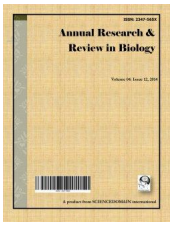

Annual Research & Review in Biology

19(3): 1-9, 2017; Article no.ARRB.37318
ISSN: 2347-565X, NLM ID: 101632869

Comparison of Regeneration between Human Bone Marrow and Adipose Mesenchymal Stem Cells on Treatment of Critical Size Bone Defect in Sprague Dawley Rats

Ismail Hadisoebroto Dilogo[1,2,3*], Bagus Pramantha[1], Evelina Kodrat[4] and Ria Anggraini[2]

[1]Department of Orthopaedic and Traumatology, Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[2]Integrated Service Unit Stem Cells Medical Technology, Cipto Mangunkusumo General Hospital, Indonesia.
[3]Stem Cells and Tissue Engineering Research Center, IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[4]Department of Anatomical Pathology, Cipto Mangunkusumo General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

Authors' contributions

This work was carried out in collaboration between all authors. Author IHD designed the study, wrote the protocol, prepared the stem cells and supervised the writing of the manuscript, the one who was given grant for this research project. Author BP managed the experimental process, performed analysis of histomorphometry, immunohistochemistry and wrote the manuscript. Author EK supervised the histomorphometry and immunohistochemistry analysis. Author RA managed the stem cells processing used in this study. All authors have read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/37318
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Hameed A. Al-Timmemi, Universiti Putra Malaysia, Malaysia.
(2) Lyubov F. Koroleva, The Russian Academy of Sciences, Russia.
Complete Peer review History: http://www.sciencedomain.org/review-history/21775

Original Research Article

Received 10th October 2017
Accepted 1st November 2017
Published 7th November 2017

ABSTRACT

Aims: This study was to investigate the difference of osteogenic capacity between BMSC and ASC by quantifying the expression of Bone Morphogenetic Protein (BMP)-2 and BMP receptor (BMPR) II also the bone healing process by histomorphometry measurement.

**Corresponding author: E-mail: ismailortho@gmail.com;*
E-mail: bagusortho@gmail.com;

Study Design: This study was experimental study on animal (Sprague Dawley Rat).
Place and Duration of Study: This study took place in Animal Laboratory of Indonesian Health Minister, Faculty of Medicine Universitas Indonesia, Department of Orthopaedic and Traumatology, Stem Cell Unit, and Department of Anatomical Pathology Cipto Mangunkusumo Hospital, and also Primate Study Center of Bogor Institute of Agriculture.
Methodology: Eighteen Sprague dawley (SD) healthy rats were induced with 5 mm femoral bone defect, then divided into three equal groups (n=6) consist of Control, Implementation of $2x10^6$ BMSC + 5 cc Hydroxypatite (HA), and Implementation of $2x10^6$ ASC + 5cc HA. They were sacrificed after 2 weeks, then underwent histomorphometry assessment with Image-J. The measured paramater were total area of callus, % of osseous area, % of cartilage area, and % of fibrous area. The immunohistochemistry (IHC) measurement was assessed by staining intensity and immunoreactivity score (IRS).
Results: The BMSC group showed higher expression of BMPR II compare to others. The expression of BMPR II was analyzed statistically and showed significant result (*P*= .04) among groups with median 4.00 ± 2.75. Both BMSC and ASC group have significantly better bone healing process compared with control group (*P*= .001). There are no significant differences between ASC and BMSC measured in % total callus area (*P*= 1.00), %Osseous area (*P*= 1.00), %Cartilage area (*P*= .49) and %Fibrous area (*P*= .18).
Conclusion: ASC bone healing ability are similar to BMSC. BMP-2 and BMPR II are important but not sole contributing factor for bone healing.

Keywords: Bone marrow mesenchymal stem cells; adipose mesenchymal stem cells; BMP-2; BMPR II; Sprague Dawley rats.

