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Bone Regeneration Capacity of FDBB and DBBM Scaffolds Combined with Mesenchymal Stem Cells in Vertical Augmentation of Rabbit Mandibular Bone Defects

Eko Wicaksono Subagio[1], David Buntoro Kamadjaja[2*], Coen Pramono Danoediningrat[2], Muhammad Subhan Amir[2], Andra Rizqiawan[2], Pratiwi Soesilowati[3], Mohammad Zeshaan Rahman[4]

[1]*Master Program of Clinical Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*
[2]*Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[3]*Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[4]*Department Oral and Maxillofacial Surgery, Pioneer Dental College and Hospital, Dhaka, Bangladesh*

Corresponding Author:
David Buntoro Kamadjaja, Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
Email: david-b-k@fkg.unair.ac.id

ABSTRACT

Background and Aim: The mandible plays a crucial role in facial structure and function. Mandibular defects can lead to both aesthetic and functional impairments, thus requiring reconstruction using bone graft materials. Xenografts such as Freeze-Dried Bovine Bone (FDBB) are advantageous due to their availability and minimal donor site morbidity. However, their regenerative capacity may be limited. This study aims to evaluate the effectiveness of tissue-engineered scaffolds combining FDBB or Deproteinized Bovine Bone Mineral (DBBM) with Human Umbilical Cord Mesenchymal Stem Cells (HUC-MSCs) in promoting bone regeneration in rabbit mandibular defects. Methods: An in vivo experimental study was conducted using rabbits with mandibular bone defects. MSCs were seeded onto FDBB and DBBM scaffolds and implanted into the defects. A control group received DBBM without MSCs. Specimens were harvested at the 4th and 8th weeks post-implantation. Woven bone formation was assessed using histomorphometric analysis. Statistical analyses included Shapiro-Wilk test for normality, Levene's test for homogeneity, ANOVA, and Tukey's post-hoc test to determine differences between groups. Results: The DBBM-MSC group demonstrated the highest percentage of woven bone area at both time points, followed by the FDBB-MSC group, with the control group showing the least. A statistically significant difference was observed only between the DBBM-MSC and control groups at week 8. Conclusions: The combination of MSCs with DBBM and FDBB scaffolds enhances bone regeneration. FDBB-MSC scaffolds showed comparable efficacy to DBBM-MSC, suggesting their potential as an alternative xenograft material. These findings support the application of tissue engineering in maxillofacial reconstruction.

KEYWORDS: Augmentation, Bone Regeneration, Scaffold, Stem Cells, Woven Bone, Xenograft

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INTRODUCTION

The mandible, morphologically, forms the lower third of the face (1). Defects occurring in the mandible can adversely affect patients in terms of function, aesthetics, or both, leading to a decrease in an individual's quality of life. These defects result in a loss of bone volume and can be caused by various factors, including bone atrophy, congenital factors, tumor excision, trauma, infections involving periodontal and bone tissues, iatrogenic procedures such as tooth extraction, or radiation therapy [2–4].

Ideally, bone defects in the mandible should undergo reconstructive treatment to restore both function and aesthetics [5]. Implant placement is currently a popular practice in modern dental medicine. Adequate alveolar bone dimensions are crucial for the success of dental implant placement. Limitations in bone dimensions can lead to implant failure, necessitating ridge augmentation as a preparation for implant placement [6].

The reconstruction and augmentation of bone defects require bone substitute materials or bone grafts [7]. Autografts, derived from the individual themselves, are still considered the best substitute material for filling bone defects. Autografts possess the ideal properties required for a bone graft, namely osteogenic, osteoinductive, and osteoconductive qualities [8]. However, autografts have limitations, including a limited supply, difficulty in obtaining graft material, increased risk of infection, elevated risk of blood loss, and additional postoperative morbidity [9].

This has led to the development of xenografts combined with tissue engineering techniques as graft materials due to their ease of availability. Deproteinized Bovine Bone Material (DBBM) is a xenograft material derived from animals (cattle) where organic

components have been removed, leaving behind inorganic components with mineral content similar to human bone [10]. DBBM exhibits osteoconductive properties, shows good biocompatibility, and acting as a scaffold. It is also stated that DBBM particles slow degradation rate up to 3-4 years and may not be completely degradable. Therefore, the particles of the vaccine integrated into the new bone, which do not completely replace the bones and do not regenerate the bones, are removed [11].

Freeze-dried bovine bone (FDBB) xenograft is a bone graft derived from bovine (cattle) bone processed by freezing and drying. This process aims to obtain organic and inorganic materials from the bone, giving it both osteoconductive and osteoinductive properties as it retains growth factors for bone growth. The presence of organic material and growth factors is expected to play a role in the osteogenic process during bone regeneration. FDBB is also mentioned to have inter-cellular organic material content similar to human bone [12,13]. Additionally, FDBB is easier to resorb compared to DBBM, allowing newly formed bone to replace the entire FDBB scaffold once the resorption process is complete (creeping substitution) [14].

