TESTING *IN VIVO* THE COMPARABILITY OF TWO DIFFERENT SCAFFOLDS IN ALVEOLAR RIDGE PRESERVATION AGAINST GEISTLICH BIO-OSS® XENOGRAFT AND CONTROL IN THE RAT MODEL

A Thesis

by

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ABSTRACT

Aim: Ridge preservation is a common procedure performed in Periodontics to prevent ridge resorption following tooth extraction. The aim of this present investigation is to compare *in vivo* the use of bioengineered Polycaprolactone (Polydopamine Coated and Uncoated) scaffolds against a commonly used xenograft, Bio-Oss®, and Control (no material) in ridge preservation procedures following first molar extraction in the rat model.

Materials and Methods: 33 male Sprague Dawley rats were used in the study. The maxillary right and left first molars were extracted and a standardized defect was created in the extraction socket using a slow speed handpiece and a #703 fissure bur. The rats were equally divided and randomly selected to receive one of four ridge preservation materials: Control (no material), Bio-Oss®, Polycaprolactone (Polydopamine Coated) scaffold, or Polycaprolactone (Uncoated) scaffold. All extraction sites were covered by a resorbable collagen membrane. Rats were randomly allocated to either be in the four week or eight week experimental groups. The rats were sacrificed, and the maxillae were dissected and hemisected into right and left halves. The tissues were processed and analyzed.

Results: In comparing radiographs, at 4 weeks, the difference in average bone height at extraction sockets was not statistically significant ($p > 0.05$) for any of the materials tested. At 8 weeks, the difference in average bone height at extraction sockets was statistically significant ($p < 0.05$) between Control and Bio-Oss®, Polycaprolactone (Uncoated), and Polycaprolactone (Polydopamine Coated). Micro-CT analysis revealed greater bone fill in the extraction sockets in the 8-week samples compared with the 4-week samples, and all samples showed a loss of residual bone height. Paraffin sections demonstrated that Control, Bio-Oss®, and Polycaprolactone (Uncoated) healed by direct ossification while Polycaprolactone (Polydopamine Coated) healed

by endochondral ossification. New mineralized tissue formation was seen inside the fibrous connective tissue in all 8-week ground section samples.

Conclusion: Both Polycaprolactone (Polydopamine Coated and Uncoated) scaffolds performed comparably to Bio-Oss® as a ridge preservation material over the Control (no material). Though new bone formation was seen with all treatment modalities, there was an improvement seen in using grafts over not using a graft.

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Dr. John Michael Bozanich, the student, was directly involved in all aspects of the study. Dr. Thomas Diekwisch performed all extraction and ridge preservation surgeries on the rat subjects. Dr. Mirali Pandya and Ms. Connie Tillberg aided in laboratory processes. Dr. Thomas Diekwisch and Dr. Mirali Pandya helped analyze and interpret the results. All other work conducted for this thesis was completed by Dr. John Michael Bozanich independently.

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NOMENCLATURE

HA	Hydroxyapatite
BMPs	Bone morphogenetic proteins
FGF	Fibroblast growth factor
IGF	Insulin-like growth factor
PDGF	Platelet-derived growth factor
RANK	Receptor activator of nuclear factor κB
RANKL	Receptor activator of nuclear factor κB ligand
OPG	Osteoprotegerin
IL-6	Interleukin 6
VEGF	Vascular endothelial growth factor
BMU	Basic multicellular unit
GH	Growth hormone
IGF	Insulin-like growth factor
ABB	Anorganic bovine bone
DBBM	Deproteinized bovine bone mineral
NCSs	Natural coral skeletons
GBR	Guided Bone Regeneration
β-TCP	Beta tricalcium phosphate
SMP	Shape memory polymer
PCL	Poly(ε-caprolactone)
GTR	Guided tissue regeneration
PTFE	Polytetrafluoroethylene

CBCT	Cone-beam computed tomography
IACUC	Institutional Animal Care and Use Committee
NIH	National Institute of Health
SEM	Scanning electron microscopy
3D	Three-dimensional
EDTA	Ethylenediaminetetraacetic acid
H&E	Hematoxylin and Eosin

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1. INTRODUCTION AND LITERATURE REVIEW

Background and Introduction

Extraction and ridge preservation is a common procedure in the field of Periodontics. When extracting non-restorable teeth without ridge preservation of some sort, the alveolar ridge will resorb and leave an insufficient amount of bone width and bone height for a final restoration.[1, 2]

Currently, there are several different graft options routinely used: autografts (bone grafts from self), allografts (bone grafts from cadavers of the same species), xenografts (bone grafts from differing species), and other bioengineered alloplasts and scaffolds.

All treatment options have advantages and disadvantages. Autografts contain "self" cells necessary for regeneration, but they require a second surgical site that can lead to post-operative pain and complications.[3] Allografts are readily available and of unlimited supply, but occasionally have variable content from graft to graft which can contribute to unpredictable results following augmentation procedures.[4,5] Furthermore, there are patients who choose not to use cadaveric bone for personal reasons.[6,7] Xenografts have similar considerations as allografts.[8] Scaffolds are advantageous over the bone grafts mentioned above as they can theoretically be bioengineered individually per clinical situation to have properties that are efficacious in the replacement of lost osseous tissue.[9] Currently, not all characteristics of the ideal alloplastic bone graft substitute have been discovered,[9] and it is through experimentation that researchers and clinicians can further develop materials and techniques to advance the practice of Periodontology.

Structure of Bone

Osseous tissue is specialized connective tissue composed of approximately one third organic and two thirds inorganic elements along with highly specialized cells that regulate its stability.[10]

The organic matrix of bone consists of primarily collagen type I (90%)[11] and 10% non-collagenous proteins, glycoproteins, proteoglycans, carbohydrates, and lipids.[10] The cell adhesion proteins, osteopontin and bone sialoprotein, are important for the adhesion of both osteoblasts and osteoclasts.[12] Other non-collagenous protein includes: osteocalcin, osteonectin, bone morphogenetic protein, phosphoproteins, and proteoglycans.[13] The principal inorganic constituents of the osseous matrix are hydrated calcium and phosphate in the form of hydroxyapatite (HA) crystals,[10] as well as hydroxyl, carbonate, citrate, and small amounts of other ions (sodium, magnesium, and fluoride).[14]

Osteoblasts synthesize the organic matrix of bone. While it is still unmineralized, it is known as osteoid. As the matrix matures, mineral nucleation occurs within the collagen fibers as calcium and phosphate are laid down to produce HA crystals. Non-collagenous proteins on the surface of collagen fibers assist in the complete mineralization of the matrix.[10]

Mineralization of osteoid typically happens a few days after the laying down of calcium and phosphate, however, it takes several months for the maturation to be complete.[10] The mineralization of bone serves two main purposes; to provide bone with rigidity and strength to resist load and protect organs and to store minerals that contribute to homeostasis of the body.[10]

Mature bone is made up of cortical (compact) and cancellous (trabecular) bone. Within the lamellar bone, individual osteons with blood and nerve supply can be observed.[15] Osteons, or haversian systems, are the basic structural unit of mature bone. They are longitudinally oriented

cylindrical structures with haversian (vascular) canals in the center[3] and are responsible for nourishing the interior bone that cannot be supplied by surface vessels. They are found primarily in the outer cortical plates and the alveolar bone proper.[16]

Four different types of cells regulate bone formation, maintenance, and repair: osteoblasts, osteocytes, bone lining cells, and osteoclasts. A sufficient blood supply is necessary for the aforementioned functions. Thus, angiogenesis is a prerequisite for not only bone formation, but for maintenance and repair as well.[3]

The osteoblast is the primary cell responsible for bone formation. They produce the organic extracellular matrix of bone and control mineralization.[10] Osteoblasts are cuboidal cells that form a single layer to cover all endosteal and periosteal surfaces where bone formation is active.[17] Bone growth occurs by the apposition of an organic bone matrix that is deposited unilaterally by osteoblasts.

The osteoblasts lack the capacity for migration and proliferation. Undifferentiated mesenchymal cells and osteoprogenitor cells (present in bone marrow, endosteum, and periosteum) migrate to a site and proliferate and differentiate to become osteoblasts.[10] This differentiation is dependent on growth factors such as bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF).[10] Osteoblasts may eventually differentiate into two different types of cells, osteocytes and bone lining cells.[10]

Osteoblasts become osteocytes by inversion of matrix secretion or by entrapment from nearby osteoblasts.[3] The osteocytes are enclosed in mineralized spaces called lacunae and maintain a network of cytoplasmic processes known as dendrites.[10] These cytoplasmic projections extend into cylindrical compartments referred to as canaliculi.[18] The canaliculolacunar system allows for the exchange of oxygen and nutrients and is essential for cell

survival because diffusion of nutrients and wastes through the mineralized bone matrix is nearly impossible.[3] The system is also an extracellular and intracellular communication channel with neighboring osteocytes, osteoblasts, and bone lining cells. This communication network is sensitive to stress caused by the inflow of fluid resulting from mechanical stimuli and bone deformation.[10] There are limitations to the canaliculolacunar transport system in mammals, and the critical distance to keep osteocytes alive is approximately 100 μm. This is the reason for why wall thickness of osteons in cancellous bone rarely exceeds 100 μm.[19]

Osteocytes orchestrate the remodeling processes in bone by translating mechanical signals into biochemical mediators that assists in the anabolic and catabolic events that occur. These signals are also important in the regulation of blood calcium levels.[20]

Bone lining cells are elongated, inactive osteoblasts that cover a large fraction of the bone surface. They are sometimes referred to as inactive or resting osteoblasts.[3] These cells are located on surfaces that are characterized by having a low modelling activity (resting surface) as well as on surfaces where osteoclasts are active. It is proposed that bone lining cells clean up after osteoclasts by engulfing and digesting demineralized bone collagen fibrils protruding from resorption lacunae. This prepares the site for bone apposition by osteoblasts, where they enter and lay down a new layer of bone matrix. Bone lining cells are vital in the sequence of events that lead to bone resorption and bone apposition.[21]

Bone formation is consistently coupled to bone resorption, and osteoclasts are the principal bone resorbing cells.[10] They are large, multi-nucleated cells,[15] and are a member of the monocyte / macrophage hematopoietic family. The most notable feature of active osteoclasts is their ruffled membrane.[22] Hydrogen ions and proteolytic enzymes are secreted from the ruffled border which dissolve the mineral crystals and degrade the organic bone matrix[3] and calcified cartilage. The matrix degradation process results in the formation of extracellular compartments

known as Howship's lacuna.[23] Another characteristic feature of osteoclasts are the presence of numerous cell surface receptors, including those for receptor activator of nuclear factor κB (RANK), macrophage colony-stimulating factor, and calcitonin.[3] The activity of osteoclasts can be regulated and modified indirectly by parathyroid hormone, or directly by calcitonin.

The periosteum is a fibrous sheath that lines the outer surface of long bones (excluding articulating surfaces) and the endosteum lines the inner surfaces of all bones. It is divided into an outer "fibrous layer" (dense, fibrous, and vascular) and an inner "osteogenic layer" (loosely arranged connective tissue) where osteoprogenitor cells originate from.[10] Osteoblasts derived from the "osteogenic layer" of the periosteum are responsible for increasing the width and size of bones.[10] Unlike osseous tissue, the periosteum has nerve endings which makes it sensitive to manipulation. It also allows the passage of blood vessels and lymphatics into and out of bone.[10]

The bone marrow is interspersed within the trabecular bone and consists of hematopoietic tissue, stroma cells, and adipose tissue. Bone marrow is comprised of both red marrow (hematopoietic tissue) and yellow marrow (adipose tissue). It is the major hematopoietic organ responsible for the production of erythrocytes, granulocytes, monocytes, lymphocytes, and platelets.[10] The stroma contains mesenchymal stem cells that can differentiate into a variety of cell types such as: osteoblasts, chondroblasts, adipocytes, myocytes, beta-pancreatic islet cells, neuronal cells, and hematopoietic stem cells that give rise to erythrocytes, leukocytes, and platelets.[10]

Bone Development

During embryogenesis, bones of the skeleton form either by a direct or an indirect ossification process.[10] In a process known as intramembranous osteogenesis, an ossification

center develops directly by mesenchymal condensation. The extracellular matrix matures, and osteoprogenitor cells differentiate into osteoblasts. As appositional growth occurs, osteoblasts become entrapped in the mineralizing matrix giving rise to the osteocyte lacunocanalicular network.[10] The skull, mandible, maxilla, and clavicle are all formed directly through intramembranous osteogenesis.

In a process known as endochondral osteogenesis, bones develop indirectly by a cartilaginous template that becomes mineralized, later is resorbed, and ultimately is replaced by bone.[10] This leads to the formation of primary and secondary ossification centers that are separated by cartilaginous growth plates.[10] The long bones, mandibular condyle, and vertebrae are all formed indirectly through endochondral osteogenesis.[10]

The Alveolar Process and the Alveolar Bone Proper

The alveolar process is the portion of the maxilla and mandible that forms the dental sockets (alveoli).[16] It forms in harmony with the development and eruption of teeth, and it also disappears gradually after the loss of teeth.[24,25] The alveolar process consists of an external plate of cortical bone (formed by haversian bone and compacted bone lamellae) and an inner socket wall of thin, compact bone, called the alveolar bone proper. The alveolar bone proper is seen in radiographs as the lamina dura.[16]

At sites on the ridge where teeth erupt in a "normal" position, hard tissue will be present on both the buccal (facial) and lingual (palatal) aspects of the root. However, when teeth erupt with a more buccal (facial) inclination, the alveolar process will be thin and possibly non-existent, creating dehiscences and fenestrations.[24] It should be noted that the height and width of

the bone varies considerable from subject to subject and from site to site within the same individual.[24]

The tooth and surrounding tissues (the cementum, periodontal ligament, and bundle bone) form a functional unit. Therefore, forces occurring during mastication are transferred from the crown of the tooth, down the root, to the soft tissue attachment, and finally dispersed to the surrounding load-carrying osseous tissue.[24]

Healing of Bone: Repair and Regeneration

Repair is the healing of injured tissue that leads to the formation of new tissue that is different in morphology and function from that of the original. Regeneration is the replacement of lost tissue so that structure and function are fully restored. Regeneration can be divided into physiologic regeneration and reparative regeneration.[3] The healing of osseous tissue usually includes both repair and regeneration depending on the injury.[10]

When bone is damaged, a multistage healing process begins to facilitate repair. Tissue and cell proliferation are mediated by various growth factors, inflammatory cytokines, and signaling molecules.[10] Bone repair can be divided into three phases: inflammation, reparative, and remodeling.[26]

Immediately after tissue injury, the inflammation phase begins and lasts approximately two weeks.[27] The first step in the repair process is formation of a blood clot. Injured cells then release cytokines to recruit inflammatory cells, allowing for damaged tissue and cells to be phagocytized by macrophages. Cells from myeloid and mesenchymal cell lineages are also recruited to the injury site where they begin to differentiate into osteoblasts and chondroblasts. This causes a reduction in the RANKL-to-osteoprotegerin (OPG) ratio.[10]