1. INTRODUCTION

Stem cells are known for their capability to regenerate and differentiate to become specific mature cells. Due to their multipotent nature, rapid proliferation, and high renewing ability, mesenchymal stem cells (MSC) are very promising in the field of tissue engineering [1,2,3]. Currently, MSCs from the bone marrow (BMSC) are proven to have the biggest osteogenic regeneration potential, but the source and quantity are limited, meanwhile, the MSCs from adipose tissue (ASC) are easier to get and easier to be isolated and their cell proliferation is faster and more abundant than the ones from bone marrow, but their osteogenic potential is questionable [4,5].

Some studies show that BMSC has superior ostegenic potential compare to ASC, especially in 2D in vitro studies. In their study, Shafiee et al. [6] showed that ASC had lower Alkaline Phosphatase (ALP) activity and mineralization than BMSC during osteogenic differentiation at days 7 and 14. Jaiswal et al. [7] in his research suggests that the MSC describes the pattern and number of different ALP activities during differentiation into osteoblasts. ALP is a biochemical marker for osteoblastic activity. Compared with umbilical cord MSC (UCSC) and ASC, superior osteogenic capacity of BMSC can be predicted from higher ALP activity at all the time points.

Interestingly, several in vivo studies gain quite contrast result with ASC has better or at least similiar osteogenic properties than BMSC. In a study conducted in canine model Kang et al. [8] found the superior osteogenesis ability of canine ASC compare to BMSC based on their findings of higher ALP activity and mineralization in osteoinduced ASC, they conclude canine ASC can hypothetically replace BMSC. Another canine study performed by Chung et al. [9] showed comparable osteogenic capability between ASC and BMSC with the similar alizarin red stain and pattern of expression of specific osteoblast gene (Osterix, RunxII, and OCN). These contradictive result among in vitro and in vivo studies lead us to questions "Is the osteogenic capacity of ASC equal or much better than BMSC?

Moreover, Suzawa et al. [6] in his study states that BMP-2 expression is a growth factor secreted by mature osteoblast cells. The higher BMP-2 relative expression on days 7 and 14 in BMSC compared to ASC and UCSC is consistent with their superior osteogenic capacity. Interestingly, BMP-2 mRNAs are expressed in large quantities in non-differentiated ASC compared with BMSC and UCSC. Kloen et al proves in their study that bone morphogenic protein-2 (BMP-2) and bone morphogenic protein-2 receptor (BMPR-II) are expressed and localized in human's callus [4]. This proves that BMP-2 hold important roles in the process of

bone healing and lead us to another question "Is BMPR II expression as a receptor for BMP-2 could affect the effectivity of BMP-2 levels to phosphorylate regulatory Smad to form complex which lead to gene regulation and bone production?

Therefore, we expect by detecting BMP and its receptors that are abundant during bone healing process can give clearer picture of osteogenic potential in bone defect treated with either BMSC or ASC [10,11,12].

2. MATERIALS AND METHODS

All procedures conducted in this study have been approved by The Ethical Committee of Faculty of Medicine Universitas Indonesia No. 461/UN2.F1/ETIK/2017. This study used post test control group study design. The samples of the study were collected consecutively from 18 white Sprague Dawley male rats which had had skeletal maturation (8-12 weeks) weighing 250-350 grams. The inclusion criteria are 3-4 month-old rats weighing 250-350 grams, male and without clinical physical disabilities. The exclusion criteria are female rats, rats with lower extremities dysfunctions, implant failure, infection on the operating field and rats which died before harvesting. MSCs from bone marrow and adipose tissue had gone through process of Good Laboratory Product (GLP), and were collected from human's MSCs with informed consent collected previously. Centrifugation process and culture were done. It is estimated that the amount of MSCs exceeded 2 x 10^6 cells per power field.

The tested animals were allocated randomly into three groups, which are group 1 (Control), group 2 (BMSC intervention), and group 3 (ASC intervention). To every group of rats, 5 mm critical bone defect was created. Every group would be internally fixated with threaded intramedullary Kirschner wire 1.4 mm. Group 1 did not undergo intervention while group 2 underwent implantation of 2x10^6 human BMSC with 5cc of HA as a scaffold and Group 3 was implanted with 2x10^6 human ASC and 5cc HA. Animals are feed and given antibiotics to prevent infection at Ministry of health animal laboratory. After 2 weeks, femurs were disarticulated and histopathologic examinations (immunohistochemistry and histomophometry) were performed to assess the expression of BMP2, BMPR II and fracture healing that are described in total % of callus area, % cartilage area, % osseous area, and % fibrous area.

