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Mechanical Evaluation of Mandibular Defects Restored with rhBMP-2: A Finite Element Model

Jelson Yalung
Loma Linda University

LOMA LINDA UNIVERSITY
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in conjunction with the
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Mechanical Evaluation of Mandibular Defects Restored with rhBMP-2: A Finite Element Model

by

Jelson Yalung

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Master of Science in Orthodontics and Dentofacial Orthopedics

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August 2012

Each person whose signature appears below certifies that this dissertation in his opinion is adequate, in scope and quality, as a dissertation for the degree Master of Science.

_____, Chairperson
Joseph M. Caruso, Professor of Orthodontics and Dentofacial Orthopedics

_____
Alan S. Herford, Professor of Oral and Maxillofacial Surgery

_____
Kitichai Rungcharassaeng, Associate Professor of Orthodontics and Dentofacial Orthopedics

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ABBREVIATIONS

ACS	Absorbable Collagen Sponge
ß-TCP	Beta-Tricalcium Phosphate [$Ca_3(PO_4)_2$]
BCP	Biphasic Calcium Phosphate
BMP	Bone Morphogenetic Protein
CCS	Collagen Ceramic Sponge
CRM	Compression Resistant Matrix
CT	Computed Tomography
CBCT	Cone Beam Computed Tomography
DBM	Demineralized Bone Matrix
HA	Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]
rhBMP-2	recombinant human Bone Morphogenetic Protein-2
ROI	Region of Interest

ABSTRACT OF THE DISSERTATION

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Master of Science in Orthodontics and Dentofacial Orthopedics
Loma Linda University, August 2012
Dr. Joseph M. Caruso, Chairperson

Introduction: The utilization of recombinant human Bone Morphogenetic Protein-2 (rh-BMP2) to form new bone has been shown to be a promising alternative to autogenous bone grafts. Understanding the biomechanical properties of rhBMP-2 restored mandibular defects would provide useful knowledge in the future success of orthopedic and dental treatment in patients who have had restoration of mandibular defects with rhBMP-2. The aim of the study was to evaluate and compare the biomechanical characteristics of rhBMP-2 regenerated bone in mandibular defects using 2 different concentrations of rhBMP-2 with a given carrier in non-human primates

Material and Methods: Critical-sized defects (approximately 2.5 cm) were created in the mandibles of 6 adult male non-human primates. Each side of the mandibles received one of 2 carrier types: 1) 1.35 mg/mL rhBMP-2 combined with a collagen ceramic sponge (CCS) and 2) 0.75 mg/mL rhBMP-2 combined with CCS. All defects were stabilized with a titanium reconstruction plate. Young's modulus of 10 bone samples was calculated using the results from a tensile test of the samples. Density of the 10 samples was determined with a pycnometer. A 2-dimensional model of the mandible was virtually created to simulate the mandible for a given BMP/carrier group. Subdomains of the

model included cortical bone, regenerated bone, periodontal ligament, enamel, and cementum. Boundary conditions of the subdomains were assigned using the biomechanical properties determined in the literature and the regenerated bone values were assigned based on prior testing. Finite Element Analysis was performed with the COMSOL Finite Element Modeling software to measure peak Von Mises stresses and surface displacement of the newly regenerated bone in response to a 150 N force of the masseter muscle.

Results: There was no statistical difference between the mechanical properties among treatment groups. However, both treatment groups showed a statistically significant difference in stress distribution, displacement in the x-axis, and displacement in the y-axis when compared to the control group ($p < 0.05$) but no statistically significant difference when compared to each other ($p < 0.05$).

Conclusions: The differences in stress distribution and displacement when comparing the treatment group to the control group indicate that the treatment group regenerated bone was less stiff which lead to more displacement in the mandible and higher stress in response to function. This may be attributed to the 6-month follow-up period where the regenerated bone did not complete mineralization. The similarity in mechanical properties, stress, and displacement between treatment groups indicate that a rhBMP-2 concentration of 0.75 mg/mL produced bone that was biomechanically comparable to a concentration of 1.35 mg/mL.

CHAPTER ONE

EXPANDED REVIEW OF THE LITERATURE

To date, autogenous bone grafts are considered the "gold standard" for bone grafting, which all other grafts have been compared. Autogenous bone grafts are harvested from a donor site of either iliac crest or rib, which require a second site of surgery to the patient and carry an increased risk of complication. These risks include pain, paresthesia, gait disturbances, scarring, and infection. To reduce the surgical morbidity associated with autogenous bone grafts, a number of studies have been conducted to develop alternative grafting techniques.[1-10] In 1965, Urist reported a case in which he induced ectopic bone formation in an animal model using an osteoinductive growth factor, which he later termed Bone Morphogenetic Protein (BMP).[10] Since its discovery, use of BMP has been studied in laboratory and clinical settings and is now produced using recombinant DNA technology (rhBMP) for its potential use in regeneration of osseous defects in the maxillofacial region.

With an increasing prevalence of dental patients having undergone restoration of maxillofacial osseous defects, it is important to know the biomechanical characteristics of the BMP "regenerated bone" to decipher the limitations that may occur during orthopedic or dental treatment. While previous studies have shown the success of osseous reconstruction using BMP,[1,3-5] the literature has yet to report the biomechanical response of this regenerated bone to stress and strain. This literature review will examine

reconstruction of osseous defects using BMP and the analysis of the regenerated bone's biomechanical characteristics.

rhBMP-2 and Induction of Bone Formation

rhBMP-2 is a growth factor that has been shown to induce osteoid production by converting uncommitted mesenchymal cells into an osteoblastic lineage of cells.[11] Urist was the first to discover the bone-induction principle of BMP in 1965.[10] In this study he took long bones from adult rabbits and decalcified them in HCl. This decalcified bone matrix was then implanted into muscle pouches of rabbits, rats, mice, and guinea pigs. He found that a component of the implanted bone matrix caused generation of new bone by the induction of stem cells into bone producing osteoblasts. He identified the source of the cell transformation as a growth factor, which he later called BMP.[10] This finding was a key discovery because it was the first to identify that a specific protein could induce bone formation by induction of mesenchymal cells.

Another study was done by Wang et al in 1989 to further define the factors of bone formation using BMP-2.[12] In this study, BMP-2 was purified and recombinant human BMP was made from Chinese Hamster ovary cells. rhBMP-2 was then implanted in rats at varying concentrations. Cartilage formation was observed at day 7 and bone formation at day 14. They found that the timing of bone formation was dependent on the amount of rhBMP-2 implanted, which was confirmed by bone formation in only 5 days with the highest concentration of implanted rhBMP-2. Their histological analysis of rhBMP-2 regenerated bone showed no significant difference from the original bone extracts. With their findings, Wang concluded that rhBMP-2 from Chinese Hamster Ovary has the

potential for de novo bone formation in humans.[12]

With the regenerative potential of rhBMP-2 being investigated in animal and clinical models, the biomaterials used to deliver rhBMP-2 have been observed to play a critical role in the osteoinductive activity of BMP.[31] A study done by Zellin and Linde[13] aimed to investigate whether the choice of carrier/delivery system might be crucial for rhBMP-2 induced osteogenesis. The authors implanted rhBMP-2 into 5mm transosseous mandibular defects in rats using either a collagen sponge or bioabsorbable poly beads (PLA/PGA) w/ allogenic blood. The implanted rhBMP-2/carrier matrix was then placed with either a membrane or no membrane. Their findings showed that when comparing the use of a BMP carrier with a membrane to membrane alone, there was enhanced osteogenesis with the addition of the BMP and carrier. This is validated in their result of complete regenerated bone bridging with use of rhBMP-2 and PLA/PGA under a membrane but only 53% bone bridging when membrane was used alone. Furthermore, when comparing the two carrier systems overall, the PLA/PGA carrier was superior to the collagen carrier in the presence of a membrane. They concluded that the delivery system used can enhance the amount of bone formation obtained.[13]

Another study done by Uludag[14] attempted to describe the pharmakokinetics of rhBMP-2 with biomaterial used as a carrier and a correlation it has with its osteoinductive ability. The basis of their idea was the observation in other studies that when a biomaterial was used with rhBMP-2, (i) induction of bone was in close proximity to biomaterial (ii) the dose of rhBMP-2 needed for bone induction was significantly less with biomaterial (iii) the bone induction cascade was improved. To test their idea, they used a rat animal model and implanted rhBMP-2 and rhBMP-4 at varying concentrations

with an absorbable collagen sponge. The sponges were grouped as untreated, formaldehyde treated, and formaldehyde and ethylene oxide treated. Their results show varying retention of rhBMP-2 to the biomaterial based on the physical characteristics of the sponge. The group with the sponge treated with formaldehyde and ethylene oxide saw the highest retention of rhBMP-2. Their findings identified that the biomaterial was a critical component to the osteoinductive properties of rhBMP-2 and was also seen to modulate local protein pharmacokinetics.

