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# Osseodensification-induced bone modification in mouse calvaria cultures: dynamic conditions

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HENRY M. GOLDMAN SCHOOL OF DENTAL MEDICINE

THESIS

**OSSEODENSIFICATION-INDUCED BONE MODIFICATION IN MOUSE  
CALVARIA CULTURES: DYNAMIC CONDITIONS**

by

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**KHALED SALEH**

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**ABSTRACT**

**INTRODUCTION:** Osseodensification is an innovative technique in dentistry to create osteotomies in bone to enhance bone density. Developed by Huwais in 2013, this method has been used to increase the primary stability around dental implants, help in indirect sinus lifts and achieve desired expansion in bone.

**AIM:** The main objective of this study was to examine the effects of osseodensification-induced bone modification in mouse ex vivo cultures under dynamic conditions.

**MATERIALS AND METHODS:** Thirty calvaria were surgically extracted from 7-9 days old mice and divided into groups. Densah burs were utilized to create the defects. Clockwise action of the bur produced conventional osteotomies whereas counter-clockwise action created osseodensification effects. Photomicrographs were taken on days 7,14 and 28. Image J software was used to trace the defects and all data were transferred into Microsoft Excel to generate graphs. Statistical analysis and tests of association were done using SPSS software.

**RESULTS:** Increased defect closure was evident in the “Osseodensification” group compared to the “Conventional Osteotomy” group. Defect closure was highest in the first week. There was no statistical significance between groups in original defect size (P-value: 0.6097). Comparing both groups, defect closure was statistically significant on day 7 (P-value: 0.0313).

**CONCLUSION:** Osseodensification has proven to be superior to conventional osteotomies in terms of healing around bony defects and enhancing the primary stability of dental implants. Dynamic conditions during the initial phase of wound healing could hinder the healing cascade and result in a delay of the normal healing process.

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# INTRODUCTION

## CHAPTER 1

### 1.1. Implant Dentistry

Dental implant replacement in the edentulous area is one of the most rapidly developing areas of dentistry as the field continues to embrace modern technology. The dental implant has emerged as a solution to people missing teeth either in the upper or lower jaw due to diseases, accidents, or congenital disabilities. The difficulties associated with conventional dentures have encouraged alternative solutions for individuals with dental problems. Implant dentistry has revolutionized the replacement of lost teeth (Misch., 2008).

### 1.2. Bone

Bone can be defined as a tissue responsible for support. To achieve this role, bone is composed of a hardened extracellular matrix. There are two bone categories, trabecular and cortical bone, based on both their function and structure. (Iolascon., 2013). Trabecular bone has metabolic functions and is found between the layers of cortical bone. Trabecular bone is usually thin and porous while cortical bone is thick and dense and provides attachment for tendons and ligaments. (Iolascon., 2013).

The three main cells of bone tissue are osteoblasts, osteocytes and osteoclasts. They are derived from different cell lines and vary in structure and function. Osteoblasts develop from osteoprogenitor stem cells. They line the surface of the bone and secrete collagen and osteoid which calcifies. (Delgado-Calle and Bellido., 2015).

### **1.3. Osteocytes**

Osteocytes are found in calcified matrix in the lacunae. Long processes of the cells extend in channels referred to as canaliculi that have important functions for cell survival including transportation of both nutrients and waste substances (Gogakos et al., 2009). Osteocytes have gap junctions that facilitate communication from one osteocyte process to another (Gogakos et al., 2009).

### **1.4. Osteoclasts**

Osteoclasts are large stellate-shaped multinucleated cells that are responsible for bone resorption during remodeling. They are involved in growth and repair as well as bone remodeling. Unlike osteocytes which are derived from osteoprogenitor cells, osteoclasts are derived from hematopoietic cells that collect in bone resorption sites and fuse to form multinucleated osteoclasts. (Gogakos et al., 2009).

### **1.5. Cellular Interactions for Bone Turnover**

Bone turnover is governed by the balance between osteocytes and osteoclasts (Delgado-Calle and Bellido., 2015). This balance is critical to ensure that overproduction as well as over-degradation of bone are kept in check.

Gap junctions are integral in coordinating the activities of the different cell types. Osteocytes create the gap junctions which are mainly made of connexin protein monomers (Buo et al., 2014). They provide aqueous conduits between adjacent osteocytes and allow material exchanges including nucleotides, ions and other small molecules. Gap junctions usually occur within groups of cells which contain thousands of

channels and facilitate passage of second messengers between adjacent cells (Buo et al., 2014).

The messengers are produced by target cells responding to various stimuli. The signaling process continues as messengers participate in the downstream signaling pathway (Buo et al., 2014). The initial step provokes a response from neighboring cells. Stability of bone requires gap junction protein Connexin-43 (CX43) regulation to control osteoblast differentiation (Buo et al., 2014). The process occurs through expression of factors including osteoprotegerin and RANK-L.

## **1.6. Bone Remodeling**

Bone remodeling is a life-long process in which mature bone is removed and new bone is formed. Even though bone may appear to be stable and unchanging, remodeling ensures that bone is continuously rebuilt to repair microdamage. The process of bone remodeling is based on the specific properties and functions of bone cells. Osteoblasts play a critical role in bone formation through production of type 1 collagen (Gogakos et al., 2009). Osteoclasts are vital for bone resorption and remodeling to repair microfractures.

Bone resorption may be triggered by the need for calcium in the extracellular fluid (Raisz., 1999). Bone resorption is stimulated by parathyroid hormone secreted in response to hypocalcemia. The hormone may stimulate generation of new osteoclasts (Allen et al., 2014). The membrane becomes a ruffled membrane that attaches to the bone. The compartments adjacent to the bone are then acidified. Minerals in the bone are

dissolved by the acid to allow for enzymatic breakdown of type I collagen (Allen et al., 2014). The final stage in bone resorption is the loss of osteoclasts by apoptosis.

The process of osteoclast formation, popularly known as osteoclastogenesis, is stimulated by high levels of PTH which cause osteoclast precursors to undergo fusion and activation. Osteoclasts express a cell-surface receptor known as receptor activator of NF-kappa b (RANK) while osteoblasts express rank ligand (RANK-L). Differentiation and survival of the osteoclasts are dependent on binding of RANK and RANK-L. Osteoprotegerin, which is essential in regulating bone resorption, is a decoy receptor for RANK and thus inhibits osteoclastogenesis. (Raggatt et al., 2010).

Bone mineralization is an important process of remodeling whereby vital minerals are laid down on the bone matrix. The process determines the mineral content of the bone which is vital in implant dentistry. A large percentage of the bone mineral is hydroxyapatite crystals of calcium and phosphorus. (Allen et al., 2014). However traces of other minerals such as magnesium and carbonate are also present. The mineralization process is highly regulated. The osteoblasts produce crystals of calcium phosphate required in mineralization. They are then deposited within the fibrous matrix (Allen et al., 2014). Defects in the process of mineralization can result in either excessive or inadequate deposition of minerals.

The calcification process is life-long. Both the organic and inorganic components are formed and secreted by the osteoblasts including osteopontin and osteocalcin which may regulate the amount of deposited minerals. Regulation of the bone mineralization process

requires cell products, systemic hormones and bone cells (Allen et al., 2014). The extracellular levels of both inorganic phosphate and pyrophosphate must be kept in balance for mineralization to take place effectively.

The quiescent phase, also known as the resting phase, refers to the initial stage of bone remodeling whereby the inactive osteoblasts are attached to the bone surface. The cells participate in occasional calcium release. However some of the osteoblasts become embedded osteocytes that are connected by long cell processes (Allen et al., 2014).

### **1.7. Clinical Approach for Regenerative Surgery**

The surgical approach of bone regeneration has become one of the essential areas in dentistry. Previously, the process relied on bone grafts taken from external sources (Dimitriou et al., 2011). However, the autogenous bone graft is challenging because of the difficulties of adequate bone harvesting from a single operation. Thus, dentists have used bone or bone-like particles from several resources (allografts, xenografts, alloplasts) to obtain a sufficient amount of materials for bone regeneration. Coral or marine sponges have also been introduced as new materials of scaffolds to support bone regeneration (Dimitriou et al., 2011). These two materials are preferred for bone regeneration because not only do they resemble bone composition, but they can also integrate into the existing bone.

The process of clinical bone regeneration begins with preparation of scaffolds that can fit the required region. Marrow stromal cells, together with the scaffolds, are inserted into the bone section undergoing repair (Henkel et al., 2013). The bone then extends into the

scaffold. The extent of regeneration of the bone depends on whether stem cells or marrow stromal cells were added into the scaffolds. The scaffolds become fully integrated into the bone upon healing making this strategy of bone regeneration efficient (Henkel et al., 2013).

### **1.8. Tissue Engineering Concepts for Regenerative Medicine**

Tissue engineering is another widely studied area in dentistry. Unlike tissue regeneration tissue engineering seeks to develop and implant synthetic devices capable of performing the functions of the organ (Vasita et al., 2006). More advanced methods of tissue engineering rely on the role of growth factors to induce cell responses. The purpose of including growth factors is to encourage accelerated tissue regeneration (Vasita et al., 2006). Tissue engineering has grown rapidly as a result of the converging disciplines dealing with the technology including biochemistry, cell biology, material science, medicine and engineering (Hassanzadeh., 2012).

Different approaches to tissue engineering include histioconductive, histioinductive and substitutive approaches. Histoconductive strategies focus on replacement of a damaged or a missing body based on ex vivo constructs (Hassanzadeh., 2012). Histoinductive methods rely on gene therapy as a facilitator to self-repair. The substitutive strategy aims at replacing the entire organ (Hassanzadeh., 2012). Medical applications of tissue engineering in dentistry include engineering of both cartilage and bone. Individuals with degenerative disorders or victims of an accident can benefit from the technology, in

which the tissue-engineered bone provides a suitable microenvironment for osteocyte differentiation (Hassanzadeh., 2012).

For tissue-engineering to be successful there are some conditions that are necessary. (Hassanzadeh., 2012). The first condition is that an adequate number of cells must be produced to facilitate filling of the defect. Secondly, the cells that are generated by tissue engineering must be compatible with the native cells both structurally and mechanically to minimize the possibility of immunological reactions. Lastly, three dimensional structural support must be supplied for the cells (Hassanzadeh., 2012).

