EVALUATION OF A SEGMENTAL TITANIUM IMPLANT FOR MANDIBULAR RECONSTRUCTION USING A CRITICAL SIZE DEFECT MODEL IN THE DOG—A PILOT STUDY

By

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EVALUATION OF A SEGMENTAL TITANIUM IMPLANT FOR MANDIBULAR RECONSTRUCTION USING A CRITICAL SIZED DEFECT MODEL IN THE DOG—A PILOT STUDY

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CHAPTER I

INTRODUCTION

Craniomaxillofacial trauma and tumor resection are common causes of large mandibular defects in human beings and animals. Restoration of function and occlusion via rigid fixation is the goal of reconstructive procedures for unstable fractures and segmental defects. Multiple techniques have been described to achieve this end and include but are not limited to interfragmentary wiring, external skeletal fixation, interdental wiring, intraoral acrylic splinting, and bone plating (1) (2) (3). While rigid fixation and autogenous bone grafting remains the gold standard for the repair of mandibular segmental defects, harvesting autogenous bone requires longer procedures and a second surgical site with increased patient morbidity. Alternative reconstructive techniques described for the canine mandible that avoid morbidity associated with autograft procedures include defect augmentation with allografts (4), coralline hydroxyapatite blocks (5), recombinant human bone morphogenetic protein-2 (rhBMP-2) (6) (7), polylactic acid:polyglycolic copolymer (8), poly L-lactide mesh (9), and bioactive glass (4). The purpose of this preliminary study was to describe the use of the Regenerex® porous titanium alloy implant (Ti-6Al-4V) in comparison to the standard cortical strut allograft techniques for canine mandibular reconstruction using a critical size defect (CSD) model. Furthermore, we hoped to report upon the presence of osteointegration
between the Regenerex® alloy and the animal’s tissue. The term “critical size defect” refers to the smallest size intraosseous wound, in a particular bone and species of animal, that will not heal spontaneously during the lifetime of the animal. Hollinger and Kleinschmidt developed this model to test new bone repair materials and standardize research models. The ideal model would be one in which the defect progresses to osseous union only in the presence of the bone repair material (10). The actual dimension of a mandibular body critical sized defect in the dog is unknown and likely varies with age, size, and breed of dog. Research focused on establishing the CSD for adult mongrel dog mandibles has demonstrated that the CSD is probably between 20mm and 40mm, with 40mm being the maximum sized defect that can be conveniently created (11). Recent studies have used 30mm segmental defects for the mongrel dog mandible CSD model (5) (6) (7). To the authors’ knowledge, there are no reports for the use of a porous titanium alloy in the repair of critical size defects of the canine mandible. Porous implants are designed to facilitate osteointegration and similar technology is currently being utilized in canine total hip and elbow arthroplasty. The Regenerex® material has an average porosity of 67% and pore sizes ranging from 100-600 microns in diameter with an average of 300 microns. This material is currently utilized in human total hip arthroplasty and cruciate sparing total knee arthroplasty procedures. It can be custom milled to virtually any three-dimensional specification making it an attractive potential biomaterial for maxillofacial applications. We speculated that Regenerex® would provide a 3-dimensional scaffold for osteointegration and vascularization as well as provide long term rigid fixation for canine mandible reconstruction.
CHAPTER II

MATERIALS AND METHODS

Adult purpose-bred male (n-5) and female (n-5) mongrel dogs aged 2-3 years old, weighing between 20 to 25 kg were acquired from a USDA approved breeding facility. Physical examination, complete blood count, serum biochemistry profile, and skull radiography were performed on each dog before study entry. Approval was obtained from the Oklahoma State University Institutional Animal Care and Use Committee and all procedures conformed to the National Institutes of Health Guidelines for the Care of Laboratory Animals.