Collectively, the findings of these studies validate the use of rhBMP-2 in regenerating bone. Under the proper conditions, rhBMP-2 has the potential to be used in restoration of osseous defects in medicine and dentistry. With the positive results stemming from rhBMP-2 in laboratory studies, a wave of clinical trials began that addressed using rhBMP-2 to restore osseous defects in the maxillofacial region.

rhBMP-2 Clinical Use in Restoration of Mandiular Defects

Initially, the first studies involved an animal model to test the capability of rhBMP-2 to restore defects in the mandible.[6- 9,15,16] In a study done by Toriumi in 1991, they evaluated the ability of BMP-2 implants to form bone and restore mandibular continuity and provide functional stability.[16] Their sample included 26 dogs who had 3-cm mandibular defects. They treated 3 groups, where group 1 was treated with BMP-2 and a matrix carrier, group 2 was treated with carrier alone, and group 3 was given no treatment. Their results showed that in group 1, a histomorphometric analysis revealed 68% replacement of the BMP-2 implant with mineralized bone whereas group 2 and 3 showed very little bone formation. They also found an average bending strength of group

1 to be 27% of the contralateral mandible where strength increased significantly from post-operative months 3-6 which the others attribute to an increase in the degree of mineralization and thickness of bone bridging. This study signifies the success of BMP-2 in restoring mandibular discontinuity defects in an animal model.

Another study done by Marukawa in 2002 observed reconstruction of a primate mandible using rhBMP-2 over the period of a year. Their goal was to evaluate the long-term functional properties of bone regenerated with rhBMP-2.[7] In this study, they restored 30mm mandibular defects in 6 primate models using rhBMP-2 with poly-D, L-lactic glycolic acid-coated gelatin sponge. They then placed dental implants in the regenerated mandible 20 weeks after surgery and loaded the implants 8 weeks after implant placement. Using histological and radiographic analysis of the newly generated bone, they found that the resected mandibles were completely regenerated with rhBMP-2 induced bone. The bone maintained its functionality for 1 year and demonstrated its use as a viable option for mandibular reconstruction to autogenous bone grafts.[7]

Toriumi carried out another study in 1999 to follow mandibular reconstruction using rhBMP-2 for an even longer period of time, thirty months. His goal was to determine the degree of bone resorption and stability of 3-cm, full thickness canine mandibular defects restored with rhBMP-2 and a poly bioerodable carrier.[9] In his sample, he used nine dogs divided into three groups, which included six dogs that were restored with rhBMP-2 and carrier, of which three were sacrificed at three months and 3 at 30 months. The other 3 animals served as control and only received carrier alone and were sacrificed at 3 months. The results of their study showed that control animals did not show any bone formation across the defect while the short term animals showed 41%

mean area density of defect regeneration. The long-term animals showed d 56.5% mean area density of restored defect and stabilization at 11 months with no indication of resorption. Over time, the authors observed an increase in the density of the regenerated defect in the long-term animals. These findings suggest that rhBMP-2 regenerated mandibular defects successfully integrate with host bone and are capable of responding to normal masticatory function with no signs of significant resorption.

A number of authors have used the success of rhBMP-2 from animal studies and observed the results of using rhBMP-2 in a human model. In 2008, Herford conducted a study on 14 human patients that had mandibular continuity defects caused by neoplastic and pathologic conditions.[5] The author's goal in the study was to observe the effects of rhBMP-2 in a collagen carrier in restoring critical sized defects of the mandible. His findings showed that all patients exhibited radiographic evidence of bone formation at three to four months after surgery and regained continuity of the mandible seen both radiographically and clinically.[5] This study shows that rhBMP-2 can be used to restore mandibular continuity defects successfully without the need of calcified graft system. Another study done by Heford in 2007 used rhBMP-2 with a collagen sponge to restore premaxillary clefts.[4] In this study, ten patients had unilateral premaxillary clefts restored with rhBMP-2/carrier and were compared with two patients who had unilateral premaxillary clefts restored with autogenous bone grafts from the anterior iliac crest. The volume of each defect was calculated pre-operatively and compared to the post-operative volume of the regenerated bone. At four months post-operatively, volume ratios of rhBMP-2 restored defects ranged from 24.1-90.6% with an average of 71.7% while patients who received autogenous bone grafts had volume ratios ranging from 71.3-

84.9% with an average of 78.1%. This study compares the use of rhBMP-2 in regenerating premaxillary clefts with what is still considered the "gold standard" of grafting in autogenous bone grafts. These findings suggest that rhBMP-2 is an effective alternative to autogenous bone grafts without the risks associated with the surgical morbidity of autogenous bone grafts.[4]

These pre-clinical and clinical trials show the effectiveness of using rhBMP-2 to restore defects in maxillofacial region.[1, 3, 4, 6- 9,15,16] However, in regenerating osseous defects, it is important to know the biomechanical characteristics of the regenerated bone and if the physical and biological response of the bone will be similar to native bone.

Finite Element Analysis Using a Mandibular Model

Determining the response of bone to a given force is important in determining its response to functional, physiologic, and even therapeutic forces. Several authors have sought to measure the strength of mandibular bone using a finite element model (FEM). FEM consists of a numerical procedure that simulates strain and stress response of a material based on the physical characteristics of that material. A 3-D reconstruction of a sample is made using a type of scan which can include cone beam CT or conventional CT, and the 3-D model is divided into voxels which are assigned a value based on the density reading provided by the scan. Using a set of equations, values simulating an external force can be input to the model which will in turn produce an output that correlates to stress and strain of the sample. FEM has been shown to be a useful tool in modeling functional forces of the TMJ,[17,18] forces applied to bone surrounding implants during loading,[19,20] and even effects of third molar removal on the mandible.[20] A study

done by Al-Sukhun tested the validity of FEM in analyzing mandibular strain by comparing its values to mandibular surface strain calculated by strain gauges. He found good agreement between predicted values by FEM and measure strain values deeming FEM a valid tool in measuring strain in the mandible.[21] In 2000, Vollmer carried out a similar experiment seeking to define mandibular deformation under a given load by comparing FEM to measured surface strain. In this study he also found agreement between the FEM and measured strain. With this he concluded that FEM is a valid and accurate, non-invasive method to predict biomechanical behavior of the mandible.[22]

Tie conducted a study using FEM that compared the biomechanical effects of restoring a mandibular defect with autogenous bone compared to native bone. In this study, computerized tomography scans of the mandible, fibula, and iliac crest were collected and used to model the FEM. A masticatory force modeling the TMJ and masticatory muscles was simulated and applied to the sample. FEM showed that the iliac bone graft compared to fibula had the most similar distribution of Von Mises stresses as the normal mandible. Most of the stress on the autograft was in the form tensile and compressive and was overall less in the autograft from the iliac crest. Using FEM, they were able to conclude that mandibles repaired with iliac crest grafts are more similar to normal bone biomechanically.[23]

These studies show that FEM can be an effective tool in modeling the mandible and functional forces that act on the mandible. While a number of studies have documented the biomechanical characteristics of the mandible and autogenous grafts using FEM, there is little, if any, literature that models the strength of BMP restored

defects compared with native bone. A study of this nature would provide information helpful in restoring a functional occlusion both dentally and orthopedically.

The literature shows that restoration of osseous defects in the maxillofacial region can be done successfully with rhBMP-2. In addition, grafting with rhBMP-2 does not carry the surgical morbidity associated with autogenous bone grafts and may serve as a more desirable treatment option to patients. With the refinement of grafting techniques and the increasing familiarity surgeons will have with rhBMP-2, it is expected that the number of patients receiving rhBMP-2 bone grafts will increase in the coming years. As dentists, it will be important to know the biomechanical characteristics of the regenerated bone to aid in making prudent treatment planning decisions. Examples include scenarios such as the role functional forces may have in loading the grafted area, which may lead to resorption or apposition in the defect site, or even the effect skeletal or dental expansion may have in a grafted premaxillary site of cleft palate patients. While the literature supports the use of modeling forces of the mandible using a Finite Element Model, it has yet to use this model to quantify the stress and strain response of rhBMP-2 restored defects in the mandible. This model would provide a valid, non-invasive approach to learning more about the biomechanical properties of rhBMP-2 restored defects.

CHAPTER TWO

INTRODUCTION

Statement of the Problem

Reconstruction of osseous defects in the maxillofacial region with rhBMP-2 has been shown to be a viable alternative to autogenous bone grafting. Autogenous bone grafting, which is considered to be the current gold standard in regenerating bone, suffers several drawbacks, including most notably donor site morbidity and difficulty with obtaining a sufficient donor supply. Given the novel nature of grafting with rhBMP-2, the biomechanical properties of the regenerated bone have not been addressed in the literature. What has historically been limited in treatment options, osseous defects of the mandible now have alternatives for restoration, which include Bone Morphogenetic Protein. Understanding the biomechanical characteristics of the BMP "regenerated bone" would be important to decipher the limitations that may occur during orthopedic or dental treatment and serve as an aid in making prudent treatment planning decisions.

