalline materials. Hence use of polymers that have high crystallinity is usually avoided. Membranes that resorb too quickly can be detrimental to the success of the graft. The inflammation caused by rapid degradation process could result in instability and movement of the grafted material and thus resorption of the graft.

Polyhydroxybutyrate (PHB) is another resorbable polymer that has shown high biocompatibility when implanted into pericardium [78, 79]. It is reported that the degradation time of PHB can be adapted for different purposes when it is copolymerized with hydroxyvalerate (HV) [80]. When PHB is reinforced with polyglactin 910 (PG) it could catalyze the degradation process and make the material easier to handle. Gotfredson et al reported that the PHB-HV-PG membrane did not prevent the physiologic resorption of bone in the immediate post-extraction phase, and also the membrane did not prevent the ingrowth of connective tissue in the peri-implant crestal area. They noted this biodegradable membrane induced an increased inflammatory reaction, which inhibited the bone fill around implants and produced a granulomatous foreign body reaction [81]. Degradation of resorbable polymer membranes could lead to an increase in pH manifested by local fluid accumulation, increased osmotic pressure or even transient sinus formation. This is often associated with an increased release of by-products, which can cause inflammatory reactions and thereby considerably disturb the healing process and bone formation underneath the membrane [82, 83].

Collagen has been a topic of increasing interest as a membrane material. It has been shown to have superior tissue integration characteristics when compared to synthetic polymers. However, the use of collagen membrane for guided bone regeneration procedures is very limited, first because of rapid degradation kinetics and second because of its lack of rigidity [84]. Collagen devices usually need a second supportive material to fulfill the above fore-mentioned criteria for an ideal barrier membrane. Most human data on guided bone regeneration have been derived from case reports that have used resorbable and nonresorbable membranes but none specify the surface and mechanical characteristics of the material used.

There are several disadvantages with resorbable membranes which include; unpredictability, potential for antigenicity, difficult to stabilize, and difficulty with orientation. The resorbable and biologic membrane barriers have shortcomings like lack of rigidity, immunogenic and comparatively short track record. However, dehiscence and infection have been less problematic with resorbable membranes.

RATIONALE AND OBJECTIVE OF THE STUDY

The value of titanium mesh barriers has been shown to be reliable in vertical ridge augmentation procedures. However, little literature supports the role of pore size of barrier membranes to allow adequate vascular ingrowth and in preventing soft tissue ingrowth. There has always been a layer of connective tissue adhering to the local newly regenerated bone. In an attempt to shed light on this topic,

We propose the following hypothesis:

1. Pore size definitely has an effect on the quality of regenerated bone and predictability of graft intake
2. There may be an optimal pore size that allows for vascular ingrowth.
3. There is a critical size that prevents soft tissue ingrowth into the graft material or the graft site.
4. The presence of cortical perforations has a positive impact on the regeneration process.
5. The grafting technique has a role in the ingrowth of soft tissue.

This hypothesis is based on the thought that an occlusive barrier would prevent vascular ingrowth, thus taking longer time and less reliable pattern of bone regeneration. There would probably be an optimal pore size which facilitates angiogenesis, allows

osteoblasts to pass through the barrier membrane into the graft site from the overlying periosteum, and importantly prevent ingrowth of soft tissue cells into the graft site.

SPECIFIC AIMS

To test the above hypothesis, the following specific aims are proposed:

1. Prove the presence of an optimal pore size that facilitates qualitative bone regeneration.
2. Identify the critical pore size that excludes soft tissue ingrowth into regenerative sites.
3. Reiterate that bone graft containment is an important parameter for a successful regeneration.

Alveolar bone loss with edentulism continues to be a major problem. Clinicians are still not sure how to prevent soft tissue ingrowth, prevent exposure of barrier titanium mesh, and the value of pore size of barrier membranes in the reconstruction of alveolar bone. This research would help to answer the above critical problems, document a small research tool, and may identify the best material that might be valuable for reconstruction procedures. This would have a dramatic impact in the reconstruction procedures, not only in the maxillofacial region but in the field of orthopedics as well.

ANTICIPATED RESULTS

Based on our clinical experience, we expect to see a significantly greater amount of soft tissue ingrowth into the sites without barriers membranes. There might be minimal or no connective tissue ingrowth in the sites which received a microporous mesh. A relatively greater amount of ingrowth would occur in macroporous mesh sites compared

to the microporous mesh. Also there might be a defective quality of bone formed in the side that didn't receive cortical perforations. Although it has been shown that periosteum plays a major role in providing blood supply to the underlying graft material. We expect a good quality of bone in the experimental sites compared to the control sites, which were prepared with 1mm holes to provide way for angiogenesis and vascular ingrowth from the trabecular bone.

Our suspicion is that there is a definite role of pore size in barrier membranes that allows of vascular ingrowth and preventing soft tissue ingrowth at the same time. In our study we don't expect to see any dehiscence or exposure of titanium mesh, since it is more often due to improper flap design, intraoral approach, and sharp edges than the property of the material. Future efforts will be to identify the proper material characteristic with the optimal pore size to achieve superior quality of regenerated bone.

If successful this research will demonstrate that there is an optimal pore size of barrier membranes, which allows for angiogenesis and simultaneously preventing soft tissue ingrowth. This information could lead to identification of the necessary changes that could be incorporated into material design which allows for superior characteristics that would lead to qualitative and quantitative applications in maxillofacial, orthopedic, and other surgical fields.

MATERIALS AND METHODS

This study analyzed three different mesh materials and compared them to controls. Specifically autogenous particulate bone graft was utilized to augment the lateral mandibular ramus in hound dogs. The bone graft was protected with two different titanium meshes (macroporous & microporous), and a resorbable polylactide mesh. There

were also control sites where no barrier membrane was used. The subjects were serially sacrificed and the sites were evaluated using histomorphometry.

SUBJECT SELECTION

Adult hound dogs were used as experimental subjects in this study. This animal model was chosen because of the well documented interactions between biomaterials and healing. These results were then applied to clinical use in humans. The study was performed according to guidelines of the University of Alabama Animal Resource Program (IACUC).

GROUP DISTRIBUTION

The study involved the use of 5 adult hound dogs that were randomly divided into 3 groups. Group 1 consisted of 1 animal which was sacrificed at the end of one month after the surgical procedure. Groups 2 and 3 consisted of 2 dogs each and were sacrificed at 2 months and 4 months respectively. All dogs had the same procedures performed.

PRE-FORMED MESHES

The titanium and resorbable meshes used for this experiment were pre-formed into the shape to a cube with one face open (figure1). Each side of the cube measured approximately 10 millimeters in size. The cubes were open on one side to facilitate packing of the bone graft material into them. The titanium meshes are manufactures from commercially pure grade titanium. The macroporous mesh has an average pore size of 1.2mm (Stryker-Leibinger, Kalamazoo, MI). The microporous mesh

has a pore size of 0.6mm (Stryker-Leibinger, Kalamazoo, MI). The resorbable mesh is made from polylactic acid (70/30 copolymer of poly[L-lactide-co-D,L-lactide] with a pore size of 1.0mm (Macropore Inc., San Diego, CA).

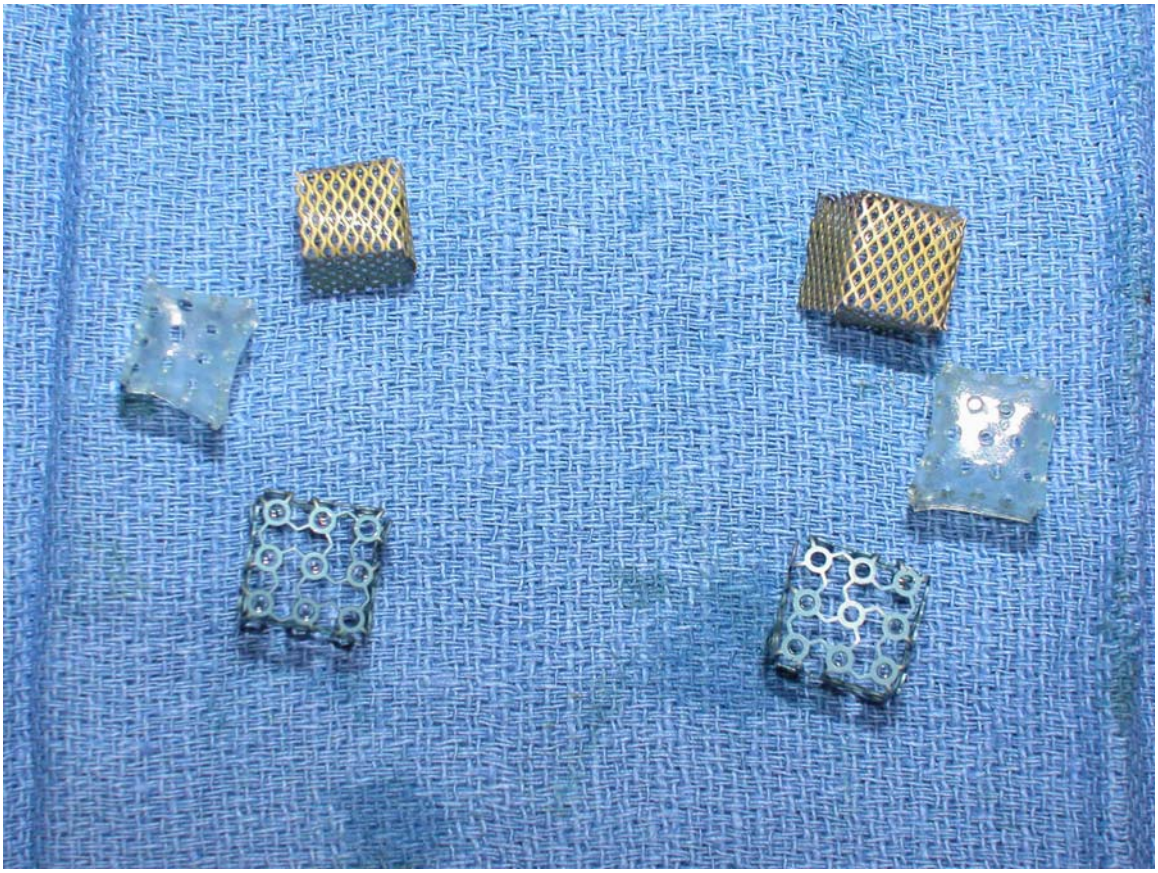

Figure 1. Preformed macroporous, microporous, and resorbable meshes.

SURGICAL TECHNIQUE

Preoperative Care

Animals were maintained in an AAALAC accredited animal care and use program in accordance with the standards of the Guide for the care and use of Laboratory animals (National research council, 1996). All animals were acclimatized for a period of one week. The animals were also evaluated for any infectious disease process prior to

undergoing anesthesia. Trained animal technicians under the supervision of veterinarians provided veterinary care. All animals were checked twice daily until fully recovered

following surgery. The protocol included analgesic administration to the animals after surgery, as well as close post-operative monitoring.

Peri-operative Procedure

All the animals were prepped for surgery. They were transferred from the housing facility to the preoperative holding area. Intravenous access was obtained for all the animals. Preoperative sedation was administered for all animals with ketamine. Subsequently, the animals were transferred to the operating room. On the operating table, the animals were placed in a supine position and appropriate monitors were placed. All the animals underwent general anesthesia under Isoflurane with oral endotracheal intubation (figure 2). Once the animals were properly anesthetized, the surgical sites were prepped by removing hair with surgical clippers and srubbed thoroughly with betadine solution. The animals were then draped in a sterile fashion to present a surgical field. Prior to incision, all the animals received preoperative antibiotics in the form of first generation Cephalosporin at a dose of 20 mg per kilogram. Two surgical teams prepared simultaneously to harvest the tibia and then perform the bone graft procedure to the mandible.