2.1 Histopathologic Examination by Histomorphometry

After euthanasia, the right femur was resected immediately. By maintaining a K-wire inside, harvested femur was fixed in 10% neutral buffered formalin for 48 hours. They were decalcified with Plank Rychlo's solution (Wako Pure Chemical Industries Ltd., Osaka, Japan) [13,14] Samples were embedded in paraffin and cut transversely with a microtome 5 μm section, and stained with Safranin O/Fast green. Then, they were examined with a Leica Microsystems microscope.

The histological images were taken by digital microscope camera and photo-stitched using PTGUI® software. The width and diameter of the callus, total callus area, osseous, cartilage, and fibrous tissue area (Fig. 1) were evaluated using Image J® software (Fig. 2) [15].

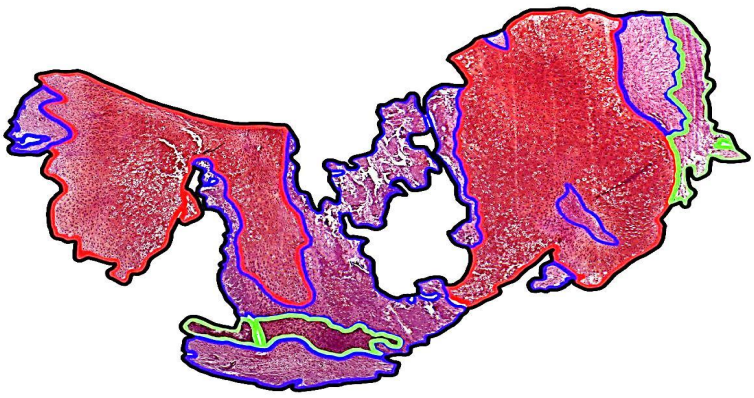

Fig. 1. Determination of assessment area on Safranin-O / Fastgreen slide with 40x magnification using image J software after image stitching using PT-GUI software. Black line: total callus area, orange line: Osseous area, blue line: area of fibrosis, red line: cartilage area

