Potential Proliferation of Stem Cell from Human Exfoliated Deciduous Teeth (SHED) in Carbonate Apatite and Hydroxyapatite Scaffold

Tania Saskianti[1], Rianita Ramadhani[1], Els S Budipramana[1], Seno Pradopo[1], Ketut Suardita[2]*

1. Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia.
2. Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia.

Abstract

Stem cell from Human Exfoliated Deciduous Teeth (SHED) is a source of stem cells that is easy to obtain. The application of SHED can be used for repairing damaged teeth, bone regeneration, and treatment of neural tissue injury. Scaffold can be used as a carrier for application of SHED and also increase the cell growth and regeneration of damaged tissue. Carbonate apatite and hydroxyapatite are material which commonly used as scaffold.

The aim of this study was to evaluate the potential proliferation of SHED in carbonate apatite scaffold and hydroxyapatite scaffold as candidate biomaterials in bone tissue engineering.
SHED was obtained from dental pulp of primary extracted teeth. SHED was seeding in carbonate apatite and hydroxyapatite scaffold for 7 days. The proliferation of SHED were determined using Scanning Electron Microscope (SEM).

The average number of SHED was higher in carbonate apatite scaffold compared to hydroxyapatite scaffold.

Combination of SHED and carbonate apatite scaffold has higher proliferation potency compare with SHED and hydroxyapatite.

Experimental article (J Int Dent Med Res 2017; 10(2): pp. 350-353)

Keywords: SHED, carbonate apatite, hydroxyapatites, scaffold.

Received date: 18 April 2017 **Accept date:** 20 May 2017

Introduction

Bone defect is still become a serious health problem in dentistry. Bone defect can be caused by trauma, congenital defect, and acquired diseases (cancer and periodontal disease).[1,2]

Biomaterial innovation is needed for bone substitution which can help the osteogenesis process on tissue regeneration.[3] The current therapy for bone defect is using bone graft. Bone graft is one of new method which offers replacement for damaged tissue. Bone graft are divided into autograft, allograft, and synthetic materials (alloplast).[4]

Bone graft has promising result, but it has some limitations. Autograft, though it is a gold standard for bone regeneration, may cause donor site morbidity, additional surgery procedure, increase in operative time and cost, and increase risk of trauma and infection.[4] Allograft is the alternative of autograft. The complication of these graft are nonunion, fracture, infection, and graft disease transmission.[5,6] Beside that, the risks of allograft involve mandatory lifelong immunosuppression since immunosuppressive agents are the front line in limiting rejection following transplantation.[7] A new approach that still develop to eliminate these difficulties is tissue engineering.[8] Tissue engineering can restore, maintain and improve tissue function following damage either by disease or traumatic process.[9] The strategies of tissue engineering in regenerating the damage tissue are cell injection therapy, cell induction therapy, and cell seeded scaffold.[10]

Stem cell from Human Exfoliated Deciduous Teeth (SHED) is a source of stem cells that is easy to obtained. The application of SHED can be used for repairing damaged teeth, bone regeneration, and treatment of neural tissue injury. Scaffold can be used as a carrier for

***Corresponding author:**
Tania Saskianti, DDS, PhD
Department of Pediatric Dentistry,
Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjend Prof. Dr. Moestopo No. 47 Surabaya-Indonesia.
E-mail: tania-s@fkg.unair.ac.id

application of SHED and also increase the cell growth and regenerate the damaged tissue.[11] The previous study showed that stem cells from pulp of primary teeth are proven to have good viability and potential proliferation.[12]

Scaffold can be used as a carrier for application of SHED and also increase the cell growth and regenerate the damaged tissue. Carbonate apatite and hydroxyapatite are material which commonly used as scaffold.[13,14]

This study used GAMA CHA and BioHydrox which both are Indonesian product of scaffold. GAMA CHA that contains carbonate apatite and gelatin is clinically proven to be a bone scaffolds in regenerative procedure. GAMA CHA is used by dentist, oral surgeon, pediatric dentist, and orthopedic specialist to accelerate bone growth.[15] BioHydrox is biomaterials that contain hydroxyapatite from bovine bones. It has microarchitecture and mineral composition that similar to human bones.[16]

The aim of this study was to evaluate the potential proliferation of SHED in the carbonate apatite scaffold and hydroxyapatite scaffold as candidate biomaterials in bone tissue engineering.

Materials and methods

The type of this research was experimental laboratories with post-test only control group design. SHED was isolated from the dental pulp of primary teeth that extracted due to persistence or orthodontic treatment purposes.