With the increasing prevalence of dental patients having undergone restoration of maxillofacial osseous defects with BMP, it is important to understand the biomechanical characteristics of the BMP "regenerated bone" to decipher the limitations that may occur during orthopedic or dental treatment. Those who have undergone neoplastic tumor resection, were born with incomplete fusion of sutures, seen in cleft palate, and even localized bone loss caused by periodontal disease are a population who seek novel ways to regenerate bone and restore a functional occlusion. While previous studies have shown

the success of osseous reconstruction using BMP,[1-4] the literature has yet to report the biomechanical response of this regenerated bone to stress and strain.

The purpose of this study is to use Finite Element Analysis to evaluate and compare the biomechanical characteristics of the rhBMP-2 regenerated bone in mandibular osseous defects using 2 different concentrations of rhBMP-2 with a given carrier in non-human primates (*Macaca fascicularis*).

Hypothesis

The null hypothesis is there is no difference in peak stresses within a regenerated mandible between the 2 concentrations of rhBMP-2/carrier when exposed to forces of the masticatory musculature.

The alternative hypothesis is that a mandible with defects restored with a higher concentration of rhBMP-2/carrier will exhibit a different distribution/magnitude of peak stresses and displacement when exposed to forces of the masticatory musculature.

Materials and Methods

Surgical Procedures

This study was approved by the Institutional Animal Care and Use Committee. Six adult male non-human primates (*Macaca Fascicularis*) were used in this study to produce 12 mandible-halves for evaluation. Each animal underwent two separate surgical procedures. In the first surgical procedure, the canines, 1st and 2nd premolars, and 1st and 2nd molar teeth were bilaterally extracted to produce a smooth, edentulous ridge in preparation of the mandibular discontinuity defects. Six weeks later, bilateral

(approximately 2.5 cm) critical sized defects were created with immediate reconstruction with a reconstruction plate and simultaneous implantation of a graft material. Before each surgery, each animal was given 0.2 mg/kg Ketamine intramuscularly IM and local anesthesia of 2% lidocaine with 1:200,000 Epinephrine. During surgery, each animal was given intravenous 2 mg/kg Ketamine every 25 minutes, 2% lidocaine with 1:200,000 Epinephrine, oral endotracheal Isofluorane, and 0.5% Marcaine with 1:100,000 Epinephrine.

Long term care with a soft diet and exercise was observed for 6 months. Tetracycline labels to produce intravital fluorochrome for bone labeling were administered at the following intervals:

- 3 weeks post-resection and reconstruction to indicate initial bone matrix deposition.
- 16 weeks post-resection and reconstruction to label bone turnover

At the 6th post-operative month, the animals were euthanized and underwent cannulation of bilateral carotid arteries and were perfused with 10% formalin. The mandibles were subsequently harvested. All 6 mandibles were then radiographically scanned using the Newtom 5G CBCT machine (QR Srl, Verona, Italy).

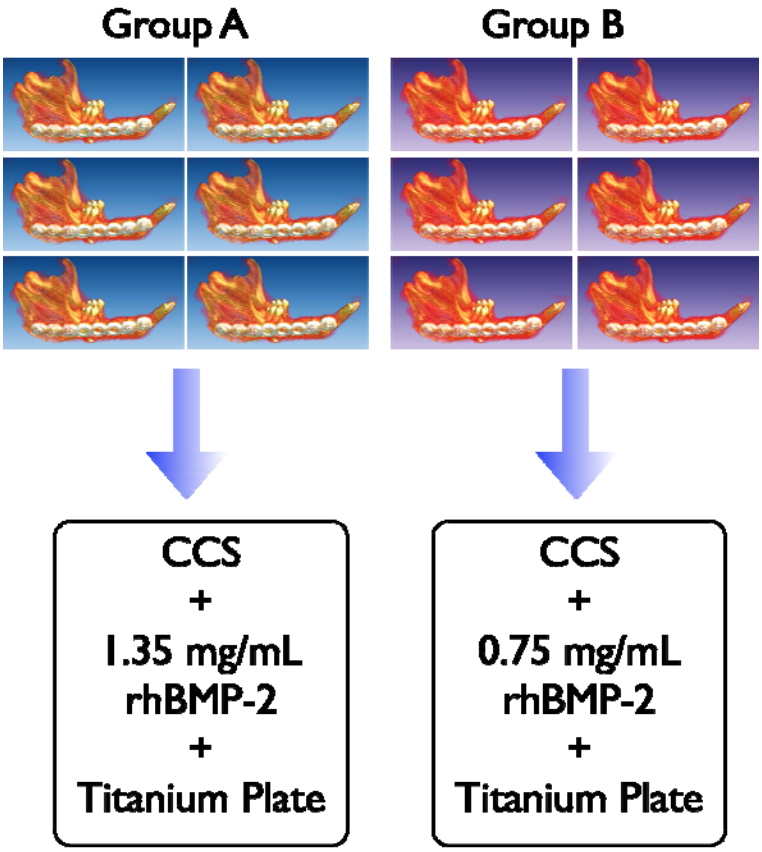


Figure 1. Distribution of treatment groups.

Graft Materials

In restoring the critical-sized defects, a split mouth study design was used where each mandible-half was selected to receive one of 2 types of carrier/rhBMP-2 dose combinations, resulting in six defects per carrier group. The bulking agent used was a collagen ceramic sponge comprised of bovine type I collagen sponge impregnated with 15% hydroxyapatite/85% B-tricalcium phosphate ceramic granules. Group A received 1.35 mg/mL rhBMP-2 combined with CCS received (Figure 1). Group B received 0.75 mg/mL rhBMP-2 combined with a CCS. The animals were divided into 2 groups (n=6 for each group). Because the defects varied in size slightly, the doses were calculated after

the bone was removed from the defect and measured. In order to calculate the total dose for the groups the dry volume (length x width x height) was multiplied by the solution concentration (either 1.35 mg/mL—Group A, or 0.75 mg/mL—Group B) and the soak load (0.5 mL for both). All of the defects were stabilized by a 2.4 cm locking reconstruction plate (Synthes, Paoli) to provide rigid fixation after creation of the defect.

