

21. Addition Of Adipose

by Indri Lakhsmi Putri

Submission date: 14-May-2023 01:08PM (UTC+0800)

Submission ID: 2092468376

File name: 21._Addition_Of_Adipose.pdf (150.55K)

Word count: 2902

Character count: 15958

ORIGINAL ARTICLE

Addition Of Adipose Derived Stem Cell To Beta Tricalcium Phosphate and Human Cancellous Bone for Craniofacial Bone Tissue Engineering: An In Vitro Study

Indri Lakhsmi Putri¹, Arif Rachman², Pratiwi Soesilawati³, Lukman Hakim^{4,5}, Fedik Abdul Rantam⁵, David Sontani Perdanakusuma¹

¹ Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

² Doctoral Program, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

³ Department of Oral Biology, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia

⁴ Department of Urology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

⁵ Stem Cell Research and Development Center, Airlangga University, Surabaya, Indonesia

ABSTRACT

Introduction: Autologous bone graft remains the method of choice for correction of osseous defects despite its shortcomings related to its limited availability, donor side effects and post-surgical potential complications of the recipient. It is imperative to develop more innovative substitute that offers little to no adverse effects. We aimed to assess the impact of addition human adipose derived stem cell to Beta tricalcium phosphate (β TCP) and human cancellous bone in vitro. **Methods:** Experimental study was carried out in vitro, where β TCP and human cancellous freeze-dried bone graft were seeded onto a 24-well microplate (each well containing 2×10^6 hADSCs). A colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide/MTT) was carried out for three days using the second passage of hADSCs to calculate the cell viability using ELISA reader at optical density (OD) 590nm. **Results:** MTT Assay showed that the percentage of viable cells in both groups were more than 70%, of which the β TCP showed significantly higher percentage than cancellous bone groups. **Conclusion:** This study proved that the addition of human adipose derived stem cell to β TCP and human cancellous bone in vitro is harmless and significantly improve cell viability in vitro.

Keywords: Bone Tissue Engineering, Mesenchymal Stem Cells, Adipose Derived Stem Cells, Beta Tricalcium Phosphate, Human Cancellous Bone

Corresponding Author:

Lukman Hakim, PhD

Email: lukman-h@fk.unair.ac.id

Tel: +6231 5501318

INTRODUCTION

Autologous bone graft is a regular method to treat osseous defects as it merges all the characteristics of bone material (1). Risk of infection, rejection or immunoreaction are diminished since it is histocompatible and non-immunogenic and due to neovascularization and integration with surrounding tissues (2). Disadvantages of autologous bone graft are the occurrence of a donor defect, inadequate donors, requirement for a follow-up surgery with the concerns of postoperative discomfort, and potential postoperative problems (3,4).

Osteogenic differentiation can be found in mesenchymal stem cells (MSCs) (5). MSCs that are best suited for therapy must have the following properties: they can be derived with minimal morbidity to the patient, abundant

in reserve, have the ability to be cultured, and can potentially differentiate into several cell lineages. It has been proven that adipose-derived stem cells (ADSCs) are superior over bone marrow MSCs (BM-MSCs) because they are more abundant (6) and can be extracted by simpler and less invasive methods such as liposuction, subcutaneous adipose tissue fragments, or during other surgical procedures. MSCs have been proven to be very beneficial in allogenic stem cell therapy as they carry the potential to diminish the immunogenicity and immunosuppressive potential of a material, two excellent hallmark of MSCs (6,7). The ADSCs are MSCs deriving from adipose tissue. Several studies have explained bone regeneration using ADSCs (8–14). Recent bone tissue engineering approach, combine a scaffold with mesenchymal stem cells then is inserted into osseous defects.

Over the past years, techniques in tissue modification have been implemented to enhance bone healing, taking advantage of ideal bone tissue biocompatibility (Roohani-Esfahani et al., 2012, Collins et al., 2009,

Bretcanu et al., 2009). These techniques commonly involve the incorporation of cultured mesenchymal stem cells (MSCs) into a biomaterial framework before its implantation into a tissue defect (Lu et al., 2010).