Tissue engineering procedures, combining scaffolds, cells, and growth factors, are currently used to address the limitations of autografts and allografts. Block-shaped scaffolds are often used, serving as a three-dimensional (3D) framework (osteoconductive) for cell attachment, proliferation, differentiation (osteoinductive), and the formation of new bone (osteogenic) [15].

Tissue engineering procedures also utilize a cell-based approach by combining scaffolds with adult stem cells. Stem cells offer unlimited potential as a cell source. Mesenchymal stem cells (MSCs) can differentiate into all connective tissue derivatives, including cartilage and bone [16]. One commonly used source of MSCs is human umbilical cord, as these stem cells are obtained from discarded biological tissue, pose no ethical issues, and can be easily isolated and stored [17].

The research questions of this study are: Is the combination of FDBB scaffold with human umbilical cord mesenchymal stem cells (HUC-MSC) more effective in stimulating new bone formation than the combination of DBBM scaffold with HUC-MSC in mandibular bone defect model?

The aim of this study was to evaluate and compare the new bone formation ability of FDBB-MSC and DBBM-MSC block scaffolds in a vertical augmentation procedure of mandibular bone defects in vivo.

The scope of this article includes in vivo tests using animal models (rabbits), scaffold characterization, histological observations of the bone regeneration process, and analysis of the effectiveness of both material and stem cell combinations. The results of this study are expected to make a scientific contribution in the development of biomaterials and tissue engineering techniques for clinical applications in the field of maxillofacial bone reconstruction surgery.

METHOD

This study used a Post-Test Only Control Group Design with a split-mouth approach to evaluate the area of woven bone formed in rabbit mandibular defects after scaffold implantation of Freeze-Dried Bovine Bone (FDBB) combined with Human Umbilical Cord Mesenchymal Stem Cell (HUC-MSC), Deproteinized Bovine Bone Material (DBBM) combined with HUC-MSC, and DBBM without HUC-MSC as the control group. Evaluation was performed at week 4 and 8 post-implantation using histomorphometric techniques. The research subjects were male New Zealand White rabbits, approximately 6 months old with body weight between 2500-3500 grams. Rabbits were obtained from the Institute of Tropical Disease (ITD), Airlangga University. A total of 24 mandibular bone defects were made on the right and left sides of the rabbit mandible, which allowed for six treatment combinations with a total of four replicates for each group (n=4).

The independent variable in this study was the type of scaffold used, namely FDBB + HUC-MSC and DBBM + HUC-MSC. The dependent variable is the percentage of woven bone area formed. Control variables included the type and size of the graft material used, the viability of HUC-MSC, and the size and shape of the mandibular defect in rabbits which were made uniform to maintain consistency between samples.

Surgical procedures and animal care up to the euthanasia stage were carried out at the Institute of Tropical Disease (ITD), Airlangga University, Surabaya. The decalcification process and histology slide preparation were carried out at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga. Meanwhile, observation and analysis of the area of woven bone was carried out at the Research Center, Faculty of Dentistry, Universitas Airlangga.

The data obtained was quantitative data, namely the percentage of woven bone area. Data normality test was conducted with Shapiro-Wilk test. If the test results show $p > 0.05$, then the data is considered normally distributed. Furthermore, the homogeneity test was carried out with the Levene test. If the data is homogeneous ($p > 0.05$), then the analysis is continued with a comparison test between groups using one-way ANOVA (One-Way ANOVA), followed by a post hoc follow-up test using Tukey's Multiple Comparison to see significant differences between treatment groups.

RESULT

In this study, experiments were conducted on 12 New Zealand White rabbits, divided into 3 groups: 1 control group with DBBM application and 2 treatment groups, namely DBBM-MSC and FDBB-MSC. Each group was further subdivided into 2 observation periods, namely at the 4th week and 8th week. Each rabbit mandible was utilized for 2 samples (split-mouth design). Sample collection involved the removal of the rabbit mandible, freeing it from skin and muscle tissues covering it. Subsequently, the right and left mandibular samples were separated and placed in labeled specimen containers. The sample results can be seen in the figure below (Figure 1).