During the reparative phase, osteoblasts and chondroblasts produce a protein scaffold that initially forms a soft callus, eventually becoming mineralized to form a hard callus. The hard callus is comprised of immature wove bone. The formation of the hard callus is mediated through up regulation of interleukin 6 (IL-6), OPG, vascular endothelial growth factor (VEGF), and BMPs. It takes approximately six to twelve weeks to transition from soft to hard callus following bone injury.[10]

During the final remodeling stage, the woven bone matrix and cartilage are remodeled into mature lamellar bone. This is done through normal bone turnover from osteoblast-osteoclast coupling. The time for remodeling varies depending on an individual's bone metabolism, but usually requires months from the injury.[10]

Bone is a dynamic tissue that is constantly remodeled in response to mechanical loading, serum calcium levels, and in response to multiple paracrine and endocrine factors in a process known as physiologic regeneration. This process occurs in both cortical and cancellous bone.[28]

Reparative regeneration replaces tissues that are lost to injury or disease. Osseous tissue has a remarkable regenerative potential to restore its original mechanical properties and architecture. The reconstruction of the original tissue occurs sequentially and closely repeats the pattern seen during development and growth. There are challenges to this process such as needing sufficient blood supply, having mechanical stability, and competing with other tissues of highly proliferative activity.[3]

Bone lesions activate localized bone regeneration by the release of growth factors and other signaling molecules. In orthotopic bone formation, osteoprogenitor cells from nearby bone marrow stroma, periosteum, endosteum, and intracortical canals respond to the inductive signals and proliferate and differentiate into osteoblasts. The lag phase is roughly one to three days, and newly formed bone is laid down directly on the preexisting bony surface.[3]

There are two steps in the regeneration of bone, osteogenesis, the predominant factor, and resorption. Resorption removes damaged and necrotic bone and remodels newly deposited bone, particularly in the conversion of immature woven bone to mature lamellar bone.[29] This process involves osteoblastic-osteoclastic coupling of bone formation and bone resorption in a "basic multicellular unit" (BMU).[28] In BMUs, osteoclasts first resorb bone over a period of three to four weeks. Cellular signals are then sent to osteoblasts to be recruited to the area. It takes osteoblasts roughly three to four months to reform the bone.[10]

Treatment to promote bone regeneration include bone grafting from different materials, epithelial-occlusive barrier membranes, anabolic agents, anti-resorptive agents, and growth factors to promote osteoblastic differentiation and proliferation.[10]

Dimensional Changes Following the Extraction of Teeth

The loss of teeth will result in a series of adaptive changes in the newly edentulous site of the ridge. Pietrokovski and Massler (1967) studied the dimensional changes in the ridge following the removal of a single tooth. They looked at dental casts where one tooth was missing on one side of the jaw. They concluded that the amount of hard and soft tissue resorption following the loss of a single tooth was significant and that the reduction of the ridge was twice as big on the buccal or facial aspect as it was along the lingual or palatal aspect in all sites studied. Tissue modeling shifted the center of the edentulous site towards the lingual or palatal aspect of the ridge.[1] Similar findings were found by Schropp *et al.* (2003). Through the use of dental stone casts and clinical examinations, they observed the hard and soft tissue changes following the extraction of premolars and molars over the course of a year. They found that at three months, the bucco-lingual / -palatal dimensions were reduced by 30%, and by

twelve months it was reduced by 50% of the original width. Furthermore, the vertical height of the buccal bone was also reduced.[2]

The amount of buccal bone resorption is also dependent on the thickness of the buccal plate. In early resorption studies of less than four months, Sanz *et al.* (2010) observed that buccal plates that were less than 1 mm wide had substantially more dimensional changes horizontally and vertically than buccal plates that were greater than 1 mm.[30] One must also consider that buccal bone in the anterior region is frequently less than 1 mm wide.[31] As exemplified through CBCT analysis, Kan *et al.* (2011) has shown that 81.1% of all maxillary anterior roots are positioned against the labial cortical plate, and 0.7% of the time maxillary anterior roots are positioned against the palatal cortical plate.[32] Therefore, it can be anticipated that if there is tooth loss in the anterior region, marked dimensional change in the horizontal and vertical dimensions will occur, and that will cause esthetic concerns.[24]

Alterations that Occur in the Alveolar Process Following Extraction

The earliest studies of the healing response following dental extractions used the animal canine model. Schram (1929) used dogs with sound dentition in his experiment to study the repair process following surgical intervention in the maxillary dentition. The maxillary first premolars were removed by simple forceps extraction. The animals were euthanized at various times, and the extraction sites were studied histologically. In looking at the simple extractions at eight days, the socket was filled with a blood clot, and the surface was completely covered with epithelium. Connective tissue extended from the bony walls of the socket, and the central part of the clot contained red blood cells in all stages of degeneration. Schram noted absorption taking place along the entire alveolar wall with evidence of new bone being laid down. At fourteen

days, the connective tissue projecting from the bottom and sides of the socket was calcified and had been converted into new spongy (cancellous) bone. At twenty-one days, the socket was almost completely filled with cancellous new bone while some remnants of the original clot remained in the center of the wound. At forty-eight days, the rebuilding of the socket was essentially complete.[33]

Claflin (1936) also looked at histologic findings concerning the undisturbed healing of extraction wounds in dogs and came up with similar observations and conclusions. The formation of a blood clot was the first step in tissue regeneration and healing. Fibroblasts began to proliferate into the blood clot by the end of the third day. Osteoclasts were also found at the alveolar crest on the third day. Bone regeneration was first noted in the apical one third of the socket beginning on the fifth day, and the sockets were completely filled with new bone thirty-one days after extraction. Osteoclastic action was also still seen taking place below the cribriform plates of the socket after the extraction wound had healed for thirty-one days.[34]

Cardaropoli *et al.* (2003) provided some of the most in-depth detail of the phases of socket healing, including modeling and remodeling, in the dog model. Nine mongrel dogs were used in the study. The distal root of the mandibular fourth premolars were extracted, and biopsies of the experimental sites were taken at various times up to six months.[35] It is important to note that the phases of healing in the dog model are more rapid than that in humans. The authors saw that oftentimes the socket was completely healed within two to three months.[24]

Blood from severed vessels immediately filled the socket following extraction. Proteins from the damaged cells and vessels led to the formation of a fibrin network which entrapped erythrocytes and platelets to form a clot.[35] The blood clot acted as a physical matrix that directed cellular movement. The blood clot also contained growth factors that were important in the healing process.[24] Although, the blood clot was crucial during the initial phase of healing, its

removal was necessary to allow the formation of new tissues. Within a few days following extraction, the blood clot was broken down in a process called fibrinolysis.[24]

During days one to three, neutrophils and macrophages migrated to the extraction socket to rid the site of damaged cells and bacteria before new tissue could be formed. The macrophages also released growth factors and cytokines that promoted the migration, proliferation, and differentiation of mesenchymal stem cells.[24] Once the site had been cleansed, the neutrophils underwent apoptosis (programmed cell death) and were removed by the macrophages. The macrophages then exited the site.[24]

After three days, the blood clot had been replaced by granulation tissue. The granulation tissue was comprised of vascular structures and fibroblast-like cells from the bone marrow and periodontal ligament.[35] After seven days of healing, the wound had undergone marked changes.[35] During this time, the fibroblast-like cells released growth factors, had proliferated, and deposited a new extracellular matrix (fibroplasia) that guided the ingrowth of cells. The vascular structures expanded (angiogenesis) and provided the oxygen and nutrients needed for increasing the number of new cells in the tissue. Fibroplasia and angiogenesis were needed to establish a provisional connective tissue that replaced the granulation tissue.[24]

After fourteen days of healing, the marginal portion of the socket was lined by connective tissue that was rich in vasculature and inflammatory cells.[35] Osteoprogenitor cells migrated to these vessels and differentiated into osteoblasts to produce the osteoid. As osteoblasts continued to lay down bone, these cells became entrapped within the matrix eventually becoming osteocytes. The first set of osteons, the primary osteons, were organized in the newly formed woven bone.[24]

At thirty days of healing, it was observed that the marginal soft tissue harbored a fibrous connective tissue lined by keratinized epithelium. Most of the socket was lined by newly formed

woven bone (primary bone spongiosa) that was continuous with the old bone of the extraction socket walls.[35] The primary bone spongiosa was important for tissue modeling because it offered a stable scaffold, a source of osteoprogenitor cells, and an ample blood supply. At this point, the initial phase of healing, tissue modeling, was now complete.[24]

At sixty days of healing, it was observed that woven bone with its primary osteons had been replaced with lamellar bone and bone marrow through the actions of BMUs. Osteoclasts created a resorption front known as the reversal line, and it was from this line that new bone was formed with secondary osteons. It took several months until all woven bone in the extraction socket had been replaced by lamellar bone.[24]

Healing became complete by the formation of a hard tissue cap that closed the entrance to the socket. The cap was initially comprised of woven bone but was later remodeled and replaced by lamellar bone to become continuous with adjacent cortical bone in a process known as corticalization.[24] The site continued to adapt to functional demands, and since it was not subject to forces of mastication and occlusal contacts, it was remodeled mainly into marrow.[24]

Huebsch *et al.* (1952) examined the healing response radiographically as well as histologically after the simple extraction of mandibular left first molars in the Long-Evans strain of rats. They came up with the following observations: the blood coagulum began twenty-three hours after the extraction by evidence of capillary ingrowth. Fibrocytes invaded the socket three days after extraction and began forming immature connective tissue. Initial bone formation was seen on the fifth day in the fundus of the alveolus. By the thirteenth day, young bone almost completely filled the socket. On the twenty-fifth day, new bone had formed a cortical layer between the lingual and buccal crests. By this time, bone healing had been complete. Bone formation was first visible radiographically on the sixteenth day at the apical portion of the socket, while the coronal portion of the socket was still somewhat radiolucent. At thirty days

following extraction, the socket showed a homogenous continuity with the adjacent part of the mandible. Huebsch *et al.* found the characteristics of histogenesis of repair to be in accordance with observations of other species.[36]

Glickman *et al.* (1947) studied the effects of extraction socket healing when leaving root remnants or bone fragments in the rat model. Much of the previous studies focused on uncomplicated extraction healing. Glickman *et al.* wanted to observe what happens when complications arise. Glickman *et al.* extracted the maxillary right first molar only, leaving some root remnants behind in fourteen rats and compared it to four rats where they extracted all crown and root remnants of the maxillary right and left first molars. The rats were sacrificed at varying time points up until one hundred and eight days. They concluded that retained root remnants and bone fragments slow the progress of post-extraction healing by interfering with surface closure and / or secondary inflammatory changes. Of note is that it is the position of the root remnant in relation to the surface which determines the response of the adjacent tissues. Root remnants deep in the wound are generally well tolerated by the surrounding tissues, whereas remnants close to the surface prevent epithelial closure by increasing inflammation. Glickman *et al.* however noted that with deep root remnants, there could be pathologic changes in the presence of an intact healed epithelial surface that makes the retention of theses root remnants undesirable.[37]

Steinhardt (1932) studied the histology in human jaws obtained at necropsy and made comparisons to the healing sequence of other animal models. Steinhardt reported his findings on three subjects with known time of elapse from extraction to death. The first patient died three weeks after extraction of his teeth. Steinhardt observed that the wounds were covered with epithelial tissue, the sockets were filled with young granulation tissue, and the fundi with uncalcified bone. Steinhardt compared this to a nine- or ten-day old wound in a dog. The

second patient died three and a half months after extraction of a lower bicuspid. The depth of the socket was reduced by the presence of new bone, the epithelium had covered the wound completely, and the central portion of the socket contained remnants of the original blood clot. Steinhardt stated this would correspond to an eight-week-old extraction in a dog. The third patient had a lower second molar extracted six weeks before death. The socket revealed new bone formation and was filled with granulation tissue. Epithelium had only partially covered the socket.[38] In reviewing Steinhardt's 1932 paper, Claflin (1936) concluded that the human healing processes in extraction wounds are the same as in dogs, but they occur at a slower rate.[34]

Amler *et al.* (1960) studied socket healing following dental extractions in healthy human beings at two- to three-day intervals up to a period of fifty days. They observed that the same histochemical reactions seen for histogenesis of bone observed in animal studies were also found in human alveolar socket healing. Amler *et al.* observed the following healing sequence: by twenty-four hours, blood clot formation filled the entire alveolar socket. At two to three days post-extraction, granulation tissue then began to arise from the periphery of the socket. This granulation tissue was accompanied by a metachromatic ground substance, glycoprotein, and alkaline phosphatase. The granulation tissue invaded the blood clot and replaced it by the seventh day. By the fourth day, young connective tissue was found at the periphery along with a metachromatic ground substance, alkaline phosphatase, and a glycoprotein framework. Connective tissue encroached upon the granulation tissue and replaced it by the twentieth day. Also, epithelialization was evident and associated with glycogen and glycoprotein on the fourth day. Bone formation was first seen at the base of the socket on the seventh day and at least two thirds of the socket was filled by the thirty-eighth day.[39]

Amler *et al.* noted the following factors to affect the time for complete epithelialization: 1) diameter of the socket, 2) age of the patient, 3) laceration of the gingival, 4) height of the

alveolar crest, 5) foreign bodies in the socket (bone particles or root splinters). They classified these as normal factors to affect healing. They also noted the following pathologic factors that could affect complete epithelialization: 1) periodontal condition (pre-operative), 2) local infection (post-operative), 3) nutritional and systemic factors.[39]

Amler *et al.* (1960) was of short duration and only evaluated healing events that took place in the marginal portion of the socket. Their research also did not include the healing processes of remodeling that occur in the later phases of socket healing. It is this reason that the tissue composition of the fully healed extraction socket could not be documented.[24]

In a longer-term study of six months, Trombelli *et al.* (2008) examined socket healing in humans. They confirmed most of the early healing findings from Amler *et al*. (1960). In biopsies taken from later samples, they saw that when woven bone was replaced by lamellar bone, the process of remodeling, it was slow and varied greatly from individual to individual. In only a few of the specimens at six months had woven bone been replaced by trabeculae and bone marrow of lamellar bone. They concluded that the modeling process that occurs during the first weeks of healing is consistent, but the interval during which remodeling takes place is slow and may take several years to complete.[40]

Lindhe *et al.* (2012) studied human biopsy samples from the bony part of edentulous ridges to examine their composition. Core samples were taken from subjects prior to implant placement with fully healed edentulous regions. What they found was that the peripheral borders of the ridge were lined with dense cortical bone. The central portion consisted mostly of cancellous bone with trabeculae of lamellar bone embedded in bone marrow. The composition of the samples was roughly 47% lamellar bone, 8% woven bone, 4% osteoid, 17% bone marrow, and 20% fibrous tissue.[41]

After the extraction of a tooth, sockets heal by secondary intention, and it takes many months before it becomes difficult radiographically to distinguish the extraction socket from the surrounding bone.[42] Schropp *et al.* (2003) studied bone formation in single extraction sockets using subtraction radiography at baseline, three, six, and twelve months post-extraction. All radiographs were standardized. What they found was that initially, vertical bone loss took place in the alveolar crest region. Most bone gain in the socket occurred in the first three months, with some additional gain in months three to six. Remodeling was seen between months six and twelve with the amount of mineralized tissue being reduced.[2] As evident by many students, the initial phases of healing follow a pattern, but the later phases of remodeling varies from individual to individual.

