INTRODUCTION

1.1 Normal bone formation

Bone formation occurs by two separate processes: endochondral and intramembranous ossification. Endochondral bone formation occurs on a cartilage model, on which embryonic mesenchymal stem cells differentiate into osteogenic phenotype, depositing osteoid and bone. This type of bone formation is responsible for the formation of short and long bones and those bones ending in joints, including the head of the mandible and base of skull.[1-5]

When ossification occurs directly, within a connective tissue membrane, it is termed intramembranous ossification. [1-5] This occurs during the formation of the cranial vault, facial skeleton, body of the mandible, the clavicle and scapula. The mandible, therefore, has both endochondral and intramembranous ossification centres.[1, 4]

Bone is composed of a specialised rigid mineral component consisting of 67% inorganic matrix, namely, hydroxyapatite and 33% of an organic component made up of 28% Type I collagen and 5% non-collagenous protein.

The cellular component is subdivided into 3 distinct cell types: the osteoblast, the osteocyte, and the osteoclast, which are responsible for formation, maintenance and resorption of bone respectively.[1, 2]

Osteoblasts are derived from a multipotent mesenchymal cell and synthesize collagenous and non-collagenous bone proteins. Osteoblasts secrete several bone morphogenetic

proteins (BMP), Transforming Growth Factor beta (TGF-β), in addition to insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF) and fibroblastic growth factor beta (FGF-β). The process of mineralization takes place continually when osteoblasts form and secrete the inorganic and organic constituents of the extracellular matrix. Osteoblasts display high levels of alkaline phosphatase on their plasma membranes, which together with other phosphoproteins are critical to the mineralisation of bone. [1, 2, 4]

The osteocyte is a mature osteoblast that becomes entrapped within a bony lacunae. It controls osteoblast and osteoclast function, and therefore plays an important role in maintaining strength and health of the bone matrix. Osteocytes communicate with adjacent osteocytes, osteoblast and osteoclasts via tentacle-like dendrites through multiple canaliculi. Osteocytes produce a protein called sclerostin, which inhibits osteoblast activity while stimulating osteoclast activity. [1, 6, 7]

Osteoclasts are large multinucleated giant cells of haematopoetic origin that initiate normal bone remodelling and mediate bone loss in pathologic conditions by increasing their resorptive activity. The osteoclast precursors are attracted to bone sites destined for resorption and are under the influence of osteoblastic cells in the bone marrow. [2, 8-10]

1.2 Bone healing processes

Bone is a remarkable connective tissue that heals entirely by regeneration and the production of a mineral matrix. Injured bone can repair itself and return to full function without deformity or scarring.[11] The host bed, vascular supply, oxygen tension and stability of the bony segments or graft, dictates the pattern and speed of bone healing.[12]

When bone healing occurs directly, it is known as primary bone healing and secondary bone healing occurs with an intermediate cartilaginous phase. [13, 14]

The following events occur during healing of a fractured bone: haematoma or clot formation with release of bone inducing factors and cellular recruitment, inflammation with the development of fibrovascular tissue and vascular invasion, followed by focal resorption of osteocytes and the subsequent new bone formation and remodelling.[1, 2, 15, 16] When bone is fractured, its matrix is destroyed and the bone cells adjacent to the fracture die. The damaged and broken blood vessels produce haemorrhage and subsequent clot formation. During the repair phase, macrophages remove the blood clot, cells and damaged bone matrix. The periosteum and endosteum respond by vigorous proliferation of osteoprogenitor cells, which penetrate between the fragments of the fractured bone. Primary bone is formed by endochondral ossification of small cartilage fragments. Bone is also formed by intramembranous ossification within the fracture. This bony repair progresses by forming irregular trabeculae of primary bone that temporarily unite the ends of the fracture and is called a bony callus. Normal stresses imposed during healing and by the patients return to normal function causes remodelling, which reconstitutes the bone as it was before the fracture.[1]

1.3 Bone grafting

A bone graft is required when the size of a defect exceeds the body's natural ability to restore the missing bone. This is known as a critical size defect.[17] The volume of bone required for grafting a defect will determine the donor site. If a large volume of bone is required, sites such as the iliac crest (anterior or posterior), the calvarium, the rib, and the proximal tibia may be used.[18] For the reconstruction of large continuity defects, the

posterior ilium provides the best bone in both quality and quantity of cortical and cancellous bone, with less morbidity than in a lateral approach of the anterior ilium.[19-25] Importantly, one should know how much bone is available from these individual sites in order to properly plan and manage the reconstruction of an oro – facial defect.

1.3.1 Types of bone grafts

Bone grafts can be classified as autogenous, allograft, xenograft, and alloplastic material, and can even include osteoinductive morphogens.

1.3.1.1 Autografts

This is a graft taken from an individual and transferred to a different site in the same individual. Autogenous bone grafting is the gold standard by which all grafting techniques of osseous reconstruction should be judged. An important advantage of autogenous bone grafts is that they do not trigger an immune response. Furthermore, autogenous cancellous bone grafts produce the most successful and predictable results and remain the most effective option for mandibular reconstruction.[20, 26] Autografts are osteoconductive, osteoinductive and osteogenic. The major disadvantage of autogenous bone graft is the need for a second surgical site and therefore, increased morbidity and surgery time.

1.3.1.2 Allografts

Allogenic bone is bone obtained from one individual transferred to another individual within the same species.[22, 27] There are three forms of allogeneic bone: fresh frozen, freeze-dried and demineralized bone matrix (DBM). Fresh frozen grafts are not used because of concerns related to transmission of viral diseases. Freeze-dried allogeneic bone is produced by dehydrating bone. It is osteoconductive but has no osteogenic or osteoinductive

capabilities, and therefore requires a source of osteo-competent cells for new bone formation to occur, which may potentially be a disadvantage of this particular form of allograft. Freeze-dried allogenic grafts are stronger than DBM and can be used as onlay grafts or as cribs together with autogenous bone grafts. [28, 29] By demineralising freeze-dried bone, DBM is created. This process gives DBM the advantage of exposing osteoinductive proteins but the disadvantage of decreasing its mechanical strength.[29]

1.3.1.3 Xenografts

A xenograft is animal derived tissue used to replace or fill defects in the human body. These grafts are derived either from mammalian skeleton or coral exoskeletons.[27, 30] Examples of these grafts are bovine or porcine bone. The advantage of using a xenograft is that it reduces donor site morbidity. However, xenografts are osteo-conductive without the osteo-inductive qualities.

1.3.1.4 Synthetic (alloplastic) materials

There are three types of alloplastic materials in routine clinical use: hydroxyapatite (HA) (a ceramic), other ceramics and polymers. Hydroxyapatite is a ceramic which can be used as a filler for bony defects or as a bone expander when combined with autogenous bone during ridge augmentation and maxillary sinus floor augmentation.[31] Other types of ceramics are tricalcium phosphate (TCP), bioactive glass and calcium sulphate.[27] Polymers are materials that can be fashioned into various configurations. Combinations of polyglycolic acid and polylactic acid have been used in bioresorbable sutures. These materials are useful as bioactive scaffolds.[32]

1.3.1.5 Osteoinductive morphogens

Osteoinductive morphogens are proteins that have the ability to stimulate the differentiation of osteoblasts and bone formation. They are classified as osteoinducers, osteopromoters or bioactive peptides.