Tricalcium phosphate (TCP) is one kind of bone substitute material that can provide significant biocompatibility, is highly porous, absorbs and conducts rapidly (15). Beta Tricalcium phosphate (β TCP), the latest generation of TCP, results in more biodegradation and formation (15,16), and more permanent because of its lower solubility as compared to β TCP (17). β TCP is also Furthermore, as it is absorbed more slowly, preserve more bone filling volume in the defect area (18). β TCP is also thermogenically more stable in normal temperature (15) and highly porous, facilitating the advancement of microcirculatory growth, penetration by proliferating stem cells (19), nutrient diffusion and transfer of fluid pressure (20), so as to promote bone growth.

14

In this study, we aimed to assess the impact of addition human adipose derived stem cell to β TCP and human cancellous freeze-dried bone graft in vitro. The addition was expected to improve cell viability in vitro, a promising future method of choice in bone tissue engineering.

MATERIALS AND METHODS

Stem cells were harvested from the adipose tissue of pregnant women undergoing Caesarean section (C-section) because of utero fetal malposition or multiple history of caesarean section exclusive of other pathological anomalies. In consensus with the guidelines of the Ethical Committee of Airlangga University Hospital (permit numbers 106/KEH/2018), we made sure that signed informed consent forms were taken before the procedure from each subject.

Cell culture, propagation and differentiation of cells under controlled environment were carried out at Stem Cell Research and Development Center Laboratory, Airlangga University, Surabaya. The results of stem cell differentiation showed that the cultured cells have differentiated into their adipogenic and osteogenic phenotypes. Further on, characterization was done at the Regenic Laboratory, Stem Cell and Cancer Institute, Kalbe Farma Tbk, Jakarta. Percentage of expression of MSC markers in accordance with expected specifications; express CD105 (97.4%), CD73 (99.56%), and CD90 (97.61%), lack CD45 (1.42%), CD34 (1.45%), CD14 (0.74%), CD19 (0.83%) and HLA-DR (0.84%) expression. Second passage human adipose derived stem cell (hADSC) was used for combination with β TCP and human cancellous bone (Figure 1).

The β TCP was obtained from Kasios (Kasios TCP Dental HP; Kasios, L'Union, France), in the form of 2-3 mm granules. Permission from the Food and Drug

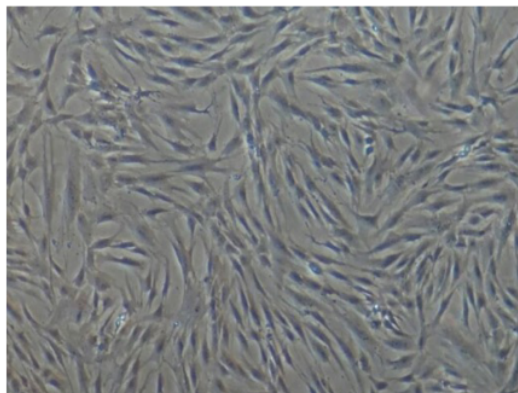


Figure 1: Second passage human adipose derived stem cell. A representative of fibroblast-like cells differentiated from human adipose derived stem cells passage-2

Administration (FDA) regulation number (21 CFR 888.3045) was obtained beforehand. In its properties, Kasios is very porous, thus facilitating osseointegration and resorption. It is biocompatible and synthetic, diminishing the risk of immunological rejection and microbial transmission. It is also bioresorbable, which allows for its replacement by autogenous bone. Its osseoconductive and interconnected porosity facilitate osseointegration. Furthermore, it is ready to use and radioopaque, allowing for shorter duration of surgery and possible long term follow up respectively. The granule form allows us to fill in irregular defects, which add up to its advantages.

Human cancellous freeze dried bone graft was obtained from the Center of Biomaterial and Tissue Bank Dr. Soetomo General Hospital, Surabaya, taken from living donors who have been previously screened for HIV.

Each of the β TCP and human cancellous freeze-dried bone graft was seeded onto a 24-well microplate, each group has five wells, each well containing 2×10^6 hADSCs. Before transplantation was carried out, 1 mL of complete medium DMEM was administered to the cell and incubation is carried out in a 5% CO_2 incubator at 37°C for 24 hours to ensure homogenization.