### **1.9. Growth Factors**

Incorporation of growth factors during tissue engineering is based on specific physiological activities of the wound healing cascade (Chen et al., 2010). It is possible to replicate the cells, synthetic materials and growth factors to create a suitable environment for tissue growth (Chen et al., 2010). The growth factors selected for specific procedures should be capable of regulating critical cellular activities such as proliferation, cell division and migration (Chen et al., 2010). However growth factors are rarely used in tissue engineering due to their instability in vivo as well as their short half-life.

### **1.10. Periodontal Ligament**

The periodontal ligament is a fibrous connective tissue that maintains the masticatory apparatus and serves as a shock absorber by providing resistance to masticatory forces (Panagiotopoulou et al., 2011). It is heavily supplied by the myelinated dental nerve endings thus serving as a receptor for both pressure and pain (McCormack et al., 2014).



In its remodeling role the periodontal ligament provides cells that form and resorb the tissues in the attachment apparatus. The periodontal ligament also has a nutritive function because it is well supplied by the dental arteries.

### **1.11. Mechanical Stress Activates Cellular Response**

As a new technique piezosurgery has become popular in dentistry especially in oral surgery, implantology and periodontics. Piezosurgery is based on piezoelectricity which is a concept that was discovered in 1881 (Rahnama et al., 2013). An ultrasound transducer converts piezoelectricity into ultrasonic vibrations. Piezosurgery has revolutionized biological bone modification procedures because the device provides safety and precision when cutting bone while ensuring perfect ergonomics.

Similar to piezosurgery osseodensification is a relatively new concept in dentistry. It has revolutionized dental surgery especially in terms of site preparation in implantology (Kanathila and Pangi., 2018). Osseodensification was developed by Huwais in 2013 and it helps in bone densification, osteotomy preparation, and indirect sinus elevation (Kanathila and Pangi., 2018). As opposed to other bone drilling technologies osseodensification is preferred because it does not excavate the bone. The uses of osseodensification range from elevation of the sinus floor to expansion of the alveolar ridge (Kanathila and Pangi., 2018).

Mechanical stress can change the shape of the bone during bone remodeling. Osteocytes in the bone matrix are important mechanosensors after which the stress is converted into a cascade of biochemical reactions that activate both the osteoblasts and osteoclasts to

initiate bone resorption (Nomura and Takano-Yamamoto., 2010). Osseodensification uses burs to place dental implants. The burs are rotated in reverse at 800 to 1500 rpm (Kanathila and Pangi., 2018). Osseodensification is critical in implant dentistry because it provides primary stability to the bone in contact with the implant.

### **1.12. Osseodensification**

Osseodensification increases the insertion torque and early loading while minimizing implant micromotion (Nomura and Takano-Yamamoto., 2010). Osseodensification helps avoid bone destruction that is common with conventional drilling. The procedure also prevents fracture of the trabeculae, an occurrence known to delay bone growth. Various osseodensification techniques improve bone expansion for insertion of wider diameter implants within the narrow ridge while minimizing the risks of creating bone fenestrations (Nomura and Takano-Yamamoto., 2010).

Osseodensification enhances bone density. The use of Densah burs enhances both condensation and preservation of bone as well as increases the density of the bone minerals. Osseodensification accelerates the healing process substantially since the cells, biomolecules and bone matrix are maintained. (Kanathila and Pangi., 2018). Other advantages associated with osseodensification include increasing implant stability, expansion of the residual ridge, increasing residual strain and compact autografting. Compared to the traditional drills osseodensification statistically raises the biomechanical value of the implants by 30 to 40% (Trisi et al., 2016).

The Densah bur is a new surgical instrument which has replaced the traditional use of standard drills when preparing dental implant osteotomies (**Figure 1**).

Osseodensification is a novel bio-mechanical site preparation technique by the Densah bur. It produces low plastic deformation leading to preservation of the bone and enhances the host bio-reaction (Trisi et al., 2016). The design of the Densah burs gives them a minimum of four lands and flutes allowing the burs to be guided precisely through the bone which is important in minimizing the risk of chatter (Trisi et al., 2016).

This technique is classified as a non-extraction technique due to preserving bone by condensing it. On the other hand, conventional standard drilling techniques is classified as an extraction technique due to removing bone while drilling (Huweis and Meyer, 2017). According to a meta-analysis of different drilling techniques, the osseodensification bur significantly reduced alveolar crest loss (Tretto et al., 2018).

The ideal rotational speed of the Densah bur is 800 to 1500 revolution per minute (RPM) with sufficient irrigation to prevent bone overheating (Trisi et al., 2016). This bur has two different functions depending on the direction of rotation, as counterclockwise rotation utilizes four tapered flutes with a negative rake angle to create a layer of compact or dense bone while clockwise rotation utilizes a positive rake angle and extracts bone as in conventional osteotomy (Lahens et al., 2016).

When rotated counterclockwise, non-cutting direction in densifying mode, downward pressure with adequate external irrigation creates a gentle compression wave inside the

osteotomy along the fluted structure. This hydrodynamic process generates a densified layer through compaction with autogenous bone remnant as an autografting material derived from the surrounding bone. This creates a plastically expanded bony ridge (Meyer and Huwais et al., 2014; Oliveira et al., 2018).



**Figure 1. The Geometric Configuration of Osteotomy Drills.**

These CAD images illustrate geometric configuration of (a) conventional osteotomy bur and (b) Densah bur™. Reprinted from Journal of Orthopaedic Research, Volume: 36, Issue: 9, Pages: 2516-2523, First published: 14 March 2018, DOI: (10.1002/jor.23893)

The Densah burs may provide versatilities for dental surgery with a new surgical concept called osseodensification. The bur diameter is increased in sequence during the surgery. This allows for the cylindrical osteotomy to be expanded; it condenses bone leading to increased peri-implant bone density which is essential for implant success (Trisi et al., 2016). The technology is easily combined with the standard surgical engine making it easier to use. Osseodensification using these burs improved the primary stability of the implant (Jimbo et al., 2014). The use of Densah burs does not obstruct the healing process following condensation of the bone (Trisi et al., 2016).

Also, a sheep ilium study demonstrated the primary stability of endosteal dental implants. The osseodensification procedure showed abundant bone remnants surrounding the implant surface with a higher insertion torque level. Conventional osteotomy rarely found bone remnants. (Lahens et al., 2016). The osseodensification produced higher bone-to-implant contact (BIC) observed from histological analysis, representing more robust implant stability (Stokholm et al., 2014).

### **1.13. Hypothesis and Aim**

According to our literature review, the theory of osseodensification is a promising surgical approach for cellular activation under proper maintenance protocols. However, it is still crucial to provide proper post-operative protocol on the surgical site.

We hypothesized that proper post-operative maintenance induces cellular activities in the recipient site to accelerate new bone formation. For example, a static condition is an essential role for proper wound healing. In this study, we investigated bone healing processes after osseodensification under an unstable condition using mouse calvaria *ex-vivo* culture in a dynamic condition.

## MATERIALS AND METHODS

### CHAPTER 2

#### 2.1. Mouse Calvaria

A 28-day study was designed to determine the effect of osseodensification on activation of bone remodeling using mouse bone cultures under dynamic conditions.

Fifteen 7-9-day neonatal CD-1 mice from the Charles River Laboratories in Massachusetts were used. On the first day calvaria were surgically harvested under sterile conditions (**Figure 2**). Calvaria were then cultured in a bone formation medium consisting of 1000 ml of DMEM in the absence of fetal calf serum mixed with 1% Penicillin-Streptomycin as well as 5% (5mg/ml) Bovine Serum Albumin (BSA). This medium was supplemented with 150 ug/mL of ascorbic acid. Each calvarium was then transferred into six-well plates, which were placed in an incubator set at 37°C with 5% CO<sub>2</sub>.



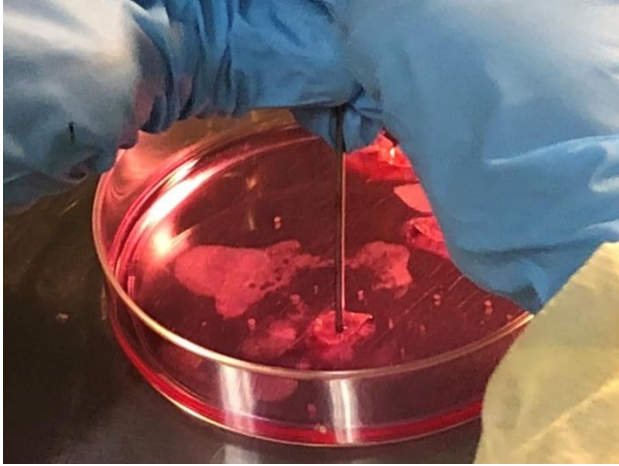


**Figure 2. Harvesting Mouse Calvaria.**

Fifteen neonatal mice were euthanized and soft tissue removed prior to harvesting calvaria under sterile conditions.

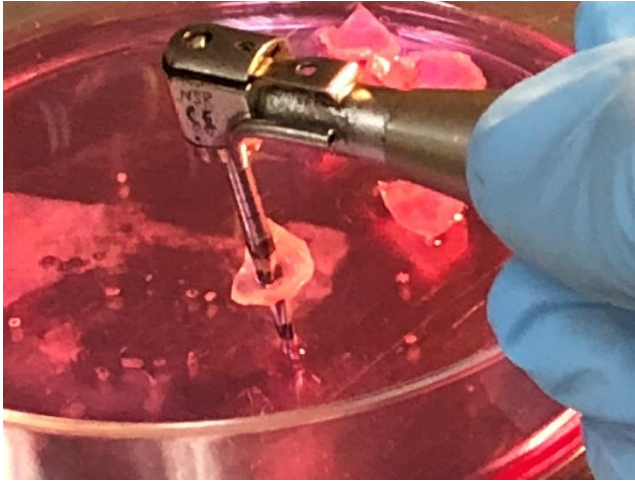
## **2.2. Surgical Defect**

On day 3 each calvarium was divided along the mid-sagittal suture. A mark was made using an indentation stick on the left and right side of each parietal bone (**Figure 3**). The osteotomy was created at the location of these marks utilizing Densah burs with a diameter of 2 mm and a speed of 500 rotations per minute. A conventional osteotomy rotated the Densah bur clockwise on the left side. On the right side osseodensification was achieved by rotating the Densah bur counter-clockwise (**Figure 4**). Thus, the cutting side was represented on the left side while the compression side was represented on the right side.



**Figure 3. Using an Indentation Stick for Demarcations.**

An initial pinhole was created on the center of parietal bone by an indentation stick (0.5 mm diameter).