Bone morphogenic proteins (BMPs) are morphogens with osteoinductive properties and belong to the Transforming Growth Factor-Beta (TGF-β) superfamily. These are considered to be osteopromoter agents that enhance bone healing.[29, 33] Critical sized defects in several preclinical models have healed with BMP, including cranial vault defects and mandibular continuity defects.[34]

Platelet-derived growth factor (PDGF) is angiogenic and stimulates the reproduction and chemotaxis of connective tissue cells and matrix deposition.[35] Insulin-like growth factor (IGF) increases bone cell mitosis and increases deposition of matrix. PDGF and IGF work together during bone healing.[36]

The advantage of using bioactive polypeptides is that they act as osteo-enhancers or osteo-inducers, while stem cells have the potential to differentiate along various pathways including the direction of bone forming osteocompetent cells.[27, 37]

1.3.2 Vascularisation of bone grafts

1.3.2.1 Vascularised bone grafts

A vascularised bone graft retains its nutrient vessels and when anastomosed to the recipient vessels at the recipient site, becomes immediately viable by providing an immediate blood supply. It is better suited to poorly vascularised beds, such as irradiated areas, or in

recipient beds with extensive scarring.[38 - 40] Advantages of using vascularised composite grafts are that they resorb less and implants can be placed immediately for dental reconstruction. Another advantage is the possibility of soft tissue and bone reconstruction with the same composite graft. This type of graft will have a remodelling process similar to normal bone. The most common vascularised bone graft donor sites are the fibula, radius, iliac crest and scapula, with the former three being the most frequently used to reconstruct mandibular defects. The disadvantage of using vascularised grafts to reconstruct mandibular defects is that the surgical harvesting and re-anastomosing of blood vessels is time-consuming, results in substantial morbidity, unsightly donor site defects, and is costly.[22, 40 – 42] Moreover, free flaps fail to reconstitute the shape, height and form of the mandible.[43]

1.3.2.2 Non-vascularised bone grafts

Non-vascularised autogenous bone grafts may be obtained from both intra-oral and extra oral sites, depending on the size of the defect being reconstructed. It may be cortical, cancellous or cortico-cancellous, and may be harvested in two forms: either particulate or block graft. Advantages of a non-vascularised graft is that large volumes can be obtained, with decreased surgery time, minimal donor site morbidity, and recipient site scarring. Non-vascularised particulate cortico-cancellous grafts can be used to restore defects of any length as the distance to soft tissue interface, (the closest angiogenic source) determines success not the distance of the defect from bony margin to bony margin.[43] A disadvantage of using non-vascularised bone graft is that the failure rate increases in poorly vascularised beds, irradiated areas or areas of excessive scar tissue and dental implant success rates vary from 60 - 90 %.[38-40]

1.3.3 Bone graft healing

Bone graft healing and regeneration occurs by three mechanisms:

Osteoconduction - new bone formation occurs from the adjacent bone, recipient bed or from the periosteum through a matrix that acts as a scaffold. Any material which allows or directs bone formation along its surface can act as a scaffold. This phenomenon is also known as 'creeping substitution'. [19]

Osteoinduction - biochemical transformation and stimulation of stem cells into bone-producing cells. Bone morphogens of the Transforming Growth Factor-β (TGF-β) superfamily are the most important in this regard. [29, 44]

Osteogenesis - the formation of new bone by transplanted osteoprogenitor cells. The numerous surviving endosteal osteoblasts are the cells within a graft which produce new bone. [19, 22, 41]

Importantly, an ideal bone graft material should incorporate all three of the above mechanisms to optimally regenerate and induce new bone formation at the graft site. The structure of the bone graft may be block or particulate which will determine the rate of healing and remodelling of the graft.

Vascularised cortical block bone grafts heal via anastomosis of blood vessels to the recipient vessels and will heal as in normal fracture healing. The implantation of autologous non-vascularised cortical, cancellous and particulate bone produces a vigorous growth of new bone, leading to the formation of bony ossicles as a result of the activity of the transplanted cellular components combined with those of the host tissue. Buchardt (1983) summarised the three differences between cortical and cancellous bone graft healing as follows:

- Cancellous grafts revascularise completely and more rapidly than cortical bone grafts.
- Cancellous grafts have an appositional bone formation phase, followed by a resorptive phase, while cortical grafts undergo a reverse creeping substitution phase.
- Cancellous grafts repair completely with time, while cortical grafts remain as an admixture of necrotic and viable bone.[45, 46]

The process of bone graft healing occurs in two phases.[13] During the first phase of bone healing, no major differences are seen between cortical and cancellous grafts. The initial regenerative process begins within hours of placing the graft. Osteoblasts begin the production of osteoid and the haematopoietic stem cells start differentiating into osteoblasts.[45] Angiogenic rates differ in cortical and cancellous grafts after this initial regenerative phase. The degeneration of the cancellous graft marrow spaces creates channels for ingrowth of new vessels from the host bed. Often, autologous cancellous grafts have completed revascularisation by 1 week while cortical grafts revascularise by ingrowth of vessels through Haversian canals after a period of 6 – 8 weeks.

During phase 1 healing of a particulate cortico-cancellous graft, new bone formation occurs as a result of the survival of transplanted osteocompetent cells, which form the foundation of new ossification.[13, 20, 43] This bone is very cellular and disorganised without meaningful structural rigidity and is called woven bone. Several angiogenic and transforming growth factors initiate the second phase of bone healing. Again, the most important growth factors belong to the transforming growth factor (TGF)-β superfamily.[29, 43, 47] Mature osteoclasts secrete coupling chemokines that cause recruitment and maturation

of osteoblasts and is a fundamental part of the remodelling of bone grafts. [48] This second phase of bone remodelling is known as lamellar compaction. This bone is more mineralised, highly organised but less cellular.[13]

Cortico-cancellous block grafts undergo greater volumetric decline than compressed cortico-cancellous particulate grafts due to the differences in the initial cellular concentration and the rapidity with which the blood vessels derived from the soft tissue can gain access to the graft. The key to successful grafting is the distance from the closest source of angiogenic invasion rather than the distance from the bony margins.[43] Failure of bone graft healing occurs when the biological parameters of the graft (handling, storage and number of transplanted cells) and the host's local or systemic factors are transgressed. The graft factors may include the volume of viable cells transplanted in the graft, the temperature of the environment, and the storage medium. The host factors that may determine success of the graft are systemic illnesses, radiation therapy to the area, intra-oral or extra-oral access to the recipient site, and immobility of the grafted bone. [20, 23, 28, 43]

There are many studies which support the use of particulate cortico-cancellous grafts to effectively regenerate bone and reconstitute continuity defects of any length and shape in the mandible more accurately than a vascularised free bone graft, with decreased morbidity and cost.[28, 49-52]

1.4 Reason for this research study

There is uncertainty regarding the structure of bone grafts required to repair mandibular defects, and the volume of bone required to optimally repair 1cm of a mandibular defect.