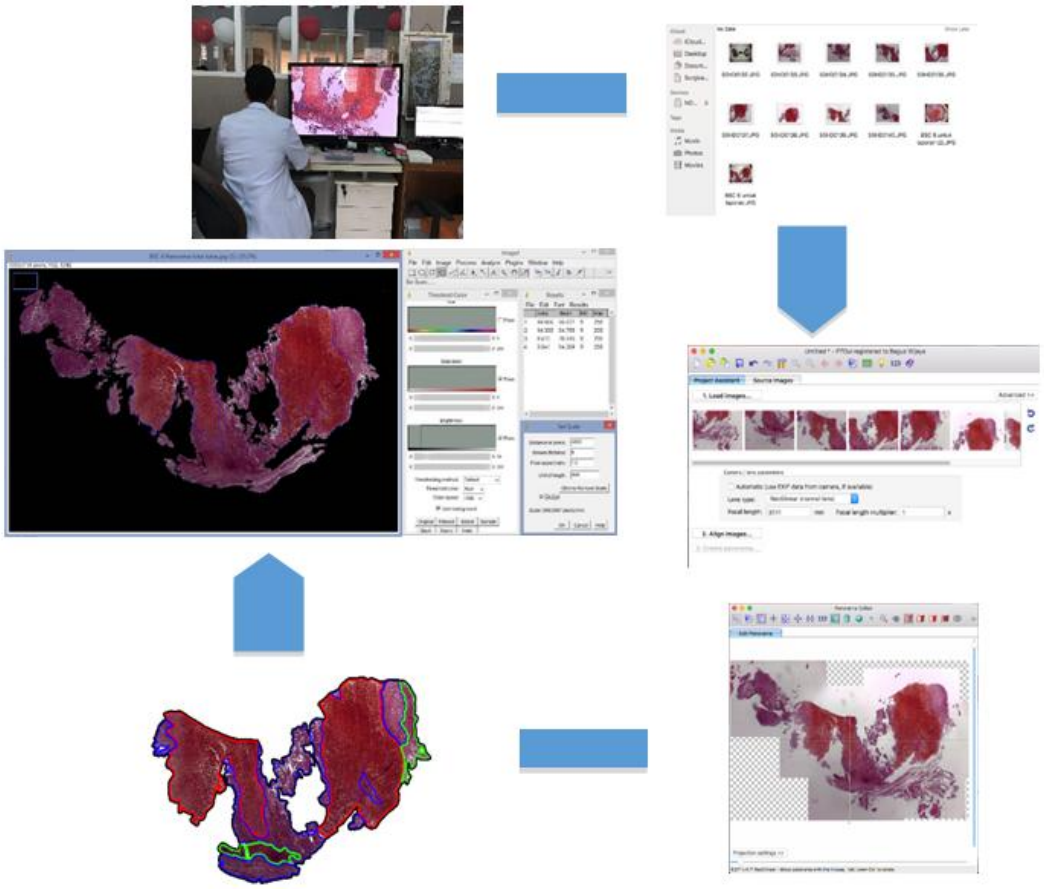

Fig. 2. Procedure of histomorphometry examination. (1) Picture was taken by Leica Microsystems microscope, 100x magnification; (2) Photomerging of picture by software PTGUI Pro 9.1; (3) Picture was analyzed with Image J sotware

2.2 Immunohistochemistry Staining of BMP 2 and BMPR II

The IHC staining was conducted by using a complex method of avidin-biotin peroxidase. Elite ABC kit with mouse IgG was used for anti BMP-2 and BMPR II antibody. The concentration of these antibody has been optimized in the preliminary study. The protocol of antibody I procollagen was using ABCAM system. The tissue from paraffin block is cut using a microtome with 4 μm thickness, dried at 37°C, and then named the sample number and antibody on each slide. Then the preparation is heated above *the slide warmer / hot plate* for 30 minutes at a temperature of 58°C.

The slide was deparaffinized in xylene I-III respectively, for 5 minutes each and dehydrated in graded alcohol (ethanol, alcohol 96% and 70%) for respectively 5 minutes then blocking with 0.5% H2O2 in methanol for 30 minutes and washed in water for 5 minutes. Pretreatment of the slide was performed with citrate buffer in microwave Cook I and Cook II for 5 minutes each, followed with blocking to non-specific antigens and then incubated for 15 minutes. Primary antibody BMP-2 and BMPR II (Mouse Monoclonal Anti-BMP 2 and anti BMPR II Antibody, ABCAM Medical, CA, US catalog number ab6285 and ab130206 respectively) were applied with 1:100 concentration followed by diluent Van Gogh Yellow, Abcam Medical, CA, US), followed by 1hour incubation. The slide then applied with universal secondary antibody to bind the primary antibody for 15 minutes. The secondary antibody was a biotinylated universal link secondary antibody (Starr Trek Universal HRP Detection System kit, Biocare Medical, USA, catalog number STUHRP700H) conjugated

to Horseradish peroxidase labeled-streptavidin (TrekAvidin-HRP, included in the kit) and then a chromogen (Betazoid DAB chromogen, included in the kit) was added, the final reaction product was diamino benzidine (DAB) which was identified as an intense brown color. After that, counterstaining was performed with haematoxylin for 1 minutes. The positive control was from human liver tissue and negative control was the same rat's fracture callus without BMP 2 and BMPR II antibody.

BMP2 and BMPR II expression was evaluated by screening 5 to 7 fields at 100x magnification to look for hotspot areas defined as an area with intense vascular structure. This study use immunoreactive score (IRS) to measure the expression of BMP 2 and BMPR II. The IRS provides a range of 0-12 as the product of multiplication between the percentage positive cell score (0-4) and the staining intensity score (0-3) [16]. IRS is used to express the broad spectrum of IHC markers (BMP and its receptors, BMPR IA, BMPR IB, BMPR II) in bone studies by Koerdt et al. For evaluation of BMP-6 reactions, IRS scores with some modifications were used by Raida et al where IRS calculations were performed by summarizing different scores [17].