Bone Grafting and Bone Grafting Substitutes

Bone grafting has been attempted for centuries with varying degrees of success.[8] There are a variety of clinical scenarios in which bone grafting has been used such as: replacing portions of bone in reconstructive surgery, repairing osseous defects through osteoconduction, stabilizing blood clots, providing mechanical membrane support, and serving as a vehicle for growth factors.[3] The ideal bone grafting materials are those that fulfill the following requirements: safe and non-toxic, biocompatible, become osseointegrated or replaced, provide mechanical support and act as an osteoconductive scaffold, allow the ingrowth of vasculature, and are relatively inexpensive and readily available.[3]

There are several types of grafts used in regenerative therapy: autograft (from self), allograft (from the same species), xenografts (from other species), alloplasts (from man-made sources), or a combination of grafts. These materials may display one or more of the following

properties: osteoconductive, osteoinductive, and osteogenic. Because of their source and the preparation used to avoid a rejection reaction in the host, the grafts have different qualities and different indications for use.

Osteoconductive materials are those that have a matrix that serves as a scaffold for bone deposition. Osteoinductive materials have proteins that stimulate and support the proliferation and differentiation of osteoprogenitor cells that later become osteoblasts. Osteogenic materials are those that contain osteogenic cells (osteoblast and osteoclast precursors) that when placed in the proper environment are capable of forming bone.[3]

Induction of bone formation requires three elements: soluble osteoinductive signals, cells that are able to respond to these and other signals, and a supporting matrix or scaffold to carry and deliver cells or growth factors.[3] BMPs are the only growth factors that have true osteoinductive properties. Other growth factors such as PDGF, VEGF, FGF, growth hormone (GH), and insulin-like growth factor (IGF) do not have true osteoinductive properties, but do play a role in enhancing bone repair and regeneration.

When a graft is transplanted from one site to another in an individual, immunologic complications usually do not occur because the tissue is recognized as "self". This is not the case when tissue is transplanted from one individual to another or from one species to another. The immune system may create a challenge for the success of the grafting procedure. If the graft tissue is recognized as a foreign substance, the host's immune response will try to destroy the graft. The host's immune response is typically a cell-mediated response by T lymphocytes. Often times, the response does not occur immediately after implantation, and at first it may appear as if the graft is integrating normally only to be attacked later on. The more similar the graft and the host are antigenically, the longer an immune response may take.[8]

There are two methods used clinically to prevent immunologic rejection of transplants and improve the chances of success in grafting procedures. The first method is the suppression of the host's immune response. Medications are commonly used in organ transplant patients to prevent the host's rejection of the new organ. This is however not commonly used in periodontal surgery procedures due to the risk of immunosuppression.[8] The second method, and one that is commonly used in periodontal surgery procedures, is altering the antigenicity of the graft so that it does not stimulate the host's immune response. There are several methods to treat the graft, and they include: deproteinization, freezing, freeze-drying, irradiating, and dry heating.[8]

Types of Grafts

Autografts, or self-grafts, are grafts taken from tissues of the same individual. Fresh autogenous bone grafts are the most ideal bone grafts available because they provide living, immunocompatible bone cells that are essential to osteogenesis. The amount of osseous tissue produced is directly related to the number of living cells that are transplanted.[8]

The bone can be taken from a variety of sites in the body. Block grafts are usually taken from the iliac crest, and contain both cortical and cancellous bone. Ribs can also be a source for block grafts. Cancellous bone and particulate marrow can be obtained from harvesting medullary bone and hematopoietic marrow. Cancellous bone and particulate marrow provide the greatest concentration of osteogenic cells. Furthermore, because of the particulate size, these grafts are more likely to survive transplantation because of their access to nutrients in the graft bed. This type graft is most often obtained from the ilium.[8]

There are two basic processes that occur when transplanting bone from one place to another in the same individual. The first process that leads to bone regeneration comes from the

cells in the graft that form new osteoid. The amount of bone regeneration is dependent on the number of transplanted cells that survive the grafting procedure. There are many cells that die during the transplantation process, and because of this, there may not be an abundant amount of bone regeneration. These cells depend on diffusion of nutrients from the surrounding area for survival due to the interrupted blood supply that results from transplantation. This phase is responsible for most of the newly regenerated bone.[8]

Beginning in the second week, the graft site also undergoes changes that leads to the second phase of bone regeneration. During this time, fibroblastic proliferation and new blood vessel formation from the graft bed begins. The fibroblasts and other mesenchymal cells differentiate into osteoblasts and begin to lay down new bone.[8] According to Urist (1972), there are several BMPs in the grafted bone that cause these changes to occur in the surrounding tissues.[43] The second phase of bone regeneration is responsible for the incorporation of the graft into the host with continued resorption, replacement, and remodeling of the bone.[8]

Advantages to using autografts are that they contain osteoconductive, osteoinductive, and osteogenic properties[3] and that no immunologic response occurs with their use.[8] Disadvantages to using autografts are that they may be of limited quantity and necessitate a second surgical site for obtaining a graft.[8] This second surgical site increases operative time and cost, pain, intra-operative blood loss, and recovery time.[3]

Allografts, or homografts, are grafts taken from members of the same species, and transplanted into a host. They are available with a variety of biologic properties and also available in many forms: mineralized bone matrix, demineralized bone matrix, cortical chips, cancellous chips, corticocancellous chip, osteochondral, and whole-bone block segments.[7] These grafts are often treated to reduce the antigenicity because the donor and the host are genetically dissimilar. Tissue banks have their own proprietary method to treat the grafts which ultimately

leads to the destruction of any remaining osteogenic cells in the graft. Because of this, allogenic bone grafts cannot participate in the first phase of osteogenesis. They do however offer an osteoconductive hard tissue matrix for phase two of induction. The host must produce all essential elements in the graft bed for the transplanted graft to be resorbed and replaced.[8] Allografts can be used in combination with autologous grafts when supply of autologous bone is limited to fill larger voids.[7] In fact, some of the most successful grafts are mixtures of autologous bone marrow and demineralized bone matrix.[44]

Demineralized bone is a commonly used allograft that is well suited for filling bone defects and cavities.[45] It has osteoconductive and osteoinductive properties and revascularizes quickly.[7] The osteoinductive capacity of demineralized bone is attributed to the activity of BMPs[46] present in the extracellular matrix that are made available to the host during the demineralization processing.[7] The osteoinductive capacity differs from donor to donor. Because of this, the United States Food and Drug Administration and American Association of Tissue Banks mandate that each batch of demineralized bone matrix be obtained from a single donor.[6]

Currently, the most commonly used allogenic bone is freeze-dried.[8] The freeze-drying and vacuum-packing preservation technique allows for the graft to undergo stages of incorporation similar to that of autogenous cancellous bone.[7] Freeze-dried allografts are osteoconductive and provide mechanical support. They are most beneficial when used in combination with autologous grafts to fill larger defects.[7]

Fresh allografts that require no preservation are also available. These grafts invoke an intense immunological reaction and have limited applications. Currently, they are mainly used for joint resurfacing.[7]

Advantages to using allografts are that they do not require a second surgical site in the host. Also, similar bone, in both type and shape, can be obtained to the bone that is being

replaced.[8] Disadvantages to using allografts are that the grafts do not provide viable cells to participate in phase one of osteogenesis.[8] There is also a very small risk in infectious disease transmission through the use of allografts.[7] However, in the three million allograft tissue transplants performed since the identification of the HIV virus, only two cases of HIV transmission have occurred, and these involved the use of fresh-frozen allografts.[6]

Xenografts, or heterografts, are grafts taken from differing species[8] or bone-like minerals derived from calcifying corals or algae[9] and transplanted into a host. Most animal-derived xenograft bone sources are bovine, porcine, or equine. The antigenic dissimilarity is much greater than that seen in an allograft. It is because of this dissimilarity that xenografts are treated more vigorously to prevent rapid rejection of the graft by the host.[8]

Anorganic bovine bone (ABB), such as Geistlich Bio-Oss®, is bovine bone that has been chemically treated to remove its organic components. It is biocompatible and has a trabecular and porous architecture similar to that of human bone.[47] It has been proposed that this bone graft has no osteoinductive properties but serves as an osteoconductive scaffold for new bone formation.[47] However, the processing has a strong influence on the graft's biologic behavior.[9]

There is controversy as to whether deproteinized bovine bone mineral (DBBM) is truly resorbable.[4] Human biopsies after sinus augmentation confirm that particles of bovine-derived bone substitutes can be found ten years post-operatively.[4] Xenografts must be selectively used as they are considered close to non-resorbable.[9]

Coralline calcium carbonates are processed natural coral skeletons (NCSs) that can serve as a resorbable bone graft substitute. Different species of calcifying coral were found to have a calcium carbonate skeleton with interconnected macropores similar to that of human cancellous bone.[9] The coralline calcium carbonate is transformed into HA by a hydrothermal exchange reaction with phosphorous, and this coralline HA is almost identical to the mineral component of

bone.[9] The material acts as an osteoconductive scaffold for the formation of new bone[47] although the osteoconductive potential is less than that of other bone graft substitutes.[5] NCSs enhance osteoblastic cell attachment and growth,[48] and this bone replacement graft material has shown long-term (5+ years) favorable clinical results in the treatment of periodontal osseous defects associated with moderate to advanced adult periodontitis.[49] Today, coralline HA are rarely used as onlay grafts in guided bone regeneration (GBR) procedures because of a high rate of late complications.[50]

Advantages to using xenografts are that they do not require a second surgical site in the host. Also, large quantities of the xenograft can be obtained.[8] Disadvantage to using xenografts are that the grafts do not provide viable cells to participate in phase one of osteogenesis and that they must be vigorously treated to reduce antigenicity and host rejection.[8]

Alloplasts are synthetic grafts or inert foreign bodies that are transplanted into a host.[47] They consist of ceramics, biocompatible composite polymers, and bioactive glass ceramics. Alloplastic bone graft substitutes are becoming more popular. They have osteoconductive properties of varying degrees and can be used to fill large defects.[3] However, two conditions must be met for successful osteoconduction: the scaffold must consist of a bioactive or bioinert material, and the shape of its external and internal surfaces should favor tissue ingrowth and bone deposition.[3] A material resembling spongious bone in terms of shape and dimension provides the ideal condition for angiogenesis that allows for bony ingrowth.[51] These materials have shown limited effectiveness in treating osseous defects around teeth, however they have been shown to be effective in procedures such as alveolar ridge preservation and ridge augmentation.[47]

Ceramics consist primarily of beta tricalcium phosphate (β-TCP) and HA.[47] HA is an abundantly available bone graft substitute that is non-toxic[52] and allows new bone to be formed

directly on its surface without any intervening layer of fibrous tissue.[53] The particles of HA can be either large or small, and dense or porous. The density determines the compressive strength and the extent of vascular ingrowth. Typically, larger particles are non-resorbable whereas smaller particles are resorbed rapidly. Larger particles are commonly used in ridge preservation and augmentation procedures, and smaller particles are used in periodontal defects.[47] β-TCP is similar to HA in that its resorption rate depends on both particle size and porosity. Human histologic studies have shown that when HA and β-TCP have been used in treating periodontal defects, there is resulting fibrous encapsulation of the graft material and pocket closure is achieved through long junctional epithelium,[54,55] indicating that ceramic fillers are unable to result in true regeneration.[47]

Biocompatible composite polymer, also known as "hard tissue replacement' is composed of poly-methylmethacrylate-poly-hydroxyl-ethyl-methacrylate beads coated by calcium hydroxide. When introduced into the body, the calcium hydroxide surface forms a calcium carbonate apatite.[47] Hard tissue replacement has been shown to be more effective than debridement alone in treating intra-osseous defects[56] and class II furcation defects,[57] but inconsistently promotes new attachment as seen through histology.[58]

Bioactive glass ceramics are made up of CaO, Na_2O, SIO_2, and P_2O_5 in the same proportions as in bone and teeth and are referred to as $_{45}S_5$ *bioactive* glass.[47] These silica-based materials were first introduced in the early 1970s.[9] The material was original introduced as an amorphous material (Bioglass).[47] Earlier primate studies with this material demonstrated bone and soft tissue regeneration around teeth.[59] The material was later produced in particulate form of varying diameters (PerioGlas and BioGran).[47] When the material comes in contact with body fluid, there is an ionic dissolution of the ceramic particles which allows a silica gel layer to form over the ceramic particles. A calcium phosphate layer forms over the silica gel layer which is

rapidly converted to a hydroxycarbonate apatite layer.[60] Studies have shown that this apatite layer is identical to bone mineral and it provides a surface for osteoblast cell attachment and bone deposition.[61] In a study Low *et al.* (1997) using bioactive glass ceramics in combination with debridement of intraosseous defects, the results indicate that statistically significant improvements were seen in probing depth reduction, clinical attachment level gain, and standardized radiographic comparisons, and these results remained stable over the course of two years.[62] Limitations of the Bioglass products are that they cannot reliably serve as space-maintaining devices because of their granular and non-porous nature.[63]

In order for a scaffold to be effective in tissue engineering, it must satisfy the following demands: be able to fit into an anatomic defect, possess mechanical properties that can bear *in vivo* loads, enhance tissue in-growth, and produce biocompatible degradation byproducts[64] to avoid the need for later surgical removal. The scaffold should also have an internal spatial structure of appropriate pore size and interconnectivity that allows for communication with cells dispersed within the scaffold.[65] Shaping the three-dimensional scaffold so as to precisely fit the irregular bone defect is limited to mold shape, post-fabrication shaping, and complex computer-aided fabrication techniques.[66,67] Because of this, scaffolds that form *in situ* (i.e. injectables) have been explored.[68] Many of these hydrogels lack the mechanical strength[69] and the cytotoxicity of the reagents may be problematic.[70] Also, *in situ* forming hydrogels often lack adequate pore size and the interconnectivity that limits cellular migration and subsequent degradation.[71] Thermoresponsive shape memory polymers (SMPs), such as Poly(ε-caprolactone) (PCL)-based SMP, was fabricated to "self-fit" into irregular cranio-maxillofacial defects.[72]

Poly(ε-caprolactone) (PCL)-based SMP have attracted attention[72] because they are an inexpensive and readily available, bioresorbable polymer scaffold with potential application for

repair of osseous defects.[64] These scaffolds are prepared in such a way that allows them to be manually compressed into an irregular shaped defect, and their expansive shape recovery occurs until the defect boundaries are met.[73] Shape memory behavior and pore interconnectivity were made possible through the unique fabrication process.[73] In addition, the PCL SMP can also be coated with a bioactive polydopamine coating that increases hydrophilicity and bioactivity. This can ultimately enhance HA formation *in vivo*.[74,75]

Advantages to using alloplastic grafts and scaffolds are they bear no risk of disease transmission because of their completely synthetic nature. Also, every material characteristic can theoretically be designed individually for a specific clinical indication.[9] Short-comings of the materials are that not all characteristics of the ideal alloplastic bone graft substitute have been identified.[9] Furthermore, technical limitations have made it impossible to design desired material characteristics such as a microporous material with a surface roughness that stimulates natural bone mineral.[9]

Barrier Membranes

GBR, the combination of a bone graft material and a barrier membrane, is one of the most widely used methods to augment bone in the oral cavity. Hurley *et al.* (1959) were the first to study the application of a barrier membrane to promote bone regeneration in orthopedic research in canines. They found that the use of a porous HA Millipore (an inert cellulose acetate plastic) barrier prevented the transmigration of cells while permitting the diffusion of extracellular fluids. They concluded that HA Millipore barrier membranes would be a valuable tool in osteogenesis.[76] The key principle behind GBR is to exclude the faster growing fibroblasts and epithelial cells from ingrowth into a defect or void in the oral cavity.[3] Through use of barrier

membranes, osteoprogenitor and stem cells are allowed to differentiate into osteoblasts in the coagulum and begin the process of laying down a bone matrix.