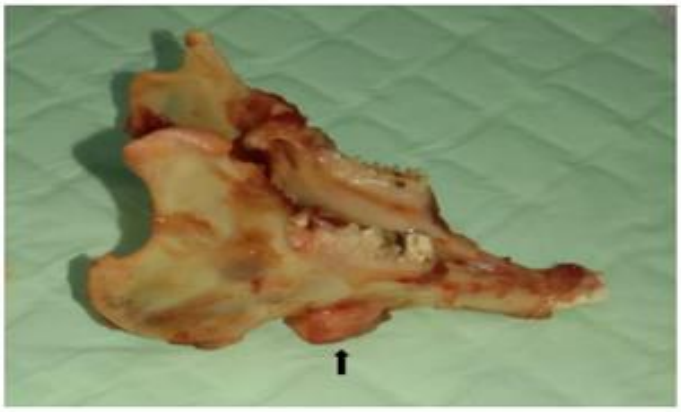

Figure 1: Post-harvesting rabbit mandible sample Scaffold (➡), showing a 5x5x3mm scaffold on the inferior border of the mandible.

Samples that have been taken are then carried out the process of decalcification, making paraffin blocks, cutting, and staining with Hematoxylin-Eosin (HE) Then the preparations are observed and analyzed the results.

New Bone Formation Observation Results

Hematoxylin and Eosin (HE) staining was performed on mandibular bone preparations of experimental animals to determine the appearance of bone matrix formed after augmentation with scaffold blocks at week 4 and week 8. A historical study of the formation of the new bone was identified, in a part stained with the HU, at the edge of the bone earth that has just appeared at the edge of the highly experienced mandibular animal.

HE staining provides a general overview of histological sections. Bone appears as a compact, dark red structure, while connective tissue appears as a cell structured by collagen fibers and tissues. The dye will be able to find different types of cells by identifying morphological structures. Osteocytes, osteoblasts, and chondrocytes are distinguished in the components of hard tissues. Histomorphometry methods analyze the area of tissue filled with immature and mature bone on the prepared slide.

Examinations were conducted using a microscope at a magnification of 40x, and the area of new bone was marked using Element-D software. Observations at the 4th week for all groups showed incomplete new bone formation. New bone formation was indicated to have started in the bone defect, while collagen fiber tissue was depicted as still filling some areas.

Percentage of Woven Bone Area in the 4th Week

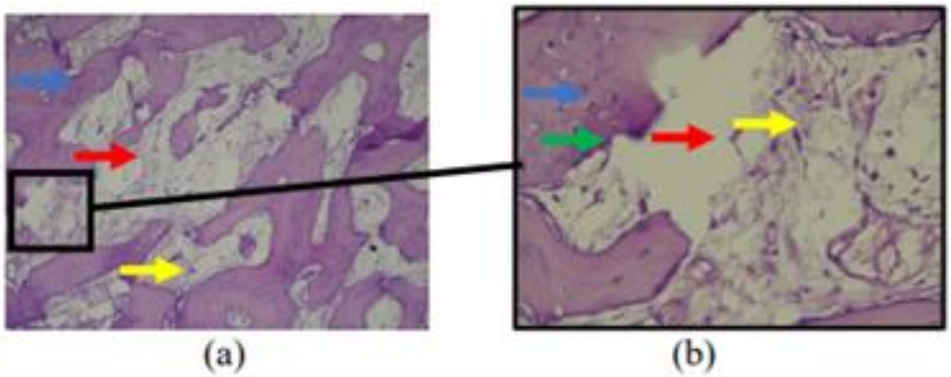

Figure 2: Post implantation microscopic image of FDBB-MSC scaffold at week 4 (a) 100x magnification, (b) 400x magnification Description: Woven bone (➡), Extracellular matrix (➡), Residual scaffold (➡), Osteoblastic lining (➡).

In the 4th-week observation (Figure 2), an extensive extracellular matrix tissue was evident, dominating over the woven bone appearance in each group. The depiction of osteoblastic lining at the edge of woven bone was still not prominently visible, and the extracellular matrix was dominated by resident cells, scaffold remnants, and connective tissue. Many areas of the defect still lack bone filling. The least amount of scaffold remnants was observed in the FDBB-MSC group compared to the DBBM and DBBM-MSC groups. Microscopic image of the woven bone trabecular area after implantation from each group are shown in figure 3 below.