Study Design

Animal pairs, consisting of one male and one female, were placed into 90, 180, 270, and 360 day treatment groups. The remaining pair was for the inclusion of a 540 day group or for the replacement of a group if an animal(s) had to be removed from the study. Following complete healing from left hemi-mandible dental extractions, as determined by five-view, mandibular radiography, each dog was either implanted with a commercially obtained cortical strut allograft or a porous titanium segmental implant following the creation of a mandibular body 40mm segmental ostectomy. Treatment pairs were scheduled for euthanasia 90, 180, 270, and 360 days later. The left mandible and both TMJ joints were collected for gross evaluation and histopathology.
Technique

Left hemi-mandible dental extractions were performed a minimum of two months before creation of the segmental ostectomy. Dogs were medicated with glycopyrolate (0.005-0.01 mg/kg intramuscularly [IM]) and morphine (1mg/kg IM). A cephalic intravenous (IV) catheter was placed and anesthesia was induced with thiopental (8-17mg/kg IV to effect) and maintained with isoflurane (baseline concentration, 2% delivered in oxygen 30ml/kg/hr). Normosol-R® (10ml/kg/hr) was administered during anesthesia. An intraoral inferior alveolar nerve block was performed using bupivicane (1-2mg/kg via local infusion). Mandibular radiographs were taken and reviewed by one of three board certified veterinary radiologists for abnormalities. Following radiography, the animal was placed in right lateral recumbency and the left mandibular canine tooth, pre molars, and molars were extracted using dental elevators, rongeurs, and a high speed dental burr under continuous irrigation. The gingiva was closed in a simple continuous pattern using 3-0 polyglactin 910® over the exposed alveoli. Digital radiography software and calipers were used to measure and approximate the average mandibular dimensions of dogs in groups 1 and 2 for fabrication of the porous titanium implants. Each implant was machine milled from a Regenerex® block according to the specifications provided to the manufacturer. Post extractions, dogs were fed a gruel diet created by blending a commercial canned adult maintenance diet with water to a paste like consistency for the remainder of the study. Dogs recovered postoperatively in the intermediate care facility and received a morphine bolus (2mg/kg IM) prior to returning to the research wards. Additionally, carprofen (4.4mg/kg IV) was given prior to recovery and continued orally.
for 7 days (2.2 mg/kg every 12 hours). Tramadol (2-6mg/kg orally every 12 hours) was started immediately following recovery and was continued for 7 days.

Segmental Ostectomy

Dogs were medicated with glycopyrolate (0.005-0.01 mg/kg intramuscularly [IM]) and morphine (1mg/kg IM). A cephalic intravenous (IV) catheter was placed and anesthesia was induced with thiopental (8-17mg/kg IV to effect) and maintained with isoflurane (baseline concentration, 2% delivered in oxygen 30ml/kg/hr). Normosol-R (10ml/kg/hr) was administered during anesthesia. An intraoral inferior alveolar nerve block was performed using bupivicane (1-2mg/kg via local infusion). Mandibular radiographs were taken and reviewed by one of three board certified radiologists a minimum of 61 days post extractions (Median 90 days) to ensure complete healing from the previous dental extractions. Following radiography, the hair was clipped and skin prepared for aseptic surgery over the left lateral and ventral surfaces of the mandible. Animals were placed in right dorso-lateral recumbency to facilitate an extraoral, ventro-lateral approach to the mandible. Hemostasis was achieved via monopolar electrosurgical scalpel cauterization and direct pressure. Careful circumferential sub-periosteal elevation of soft tissues exposed the mandibular body for creation of a 40mm segmental ostectomy; care was taken not to enter the oral cavity. A sterile marking pen and ruler were used to delineate the margins of the ostectomy. A 12-hole 2.0 locking titanium reconstruction plate was contoured and clamped to the mandible and the three cranial and caudal screw holes pre-drilled. Under continuous saline irrigation, an oscillating saw was used to create the 40mm segmental ostectomy perpendicular to the long axis of the body as marked. The critical size defect was then repaired via one of two methods:
For the Regenerex® group, the pre-fabricated implant was attached to the bone plate using four standard 2.0 self-tapping cortical screws in the manufacturer’s designated locations. The Regenerex®-plate construct was then secured to the previously drilled holes using three 2.0 self-tapping locking screws in the cranial and caudal segments.