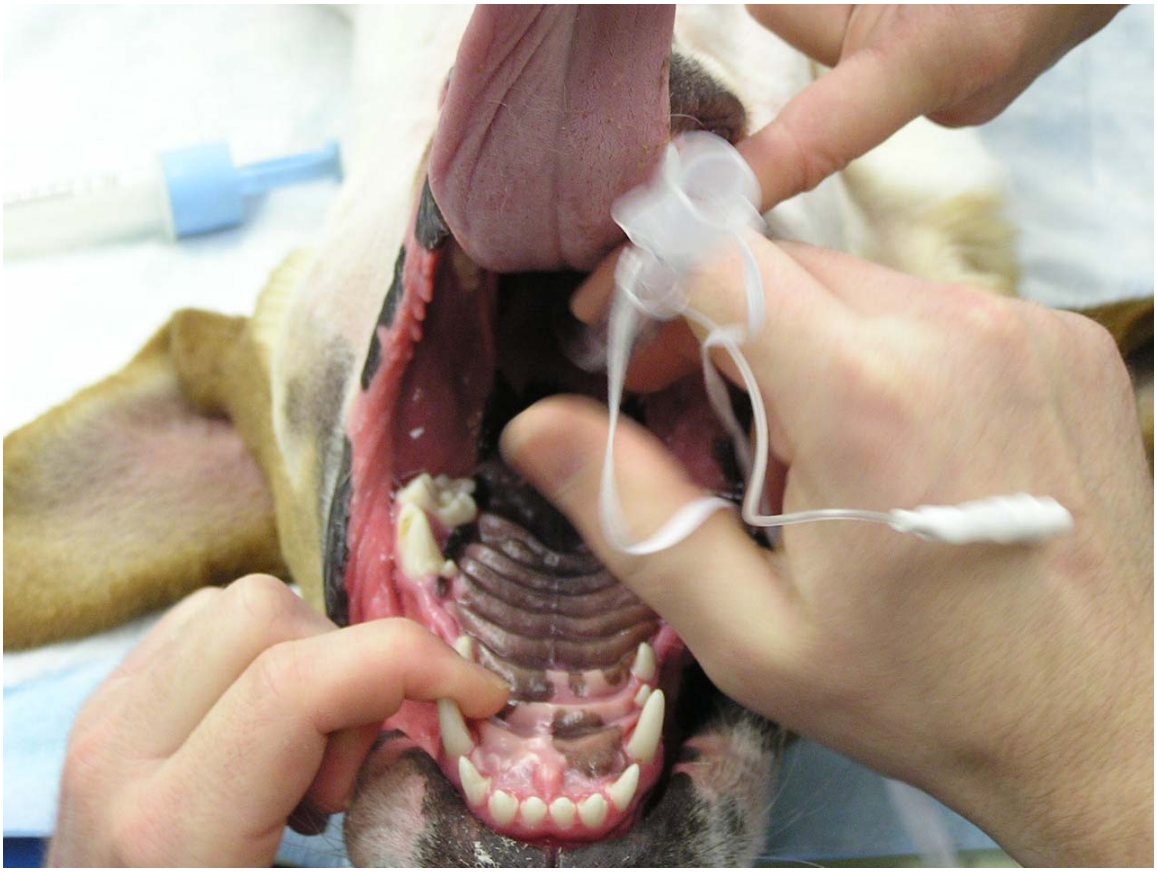

Figure 2. Experimental subjects undergoing intubation for general anesthesia

Tibial bone graft harvest technique:

A craniocaudal incision is made on the medial aspect of the tibia in each dog. The incision is carried deep to the skin. Blunt dissection was then performed to avoid iatrogenic injury to the region's soft tissue and neurovascular structures. Subsequently, the tibial cortex was exposed and identified. A surgical drill with a carbide fissure bur was then used to make cortical perforations. A tibial strut measuring 7 seven centimeters x 1 centimeter was then harvested (figure 3). A small bone graft curette was then introduced into the harvest site and cancellous bone was harvested. This was performed

without compromising the structural integrity of the animal's extremity. Hemostasis was achieved with the help of a surgical cautery. Prior to closure, the site was irrigated with normal saline solution to remove any surgical debris. The wound was then closed in subcutaenous layers with 3.0 Vicryl. The skin was closed with 2.0 Nylon in a continuous horizontal mattress pattern.

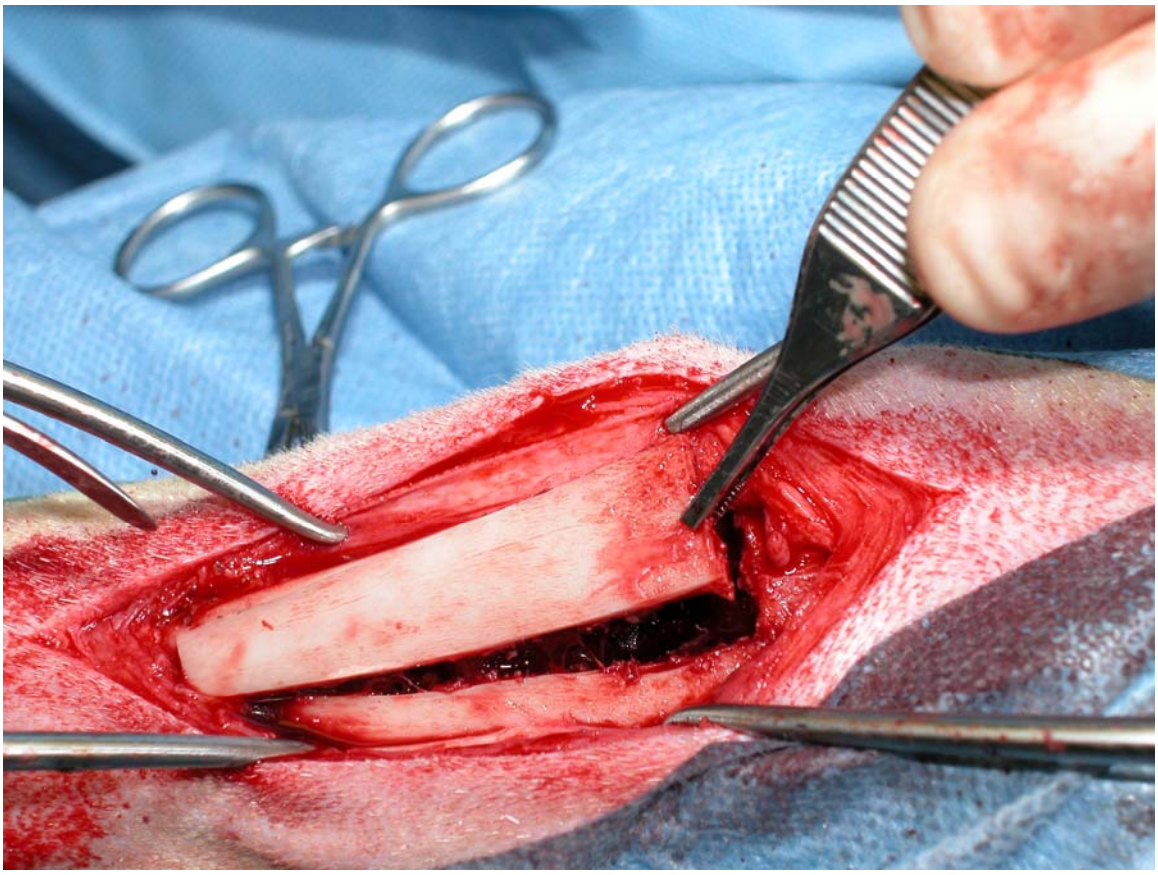

Figure 3. Harvesting the Tibial bone graft

To achieve consistency in the experimental procedure, the right tibia was randomly chosen for graft harvest. The graft was harvested under copious irrigation with saline and morselized into particles less than 1mm in size with the use of a bone mill /

ronguers (figure 4). The graft was then stored in normal saline until the recipient site is ready for grafting.

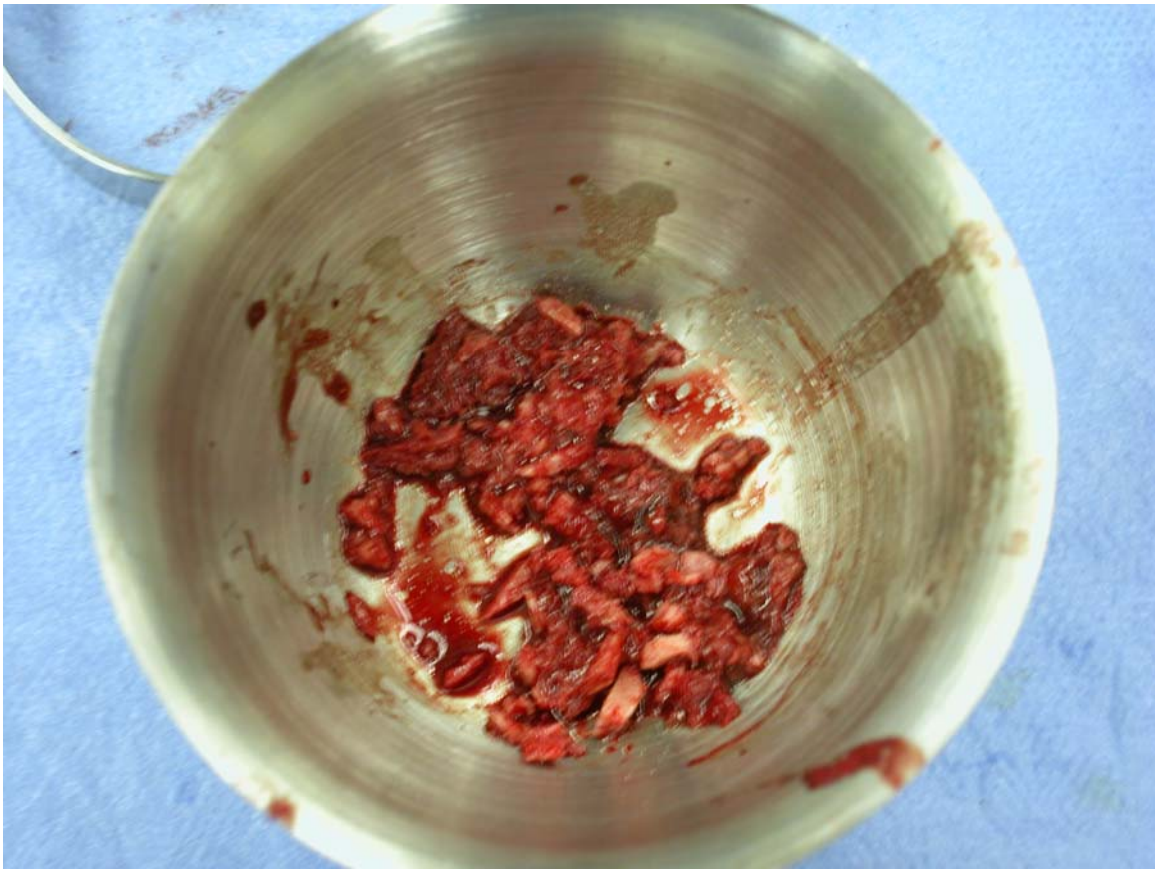

Figure 4. Morselized tibial bone graft strut into corticocancellous bone

Bone grafting technique

An incision was made with a # 10 bard-parker blade on the inferior border of the mandible through the skin and subcutaneous tissue. A blunt dissection was performed in layers to avoid injury to the soft tissue and neurovascular structures. During the dissection process, if the facial artery is identified, this was separated and ligated. The dissection was performed until the masseter muscle was identified. At this point, the periosteum was incised along the inferior border of the mandible. A subperiosteal

dissection was then performed to expose the lateral aspect of the body and ramus of the mandible (figure 5). Similar exposure was performed on the contralateral site. Four sites of regeneration were prepared on each side along the lateral body and ramus of the mandible. This was done by subperiosteal exposure of the lateral body of the mandible. Care was taken not to damage the periosteum. On the left side, the cortex along the proposed site of graft placement was perforated with a 0.8 millimeter round carbide burr under copious irrigation with normal saline (figure 6).

The harvested particulate graft was then packed into the pre-formed mesh cubes. The meshes were then overlayed along the lateral border of the mandible. This was done in such a way that the open end of the mesh cube faced the lateral cortex of the mandible. Each mesh was then secured with approximately 1.1 mm diameter titanium screws of a depth sufficient to pierce the cortex, but not pierce the opposite or the lingual cortex. This was also performed under copious irrigation with normal saline. For the control site, an equivalent amount of the bone graft material was used as in the mesh cubes. The control sites were not covered by a barrier. Once the grafts were secured, the wound was closed and the opposite side was addressed. The site preparation was the same, except that no holes were drilled through the cortex of the ramus. The mesh cubes with bone graft were secured in a similar fashion as described above. After the bone grafting procedure was complete, the surgical sites were irrigated with saline to remove any surgical debris. A penrose drain was inserted into the surgical site to prevent wound seroma. Closure of the wound was performed with a 3-0 Vicryl by resecuring the masseter muscle and periosteum over the surgical sites. The subcuticular layer was closed with 3-0 Vicryl and the skin was closed with a 2-0 nylon suture (figure 7). After adequate wound closure was achieved, the wound was dressed with antibiotic ointment.