**Figure 4. Using the Densah Bur.**

The Densah bur created defects. The clockwise direction is the bone cutting setting as conventional osteotomy. The counterclockwise direction is the bone densifying setting as osseodensification.

### **2.3. Ex-vivo Organ Culture Under a Dynamic Condition**

The harvested calvaria were randomly placed into three groups (A, B and C) consisting of five left halves and five right halves.

Calvaria were cultured in individual test tubes containing 2 ml of BSA media supplemented with 150 ug/ml of ascorbic acid. In addition, six test tubes containing media alone served as controls. All 36 test tubes were placed in a roller tube apparatus set at an angle of 10 degrees and a rotational speed of 5 revolutions per minute (**Figure 5**).



**Figure 5. Roller Tube Apparatus.**

The roller tube apparatus set at an angle of 10 degrees with the rotational speed of five revolutions per minute. Incubated at 37°C in the closed glass tubes and medium was changed every two days.

The culture media was collected from each test tube using sterile pipettes. The collected media were transferred into 2 ml Eppendorf tubes that were frozen at -80 °C. This process was repeated every two or three days throughout the duration of the study.

#### 2.4. Sample Collection

At the end of the culture period, the calvaria were fixed by removing the media and transferring the calvaria into glass tubes containing 2 ml of formaldehyde, and the tubes were then incubated at 4°C. After 48 hours the formaldehyde was removed, and each specimen was kept in 5 ml of 70% ethanol at 4°C.

Group A calvaria were fixed on day 7. Group B was fixed on day 14. Group C was fixed on day 28. After fixation each calvarium (total 30 calvaria halves) was analyzed microscopically and photomicrographs were taken (**Table 1**).

| Group Name | Culture Duration | Right Halves:<br>Osseodensification | Left Halves:<br>Conventional Osteotomy |
|------------|------------------|-------------------------------------|--|
| Group A    | 7 days culture   | N=5                                 | N=5                                    |
| Group B    | 14 days culture  | N=5                                 | N=5                                    |
| Group C    | 28 days culture  | N=5                                 | N=5                                    |

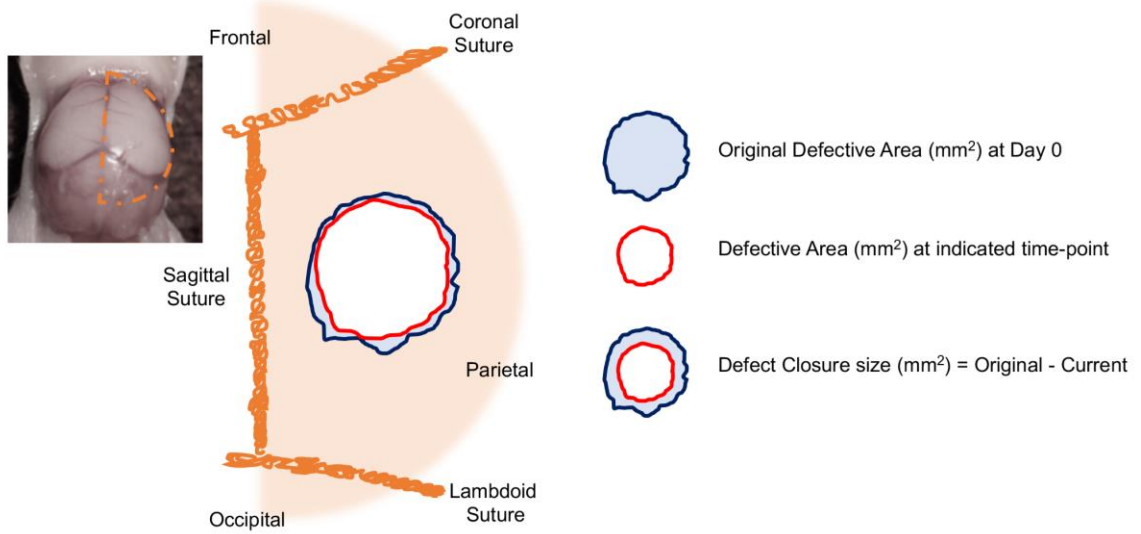
**Table 1. Experimental Group.**

Three different groups: cultured for 7, 14, or 28 days were designated as Group A, B, and C, respectively.

## **2.5. Morphological Analysis**

The photomicrographs were superimposed over a grid to serve as a scale. Analysis of these superimposed pictures via Image J computer software to trace the outline of each defect throughout the study. Microsoft Excel was used to tabulate the data and create graphs (**Figure 6**).





**Figure 6. Experimental Design on Measurement of Defect Size.**

Defect closure (mm<sup>2</sup>) was calculated by subtraction from the Original Defective Area to the Defective Area at indicated time-point (current defect).

## **2.6. Statistical Analysis**

SPSS software was used to analyze data and detect changes across time intervals. Means and standard deviations were calculated for both “Conventional Osteotomy” and the “Osseodensification” groups. Student t-test was used to assess statistically significant associations between both groups. A p-value of  $< 0.05$  was regarded as statistically significant.

## RESULTS

### CHAPTER 3

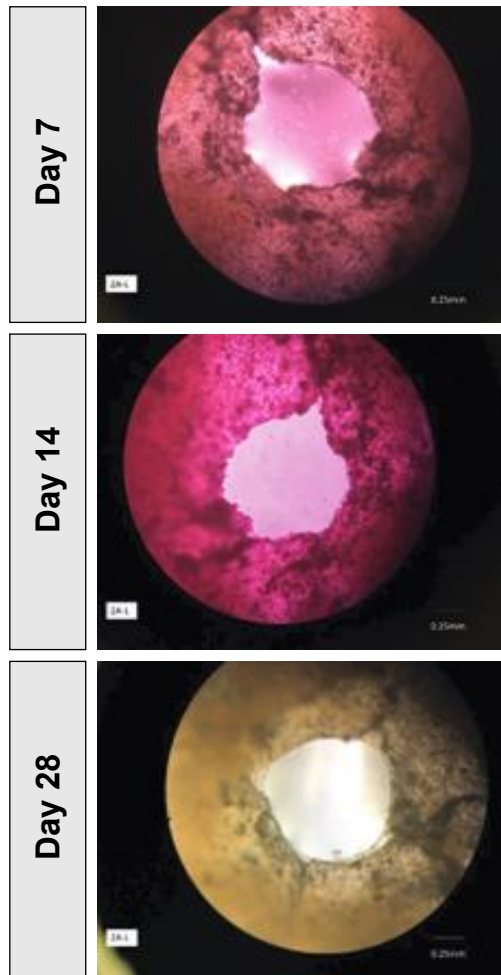
#### **3.1 AIM: Osseodensification-induced morphological change by increased cellular migration and proliferation in dynamic condition of organ culture.**

##### **3.1.1. Microscopic Image Collection**

The photomicrographic analyses are illustrated in **Figures 7 and 8**. Each calvaria was fixed with 4% formaldehyde for 48 hours and stored in 70% EtOH at 4°C as described in the materials and methods.

**Figure 7** shows the morphological changes of defect closure under dynamic organ culture condition from postoperative day 7 to 28 as the “control group” with conventional osteotomy. There was minimal cellular migration or proliferation toward the defect area from the bone side.

## Control Group: Conventional Osteotomy

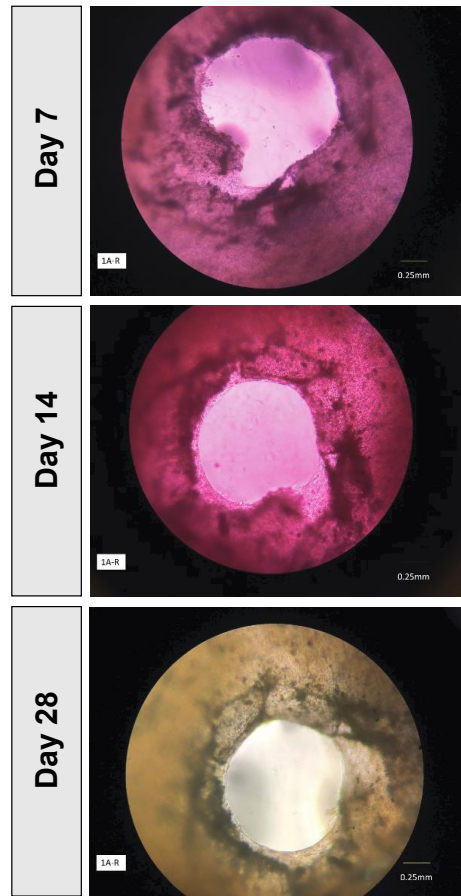


**Figure 7. Post-Op Day 7, 14 and 28 – Conventional Osteotomy (Left Side).**

This shows a series of the photomicrographs from the “Conventional Osteotomy” group. The same calvaria was photomicrographed on day 7, 14 and 28 respectively. All images were visually traced using Image J software to generate quantitative data. It is evident that in this group there is a minimal closure of the original defect throughout the study. Scale bar = 0.25mm.

**Figure 8** shows morphological changes of defect closure from postoperative day 7 to 28 as the “test group” with compressed osteotomy (osseodensification). The cellular migration or proliferation slightly increased toward the defect area from the edge of the bone.

### Test Group: Osseodensification



**Figure 8. Post-Op Day 7, 14 and 28 – Osseodensification (Right Side).**

This shows a series of the photomicrographs from the “Osseodensification” group. The Densah bur was used in a counter-clockwise motion to create the original defect in each calvaria. The same calvaria was photomicrographed on days 7, 14 and 28. All images were visually traced using Image J software to generate quantitative data. It is evident that, in this group, the original defect decreases considerably in size throughout the duration of the study. Scale bar = 0.25mm.

### 3.1.2. Statistical Analysis of Defect Size

Qualitative changes were detected in photomicrographs and analyzed by Image J software with a standardized scale (0.25 mm), as shown in **Figures 7 and 8**.

Each table represents the morphological change by the “original defect size (mm<sup>2</sup>)” at the day of surgery (Day 0), defect size at the indicated postoperative day as “current defect size (mm<sup>2</sup>)”. The defect closure size (mm<sup>2</sup>) was calculated by subtraction of the current defect size from the original defect size. The mean value, the standard deviation of the mean shown in each category (**Table 2-8**).

**Table 2** compared “original defect size” between the control group and the test group.

According to the Shapiro-Wilk W test, both group shows normal distribution **Figure 9A**.

Thus, we analyzed statistical significance by student t-test instead of the Mann-Whitney

U test (also called a Wilcoxon test). **Figure 9B** shows there were no significant

differences of the original defect size between groups (P-value: 0.6097).