Teeth were cut horizontally with sterilized fissure bur then pulp tissue was taken and put into a culture medium in 15 ml falcon tube. The pulp was isolated by using 1 mg/ml trypsin enzyme and cultured on Dulbeccos Modified Eagle Medium (DMEM), Life Technologies/GIBCO BRL) with the addition of 20% Fetal Bovine Serum (FBS, Biochrom AG, Germany), 5 mM L-glutamine (Gibco Invitrogen, USA), 100 U/ml penicillin-G, 100 µg/ml streptomycin, and 100 µg/ml kanamycin.

Primary cells were cultured until confluence. Cells were collected by trypsine versene. Cells were planted on α-MEM medium which contain 20% bovine serum albumin, 2% penicillin/streptomycin, and incubated in 5% CO_2 37°C. Medium were replaced every 3 – 4 days and sub cultured every 7 days. After that, cells were moved to small bottle with density $2x10^4$ cell/ml.

Cells were rinsed by using PBS and given trypsin-EDTA to harvest the cell, then incubated in 5% CO_2 37°C. Cells that had been harvested from α-MEM medium were split and centrifuged in 3000 rpm for 5 minutes and medium were added. The cell suspension 20 µl with density $2x10^4$cell/ml were added into carbonate apatite scaffold (GAMA CHA, P.T. Swayasa Prakarsa, Indonesia) and hydroxyapatite scaffold (BioHydrox, Tissue Bank of Dr. Soetomo Hospital, Indonesia) that had been submerged in medium for 24 hours and placed in 24-well tissue culture plate. The cells on scaffold were harvest after 7 days.

After 7 days, proliferation potency of scaffold is evaluated using SEM (TM3000 TableTop Scanning Electron Microscope, Hitachi, United States) with 2000x magnification. The data were collected and analyzed with Mann-Whitney Test.

Results

The SEM result showed that SHED could be adhered to carbonate apatite scaffold and hydroxyapatite scaffolds. The SEM result of SHED seeded in carbonate apatite scaffold and hydroxyapatite scaffold could be seen in Figure 1 and Figure 2.

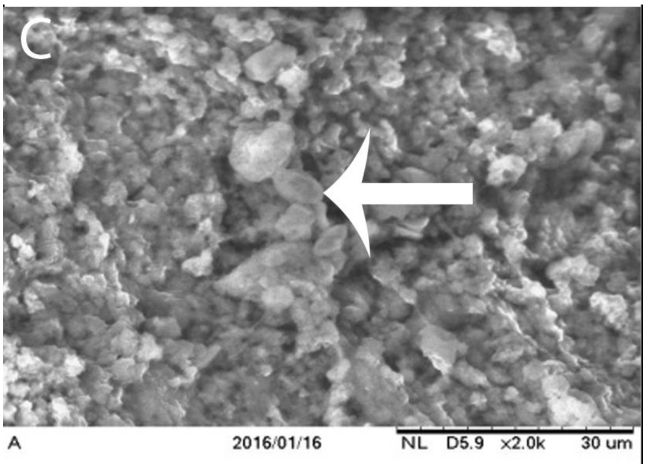

Figure 1. SHED adhesions (white arrow) on GAMA CHA by using SEM with 2000x magnification.

The average number of SHED which adhere to carbonate apatite scaffold is 2,5 and hydroxyapatite is 6,5. The data's distribution was calculated using Shapiro Wilk test. The result of that test is p=0,798 (p>0,05) for carbonate apatite scaffold and 0,216 (p>0,05) for hydroxyapatite scaffold. The data distribution was not normal so the data was analyzed using Mann-Whitney test. The result of Mann Whitney test showed p=0,021 (p<0,05).

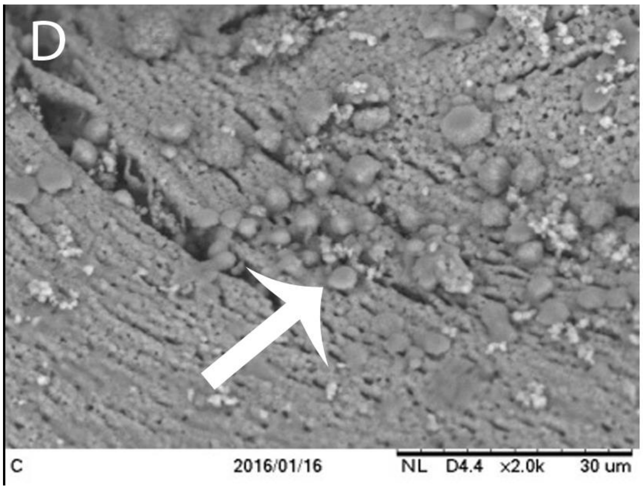

Figure 2. SHED adhesions (white arrow) on BioHydrox by using SEM with 2000x magnification.