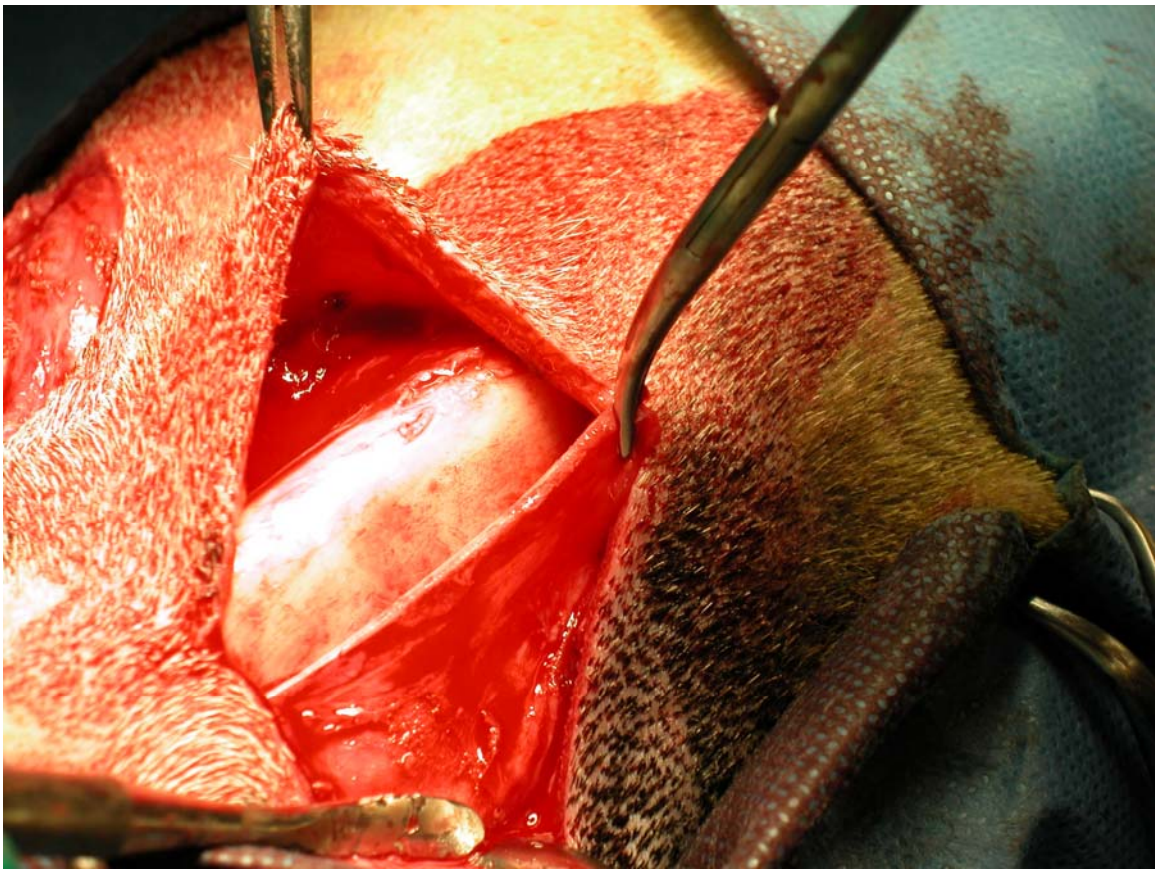

Figure 5. Extraoral approach to the body & ramus of the mandible

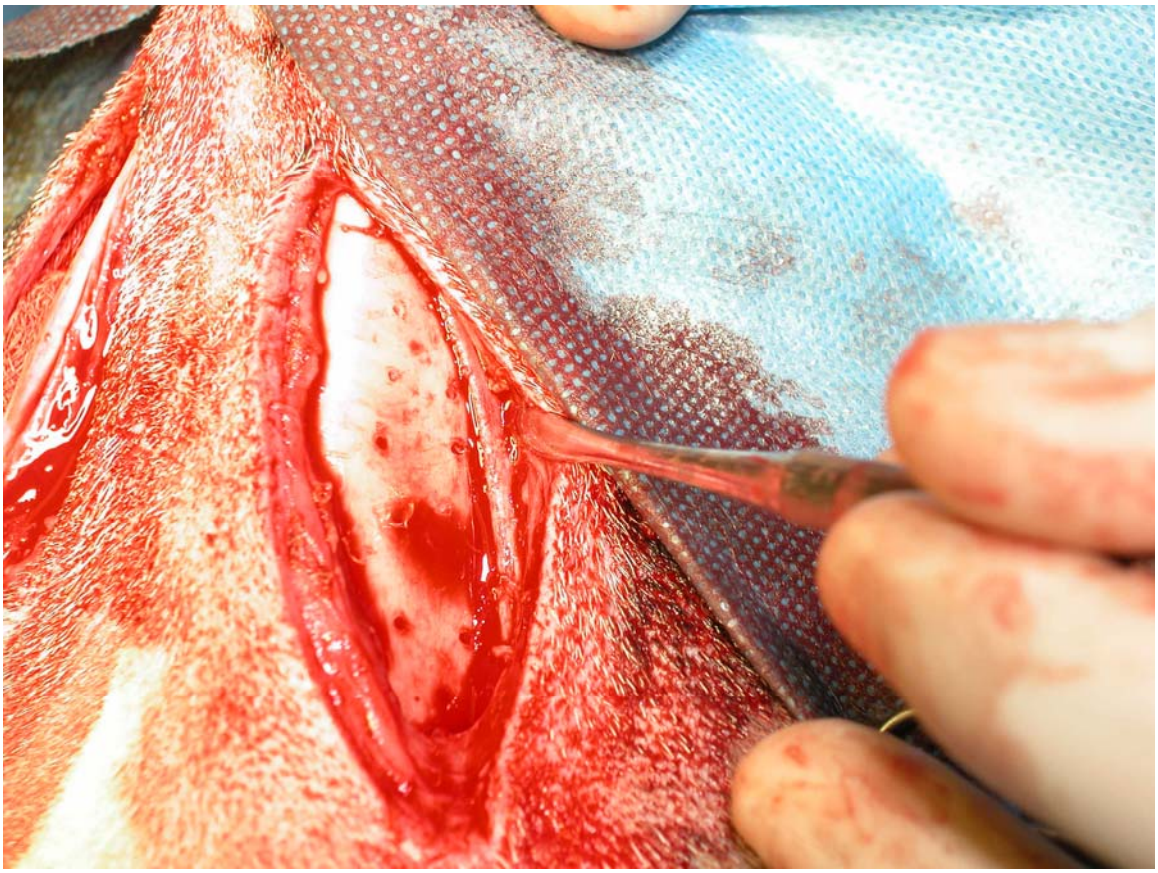

Figure 6. Cortical perforations to the external cortex on the left side of the subjects

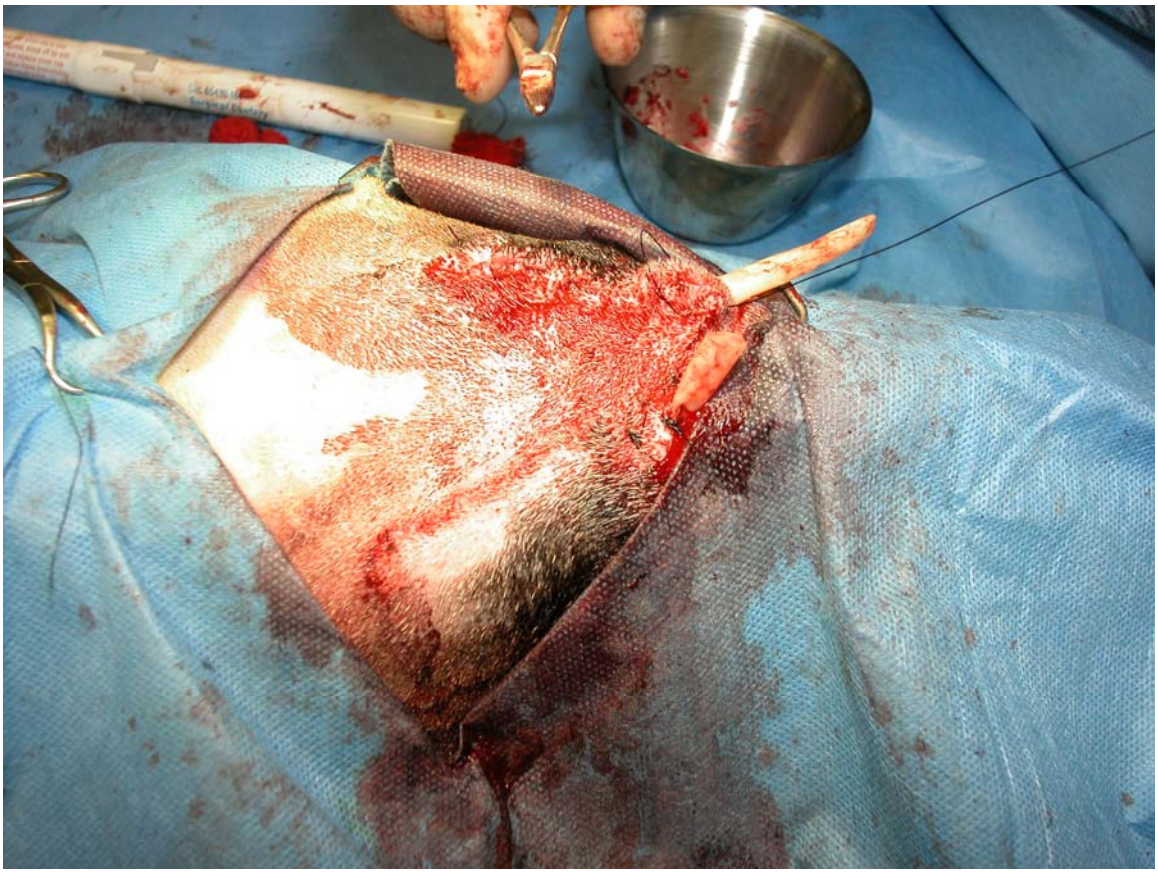

Figure 7. Wound closed in layers with penrose drain in place

Post-operative care

After recuperation from the anesthesia, the animals were extubated. The animals were then closely monitored until they were completely recovered. Post-operatively, the animals received analgesics for pain control and they were transferred to the housing unit. The animals were monitored daily for signs of wound infection, dehiscence of the surgical wound or graft exposure. Any of these events were handled according to IACUC protocols and veterinary advice. Additionally, the animals were fed with a soft mush diet

and maintained on antibiotics for a total of 5 days. The animals were also given water ad lib. The extraoral skin sutures were removed from all the animals on post-operative day 7. Prior to sacrifice, the animals received tetracycline at the dose of 25 mg/kg as a marker for appropriate staining of the regenerated bone. A total of 2 doses were administered with a 2 week interval between the doses.

Euthanasia

Euthanasia was accomplished with intravenous barbiturates, a method consistent with the American Veterinary Medical Association guidelines on euthanasia. The animals were sacrificed each according to the protocol. One animal was sacrificed at one month after the procedure. One animal was sacrificed after two months, and two animals were sacrificed at the end of four months. Intravenous barbiturate in the form of phenobarbitol was injected at the dose of 400mg per each animal. Euthanasia was confirmed by performing an open thoracotomy.

After the animal was successfully euthanized, the surgical sites were re-entered. This was performed with a #10 bard-parker blade and an incision was made along the inferior border of the mandible. The wound was open in layers until the surgical sites were approached. At this juncture, an enormous amount of scar tissue was encountered making the dissection difficult. During the process of specimen harvest for histomorphometry, several specimens were completely encompassed with a layer of bone. The meshes were identified and removed enbloc using a surgical drill. The specimens were then stored in formalin solution for analysis.

Histology

Eight specimens were harvested from each mandible for a total of 31 specimens. In group one, the resorbable mesh on the left side was excluded from the study due to improper surgical technique. All the specimens were trimmed and fixed in 10% Neutral Buffered Formalin for one month. All specimens were subjected to tissue processing, dehydration, and infiltration with methyl methacrylate (MMA) solution according to the standard operating procedure of the UAB Orthopaedic Research Laboratory, and subsequently embedded in MMA. All specimens with embedding mixture were placed under UV light for 48 hours to allow for polymerization. A buccal-lingual mid-line section was obtained from each specimen using the Exakt® macro-saw. Each mid-line section was then ground to 80–100 μm using the Exakt® grinder. Then the sections were stained with Sanderson's Bone Stain.

Histomorphometry

A region of interest was selected within the mesh including the area between the pores for subgroups with small mesh and large mesh. Four random regions of interest were selected for subgroups with resorbable mesh and no mesh. Each region was approximately 10mm from the border of the compact bone.

Histomorphometry was performed by using Bioquant Image Analysis Software® (R & M Biometrics, Nashville, TN). With this software, a two dimensional histologic section displays profiles of three dimensional structures. Three measurements were made—total tissue area, total bone area, soft tissue area. The software then calculated the indices.

Results

All the values obtained with histomorphometry were statistically analyzed with a student's t-test procedure.

Bone growth. The mean area of new bone formation for the groups of macroporous and microporous mesh was 66.26 +/- 13.78 mm^2 and 52.82 +/- 24.75 mm^2 respectively. In the group without mesh, the amount of new bone formed was 29.80 +/- 21.22 mm^2 and in the group with resorbable mesh, the area of new bone formed was 46.76 +/- 9.35 mm^2 (figures 8-11).