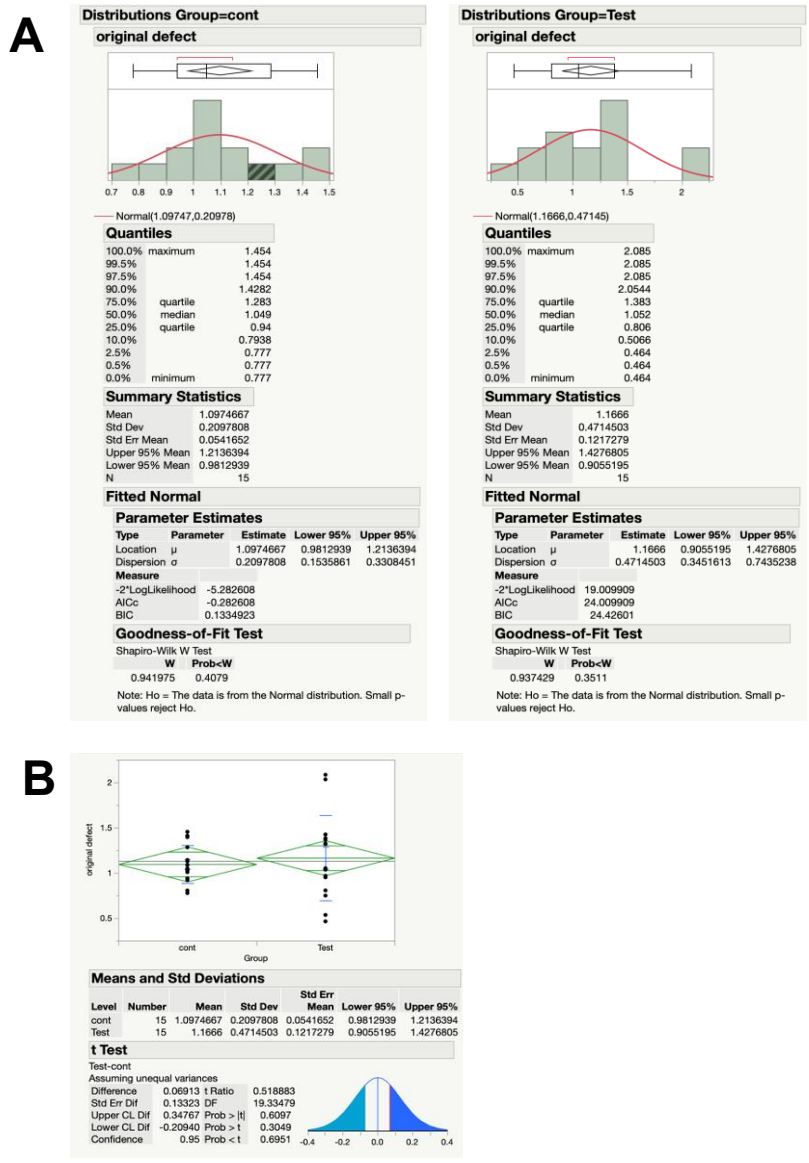
| Sample A-C     | Original Defect (mm <sup>2</sup> ) |                                    |
|----------------|------------------------------------|------------------------------------|
|                | Control Group<br>(Conventional)    | Test Group<br>(Osseodensification) |
| 1A             | 1.454                              | 2.034                              |
| 2A             | 1.128                              | 1.327                              |
| 3A             | 1.396                              | 1.425                              |
| 4A             | 0.777                              | 2.085                              |
| 5A             | 1.283                              | 1.035                              |
| 1B             | 1.411                              | 0.749                              |
| 2B             | 1.037                              | 0.535                              |
| 3B             | 1.025                              | 0.464                              |
| 4B             | 0.805                              | 0.951                              |
| 5B             | 1.049                              | 0.806                              |
| 1C             | 1.142                              | 1.317                              |
| 2C             | 0.940                              | 1.368                              |
| 3C             | 1.008                              | 0.968                              |
| 4C             | 1.088                              | 1.052                              |
| 5C             | 0.919                              | 1.383                              |
| <b>Average</b> | 1.097                              | 1.167                              |
| <b>STDEV.S</b> | 0.210                              | 0.471                              |
| <b>SEM</b>     | 0.054                              | 0.122                              |

no statistical significance (P < 0.05, compared to Control)

**Table 2. Original Defect Size.**

There were no significant differences of original defects between groups.





**Figure 9. Statistical Analysis of Original Defect Size.**

A. Shapiro-Wilk analysis shows normal distribution with the probability of 0.4079 and 0.3511 in the control and test group, respectively.

B. Student T-test for comparison original defect between groups. There were no significant differences between groups (n=15, two-tailed probability = 0.6097).

**Table 3** shows the early phase of morphological change in the 7 days culture group (Group A).

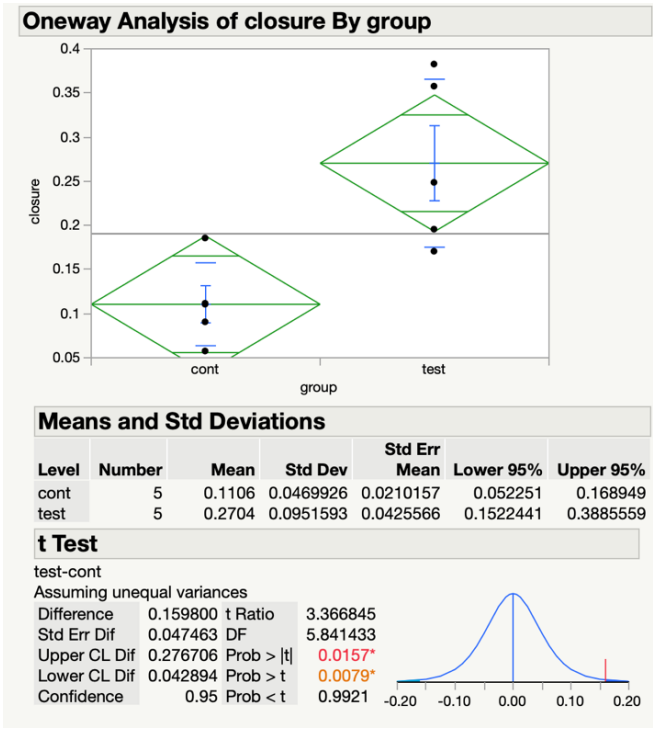
Shapiro-Wilk analysis shows normal distribution in the current defect size with the probability of 0.2341 and 0.3256 in the control and test group, respectively. The defect closure size also has normal distribution with the probability of 0.5293 and 0.3805 in the control and test group, respectively.

The osseodensification mode (test group) had significantly reduced “defect closure size” at day7 ( $0.270 \pm 0.095 \text{ mm}^2$ ) compared to the conventional group ( $0.111 \pm 0.047 \text{ mm}^2$ ), as shown by Student T-test with a two-tailed probability value of = 0.0157 (**Figure 10**).

| Sample Group A | Original Defect at Day 0 (mm <sup>2</sup> ) |                                 | Current Defect at Day 7 (mm <sup>2</sup> ) |       | Defect Closure from Day 0 to Day 7 (mm <sup>2</sup> ) |                |
|----------------|---|---------------------------------|--|-------|---|----------------|
|                | Control Group (Conventional)                | Test Group (Osseodensification) | Control                                    | Test  | Control   | Test           |
| 1A             | 1.454                                       | 2.034                           | 1.269                                      | 1.677 | 0.185   | 0.357          |
| 2A             | 1.128                                       | 1.327                           | 1.038                                      | 1.132 | 0.090   | 0.195          |
| 3A             | 1.396                                       | 1.425                           | 1.286                                      | 1.177 | 0.110   | 0.248          |
| 4A             | 0.777                                       | 2.085                           | 0.720                                      | 1.703 | 0.057   | 0.382          |
| 5A             | 1.283                                       | 1.035                           | 1.172                                      | 0.865 | 0.111   | 0.170          |
| <b>Average</b> | 1.208                                       | 1.581                           | 1.097                                      | 1.311 | 0.111   | <b>0.270 *</b> |
| <b>STDEV</b>   | 0.271                                       | 0.460                           | 0.233                                      | 0.366 | 0.047   | 0.095          |
| <b>SEM</b>     | 0.121                                       | 0.206                           | 0.104                                      | 0.164 | 0.021   | 0.043          |

\* denotes statistical significance (P < 0.05, Student T-test, compared to Control)

**Table 3. Early Phase of Morphological Change in Group A After 7 Days Culture.**



**Figure 10. Statistical Analysis of Defect Closure in Group A After 7 Days Culture.**

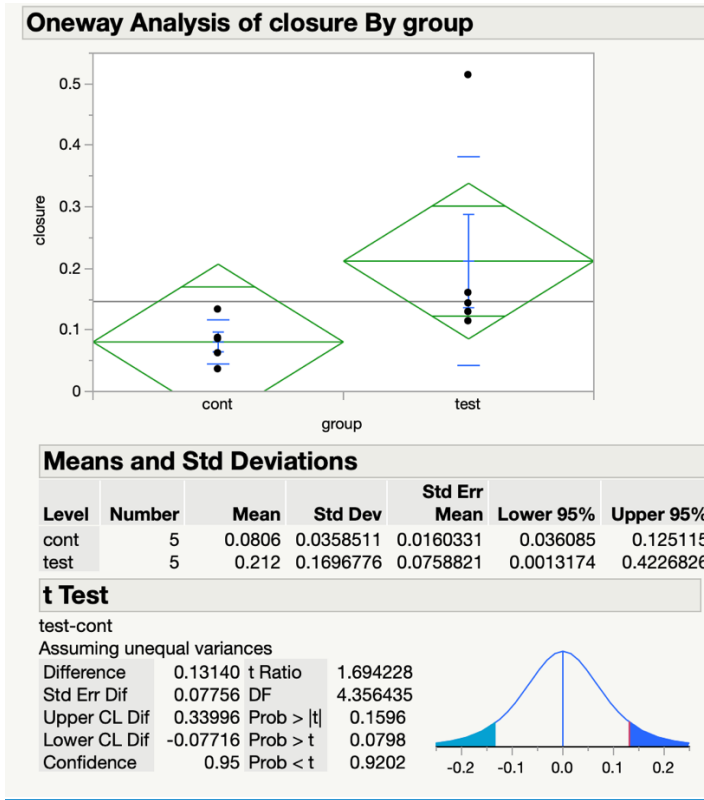
There were significant differences (a two-tailed  $P = 0.0157$  by Student t-test) which were observed in the “defect closure size” between the test and control group.