Pogrel (1997) and Foster (1999) reported that vascular free flaps should be used for the reconstruction of mandibular defects longer than 6cm.[53, 54] Ferretti *et al.*, raises the issue that Pogrel and Foster refer to block grafts only and not to particulate cortico-cancellous grafts. [43, 53, 54] Marx (1988) has estimated that to repair 1cm of a mandibular defect, 8 – 10cc of non-compressed particulate cancellous bone marrow graft is required. Although the volume of bone obtainable from the posterior iliac crest has been studied by many surgeons, only one article by Mirovsky used weight as a measurement to quantify bone grafts. [23, 55-59] Mirovsky used the iliac crest bone grafts in spinal fusion surgery, not for oro-mandibular reconstruction. No guidance is given regarding the amount of bone required for a particular defect length.

Determining bone volume intra-operatively can only be done by volume displacement method which is cumbersome and requires soaking bone in fluid which may be detrimental to cellular viability.

I am hoping to determine the mass of bone required to reconstruct a 1cm mandibular continuity defect successfully. By measuring the weight and volume of bone graft per centimetre of defect, I can calculate the volume per gram (cc/g) of harvested cortico-cancellous particulate bone graft required to repair a defect in the mandible

2. AIMS AND OBJECTIVES

2.1 Aims

The aim of this study is to quantify the mass of autogenous bone required to graft a 1cm mandibular continuity defect with a particulate graft harvested from the posterior iliac crest.

2.2 Objectives

- To quantify the wet bone mass and volume of cortico-cancellous bone graft required to repair 1cm of a mandibular continuity defect.
- To assess whether the quantity of bone available for harvest differs in male and female patients.

3. MATERIALS AND METHODS

3.1 Study design

A retrospective observational cohort study was conducted using the database of patients who had mandibular reconstruction with particulate cortico-cancellous graft from the posterior iliac crest. All patients were treated at the Department of Maxillofacial and Oral Surgery, School of Oral Health Science, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. Permission to use the database was obtained from the acting Head of the School of Oral Health Sciences and ethical clearance was obtained for the Human Research Ethics Committee of the University of the Witwatersrand (Appendix B). Patient records were obtained for the time period January 2010 to January 2015.

3.2 Selection of patients

3.2.1 Inclusion criteria

All patients with continuity defects of the mandible (secondary to resection of benign pathology, trauma or infection) requiring reconstruction were included in this study.

3.2.2 Exclusion criteria

Patients in poor general health and thus poor candidates for surgery were excluded.

Patients who did not require osseous reconstruction e.g. patients whose defects could be left edentulous without undue compromise of the patient's quality of life. These were patients who had tumours of the mandibular ramus that extended into the body with the loss of no more than 2 molars.

Patients who expressed concern about their ability to return for several follow-up operations as they were from rural areas. These patients were treated by resection or debridement only and reconstructed with a mandibular reconstruction plate.

Any patient whose defect length was of such magnitude that bone substitutes and osteo-inductive morphogens were required to augment the bone graft, were excluded.

Patients with malignant pathology, who received radiation therapy.

3.3 Stages of treatment

3.3.1 Treatment planning and resection stage

All patients were treated in two stages. The first stage involved treatment planning with computed tomographic scanning and standard radiography. A stereolithographic model was prepared following virtual treatment planning which allowed for the production of a patient-personalised prebent titanium plate (Biomet Microfixation, Jacksonville, Florida, USA). Once the tumour had been resected and the prebent plate secured to the mandible, a non-toxic medical and food grade soft silicone spacer was placed and secured to the reconstruction plate with 0.18 inch stainless steel wire (Figure 1). The operative site was then closed in layers. The patient was fed via the nasogastric tube, administered intravenous antibiotics for 7 days, and kept in inter-maxillary fixation for a period of six to eight weeks.

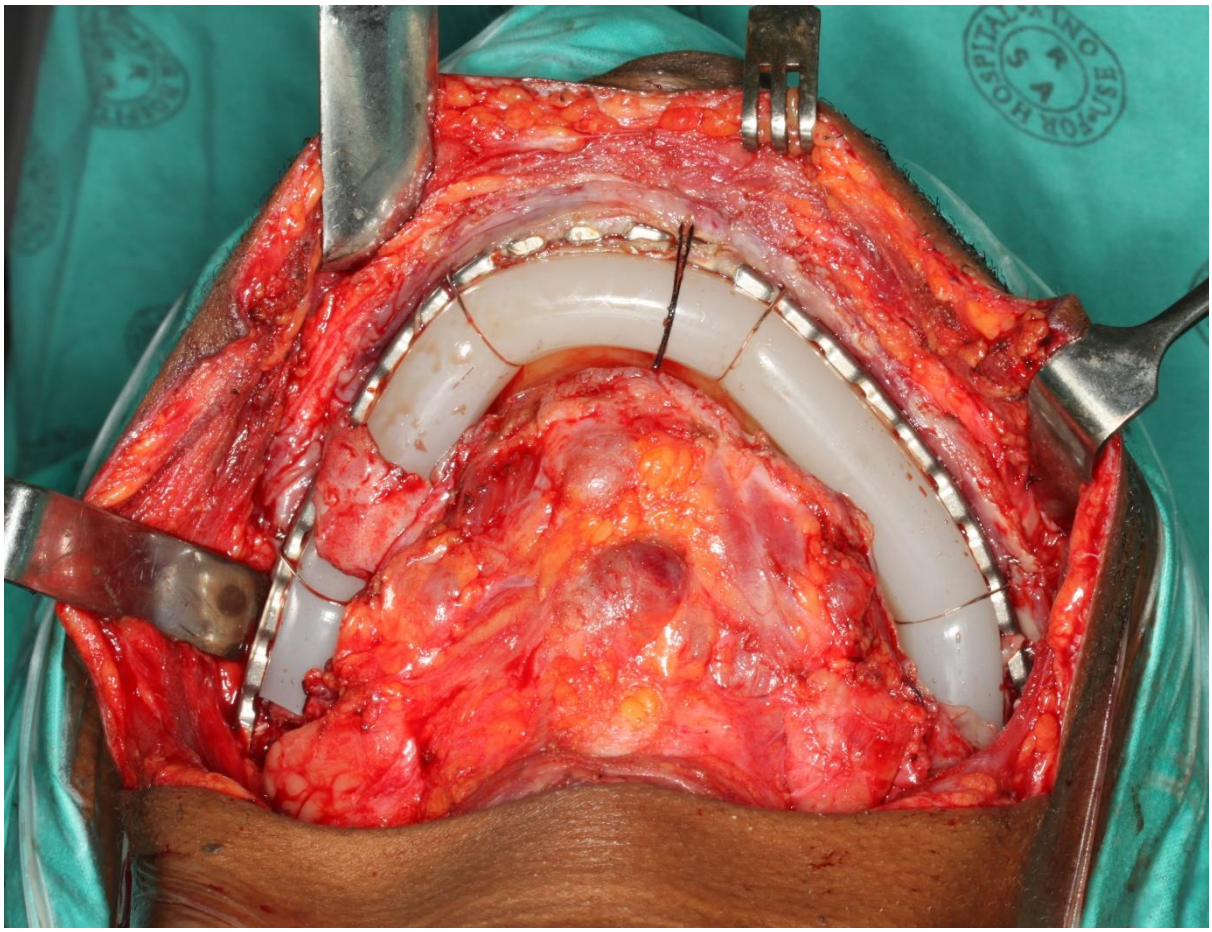

Figure 1. Silicone spacer in situ, secured with 0.18 inch stainless steel wire.