In a human study, Nyman *et al.* (1982) were the first to use the Millipore filter on a single tooth with advanced periodontal disease planned for extraction. Flaps were laid, the site was debrided, and a Millipore filter was placed over the defect. Three months later, the tooth was block sectioned and the sample was prepared for histology. What they discovered was that new cementum was found coronal to the initial defect site, and that the Millipore filter prevented the apical downgrowth of dentogingival epithelium.[77] Guided Tissue Regeneration (GTR) in the mouth was shown to be effective by using a barrier Millipore filter.

Dahlin *et al.* (1988) also studied how bony defects heal by GTR. They created standardized through and through 5 mm diameter defects with trephine burrs on both sides of a rat mandible. On one side of the jaw, the through and through defect was covered with a porous polytetrafluoroethylene (PTFE) membrane that extended 3 mm past the edges of the defect on both the buccal and lingual side. The other side received no membrane and served as the control. The rats were sacrificed at various time points throughout the experiment. What they discovered was that by the use of a PTFE membrane, virtually complete osseous healing had occurred by three weeks, whereas on the non-membrane side, connective-tissue ingrowth had prevented bone healing even after twenty-two weeks of observation. They concluded that a mechanical hindrance to soft tissue proliferation into a bone defect is a benefit for unimpeded bone healing.[78] This is one of the earliest studies to demonstrate that a chemically inert Teflon membrane was effective in GTR.

Non-resorbable membranes are highly effective in GTR and guided bone regeneration. The downside to using such materials is the need for a second surgery to remove, and this puts an extra burden on the patient. This led to the development of a second generation of

biodegradable materials,[79] the collagen membrane. Numerous studies have been conducted that have shown collagen membranes are effective in gingival connective tissue and epithelial exclusion.[80,81] Collagen membranes have been shown to be incorporated within the healing tissues by ingrowth of the host's fibroblasts and blood vessels with minimal inflammatory reactions.[82] The collagen membranes have also been shown to be degraded during the healing process[82] via the patient's own collagenase enzymes.[79] These properties make collagen membranes an effective material in GTR.

The question now becomes, is there a significant difference in the use of non-resorbable versus resorbable membranes in treatment? Blumenthal (1993) compared the effectiveness in treating mandibular class II buccal furcation defects with either resorbable collagen membranes or non-resorbable e-PTFE membranes over the course of a twelve-month healing period. Blumenthal discovered that both materials were effective in gaining vertical and horizontal probing new attachment and concluded that the intrinsic properties and ease of handling made collagen membranes a feasible alternative in GTR.[79] Cortellini *et al.* (1996) also compared the use of bioresorbable membranes and non-resorbable e-PTFE membranes against open flap debridement alone in the treatment of deep interproximal Intrabony defects. They concluded that clinically significant clinical attachment level gains can be obtained with GTR procedures using both bioresorbable and non-resorbable membranes. However, the patient's morbidity was lower in the group treated with bioresorbable membranes.[83]

It has been thoroughly discussed that tooth extraction (or loss) results in alveolar ridge resorption. Therefore, clinicians should intervene at the time of extraction to preserve as much alveolar bone as possible. In an experimental animal study, Becker *et al.* (1995) showed that by using a non-resorbable e-PTFE barrier membrane, more original bone volume could be maintained in grafted and ungrafted extraction sockets compared to mucoperiosteal flap

coverage alone.[84] Several human studies have also shown the clinical effectiveness of using non-resorbable e-PTFE membranes[85] and bioabsorbable membranes[86] in preserving alveolar ridges following tooth extraction based on the principles of GBR. These studies demonstrated that the use of membrane barriers resulted in minimal resorption of the alveolar ridge in both size and shape.[87]

In most of the earlier studies that treated extraction sockets using a membrane, they were performed without achieving primary closure of the flap (i.e., an exposed membrane that covered a grafted socket). More recent studies have indicated that more bone fill may be obtained by primary closure of the flap over the barrier membrane because early exposure hinders the effectiveness of GBR.[88,89] The down-side to advancing a flap would be the soft-tissue changes, such as mucogingival junction discrepancies and esthetic problems. Therefore, the decision to achieve primary closure with flap advancement must be weighed against the soft tissue changes that follow.[87]

Alveolar Ridge Preservation and Management of Extractions

Often times the loss of teeth results in alveolar ridge resorption. Because of this, the preservation of bone volume at the time of extraction is desirable.[87] Carlsson *et al.* (1967) showed that when teeth were extracted from the maxillary anterior, the alveolar process diminished greatly during the first six months. Reduction continued at the site at a reduced rate, but was still noticeable at the five-year follow-up.[90] It is important that clinicians intervene at the time of extraction to preserve alveolar bone. Clinicians must also conservatively manage the extraction sites to lessen the need for advanced augmentation procedures in the future.[87]

Iasella et al. (2003) studied whether ridge preservation would prevent the typical horizontal and vertical resorptive changes seen clinically following extraction. Non-molar teeth were extracted and randomly selected to receive ridge preservation using freeze-dried bone allograft and a collagen membrane or no material. The sites were allowed to heal for four to six months. In evaluating horizontal changes, they found that although both groups lost ridge width, an improved result was seen in the sites grafted at the time of extraction. Furthermore, most of the resorption took place on the buccal, and maxillary sites lost more width than mandibular sites. In analyzing vertical changes, they found that grafted sites gained vertical height, whereas the non-grafted sites lost vertical height.[91]

Walker *et al.* (2017) sought to evaluate alveolar ridge changes following molar extraction with and without the use of ridge preservation. After extraction, sites were either grafted with freeze-dried bone allograft covered by a non-resorbable dPTFE membrane or allowed to heal naturally. Cone-beam computed tomography (CBCT) images were taken immediately after extraction and three months post-extraction. They found that there was significantly greater loss in alveolar ridge height in the molars that were not grafted at the time of extraction. They did not find a significant difference in ridge width loss between the two groups. Interesting to note is that when ridge preservation was performed, the width diminished evenly between the buccal and lingual aspects, whereas when ridge preservation was not performed, the majority of ridge width loss occurred on the buccal.[92] Considering this finding, placement of future restorations could be compromised due to the uneven loss of osseous tissue.

The evidence overwhelmingly supports that ridge preservation is beneficial in preventing dimensional changes that occur soon after dental extractions. The clinician has an abundant variety of materials to choose from in grafting extraction sockets and must take into consideration clinical and patient related factors. The aim of this present investigation is to

compare *in vivo* the use of bioengineered Polycaprolactone (Polydopamine Coated and Uncoated) scaffolds against a commonly used xenograft, Geistlich Bio-Oss®, and Control (no material) in ridge preservation procedures following first molar extraction in the rat model. The study hypothesis is that Polycaprolactone (Polydopamine Coated and Uncoated) scaffolds will perform comparable to Geistlich Bio-Oss® and show an improvement over the Control (no material).

2. MATERIALS AND METHODS

Surgical Protocol

The Institutional Animal Care and Use Committee (IACUC) of Texas A&M University College of Dentistry, Dallas, Texas, reviewed and approved the protocol for this *in vivo*, prospective, randomized clinical trial. A total of 33 male Sprague Dawley rats were used in the study between October 2017 and May 2018.

Rats were weighed and anesthetized with a mixture of ketamine/xylazine 10:1 via intraperitoneal injection. Upon profound anesthesia, the right and left maxillary first molars were extracted as atraumatically as possible using curettes and a rongeur. Using a slow speed handpiece, a Brasseler #703 flat-end taper cross-cut fissure 44.5 mm bur was drilled to the depth of 3 mm in the extraction sockets to create standardized sized defects. Rats were randomly selected to receive one of four ridge preservation materials to be placed in the right and left maxillary first molar extraction sockets: Control (no material), Geistlich Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland), Polycaprolactone (Polydopamine Coated) scaffold (Texas A&M University, College Station, TX), or Polycaprolactone (Uncoated) scaffold (Texas A&M University, College Station, TX). Geistlich Bio-Oss® was hydrated per manufacturer's instructions and the Polycaprolactone discs (Polydopamine Coated and Uncoated) were trimmed and prepared for placement. Enough graft material was placed to completely fill the socket. A Cytoplast RTM Collagen Membrane (Osteogenics Biomedical, Lubbock, TX) was trimmed to completely cover the extraction site and placed over the graft material, tucking the membrane under the gingiva to prevent dislodgement. A drop of PeriAcryl (GluStitch Inc., BC, Canada) was placed over the membrane. The same surgical protocol was performed on the control rats,

with the exception of having no graft material placed in the extraction sockets. Following recovery, the rats were returned to their quarters and fed a soft diet for three days. The rats were randomly allocated to either be in the four-week experimental group or the eight-week experimental group.

The rats were sacrificed in accordance with guidelines of the National Institute of Health (NIH) via CO_2 overdose. The maxillae were dissected, removing all soft tissue remnants. Afterwards, the maxillae were hemisected into right and left halves. The specimens contained the surgical site of the maxillary first molar, along with the second and third molars.

Scanning Electron Microscopy (SEM)

A Polycaprolactone (Polydopamine Coated) disc, a Polycaprolactone (Uncoated) disc, and Geistlich Bio-Oss® particles were sputter coated with Au/Pd alloy (60 seconds for Polycaprolactone discs, 120 seconds for Geistlich Bio-Oss® particles). The samples were analyzed using a scanning electron microscope (JEOL JSM-6010LA) at 17X, 100X, 500X, and 1000X.

Radiographs

Right and left hemisected maxillae were fixed in 10% formalin. Comparative radiographs were taken of all samples in a similar sagittal orientation using a Faxitron MX-20 Specimen Radiography System (Faxitron X-ray Corp., IL) at a standardized distance, at 26 kVp for 6 seconds.

Micro-CT Analysis

A Micro-CT 20 Scanco Medical Scanner (Zürich, Switzerland) was used to individually image eight samples (one sample for each treatment modality per experimental time point). Samples were scanned with an x-, y-, and z-axis resolution mode of ~10 μm, a voltage of 55 kVp, and 800 ms exposure time. Three-dimensional (3D) reconstructed images were prepared using the micro-CT scanner software. Images were displayed to include both an overall gross image of the specimen as well as a cut image to see approximately midway through the surgical site.

Paraffin Sections

Right and left hemisected maxillae were fixed in 10% formalin and decalcified with 10% ethylenediaminetetraacetic acid (EDTA) (pH 8) for 5 days using an EMS-820 Precision Pulsed Laboratory Microwave Oven (Electron Microscopy Sciences, PA). The samples were then paraffin embedded and sectioned horizontally at 6 μm. One half of the samples were subsequently stained with Hematoxylin and Eosin (H&E) and the other half with Masson's Trichrome using established methods. Stained sections were analyzed using a Leica DMR light microscope (Nuhsbaum, IL).

Ground Sections

Right and left hemisected maxillae were fixed in 10% formalin and processed for ground sections. The hemisected maxillae were subjected to a series of different gradients of alcohol as

well as a mixture of alcohol/Technovit 7200 per the EXAKT company protocol in preparation for ground sections. The samples were polymerized and embedded in 100% light cure Technovit 7200 (Technovit 7200, EXAKT). A diamond bandsaw (EXACT 300 CP) was used to grossly section the samples. The samples were then polished to produce 20 μm thin sections. The samples were stained with Von Kossa stain and were analyzed using a Leica DMR light microscope (Nuhsbaum, IL).

Statistical Analysis

Comparative radiographs from three samples were randomly chosen from each treatment modality at both time points to measure the bone height in the middle of the extraction, ridge preservation site using ImageJ software. The values were averaged and compared to each other. The data analysis was conducted using student's *T*-test, and the significance value was set at $p < 0.05$.

3. RESULTS

Scanning Electron Microscopy (SEM)

Geistlich Bio-Oss®: At 17x magnification, the particles displayed irregular, sharped edge shapes. Porosity was seen in several of the particles examined. At higher magnifications of 100x and 500x, interparticle pores could be observed. See Figure 1.

Polycaprolactone (Uncoated): At 17x magnification, a highly porous specimen is observed. At higher magnifications of 100x and 500x, an interconnection network of pores can be seen. At 1000x, there appears to be irregularly arranged microscopic pores on the flat surfaces. See Figure 2.

Polycaprolactone (Polydopamine Coated): At 17x magnification, a highly porous specimen is observed. At higher magnifications of 100x and 500x, an interconnection of the pores can be seen. At 1000x, the irregularly arranged microscopic pores seen on the flat surfaces of the Polycaprolactone (Uncoated) sample are absent. See Figure 3.

Radiographs

At 4 weeks, the average bone height in the middle of the extraction socket was: Control 9.167 mm ± 1.312 mm, Geistlich Bio-Oss® 10.667 mm ± 1.247 mm, Polycaprolactone (Uncoated) 10.500 mm ± 0.707 mm, and Polycaprolactone (Polydopamine Coated) 10.667 mm ± 0.624 mm. The difference in the values of average bone height was not statistically significant ($p > 0.05$) for any of the materials tested at 4 weeks. See Table 1 and Figure 4.

At 8 weeks, the average bone height in the middle of the extraction socket was: Control 9.167 mm ± 0.235 mm, Geistlich Bio-Oss® 12.667 mm ± 0.849 mm, Polycaprolactone (Uncoated) 11.833 mm ± 0.236 mm, and Polycaprolactone (Polydopamine Coated) 11.333 mm ± 0.471 mm. The difference in the values of average bone height was statistically significant ($p < 0.05$) between Control and Geistlich Bio-Oss®, between Control and Polycaprolactone (Uncoated), and between Control and Polycaprolactone (Polydopamine Coated) at 8 weeks. The difference in the values of average bone height was not statistically significant ($p > 0.05$) between Geistlich Bio-Oss® and Polycaprolactone (Uncoated) and Polycaprolactone (Polydopamine Coated) at 8 weeks, and between Polycaprolactone (Uncoated) and Polycaprolactone (Polydopamine Coated) at 8 weeks. See Table 2 and Figure 5.