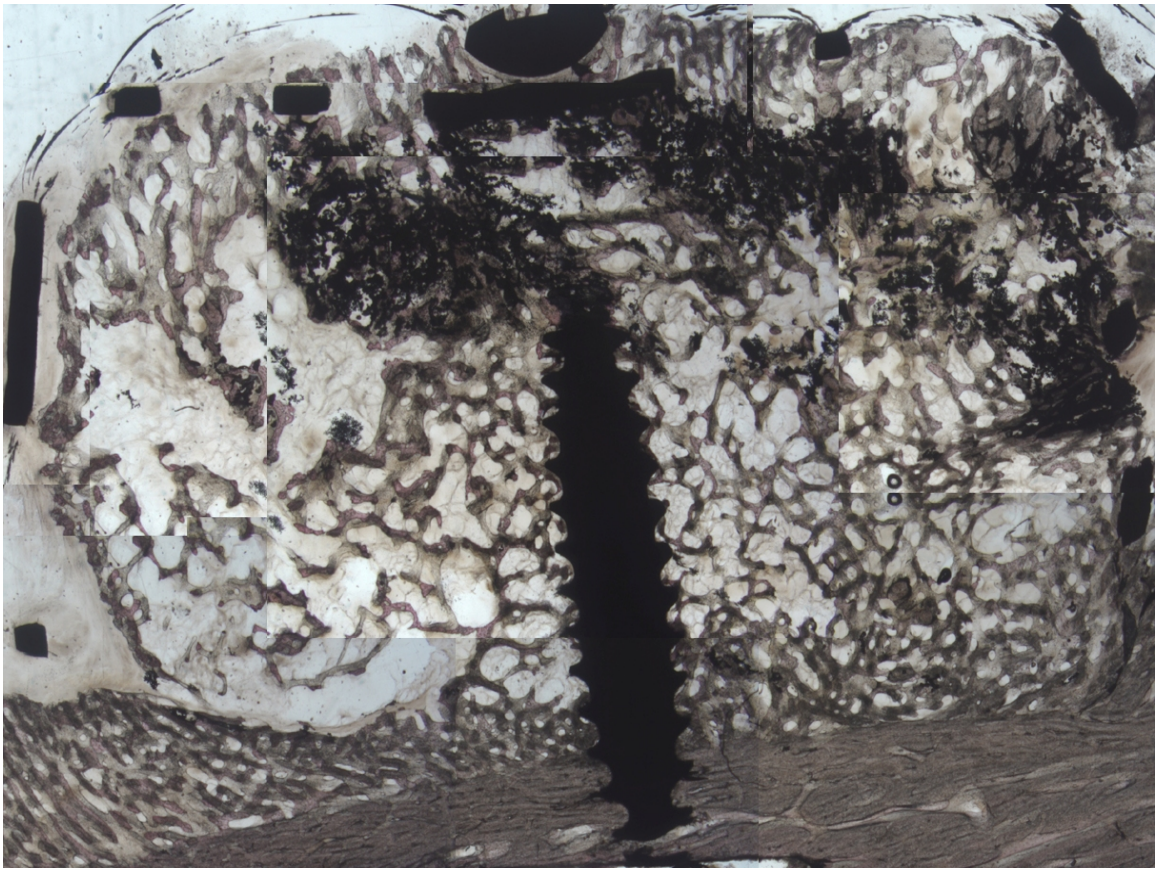

Figure 8. Microsection revealing bone formation with macroporous mesh

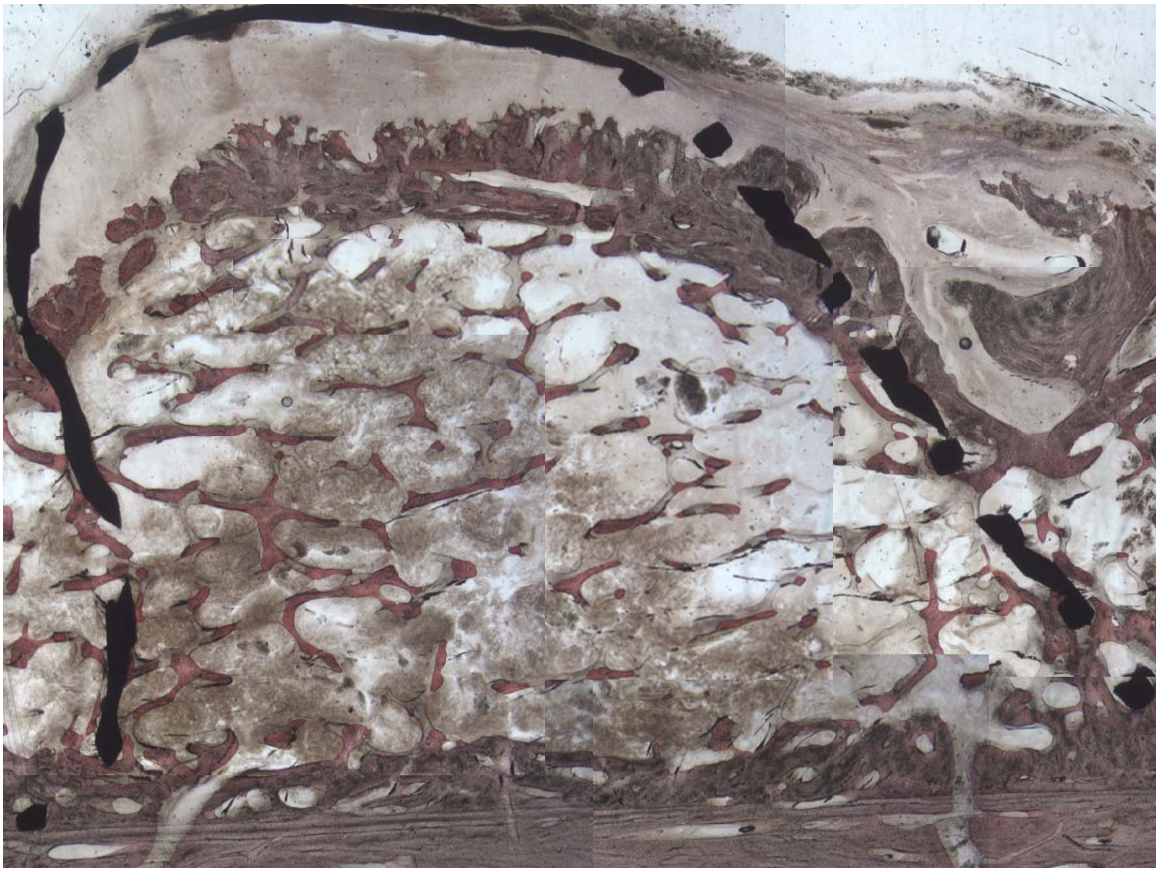

Figure 9. Microsection revealing bone formation with microporous mesh

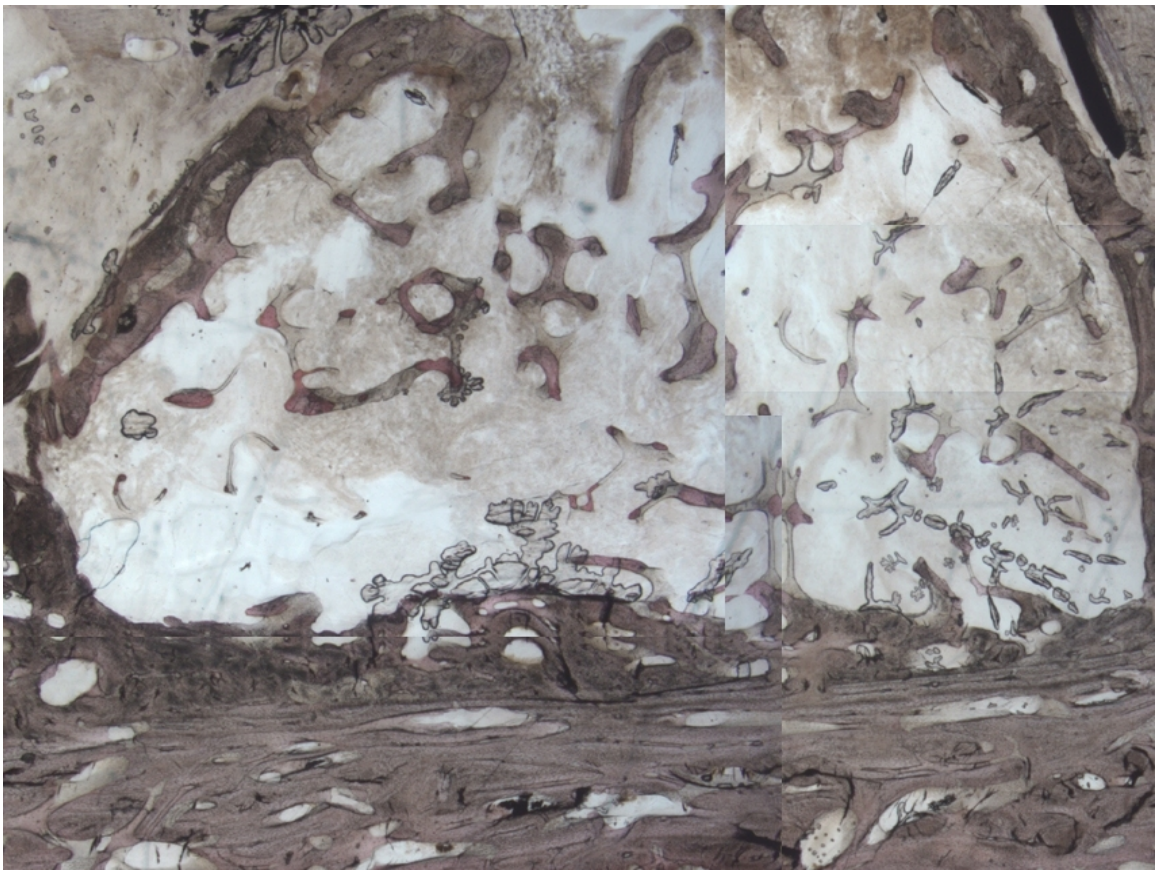

Figure 10. Microsection revealing bone formation with resorbable mesh

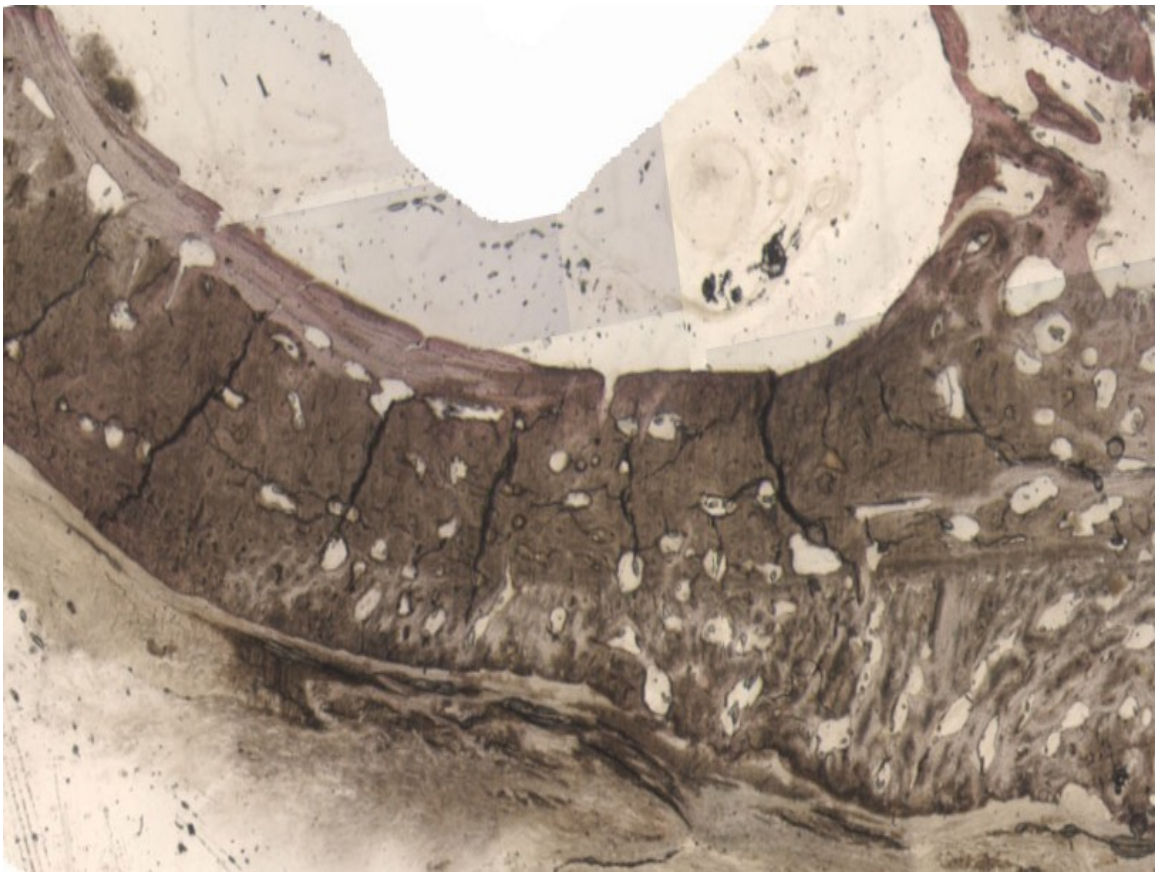

Figure 11. Microsection revealing minimal bone formation in the site without any containment

Among the four groups analyzed, new bone formation in the group with macroporous titanium mesh was significantly higher than the other groups. This was followed by the group with microporous mesh and then the resorbable mesh group. However, as expected the group without mesh failed to have any significant bone formation (figure 16, 17). There was no difference in the amount of bone formation between the left and right sides ($p=0.3172$) (table 1).