3.3.2 Reconstruction stage

After a six to eight week healing period, a bone graft was harvested from the posterior iliac crest, (either unilateral or bilateral) while the patient was in the prone position. The bone was weighed on a kitchen scale and recorded as the wet weight in grams (Figure 2). Once harvested, the bone was stored on ice to maintain cellular viability. The patient was returned to the supine position and the mandibular defect was exposed via an extra-oral submandibular approach. The defect length was measured with a flexible ruler in centimetres and recorded (Figure 3). The spacer was removed, the recipient soft tissue bed and the osseous interfaces carefully debrided and refreshed (Figure 4). The harvested bone stored on ice was milled

with a power bone mill (Stryker Leibinger, Michigan, USA). The milled particulate cortico-cancellous bone was transferred into 20ml plastic syringes and maximally manually compressed to expel all blood, fat and air (Figure 5). The compressed bone volume was recorded. Compressing the particulate graft produced a compact graft that was easily contoured to the shape of the mandibular defect (Figure 6). The compressed cortico-cancellous graft was syringed into the recipient bed. Careful attention was given to adapting the particulate graft to the mandibular bony interface. Suspension sutures were placed through the reconstruction plate from buccal to lingual to prevent graft subsidence and the site closed in layers. The patient was fed via the nasogastric tube and given intravenous antibiotics for 7 days. The patient was placed into inter-maxillary fixation for a period of 6 weeks to allow for graft consolidation.

Figure 2. Harvested cortico-cancellous bone from the posterior iliac crest weighed.

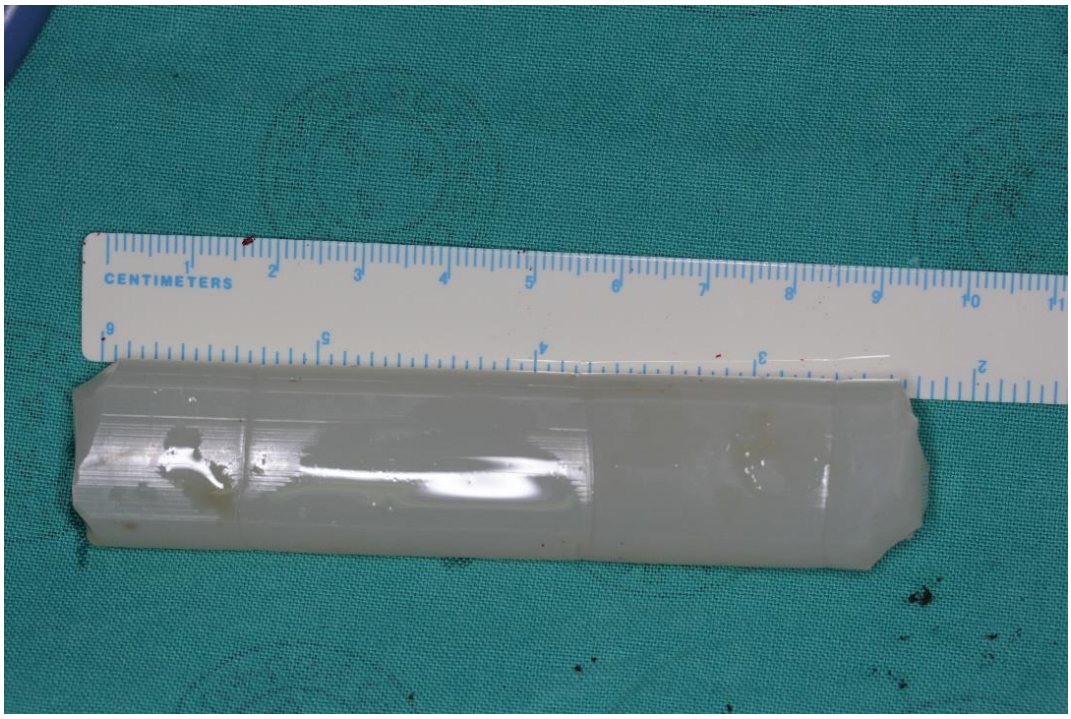

Figure 3. Silicone spacer removed at reconstruction phase and measured in centimetres.

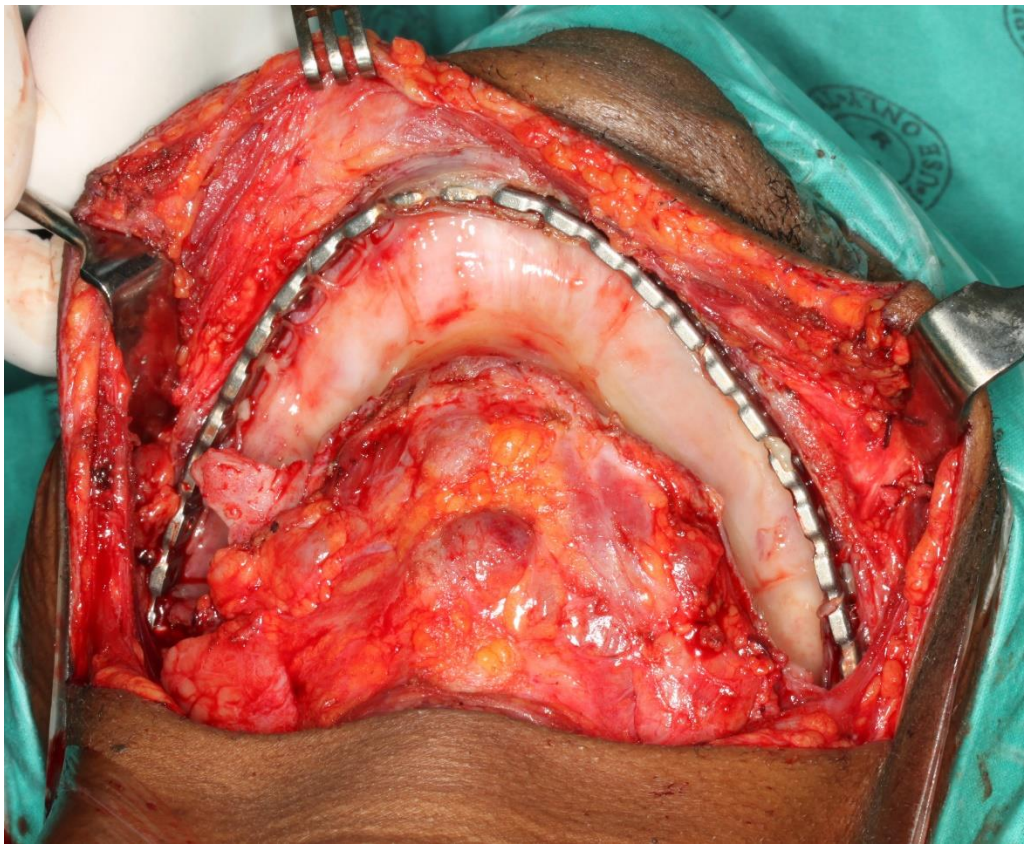

Figure 4. Graft bed and bone margins refreshed before placement of particulate cortico-cancellous graft.