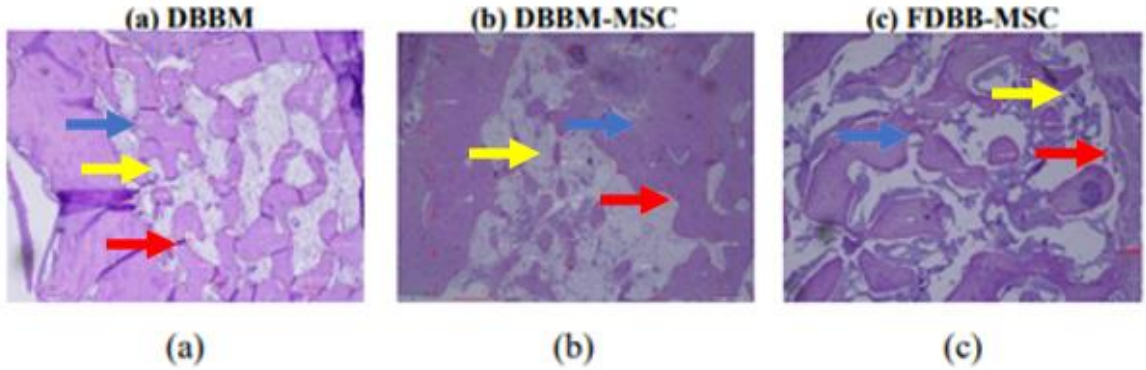

Figure 3: Microscopic depiction of HE staining of the woven bone trabecular area post-implantation of the scaffold in the 4th week (magnification 40x). (a) DBBM group, (b) DBBM-MSC group, (c) FDBB-MSC group.
Description: Woven bone ( ), Extracellular matrix ( ), Residual scaffold ( )

Percentage of Woven Bone Area in the 8th Week

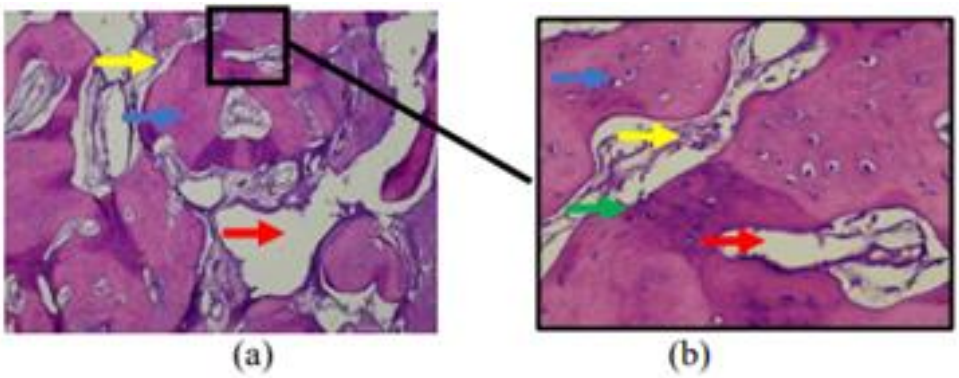

Figure 4: Microscopic depiction of HE staining after implantation of FDBB-MSC scaffold in the 8th week (a) at a magnification of 100x, (b) at a magnification of 400x.
Description: Woven bone ( ), Extracellular matrix ( ), Scaffold remnants ( ), Osteoblastic lining ( )

Observations in the eighth week for all groups indicate more substantial new bone formation compared to the fourth week. In general, a wider area of woven bone is observed with osteoblastic lining and a larger area of connective tissue/fibroblast compared to the fourth-week observation. Some scaffold remnants are still visible in certain preparations (Figure 4). In the DBBM-MSC and FDBB-MSC groups, the woven bone and extracellular matrix components appear to be more extensive than in the DBBM group. Microscopic image of the woven bone trabecular area after implantation from each group are shown in figure 5 below.

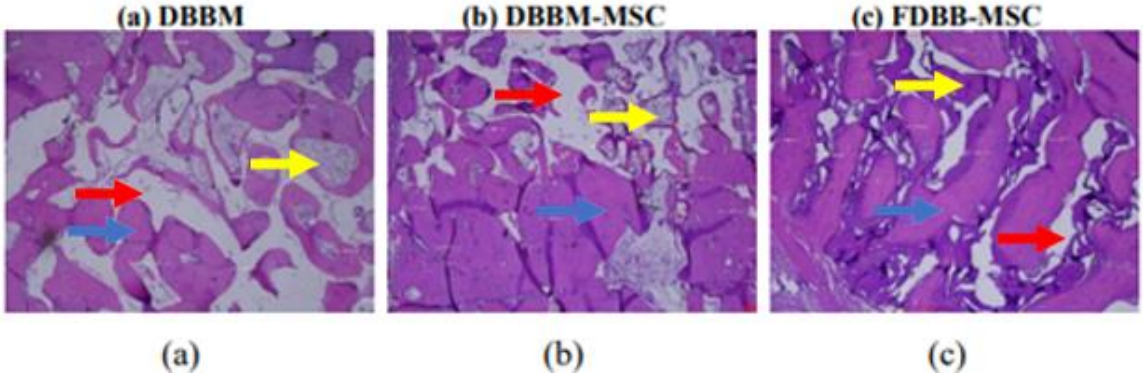

Figure 5: Microscopic depiction of the woven bone trabecular area post-implantation of the scaffold in the 8th week (magnification 40x). (a) DBBM group, (b) DBBM-MSC group, (c) FDBB-MSC group.
Description: Woven bone ( ), Extracellular matrix ( ), Scaffold remnants ( )

