Perspective

SHED, PRF, and Chitosan as Three-Dimensional of Tissue Engineering for Dental Pulp Regeneration

Kelvin Alfan Nola Anggrarista[1], Pamela Handy Cecilia[1], Ayu Anggraini Broto Nagoro[1], Tania Saskianti[2], Meircurius Dwi Condro Surboyo[3]

[1]Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Department of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, [3]Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

The gold standard for pulpitis irreversible treatment is root canal treatment. However, it caused the loss of tooth vitality. To restore tooth vitality, materials that have regenerative ability in the pulp is needed. The exfoliated deciduous teeth stem cells (SHED) not only expressed specific markers for mesenchymal stem cells (MSC) but also induced the odontoblastic differentiation, and stimulating the formation of endothelium and fibroblast. The combination SHED with platelet rich fibrin (PRF) and chitosan were able to facilitate and increase the migration, proliferation and odontoblastic differentiation of dental pulp cells. Based on that fact, the combination of SHED, PRF and chitosan as three-dimensional tissue engineering is promising as new modality to pulp regeneration in the clinical setting. The purpose of this review is describing the potential combination of SHED, PRF, and chitosan scaffold as three-dimensional tissue engineering for pulp regeneration.

Keywords: Chitosan, dental pulp, PRF, SHED, tissue engineering

Introduction

Pulpectomy, a procedure to remove the dental pulp, is mainly done to prevent the continued inflammation of the pulp that can cause root channel infections and pain related. Even though pulpectomy followed by root canal treatment can remove the primary cause of pulpitis, it cannot regenerate the already nonvital tooth. External stimuli tolerance declines in nonvital teeth due to total loss of immune and perception systems, and the tooth is vulnerable due to metabolic capability loss.[1] Moreover, the root canal retreatment success rate is not high and often has to be repeated. The repeated treatment makes the teeth more fragile and causes the roots to crack or fracture, which leads to decrease of the well-being.[2]

The gold standard for sealing material after pulpectomy and root canal treatment is calcium hydroxide. This material has disadvantages and low properties, such as has higher solubility, able to dissolve within saliva, and unable completely setting, to completely closing the pulp cavity and sealing the root canal after application.[3] In some cases, calcium hydroxide showed internal resorption that confined to the tooth and did not show any clinical symptoms. The internal resorption of root systems, increase in interradicular radiolucency, and also presented with clinical symptoms of abscess and mobility.[4]

To restore tooth vitality, material that has regenerative ability in the pulp is needed. Tissue engineering is creates three-dimensional functional tissues combining cells, bioactive molecules, and scaffolds. This uses the basic principles of engineering and life sciences to establish biological replacement that improve, maintain, or restore the functions of tissue. The fundamental principle of tissue engineering started with cells that were isolated from a source and expanded into a cell culture system, then seeded onto a matrix that provides structural support along with addition of appropriate growth factor. The cells differentiate, proliferate, and migrate to carriers and

Access this article online

Quick Response Code:

Website: www.dentalhypotheses.com

DOI: 10.4103/denthyp.denthyp_121_20

Address for correspondence: Meircurius Dwi Condro Surboyo, Department of Oral Medicine. Faculty of Dental Medicine, Universitas Airlangga. Surabaya, Indonesia.
E-mail: meircurius-2015@fkg.unair.ac.id

Received: 13 August 2020 **Revised:** 23 August 2020
Accepted: 5 September 2020 **Published:** 2 March 2021

How to cite this article: Anggrarista KN, Cecilia PH, Nagoro AB, Saskianti T, Surboyo MC. SHED, PRF, and Chitosan as Three-Dimensional of Tissue Engineering for Dental Pulp Regeneration. Dent Hypotheses 2021;12:43-6.

forming new tissues, replacing the old one.[5] In irreversible pulpitis treatment, tissue engineering is convinced to be a new innovation because it can form new tissue with its complex properties, so it can regenerate the dental pulp.

Human exfoliated deciduous teeth stem cells (SHED) have the ability to form odontoblast-like cells to reparative dentin building and pulp repair. These stem cells are combined with growth factor in form of platelet-rich fibrin (PRF) to direct the growth and differentiation of stem cells and planted in with chitosan scaffold to facilitate required tissue pulp formation, establishing the three-dimensional of tissue engineering that has the ability to optimize pulp regeneration. This treatment aims to accelerate the regeneration of pulp tissues by *in vivo* regeneration of complicated functional tissues that consist of a cell, growth factor, and scaffold produced from natural materials for its structural support and proper medium for its differentiation and proliferation.