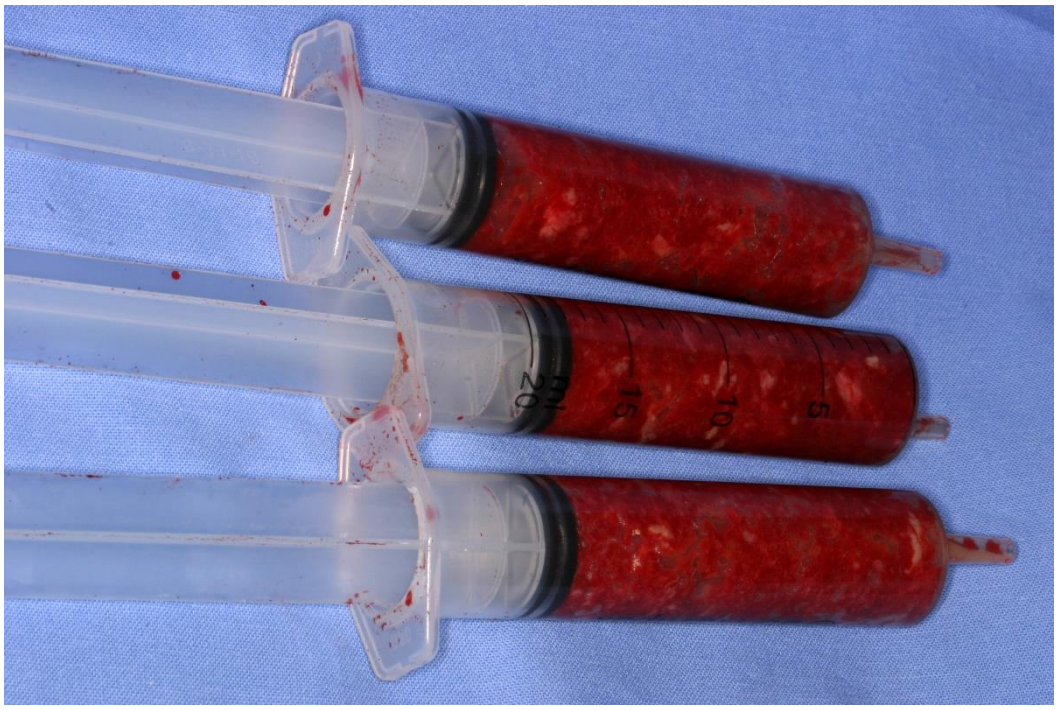

Figure 5. Particulate cortico-cancellous graft compressed in 20ml syringes.

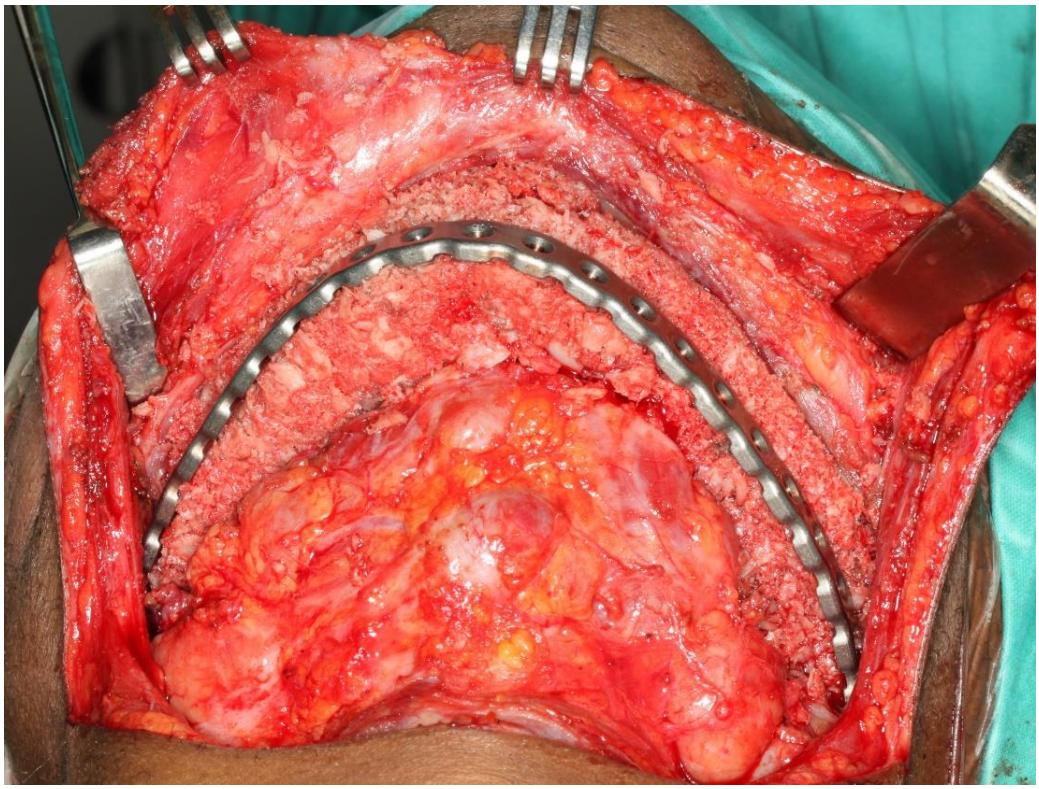

Figure 6. Compressed cortico-cancellous graft syringed onto graft bed, moulded into shape of mandible.

3.4 Data collection

Patient clinical records were obtained from the patient data base and their clinical records were allocated a study number to maintain anonymity.

The patient's age, gender and cause of mandibular continuity defect was obtained from the clinical records. The length of the defect (cm), wet bone mass in grams (g), compressed bone volume in cubic centimetres (cc) and the compressed bone volume used (cc), was recorded on the date of reconstruction. From this data the mass of wet bone used for 1cm of mandibular defect (g/cm) was calculated. The compressed bone volume per centimetre of mandibular defect (cc/cm) and the compressed bone volume per gram (cc/g) were also calculated. (Table 1, 2, and 3)

3.5 Statistical analysis

Data were captured using an Excel spreadsheet, and was imported into the Stata 13.1 (Microsoft Corporation, Redmond, Washington) programme for further analysis. The baseline characteristics of the patients were described by using frequencies and percentages for sex, and means, standard deviations and ranges for all variables. Skewness test was used to determine the distribution of the data, and the independent Students t-test was used to determine variability between the males and females. A p-value of ≤ 0.05 was chosen as an indication of statistical significance. All variables were normally distributed in the population; therefore no non-parametric tests were used.

4. RESULTS

Thirty one patient records were included in this study of which 11 were males and 20 were females. The defects were the results of resections of ameloblastoma (n=25), cemento-ossifying fibroma (n=2), ameloblastic carcinoma (n=1), odontogenic myxoma (n=1), central giant cell granuloma (n=1), and gun-shot injury (n=1).

The mean age of all 31 patients was 27.43 with a SD of 10.63 and a range of 12 - 62 years. All the ensuing data is reported as mean ± SD and (range). Combined data refers to male and female data together.

4.1 Wet bone mass (g)

The wet bone mass in males was 70.82 ± 16.73 g (47 – 98 g), and in females was 62.2 ± 15.97 g (32 – 100 g). The combined wet bone mass was 65.26 ± 16.50 g (32 – 100 g). There was no statistical difference between males and females.

4.2 Compressed bone volume (cc)

The compressed bone volume in males was 38 ± 10.49 cc (23 – 52 cc) and in females was 29.45 ± 6.61 cc (21 – 42 cc). The combined compressed bone volume was 32.48 ± 9.03 cc (21 – 52 cc). There was a statistically significant difference in the compressed bone volume between males and females with a *p-value* of 0.01.