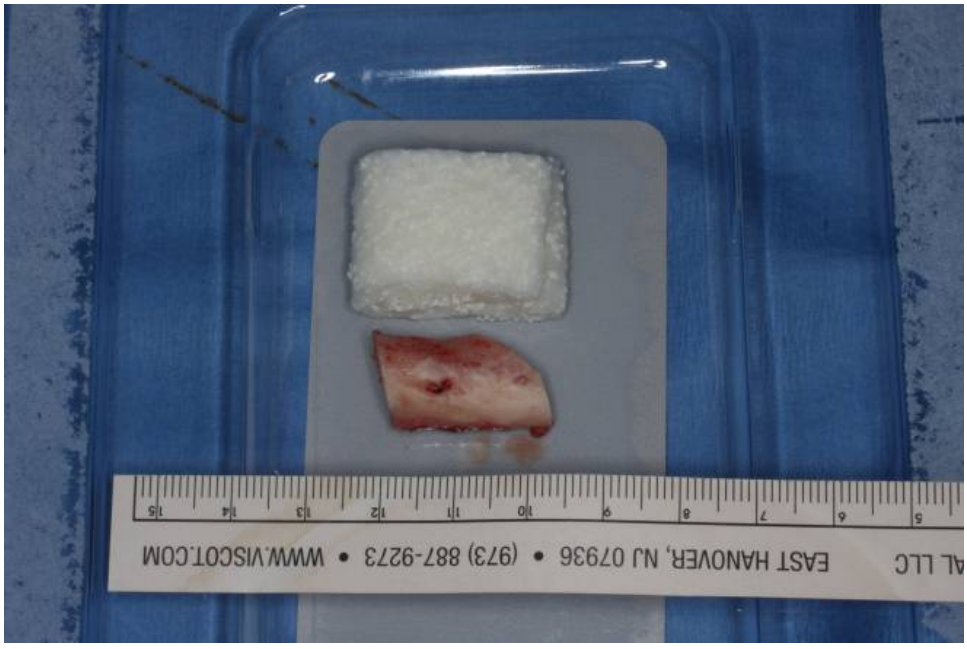


Figure 2. Resected bone with rhBMP-2 and CCS

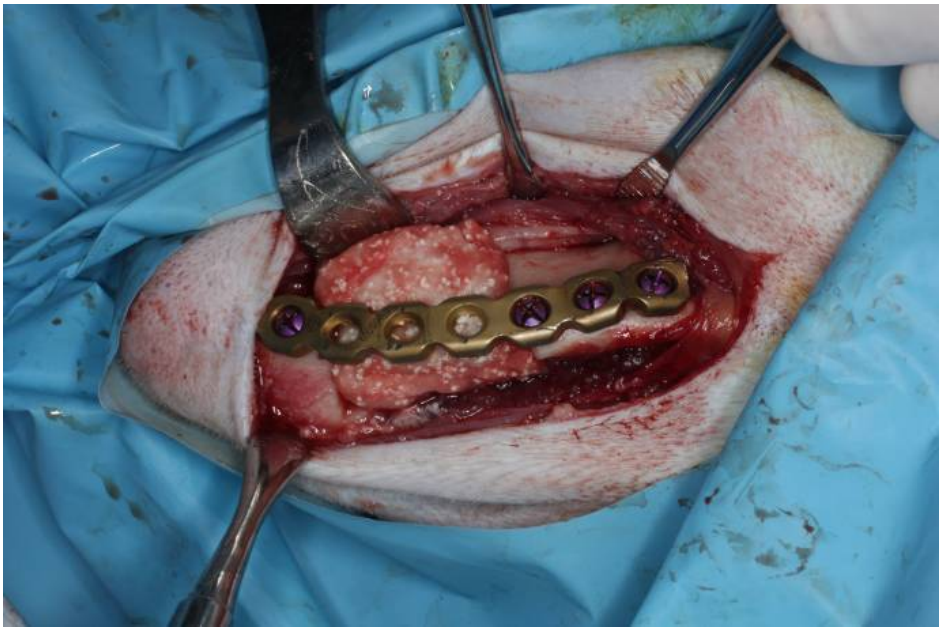


Figure 3. Group A carrier (CCS with 0.75 mg/mL rhBMP-2) stabilized with titanium plate.

Tensile Testing

Bone specimens from each treatment group were collected by slicing a sagittal section through the defect site. Twelve samples were collected and placed in a formalin solution. To prepare for tensile testing with the MTS mechanical testing machine (MTS Systems, Eden Prairie, MN), soft tissue was removed from the bone leaving a solid piece of intact bone. Out of the 12 samples that were collected, Ten samples, five from each treatment group, were prepared for use in this study. The remaining 2 samples had excess soft tissue and did not have enough bone, as a result of a poor slice. The bone from each sample was then sectioned within the defect site between the remaining molar and the lateral incisor using a diamond disc to achieve a dimension compatible with the functional components of the MTS machine.

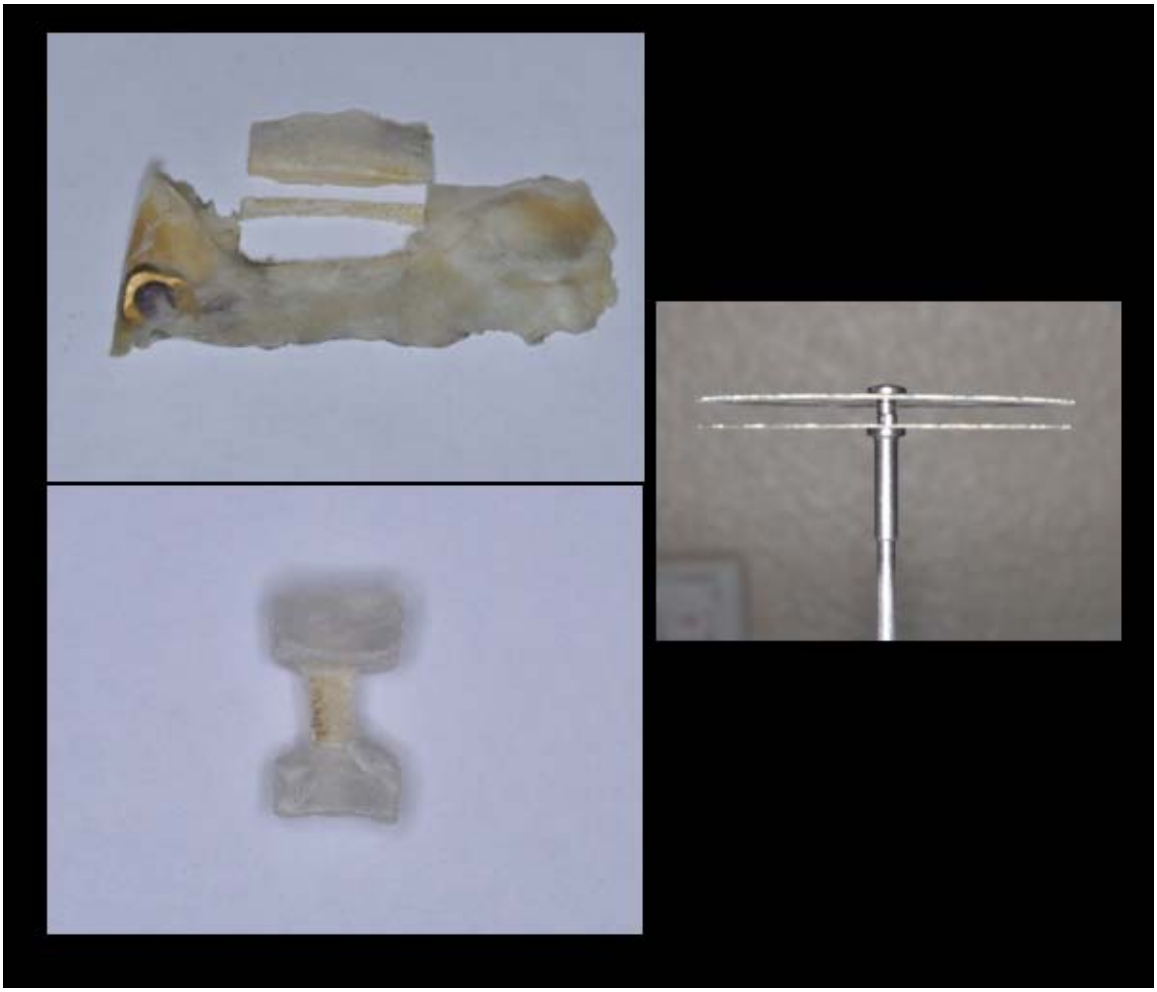


Figure 4. Regenerated bone from the defect site was sectioned after sacrifice of the animals. From each section, regenerated bone was further cut to be tested with an MTS machine.

The tensile testing of our bone samples in this study followed the protocol outlined by Jonas et al. [43] Each bone sample was sectioned using two diamond discs (Komet, Rock Hill, SC) separated by two 1 mm spacers attached to an electric handpiece. The length of each specimen was then cut to a length of 8-10 mm. Undercuts at the end of each of the bone samples were made with a cutting bur and then embedded in dental acrylic to function as a grip for the MTS machine. Once each sample was prepared, the width and length measurements were collected and recorded. Each sample was then

placed in the jaws components of the MTS machine and analyzed using the tensile test module of the Testworks software (Testworks V4.12) Young's modulus for each bone sample was calculated using the following equation:

Young's modulus = (force/cross section of area)/(change in length/original length). The force value was selected on the stress-strain curve within the sample's elastic limit.

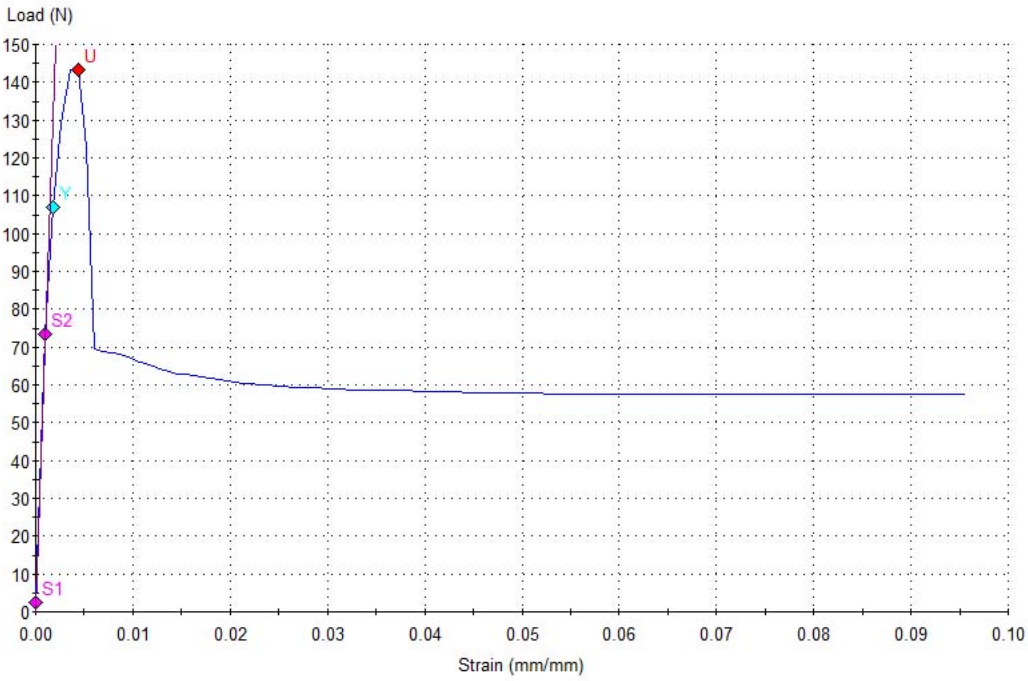


Figure 5. Stress-strain curve of a regenerated bone sample.