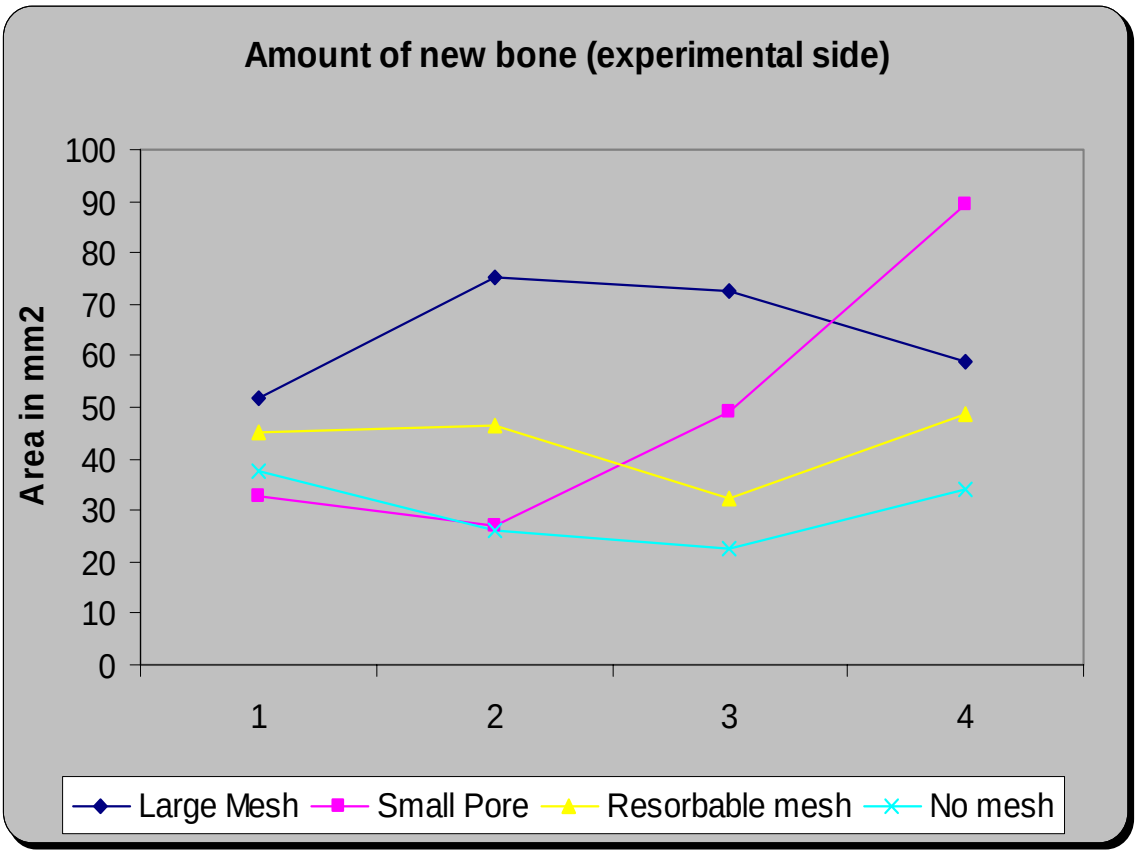

Figure 12. New bone formation in the sites (experimental) with perforations

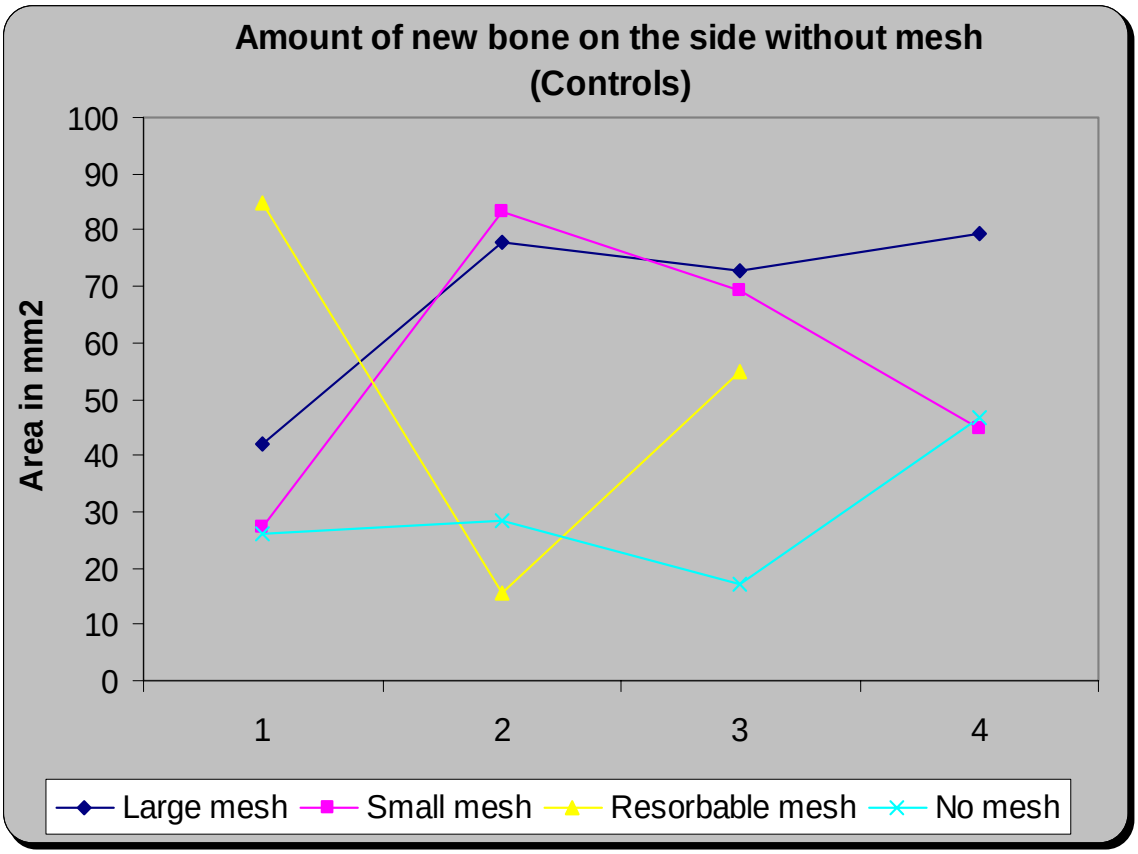

Figure 13. New bone formation in the sites (controls) without perforations

Table 1

Statistical difference in bone formation between groups as noted by p-value

	Macroporous mesh	No Mesh	Resorbable mesh	Microporous mesh
Macroporous mesh		0.0004	0.0480	0.1512
No Mesh	0.0004		0.0828	0.0175
Resorbable mesh	0.0480	0.0828		0.5250
Microporous mesh	0.1512	0.0175	0.5250	

Soft tissue ingrowth. The resorbable mesh had significant soft tissue ingrowth (23.47 mm^2) compared to macroporous esh (16.96 mm^2), and microporous mesh (22.29 mm^2). The controls had least amount of soft tissue ingrowth (9.41 mm^2) (figures 18, 19).The amount of soft tissue ingrowth into the mesh was not statistically different between the right and the left sides as well (p=0.2301) (table 2). The amount of bone growth as compared to the soft tissue ingrowth was statistically higher in all groups combined (p=0.0043).

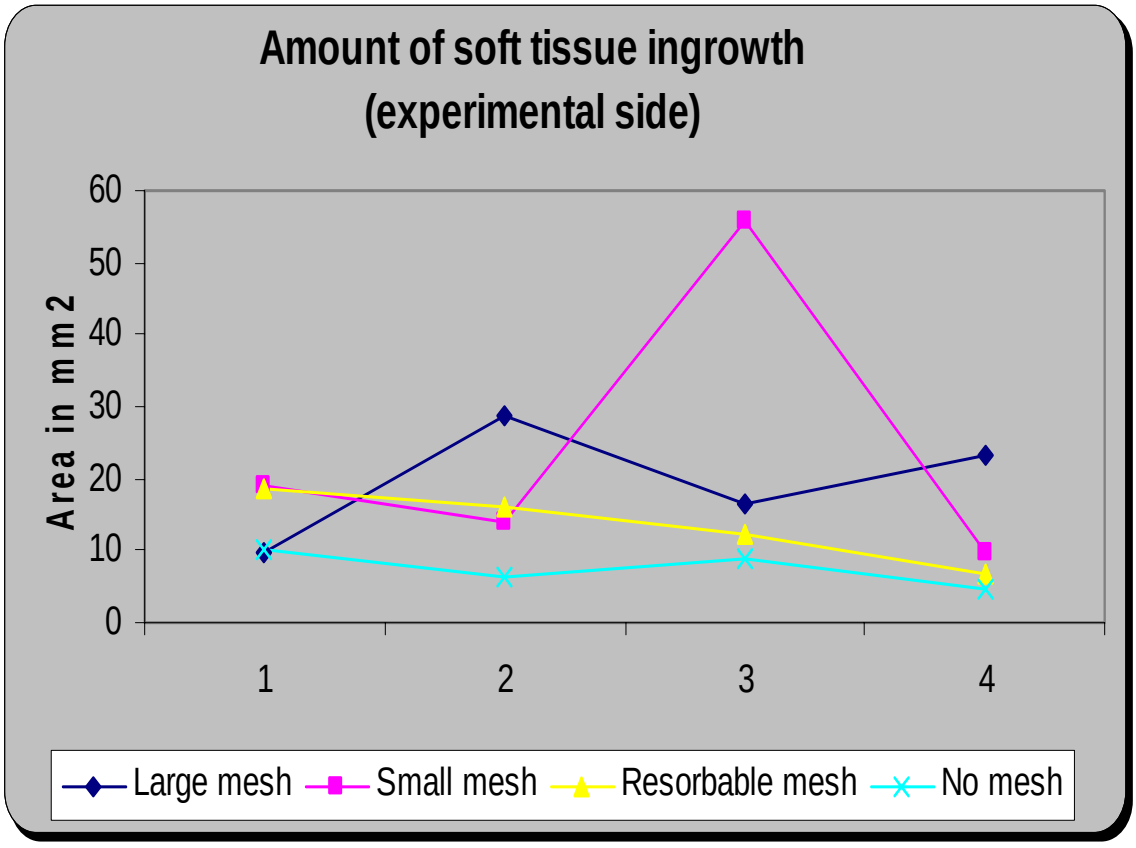

Figure 14. Amount of soft tissue ingrowth in the experimental sites

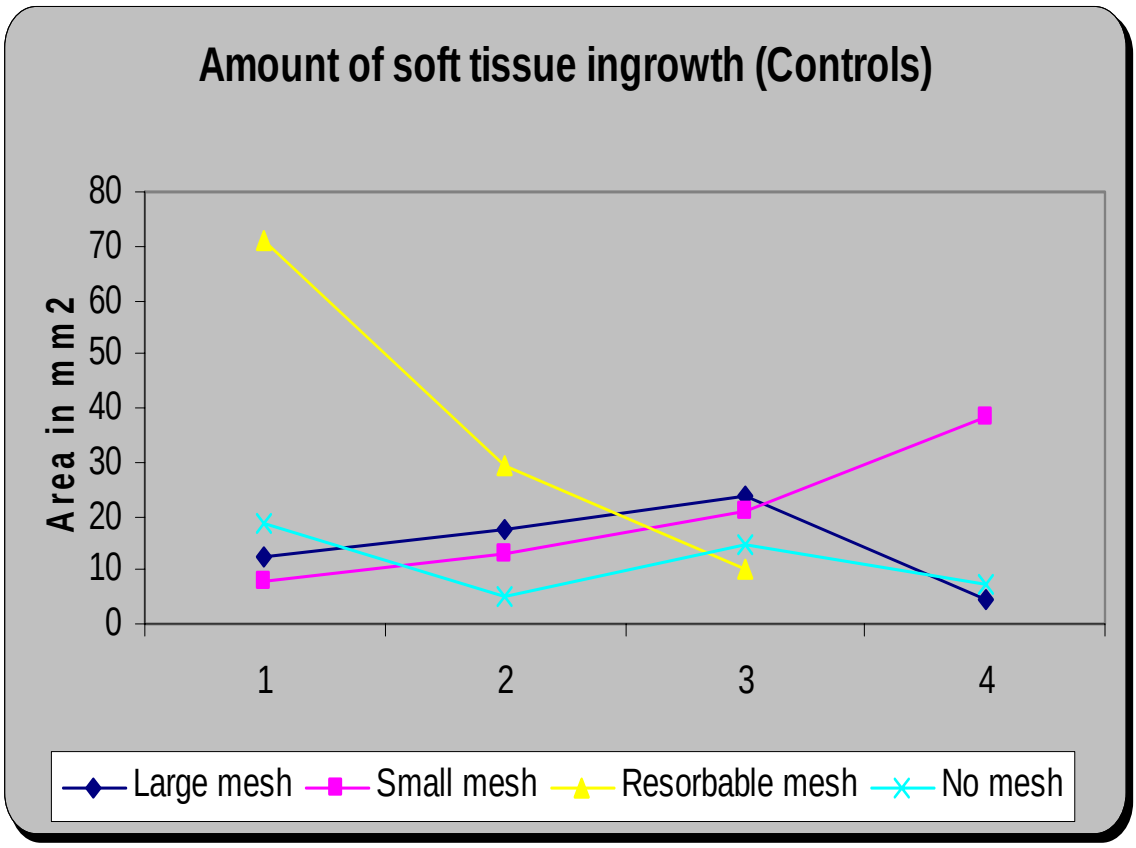

Figure 15. Amount of soft tissue ingrowth in the control sites

Table 2

Statistical difference in soft tissue formation between groups as noted by p value

	Macroporous mesh	No Mesh	Resorbable mesh	Microporous mesh
Macroporous mesh		0.2985	0.3853	0.4613
No Mesh	0.2985		0.0673	0.0818
Resorbable mesh	0.3853	0.0673		0.8737
Microporous mesh	0.4613	0.0818	0.8737	