**Table 4** summarizes the defect sizes of calvaria in Group B which were fixed on day 14. Both groups showed normal distribution in each parameter. There was no significant difference (a two-tailed P = 0.1596) in the “defect closure size” between the test and control group,  $0.212 \pm 0.170 \text{ mm}^2$  and  $0.081 \pm 0.036 \text{ mm}^2$ , respectively (**Figure 11**).

| Sample Group B | Original Defect at Day 0 (mm <sup>2</sup> ) |                                 | Current Defect at Day 14 (mm <sup>2</sup> ) |       | Defect Closure from Day 0 to Day 14 (mm <sup>2</sup> ) |       |
|----------------|---|---------------------------------|---|-------|--|-------|
|                | Control Group (Conventional)                | Test Group (Osseodensification) | Control                                     | Test  | Control  | Test  |
| 1B             | 1.411                                       | 0.749                           | 1.326                                       | 0.620 | 0.085  | 0.129 |
| 2B             | 1.037                                       | 0.535                           | 0.950                                       | 0.375 | 0.087  | 0.160 |
| 3B             | 1.025                                       | 0.464                           | 0.892                                       | 0.321 | 0.133  | 0.143 |
| 4B             | 0.805                                       | 0.951                           | 0.769                                       | 0.437 | 0.036  | 0.514 |
| 5B             | 1.049                                       | 0.806                           | 0.987                                       | 0.692 | 0.062  | 0.114 |
| <b>Average</b> | 1.065                                       | 0.701                           | 0.985                                       | 0.489 | 0.081  | 0.212 |
| <b>STDEV</b>   | 0.218                                       | 0.200                           | 0.208                                       | 0.160 | 0.036  | 0.170 |
| <b>SEM</b>     | 0.097                                       | 0.089                           | 0.093                                       | 0.072 | 0.016  | 0.076 |

no statistical significance (P < 0.05, student T-test, compared to Control)

**Table 4. Intermediate Phase of Morphological Change in Group B After 14 Days.**



**Figure 11. Statistical Analysis of Defect Closure in Group B After 14 Days Culture.**

There were no significant differences (a two-tailed  $P = 0.1596$  by Student t-test) observed in the “defect closure size” between the test and control group.

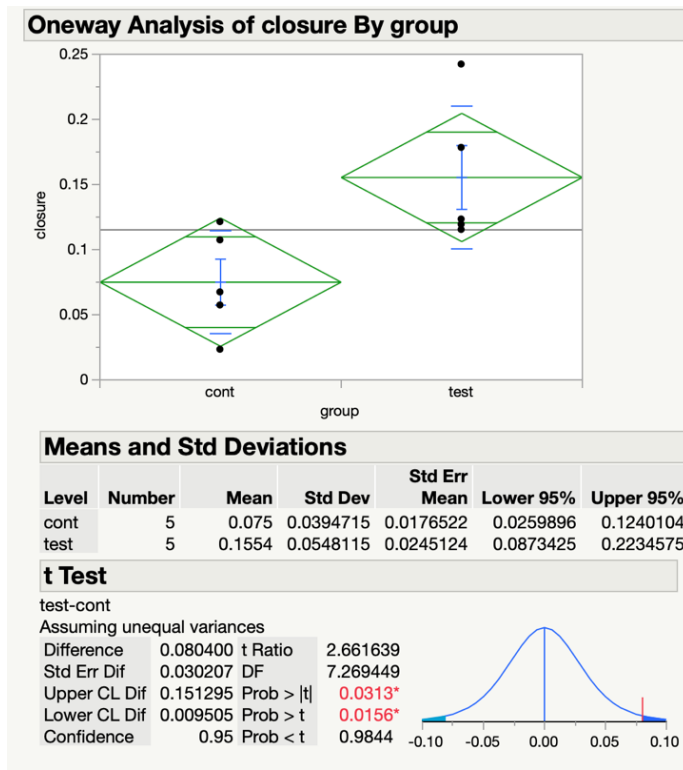
In the **Table 5-7**, we demonstrated the three phases (early, intermediate, and late phase) of morphological change from the 28 days culture group (Group C).

**Table 5** shows the early phase of morphological change after seven days culture. The test group (osseodensification mode) has significantly reduced defect size (defect closure size =  $0.155 \pm 0.049 \text{ mm}^2$ ) compared to control group (conventional osteotomy mode,  $0.075 \pm 0.035 \text{ mm}^2$ ) with two-tailed probability value of 0.0313 (**Figure 12**). This result is similar to Group A described in **Figure 10**.

| Sample Group C | Original Defect at Day 0 (mm <sup>2</sup> ) |                                 | Current Defect at Day 7 (mm <sup>2</sup> ) |       | Defect Closure from Day 0 to Day 7 (mm <sup>2</sup> ) |                |
|----------------|---|---------------------------------|--|-------|---|----------------|
|                | Control Group (Conventional)                | Test Group (Osseodensification) | Control                                    | Test  | Control   | Test           |
| 1C             | 1.142                                       | 1.317                           | 1.085                                      | 1.202 | 0.057   | 0.115          |
| 2C             | 0.940                                       | 1.368                           | 0.833                                      | 1.190 | 0.107   | 0.178          |
| 3C             | 1.008                                       | 0.968                           | 0.985                                      | 0.845 | 0.023   | 0.123          |
| 4C             | 1.088                                       | 1.052                           | 1.021                                      | 0.933 | 0.067   | 0.119          |
| 5C             | 0.919                                       | 1.383                           | 0.798                                      | 1.141 | 0.121   | 0.242          |
| <b>Average</b> | 1.019                                       | 1.218                           | 0.944                                      | 1.062 | 0.075   | <b>0.155 *</b> |
| <b>STDEV</b>   | 0.085                                       | 0.173                           | 0.111                                      | 0.146 | 0.035   | 0.049          |
| <b>SEM</b>     | 0.038                                       | 0.077                           | 0.049                                      | 0.065 | 0.016   | 0.022          |

\* denotes statistical significance (P < 0.05, compared to Control)

**Table 5. Early Phase of Morphological Change in Group C After 7 Days Culture.**



**Figure 12. Statistical Analysis of the Defect Closure in Group C After 7 Days Culture (End of Early Phase).**

There was statistically significance between groups as shown by the student t-test with a two-tailed probability value of 0.0313. Defect closure was found to be, on average, 0.155 mm<sup>2</sup> (SD: 0.055) for the “Osseodensification” group in comparison to 0.075 mm<sup>2</sup> (SD: 0.039) for the “Conventional Osteotomy” group.

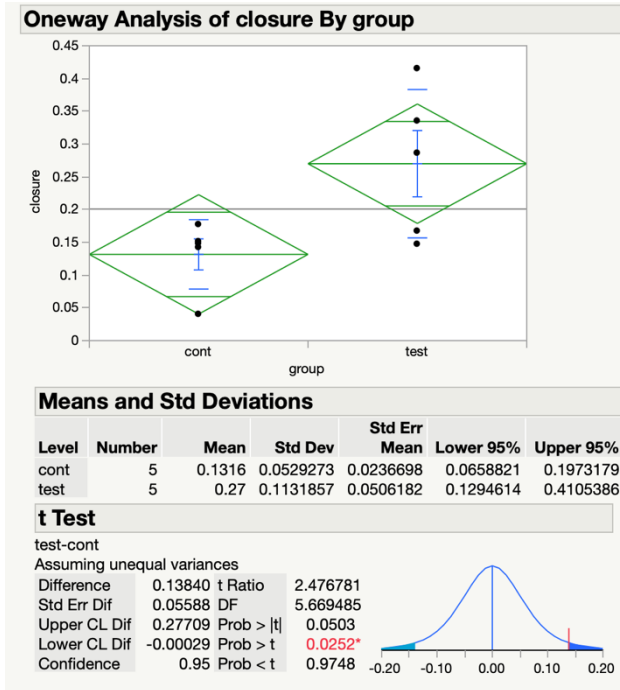


**Table 6** summarizes the intermediated phase of morphological change after fourteen days culture. The defect closure sizes in the test group ( $0.270 \pm 0.113 \text{ mm}^2$ ) was not significant difference compared to the control group ( $0.132 \pm 0.047 \text{ mm}^2$ ) with two-tailed probability value of 0.0503 by Student t-test.

| Sample Group C | Original Defect at Day 0 (mm <sup>2</sup> ) |                                 | Current Defect at Day 14 (mm <sup>2</sup> ) |       | Defect Closure from Day 0 to Day 14 (mm <sup>2</sup> ) |       |
|----------------|---|---------------------------------|---|-------|--|-------|
|                | Control Group (Conventional)                | Test Group (Osseodensification) | Control                                     | Test  | Control  | Test  |
| 1C             | 1.142                                       | 1.317                           | 0.994                                       | 0.982 | 0.148  | 0.335 |
| 2C             | 0.940                                       | 1.368                           | 0.798                                       | 0.953 | 0.142  | 0.415 |
| 3C             | 1.008                                       | 0.968                           | 0.968                                       | 0.821 | 0.040  | 0.147 |
| 4C             | 1.088                                       | 1.052                           | 0.911                                       | 0.885 | 0.177  | 0.167 |
| 5C             | 0.919                                       | 1.383                           | 0.768                                       | 1.097 | 0.151  | 0.286 |
| <b>Average</b> | 1.019                                       | 1.218                           | 0.888                                       | 0.948 | 0.132  | 0.270 |
| <b>STDEV</b>   | 0.085                                       | 0.173                           | 0.090                                       | 0.093 | 0.047  | 0.101 |
| <b>SEM</b>     | 0.038                                       | 0.077                           | 0.040                                       | 0.042 | 0.021  | 0.045 |

no statistical significance (P < 0.05, compared to Control)