Further, the average woven bone area at week 4th and week 8th observations can be seen in Figure 6 below:

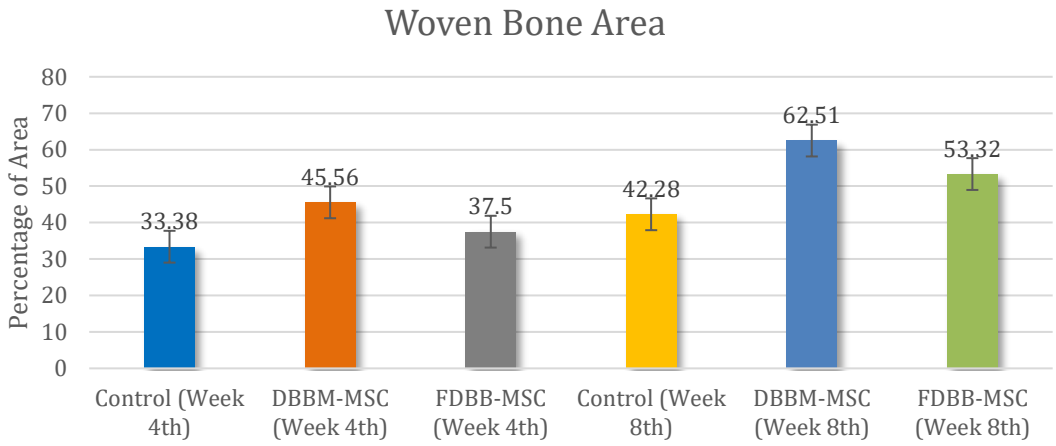

Figure 6: Bar Chart of Woven Bone Area Percentage in 4th and 8th Weeks.
Notes: Parentheses and * indicate significant differences between groups.

Description of Woven Bone Area

In Group 1 (DBBM-MSC), it is observed that there is a rise in the extent of woven bone from the 4th week to the 8th week. The mean value of woven bone area in the 4th week is 45.5561, with a standard deviation of 18.807. In the 8th week, the average value increases to 62.5055, accompanied by a standard deviation of 8.307.

Similarly, in Group 2 (FDBB-MSC), there is an increase in the area of woven bone from the 4th week to the 8th week. The average value in the 4th week is 37.5048, with a standard deviation of 13.317. By the 8th week, the average value rises to 55.3186, with a standard deviation of 7.632. Likewise, in the control group, the woven bone area also experiences an increase, although not as substantial as in Groups 1 and 2. In the 4th week, the average is 33.3751, with a standard deviation of 2.186. In the 8th week, the average value reaches 42.2837, accompanied by a standard deviation of 4.194.

All groups demonstrate an augmentation in the percentage area of woven bone as the treatment duration progresses, both in the 4th and 8th weeks. The DBBM-MSC group exhibits the highest average area of newly formed woven bone, followed by the FDBB-MSC group, while the control group displays the lowest average area of woven bone.

Statistical Test Results

Results of Data Normality Test

The normality testing results conducted using the Shapiro-Wilk test indicate that the variables, namely the area of Woven Bone (4 weeks and 8 weeks), have significance values greater than α (0.05), suggesting that the data is normally distributed. Therefore, inferential analysis can proceed with parametric statistics, specifically the ANOVA test.

Homogeneity Test Results

The homogeneity test with Levene's test of Equality of Error Variances for each observation week (4 weeks and 8 weeks) yields significance values greater than 0.05 ($p > 0.05$), indicating that the data variance among treatment groups is equal (homogeneous). Therefore, further testing using the one-way ANOVA can be conducted.

Analysis of the Difference in Woven Bone Area

Analysis of the Difference in Woven Bone Area at Week 4

Based on the analysis, the highest average area of Woven Bone in the 4th-week observation is in the DBBM-MSC treatment group at 45.56±18.81%, while the lowest average area of Woven Bone is in the control group at 33.38±2.19%. To verify whether there is a statistically significant difference in the average Woven Bone area in the 4th-week observation, a further one-way ANOVA statistical analysis will be conducted.

Based on the results of the one-way ANOVA test in the 4th-week observation, the F value is smaller than the F table ($0.859 < 4.256$), and the p-value is larger than α ($0.455 > 0.050$). Therefore, it can be concluded that there is no significant difference in the average Woven Bone area (4 weeks) among the treatments. It can be observed that the average values of the Woven Bone area (4 weeks) are relatively similar among the treatments, indicating that the values among the treatments are not significantly different.

Analysis of the Difference in Woven Bone Area at Week 8

Based on the analysis, the highest average area of Woven Bone in the 8th-week observation is in the DBBM-MSC treatment group

at 62.51±8.31%, while the lowest average area of Woven Bone in the 8th-week observation is in the control group at 42.28±4.19%. To verify whether there is a statistically significant difference in the average Woven Bone area in the 8th-week observation, a further one-way ANOVA statistical analysis will be conducted.