Mineral apposition rate (MAR). The rate of mineral apposition was calculated by dividing the distance between the 2 tetracycline markers by the time interval between their administrations. The MAR was observed to be higher in the resorbable mesh group with a mean value of 2.41µ/day, followed by the group with microporous mesh which corresponded to 2.25µ/day. In the group without mesh, MAR is 2.2µ/day. The least value was noted in the group with macroporous mesh, 1.09µ/day. (figures 5-8)

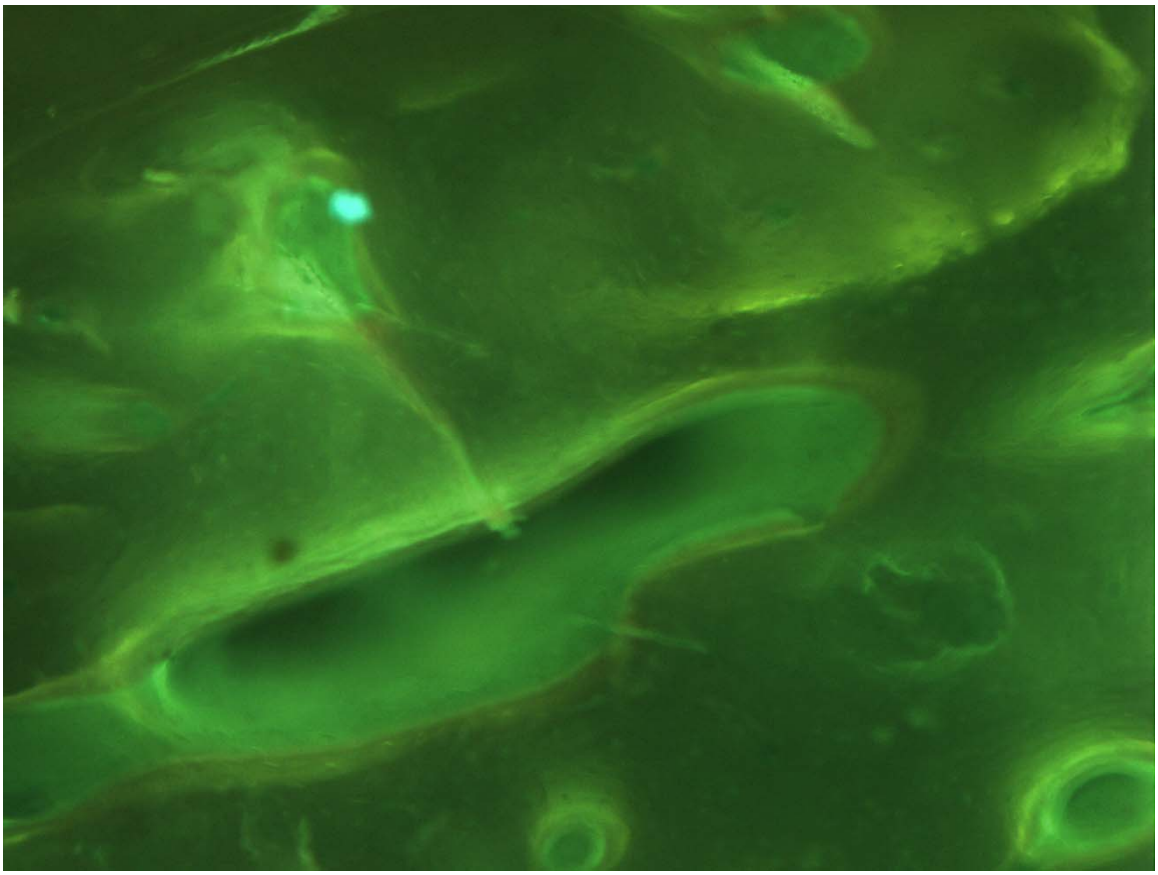

Figure 16. Tetracycline stained histological section revealing new bone formation with macroporous mesh

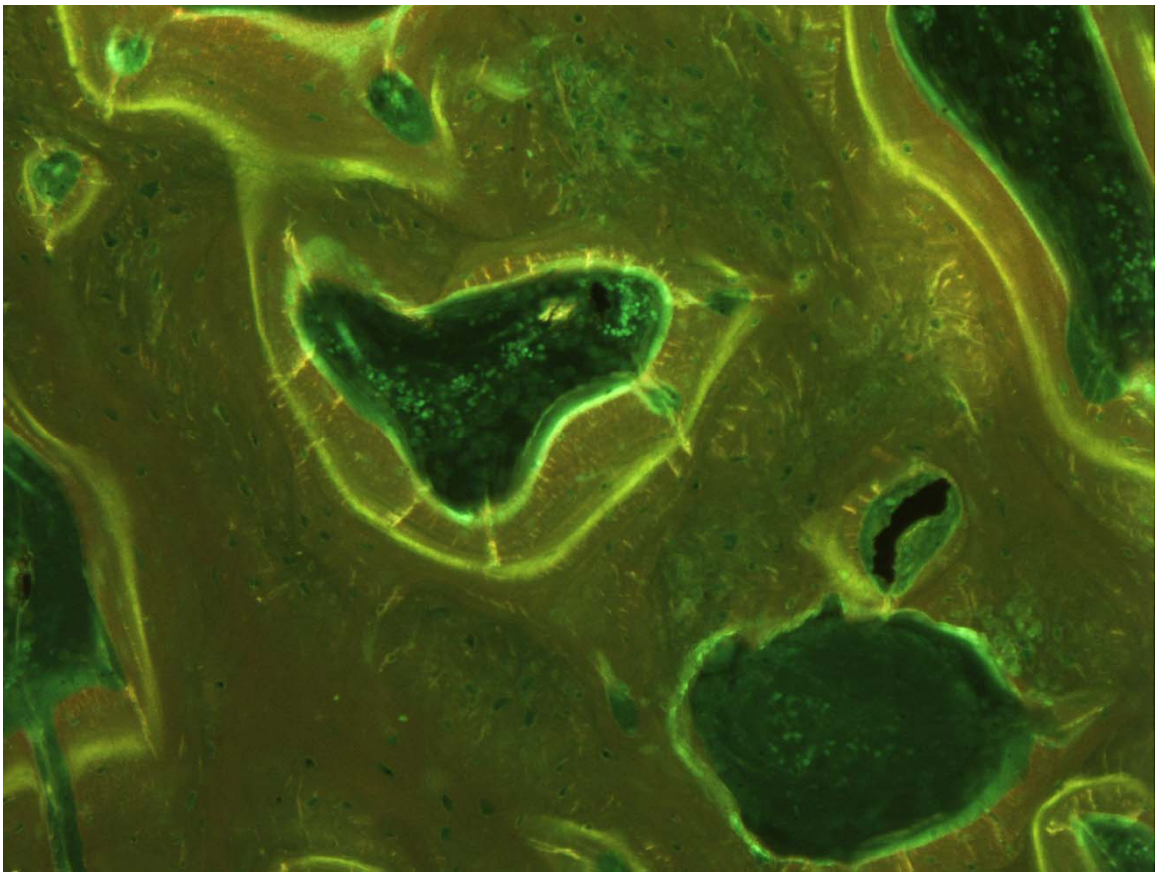

Figure 17. Tetracycline stained histological section revealing new bone formation with microporous mesh

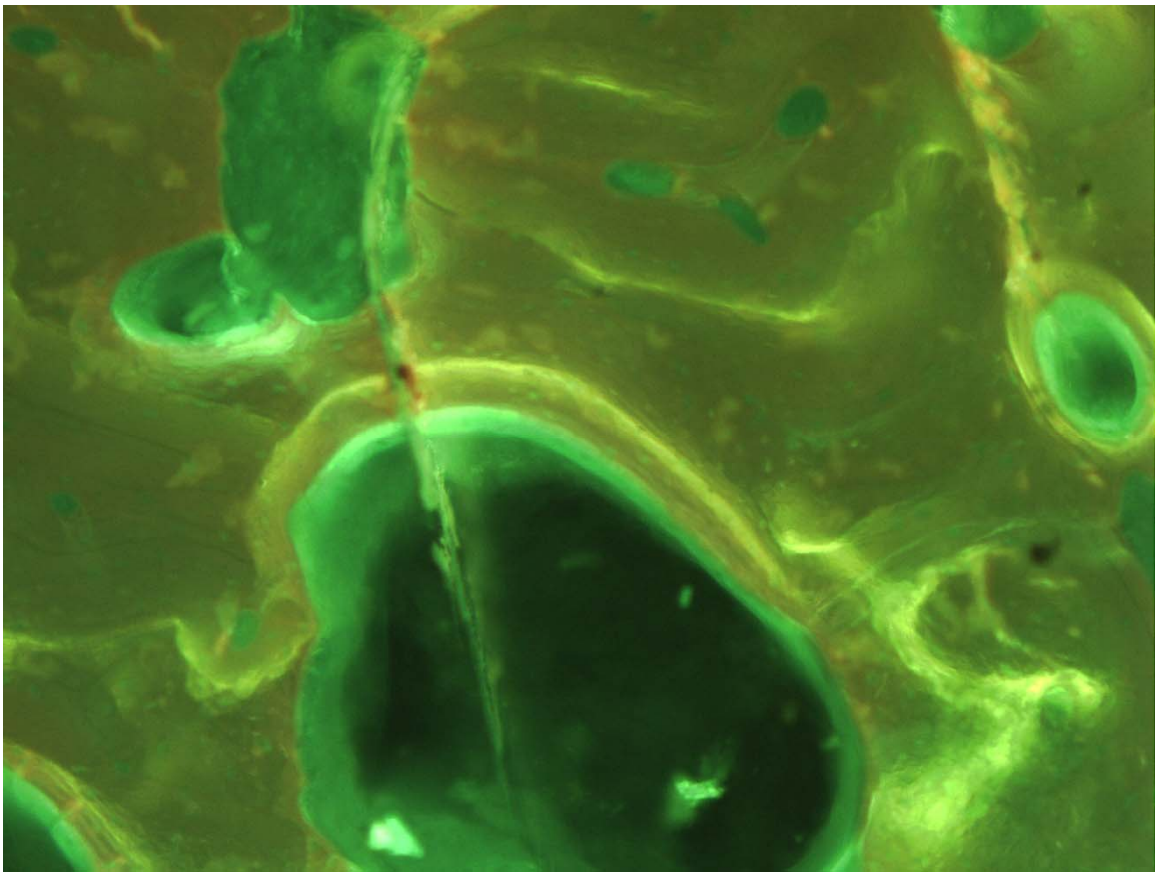

Figure 18. Tetracycline stained histological section revealing new bone formation with resorbable mesh

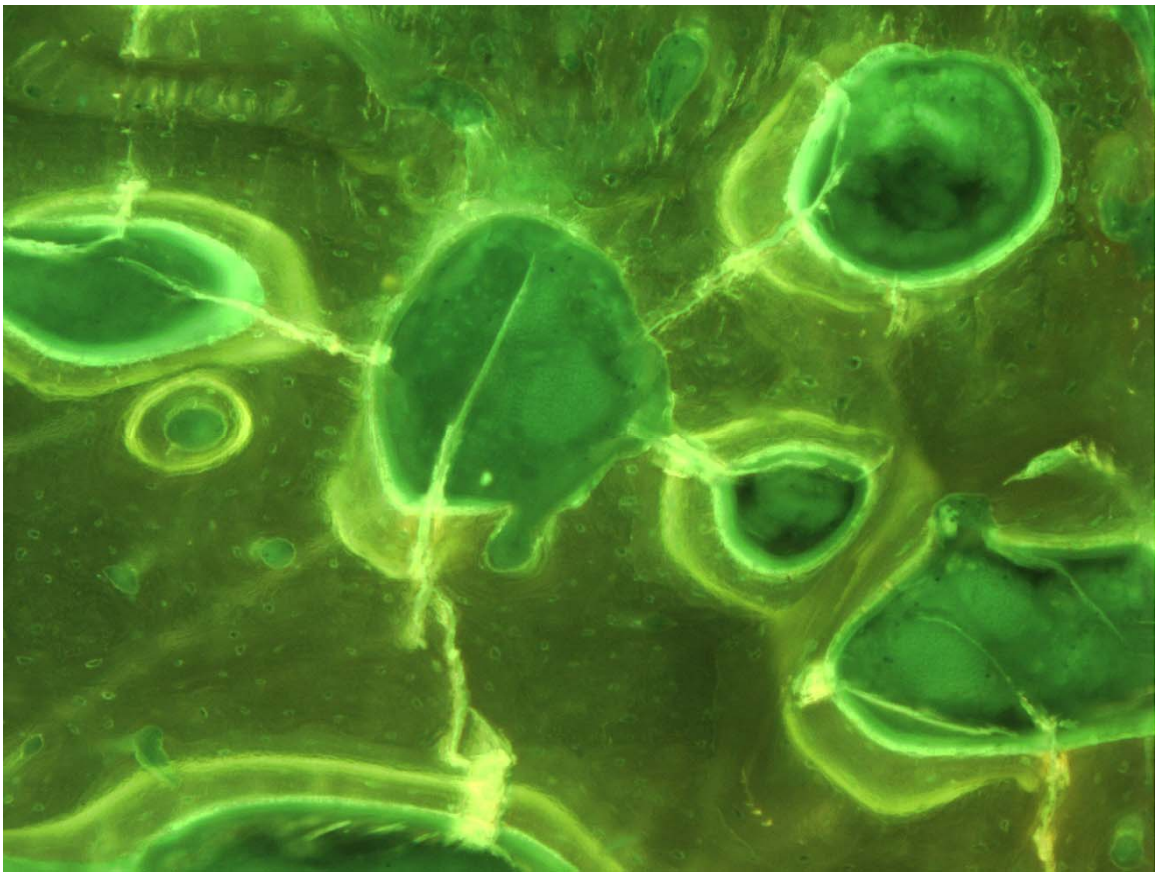

Figure 19. Tetracycline stained histological section revealing new bone formation in the site without any mesh

DISCUSSION

Reports in the literature on the effect of pore size on fibrous tissue ingrowth into porous barrier membranes are remarkably few in number. In subcutaneous implantation experiments in rats, Salvatore et al examined the soft tissue response to polyurethane sponges in six pore sizes ranging from 280µm-3.2mm [85]. He reported that implants with the smallest pore size became rapidly filled with collagen and vascular tissue. Chvapil et al suggested that pores in excess of 100µm are required for the rapid penetration of highly vascular connective tissue and small pores tend to become filled

with more avascular tissue [86]. Taylor and Smith tested two types of porous methylmethacrylate implants with average pore sizes of 42µm and 361µm [87]. They found that the smaller pore size was inadequate for the penetrations of capillaries. There is no information in the maxillofacial literature on the optimal pore size of barrier membranes for prevention of soft tissue ingrowth and allowing vascular penetration.