**Table 6. Intermediate Phase of Morphological Change in Group C After 14 Days Culture.**



**Figure 13. Statistical Analysis of the Defect Closure in Group C After 14 Days Culture (End of Intermediate Phase).**

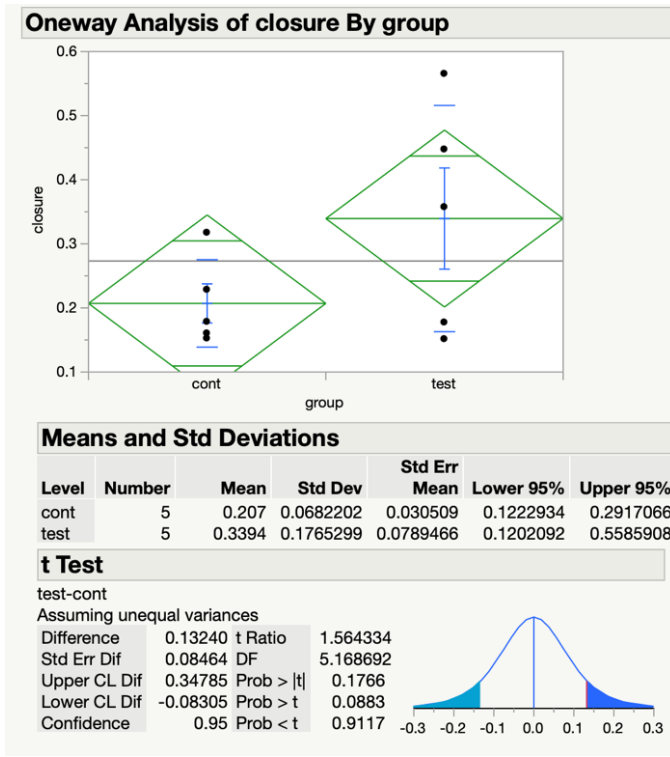
No statistically significant differences were seen between groups as shown by the student t-test with a two-tailed probability value of 0.0503. Defect closure was found to be, on average, 0.270 mm<sup>2</sup> (SD: 0.113) for the “Osseodensification” group in comparison to 0.132 mm<sup>2</sup> (SD: 0.053) for the “Conventional Osteotomy” group.

**Table 7** summarizes the intermediated phase of morphological change after fourteen days culture. The defect closure sizes in the test group ( $0.339 \pm 0.158 \text{ mm}^2$ ) was not statistically significantly different compared to the control group ( $0.207 \pm 0.061 \text{ mm}^2$ ) with two-tailed probability value of 0.1766 by Student t-test.

| Smample Group C | Original Defect at Day 0 (mm <sup>2</sup> ) |                                 | Current Defect at Day 28 (mm <sup>2</sup> ) |       | Defect Closure from Day 0 to Day 28 (mm <sup>2</sup> ) |       |
|-----------------|---|---------------------------------|---|-------|--|-------|
|                 | Control Group (Conventional)                | Test Group (Osseodensification) | Control                                     | Test  | Control  | Test  |
| 1C              | 1.142                                       | 1.317                           | 0.982                                       | 0.960 | 0.160  | 0.357 |
| 2C              | 0.940                                       | 1.368                           | 0.788                                       | 0.803 | 0.152  | 0.565 |
| 3C              | 1.008                                       | 0.968                           | 0.780                                       | 0.817 | 0.228  | 0.151 |
| 4C              | 1.088                                       | 1.052                           | 0.771                                       | 0.875 | 0.317  | 0.177 |
| 5C              | 0.919                                       | 1.383                           | 0.741                                       | 0.936 | 0.178  | 0.447 |
| <b>Average</b>  | 1.019                                       | 1.218                           | 0.812                                       | 0.878 | 0.207  | 0.339 |
| <b>STDEV</b>    | 0.085                                       | 0.173                           | 0.086                                       | 0.062 | 0.061  | 0.158 |
| <b>SEM</b>      | 0.038                                       | 0.077                           | 0.039                                       | 0.028 | 0.027  | 0.071 |

no statistical significance (P < 0.05, compared to Control)

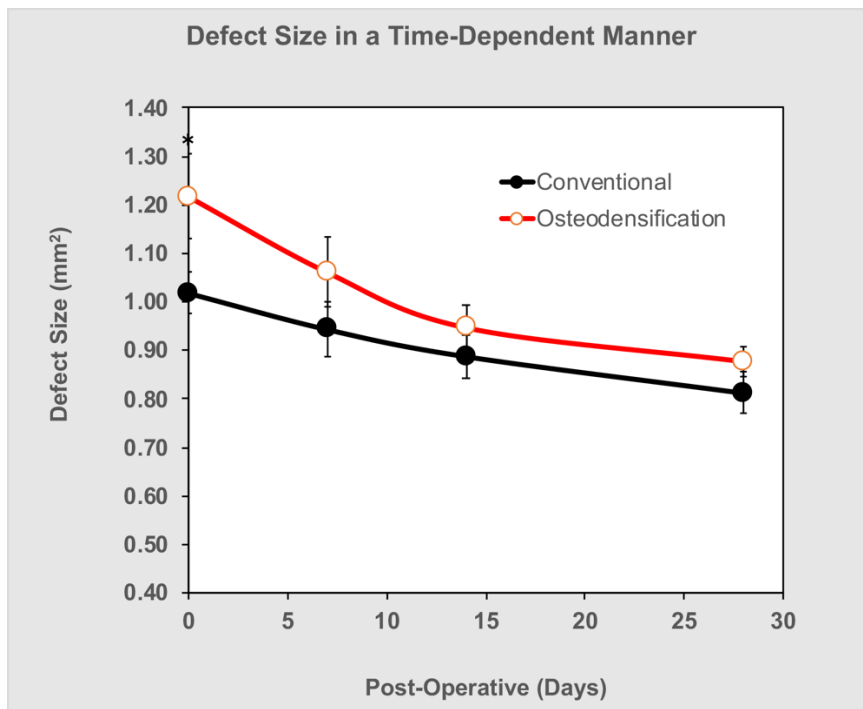
**Table 7. Late Phase of Morphological Change in Group C After 28 Days Culture.**



**Figure 14. Statistical Analysis of the Defect Closure in Group C After 28 Days Culture (End of Late Phase).**

No statistically significant differences were seen between groups as shown by the student t-test with a two-tailed probability value of 0.1766. Defect closure was found to be, on average, 0.339 mm<sup>2</sup> (SD: 0.158) for the “Osseodensification” group in comparison to 0.207 mm<sup>2</sup> (SD: 0.061) for the “Conventional Osteotomy” group.

**Figure 15** illustrated the time-dependent defect closure in the Group C (28 days culture group) compared with the test group (osseodensification) and control group (conventional osteotomy). The defect size was not significantly different in the test group compared to the control group at day 0, 7, 14 and 28, as the two tailed p-value of 0.0869, 0.236, 0.384 and 0.255 by student-t test, respectively.

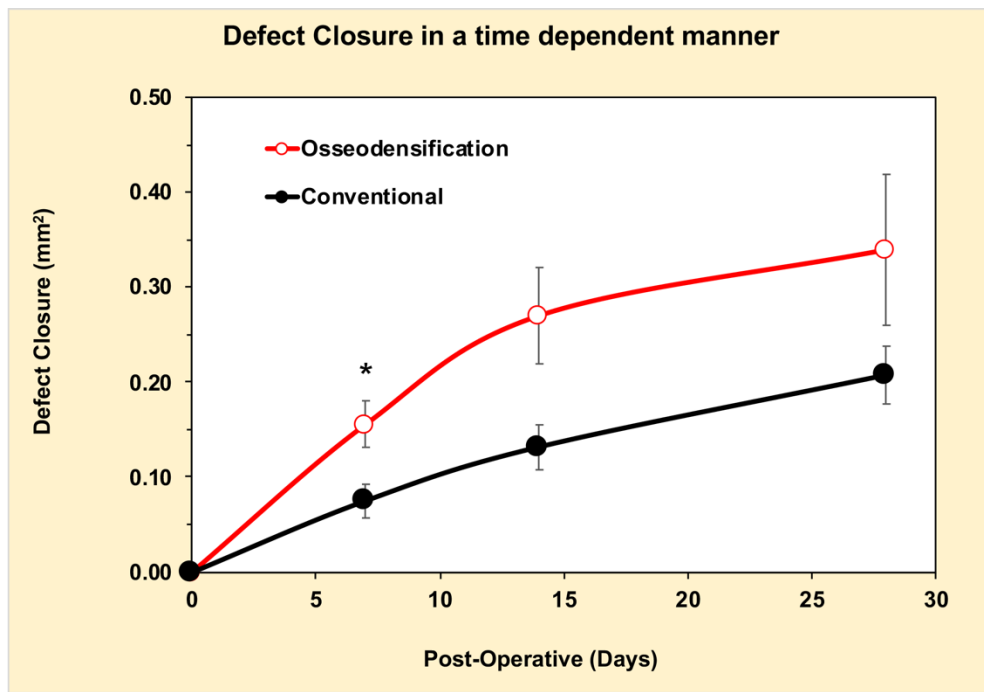


**Figure 15: Defect Size Comparison Between Conventional Osteotomy and Osseodensification (Densah) from Day 0 to Day 28.**

This graph shows changes of the defect in Group C throughout the study. The current defect was calculated from visual tracings made by Image J software to gain quantitative data which were converted into a graph. The defect size was reduced over time for both groups. This reduction is greater during the first week in the “Osseodensification” group

when compared to the “Conventional Osteotomy” group. \* denotes statistical significant with the one tailed p-value of 0.043 (two tailed p-value = 0.0870).

**Figure 16** illustrates the defect closure size calculated by subtracting the defect size at indicated time points (day 7, 14 and 28) from the previous time point; e.g., subtracted the defect size at day 14 from the defect size at day 7. The test group (osseodensification) only significantly improved healing with greater defect closure than control group at day 7.



**Figure 16. Group A - Timeline of Defect Closure Comparison Between Conventional Osteotomy and Osseodensification (Densah) from Day 0 to Day 28.**

The defect closure is measured as the current defect at each point in time subtracted from the original defect at the beginning of the study. It can be seen that more defect closure was evident between the time interval of day 0 to day 7 in the “Osseodensification” group as shown by the steep curve. Defect closure continues to be constantly higher in the

“Osseodensification” group in comparison to the “Conventional Osteotomy” group throughout the study.