For the allograft group, a 40mm by approximately 10mm cortical strut allograft was created by cutting the strut with a sagittal saw. It was fastened to the bone plate using four 2.0 self-tapping cortical bone screws using standard AO technique. The allograft-plate construct was then secured to the previously drilled holes using three 2.0 locking screws in the cranial and caudal segments.

Following implantation, the surgical site was lavaged and closed in a standard three layer fashion and post operative digital radiographs taken to confirm appropriate implant placement.

*Post-operative Care*

All Dogs recovered in the intensive care unit and received intravenous morphine (1-2mg/kg IV every 6 hours) for the first 12-24 hours. Additionally, carprofen (4.4mg/kg IV) was given prior to recovery and continued orally for 7 days (2.2 mg/kg orally every 12 hours). Tramadol (2-4mg/kg orally every 12 hours) was started immediately following recovery and was continued for 7 days. Clindamycin (11-15mg/kg orally every 12 hours) was given until the end of the study. All dogs were monitored daily for complications until euthanasia. They were euthanatized with a barbituate overdose (pentobarbital sodium 100mg/kg IV to effect). After sacrifice, a complete oral exam was performed and the left mandibular body harvested using an extraoral approach and oscillating bone saw. After gross evaluation and photo documentation the segment was placed in 10% neutral
buffered formalin for histopathology. Both tempromandibular joints were also harvested for future study.
CHAPTER III

RESULTS

The results from each treatment group are summarized in table 1.

<table>
<thead>
<tr>
<th>DOG</th>
<th>Extraction to implantation interval (days)</th>
<th>Time with Implant (days)</th>
<th>Implant</th>
<th>Gross Outcome</th>
<th>Mucoid Discharge</th>
<th>Intact Oral Mucosa</th>
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<td>1C</td>
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<td>90</td>
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<td>Yes</td>
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<td>2R</td>
<td>137</td>
<td>180</td>
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<td>Rostral Instability</td>
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<td>No</td>
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<tr>
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Post-mortum gross evaluation

Porous Titanium Implant Group

At the time of euthanasia all but the one Regenerex® implanted animal (1R) had grossly significant loosening of at least one of the implant-bone interfaces. All of the
Regenerex® implanted animals experienced oral erosions over the surface of the implant characterized by exposure of the porous titanium metal and a malodorous, mucoid, oral discharge (Figure 1). Dogs 4R and 5R were euthanized prior to their scheduled end date due to implant failure.
Figure 1: (Left Picture) Postmortem photograph (Dog 1R) demonstrating gingival erosion 3 months post Regenerex implantation. (Right Picture) Postmortem photograph (Dog 2R) demonstrating gingival erosion 6 months post Regenerex implantation. Rostral (Ro); Caudal (Cd); Buccal (B); Lingual (L)

Cortical Strut Allograft Group
All dogs in the cortical strut treatment group maintained an intact oral gingiva and subsequently did not experience the mucoid discharge associated with a breakdown in this barrier. It is also interesting to note that even though dog 4C’s plate broke at the caudal aspect and developed a loose implant-bone interface of the caudal segment, this did not result in a subsequent breach in the integrity of the oral mucosa overlying the implant or cause the dog any clinically appreciable discomfort. All other dogs within the cortical strut treatment groups did not experience post-operative complications and maintained rigidity.