In on our study, we observed that there was increased quantity of bone formation in the large pore mesh compared to the small pore mesh. This finding is consistant with Bobyn et al who reported in their study that implants with the large pore size initially had greater ingrowth [88]. But at the end of 52 weeks of their study they concluded that the difference in pore size has no influence on the healing response and on clinical consequence. In their study the authors used cylindrical tantalum implants with 2 different pore sizes, the smaller pore size averaged 430µm and the larger pore size averaged 650µm. The volume porosity was about 75%-80%. The reason for using high volume porosity is to increase the maximum interfacial strength that can develop by bone ingrowth. This is in contrast to the fiber, sintered, and beaded metal coatings that have a limited volume of porosity of 30%-50%, which limits the maximum interfacial strength. In our study the pore size of the macroporous mesh was 1.2 mm. The microporous and resorbable meshes consisted of 600µm and 1000µm pore sizes respectively. The amount of bone growth between the smaller pore size meshes was not significant statistically ($p=0.5250$). Similarly, there was no statistical difference in the amount of bone growth between the small and large pore sized meshes ($p=0.1512$).

Several investigators have studied bone ingrowth into systems with different pore sizes. Klawitter et al observed bone ingrowth into porous high-density polyethylene possessing a pore size as small as 40µm [89]. Spector et al demonstrated bone ingrowth

in porous high-density polyethylene containing an average pore size of 450μm [90]. Nilles et al showed excellent bone attachement to stainless steel-void metal composites with an average pore size of 460μm [91]. They showed that a pore size of 100μm allows bone ingrowth, but a pore size greater than 150μm is required for osteon formation. Studies on the rate of bone ingrowth have mentioned that bone ingrowth would occur if the pore size is greater than 50μm [92]. These studies indicate that the optimum pore size required for bone ingrowth remains undefined; but for osteon formation the pore size should be greater than 150μm. An interesting finding in the present study is the mineral apposition rate in the mesh with large pore size. Although, this group had greater amount of bone formation compared to the other groups, the MAR was only 1.09μm. Based on this observation, we are speculating a finding that is consistent with Bobyn et al's study [88]. There might have been a faster ingrowth of bone forming cells into the mesh with large pore size. The mineral apposition might have been slower due to increased surface area through the large pores.

Micromovement between bone and implanted material has been shown to prevent bony ingrowth and result in the development of a fibrous tissue membrane, particularly if this occurs during the healing process after implantation [92-95]. During the initial 3-week healing period there should be minimal stress on the implanted barrier membrane to prevent any fibrous ingrowth. With sufficient initial stability, the early tissue infiltrate through the pores will differentiate to bone by either direct bone formation or appositional bone growth from the adjacent bone. This has been described by Spector based on his observations of tissue ingrowth into porous polymer systems [96]. Pilliar and others have demonstrated that bone can form within porous implants even with limited initial movement, provided that the site is sufficiently vascular and that no local

inflammatory reactions occur. The extent of this movement is less than 150μm [97]. In contrast to the above studies, with excellent blood supply to the maxillofacial region and despite using rigid fixation for the titanium mesh or other membranes, there have been reports associated with a thick fibrous tissue beneath the membrane [52, 54, 98].

In our study, the meshes had been secured well with a titanium screw. Also, the extraoral approach helps with stability during masticatory function. Another distinct advantage with this approach is the absence of mesh exposure. As reported in the literature, we also had noted a significant amount of soft tissue ingrowth into the mesh. However the amount of this soft tissue is found to be greater with the resorbable mesh group. This is against our expectations that the group without mesh would have more soft tissue ingrowth. In the group without mesh, the bone graft material was significantly displaced underneath periosteal flap. The displacement of bone graft material beyond the margins of the site might have also played a role in the decreased amount of bone formation. Another interesting phenomenon noted in this study is the faster mineral apposition rate associated with the group without mesh. Vascular ingrowth into the bone graft directly from the intact periosteum might have caused this phenomenon.

An area of controversy is the need for cortical perforation during guided bone regeneration for vascular supply. In one study it has been advocated that perforations in the cortical bone of the mandible provides access for bone forming cells from the bone marrow to repopulate the space created by the membrane [43]. In another study the authors noted that bone formation took place from a non-injured cortical bone surface. They have indicated that perforations are not prerequisite for new bone formation [37]. In our study, the left side of the mandible received 0.8mm cortical perforations and the right side did not receive any perforations. The results in our study support the latter theory.

We did not find any statistical difference between the amount of bone formed in the left as compared to the right side.

Many studies have used membranes to regenerate bone, but qualitative and quantitative measurements are not always recorded. Very few studies have reported on the thickness of soft tissue ingrowth following guided bone regeneration. Becker et al, Jovanovic et al, and Simion et al have reported that the soft tissue layer under the membrane and overlying the regenerated bone is thin and rarely exceeds 1mm in thickness [52, 54, 98] . Simion et al, in a clinical and histological study in humans have demonstrated that the use of titanium-reinforced e-PTFE membranes for vertical ridge augmentation resulted in bone regeneration under the membrane that was incomplete [52]. Histologic examination showed a layer of loose connective tissue about 2.1mm in mean thickness. Some of the possible explanations for the incomplete new bone formation proposed by the authors were: (1) the shrinkage of the blood clot under the membrane during the initial stage of healing, (2) the entrapment of air under the membrane; (3) micromovement of the membrane; and (4) an insufficient healing period. In our study, all the specimens had incomplete bone formation and all the reasons cited above might have played a role.

An important observation in our study is the envelope of bone formed around the mesh. This finding is noted in most of the non-resorbable mesh. This is a supportive finding and is consistent with the tent-pole effect as reported extensively in the literature. However a layer of soft tissue ingrowth was present between the mesh and the graft material. At present it is not known if the soft tissue beneath the membrane undergoes mineralization if left for a long period or if the presence of the membrane barrier is a prerequisite for the completion of mineralization. Some studies have reported the soft

tissue underneath the membrane to be a periosteum like tissue and others have reported it to be a fibrous tissue [50, 52, 53]. The vascularity of this tissue has been variable. Some authors have suggested that this tissue should be left in place after membrane removal [35]. Others propose eliminating this soft tissue layer to expose the new bone [51, 57]. In our study we found the soft tissue to be fibrous in nature with very few capillaries. This does not support earlier theories that noted the soft tissue to be periosteum like tissue. There is no evidence in this study towards mineralization of the tissue if left longer, since there is an incomplete layer of bone formed over the mesh based on the tent-pole effect.

In the resorbable group, there was evidence of incomplete hydrolysis of the mesh at the time of specimen harvest. This finding was also consistent with reports in the literature. However, complete hydrolysis of PLA/PGA polymers may last up to 36 months.

CONCLUSION

The macroporous membranes facilitated greater bone regeneration compared to microporous and resorbable membranes. The macroporous mesh also prevented significant soft tissue ingrowth compared to other meshes. Containment of the bone graft is the most critical issue in successful bone regeneration. The presence of cortical perforations did not have any effect on the quality or the quantity of regenerated bone. Further research should be directed towards identifying a critical pore size and manufacturing a reliable mesh that would prevent excessive soft tissue ingrowth in ridge augmentation procedures.

REFERENCE

1. Marcus S, Drury T, Brown L, Zion G: Tooth retention and tooth loss in the permanent dentition of adults: United States, 1988-1991. *J Dent Res* 1996;75 Spec No:684
2. Parkinson C: Similarities in resorption patterns of maxillary and mandibular ridges. *J Prosthet Dent* 1978;39:598
3. Worthington P, Rubenstein J: Problems associated with the atrophic mandible. *Dent Clin North Am* 1998;42:129
4. Callan D: Maintaining cosmetics and marginal bone with a dental implant. *Implant Dent* 2000;9:154
5. von Wowern N, Gotfredsen K: Implant-supported overdentures, a prevention of bone loss in edentulous mandibles? A 5-year follow-up study. *Clin Oral Implants Res* 2001;12:19
6. Perry R: Reconstruction of advanced mandibular resorption with both subperiosteal and root-form implants. *Implant Dent* 1998;7:94
7. Adell R, Lekholm U, Rockler B, Brånemark P: A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg* 1981;10:387
8. Adell R, Eriksson B, Lekholm U et al.: Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants* 1990;5:347

9. Buser D, Mericske-Stern R, Bernard J et al.: Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. *Clin Oral Implants Res* 1997;8:161

10. PK M: Clinical experience with osseous site development using autogenous bone, bone substitutes, and membrane barriers. *Oral and Maxillofacial Surgery Clinics of North America* 2001;13:493

11. Becker W, Urist M, Tucker L et al.: Human demineralized freeze-dried bone: inadequate induced bone formation in athymic mice. A preliminary report. *J Periodontol* 1995;66:822

12. Urist M: Bone: formation by autoinduction. *Science* 1965;150:893

13. Reddi A, Muthukumaran N, Ma S et al.: Initiation of bone development by osteogenin and promotion by growth factors. *Connect Tissue Res* 1989;20:303

14. Burchardt H: Biology of bone transplantation. *Orthop Clin North Am* 1987;18:187

15. Burchardt H: The biology of bone graft repair. *Clin Orthop Relat Res* 198328

16. Simion M, Dahlin C, Trisi P, Piattelli A: Qualitative and quantitative comparative study on different filling materials used in bone tissue regeneration: a controlled clinical study. *Int J Periodontics Restorative Dent* 1994;14:198

17. Fazili M, von Overvest-Eerdmans G, Vernooy A et al.: Follow-up investigation of reconstruction of the alveolar process in the atrophic mandible. *Int J Oral Surg* 1978;7:400

18. Mecall R, Rosenfeld A: Influence of residual ridge resorption patterns on implant fixture placement and tooth position. 1. *Int J Periodontics Restorative Dent* 1991;11:8

19. A B: Contribution to the technique in fusion operations on the spine. *Acta Orthop Scand* 1947;1:17

20. HURLEY L, STINCHFIELD F, BASSETT A, LYON W: The role of soft tissues in osteogenesis. An experimental study of canine spine fusions. *J Bone Joint Surg Am* 1959;41-A:1243

21. PJ B: Regeneration of alveolar bone beneath cellulose acetate filter implants. *J Dent Res* 1964;43:827

22. Boyne P, Mikels T: Restoration of alveolar ridges by intramandibular transposition osseous grafting. *J Oral Surg* 1968;26:569

23. Boyne P: Restoration of osseous defects in maxillofacial casualities. *J Am Dent Assoc* 1969;78:767

24. MURRAY G, HOLDEN R, ROSCHLAU W: Experimental and clinical study of new growth of bone in a cavity. *Am J Surg* 1957;93:385

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

26. MELCHER A, DREYER C: The experimental use of polytetrafluorethylene as an implant material. *S Afr J Med Sci* 1961;26:125

27. Triplett RG CS, Fields RT: Bone augmentation with and without biodegradable and nonbiodegradable microporous membranes. *Oral and Maxillofacial Surgery Clinics of North America* 2001;13:411

28. Kahnberg K: Restoration of mandibular jaw defects in the rabbit by subperiosteally implanted Teflon mantle leaf. *Int J Oral Surg* 1979;8:449

29. Aukhil I, Iglhaut J: Periodontal ligament cell kinetics following experimental regenerative procedures. *J Clin Periodontol* 1988;15:374

30. Hämmerle C, Schmid J, Olah A, Lang N: A novel model system for the study of experimental guided bone formation in humans. *Clin Oral Implants Res* 1996;7:38

31. Hjorting-Hansen E, Worsaae N, Lemons J: Histologic response after implantation of porous hydroxylapatite ceramic in humans. *Int J Oral Maxillofac Implants* 1990;5:255