To determine the density of the regenerated bone specimens, each sample was placed in a pycnometer and the amount of water displaced was measured. Poissons ratio of the regenerated bone was assigned a value of 0.33.

Figure 6. MTS machine was used to complete tensile testing of our bone specimens.

Model Acquisition

The model acquired and imported to COMSOL Multiphysics (COMSOL, Burlington, MA) was processed through three different steps as shown in Figure 7.

Step 1: Noise from post-treatment radiographic scans was eliminated using Amira® software (Amira 5.2.2; Visage Imaging, Inc, Carlsbad, CA).

Step 2: A virtual model was drawn using AutoCAD® (San Rafael, CA) referencing images from post-treatment radiographic scans to reproduce a 2-dimensional sagittal view of our mandible sample.

Step 3: Finite element analysis was carried out using the Structural Mechanics Module of the COMSOL Multiphysics® software (Burlington, MA). The COMSOL Multiphysics®

model was meshed and solved for displacement of the regenerated bone for the twelve samples as shown in Figure 8.

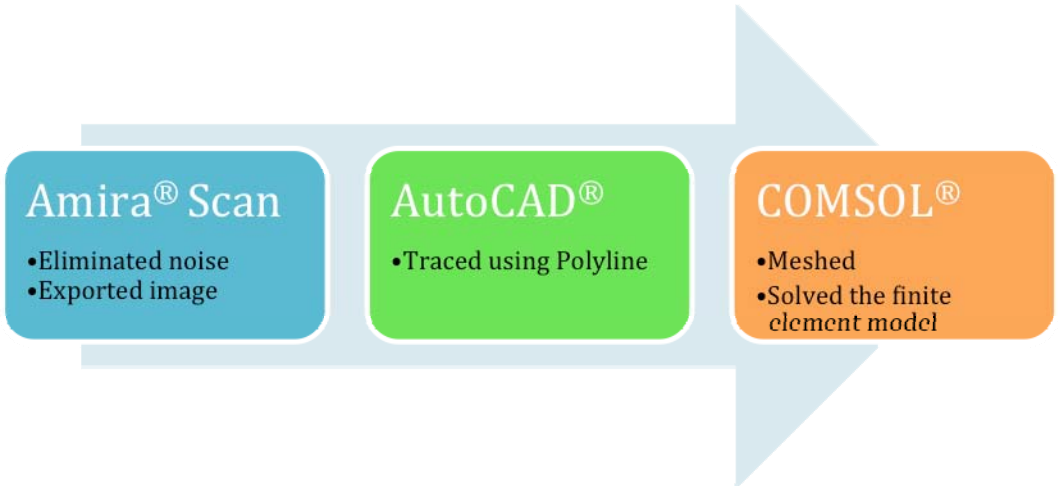


Figure 7. The process flow developed to accurately import the image for finite element modeling.

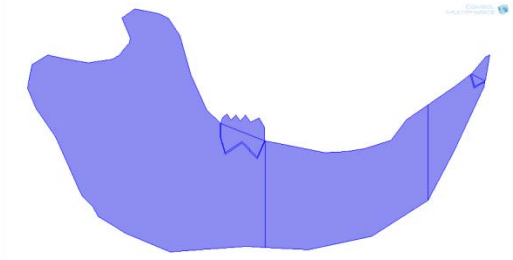


Figure 8. Virtual model of regenerated mandible

Subdomain Conditions

This model was then imported into COMSOL Multiphysics® where the subdomain boundaries were identified. The following subdomains were created in our 2-dimensional model: a) cortical bone b) regenerated bone c) cementum d) enamel 5) periodontal ligament.

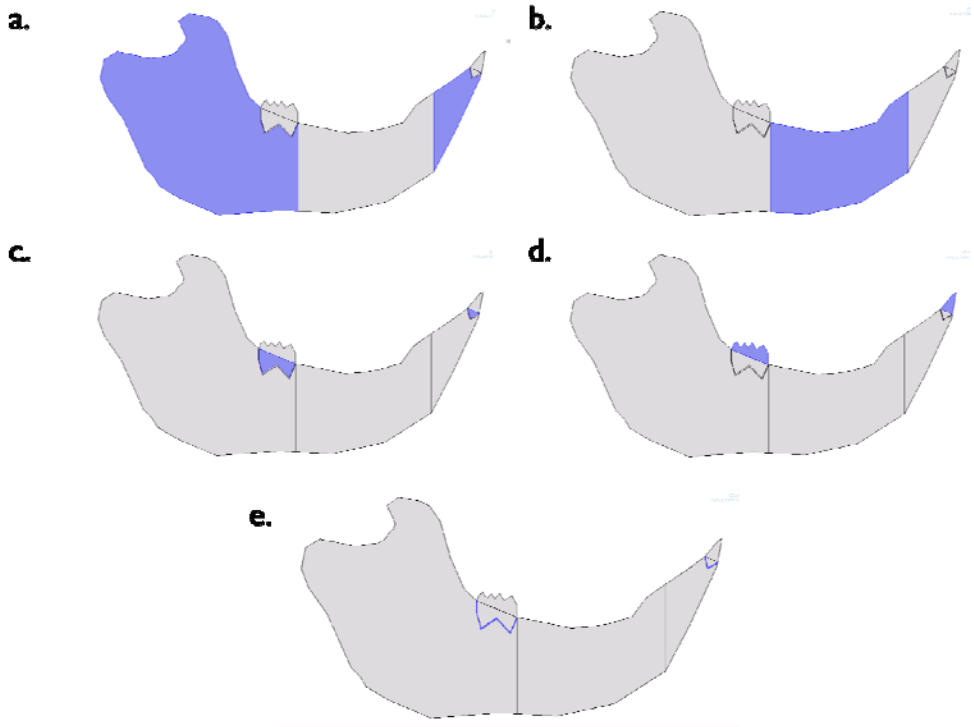


Figure 9. The figures above illustrate the locations of each subdomain. a). Cortical bone b). regenerated bone (from tensile testing) c). cementum d). enamel e). periodontal ligament

Values for Young's modulus and Poissons ratio for cortical bone, periodontal ligament, cementum, and enamel were collected from studies done by Middleton et al. [27] and Nagasao et al[28]. Young's modulus for the regenerated bone was drawn from the results of the tensile testing and the density was found using a pycnometer. The titanium plate was extracted from the model in order to simulate a continuous mandible with the biomechanical properties of the regenerated bone in the defect site.

Mesh Sizing

Once the model was completed, a mesh was generated with the COMSOL Multiphysics® software. Two different mesh sizes were created: (1) approximately

14,800 triangular elements and (2) approximately 900,000 triangular elements. A variation in mesh sizing was done in order to see if there was a difference in the resolution. The models were independently simulated with both the 900,000 and 14,800 elements with no significant differences in the results. As shown in Table 1, a Tolerance Test was performed to check for mesh independency. It was therefore decided that the 14,800 mesh element model would be used for our study in order to increase the efficiency of solving the differential equations.

Table 1. Tolerance Test with 14800 mesh elements to 183000 mesh.

Number of elements	**Time (s)**	**Deformation, δ (cm x 10^{-6})**	**Tolerance** $\frac{\delta_{finest\ mesh} - \delta_{coarse\ mesh}}{\delta_{finest\ mesh}}$
14.8K	10	7.48	0.0053
26K	46	7.49	0.0040
49.7K	9	7.50	0.0027
85.2K	13	7.50	0.0027
183K	21	7.52	0

Boundary Conditions

To illustrate a functional activity of the mandible, the activity of the masseter muscle was simulated virtually. Activity of the masseter muscle was simulated by applying a force with a vector of 130 N in the X direction and 75 N in the Y direction. The total magnitude of force by the masseter muscle was simulated to be 150 N at a 30° angle.

Figure 10. The figures above illustrate the boundary conditions applied to the mandible. The first model shows a force from the masseter muscle. The second model shows the location of the pivot point from the temporomandibular joint. The last model shows the location of the fixed point applied to the lower incisor.