Histopathology
The specimens were harvested and placed in ten percent (10%) neutral buffered formalin. Upon receipt in the Hard Tissue Research Laboratory the canine mandibles were transected according to protocol specifications to separate the rostral and caudal interfaces. Cut specimens were then dehydrated with a graded series of alcohols for nine (9) days. Following dehydration, specimens were infiltrated with a light-curing embedding resin. Following twenty (20) days of infiltration with constant shaking at normal atmospheric pressure, the specimens were embedded and polymerized by 450 nm light with the temperature of the specimens never exceeding 40°C. The specimens were
then prepared by the cutting/grinding method of Donath (12) (13). Specimens were cut to a thickness of 150 µm on an EXAKT cutting/grinding system. Following this, cores were polished to a thickness of 45-65 µm using a series of polishing sandpaper discs from 800 to 2400 grit in the EXAKT micro-grinding system followed by a final polish with 0.3 micron alumina polishing paste. The slides were stained with Stevenel's blue and Van Gieson's picro fuchsin and coverslipped for histologic analysis by means of bright field and polarized microscopic evaluation.

Porous Titanium Implant Group

Dog 1R (90d):

Rostral interface- The most striking feature of this specimen was the proliferation of new bone within the spaces of the metal (Figure 2). The porous area of the Regenerex implant had filled with granulation tissue that had transitioned to become fibrous connective tissue. Trabecular bone was formed within the surgical defect area and no inflammation was seen.
Figure 2: Photomicrograph of the Regenerex titanium alloy (white asterix) with cortical bone dispersed within the pores of the material (white arrows).

Caudal interface- Some new bone formation was seen at the end of the bone by the surgical defect but this did not reach the Regenerex® implant. All porous spaces have been filled with granulation tissue that has become connective tissue, but it had not transformed into bone. Some new trabeculae were found in the surgical defect space.

Dogs 2R (180d), 3R (270d), 4R (321d), 5R (230d):

Specimens 2R, 3R, and 5R: At the time of dissection for gross preparation, the implants were not connected to the tissue of the specimen and seemed attached only by the fixation plate. Soft, gel-like, necrotic material was present between the implant and the tissue. Microscopic evaluation was characterized by new bone formation in the surgical defects however there was a lack of connective tissue within the porosities of the metal implant. Epithelium was present between the Regenerex material and the surrounding bone and soft tissues.

Cortical Strut Allograft Group

Dog 1C (90d):

Rostral interface- Very active new bone formation was present at the margin of the surgical defect. The new bone extended onto the periosteal surface and was present in the periosteal connective tissue. Numerous trabeculae were present within the surgical defect. Blood in the vessels stained dark blue within the surgical defect area. No inflammation was seen in the surgical defect area, and the observed fixation screw was well integrated into the host bone.
Caudal interface- As in the rostral segment, very active new bone formation was present at the margin of the surgical defect and, as in the rostral segment, the new bone extends to the periphery of the allograft. No inflammation is present in the surgical defect area and new bone has formed around the fixation screws.

Dog 2C (180d):
Rostral Interface- The host cortical bone was undergoing remodeling. A very large amount of new bone formation was seen in the surgical defect area. No inflammation was present in the fatty marrow area. The fixation plate and screw were not integrated with the bone in this specimen.

Caudal interface- The host cortical bone was undergoing remodeling. A very large amount of new bone formation was seen in the surgical defect area. No inflammation was present in the fatty marrow area. This specimen shows significant new bone formation in the periosteum.

Dog 3C (270d):
Rostral interface- This specimen demonstrated some integration of the fixation plate and shaft of the fixation plate screw. New bone formation was identified in the surgical defect. Also, remineralizing particles of demineralized allograft were noted that were becoming vital bone. Some dense collagen had a pattern that appeared to be osteoid that would calcify and become bone at a later time.

Caudal interface- The fixation plate and screw were well integrated to the bone. The surgical site contained new bone formation and no inflammation was present in the fatty marrow. The mature cortical bone was being remodeled as well.

Dog 4C: (360d):
Rostral interface- Very dense new trabeculae were seen in the surgical defect. Demineralized allograft was present; it was becoming recalcified by the deposition of small deposits of calcium in droplets that would coalesce resulting in eventual recalcification of the demineralized allograft. Very thick trabeculae of new bone formation nearly fill the surgical defect area.