Discussion

Stem cells give a new hope in tissue engineering. Stem cells are unspecialized cells that have two components that able to differentiate into various cell and capable of regenerating. In the healing process, stem cell or progenitor cell is an important factor. These cells will proliferate and immigrate to area of wounds, and then differentiate into the specific cells that will replace cells that die because of injury.[17]

Stem cells can also be found in dental pulp tissue which has restorative potential. SHED represent multipotent stem cells that may be more mature compared to the population of post natal stromal stem cells previously observed.[17,18] SHED is an ideal resource of stem cells to repair damaged tooth structure, induce bone regeneration, cartilage tissue engineering and possibly treat neural tissue injury of denegerative disease.[11] SHED can induce formation of a bone-like matrix with a lamellar structure by recruiting host cells.[19] This theory is proven with new bone formation that formed surrounding the resorbed root of primary teeth.[20]

Hydroxyapatite is calcium phosphate compound containing hydroxide. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a part of apatite mineral.[21] The chemical structure is similar to the chemical structure in bone's mineral component. This similarity makes hydroxyapatite is able to replace damaged bone tissue without causing harm to other healthy tissue.[16,22]

Hydroxyapatite can be found in the form of powder and composite scaffold. Hydroxyapatite scaffold has pores with variation of size depending on the volume and the process of scaffold's production. The porosity of hydroxyapatite scaffold allows cells to move through the existing pores. Porosity of scaffold is important for transport nutrient and vascularization.[23,24] The porous hydroxyapatite can bind strongly to the bone tissue. Hydroxyapatite structure has regular porosity that is similar to natural structure of the bone tissue. The pores have an open structure and biocompatible surface which has ideal condition for cell growth and tissue differentiation.[16,25]

Hydroxyapatite is a main inorganic component of the bone beside carbonate, sodium, and magnesium.[26] Hydroxyapatite contains calcium and phosphate which can lead to increasing affinity in bone tissue, thus causing ion exchange on calcium phosphate and interaction between surface of calcium and cell. Ion exchange takes place on the surface of phosphate compound (solid phase) to its surrounding fluid (liquid phase). The ion exchange happens through the surfaces of Ca^{2+}, $PO4^{3-}$, and the existing impurities such as CO_3^{2-}, Cl^- or F^-. Fibronectin also play an important role in SHED adhesion process.[16]

This study showed higher SHED adhesion and proliferation in hydroxyapatite scaffold. Ions are able to penetrate in hydrophobic membrane. The cell membrane is coated by a skeleton in the cytoplasm. Skeleton can create organelles such as cilia or filopodia which are able to expand the surface area in order to increase cell nutrient absorption. Surface area of the hydroxyapatite is cuboid so the nutrient absorption is higher, resulting in more cellular activity. This cellular activity later results in the more SHED adhesion and proliferation.[27]

Carbonate is one of the most abundant impurity ions. Bone content about wt 4-8% of carbonate ion.[28] Carbonate apatite has cylindrical surface area so the absorption of nutrients for the cells is not as much as absorption of nutrients that occurs on hydroxyapatite. It resulted in less cellular activity of the cells, causing less SHED adhesion and proliferation on the carbonate apatite.[27]

Conclusions

SHED adhesion and proliferation occurs in carbonate apatite scaffold and hydroxyapatite scaffold. SHED combined with carbonate apatite or hydroxyapatite scaffold can be biomaterial candidate for bone tissue engineering. The amount of SHED adhesion and proliferation on hydroxyapatite scaffold was found higher than the amount of SHED adhesion and proliferation on carbonate apatite scaffold.

Acknowledgements

This study was funded by grant from Universitas Airlangga.

Declaration of Interest

The authors report no conflict of interest.