Perspective

SHED are stem cells extracted from pulp tissue in healthy human deciduous teeth.[6] Using an exfoliative deciduous tooth as a source of stem cell will not face ethical problems. SHED expressed positive expression a specific marker for mesenchymal stem cells (MSCs) such as CD19,[7] CD29,[8] CD31,[7] CD34,[7] CD90,[9] CD146,[10] CD105,[10] CD73,[11] CD44,[12] and CD10.[13] SHED are able to induce the odontoblastic differentiation by increased factor related to differentiation such as dentin matrix protein-1 (DMP-1), and dentin sialophosphoprotein (DSPP). Both are the key of noncollagenous proteins involved in dentin mineralization and main factor for the pulp tissue forming.[14]

SHED display a similar pattern of expression for a number of endothelium-associated markers such as the vascular cell adhesion molecule 1,[13] smooth muscle such as α-smooth muscle actin,[13] bone such as alkaline phosphatase (ALP),[15] type I collagen (COL-1),[13] type II collagen (COL-2),[10] osteonectin (ONN),[13] osteopontin (OPN),[13] osteocalcin (OCN),[10,13] bone sialoprotein (BSP),[9,10] runt-related transcription factor-2 (Runx2),[8] fibroblasts such as type III collagen (COL-3),[13] and fibroblast growth factor 2 (FGF-2).[13]

Other factor is that SHED are able to induce the secretion of proinflammatory cytokine such as interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor-α . SHED are also capable of inhibiting the CD178 of the lymphocytes, suppressing the proliferation of lymphocyte, and decreasing the secretion of IL-4 and IFN-γ while sequentially increasing the number of T-reg cells.[16]

Further research has shown that SHED can be induced into functional odontoblast *in vitro*. It can differentiate into odontoblast-like cells and regenerate the tissue with

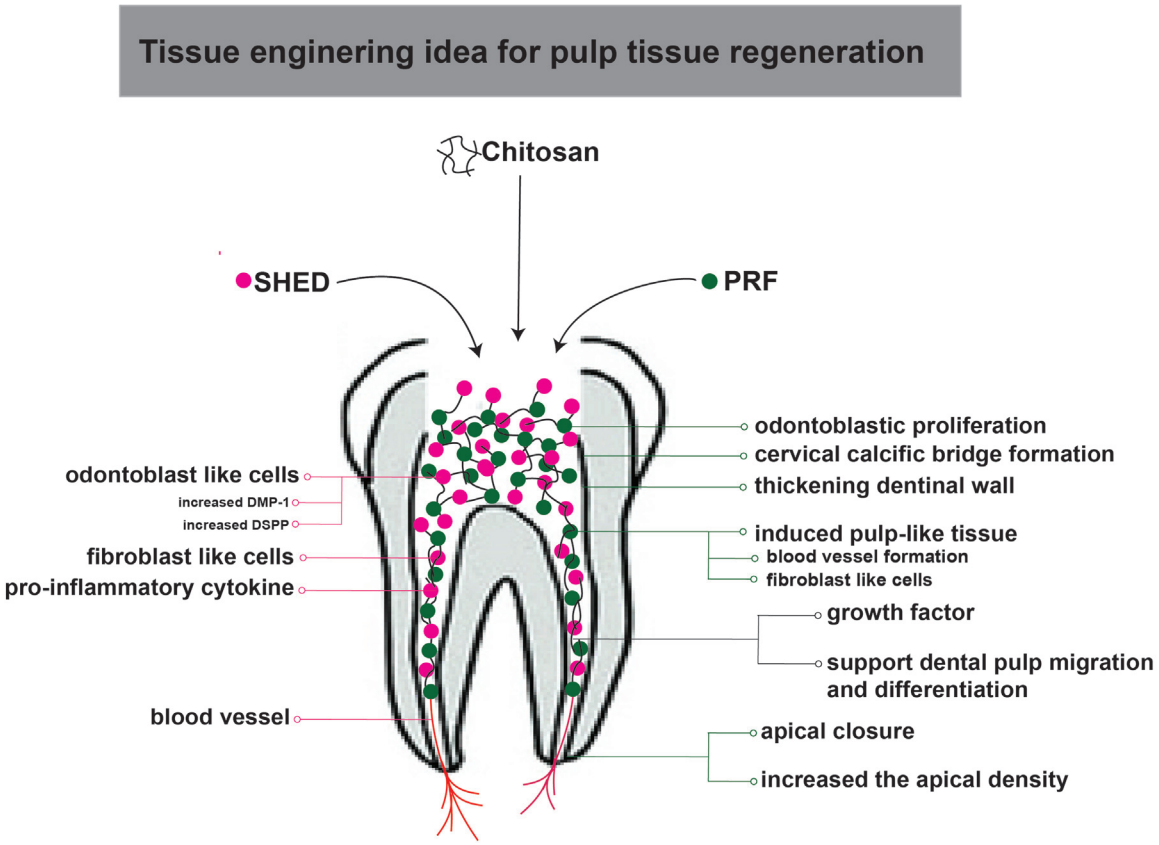

Figure 1: The combination of SHED, PRF, and chitosan for dental pulp regeneration. SHED, Human exfoliated deciduous teeth stem cells; PRF, platelet-rich fibrin.