Based on the results of the one-way ANOVA test, the F value is greater than the F table (8.494 > 4.256), and the p-value is smaller than α (0.008 < 0.050). Therefore, it can be concluded that there is a significant difference in the average Woven Bone area (8 weeks) among the treatments. To identify the location of the difference, a post hoc Tukey test is conducted.

Based on the analysis results, it is known that the highest average area of Woven Bone in the 8th-week observation in the DBBM-MSC treatment is significantly different from the control treatment, but the DBBM-MSC treatment is not significantly different from the FDBB-MSC treatment. The lowest average area of Woven Bone in the 8th-week observation in the control treatment is significantly different from the DBBM-MSC treatment, but the control treatment is not significantly different from the FDBB-MSC treatment.

DISCUSSION

The placement of dental implants often requires bone grafting procedures for bones that have defects, whether vertically or horizontally. Moreover, an ideal bone condition supports the success of dental implant placement. Ideally, grafts used should be sourced from the individual (autograft) because they possess osteogenic, osteoinductive, and osteoconductive properties. However, to minimize morbidity, grafts from other sources are often utilized. Tissue engineering techniques have been extensively developed to overcome the limitations of autografts while maintaining equivalent regenerative capabilities. The development of tissue engineering, involving a combination of scaffold, cells, and growth factors, is expected to yield an ideal scaffold [18]. Three-dimensional scaffolds are necessary for bone augmentation, providing a surface or space for cell attachment, migration, proliferation, and differentiation. Porosity or cavities serve as sites for new vascularization, transport systems, the formation of new bone, and ultimately remodeling [19].

Theoretically, FDBB scaffold is considered superior to DBBM scaffold as it still retains organic content, namely collagen and non-collagen fibers, and growth factors that are proliferative, angiogenic, and osteoinductive. Additionally, FDBB contains several osteogenic growth factors, such as transforming growth factor β (TGF-β) and BMP-2. Furthermore, FDBB undergoes complete degradation to support bone regeneration. The purpose of this study is to evaluate FDBB block scaffold osteogenesis effectiveness for bone augmentation and to evaluate MSC addition effectiveness for bone formation.

This research employed a post-test only control group design with the implantation of DBBM and FDBB scaffolds previously enriched with HUC-MSC in the mandibular defect of New Zealand white rabbits. The use of HUC-MSC is based on its multipotent nature, making it suitable for inducing osteoblastic direction, resistant to senescence, and exhibiting high osteoblast differentiation [20]. Moreover, HUC-MSC is abundant, non-destructive, and has low immunogenicity [21]. HUC-MSC has been utilized in conjunction with 3D scaffolds to accelerate bone repair and regeneration [22].

In this study, the observed aspect is the comparison of new bone formation by calculating the area of woven bone using histomorphometry examination at the 4th and 8th weeks after treatment. Histological examinations conducted at the 4th and 8th weeks respectively revealed an increase in the area of Woven Bone in each group. The extent of woven bone observed in this study is one of the indicators of bone regeneration, suggesting that a larger area of woven bone formation indicates a better bone regeneration capacity. In the control group, both at the 4th and 8th weeks, the percentage area of woven bone formation was the lowest compared to the treatment groups with scaffolds enriched with MSC.

This study proves that the administration of HUC-MSC on FDBB and DBBM scaffolds can have a direct effect on the osteogenesis process, as indicated by the significant differences between scaffolds enriched with HUC-MSC and those without. This aligns with Bahar [23] on the osteogenesis process between FDBB and DBBM scaffolds enriched with bone marrow, implanted subcutaneously in experimental animals. The results at the 4th week post-implantation showed osteoblastic lining on the FDBB scaffold.

The mechanism of MSC in bone reconstruction and repair has numerous benefits, including immunomodulatory effects, angiogenesis stimulation, anti-apoptosis in osteoblastic lineage cell changes, endogenous MSC recruitment, and stimulation of differentiation into osteoblasts. MSC also help stimulate the differentiation of preosteoclasts into osteoblasts by induces osteo-chondro progenitors, which further develop into osteo-precursors under Runx2 [24]. MSC has the potential to differentiate into various cell types, such as osteoblasts. MSC reduces TNF-α, and TNF-α itself can inhibit osteoblast differentiation by inhibiting the expression of key transcription factors in bone formation, namely RUNX2 and Osterix. MSC has a direct effect on osteoblast differentiation and bone regeneration. The therapeutic effect of MSC aims to provide stability in the microenvironment, in addition to their ability to differentiate and incorporate into host tissue. Recruitment of endogenous MSCs will further enrich and strengthen the effects of HUC-MSCs that have been seeded on the previous scaffold by releasing cytokines and growth factors to increase cell repair function. Specifically, their role in scaffold implantation, recruited endogenous MSCs have the capacity to modulate immune responses, crosstalk with endothelial cells for vascularization, and even directly differentiate into the required cellular lineages. All the capabilities of MSC mentioned above are consistent with the findings of this study, where scaffolds enriched with MSC show a greater area of formed woven bone.