An in vitro MTT Assay was conducted to determine the percentage of cell viability. The MTT results considered as non-toxic if the percentage of living cells is more than or equal to 70%, allowing good cell expansion (21). The MTT test is carried out for three days using the second passage (P2) of hADSCs. An ELISA plate reader was used to read absorbance at optical density (OD) 590nm. The mean OD of all wells in each group were used to calculate the percentage of cell viability as follows :

$$\text{percentage of cell viability} = \frac{(A_{\text{treatment}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100\%$$

(where, A = absorbance)

Statistical data were expressed as means \pm standard deviation. Statistical analysis was done using the independent sample T test to test for any significant difference between the variables in each group. A value of $p < 0.05$ shows statistical significance. The Statistical Package for Social Science (IBM Corp. Released 2016. IBM SPSS Statistics for Macintosh, Version 24.0. Armonk, NY: IBM Corp) was used in the statistical computation and evaluation.

RESULTS

Results of the MTT combination test (Table I) showed that both groups is proven to be non-toxic since the percentage of living cells is more than 70%. The matrix was considered favourable for growth as cells were able to proliferate. The results also showed that the percentage of living cells in β TCP was significantly higher than in human cancellous freeze-dried bone graft.

Table I: MTT Assay Results in percentage

NO	β TCP-hADSC	HCFCDBG-hADSC	P VALUE
1	104,49	88,31	
2	102,11	88,75	
3	102,93	89,32	
4	102,8	87,98	
5	103,99	89,07	
MEAN	103 \pm 1	89 \pm 1	0.00

DISCUSSION

This study focuses on the percentage of cell viability in addition of hADSC to β TCP and human cancellous freeze dried bone graft in vitro.

Sananta et al studied cell viability from stromal vascular fraction (SVF) with bone substitute material, showed that the combination SVF-calcium phosphate group had viable cells 47,46%, SVF-hydroxyapatite-calcium sulfate group had viable cells 69,73% and SVF-bovine bone cancellous group had viable cells 77,80% (22).

Housmand et al in their study on the proliferation of human dental pulp stem cells (hDPSCs) on biphasic hydroxyapatite/ beta-tricalcium phosphate granules of macro-porous biphasic calcium phosphate (MBCP) revealed that the highest values of MTT were found in hDPSCs + Metformin group (> 70%) followed by the hDPSCs group (> 70%) and hDPSCs + MBCP + Metformin (< 60%) and hDPSCs + MBCP groups (< 40%) (23).

Liu et al studied cellular viability of human exfoliated deciduous teeth stem cells (SHEDs) in chitosan biomaterial framework with or without magnesium borate, zinc borate and boric acid using MTT assay. The results revealed that these supplements proved harmful for SHEDs cells (< 60%) (24).

The results of our study showed the addition of human adipose derived stem cell to both group are proven to be non-toxic and improved cell viability in vitro.

CONCLUSION

The addition of adipose derived stem cell to beta tricalcium phosphate and human cancellous freeze dried bone graft are proven to be non-toxic and improved cell viability in vitro with the percentage of living cells significantly higher in β TCP. Future in vivo studies in craniofacial bone tissue engineering are awaited, in order to develop more innovative substitute that offers little to no adverse effects compare to autologous bone graft.

REFERENCES

- Dimitriou R, Jones E, McGonagle D, Giannoudis P V. Bone regeneration: current concepts and future directions. *BMC Med.* 2011 May;9:66.
- Rogers GF, Greene AK. Autogenous bone graft: basic science and clinical implications. *J Craniofac Surg.* 2012 Jan;23(1):323-7.
- Baqain ZH, Anabtawi M, Karaky AA, Malkawi Z. Morbidity from anterior iliac crest bone harvesting for secondary alveolar bone grafting: an outcome assessment study. *J Oral Maxillofac Surg [Internet].* 2009 Mar;67(3):570-5. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0278239108015036>
- Goudy S, Lott D, Burton R, Wheeler J, Canady J. Secondary alveolar bone grafting: outcomes, revisions, and new applications. *Cleft Palate Craniofac J [Internet].* 2009 Nov;46(6):610-2. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19860505>
- Halim D, Murti H, Sandra F, Boediono A, Djuwantono T, Setiawan B. Keragaman jenis stem cell : sumber, teknik isolasi, kulturisasi, diferensiasi dan kriopreservasinya. In: *Stem Cell Dasar Teori dan Aplikasi Klinis.* Erlangga; 2010.
- Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells Int [Internet].* 2012;2012:812693. Available from: <http://www.hindawi.com/journals/sci/2012/812693/>
- Siegel G, Schafer R, Dazzi F. The immunosuppressive properties of mesenchymal stem cells. *Transplantation.* 2009 May;87(9 Suppl):S45-9.
- De Girolamo L, Sartori MF, Arrigoni E, Rimondini L, Albisetti W, Weinstein RL, et al. Human adipose-derived stem cells as future tools in tissue regeneration: osteogenic differentiation and cell-scaffold interaction. *Int J Artif Organs [Internet].* 2008 Jun;31(6):467-79. Available from: <http://www.embase>.