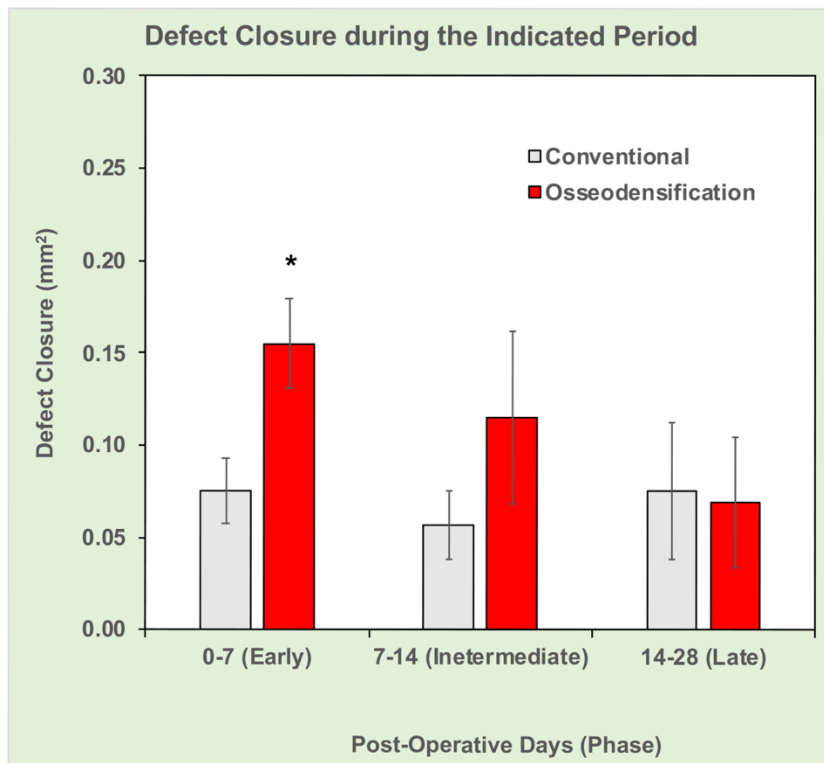
**Table 8** summarizes the statistical analysis of the defect closure size comparison between the test group and control group.

| Sample                      | Defect Closure -D7           |                                 | Defect Closure -D14 |        | Defect Closure -D28 |        |
|-----------------------------|------------------------------|---------------------------------|---------------------|--------|---------------------|--------|
|                             | Control Group (Conventional) | Test Group (Osseodensification) | Control             | Test   | Control             | Test   |
| 1C                          | 0.057                        | 0.115                           | 0.148               | 0.335  | 0.160               | 0.357  |
| 2C                          | 0.107                        | 0.178                           | 0.142               | 0.415  | 0.152               | 0.565  |
| 3C                          | 0.023                        | 0.123                           | 0.040               | 0.147  | 0.228               | 0.151  |
| 4C                          | 0.067                        | 0.119                           | 0.177               | 0.167  | 0.317               | 0.177  |
| 5C                          | 0.121                        | 0.242                           | 0.151               | 0.286  | 0.178               | 0.447  |
| <b>Average</b>              | 0.075                        | <b>0.155 *</b>                  | 0.132               | 0.270  | 0.207               | 0.339  |
| <b>STDEV</b>                | 0.039                        | 0.055                           | 0.053               | 0.113  | 0.068               | 0.177  |
| <b>SEM</b>                  | 0.018                        | 0.025                           | 0.024               | 0.051  | 0.031               | 0.079  |
| <b>P-value (two-tailed)</b> |                              | <b>0.0313</b>                   |                     | 0.0503 |                     | 0.1766 |

\* denotes statistical significance (P < 0.05, Student T-test, compared to Control)

**Table 8. Timeline of Defect Closure Size in Group C.**

We also analyzed defect closure size during the indicated phase as early (post-operative day 0 – 7), intermediate (day 7-14) and late (day 14 – 28). In the early phase, the defect closure size in the test group was significantly greater than the control group with two-tailed probability value of 0.0031 by Student t-test. The closing ratio was gradually reduced in the following phase, however there were no significant differences between each phase due to the small sample number (**Figure 17**).



**Figure 17. Defect Closure in a Time-Dependent Manner.**

This graph compares the defect closure between the “Osseodensification” group and the “Conventional Osteotomy” group in a time-dependent manner. These data were

calculated by subtracting the defect closure at different points in the study. It is evident that the highest amount of defect closure for the “Osseodensification” group occurred during the first week of the study. In addition it can be seen that the “Osseodensification” group has more defect closure on days 7 and 14 of the study. However the amount of defect closure between both groups is roughly similar on day 28.

**Table 9** shows the highest defect closure occurring in the “Osseodensification” group during the first week of the study, ie, day 0 to day 7. In comparison, the amount of defect closure taking place in the “Conventional Osteotomy” group is almost half of that of the other group during the same time period. Defect closure remains higher in the “Osseodensification” group between day 7 to day 14. However during day 14 to day 28 the amount of defect closure is roughly similar between the two groups.

| Sample                      | Early Phase Closure (Day 0-7) |                    | Intermediate Phase Closure (Day 7-14) |                    | Late Phase Closure (Day 14-28) |                    |
|-----------------------------|-------------------------------|--------------------|---------------------------------------|--------------------|--------------------------------|--------------------|
|                             | Conventional                  | Osseodensification | Conventional                          | Osseodensification | Conventional                   | Osseodensification |
| 1C                          | 0.057                         | 0.115              | 0.091                                 | 0.220              | 0.012                          | 0.022              |
| 2C                          | 0.107                         | 0.178              | 0.035                                 | 0.237              | 0.010                          | 0.150              |
| 3C                          | 0.023                         | 0.123              | 0.017                                 | 0.024              | 0.188                          | 0.004              |
| 4C                          | 0.067                         | 0.119              | 0.110                                 | 0.048              | 0.140                          | 0.010              |
| 5C                          | 0.121                         | 0.242              | 0.030                                 | 0.044              | 0.027                          | 0.161              |
| <b>Average</b>              | 0.075                         | <b>* 0.155</b>     | 0.057                                 | 0.115              | 0.075                          | 0.069              |
| <b>STDEV</b>                | 0.039                         | 0.055              | 0.041                                 | 0.105              | 0.083                          | 0.079              |
| <b>P-value (two-tailed)</b> |                               | <b>0.031</b>       |                                       | 0.299              |                                | 0.910              |

\* denotes statistical significance (P < 0.05, compared to Control)

**Table 9. Defect Closure in a Time-Dependent Manner in Group C.**

## **DISCUSSION**

### **CHAPTER 4**

#### **4.1. Role of Wound Healing**

This study compared healing of osseodensification-induced defects versus conventional osteotomies in mouse calvaria under a dynamic condition.

The process of wound healing is complex during which the missing and devitalized cells and tissues are replaced. This is a natural physiological reaction to tissue injury involving an elaborate interplay between cells, mediators, cytokines and vascular systems (Wallace et al., 2018). Platelet aggregation and vasoconstriction of the blood vessels stop bleeding before initiating a cascade of reactions to heal the wound following surgery (Wallace et al., 2018). The healing cascade begins with the influx of inflammatory cells such as neutrophils that are responsible for activating other mediators which in turn promote thrombosis and angiogenesis.

The lag period is the inflammatory phase of healing during which the wound is cleared of contaminants as well as necrotic tissues (Boese., 1957). During this phase arteries and adjoining capillaries to the wound are dilated to maximize the flow of blood into the wound. The capillaries become permeable to allow plasma proteins and other blood constituents to escape into the wound (Boese., 1957). A fibrin clot develops between the edges of the wound to maintain the strength of the wound covering and to minimize

bleeding. The fibrin clot also forms a scaffold for cells such as polymorphonuclear leukocytes that phagocytose contaminants and dead tissue (Boese., 1957).

The next phase of healing involves deposition of granulation tissue and collagen formation to increase the wound's tensile strength. The length of the lag phase may affect this phase (Boese., 1957). Surgical wounds are usually clean thus granulation tissue appears within 48 hours. Capillaries also bud to provide the cells around the wound with nutrients. Collagen is produced in either the fourth or the fifth day in a surgical wound (Boese., 1957).

As collagen contracts the epithelium grows from the wound periphery towards the center in the proliferative phase. Contraction of the wound occurs as new tissues are built. The myofibroblasts play a key role in wound contraction. The granulation tissue in the wound is red or pink in color if the healing process is healthy. The occurrence of dark granulation tissue is a sign of poor perfusion, infection or ischemia (Wallace et al., 2018). Epithelialization occurs in order to cover the wound in the final stages of healing. This process is faster if the wound is moist and hydrated (Wallace et al., 2018).

The final phase of wound healing is the maturation phase, which is also known as the remodeling phase and occurs about 21 days post-surgery. The wound closes fully with synthesis of collagen type III switching to collagen type I. Cells that are not required are removed via apoptosis (Buo et al., 2014). The collagen fibers align along the tension lines in this phase. The essence of this arrangement is to reduce the thickness and make the skin around the wound stronger (Buo et al., 2014).

#### **4.2. Growth Factors and Wound Healing**

Growth factors are among the essential elements in the process of wound healing. They are used to control the remodeling process (Blitstein-Willinger., 1991). Components of the immune system directly involved in the healing process include matrix elements and cytokines which are important in the activation, recruitment and differentiation of the cells. Platelets transform the alpha granules into growth factor beta in order to attract macrophages and neutrophils which clean up the debris on the wound through phagocytosis (Blitstein-Willinger., 1991).

#### **4.3. Wound Stability and the Healing Process**

Wound stability is an essential factor for wound healing after surgery. It is strengthened in the fibroplasia phase and occurs between 2 days and 6 weeks (Pudner., 2010). Swelling decreases as the wound size is reduced. In the fibroplasia phase, collagen synthesis is critical to the wound tensile strength as well as for scar formation (Pudner., 2010).

All calvaria in this study were cultured in roller tubes so that a dynamic environment was maintained. This influenced cellular migrations involved in healing of the defect.

#### **4.4. Sterile Conditions and the Healing Process**

Sterile conditions are also mandatory for an efficient wound healing process. A moist and humid wound environment is important in speeding up the healing process (Pudner., 2010). Cellular migration can be halted if the environment is not moist (Boese., 1957).

A moist sterile environment was maintained for the calvaria. Contamination of the calvaria would have resulted in significant changes in defect closure and bone healing. The media was supplemented with antibiotics to prevent microbial contamination.

#### **4.5. Other Crucial Factors for Proper Healing**

Other external factors that are important in wound healing include the age of the patient, infection, type of wound, nutrition, chronic disease and blood circulation. Age-related physical changes can lead to reduced inflammatory responses and slowing of epithelialization and angiogenesis (McCulloch et al., 2010). Older people also have reduced rate of collagen synthesis and thus scar tissue forms slowly. Larger wounds heal more slowly than smaller wounds. There are also differences in the healing time based on the shape of the wound. The slowest to heal are circular wounds followed by rectangular and finally the linear wounds (McCulloch et al., 2010).