32. Goodman S: The effects of micromotion and particulate materials on tissue differentiation. Bone chamber studies in rabbits. *Acta Orthop Scand Suppl* 1994;258:1

33. Triplett R, Schow S: Autologous bone grafts and endosseous implants: complementary techniques. *J Oral Maxillofac Surg* 1996;54:486

34. Lang N, Hämmerle C, Brägger U et al.: Guided tissue regeneration in jawbone defects prior to implant placement. *Clin Oral Implants Res* 1994;5:92

35. Kostopoulos L, Karring T: Augmentation of the rat mandible using guided tissue regeneration. *Clin Oral Implants Res* 1994;5:75

36. Assenza B, Piattelli M, Scarano A et al.: Localized ridge augmentation using titanium micromesh. *J Oral Implantol* 2001;27:287

37. Schliephake H, Kracht D: Vertical ridge augmentation using polylactic membranes in conjunction with immediate implants in periodontally compromised extraction sites: an experimental study in dogs. *Int J Oral Maxillofac Implants* 1997;12:325

38. Augthun M, Yildirim M, Spiekermann H, Biesterfeld S: Healing of bone defects in combination with immediate implants using the membrane technique. *Int J Oral Maxillofac Implants* 1995;10:421

39. Lekholm U, Becker W, Dahlin C et al.: The role of early versus late removal of GTAM membranes on bone formation at oral implants placed into immediate extraction sockets. An experimental study in dogs. *Clin Oral Implants Res* 1993;4:121

40. Aukhil I, Simpson D, Schaberg T: An experimental study of new attachment procedure in beagle dogs. *J Periodontal Res* 1983;18:643

41. Magnusson I, Batich C, Collins B: New attachment formation following controlled tissue regeneration using biodegradable membranes. *J Periodontol* 1988;59:1

42. Boyne PJ HA: An Algorithm for reconstruction of alveolar defects before implant placement. *Oral and Maxillofacial Surgery Clinics of North America* 2001;13:533

43. Buser D, Brägger U, Lang N, Nyman S: Regeneration and enlargement of jaw bone using guided tissue regeneration. *Clin Oral Implants Res* 1990;1:22

44. Buser D, Dula K, Hirt H, Schenk R: Lateral ridge augmentation using autografts and barrier membranes: a clinical study with 40 partially edentulous patients. *J Oral Maxillofac Surg* 1996;54:420

45. Misch C: Comparison of intraoral donor sites for onlay grafting prior to implant placement. *Int J Oral Maxillofac Implants* 1997;12:767

46. Raghoebar G, Batenburg R, Vissink A, Reintsema H: Augmentation of localized defects of the anterior maxillary ridge with autogenous bone before insertion of implants. *J Oral Maxillofac Surg* 1996;54:1180

47. Simion M, Scarano A, Gionso L, Piattelli A: Guided bone regeneration using resorbable and nonresorbable membranes: a comparative histologic study in humans. *Int J Oral Maxillofac Implants* 1996;11:735

48. Lundgren A, Sennerby L, Lundgren D: Guided jaw-bone regeneration using an experimental rabbit model. *Int J Oral Maxillofac Surg* 1998;27:135

49. Caffesse R, Smith B, Castelli W, Nasjleti C: New attachment achieved by guided tissue regeneration in beagle dogs. *J Periodontol* 1988;59:589

50. Buser D, Dula K, Belser U et al.: Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. *Int J Periodontics Restorative Dent* 1995;15:10

51. Mattout P, Mattout C: Conditions for success in guided bone regeneration: retrospective study on 376 implant sites. *J Periodontol* 2000;71:1904

52. Simion M, Trisi P, Piattelli A: Vertical ridge augmentation using a membrane technique associated with osseointegrated implants. *Int J Periodontics Restorative Dent* 1994;14:496

53. Simion M, Jovanovic S, Trisi P et al.: Vertical ridge augmentation around dental implants using a membrane technique and autogenous bone or allografts in humans. *Int J Periodontics Restorative Dent* 1998;18:8

54. Becker W, Becker B, McGuire M: Localized ridge augmentation using absorbable pins and e-PTFE barrier membranes: a new surgical technique. Case reports. *Int J Periodontics Restorative Dent* 1994;14:48

55. Chiapasco M, Abati S, Romeo E, Vogel G: Clinical outcome of autogenous bone blocks or guided bone regeneration with e-PTFE membranes for the reconstruction of narrow edentulous ridges. *Clin Oral Implants Res* 1999;10:278

56. Becker W, Dahlin C, Becker B et al.: The use of e-PTFE barrier membranes for bone promotion around titanium implants placed into extraction sockets: a prospective multicenter study. *Int J Oral Maxillofac Implants* 1994;9:31

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

58. Nowzari H, Slots J: Microbiologic and clinical study of polytetrafluoroethylene membranes for guided bone regeneration around implants. *Int J Oral Maxillofac Implants* 1995;10:67

59. Gongloff R, Cole M, Whitlow W, Boyne P: Titanium mesh and particulate cancellous bone and marrow grafts to augment the maxillary alveolar ridge. *Int J Oral Maxillofac Surg* 1986;15:263

60. Stringer D, Boyne P: Modification of the maxillary step osteotomy and stabilization with titanium mesh. *J Oral Maxillofac Surg* 1986;44:487

61. Zablotsky M, Meffert R, Caudill R, Evans G: Histological and clinical comparisons of guided tissue regeneration on dehisced hydroxylapatite-coated and titanium endosseous implant surfaces: a pilot study. *Int J Oral Maxillofac Implants* 991;6:294

62. Degidi M, Scarano A, Piattelli A: Regeneration of the alveolar crest using titanium micromesh with autologous bone and a resorbable membrane. *J Oral Implantol* 2003;29:86

63. Eisig S, Ho V, Kraut R, Lalor P: Alveolar ridge augmentation using titanium micromesh: an experimental study in dogs. *J Oral Maxillofac Surg* 2003;61:347

64. Van Steenberghe D, Johansson C, Quirynen M et al.: Bone augmentation by means of a stiff occlusive titanium barrier. *Clin Oral Implants Res* 2003;14:63

65. Schopper C GW, Moser D, Spassova E, Watzinger F, Ewers R: Long term results after guided bone regeneration with resorbable and microporous titanium membranes. *Oral and Maxillofacial Surgery Clinics of North America* 2001;13:449

66. Watzinger F, Luksch J, Millesi W et al.: Guided bone regeneration with titanium membranes: a clinical study. *Br J Oral Maxillofac Surg* 2000;38:312

67. Becker W, Becker B, Mellonig J et al.: A prospective multi-center study evaluating periodontal regeneration for Class II furcation invasions and intrabony defects after treatment with a bioabsorbable barrier membrane: 1-year results. *J Periodontol* 1996;67:641

68. Black B, Gher M, Sandifer J et al.: Comparative study of collagen and expanded polytetrafluoroethylene membranes in the treatment of human class II furcation defects. *J Periodontol* 1994;65:598

69. Sevor J, Meffert R, Cassingham R: Regeneration of dehisced alveolar bone adjacent to endosseous dental implants utilizing a resorbable collagen membrane: clinical and histologic results. *Int J Periodontics Restorative Dent* 1993;13:71

70. Zitzmann N, Naef R, Schärer P: Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. *Int J Oral Maxillofac Implants* 1997;12:844

71. Carlson-Mann L, Ibbott C, Grieman R: Ridge augmentation with guided bone regeneration and GTAM case illustrations. *Probe* 1996;30:232

72. von Arx T, Hardt N, Wallkamm B: The TIME technique: a new method for localized alveolar ridge augmentation prior to placement of dental implants. *Int J Oral Maxillofac Implants* 1996;11:387

73. Sumi Y, Miyaishi O, Tohnai I, Ueda M: Alveolar ridge augmentation with titanium mesh and autogenous bone. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;89:268

74. von Arx T, Wallkamm B, Hardt N: Localized ridge augmentation using a micro titanium mesh: a report on 27 implants followed from 1 to 3 years after functional loading. *Clin Oral Implants Res* 1998;9:123

75. Malchiodi L, Scarano A, Quaranta M, Piattelli A: Rigid fixation by means of titanium mesh in edentulous ridge expansion for horizontal ridge augmentation in the maxilla. *Int J Oral Maxillofac Implants* 1998;13:701

76. Maiorana C, Santoro F, Rabagliati M, Salina S: Evaluation of the use of iliac cancellous bone and anorganic bovine bone in the reconstruction of the atrophic maxilla with titanium mesh: a clinical and histologic investigation. *Int J Oral Maxillofac Implants* 2001;16:427

77. Breine U, Brånemark P: Reconstruction of alveolar jaw bone. An experimental and clinical study of immediate and preformed autologous bone grafts in combination with osseointegrated implants. *Scand J Plast Reconstr Surg* 1980;14:23

78. Malm T, Bowald S, Bylock A, Busch C: Prevention of postoperative pericardial adhesions by closure of the pericardium with absorbable polymer patches. An experimental study. *J Thorac Cardiovasc Surg* 1992;104:600

79. Malm T, Bowald S, Bylock A et al.: Regeneration of pericardial tissue on absorbable polymer patches implanted into the pericardial sac. An immunohistochemical, ultrastructural and biochemical study in the sheep. *Scand J Thorac Cardiovasc Surg* 1992;26:15

80. Miller N, Williams D: On the biodegradation of poly-beta-hydroxybutyrate (PHB) homopolymer and poly-beta-hydroxybutyrate-hydroxyvalerate copolymers. *Biomaterials* 1987;8:129

81. Gotfredsen K, Nimb L, Hjørting-Hansen E: Immediate implant placement using a biodegradable barrier, polyhydroxybutyrate-hydroxyvalerate reinforced with polyglactin 910. An experimental study in dogs. *Clin Oral Implants Res* 1994;5:83

82. Bergsma J, de Bruijn W, Rozema F et al.: Late degradation tissue response to poly(L-lactide) bone plates and screws. *Biomaterials* 1995;16:25

83. Böstman O, Hirvensalo E, Mäkinen J, Rokkanen P: Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. *J Bone Joint Surg Br* 1990;72:592

84. Hutmacher D, Hürzeler M, Schliephake H: A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. *Int J Oral Maxillofac Implants* 1996;11:667

85. SALVATORE J, GILMER WJ, KASHGARIAN M, BARBEE W: An experimental study of the influence of pore size of implanted polyurethane sponges upon subsequent tissue formation. *Surg Gynecol Obstet* 1961;112:463

86. Chvapil M, Holusa R, Kliment K, Stoll M: Some chemical and biological characteristics of a new collagen-polymer compound material. *J Biomed Mater Res* 1969;3:315

87. Taylor D, Smith F: Porous methyl methacrylate as an implant material. *J Biomed Mater Res* 1972;6:467

88. Bobyn J, Stackpool G, Hacking S et al.: Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *J Bone Joint Surg Br* 1999;81:907

89. Klawitter J, Bagwell J, Weinstein A, Sauer B: An evaluation of bone growth into porous high density polyethylene. *J Biomed Mater Res* 1976;10:311

90. Spector M, Flemming W, Kreutner A: Bone growth into porous high-density polyethylene. *J Biomed Mater Res* 1976;10:595

91. Niles J, Coletti JJ, Wilson C: Biomechanical evaluation of bone-porous material interfaces. *J Biomed Mater Res* 1973;7:231

92. Welsh R, Pilliar R, Macnab I: Surgical implants. The role of surface porosity in fixation to bone and acrylic. *J Bone Joint Surg Am* 1971;53:963

93. Cameron H, Pilliar R, MacNab I: The effect of movement on the bonding of porous metal to bone. *J Biomed Mater Res* 1973;7:301

94. Ducheyne P, De Meester P, Aernoudt E: Influence of a functional dynamic loading on bone ingrowth into surface pores of orthopedic implants. *J Biomed Mater Res* 1977;11:811

95. Heck D, Nakajima I, Kelly P, Chao E: The effect of load alteration on the biological and biomechanical performance of a titanium fiber-metal segmental prosthesis. *J Bone Joint Surg Am* 1986;68:118

96. M S: Bone ingrowth into porous polymers. In *Biocompatibility of orthopedic implants*, (ed. Boca Raton: CRC Press, 1982:55.

97. Pilliar R, Cameron H, Welsh R, Binnington A: Radiographic and morphologic studies of load-bearing porous-surfaced structured implants. *Clin Orthop Relat Res* 1981249

98. Jovanovic S, Nevins M: Bone formation utilizing titanium-reinforced barrier membranes. *Int J Periodontics Restorative Dent* 1995;15:56

APPENDIX

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Office of the Provost

NOTICE OF APPROVAL

DATE: March 10, 2005

TO: Patrick J. Louis, D.D.S., M.D.
SDB-419 0007
FAX: 975-6671

FROM: Suzanne M. Michalek, Ph.D., Vice Chair
Institutional Animal Care and Use Committee

SUBJECT: Title: The Effect of Varyng Pore Size of Barrier Membranes on Bone Graft Healing in Beagle Dogs
Sponsor: Internal
Animal Project Number: 050207461

On February 23, 2005, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

Species	Use Category	Number in Category
Dogs	B	10

Animal use is scheduled for review one year from February 2005. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 050207461 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.

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