4.3 Compressed bone volume used (cc)

The compressed bone volume used in males was 36.32 ± 9.77 cc (23 – 50 cc) and in females was 29.2 ± 6.19 cc (20 – 42 cc). The combined compressed bone volume used was

31.73 ± 8.25 cc (20 – 50 cc). There was a statistically significant difference in the compressed bone volume used between males and females with a *p-value* of 0.02.

4.4 Defect length (cm)

The defect length for males was 10.14 ± 3.16 cm (6.5 – 16 cm), and in females was 8.65 ± 2.12 cm (6 - 12.5 cm). The combined defect length was 9.15 ± 2.61 cm (6 – 16 cm). There was no statistical difference between males and females for defect length.

4.5 Gram per centimetre of defect (g/cm)

The wet bone mass (g) per centimetre of mandibular defect in males was 7.4 ± 2.04 g/cm (4.09 - 11.38 g/cm), and in females was 7.39 ± 1.84 g/cm (4.67 - 10.72 g/cm). The combined wet bone mass per centimetre of mandibular defect was 7.43 ± 1.89 g/cm (4.09 - 11.38 g/cm). There was no statistical difference between males and females.

4.6 Compressed bone volume per centimetre of defect (cc/cm)

The compressed bone volume per centimetre of mandibular defect in males was 3.74 ± 0.75 cc/cm (2.27 - 4.88 cc/cm), and in females was 3.49 ± 0.72 cc/cm (2.2 - 4.67 cc/cm). The combined compressed bone volume per centimetre of mandibular defect was 3.57 ± 0.73 cc/cm (2.2 - 4.88 cc/cm). There was no statistical difference between males and females.

4.7 Compressed bone volume per gram of wet bone (cc/g)

The compressed volume obtained from 1g of wet bone mass in males was 0.54 ± 0.09 cc/g (0.40 - 0.74 cc/g), and in female was 0.48 ± 0.07 cc/g (0.37 - 0.63 cc/g), while the

combined compressed bone volume per gram was 0.50 ± 0.08 cc/g (0.37 - 0.74 cc/g). There was no statistical difference between males and females.

5. DISCUSSION

This study investigated the mass and volume of particulate cortico-cancellous bone grafts from the posterior iliac crest, needed to repair mandibular defects. Data was collected from 31 patient records, which included: gender, age, reason for resection, wet bone mass, compressed volume, compressed volume used, and defect length. From the recorded data, the following variables could be calculated, grams per centimetre, volume per centimetre, volume per gram.

5.1 Wet bone mass harvested

At the reconstruction phase, cortico-cancellous bone was harvested from the posterior iliac crest (unilateral or bilateral). Wet bone is the quantity of bone harvested from the donor site, without any blood removed and no fluids added. There was no statistically significant difference in the wet bone mass harvested between males and females, thus the combined figure of 70.82 g of wet bone is the mean that can be expected from bilateral posterior iliac crest harvest.

5.2 Compressed bone volume and compressed volume used

This study found a statistically significant increase between the compressed bone volume and the compressed bone volume used in males as compared to females. The mean and the range of compressed bone volume in males (in cubic centimetres) was 38 cc (23 – 52 cc), and 29.45 cc (21 – 42 cc), in females. The mean and range of compressed bone volume used in males was 36.32 cc (23 – 50 cc), and 29.2 cc (20 – 42 cc) in females.

To corroborate my impression that female bone may be more compressible than male bone (due to decreased trabecular density), a compressibility index was developed. The compressibility index was calculated by dividing the compressed bone volume by the wet bone mass. The male mean wet bone mass was 70.82 g and the mean compressed bone volume of 38 cc yielding a compressibility index for male bone of 0.54. The female mean wet bone mass was 62.2 g and the compressed bone volume was 29.45 cc yielding a compressibility index for female bone of 0.47. This data further supports the contention that particulate cortico-cancellous bone of female patients is more compressible (or less dense), than that of males. Thus female bone is about 12% more compressible (100 - 47/54 = difference in bone compressibility in percent), and females may require 12% more wet bone mass than the male mean wet bone mass.

Importantly, in 4 patients not all the compressed volume of bone was used. This is only one of the reasons why it is so important to quantify the mass and volume of bone graft required per centimetre of defect. This quantification will decrease morbidity rates and bone wastage.

5.3 Quantitative comparison of mass/centimetre, volume/centimetre and volume/ mass of defect

The combined data found that for each centimetre of mandibular defect 7.43 ± 1.89 g of wet bone mass was used, and 3.57 ± 0.73 cc of compressed particulate bone was used. The combined mean volume of compressed bone obtained from 1 gram of wet bone was 0.50 ± 0.08 cc.

In males, for each centimetre of mandibular defect 7.4 g/cm of wet bone mass was used and 3.74 cc/cm of compressed particulate bone was used. The mean volume of compressed

bone obtained from 1 gram of wet bone was 0.54 cc/g. In females, for each centimetre of mandibular defect 7.39 g/cm of wet bone mass was used, and 3.49 cc/cm of compressed particulate bone volume used. The mean volume of compressed bone obtained from 1 gram of wet bone was 0.48 cc/g. There is no statistically significant difference for the above variables between males and females.

The combined range of wet bone mass used per centimetre of mandibular continuity defect (4.09 – 11.38 g/cm) was larger than expected. Although this study did not assess the volume of the final ossicle, I have to surmise that the volume of bone implanted would have affected the final ossicle size. The low value of 4.09 g/cm, which I would expect from either small patients or for large defects, would result in a low volume ossicle (Figure 7). The high value (11.38 g/cm), which I would expect from smaller defects or larger patients would result in high volume ossicle (Figure 8). Nevertheless, all defects reconstructed (even those with 4.09 g/cm) yielded ossicles adequate for endosseous fixture placement.

5.4 Limitations of the current study

A larger sample size may have resulted in a better statistical correlation between the variables. Correlation of compressed bone volume implanted to final ossicle size would have provided important information regarding volumetric changes during bone graft maturation.