Micro-CT Analysis

Comparison with gross samples and cut sections reveal that greater bone fill was evident with the 8-week samples compared with the 4-week samples. In the 4-week samples, the greatest fill of the extraction socket appears most evident with Polycaprolactone (Polydopamine Coated) samples, while the Control, Geistlich Bio-Oss®, and Polycaprolactone (Uncoated) demonstrate comparable results. There is a loss of ridge height present at the extraction site in all 4-week samples. Bone loss extending to or past the furcation is noted in the remaining maxillary 2nd and 3rd molars of all 4-week samples. In the 8-week samples, it appears that the extraction sockets of the Geistlich Bio-Oss®, Polycaprolactone (Uncoated), and Polycaprolactone (Polydopamine Coated) are filling with osseous tissue. There is a loss of ridge height present at the extraction site in all 8-week samples. Residual ridge height of Geistlich Bio-Oss® and Polycaprolactone (Uncoated) is comparable in the 8-week samples. Bone loss

extending to or past the furcation is noted in the remaining maxillary 2nd and 3rd molars of all 8-week samples. See Figures 6, 7.

Paraffin Sections

H&E: New bone formation was seen in both 4-week and 8-week samples for all materials tested. Under low magnification (2x), more soft tissue was present in the defect sites of the 4-week samples compared to the 8-week samples. See Figures 8 and 9.

In viewing the samples at higher magnification (20x), direct ossification via bone lining cells, osteoblasts, and osteocytes was present in the Control, Geistlich Bio-Oss®, and Polycaprolactone (Uncoated) samples at both 4- and 8-weeks. However, the Polycaprolactone (Polydopamine Coated) samples demonstrated evidence of endochondral ossification at both 4- and 8-weeks. See Figures 10 and 11.

Masson's Trichrome: New bone formation was evident within the extraction socket connective tissue at 8 weeks for all samples viewed at low magnification (5x). Geistlich Bio-Oss® samples demonstrated the most regular appearance of new bone formation. Both Polycaprolactone (Uncoated) and (Polydopamine Coated) samples displayed more new bone formation over the Control, which was erratic in appearance. See Figure 12.

Ground Sections

Von Kossa staining: In observing the 4-week samples at 10x magnification, no evidence of new mineralized tissue inside the fibrous connective tissue of the alveolar socket was observed for any of the materials studied. There was a clear demarcation between the border of

the fibrous connective tissue and osseous tissue. See Figure 13. In observing the 8-week samples at 10x magnification, new mineralized tissue formation was seen inside the fibrous connective tissue of the alveolar socket of all materials studied. See Figure 14.

4. DISCUSSION AND CONCLUSION

Discussion

In this study, thermoresponsive SMP, Polycaprolactone (Polydopamine Coated and Uncoated), were assessed and compared against Geistlich Bio-Oss® and Control (no material) in extraction, ridge preservation procedures in the rat model. Polycaprolactone is advantageous because of its elasticity, biocompatibility, and biodegradability.[72] Furthermore, the scaffold can be bioengineered by the application of a bioactive polydopamine coating to enhance hydrophilicity and HA formation when placed in osseous defects.[72]

Zhang *et al.* (2014) tested the same Polycaprolactone (Polydopamine Coated and Uncoated) scaffolds used in this study and found an advantage to having a bioactive coating in *in vitro* studies. They found that the Polydopamine Coated scaffolds showed significant HA mineralization and enhanced capacity for osteoblast adhesion and proliferation over the Uncoated scaffolds.[72] Our *in vivo* study showed that both the Polydopamine Coated and Uncoated treated sites had comparable results at both time points. The scaffolds did differ in that the Polydopamine Coated samples showed evidence of endochondral ossification, whereas the Uncoated samples showed evidence of direct ossification, which was in alignment with the Geistlich Bio-Oss® and Control samples.

In all micro-CT images analyzed, it was noted that there appeared to be loss of vertical height at the ridge preservation site. This finding is consistent with other studies that show extraction sites, whether grafted or not grafted, still lose vertical height from the initial baseline measurements.[92] One factor to consider was that it was not possible for us to obtain baseline

micro-CT measurements, so it is unknown how much of a vertical loss in hard tissue height resulted.

The surgeon (TD) used the same microscope during all extraction, ridge preservation surgeries to enhance visualization. To the best of the surgeon's ability, it was assumed that all tooth and root fragments had been extracted. However, in analyzing a few samples after processing, it had been discovered that some samples contained root fragment remnants. Glickman *et al.* (1947) concluded that retained root remnants and bone fragments slow the progress of post-extraction healing. They did find that root remnants deep in the wound are well tolerated by the surrounding tissues, whereas remnants close to the surface prevent epithelial closure which increased inflammation.[37] It is possible that the retained root fragments could have altered the results of this study.

Conclusion

In conclusion, both Polycaprolactone (Uncoated) and (Polydopamine Coated) scaffolds performed comparably to Geistlich Bio-Oss® as a ridge preservation material over the Control (no material). Though new bone formation was seen with all treatment modalities, there was an improvement seen in using a graft over not using a graft. Alloplastic scaffolds have the added advantage over allografts and xenografts in that they can be bioengineered to promote regeneration. As newer technology develops, they have the potential to perform even better than what is currently used. The use of Polycaprolactone scaffolds was shown to be beneficial in ridge preservation procedures, thus warranting the possibility of future investigations of these materials.

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APPENDIX

Figures

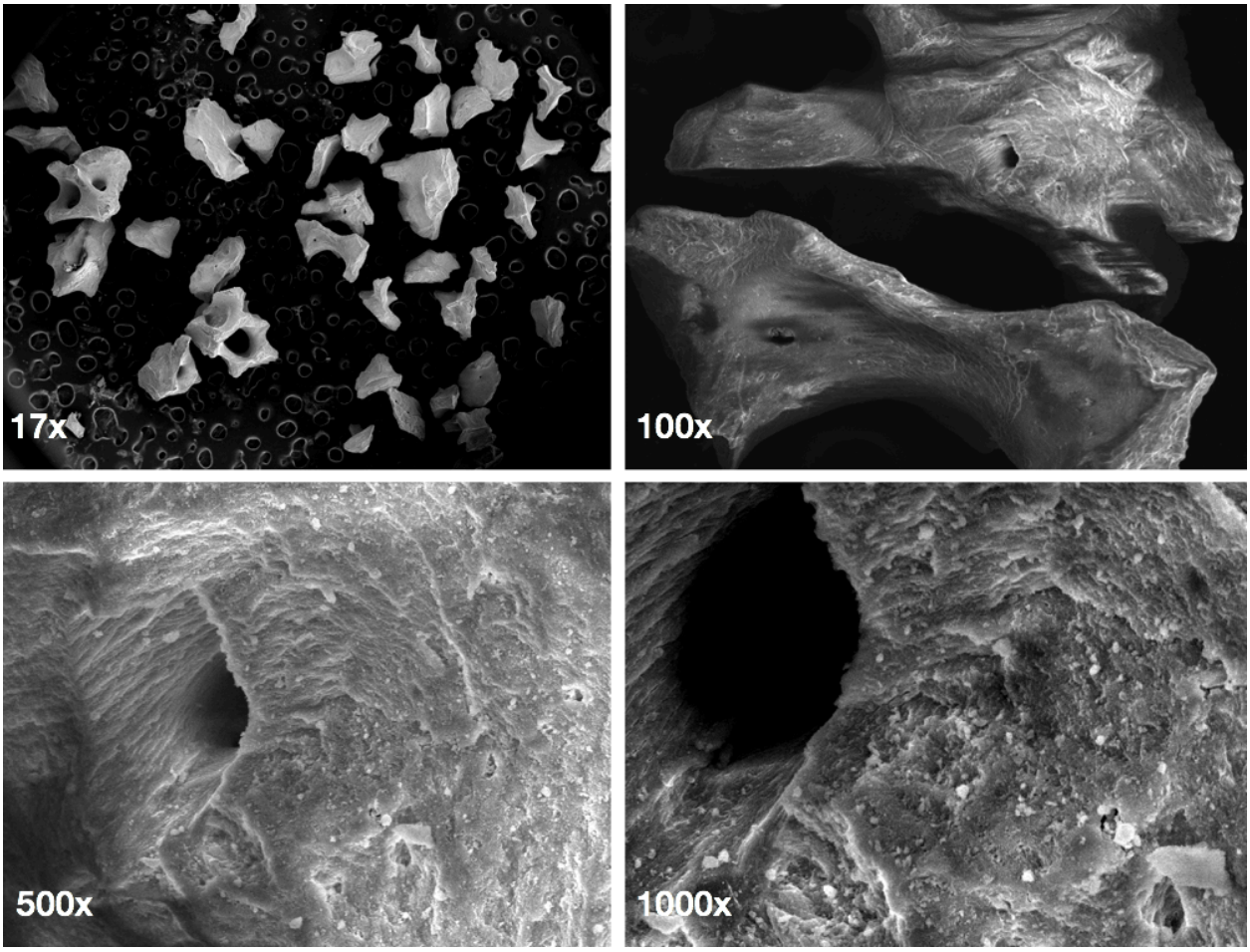

Figure 1. SEM of Geistlich Bio-Oss®. 17x, 100x, 500x, and 1000x.

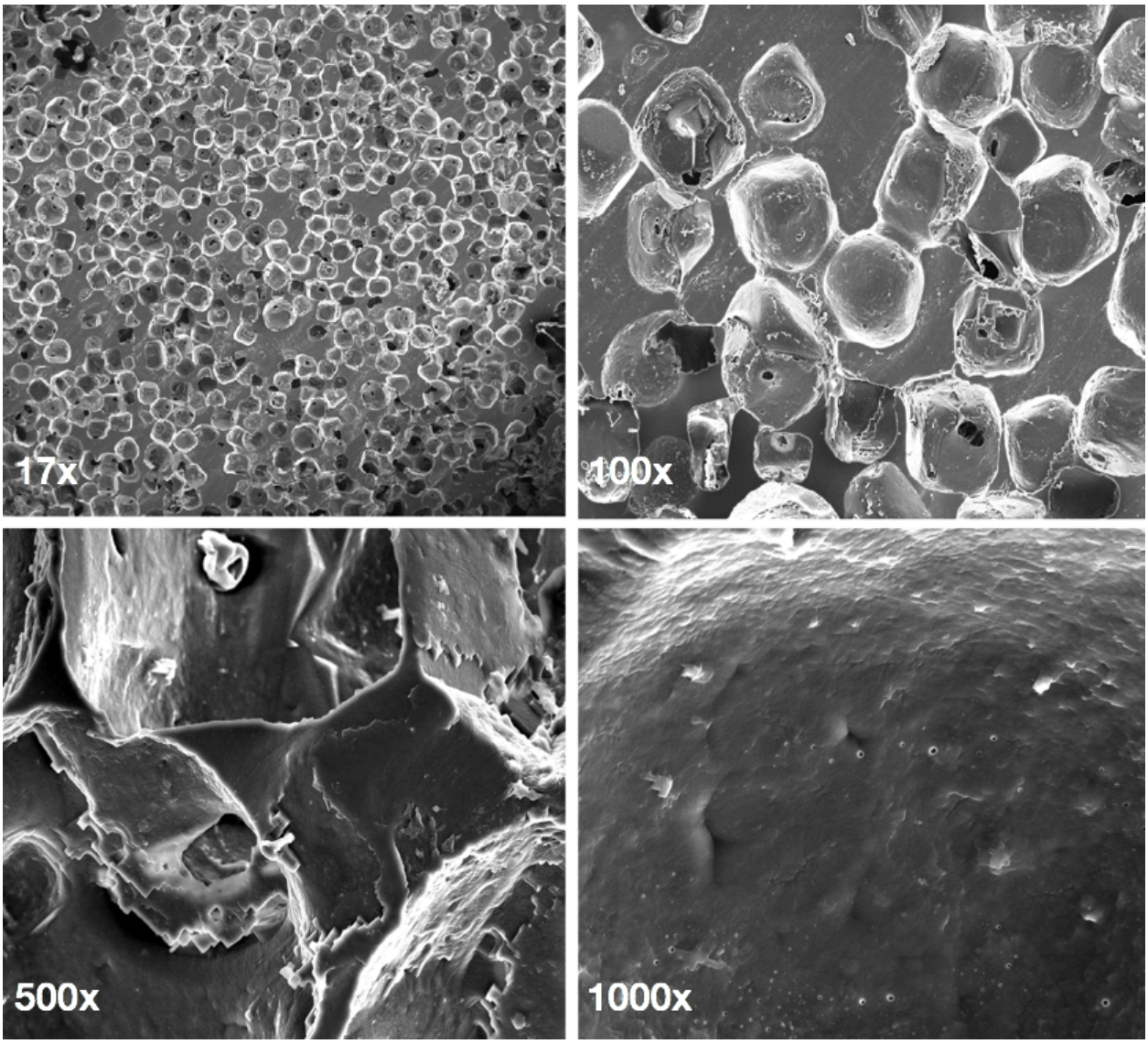

Figure 2. SEM of Polycaprolactone (Uncoated). 17x, 100x, 500x, and 1000x.

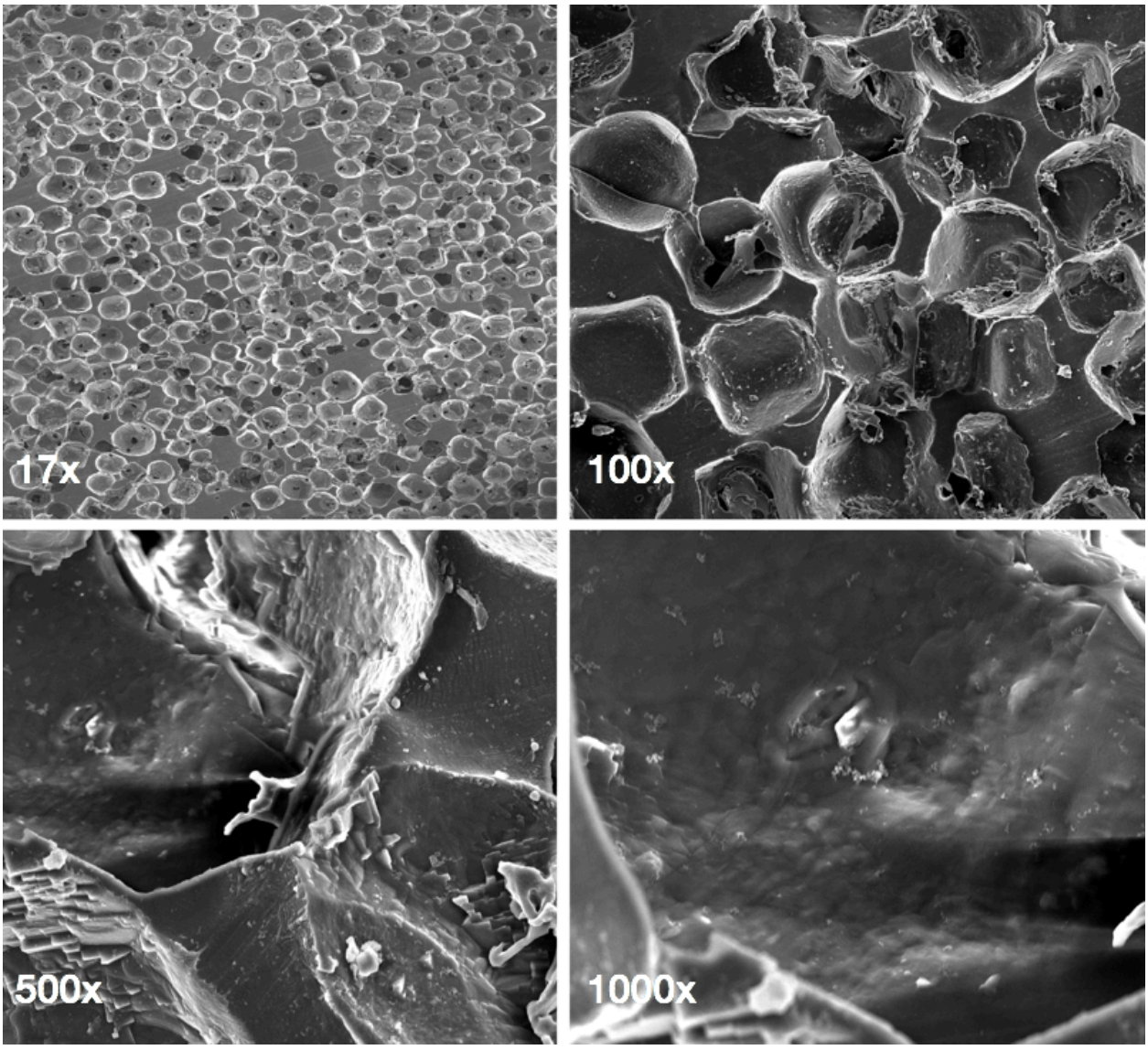

Figure 3. SEM of Polycaprolactone (Polydopamine Coated). 17x, 100x, 500x, and 1000x.