Poor nutrition and blood circulation can be combined because poor blood circulation can lead to a poor supply of nutrients (McCulloch et al., 2010).

#### **4.6. Surgical Intervention and the Inflammatory Process**

Periodontal surgery routinely removes microbial deposits (Dibart., 2011). In addition surgeries may include tissue to replace periodontal structures. Surgery triggers inflammatory reactions that are consistent with those of early wound healing (Dibart., 2011). Healing in periodontal wounds starts with a blood clot in the closed space after suturing regardless of the modality or intention of the surgery (Kaner et al., 2017). Neutrophils and monocytes appear immediately following surgery. The healing process



adheres to the normal cascade ranging from inflammation to remodeling and maturation. Following periodontal surgery factors such as tumor necrosis factor-alpha, IL-1 beta and IL-6 are elevated regardless of the treatment methods. These inflammatory mediators are important because they induce the expression of chemokines and adhesion molecules. Lack of these factors results in delayed and impaired wound healing (Kaner et al., 2017).

General periodontal surgery activates the normal wound healing cascade. The amount of GCF/WF (gingival crevicular fluid/wound fluid) as well as inflammatory cytokines increase significantly during the initial stages. However unlike other wounds a periodontal surgical wound has a flap whose closure limits the number of factors that can access the wound. Flap dehiscence is associated with prolonged inflammatory exudation (Kaner et al., 2017).

#### **4.7. Osseodensification-Induced Cellular Activity**

In this study the most significant defect closure for both “Conventional Osteotomy” and “Osseodensification” groups occurred from day 0 to day 7. Maintaining a stable wound especially during the first week after periodontal surgery results in faster and more efficient healing.

Cell number depends on the balance between cell division and loss of cells from either apoptosis or normal cell death. Treatment of bone-related diseases depends on factors that regulate their growth and remodeling (Lian et al., 1992). The mechanisms underlying bone tissue formation, stability and maturation are based on the activity of the

osteoblasts, osteoclasts, and osteocytes. In bone, a pluripotent mesenchymal cell is the progenitor stem cell of the osteoblast (Lian et al., 1992). However before commitment the stem cells can develop into either chondrocytes or osteoblasts. Bone marrow cultures can be used to study the differentiation of the progenitors.

The first step in the proliferation of the bone cells involves the release of mesenchymal cells from the bone as outgrowths. Although a mixture of cells is produced at this stage they are later committed to the osteoblast lineage (Zanello et al., 2006). Osteoblasts have specific properties that defines them such as responsiveness to PTH as well as the ability to synthesize bone-related proteins. Proliferation allows the normal process of bone formation after resorption (Zanello et al., 2006). Although the process is not well understood the mononuclear phagocytes that are present at the site of bone remodeling play a significant role in bone formation. Proliferation is increased when the cells produce prostaglandins. In contrast indomethacin inhibits the process (Gowen et al., 1985).

In this study photomicrographs of calvaria revealed cellular bands around the defect periphery, particularly in the “Osseodensification” group. This can be attributed to the increase in cellular proliferation and migration that occur during initial stages of wound healing. Histological analysis may help us better understand the defect changes at the cellular level.

#### **4.8. Tissue Engineering Concept for Regenerative Medicine**

Tissue engineering is an emerging yet widely adopted biomedical innovation. The process focuses on the regeneration of tissue in defects that are unable to undergo self-repair (Steenberghe et al., 2000). In addition tissue engineering is used to generate new synthetic tissues to replace damaged ones. The process of tissue engineering employs cells that have the potential for proliferation and differentiation. In addition, an artificial environment is employed to enable the cells to undergo regeneration (Steenberghe et al., 2000).

There are various methods that are applicable to tissue engineering to create the artificial environment for the tissues to regenerate (Tabata., 2004). One method uses scaffolds to guide cell proliferation and differentiation. Growth factors can also be used to induce the desired cellular changes. A combination of both scaffolds and growth factors is used in tissue engineering. The role of the growth factors in tissue engineering is to promote the process of regeneration since they have the potential to induce angiogenesis (Tabata., 2004). The growth of new blood vessels through angiogenesis supplies oxygen and nutrients to the cells to ensure that normal biological functions are not interrupted. Studies of drug and growth factors interactions in tissue engineering will be critical (Tabata., 2004).

#### **4.9. Angiogenesis in Bone Healing**

The basis of angiogenesis is the migration, growth and differentiation of the endothelial cells. Endothelial cells develop from the angioblasts (Demidova-Rice et al., 2011).

Angiogenesis differs from vasculogenesis. In the latter blood vessels are formed from the embryonic precursors. Hypoxia is a factor that contributes to the activation of angiogenesis. In a surgical wound blood supply to the site is temporarily halted or is inadequate as a result of the destruction of the surrounding blood vessels (Demidova-Rice et al., 2011).

Angiogenesis occurs at a rate of less than 1mm/day during wound healing. Angiogenesis occurs spontaneously within the implanted scaffolds in response to inflammatory reactions during wound healing (Adair and Montani., 2011). Capillary formation is usually semi-permanent because they revert after several weeks. Once the vascular system has developed nutrients, oxygen and waste are easily transported over a short distance through diffusion. Since bone is metabolically active it is constantly being remodeled and repaired by the osteoclasts, osteocytes and osteoblasts. When the scaffolds are implanted they mimic the complex tissues in the body hence becoming a proper site for cell proliferation, cell attachment and formation of the tissues (Adair and Montani., 2011).

#### **4.10. Osseinduction**

Osseinduction refers to the process of inducing osteogenesis (Albrektsson and Johansson., 2011). Osseinduction is important in almost all injuries of the bone including fractures. Basically osseinduction can be divided into two phases; recruitment of immature cells and cell stimulation to differentiate into preosteoblasts (Albrektsson and Johansson., 2011).

Thus, osseointegration occurs during the initial stages of bone healing and stimulates the pluripotent cell lineages to develop into lineages that are bone forming. While differentiated bone cells such as osteocytes, osteoclasts and osteoblasts are useful in the healing process, undifferentiated cells are most important in bone healing because they are easily recruited to form osteoprogenitor cells (Albrektsson and Johansson., 2011). Therefore osseointegration relies on the availability of a good stimulus to transform the mesenchymal cells into preosteoblasts.

#### **4.11. Osseointegration**

In contrast osseointegration means that bone grows on the surface. This property is important since it allows for bone growth on the implant surface. Osseointegration is guided by the activities of the differentiated cells (Gunzburg., 2002). As the second phase of the bone healing process, osseointegration depends heavily on osseointegration. Bone formation requires different growth factors as well as a good network of capillaries for blood supply (Gunzburg., 2002). Based on this property it has been suggested that many growth factors are both angiogenic and mitogenic (Albrektsson and Johansson., 2011).

Osseointegrative is one of the necessary characteristics of a good bone graft. There are different commercially available bones for applications in orthopedics and dentistry. They are useful in the reconstruction of cavitary bone defects as well as in the augmentation of bone loss (Gunzburg., 2002).

Modern studies have explored the reason for non-healing chronic wounds (Demidova-Rice et al., 2011). Reduced bioavailability of both receptors and growth factors may be a factor. Other causes include lack of proliferative capacity especially in the local cells, impaired wound perfusion, and modification of the matrix proteins. However a new treatment option is being explored in order to circumvent the problem. An example is the application of the exogenous fetal or adult progenitor cells to the chronic wound beds (Demidova-Rice et al., 2011). This treatment option is meant to trigger cells to differentiate into perivascular and vascular endothelial cells. Despite the initial success of these treatment options there are still safety and efficacy concerns that need to be addressed (Demidova-Rice et al., 2011).

#### **4.12. Osseodensification**

Osseodensification allow horizontal ridge expansion to improve the stability of the implants and support bone regeneration. The thick ridges developed by osseodensification maintains bone for easier and more stable placement of the implant (Jokstad., 2009). Bone regeneration in osseodensification does not have a significant variation with other methods of implant dentistry. The ability to use biomimicry from sponges and scaffolds increases the process while improving the quantity of the bone at the site of implantation (Jokstad., 2009).

After osseodensification the stability of the implant can be estimated histologically by calculating bone-to-bone contact percentage. The insertion torque can be measured to determine the implant stability level. Primary stability is vital because it directly affects

the secondary stability (Jokstad., 2009). Osseodensification has effects besides increased primary stability. Using this technology, the implant success rate has been more than 95%. Osseodensification leads to the development of mature tissues with soft collars (Jokstad., 2009).

#### **4.13. Bone Healing Under Dynamic Conditions**

Under dynamic conditions regeneration of bone is slower due to the changing conditions of the microenvironment around the site of the implantation (Sammarco et al., 2014). The oxygen supply is one of the factors affecting bone regeneration under dynamic conditions. In osseodensification the scaffolds resemble the complex tissues thus making it easier to provide a surface for attachment of blood vessels during angiogenesis. Under dynamic conditions bone regeneration depends on oxygen in the site of implantation (Sammarco et al., 2014). The ability to control the oxygen tension in vivo is an important part of regeneration.

Tissue oxygen supply is vital in regeneration as well as in successful wound healing. Oxygen in the tissues provides the required biochemical energy to protect against bacterial infections. Initial hypoxia is vital in regeneration because it activates a cascade of processes leading to angiogenesis (Sammarco et al., 2014).

This may explain the relatively small difference in defect closure between the “Osseodensification” group and the “Conventional Osteotomy” group. These calvaria were constantly under dynamic conditions in the roller tubes. Oxygen supply may not

have been sufficient to provide an ideal medium for enhanced bone regeneration and subsequent defect closure.

#### **4.14. Future Approach**

Future studies should be done using a larger sample size with careful examination of the calvaria. A daily analysis of the microscopic pictures with subsequent tracing of the defects should reveal additional information pertaining to the defect size closure. Moreover it would help us better understand osseodensification in terms of the specific timeline in which significant bone regeneration takes place.



## **CONCLUSION**

Osseodensification using Densah burs is a newly developed technique that plays a major role in enhancing the primary stability of the dental implant. Comparisons between conventional osteotomies and osseodensification with Densah burs revealed more bone defect closures with the latter technique. One of the reasons is the capability of the Densah burs to create an osseodensification crust around the defect site which may lead to an increase in the number of bone cells around the area and the subsequent activation of the healing process.

Closure of the bone defects was more prominent within the first week. This finding reinforces the fact that maintaining wound stability during the initial period of the wound healing process is of paramount importance for creating an ideal medium for cellular migrations.

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**CURRICULUM VITAE**