2.3 Statistical Analysis

Normality test of Saphiro Willk is done for each group before the analysis. Statistical analysis was performed with One Way ANOVA test using SPSS software version 24 for Macintosh. Any significant difference found by one-way ANOVA was then analyzed by either Bonferroni or Mann Whitney post hoc test to assess the significance of each group compared to control. Further evaluation was performed using spearman correlation test to evaluate correlation between variables.

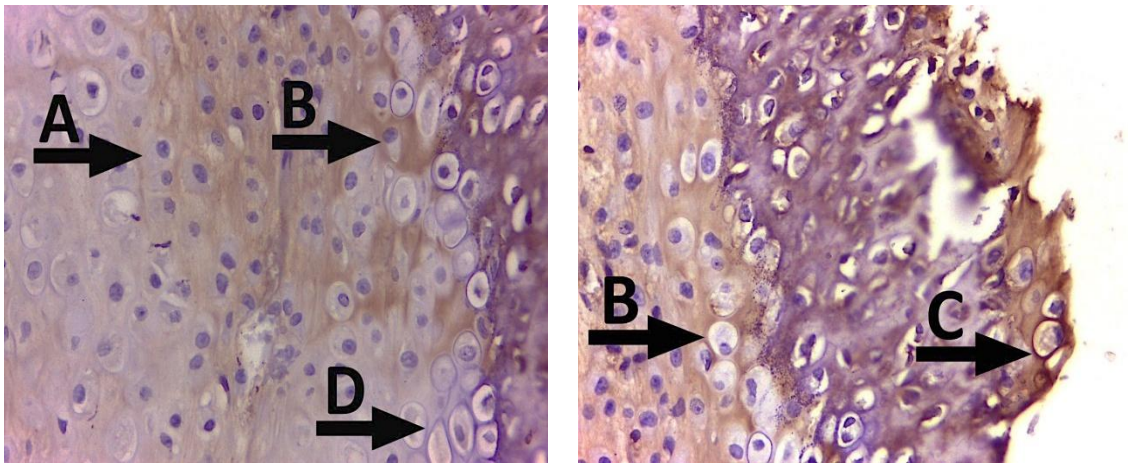

Fig. 3. BMPR II expression of BMSC group using 100x magnification (A). The weakly positive intensity staining (B). Moderately positive intensity (C). Strongly positive intensity (D). Negative staining

Table 1. Methods of immunohistochemistry measurement. IRS score was used to calculate the expression of BMP 2 and BMPR II

Percentage of positive cells (A)	Intensity of staining (B)	IRS score (Multiplication of A and B
0 = no positive cells	0 = no color reaction	0-1 = negative
1 = <10% of positive cells	1 = mild reaction	2-3 = mild
2 = 10-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4 = >80% positive cells	**Final IRS score (AxB): 0-12**	

3. RESULTS AND DISCUSSION

3.1 Histomorphometry Evaluation

ANOVA test showed a significant difference in the % osseous area (*P*= .001). The Bonferroni post hoc test concluded a significant differences of osseous area between MSC group and control groups (*P*= .001). But there was no significant difference between BMSC and ASC (*P*= 1.00). By using one-way ANOVA, it is concluded that there is no significant cartilage percentage between study groups (*P*= .49). Therefore, MSC types that are given do not influence the result of study.

Kruskal wallis test showed a significant difference in the % fibrous area (*P*= .001). The Mann-Whitney post hoc test shows significant difference of % fibrous area between ASC group and control (*P*= .002) and BMSC and control (*P*= .002). There was no difference of fibrous area between ASC group and BMSC group (*P*= .18).

3.2 Immunohistochemistry Evaluation

We were able to obtain an accurate measurement of the effect of BMP2 and BMPR II during healing process in critical size defect by detecting their expression during early phase bone healing. This study compares the expression of BMP2 and BMPR II of the three study groups using IRS Scoring. Kruskal wallis test shows no difference between IRS score of the BMP2 expression of the three study groups (*P*= .345). In the analysis of BMPR II, kruskal wallis test shows significant difference of IRS Scoring on the expression of BMPR II between study groups (*P*= .004). Mann Whitney post Hoc test shows significant difference of BMPR II expression between BMSC group compared to ASC group (*P*= .026) and control group (*P*= .004).