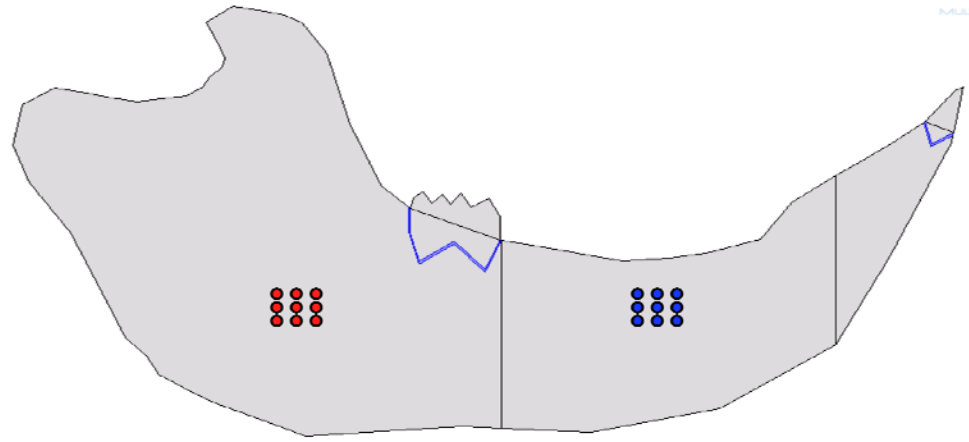
The temporomandibular joint of this model was designated the pivot point of the model, while the lower incisor was assigned as a fixed point, toward which the masseter muscle is lifting the mandible.

Methodology of the Measurements

A total of ten models were created to represent each treatment sample. An eleventh model was created to represent the control sample, where the regenerated bone

subdomain was given biomechanical values identical to cortical bone.[28] All eleven models were run individually using the 14,800 mesh elements. Data was collected for von Mises stress, displacement in the X-direction, and displacement in the Y-direction. To gather a varied distribution of data from our model, four domains were chosen. One domain was completely within the cortical bone subdomain, one was completely within the regenerated bone subdomain, and the remaining two were at the anterior and posterior cortical bone-regenerated bone boundary (Figure 11). In each domain, a three by three grid was created to reduce sampling error. Each point within a domain was assigned a coordinate. Values for the variables von Mises stress, displacement-x, and displacement-y were collected using the COMSOL Multiphysics® software.

a)



b)

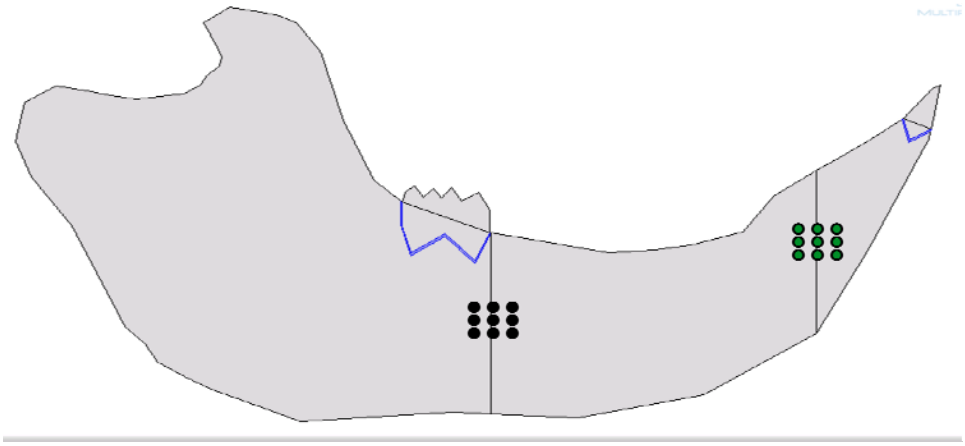


Figure 11. Four domains were created for comparisons of strain, displacement-x, and displacement-y. a) 2 domains were within subdomains b) The remaining 2 were at subdomain boundaries. Within each domain, a 3x3 grid was created to provide a larger sample of data.

Figure 12. Diagrammatic representation of the regions of interest (ROIs) in 2-D arranged from posterior to anterior. First digit indicates domain location. Second digit indicates orientation within domain.

Statistical Analysis

For each mandible, 36 data points were collected for the 3 variables which included stress, displacement-x, and displacement- y. Means and standard deviations of all variables were calculated. Friedman's two-way analysis of variance by ranks was performed to compare differences in stress and displacement of the mandible among the treatment groups and the control. Further analysis included the Wilcoxon Signed Rank Test omitting data points in the domains that were on the boundary of bordering subdomains to account for any discrepancy in the differential equations at the borders. The Independent Wilcoxon Signed Rank Test was also used to detect any clinically significant differences in the 3 variables among the control group and among regions completely within its subdomain. All statistical analyses were performed with SAS v. 9.1.3 **(**SAS Institute, Cary, North Carolina) at the significance level of $\alpha = 0.05$.

CHAPTER THREE

RESULTS

The biomechanical properties from each sample are summarized in Table 2. The average modulus of elasticity of regenerated bone from group's A and B were 40.0% (range = 31-84%) and 20.7% (range = 2-76%) of cortical bone, respectively. The value for Young's modulus of elasticity for cortical bone was referenced as 150 x 10^8Pa.[27]

Table 2. Modulus of elasticity and density values tests from each treatment group.

Sample	Young's Modulus (10^8 Pa)	Density (g/cm^3)
Group A	60.00 ± 39.95	1.00 ± 0.10
Group B	31.01 ± 46.83	1.00 ± 0.50

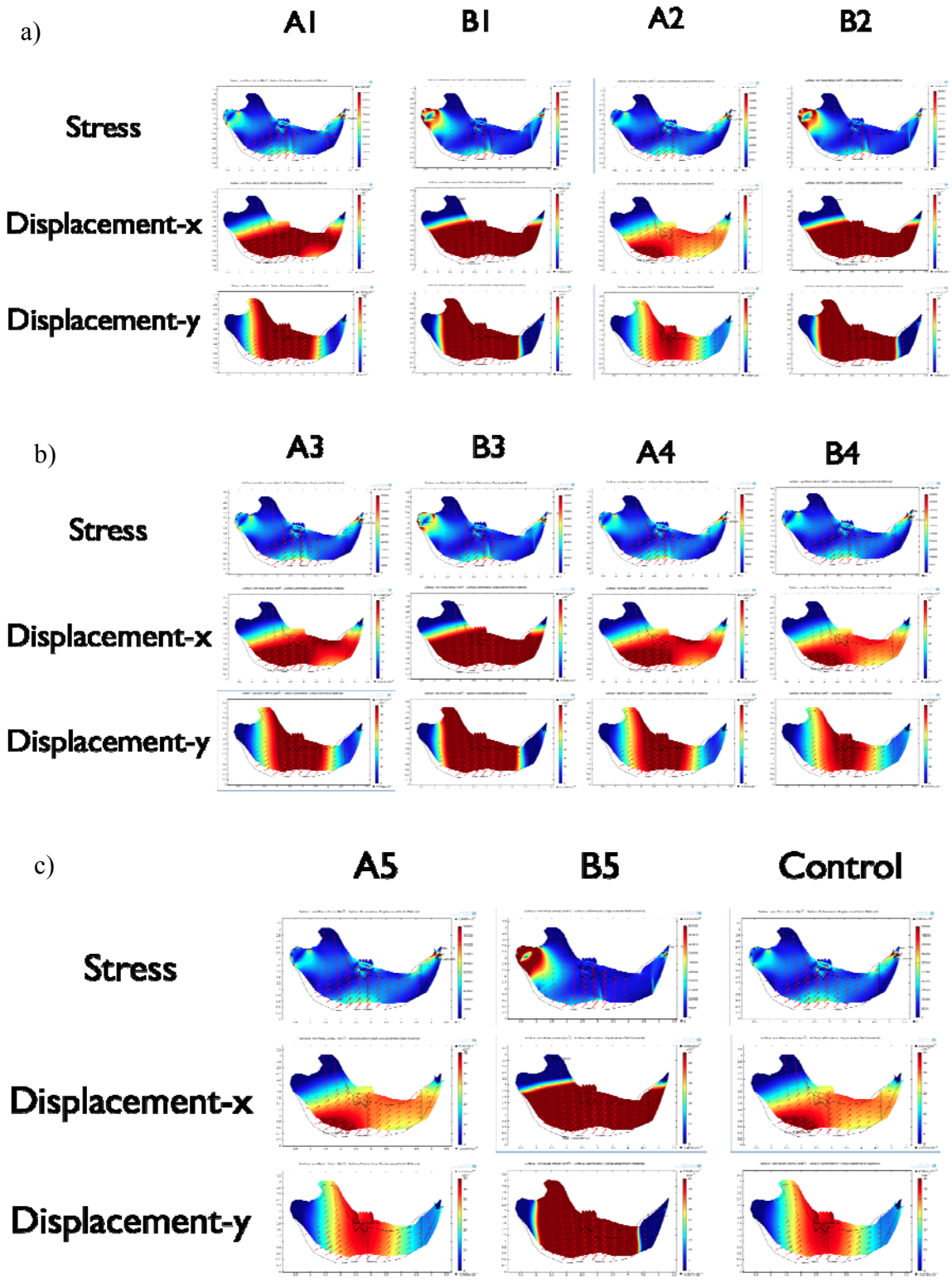


Figure 13. Color map shows the magnitude of strain, displacement-x, and displacement-y in the treatment groups. Blue color indicates lower values while the red indicates greater values.