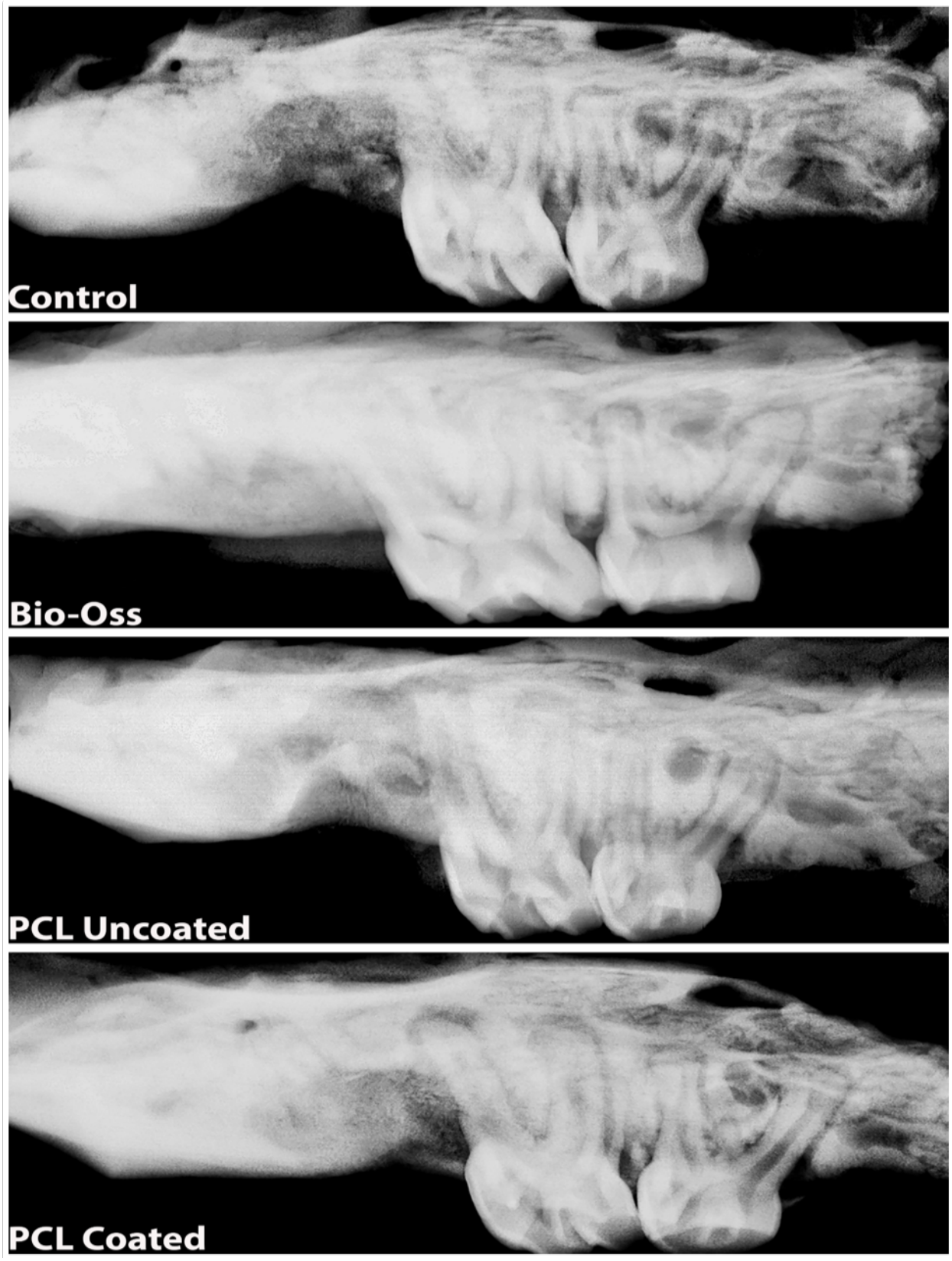

Figure 4. Radiographs, 4 Weeks.

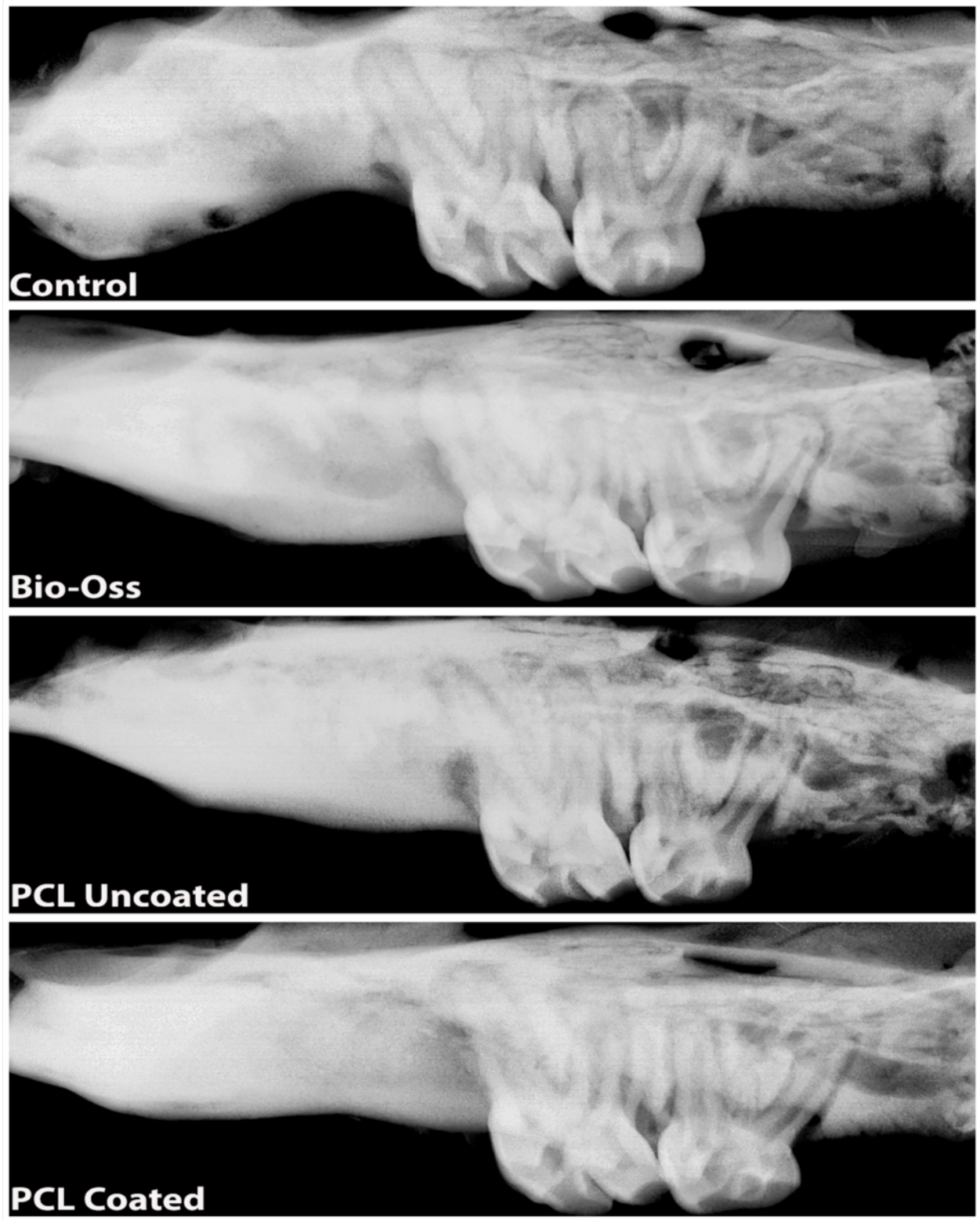

Figure 5. Radiographs, 8 Weeks.

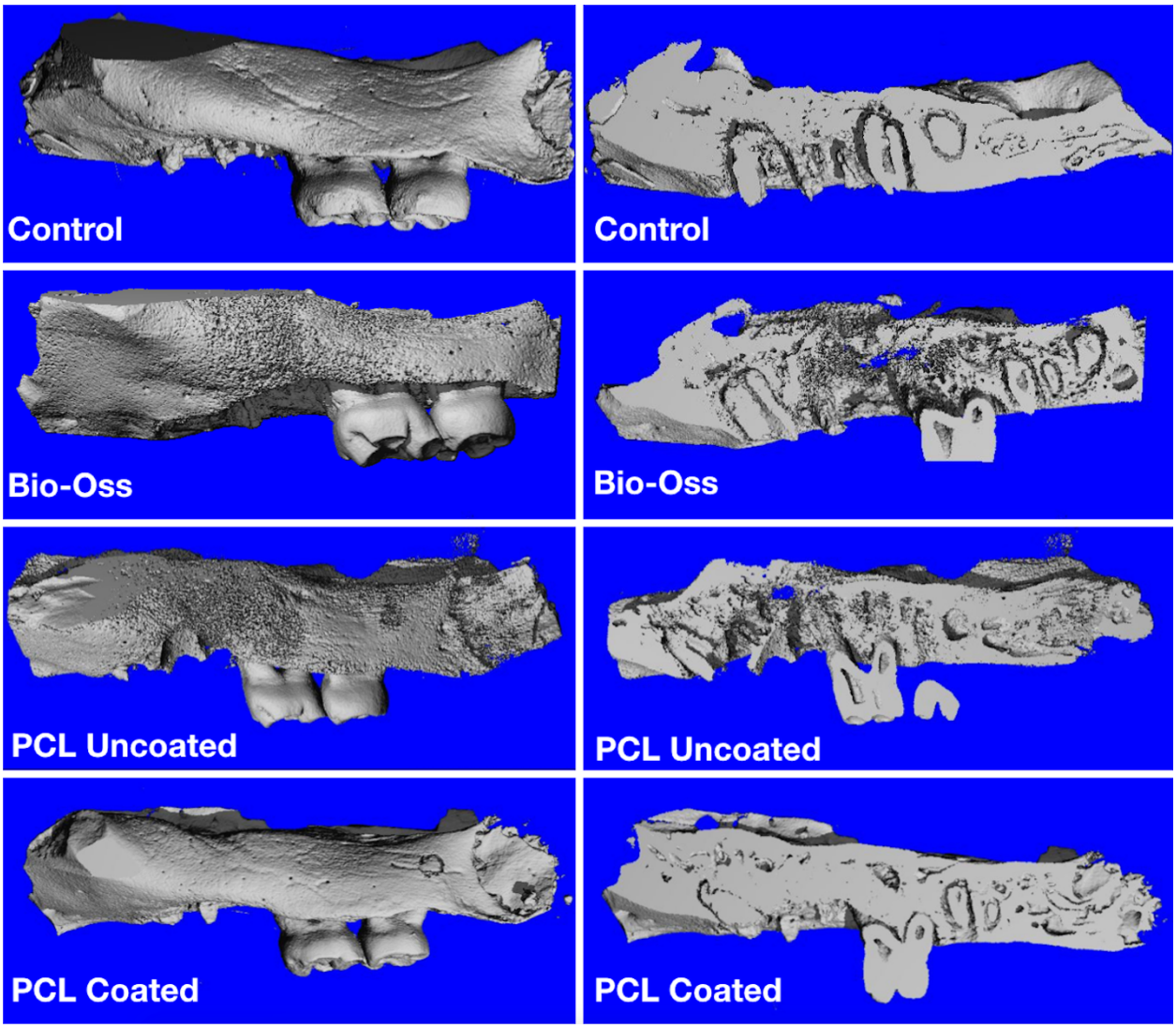

Figure 6. Micro-CT, 4 Weeks.