Furthermore, to reveal the effect of BMP2 and BMP II on bone healing process we performed spearman correlation test between % total callus area, % cartilage area, and % of fibrous area and IRS score that represent BMP2 and BMPR II expression. Spearman correlation test shows significant relationship between % osseous area and BMPR II expression (*P*= .034), and r value of 0.501 that indicates linear and moderate relationship. Our result shows the increase % of osseous area will be followed by higher level of BMPR II expression. However, there was no correlation between % osseous area and BMP2 expression (*P*= .746).

There is significant relationship between formation of cartilage with BMP 2 expression (*P*= .041) and r value of 0.485 which implies linear and moderate correlation. There was no relationship between % of cartilage area and BMPR II expression (*P*= .717). This study shows significant relationship between % of fibrous area and BMPR II expression (*P*= .003), and r value of -0.664 that indicates reverse and moderate relationship, and no relationship with BMP2 expression (*P*= .071). Lastly, spearman correlation test shows no correlation between total callus area with the expression of BMP2 (*P*= .781) and BMPRII (*P*= .746).

This study is aimed to elucidate the controversies among in vitro and vivo studies which stated inconclusive theory of osteogenic abilities between BMSC and ASC. Some studies also state that bone marrow MSCs are superior compared to adipose tissue MSC in the osteogenic potential, especially in vitro. [12] But in some in vivo studies, adipose tissue MSCs have comparable potential with bone marrow MSCs by microradiology evaluation and histomorphometry.

De Ugarte et al. in [14] showed no signicant difference of osteogenesis between human ASCs and BMSCs by ALP activity and calcium content assay. Other in vivo study by Wen et al. compared the bone regeneration capacity on 5 mm cranial defect of SD rat between human ASC and BMSC which combined with collagen gel. They found no significant difference of new bone regeneration between two groups by X-ray and histology analysis. These findings contrast other findings by Toupadakis et al. [18] that overview the osteogenic gene expression between osteoinduced equine BMSC and ASC and revealed that BMSCs had the highest overall expression of the osteogenic genes *Cbfα1*, *Osteorix*, and *OMD*. Other study conducted by Hayashi et al. [19] found the rat BMSC depicts greater osteogenic potential than rat ASC by mineralization, AP activity and osteocalcin secretion.

Our result support studies that show osteogenic potency of ASC is resembling BMSC seen by the histomorphometry result of bone healing process in which percentage of osseous, cartilage and fibrous area did not differ significantly among both groups. Furthermore, ASC also has better formation of total callus area, although it was not statistically significant.

Table 2. Evaluation of histomorphometry result (*) shows statistically significant result *P* ≤ .05. Control (C), Bone Marrow Stem Cell (BMSC), Adipose stem Cell (ASC)

No.	Parameter	Group	Comparative test	*P* value	POST-HOC Test (*P* value)
1	Total callus area	Control BMSC ASC	One Way Anova	(*P*= .05)	C-ASC (*P*= .09) C-BMSC (*P*= 1.00) ASC-BMSC (*P*= .11)
2	% Osseous area	Control BMSC ASC	One Way Anova	(*P*= .001)	C-ASC (*P*= .001)* C–BMSC (*P*= .001)* ASC–BMSC(*P*= 1.00)
3	% Cartilage	Control BMSC ASC	One Way Anova	(*P*= .493)	-
4	% Fibrous Area	Control BMSC ASC	Kruskal Wallis	(*P*= .001)	C – ASC (*P*= .002)* C – BM SC(*P*=.002)* ASC–BMSC(*P*= .18)
5	Expression BMP 2	Control BMSC ASC	One Way Anova	(*P*= .345)	-
6	Expression BMPR II	Control BMSC ASC	One Way Anova	(*P*= .004)	C – ASC(*P*= .093) C – BMSC (*P*= .004)* ASC–BMSC (*P*= .026)*