- com/search/results?subaction=viewrecord&from=export&id=L352171992%5Cnhttp://sfx.library.uu.nl/utrecht?sid= EMBASE&issn=03913988&id=doi:&atitle=Human+adipose-derived+stem+cells+as+future+tools+in+tissue+regeneration:+Osteogenic+differentia
9. Di Bella C, Farlie P, Penington AJ. Bone regeneration in a rabbit critical-sized skull defect using autologous adipose-derived cells. *Tissue Eng Part A* [Internet]. 2008 Apr;14(4):483–90. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18370606>
 10. Grewal NS, Gabbay JS, Ashley RK, Wasson KL, Bradley JP, Zuk PA. BMP-2 does not influence the osteogenic fate of human adipose-derived stem cells. *Plast Reconstr Surg*. 2009 Feb;123(2 Suppl):158S-65S.
 11. Li H, Dai K, Tang T, Zhang X, Yan M, Lou J. Bone regeneration by implantation of adipose-derived stromal cells expressing BMP-2. *Biochem Biophys Res Commun* [Internet]. 2007 May;356(4):836–42. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/17391646>
 12. Rada T, Reis RL, Gomes ME. Adipose tissue-derived stem cells and their application in bone and cartilage tissue engineering. *Tissue Eng Part B Rev* [Internet]. 2009 Jun;15(2):113–25. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/19196117>
 13. Tapp H, Hanley EN, Patt JC, Gruber HE. Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair. *Exp Biol Med (Maywood)* [Internet]. 2009 Jan;234(1):1–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19109553>
 14. Yoon E, Dhar S, Chun DE, Gharibjanian NA, Evans GRD. In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model. *Tissue Eng* [Internet]. 2007 Mar;13(3):619–27. Available from: <https://www.liebertpub.com/doi/10.1089/ten.2006.0102>
 15. Bettach R, Guillaume B, Taschieri S, Del Fabbro M. Clinical performance of a highly porous beta-TCP as the grafting material for maxillary sinus augmentation. *Implant Dent* [Internet]. 2014 Jun;23(3):357–64. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24819814>
 16. Jiang S-D, Jiang L-S, Dai L-Y. Surgical treatment of calcaneal fractures with use of beta-tricalcium phosphate ceramic grafting. *Foot Ankle Int*. 2008 Oct;29(10):1015–9.
 17. Li Y, Weng W, Tam KC. Novel highly biodegradable biphasic tricalcium phosphates composed of alpha-tricalcium phosphate and beta-tricalcium phosphate. *Acta Biomater*. 2007 Mar;3(2):251–4.
 18. Grandi G, Heitz C, Santos LA dos, Silva ML, Sant’Ana Filho M, Pagnocelli RM, et al. Comparative histomorphometric analysis between β -TCP cement and β -TCP/Ha granules in the bone repair of rat calvaria. *Mater Res* [Internet]. 2011 Apr 1;14(1):11–6. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-14392011000100004&lng=en&tlng=en
 19. Chappard D, Guillaume B, Mallet R, Pascaretti-Grizon F, Baslir MF, Libouban H. Sinus lift augmentation and beta-TCP: a microCT and histologic analysis on human bone biopsies. *Micron* [Internet]. 2010 Jun;41(4):321–6. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0968432809002017>
 20. Allegrini (Jr) S, Koenig (Jr) B, Allegrini M, Yoshimoto M, Gedrange T, Fanghaenel J, et al. Alveolar ridge sockets preservation with bone grafting review. *Ann Acad Med Stetin*. 2008;54(1):70–81.
 21. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal / stem cells : a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *J Cytotherapy* [Internet]. 2013;15(6):641–8. Available from: <http://dx.doi.org/10.1016/j.jcyt.2013.02.006>
 22. Sananta P, Mustaqim WA, Andarini S. In vitro study of viability and apoptosis of mesenchymal stem cells from stromal vascular fraction (SVF) in combination with bovine bone cancellous as a good bone substitute material In Vitro Study of Viability and Apoptosis of Mesenchymal Stem Cells fr. In: *AIP Conference Proceedings* 2108, 020042 (2019) [Internet]. 2019. Available from: <https://aip.scitation.org/doi/abs/10.1063/1.5110017>
 23. Houshmand B, Tabibzadeh Z, Reza S, Kouhestani F. Archives of Oral Biology Effect of metformin on dental pulp stem cells attachment , proliferation and differentiation cultured on biphasic bone substitutes. *Arch Oral Biol* [Internet]. 2018;95(May):44–50. Available from: <https://doi.org/10.1016/j.archoralbio.2018.07.012>
 24. Liu Y, Su W, Chen P. Magnesium and zinc borate enhance osteoblastic differentiation of stem cells from human exfoliated deciduous teeth in vitro. *J Biomater Appl* [Internet]. 2017;0(0):1–10. Available from: <https://journals.sagepub.com/doi/10.1177/0885328217740730>