In this study, the DBBM-MSC treatment group showed the highest percentage area of formed woven bone compared to the FDBB-MSC and control groups at both the 4th and 8th weeks. Statistically, there was no significant difference among the groups at the 4th

week. However, at the 8th week, there was a significant difference between the DBBM-MSC group and the control group, but the DBBM-MSC treatment did not significantly differ from the FDBB-MSC treatment. This indicates that FDBB is also a promising scaffold candidate for MSC differentiation, compared to DBBM, which has been the "gold standard" until now. This research's findings are supported by in vitro studies conducted by Nugraha [25] on the osteoinductive potential of FDBB and DBBM scaffolds. The study mentioned that, although not significantly different, FDBB scaffolds have better potential than DBBM scaffolds, as indicated by higher expression of RUNX2 and Osterix compared to other groups. Osteoblast differentiation requires the expression of RUNX2 and Osterix to bind progenitors into preosteoclasts. Another advantage of FDBB is its inclusion of growth factors, such as BMP2, within it. The BMP present in the FDBB scaffold is part of the TGF-β superfamily or multifunctional cytokines. The SMAD pathway activated by BMP, especially BMP-2, will induce the transcription of RUNX2 and Osterix.

The attachment of MSC in a scaffold also plays a crucial role in the differentiation process into osteoblasts. The scaffold acts as a matrix and can facilitate attachment, migration, and differentiation of progenitor cells [26]. In a previous in vitro study by Nicco [27], that compared to DBBM, FDBB has better cell attachment and viability. This is possible because FDBB has an ECM involved in cell adhesion, providing biochemical and biomechanical signals for cells to migrate, differentiate, and undergo angiogenesis [28]. This study demonstrates a nearly equivalent ability for new bone growth between FDBB and DBBM, but there are some possibilities that make DBBM slightly superior to FDBB. In theory, FDBB should be superior because it still contains organic content such as collagen and non-collagen fibers, as well as growth factors that are proliferative, angiogenic, and osteoinductive. However, besides that, scaffold materials should mimic or resemble the characteristics of natural bone, facilitating biomechanical support for adhesion, migration, osteogenic differentiation, and angiogenesis [29]. The significant drawback of FDBB scaffolds is attributed to freeze-drying, which weakens structural stability and subsequently shortens degradation time, which can lead to bone reopening before adequate bone regeneration is obtained.

In terms of its structure, DBBM is a system of wide and interconnected pores that allows it to function as a physical scaffold for osteogenic cells, facilitating the migration and unity of subsequent cells. Research conducted (unpublished) also mentions that the microarchitecture morphology of DBBM scaffold is more ideal compared to FDBB scaffold. The strengths and weaknesses of the mentioned scaffolds will significantly influence the bone growth process. The hydroxyapatite crystal structure in DBBM is reported to have free calcium and phosphate ions that can initiate notch signaling, subsequently leading to increased regulation of RUNX2 and OSX expression [30]. Notch signaling mediates communication between contacting cells. In bone, the notch protein is expressed in osteoblasts, osteocytes, and osteoclasts. In-vivo studies show that notch signaling activity in osteoblast differentiation depends on the maturation stage of the cells. Notch signaling expression results in an increase in the number and proliferation of osteoblasts; on the other hand, notch signaling also promotes the final stage differentiation of osteoclasts.

Another signaling mechanism involves TGF-β and BMP, which operate on the tetramer receptor complex, altering signals in the canonical Smad-dependent pathway (TGF-β/BMP ligands, receptors, and Smads) and the non-canonical Smad-independent pathway (p38 mitogen-activated protein kinase/p38 MAPK) to regulate MSC differentiation during new bone formation and bone homeostasis. Both the Smad signaling pathway and the p38 MAPK pathway work on transcription factors. Runx2 promotes the differentiation of osteoblasts and chondrocytes from mesenchymal precursor cells. The TGF-β and BMP signaling pathways are influenced by various factors, including the ubiquitin-proteasome system, epigenetic factors, and microRNA. Disruption in the regulation of the TGF-β and BMP signaling pathways has been proven to cause bone formation disorders in humans [31].

