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

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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

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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 adiposed derived stem cell to Beta tricalcium phosphate (βTCP) and human cancellous bone in vitro. **Methods:** Experimental study was carried out in vitro, where βTCP and human cancellous freeze-dried bone graft were seeded onto a 24-well microplate (each well containing 2x106 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 βTCP showed significantly higher percentage than cancellous bone groups. **Conclusion:** This study proved that the addition of human adipose derived stem cell to βTCP 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

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

Tricalsium phosphate (TCP) is one kind of bone substitute material that can provide significant biocompatibility, is highly porous, absorbs and conduct rapidly (15). Beta Tricalsium phosphate (βTCP), the latest generation of TCP, results in more biodegradation and formation (15,16), and more permanent because of its lower solubility as compared to βTCP (17). βTCP is also Furthermore, as it is absorbed more slowly, preserve more bone filling volume in the defect area (18). βTCP 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.

In this study, we aimed to assess the impact of addition human adiposed derived stem cell to βTCP 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 urdergoing 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 βTCP and human cancellous bone (Figure 1).

The βTCP 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 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 immonological 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.

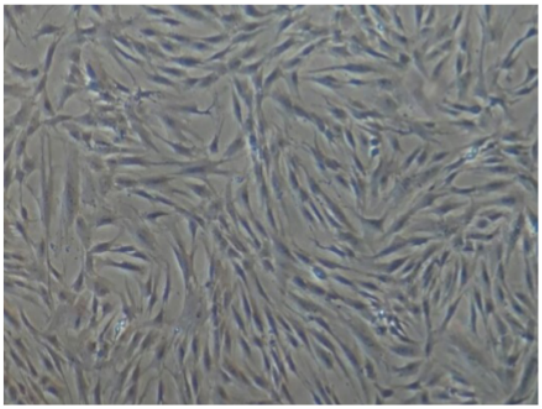

Figure 1: Second passage human adipose derived stem cell. A representative of fibroblast-like cells differentiated from human adipose derived stem cells passage-2

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 βTCP and human cancellous freeze-dried bone graft was seeded onto a 24-well microplate, each group has five wells, each well containing $2x10^6$ 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 :

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

(where, A = absorbance)

Statistical data were expressed as means ± 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 βTCP was significantly higher than in human cancellous freeze-dried bone graft.

Table I: MTT Assay Results in percentage

NO	βTCP-hADSC	HCFDBG-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 ± 1	89 ± 1	0.00

DISCUSSION

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

Sananta et al studied cell viability from stromal vascular fraction (SVF) with bone subtitute 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 adiposed 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 βTCP. 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.

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