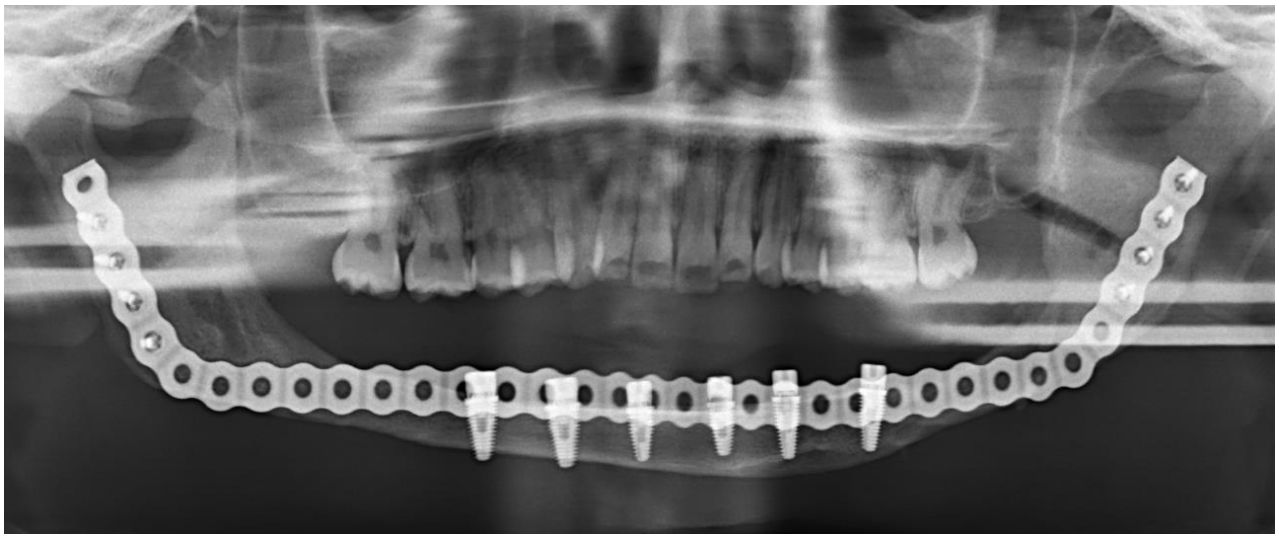

Figure 7. Low volume ossicle.

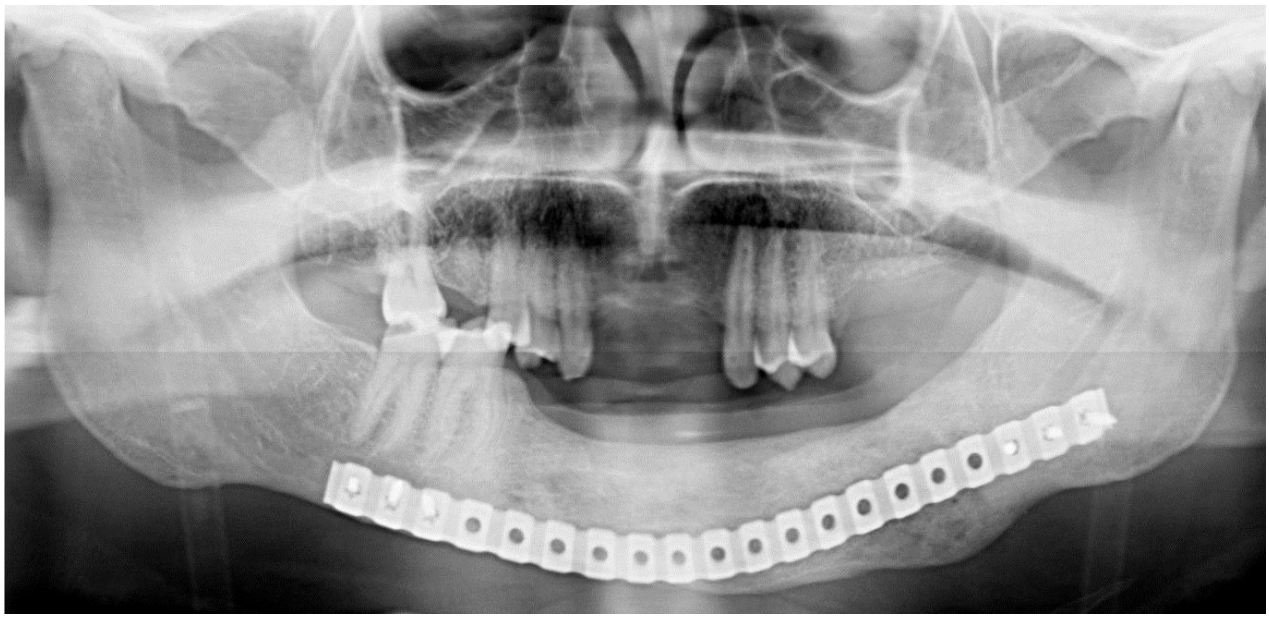

Figure 8. High volume ossicle.

6. CONCLUSIONS

The mean wet bone mass of particulate cortico-cancellous bone graft required to adequately repair 1 centimetre of mandibular continuity defect is 7.43g/cm. There was a statistically significant difference in the compressed volume and compressed volume of bone used between males and female. Female bone is about 12% more compressible than male bone. This may require 12% more wet bone mass harvested for female patients.

7. REFERENCES

1. Junqueira LC, Carneiro J, Kelly RO. Basic Histology 8th Edition. Connecticut: Appleton and Lange; 1995. 132-151.
2. Ten Cate AR. Oral Histology: Development, structure, and function. 4th Edition. Missouri: Mosby; 1994. 120-146.
3. Marks SC, Popoff SN. Bone cell biology: the regulation of development, structure and function in the skeleton. Am J of Anat. 1988; 183: 1-44.
4. Moore KL, Persaud TVN, Torchia MG. The Developing Human: Clinically Orientated Embryology. 9th Edition. Philadelphia: Elsevier Saunders 2013; 344-360.
5. Frost HM, Jee WS. Perspectives: a vital biomedical model of the endochondral ossification mechanism. Anat Rec. 1994; 240: 435-446.
6. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011; 26(2): 229-238.
7. Burgers TA, Williams BO. Regulation of Wnt/β-catenin signaling within and from osteocytes. Bone. 2013; 54(2): 244-249.
8. Boyce, BF, Yao Z, Xing L. Osteoclasts have multiple roles in bone in addition to bone resorption. Crit Rev Eukaryot Gene Expr. 2009; 19(3): 171-180.
9. Rodan GA, Martin JJ. Role of osteoblasts in hormonal control of bone resorption- a hypothesis. Calcif Tissue Int.1981; 33(4): 349-351.
10. Mundy GR. The effects of TGF-beta on bone. Ciba Found Symp.1991; 157: 137-143.
11. Salter RB. Disorders and injuries of the musculoskeletal system. Baltimore, Williams and Wilkins. 1983; 93-97.

12. Buckwalter JA, Glimcher MJ, Cooper RR. Bone biology Part 1: Structure, blood supply, cells, matrix and mineralisation. J Bone Joint Surg. 1995(a); 77A: 1256-1275.
13. Axhausen W. The osteogenic phases of regeneration of bone: a historical and experimental study. J Bone Joint Surg. 1956; 38A: 593-601.
14. Hollinger JO, Buck DC, Schmitz J. Quantitative light microscopy. A powerful tool to assess bone. Clin Plastic Surg. 1994; 21: 463-475.
15. Frost HM. The biology of fracture healing. An overview for clinicians. Part I. Clin Orthop Relat Res. 1989; (248): 286-293.
16. Frost HM. The biology of fracture healing. An overview for clinicians. Part II. Clin Orthop Relat Res. 1989; (248): 294-309.
17. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunion. Clin Orthop Relat Res. 1986; (205): 299-308.
18. Boyne PJ. Osseous reconstruction of the maxilla and mandible. Chicago Quintessence Publishing. 1997; 52-67.
19. Marx RE. Bone and bone graft healing. Oral Maxillofac Surg Clin North Am.2007; 19(4): 455-466.
20. Marx RE. Clinical application of bone biology to mandibular and maxillary reconstruction. Clin Plast Surg. 1994; 21(3): 377-392.
21. Bloomquist DS, Feldman GR. The posterior ilium as a donor site for maxillo-facial bone grafting. J Maxillofac Surg. 1980; 8(1): 60-64.
22. Oppenheimer AJ, Tong L, Buchman SR. Craniofacial bone grafting: Wolff's Law Revisited. Craniomaxillofac Trauma Reconstr. 2008; 1(1): 49-61.