Caudal interface- The surgical defect area was nearly filled with newly formed trabeculae. Sharpey’s type fibers were attaching to the new bone. No inflammatory tissue was seen in this specimen.

Dog 5C: (420d):

Rostral interface- Dense trabeculae of new bone had formed within the surgical defect. In the area where a retaining screw was seen, new bone formation had almost entirely filled the surgical defect. The polarized view demonstrated how immature the new bone was in contrast to the lamellar host bone next to the surgical defect.

Caudal interface- Retaining screws were present in several areas, generally surrounded by very active new bone formation. Very active new bone formation was present within the surgical defect as well as in the periosteal area. The bone seemed denser and seemingly filled the surgical defect more than in the specimens with the Regenerex® implant.
CHAPTER IV

DISCUSSION

In this study, reconstruction of a mandibular critical sized defect utilizing a porous titanium alloy or cortical strut allograft was evaluated grossly and histologically for evidence of osteointegration. Dogs were chosen since they are considered an acceptable animal model for studying craniomandibulofacial reconstructive techniques and novel biomaterials (11) (14) (15) (16) (17) (18) (19).

A ventrolateral approach to the body of the mandible with careful sub-periosteal elevation of soft tissues allowed for the application of orthopedic implants avoiding direct contamination from the oral cavity. The creation of a 40mm segmental ostectomy was performed to ensure creation of a true critical size defect while simultaneously allowing enough remaining bone length for the application of a 2.0mm locking reconstruction plate with six cortices engaged rostrally and caudally. During the surgical procedures particular attention was paid to implant placement, contour, and creation of a tight fitting implant-bone interface. The porosity, pore size and surface roughness of the Regenerex® material is thought to be appropriate for vascularization and osteointegration based on several studies using similar materials in veterinary species (20) (21) (22) (23) (24) (25). The Regenerex® implant was consistently oversized in relation to the rostral and caudal mandibular segments in all dimensions; thus, the creation of a smooth contour could not
be created. This was especially evident intraoperatively as the implant extended medially beyond the adjacent corticies creating an irregular contour with rough edges adjacent to the oral gingival (Figure 3).

![Figure 3: (Top) Ventral view, intra-operative photograph of Dog 3R demonstrating oversize of Regenerex implant in comparison to patient’s mandibular anatomy. (Bottom) Contrasted to the same view of a cortical strut allograft in dog 1C.](image)

This effect could have been avoided with more accurate determination of the mandibular dimensions. Ideally, computed tomography and stereolithic modeling of the edentulous mandible would allow for the custom fabrication of a Regenerex® implant by the manufacturer that would be anatomically specific to the individual. Unfortunately, this was not possible in this study. Subsequent studies using a custom, anatomically specific implant or perhaps slightly undersized implant should be performed to achieve a better implant-bone contour and potentially avoid the gingival erosion, implant exposure and subsequent failure of osteointegration seen in the Regenerex® treatment group. Initially, the study was designed with a treatment pair to be sacrificed at 12 and 18 months, however the Regenerex® implanted dogs in these groups (4R & 5R) were euthanatized.
early due to severe implant instability. The corresponding animals with cortical struts did not experience clinically evident problems associated with their implants. Animal 5C was euthanized ahead of schedule to close the study 14 months post operatively as continued evaluation would not contribute to the study’s purpose. Inclusion of the cortical strut implanted animals enabled us to evaluate the technical aspect of implanting the graft material since implantation was performed in similar fashion utilizing the same plating system. While study numbers do not allow for critical statistical evaluation, we believe technical errors in implant placement are an unlikely contributor to the outcome of the Regenerex® population since all of the cortical strut animals maintained an intact oral mucosa. Only one cortical strut animal experienced an implant related complication which was apparent only upon post-mortem exam. Results of this pilot study must be evaluated with regard to the limitations regarding the small number of animals and the poor anatomical contour of the Regenerex® implant. The decision to use the average mandibular dimensions from dogs in groups 1 and 2 for creation of the Regenerex® implants may have contributed to the poor contouring of the implants in all dogs. This method was chosen since it allowed sufficient time for the fabrication and, if necessary, the revision of the five implants by the manufacturer. Further studies using more appropriately sized Regenerex® implants and a greater number of animals to enable statistical analysis is warranted to further explore the use of porous titanium alloy implants for craniomandibulofacial reconstruction. This was not pursued initially due to lack of resources for computed tomography and stereolithic modeling. Additionally, the number of animals required for statistical validation for such a study is significant and more appropriately performed after experimental revision to
ensure the overall success of the project in effectively evaluating the application of the novel biomaterial.