21. Addition Of Adipose

ORIGINALITY REPORT

12%

SIMILARITY INDEX

10%

INTERNET SOURCES

10%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

1 Chiquita Prahasanti, Alexander Patera Nugraha, Tania Saskianti, Ketut Suardita, Wibi Riawan, Diah Savitri Ernawati. " 2%

Exfoliated Human Deciduous Tooth Stem Cells Incorporating Carbonate Apatite Scaffold Enhance BMP-2, BMP-7 and Attenuate MMP-8 Expression During Initial Alveolar Bone Remodeling in Wistar Rats (*Rattus norvegicus*)

", Clinical, Cosmetic and Investigational Dentistry, 2020

Publication

2 docksci.com 1%
Internet Source

3 Johannes Mathis, Daniel Andres, Wolfgang Schmitt, Claudio L Bassetti, Christian W Hess, David R Schreier. "The diagnostic value of sleep and vigilance tests in central disorders of hypersomnolence", Sleep, 2022 1%
Publication

4	Internet Source	1 %
5	www.freepatentsonline.com Internet Source	1 %
6	Behzad Houshmand, Zohreh Tabibzadeh, Saeed Reza Motamedian, Farnaz Kouhestani. "Effect of metformin on dental pulp stem cells attachment, proliferation and differentiation cultured on biphasic bone substitutes", Archives of Oral Biology, 2018 Publication	1 %
7	Yao-Jen Liu, Wen-Ta Su, Po-Hung Chen. "Magnesium and zinc borate enhance osteoblastic differentiation of stem cells from human exfoliated deciduous teeth in vitro", Journal of Biomaterials Applications, 2017 Publication	1 %
8	www.coursehero.com Internet Source	1 %
9	www.researchgate.net Internet Source	1 %
10	www.scielo.br Internet Source	1 %
11	Fei Xu, Fei Tan, Ziran Zheng, Xiongwen Zhou. "Effects of pre-osteogenic differentiation on the bone regeneration potentiality of marrow	<1 %

mesenchymal stem cells/poly(ethylene glycol)-diacrylate hydrogel using a rat cranial defect model", Journal of Biomaterials Applications, 2022

Publication

12

Stem Cell Biology and Regenerative Medicine, 2016.

Publication

<1 %

13

www.omicsonline.org

Internet Source

<1 %

14

www.science.gov

Internet Source

<1 %

15

Kleinjan Redelinghuys, Sebastiaan Rothmann, Elrie Botha. "Workplace flourishing: Measurement, antecedents and outcomes", SA Journal of Industrial Psychology, 2019

Publication

<1 %

16

L. Rony, E. Aguado, F. Pascaretti-Grizon, L. Hubert, D. Chappard. "Hyaluronic Acid Stimulates Osseointegration of β -TCP in Young and Old Ewes", Calcified Tissue International, 2019

Publication

<1 %

17

f1000research.com

Internet Source

<1 %

18

Susanne Kern, Hermann Eichler, Johannes Stoeve, Harald Klüter, Karen Bieback.

<1 %

"Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue", Stem Cells, 2006

Publication

Exclude quotes On

Exclude matches Off

Exclude bibliography On

21. Addition Of Adipose

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4