23. Marx RE, Morales MJ. Morbidity from bone harvest in major jaw reconstruction: a randomised trial comparing the lateral anterior and posterior approaches to the ilium. J Oral Maxillofac Surg. 1988; 46(3): 196-203.
24. Dingman RO. The use of iliac bone in repair of facial and cranial defects. Plast Reconstr Surg. 1950; 6(3): 179-195.
25. Tayapongsak P, Wimsatt JA, LaBanc JP, Dolwick MF. Morbidity from anterior ilium bone harvest. A comparative study of lateral versus medial surgical approach. Oral Surg Oral Med Oral Pathol.1994; 78(3): 296-300.
26. Clokie CML, Coulson R, Peel SAF, Sandor GKB. Approaches to Bone Regeneration in Oral and Maxillofacial Surgery. In: Davies JE. Bone Engineering. Toronto; 2000. 558-576.
27. Sandor GKB. The minimization of morbidity in cranio-maxillofacial osseous reconstruction. Bone graft harvesting and coral derived granules as a bone substitute. 2003. ISBN 951-42-6964-0.
28. Marx RE. Philosophy and particulars of autogenous bone grafting. Oral Maxillofac Surg Clin North Am. 1993; 5: 599-612.
29. Urist MR. Bone: formation by autoinduction. Science.1965; 150: 893-899.
30. Guillemin G, Patat JL, Fournie J, Chetail M. Use of coral as a bone graft substitute. J Biomed Mater Res. 1987; 21(5): 557-567.
31. Stoelinga PJW, Blijdorp PA, Poss R et al. Augmentation of the atrophic mandible with interposed bone graft and particulate hydroxylapetite. J Oral Maxillofac Surg.1986; 44: 353-360.
32. Clokie CML, Sandor GKB. Bone: Present and Future. In: Babush C. Dental Implants: The Art and Science. Philadelphia, WB Saunders Company. 2001: 59-84.

33. Sampath TK, Coughlin JE, Whetstone RM, et al. Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor- beta superfamily. J Biol Chem. 1990; 265(22): 13198-13205.
34. Lindholm TC, Lindholm TS, Alitalo I, Urist MR. Bovine bone morphogenetic protein (bBMP) induced repair of skull trephine defects in sheep. Clin Orthop Relat Res.1998; 227: 265-268.
35. Singh JP, Chaikin MA, Stiles CD. Phylogenetic analysis of platelet derived growth factor by radio receptor assay. J Cell Biol.1982; 95(2 Pt I): 667-671.
36. Howell TH, Fiorellini J, Jones A, et al. A feasibility study evaluating rhBMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation. Int J Periodontics Restorative Dent. 1997; 17: 124-139.
37. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol. 1966; 16(3): 381-390.
38. Shpitzer T, Neligan PC, Gullane PJ, et al. The free iliac crest and fibula flaps in vascularised oromandibular reconstruction: comparison and long term evaluation. Head Neck. 1999; 27(7): 639-647.
39. Shpitzer T, Neligan PC, Gullane PJ, et al. Oromandibular reconstruction with fibula free flap. Analysis of 50 consecutive flaps. Arch Otolaryngol Head Neck Surg. 1997; 123(9): 939-944.
40. Tang CL, Mahoney JL, McKee MD, et al. Donor site morbidity following vascularised fibula grafting. Microsurg. 1998; 18(6): 383-386.
41. Elsalanty ME, Genecov DG. Bone grafts in craniofacial surgery. Craniomaxillofac Trauma Reconstr. 2009; 2: 125-134.
42. Shpitzer T, Neligan P, Boyd BJ, et al. Leg morbidity and function following free fibula graft harvest. Ann Plastic Surg. 1997a; 38: 460-464.

43. Ferretti C, Muthray E, Rikhotso E, et al. Reconstruction of 56 mandibular defects with autologous compressed particulate cortic-cancellous bone grafts. Br J Oral Maxillofac Surg. 2016; 54(3): 322-326.
44. Urist MR, Mikulski A, Lietze A. Solubilized and insolubilized bone morphogenic protein. Proc Natl Acad Sci USA. 1979; 76(4): 1828-1832.
45. Buchardt H. The biology of bone graft repair. Clin Orthop. 1983; 174: 28-42.
46. Buchardt H Biology of bone transplantation. Orthop Clin North Am. 1987; 18: 187-196.
47. Sampath TK, Reddi AH. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci USA. 1981; 78: 7599-7603.
48. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003; 423: 337-342.
49. Boyne PJ, Zarem H. Osseous reconstruction of the resected mandible. Am J Surg. 1976; 132: 49-53.
50. Dumbach J, Rodemer H, Spitzer WJ, et al. Mandibular reconstruction with cancellous bone, hydroxylapatite and titanium mesh. J Craniomaxillofac Surg. 1994; 22(3): 151-155.
51. Tideman H, Samman N, Cheung LK. Functional reconstruction of the mandible: a modified titanium mesh system. Int J Oral Maxillofac Surg.1988; 27: 339-345.
52. Simon EN, Merkx MA, Kalyanyama BM, et al. Immediate reconstruction of the mandible after resection for aggressive odontogenic tumours: a cohort study. Int J Oral Maxillofac Surg. 2013; 51: 319-325.

53. Pogrel MA, Podlesh S, Anthony JP, et al. A comparison of vascularized and nonvascularised bone grafts for reconstruction of mandibular continuity defects. J Oral Maxillofac Surg. 1997; 55: 1200-1206.

54. Foster RD, Anthony JP, Sharma A, et al. Vascularized bone flaps versus nonvascularised bone grafts for mandibular reconstruction: an outcome analysis of primary bony union and endosseous implant success. Head and Neck. 1999; 324: 66-74.

55. Hall MB, Vallerand WP, Thompson D, et al. Comparative anatomic study of anterior and posterior iliac crests as donor sites. J Oral Maxillofac Surg. 1991; 49(6): 560-563.

56. Mirovsky Y, Neuwirth MG. Comparison between the outer table and intracortical methods of obtaining autogenous bone graft from the iliac crest. Spine (Phila Pa 1976). 2000; 25(13): 1722-1725.

57. Kessler P, Thorwarth M, Bloch-Birkholz A, et al. Harvesting of bone from the iliac crest – comparison of the anterior and posterior sites. Br J Oral Maxillofac Surg. 2005; 43(1): 51-56.

58. Engelstad ME, Morse T. Anterior iliac crest, posterior iliac crest, and proximal tibia donor sites: a comparison of cancellous bone volumes in fresh cadavers. J Oral Maxillofac Surg. 2010; 68(12): 3015-3021.

59. Burk T, Del Valle J, Finn RA, et al. Maximum Quantity of Bone Available for Harvest From the Anterior Iliac Crest, Posterior Iliac Crest and Proximal Tibia Using a Standardized Surgical Approach: A Cadaveric Study. J Oral Maxillofac Surg. 2016; 74(12): 2532-2548.

APPENDIX A:

Data recording table used in this study

Study no	Gender	Age	Condition	Defect length (cm)	Wet bone mass (g)	Compressed bone volume (cc)	Compressed bone volume used (cc)