There was no statistical difference between the distribution of stress in the regenerated mandibles between treatment group A and group B in response to function. However, both treatment groups A and group B showed significantly less distribution of stress when compared to the control group. Evaluating the amount of displacement in response to function, the results were similar for both displacement in the x coordinate and displacement in the y coordinate for treatment group A and group B. There was no statistical difference between group A and group B for both displacement in the x coordinate and displacement in the y coordinate. However, both treatment groups A and B showed significantly more displacement than the control group in response to function.

Table 3. Related samples Friedman's two-way analysis of variance by ranks test of strain distribution and displacement among groups.

Variable	Control	Group A	Group B	p-value
Stress Distribution (10^3Pa)	8.11 +/-3.80[a]	7.95+/- 3.65[b]	7.29+/-2.53[b]	.038
Displacement-x (10^{-5}mm)	0.35+/-0.035[a]	1.40+/-1.82[b]	1.04+/-0.50[b]	.000
Displacement-y (10^{-5}mm)	0.30+/-0.055[a]	1.46+/-1.99[b]	1.07+/-0.57[b]	.000

[a,b]: Different letters denote statistically significant differences

CHAPTER FOUR

DISCUSSION

Bone grafting with rhBMP-2 has been shown to be a viable alternative to autogenous bone grafts in restoring osseous defects in the maxillofacial region[3-5]. While these studies have shown clinical success in restoring maxillofacial defects with rhBMP-2, the mechanical properties of the restored bone are still relatively unknown. In this study, rhBMP-2 regenerated bone was tested to determine it's mechanical properties and modeled in a functional mandible using Finite Element Analysis. In this study, tensile testing of the low and high concentration rhbmp-2 regenerated bone samples showed an average of 20.7% and 40.0% the stiffness of cortical bone.[28] The disparity in stiffness between treatment groups and cortical bone likely contributed to statistically significant differences between treatment groups and control, when evaluating differences in stress distribution, displacement-x, and displacement-y. There were, however, no statistically significant differences in stress distribution, displacement-y, and displacement-x between treatment group A and treatment group B. This suggests that the load bearing capacity of a restored mandible with different concentrations of rhBMP-2 might be comparable.

These results are consistent with other studies, which have reported mechanical properties of BMP regenerated bone to have a mean stiffness of 24% (range 9-63%) the stiffness of non-operated bone.[29] Studies have suggested that a longer follow up period might result in rhBMP-2 regenerated bone with mechanical properties approaching that of cortical bone.[1, 29] The sample of regenerated bone created in our study was collected at

6 months, which may have contributed to the inferior quality compared to cortical bone. Furthermore, our tensile testing of regenerated bone yielded a large variability in Young's modulus within the same treatment group. This finding is consistent with other studies that have attempted to compare the mechanical properties of BMP-induced bone to native bone.[32] This can be attributed to variable rates of bone mineralization with BMP-induced bone. In this study, there may have also been variability in the location when sectioning the regenerated bone, yielding a different location within the defect site. Standardization of the bone section of bone samples taken for tensile testing may reduce the disparities in mechanical properties due to anatomical differences.

Studies have demonstrated that a threshold dose of BMP is needed to form bone, but significantly higher doses beyond this may not increase the volume or quality of the regenerated bone .[30,31] It has been shown that BMP's can stimulate osteoclastic activity when present at high doses,[30] and high doses are likely to turn on negative feedback mechanisms prohibiting excess bone formation.[31] The results of this study suggest that the 0.75 mg/cc rhBMP-2 dose with CCS reached the threshold dose for BMP to adequately form bone, and higher doses were not more effective. This substantiated the finding of a previous study where no significant differences ($p < .05$) in bone densities were found between bone regenerated with high (2.0 mg/mL rhBMP-2) and low concentration (0.75 mg/mL rhbmp-2) BMP.[33]

In this study, Finite Element Analysis was used to illustrate the mechanical properties of a mandible restored with rhBMP-2. The basis for our the use of finite element analysis were studies that showed agreement between the methodology of using mandibular strain gauges and finite element analysis in evaluating the stress of a

mandible in response to bending.[21,22] In this study, finite element analysis was used to illustrate a regenerated mandible. The goal of this study was to theoretically model the restored mandible, and provide a template for future studies. By using a 2-dimensional model, errors in solving the differential equations could be minimized. Future studies involving the 3-dimensionally restored mandible can potentially depict more accurately areas where high stress and displacement may occur.

Limitations of the Study

A limitation of this finite element study of new bone formation involves the non-standardized sample of regenerated bone. Each slice of bone was gathered from different segments of the mandible, which in turn may have contributed to a different rate of mineralization. This could account for the variability in Young's modulus that was observed among the samples. Precisely designed sections could provide a more accurate picture of the mechanical properties of the regenerated bone and may show less of a deviation among samples.

While the 2 dimensional nature of our model can give a snapshot of how a restored mandible would behave mechanically, it only provides a snapshot. In the future, a 3-dimensional model could provide more detail and information to specific areas that may be susceptible to high stress and displacement.

The original data set comprised 2 treatment groups with 6 samples in each group. Due to limitations in the slice of each sample, only 5 samples for each treatment group were available for use in this study. The small sample size may have contributed to variability in our data set. Future studies will benefit if more animals were included in

each treatment group, or results from multiple studies were grouped to increase the sample size.

CHAPTER FIVE

CONCLUSIONS

1. After 6 months, treatment group A and B produced regenerated bone that possessed 40.0% and 20% stiffness of cortical bone, respectively.
2. There were no statistical differences in stress distribution, displacement-x, and displacement-y between treatment group A and treatment group B ($p < .05$).
3. There was a statistically significant difference between the control group and treatment group A and treatment group B. Both treatment groups had a mean difference of 119 Pa (group A) and 814 Pa (group B). This difference is equal to approximately 0.1% and 0.69% of the peak maximum stress in the mandible exhibited in the model. While this difference is significant statistically, the small fraction of difference is clinically insignificant. Differences between treatment groups and control group in displacement were also clinically insignificant with their magnitude of difference being on the order of a thousandth of a millimeter.
4. A concentration of 0.75 mg/mL rhbmp-2 carrier with CCS produced bone with similar mechanical characteristics to a higher concentration of 1.3 mg/mL rhbmp-2 with CCS. 0.75 mg/cc rhbmp-2 is shown to fall within the threshold amount to regenerate adequate bone.
5. Finite Element Analysis is a non-invasive method where the biomechanical properties of a functional model based on the material properties of the regenerated bone can be tested.

REFERENCES

1. Boyne P. Application of Bone Morphogenetic Proteins in the Treatment of Clinical Oral and Maxillofacial Defects. Journal of Bone Joint Surgery. 2001;83:146-150.

2. Dimar JR, Glassman SD, Burkus JK, Pryor PW, Hardacker JW, Carreon LY. Clinical and radiographic analysis of an optimized rhBMP-2 formulation as an autograft replacement in posterolateral lumbar spine arthrodesis. Journal of Bone Joint Surgery. 2009;91:1377-86.

3. Herford AS, Boyne PJ, Rawson R, Williams RP. Bone morphogenetic protein-induced repair of the premaxillary cleft. Journal of Oral and Maxillofacial Surgery. 2007;65:2136-41.

4. Herford AS, Boyne PJ. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). Journal of Oral and Maxillofacial Surgery. 2008;66:616-24.

5. Herford AS. rhBMP-2 as an option for reconstructing mandibular continuity defects. Journal of Oral and Maxillofacial Surgery. 2009 Dec;67(12):2679-84.

6. Hollinger JO, Schmitt JM, Buck DC, Shannon R, Joh SP, Zegzula HD, Wozney J. Recombinant human bone morphogenetic protein-2 and collagen for bone regeneration. Journal of Biomedical Materials Research. 1998;43:356-64.

7. Marukawa E, Asahina I, Oda M, Seto I, Alam Md, Enomoto S.. Functional reconstruction of the non-human primate mandible using recombinant human bone morphogenetic protein-2. International Journal of Oral and Maxillofacial Surgery. 2002; 31: 294–302.

8. Spector DI, Keating JH, Boudrieau RJ. Immediate mandibular reconstruction of a 5 cm defect using rhBMP-2 after partial mandibulectomy in a dog. Veterinary Surgery. 2007;36:752-9.

9. Toriumi DM, O'Grady K, Horlbeck DM, Desai D, Turek TJ, Wozney J. Mandibular reconstruction using bone morphogenetic protein 2: long-term follow-up in a canine model. Laryngoscope. 1999;109:1481-9.