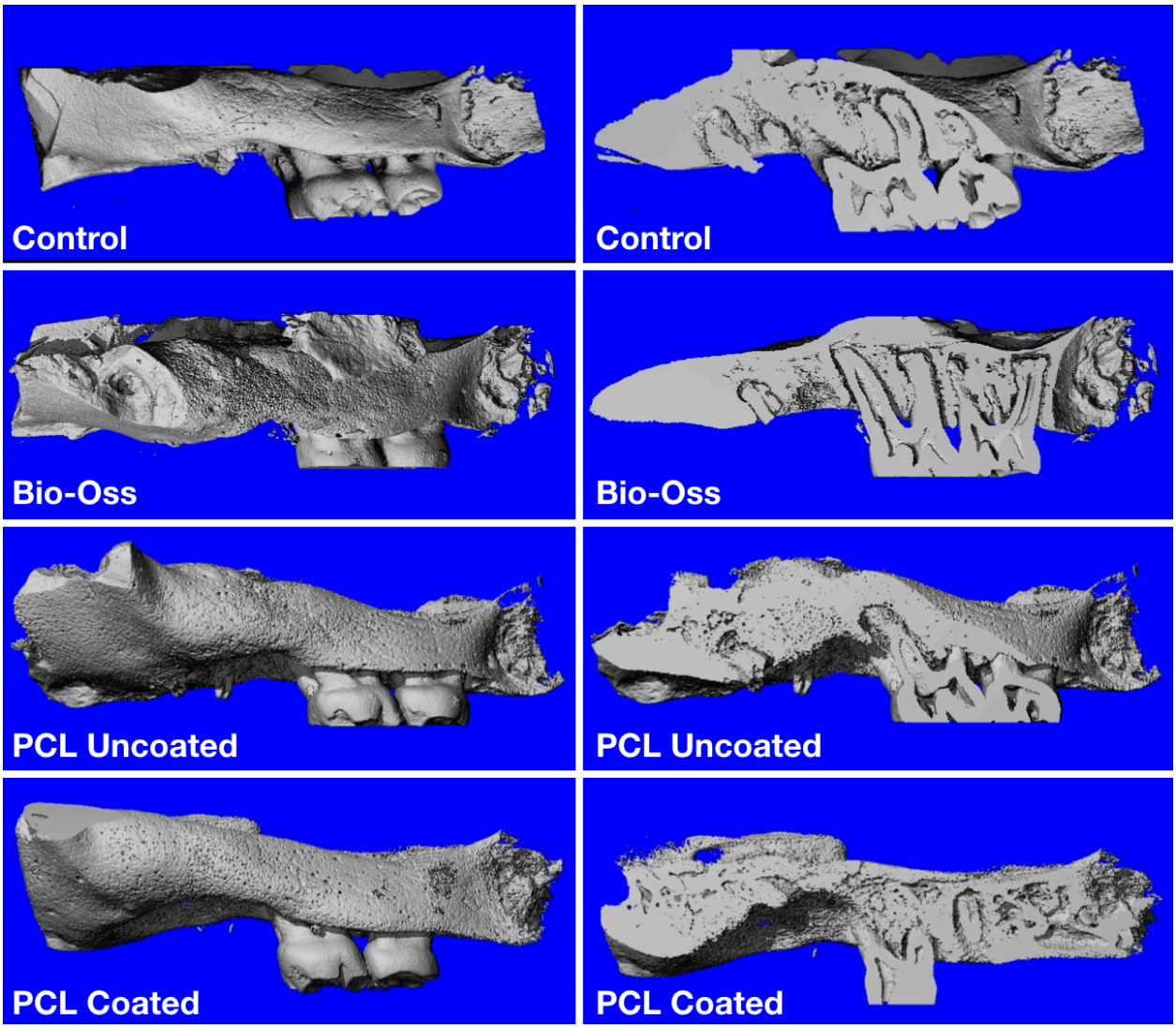

Figure 7. Micro-CT, 8 Weeks.