References

1. Masaki C, et al. Strategies for alveolar ridge reconstruction and preservation for implant therapy. J Prosthodont Res. 2015; 59(4): 220 – 8.
2. Li P, et al. Interferon-γ enhances the efficacy of autogenous bone grafts by inhibiting postoperative bone resorption in rat calvarial defects. J Prosthodont Res. 2016; 60(3): 167 – 76.
3. Soeroso Y, Endang WB, Bachtiar BM, Benso S, Siti WP. The Prospect of Chitosan on The Osteogenesis of Periodontal Ligament Stem Cells. Joural of International Dental and Medical Research. 2016; 9(2): 93 – 7.
4. Sheikh Z, Sima C, and Glogauer M. Bone replacement materials and techniques used for achieving vertical alveolar bone augmentation. Materials. 2015; 8, 2953 – 93.
5. Hung, NN. Basic Knowledge of Bone Grafting. 2012. Available at http://cdn.intechopen.com/pdfs/33456/InTech-Basic_knowledge_of_bone_grafting.pdf. Accessed November 23, 2015.
6. Vincent Y. Risk of Disease Transmission with Bone Allograft. Orthopedics. 2012; 35(8): 679 – 81.
7. Talaat, Wael. The Effect of Immunosuppression Agents on Stem and Endothelial Cells' Proliferation In Vitro. Journal of International Dental and Medical Research. 2015; 8(3): 116 – 22.
8. Chan, WD et al. Tissue Engineering Scaffolds for the Regeneration of Craniofacial Bone. Journal of the Canadian Dental Association. 2009; 75(5): 373 – 7.
9. Kim KM and Evans GRD. Tissue Engineering: The Future of Stem Cells. Topics in Tissue Engineering. 2005; 2: 1-21. Available at http://www.oulu.fi/spareparts/ebook_topics_in_t_e_vol2/abstracts/evans_0102.pdf. Accessed October 19, 2015.
10. Neel, EAA et al. Tissue Engineering in Dentistry: Review. Journal of Dentistry. 2014; 42: 915 – 28.
11. Kashyap, Rucha. SHED – Basic Structure for Stem Cell Research: Review Article. Journal of Clinical and Diagnostic Research. 2015; 9(3): 7 – 9.
12. Meyda B. Potential Differentiation of Pulp Cells of Primary Teeth: In Vitro. Undergraduate Thesis. Surabaya: Faculty of Dental Medicine Universitas Airlangga, 2013.
13. Amini AR, et al. Bone Tissue Engineering: Recent Advances and Challenges. Crit Rev Biomed Eng. 2012; 40(5): 363 – 408.
14. Yu X, et al. Biomaterials for Bone Regenerative Engineering. Adv Health Mater. 2015; 4(9): 1268 – 85.
15. Arbi, TA. Apex Resection and Bone Grafting After Enucleation of Periapical Granuloma: A Case Report. Proceeding on Bali Dental Science and Exhibition. 2016: 167 – 171.
16. Ferdiansyah, Rushadi D, Rantam FA, Aulani'am. Regeneration in massive bone defect with bovine hydroxyapatite as mesenchymal stem cell scaffold. JBP. 2011; 13: 3-15.
17. Arundina I, Ketut S, Hendrik S, Maretaningtias DA. Golden Sea Cucumbers (Stichopus hermanii) as Growth Factors of Stem Cells. Journal of International Dental and Medical Research. 2016; 9(3): 242 – 8.
18. Effendi MC, Boy MB, Endang WB, Ellyza H. The Effect of Nanoparticle Mineral Trioxide (NMT) on The Proliferation and Differentiation of Stem Cells Human Exfoliated Deciduous to Odontoblasts. Journal of International Dental Medical Research. 2015; 8(2): 68 – 76.
19. Seo BM, et al. SHED repair critical-size calvarial defects in mice. Oral Dis. 2008; 14: 428 – 34.
20. Egusa H, et al. Stem cells in dentistry – Part I: Stem cell sources. Journal of Prosthodontic Research. 2012; 56: 151 – 65.
21. Rajabi, AH, Behnamghader A, Kazamzadeh A, Mozarzadela F. Synthesis and characterization of nanocrystalline hydroxyapatite powder via sol gel method. Springerlink: Biomed 06, IFMBE 15, 2007: 140 – 51.
22. Earl JS, Wood DJ, Mine SJ. Hydrothermal synthesis of hydroxyapatite. Journal of Physics: Conference Series 26. 2006: 268 – 71.
23. Teixeira S, Rodriguez MA, Pena P, De Aza AH, Ferraz P, Monteiro FJ. Physical characterization of hydroxyapatite porous scaffolds for tissue engineering. Material Science and Engineering C. 2009: 29(5): 1510 – 14.
24. Loh, QL and Choong C. Three-Dimensional Scaffolds for Tissue Engineering Application: Role of Porosity and Pore Size. Tissue Eng Part B Rev. 2013: 19 (6): 485 – 502.
25. Tripathi, G and Bikramjit B. A porous hydroxyapatite scaffold for bone tissue engineering: Physico-mechanical and biological evaluations. Ceramics Internationals. 2012; 38: 341 – 9.
26. Boskey, AL. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. International Bone and Mineral Society. 2013; 2(447): 1 – 11.
27. MacMahon HT, Doherty DJ. Mediation, modulation and consequences of membrane cytoskeleton interactions. Annual Review of Biophysics. 2008; 37: 65 – 95.
28. Ana ID, Matsuya S, Ishikawa K. Engineering of carbonate apatite bone substitute based on composition-transformation of gypsum and calsiumhidroxide. Scientific Research Engineering J. 2010; 2: 344 – 52.