10. Urist MR. Bone: Formation by autoinduction. Science 1965; 150:893-99.

11. Lieberman J, Daluiski A, Einhorn T. The Role of Growth Factors in the Repair of Bone: Biology and Clinical Application. Journal of Bone Joint Surgery. 2002;84:1032-1044.

12. Wang E, Rosen V, D'alessandro J, Bauduy M, Cordes P, Harada T, Israel D, Hewick R, Kerns K, Lapan P, Mcquaid D, Moutsatsos I, Nove J, Wozney J. Recombinant human bone morphogenetic protein induces bone formation. Proceedings of the Indian National Science Academy. 1990; 87: 2220-2224.

13. Zellin G, Linde A. Importance of delivery systems for growth-stimulatory factors in combination with osteopromotive membranes. An experimental study using rhBMP-2 in rat mandibular defects. Journal of Biomedical Materials Research. 1997;35:181-90.

14. Uludag H, Gao T, Porter T, Friess W, Wozney J. Delivery Systems for BMPs: Factors Contributing to Protein Retention at an Application Site Journal of Bone and Joint Surgery. 2001;83:128-135.

15. Inoda H, Yamamoto G, Hattori T. Histological investigation of osteoinductive properties of rh-BMP2 in a rat calvarial bone defect model.Journal of Craniomaxillofacial Surgery. 2004;32:365-9.

16. Toriumi DM, Kotler HS, Luxenberg DP, Holtrop ME, Wang EA. Mandibular reconstruction with a recombinant bone-inducing factor. Functional, histologic, and biomechanical evaluation. Archives of Otolaryngology-Head and Neck Surgery. 1991;117:1101-12.

17. Hirose M, Tanaka E, Tanaka M, Fujita R, Kuroda Y, Yamano E, Van Eijden T, Tanne K. Three-dimensional finite-element model of the human temporomandibular joint disc during prolonged clenching. European Journal of Oral Science. 2006; 114:441–448.

18. Tanaka E, del Pozo R, Tanaka M, Asai D, Hirose M, Iwabe T, Tanne K. Three-dimensional finite element analysis of human temporomandibular joint with and without disc displacement during jaw opening. Medical Engineering and Physics. 2004;26:503-11.

19. Ihde S, Goldmann T, Himmlova L, Aleksic Z. The use of finite element analysis to model bone-implant contact with basal implants. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2008;106:39-48.

20. Koca OL, Eskitascioglu G, Usumez A. Three-dimensional finite-element analysis of functional stresses in different bone locations produced by implants placed in the maxillary posterior region of the sinus floor. Journal of Prosthetic Dentistry. 2005 Jan;93:38-44.

21. Szucs A, Bujtár P, Sándor GK, Barabás J. Finite element analysis of the human mandible to assess the effect of removing an impacted third molar. Journal of the Canadian Dental Association. 2010;76:72.

22. Al-Sukhun J., Kelleway J., Helenius M. Development of a three-dimensional finite element model of a human mandible containing endosseus dental implants. I. Mathematical validation and experimental verification. Journal of Biomedical Materials Research. 2007; 80: 234-46.

23. Vollmer D, Meyer U, Joos D. Experimental and finite element study of a human mandible. Journal of Cranio-maxillofacial Surgery. 2000; 28: 91–96.

24. Tie Y, Wang DM, Ji T, Wang CT, Zhang CP. Three-dimensional finite-element analysis investigating the biomechanical effects of human mandibular reconstruction with autogenous bone grafts. Journal of Craniomaxillofacial Surgery. 2006;34:290-8.

25. Ammar HH, Ngan P, Crout RJ, Mucino VH, Mukdadi OM. Three-dimensional modeling and finite element analysis in treatment planning for orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics. 201; 139: e59-71.

26. Bujtár P, Sándor GK, Bojtos A, Szucs A, Barabás J. Finite element analysis of the human mandible at 3 different stages of life. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2010;110:301-9.

27. Middleton J, Jones M, Wilson A. The role of the periodontal ligament in bone modeling: The initial development of a time-dependent finite element model. American Journal of Orthodontics and Dento-facial Orthopedics. 1996;109:155-62.

28. Nagasao T, Kobayashi M, Tsuchiya Y, Kaneko T, Nakajima T. Finite element analysis of the stresses around endosseous implants in various reconstructed mandibular models. Journal of Cranio-Maxillofacial Surgery. 2002;30:170-77.

29. Abu-Seriah M., Kontaxis A., Ayoub A., Harrison J., Odell E., Barbenel J. Mechanical evaluation of mandibular defects reconstructed using osteogenic protein-1 (rhOp-1)

in a sheep model: a critical analysis.

30. Franceschi RT. Biological approaches to bone regeneration by gene therapy. J Dent Res 2005; 84:1093-1103.

31. Winn SR, Uludag H, Hollinger JO. Sustained release emphasizing recombinant human bone morphogenetic protein-2. Advanced Drug Delivery Reviews 1998; 31:303-18.

32. Cook SD, Wolfe MW, Salkeld SL, Rueger DC. Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. Journal of Bone and Joint Surgery. 77: 734:50.

33. Kim J, Caruso J, Rungcharassaeng K, Herford A. Density of reconstructed bone using different rhBMP-2 carriers in critical-sized defects: A comparative microCT study. LLU Masters Thesis 2010.

34. Hart RT, Hennebel VV, Thongpreda N, Van Buskirk WC. Anderson R. Modeling the biomechanics of the mandible: a three dimensional finite element study. Journal of Biomechanics.1992;25:261-86.

35. Inoda H, Yamamoto G, Hattori T. Histological investigation of osteoinductive properties of rh-BMP2 in a rat calvarial bone defect model.Journal of Craniomaxillofacial Surgery. 2004;32:365-9.

36. Inoda H, Yamamoto G, Hattori T. rh-BMP2-induced ectopic bone for graftingcritical size defects: a preliminary histological evaluation in rat calvariae. International Journal of Oral and Maxillofacial Sugery. 2007; 36: 39–44.

37. Jovanovic SA, Hunt DR, Bernard GW, Spiekermann H, Wozney JM, Wikesjö UM. Bone reconstruction following implantation of rhBMP-2 and guided bone regeneration in canine alveolar ridge defects. Clin Oral Implants Research. 2007;18:224-30.

38. Jung JH, Yun JH, Um YJ, Jung UW, Kim CS, Choi SH, Cho KS. Bone formation of Escherichia coli expressed rhBMP-2 on absorbable collagen block in rat calvarial defects. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2010;xx:xxx.

39. Kirker-Head, CA. Potential applications and delivery strategies for bone morphogenetic proteins. Advanced Drug Delivery Reviews. 2000; 43:65-92.

40. Korioth T, Versluis A. the Mechanical Behavior of the Jaws and Their Related Structures By Finite Element (Fe) Analysis. Critical Reviews in Oral Biology & Medicine. 1997 8: 90-104.

41. Lieberman J, Daluiski A, Einhorn T. The Role of Growth Factors in the Repair of Bone: Biology and Clinical Application. Journal of Bone Joint Surgery. 2002;84:1032-1044.

42. Moon HS, Won YY, Kim KD, Ruprecht A, Kim HJ, Kook HK, Chung MK. The three-dimensional microstructure of the trabecular bone in the mandible. Surgical and Radiologic Anatomy. 2004;26:466-73.

43. Srirekha A, Bashetty K. Infinte to finite: An overview of finite element analysis. Indian Journal of Dental Research. 2010;21:425-32.

44. Jonas J, Buns J, Abel EW, Cresswell MJ, Strain JJ, CR Paterson. A technique for the tensile testing of demineralised bone. Journal of Biomechanics. 1993;26:271-76.

45. Hannam AG, Stavness I, Lloyd JE, Fels S. A dynamic model of jaw and hyoid biomechanics during chewing. Journal of Biomechanics. 2008;41:1069-76.

46. Hannam AG et al. A comparison of simulated jaw dynamics in models of segmental mandibular resection with alloplastic reconstruction. Journal of Prosthetic Dentistry. 2010; 104: 191-98.

47. Wong RC, Tideman H, Kin MA, Merkx AW. Biomechanics of mandibular reconstruction: A review. International Journal of Oral and Maxillofacial Surgeons. 2010; 39: 313-19.

48. Hannam AG. Current computational modeling trends in craniomandibular biomechanics and their clinical implications. Journal of Oral Rehabilitation. 2011;38: 217-34.

49. Curtis DA, Plesh O, Hannam AG, Sharma A, Curtis TA. Modeling jaw biomechanics in the reconstructed mandibulectomy patient. Journal of Prosthetic Dentistry. 1999; 81: 167-73.

50. Thoma DS, Jones AA, Yamashita M, Edmunds R, Nevins M, Cochran DL. Ridge Augmentation Using Recombinant Bone Morphogenic Protein-2 Techniques - an Experimental Study in the Canine. Journal of Periodontology. 2010;81:1829-38.