Table 3. Correlative analysis between histomorphometry result and immunohistochemistry result. (*)Shows statistically significant result *P* ≤ .05

No.	1st variable	2nd variable (IRS score)	r value	*P* value
1	Total callus area	BMP 2	0.071	(*P*= .781)
		BMPR II	0.082	(*P*= .746)
2	% Osseous Area	BMP 2	-0.092	(*P*= .717)
		BMPR II	0.501	(*P*= .034)*
3	% Cartilage area	BMP 2	0.485	(*P*= .041)*
		BMPR II	-0.092	(*P*= .717)
4	% Fibrous Area	BMP 2	-0.435	(*P*= .071)
		BMPR II	-0.664	(*P*= .003)*

Furthermore, Niemeyer et al in their study states that BMSC have greater osteogenic potential than ASC in the 3 cm critical bone defect on lamb's tibia but with the addition of Platelet Rich Plasma (PRP) [16,20]. However, ASC have some interesting characteristics for clinical application compare to BMSC, like abundant stem cell of lipoaspirate, the growth is faster and the morbidity is lower during operation [21,22,23].

Kloen et al proves in their study that BMP 2 and BMPR II are expressed and localized in human's callus. It proves that BMP2 holds important roles in the process of bone healing [24]. Lissenberg et al. [25] in his study stated that the presence of BMP alone does not guarantee efficient bone healing. Although the presence of BMP is essential for a number of processes during bone healing, BMP-mediated bone formation is highly dependent on the presence of various BMP activities that regulate local inhibitors and stimulators. This is evidenced in our study where BMP2 expression did not differ significantly between control group, BMSC and ASC group.

3.3 Safety of MSC Usage

In spite their advantages, there is risk of oncogenic transformation of MSC even with normal genotype. In which we did not experience it in our study. Several possible mechanisms to undergo malignant transformation were thought to be happened *in vitro* during production

phases, by interactions with tumor stromal *in vivo*, or through genetic adjustments with transgenes. More evaluation of the oncogenic risk of MSC and elucidation of molecular mechanism of their biological properties are still needed. With these, the scope of limitations of MSC based therapies can trigger the development of future methods to facilitate MSC to be use in clinical settings [26].

4. CONCLUSION

Our study demonstrates that ASC has comparable osteogenic properties to BMSC. There was no significant difference in the bone healing ability between ASC and BMSC after quantitative measurement by histomorphometric examination despite significant difference in Immunohistochemistry results. It proves although they are important but BMP-2 and BMPR II were not sole major factors in osteogenesis. Taken together, we conclude ASC could be use as safe alternative treatment for critical size bone defect due to similar osteogenic potential to BMSC. Follow up study using IHC analysis of more osteoblast markers (ALP, BMP2, BMPR II, osteocalcin, noggin) and osteoclast marker concomitant with radiology scoring over several periods of time will be essential. Hence, translational research with Randomized Controlled Trial (RCT) design needs to be conducted in order to prove the consistency of this research.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENT

This research project is being fund by the grant for International Publication (PITTA) given by Universitas Indonesia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alwattar BJ, Schwarzkopf R, Kirsch T. Stem cells in orthopaedics and fracture healing. Bull NYU Hosp Jt Dis. 2011;69:6–10.
2. Granero-moltó F, Weis JA, Miga MI, Landis B, Timothy J, Rear LO, et al. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. 2012;27:1887–98.
3. Kamal AF, Dilogo IH, Hutagalung EU, Iskandriati D, Susworo R, Siregar NC, et al. Transplantation of mesenchymal stem cells, recombinant human BMP-2, and their combination in accelerating the union after osteotomy and increasing, the mechanical strength of extracorporeally irradiated femoral autograft in rat models. Med J Islam Repub Iran; 2014.
4. Kloen P, Lauzier D, Hamdy RC. Co-expression of BMPs and BMP-inhibitors in human fractures and non-unions. Bone. 2012;51:59–68.
5. Birmingham E, Niebur GL, McHugh PE, Shaw G, Barry FP, McNamara LM. Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. Eur Cell Mater. 2012;23:13–27.
6. Shafiee A, Seyedjafari E, Soleimani M, Ahmadbeigi N, Dinarvand PGN. A comparison between osteogenic differentiation of human unrestricted somatic stem cells and mesenchymal stem cells from bone marrow and adipose tissue. Orig Res Pap. 2011;1257–64.
7. Jaiswal N. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. J Cell Biochem. 1997;295–312.
8. Kang BJ, Ryu HH, Park SS, Koyama Y, Kikuchi M, Woo HM, Kim WH KO. Comparing the osteogenic potential of canine mesenchymal stem cells derived from adipose tissues, bone marrow, umbilical cord blood, and Wharton' s jelly for treating bone defects. J Vet Sci. 2012;13:299–310.
9. Chung DJ, Hayashi K, Toupadakis CA, Wong AYC. Osteogenic proliferation and differentiation of canine bone marrow and adipose tissue derived mesenchymal stromal cells and the influence of hypoxia. Res Vet Sci. 2012;92:66–75.

10. Fan J, Park H, Tan S, Lee M. Enhanced osteogenesis of adipose derived stem cells with noggin suppression and delivery of BMP-2. PLoS One. 2013;8:e72474.
11. Fayaz HC, Giannoudis PV, Vrahas MS, Smith RM, Moran C, Pape HC, et al. The role of stem cells in fracture healing and nonunion. Int Orthop. 2011;35:1587–97.
12. Ilic D, Miere C, Lazic E. Umbilical cord blood stem cells: Clinical trials in non-hematological disorders. Br Med Bull. 2012;102:43–57.
13. Zhu Y, Liu T, Song K, Fan X, Ma XCZ. Adipose-derived stem cell: A better stem cell than BMSC. Cell Biochem Funct. 2008;26:664–75.
14. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser JHM. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs. 2003;101–9.
15. Im GI, Shin YWLK. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells. Osteoarthr Cartil. 2005;845–53.
16. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn Pathol. 2014;9:221.
17. Universita HH. Expression, regulation and clinical significance of bone morphogenetic protein 6 in esophageal Squamous-Cell Carcinoma. 1999;44:38–44.
18. Toupadakis CA, Wong A, Genetos DC, Cheung WK, Borjesson DL, Ferraro GL, Galuppo LD, Leach JK, Owens SDYC. Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord blood, and umbilical cord tissue. Am J Vet Res. 2010;71:1237–45.
19. Hayashi O, Katsube Y, Hirose M, Ohgushi HIH. Comparison of osteogenic ability of rat mesenchymal stem cells from bone marrow, periosteum, and adipose tissue. Calcif Tissue Int. 2008;82:238–47.
20. Koerdt S, Siebers J, Bloch W, Ristow O, Kuebler AC, Reuther T. Immunohistochemial study on the expression of von Willebrand factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation. Head Face Med. 2013;9:40.
21. Slyfield CR, Tkachenko E, Wilson D, Hernandez C. Three-dimensional dynamic bone histomorphometry. Physiology. 2010;27:153–8.
22. Hee HT, Ismail HD, Lim CT, Goh JCH, Wong HK. Effects of implantation of bone marrow mesenchymal stem cells, disc distraction and combined therapy on reversing degeneration of the intervertebral disc. J Bone Joint Surg Br. 2010;92:726–36.
23. Giannoudis PV, Einhorn TA, Schmidmaier G, Marsh D, Calori GMGPV. Enhancement of fracture healing with the diamond concept: The role of the biological chamber. Injury. 2011;42:1191–3.
24. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury. 2005;36:1392–404.
25. Lissenberg-Thunnissen SN, De Gorter DJJ, Sier CFM, Schipper IB. Use and efficacy of bone morphogenetic proteins in fracture healing. Int Orthop. 2011;35:1271–80.
26. Momin EN, Vela G, Zaidi HA, Quiñones-Hinojosa A. The oncogenic potential of mesenchymal stem cells in the treatment of cancer: Directions for future research. Curr. Immunol. Rev. 2010;6:137–148.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21775