In conclusion, we report the generally unsuccessful use of a porous titanium alloy in the repair of 40mm critical size defects in the canine edentulous mandible and the generally successful repair of said defects utilizing commercially available cortical strut allografts. Until further revision, the Regenerex® porous titanium alloy construct cannot be recommended for mandibular reconstruction in clinical patients. However, it is our opinion the concept of utilizing patient specific, custom fabricated, porous titanium implants for craniomandibulofacial procedures is possible and potentially efficacious, pending minor revisions to the implant directed by computed tomography and stereolithic modeling. In addition, osteointegration did occur in dog 1R (Figure 2) and supports the concept of using porous titanium implants for reconstruction of the canine mandible.
REFERENCES

aBiomet Orthopedics, Warsaw, IN, USA; bVeterinary Transplant Services, Kent, WA, USA; cEthicon, Sommerville, NJ, USA; dMerge e-Film, Merge Healthcare, Milwaukee, WI, USA; eBiomet Microfixation, Jacksonville, FL, USA; fMicroAire, Charlottesville, VA, USA; gTechnovit 7200 VLC, Kulzer, Wehrheim, Germany; hEXAKT Technologies, Oklahoma City, USA


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Master of Science

Thesis: EVALUATION OF A SEGMENTAL TITANIUM IMPLANT FOR
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Pages in Study: 21               Candidate for the Degree of Master of Science

Major Field: Veterinary Biomedical Science

Scope and Method of Study: Pilot Study

Findings and Conclusions:
Objective-- Craniomaxillofacial trauma and tumor resection are common causes of large mandibular defects in human beings and animals. Restoration of function and occlusion via rigid fixation is the goal of reconstructive procedures for unstable fractures and segmental defects. The aims of this pilot study were to describe the use of a porous titanium alloy implant (Regenerex®) in comparison to cortical strut allografting techniques in the repair of critical size defects (CSD) in the dog edentulous mandible and report upon the operative technique and complications encountered with the procedures and novel application of this porous titanium alloy.

Methods- Ten purpose-bred, male and female adult mongrel dogs between 2 to 3 years of age, weighing 20-25 kg, and without evidence of craniomaxillofacial or systemic disease had left sided dental extractions extending from the mandibular canine tooth to the last molar. Once healed, a 40mm segmental ostectomy was performed and repaired with a 2.0 locking titanium reconstruction plate augmented with either an interfragmentary cortical strut allograft (n=5) or the Regenerex® implant (n=5). Dogs were euthanized and mandibles harvested for gross evaluation and histopathology.

Results— Osteointegration occurred in the 3 month Regenerex® implanted dog only. The cortical strut allograft implanted animals demonstrated new bone formation and incorporation of the allograft in all but 1 dog that experienced plate breakage.

Clinical Relevance-- The use of a porous titanium alloy implant designed to facilitate osteointegration for canine mandibular reconstruction following creation of a critical size defect has not been previously reported. Osteointegration could not be demonstrated in patients beyond three months postoperatively. The authors suspect inadequate contouring of the implant to be responsible for the failure.