Another factor contributing to the slower new bone growth observed in FDBB compared to DBBM is the reduced osteoinductive capacity of BMP present in FDBB due to down-regulation effects caused by the interaction of MSC with growth factors. This process leads to an increase in noggin and gremlin, which are antagonists of BMP-2. Additionally, the SMAD pathway in MSC does not respond to BMP-2 induction, preventing MSC from triggering osteogenesis [32].

DBBM has the drawback of being difficult for the body to absorb and has the potential to slow down the process of new bone formation. Although FDBB has a lower percentage of Woven Bone compared to DBBM, FDBB exhibits greater osteoconductive and osteoinductive potential. This is related to the limitations of the study due to the restricted observation periods at weeks 4 and 8, where observations only cover the early stages of the remodeling phase. The bone healing process for subsequent stages requires a longer observation period. Hence, further research with an observation period exceeding 8 weeks is needed to better capture the potential of the FDBB scaffold.

The results of this study show the advantages and changes of DBBM and FDBB particles in the formation of new bone. DBBM functions as an osteoconductive scaffold to cope with recovery, with a stable character that facilitates the formation of the new bone. On the other hand, the FDBB scaffold undergoes faster graft resorption and simultaneous bone regeneration [33]. The speed of resorption can also be influenced by the sterilization method used. Research by Haimi [34] states that sterilization with gamma radiation reduces the mechanical ability of the scaffold. The moisture content of the scaffold made up to 0% is proven to have lower mechanical abilities compared to 5% moisture content. The duration of washing in scaffold production. Washing time with hydrogen peroxide is also associated with a weakened osteoinductive ability of the scaffold [35]. The current use of FDBB is washed with hydrogen peroxide for 3 hours, hence its osteoinductive ability is also estimated to decrease. It is mentioned that washing with hydrogen peroxide with the best osteoinductive properties is obtained at a washing duration of 1 hour. Hydrogen peroxide washing has been proven to reduce the osteoinductive properties of the scaffold by affecting the protein structure, especially BMP [36].

The results of this study show that FDBB scaffold material is not significantly different from DBBM. From histomorphometry

evaluation, there was an equivalent increase in new bone growth between DBBM-MSC and FDBB-MSC from the 4th week to the 8th week. So, it can be concluded that the FDBB scaffold also has the potential to become an alternative bone graft material for bone augmentation. However, further research after 8th week needs to be conducted to evaluate new bone formation between this material.
This study showed almost equal new bone growth ability between FDBB and DBBM, but there are several possibilities that make DBBM slightly superior to FDBB. In theory, FDBB should be superior because it still contains organic content such as collagen and non-collagenous fibers, as well as growth factors that are proliferative, angiogenic, and osteoinductive. However, in addition, the scaffold material should mimic or resemble the characteristics of natural bone, facilitating biomechanical support for attachment, migration, osteogenic differentiation and angiogenesis. A significant drawback of FDBB scaffolds is the freeze-drying process, which can weaken structural stability and shorten degradation time, which could lead to bone opening before adequate bone regeneration is achieved.
In terms of structure, DBBM is an extensive, interconnected pore system that allows it to serve as a physical scaffold for osteogenic cells, facilitating subsequent cell migration and fusion. Research conducted (unpublished) also mentioned that the microarchitectural morphology of DBBM scaffolds is more idealized compared to FDBB scaffolds. The strengths and weaknesses of the scaffolds mentioned will greatly affect the bone growth process. The crystal structure of hydroxyapatite in DBBM is reported to have free calcium and phosphate ions that can initiate notch signaling, which further upregulates the expression of RUNX2 and OSX.
Implications from a clinical perspective, these findings support the potential of FDBB as an effective and affordable alternative to DBBM, particularly in resource-limited settings. The use of HUC-MSC further enhances this potential by improving osteogenic outcomes. Further research should focus on long-term studies, mechanical stability assessment, and clinical trials to assess the reliability of FDBB in human applications.

CONCLUSION

This study aimed to evaluate the bone regeneration capacity of DBBM-MSC compared to FDBB-MSC and the control group, focusing on the percentage of new bone area formed. The results showed that DBBM-MSC had the highest percentage of Woven Bone area at week 4, indicating its potential in accelerating bone regeneration. However, this increase did not show a significant difference compared to the FDBB-MSC and control groups. On day 8, DBBM-MSC showed a statistically significant increase in bone tissue area compared to the control group, although there was no significant difference compared to FDBB-MSC. These findings support the hypothesis that MSC administration can enhance bone formation at an early stage. For future studies, it is suggested that additional treatment groups with FDBB or DBBM alone be added to allow for a more in-depth comparison. Further, the use of larger experimental animals and a longer duration of observation is recommended so that the process of bone healing and scaffold resorption can be monitored thoroughly. Modification of the FDBB scaffold by the H_2O_2 washing method for 1 hour is also recommended to improve its osteoinductive properties and mechanical strength, and slow down the resorption rate of the scaffold.

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