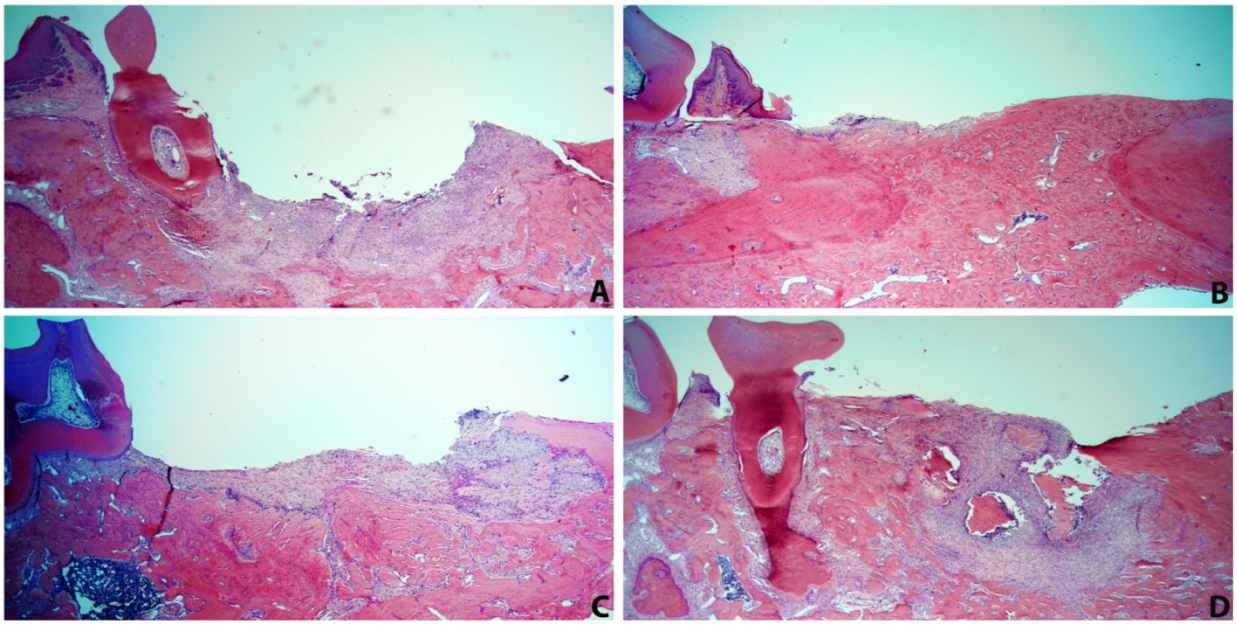

Figure 8. H&E (2x), 4 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

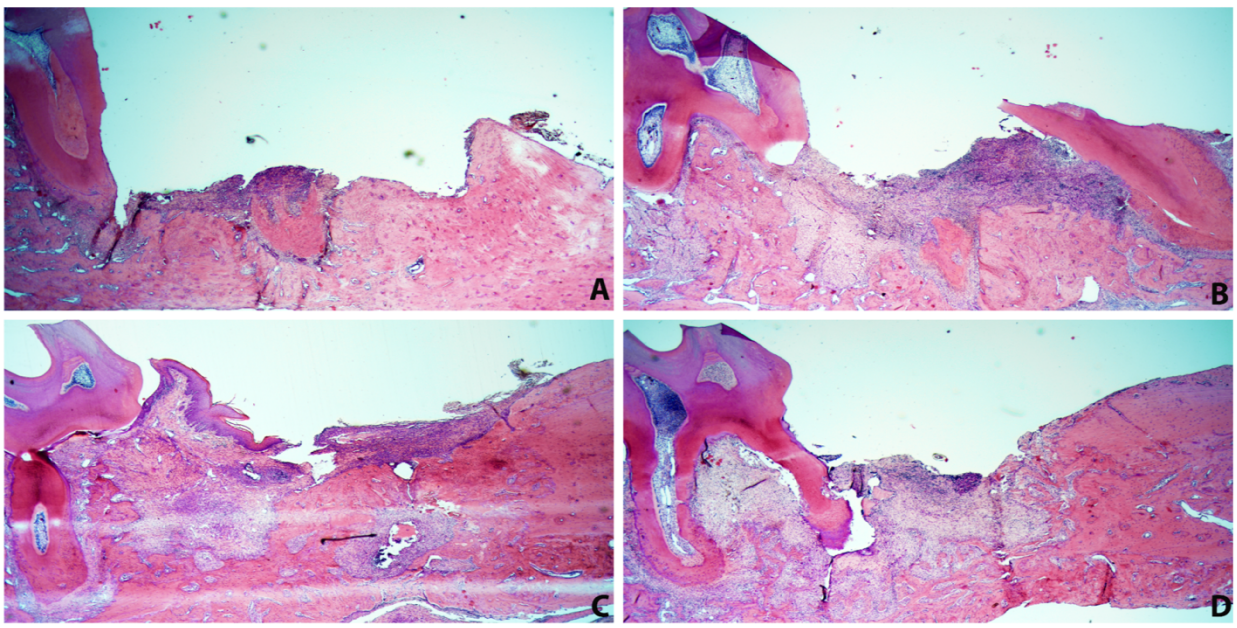

Figure 9. H&E (2x), 8 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

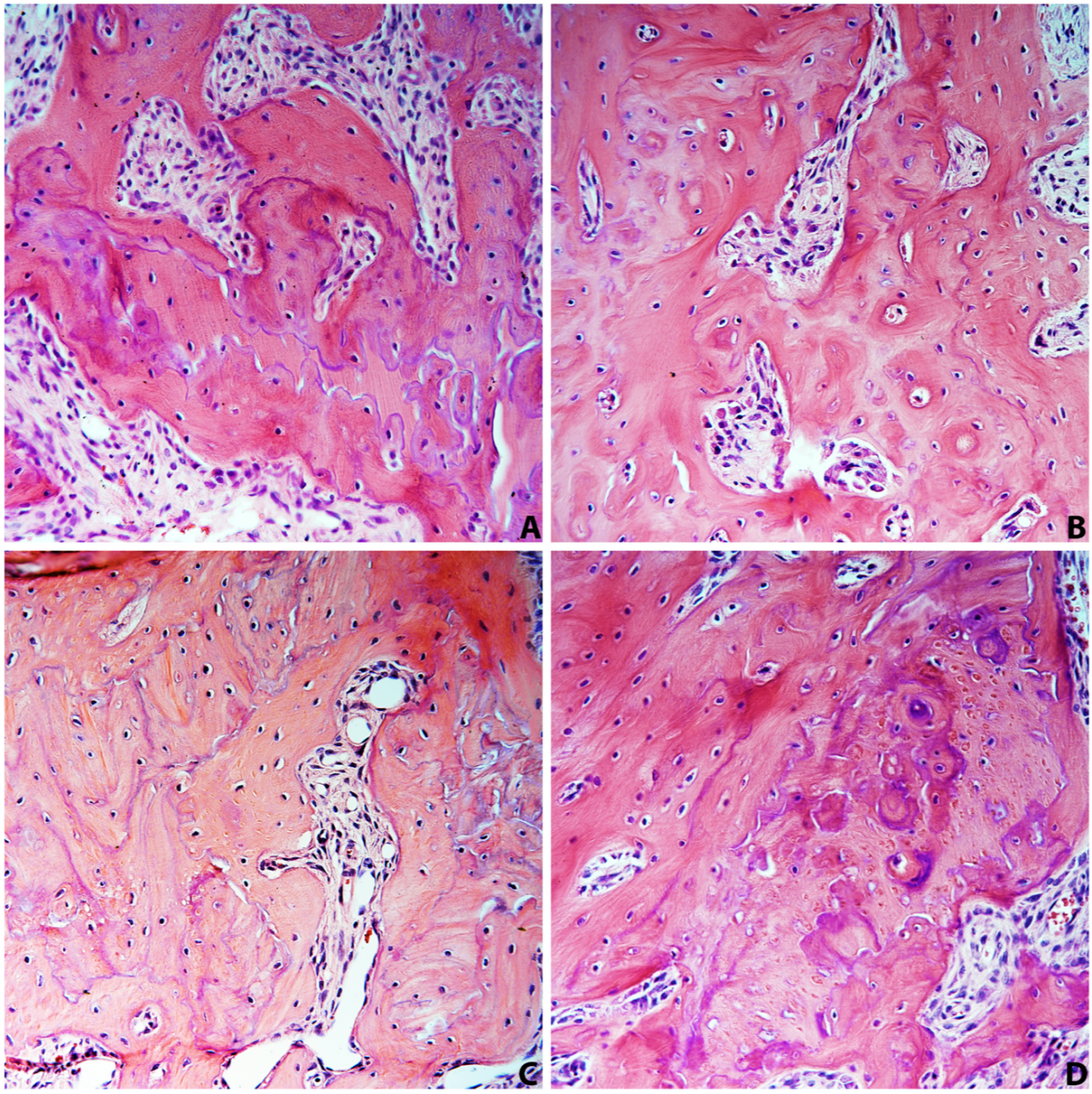

Figure 10. H&E (20x), 4 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

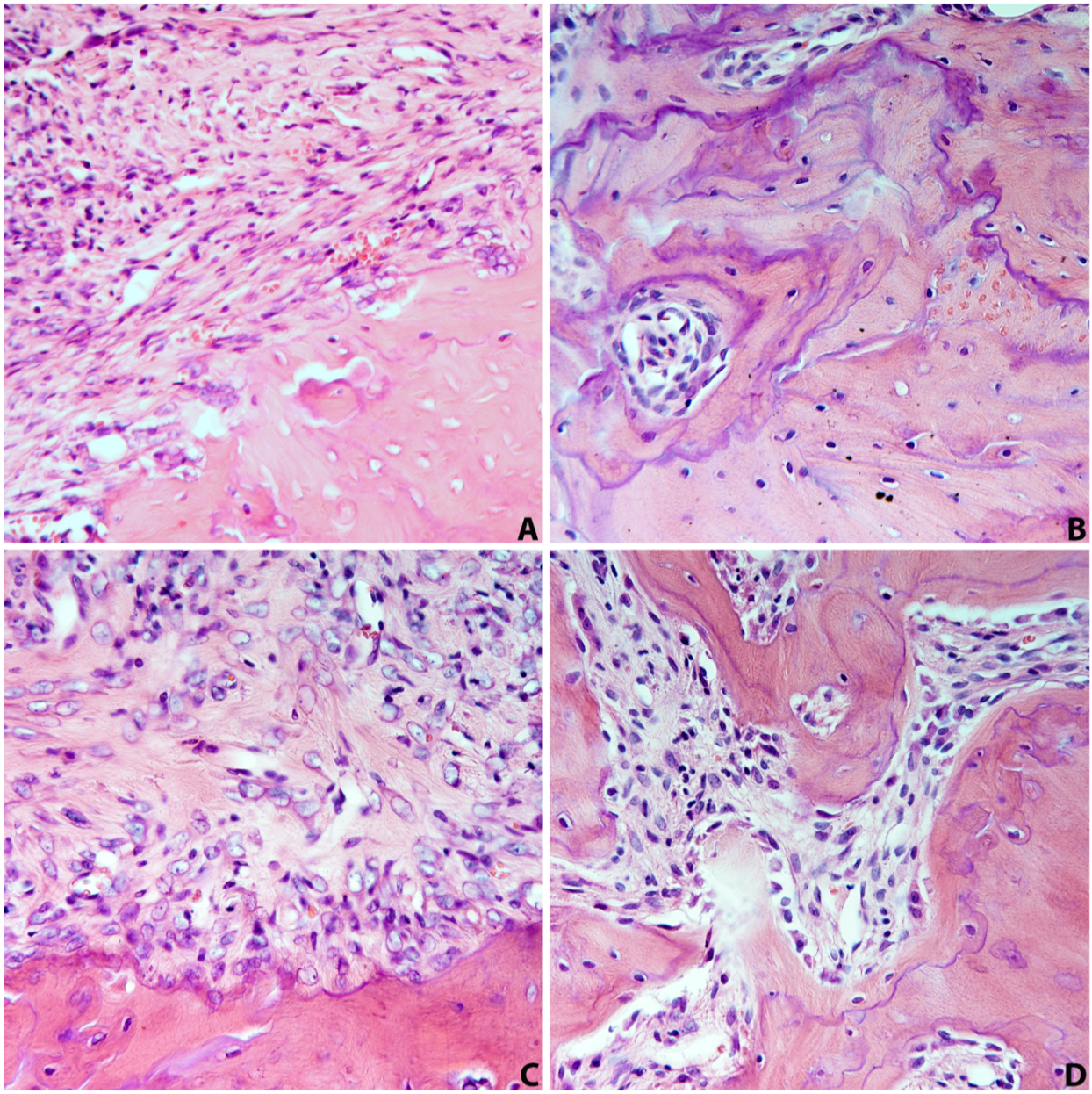

Figure 11. H&E (20x), 8 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

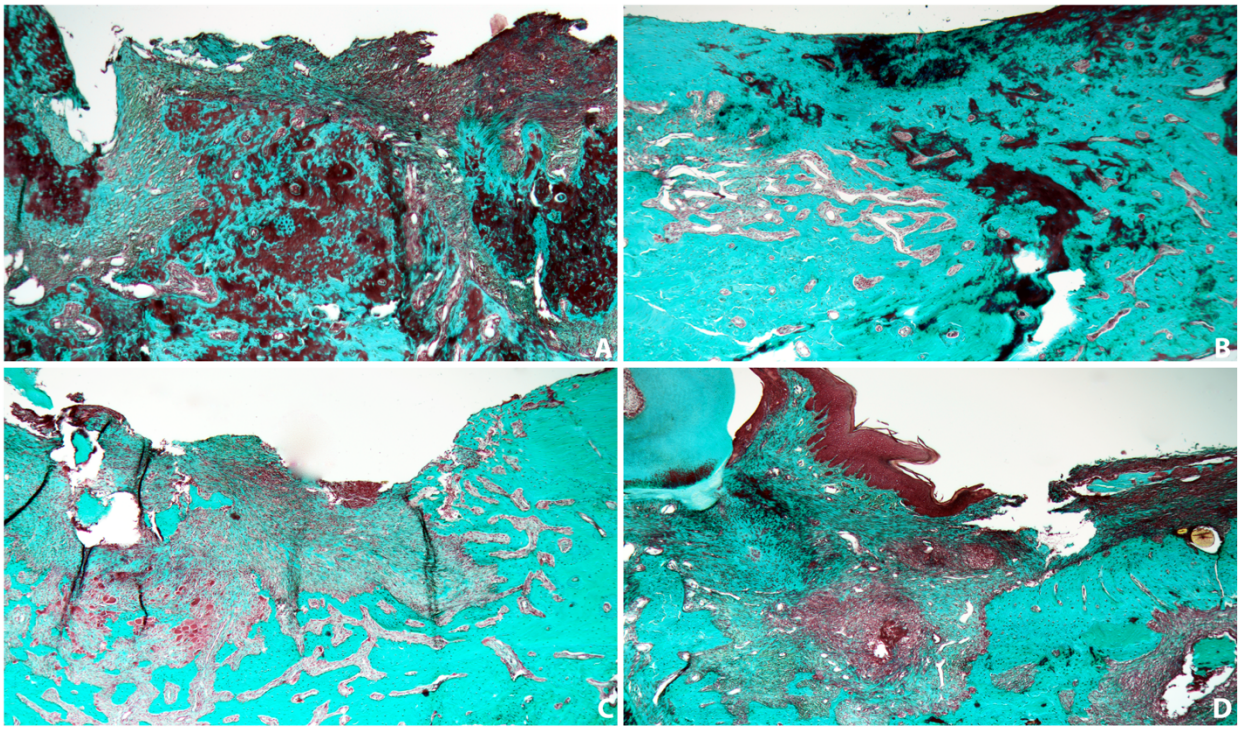

Figure 12. Masson's Trichrome (5x), 8 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

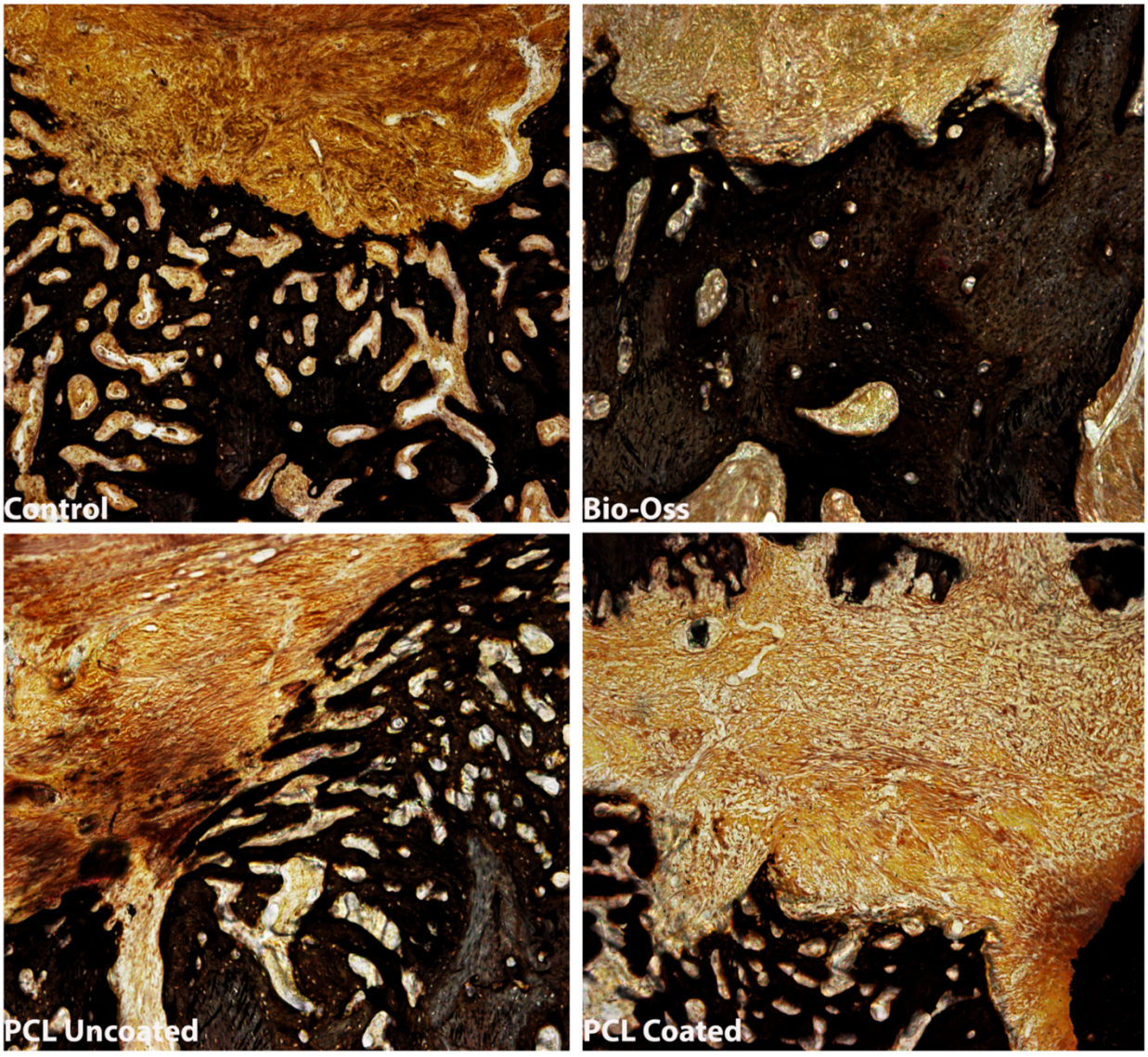

Figure 13. Von Kossa Stain (10x), 4 Weeks.

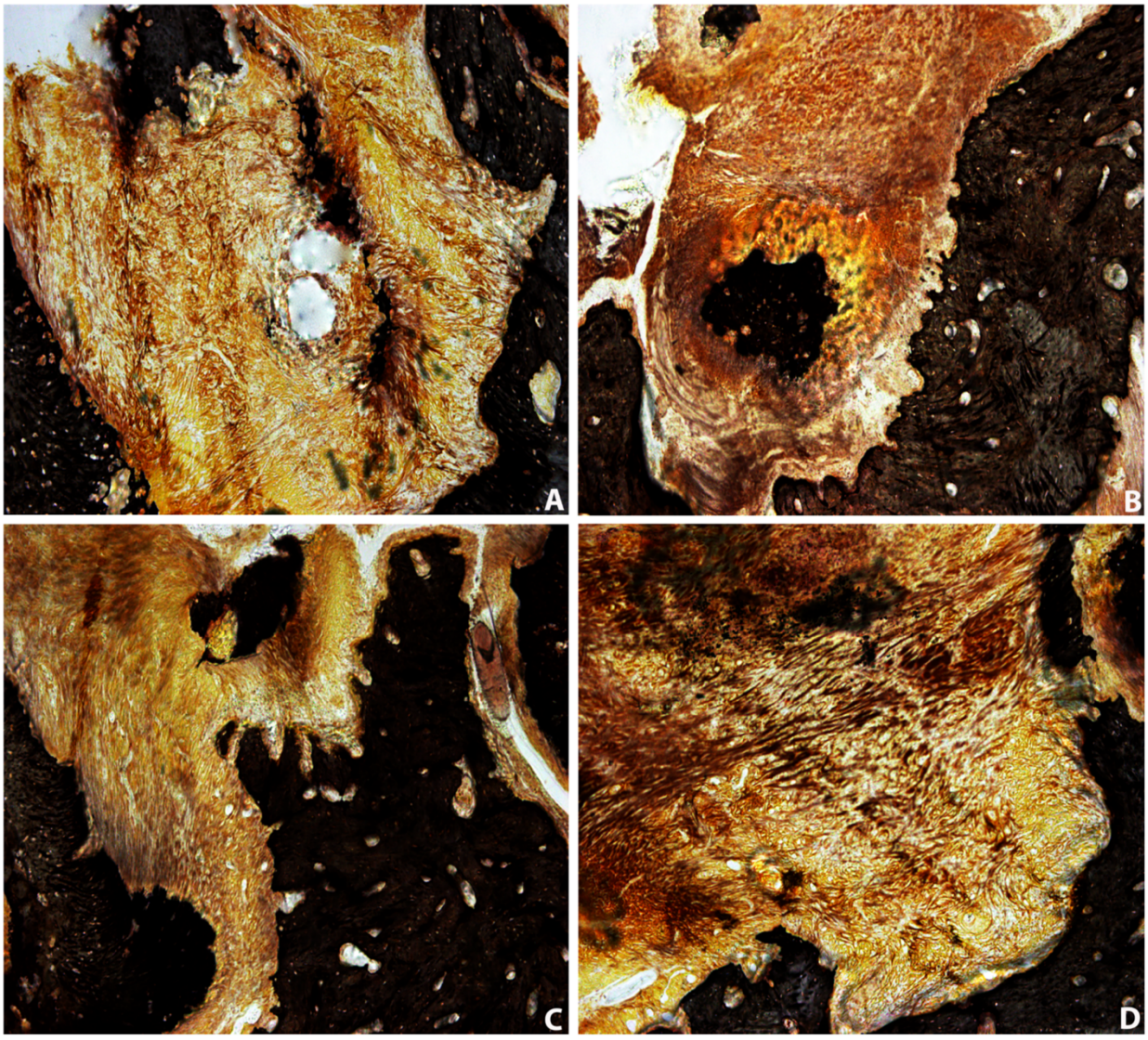

Figure 14. Von Kossa Stain (10x), 8 Weeks. (A) Control, (B) Geistlich Bio-Oss®, (C) Polycaprolactone (Uncoated), (D) Polycaprolactone (Polydopamine Coated).

Tables

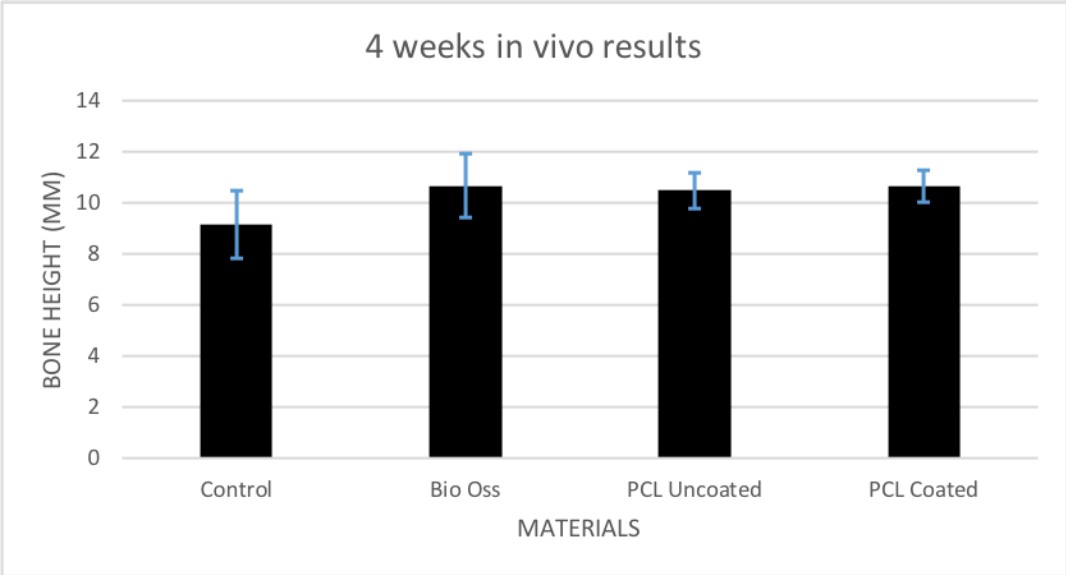

Table 1. 4 weeks *in vivo* results.

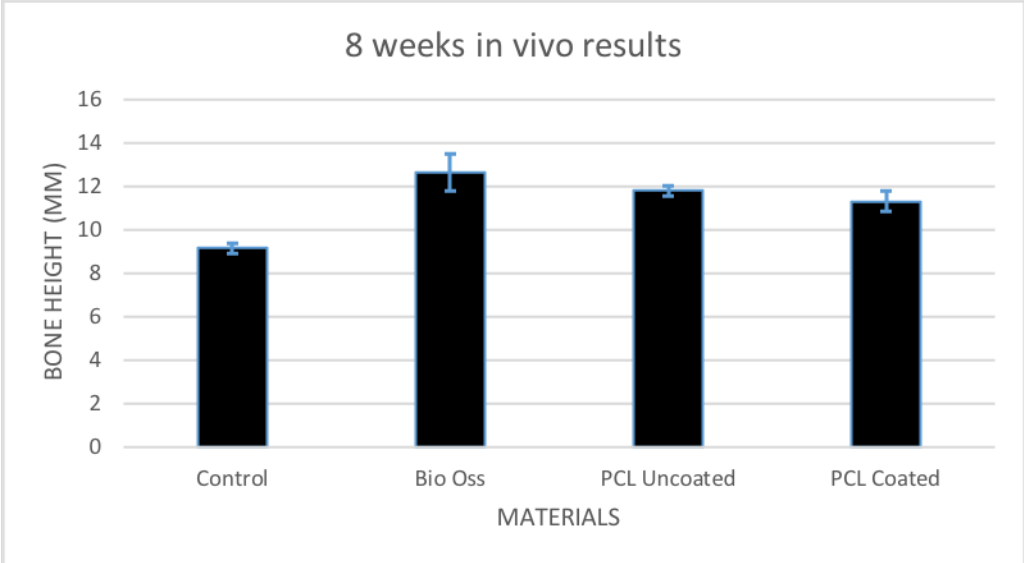

Table 2. 8 weeks *in vivo* results.