comparable cellularity and architecture to the physiological dental pulp when cultured in scaffolds.[11]

SHED are highly capable of differentiation and proliferation, and able to represent as a good cell sources for dental tissue regeneration mediated by mesenchymal stem cell (MSC) [Figure 1], which contain stem cells that can express specific gene such as pulp dan dentinal complexes while having an appropriate tissue regeneration capacity. SHED have the abilities to form odontoblast-like cells, indicated by odontoblastic differentiation markers such as DSPP and DMP-1.[17] DSPP are processed by proteases and interact with integrin 6 to induce SMAD 1/5/8 phosphorylation and nuclear translocation via P38 and ERK1/2 pathway, resulting in differentiation into odontoblast-like cells. DMP-1 is involved in maintaining dentin mineralization.[18] The regenerated pulp tissue built by SHED has the similar cellularity and architecture to the physiologic dental pulp.[6] The potential of SHED, not only able to form odontoblast-like cells but also able to suppress the inflammation by inhibit the proinflammatory cytokine production.[16]

PRF has an equilateral fibrin branch junction that makes its architecture flexible, allowing it to support cellular migration and enmeshment of cytokine. PRF can gradually release growth factors such as transforming growth factor β (TGF-β), insulin growth factor (IGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) to the tissue. TGF-β serves as the mediator for tissue regeneration by forming fibroblast and regulating odontoblast differentiation. PDGF is the main regulator for migration and proliferation of the stem cells. EGF has the ability to stimulate mitosis in stem cells. IGF and FGF enhance odontogenic differentiation via activation of MEK/ERK signaling pathway and deposition of extracellular matrix in differentiating cells. VEGF serves to increase the formation of new blood vessels, carry nutrients, and increase the blood flow direction toward the regenerating area.[19]

In various research, PRF is able to increase cervical calcific bridge formation,[20] lateral dentinal wall thickening and apical closure.[21] The root canal is also filled with the pulp-like tissue formation,[22] consisting of blood vessels and fibroblast-like cells.[23]

The characteristics of hydrogels have relatively low-viscosity formulations, and are more applicable to treatments that require flowing materials to allow easy injection into empty endodontic spaces with millimeter diameter ranges. The high porosity and hydrophilic property facilitate and enhances cell colonization and proliferation. Chitosan scaffold can promote the regeneration of dental pulp by stimulated significantly higher expressions of COL-1, ALP, DSPP, and DMP-1 that leads to about five times more mineralized matrix deposition. This condition able to induced apical closure and root growth by increasing the thickness, which is needed in dental tissue regeneration.[24]

SHED attached well to the structure of chitosan while remaining viable. Moreover, the proliferative activity of SHED on the chitosan scaffold is increased significantly by the combination of growth factor. The SHED itself-able to the enhanced the osteogenic differentiation, such as strong mineral deposition, up-regulation of ALP, BSP, COL1, and expression OCN gene/protein.[25]

The combination of SHED, PRF, and chitosan scaffold is promising as a new treatment for complex root and pulp tissue system. The three combinations support each other to form new networks. Chitosan as the main carrier for SHED and PRF is expected to be able to bring cells and growth factors to reach and fill all the root canals until they reach the apical foramen. The apical foramen is the main source of vascularity and innervation for a tooth. SHED at this point are expected to be able to differentiate into odontoblast cells that can build functional pulp tissue, differentiate into neural networks and blood vessels as an initial step to start the formation of new tissue supported by the presence of PRF as a blood vessel. With the synergy of these three materials, it is hoped that the formation of new pulp tissue can occur. The idea of these combinations is very likely to be implemented and begin to be analyzed both at the *in vitro* and *in vivo* levels. The potential of each component has been proven to have the ability and support to form a pulp and dentinal tissue system in the root system. An important key of the three components is SHED, which have the main ability to form odontoblast-like cells and fibroblasts to build pulp structures.

Financial support and sponsorship

Nil.

Conflict of interest

All authors declare no conflict of interest.

References

1. Giuroiu CL, C runtu ID, Lozneanu L, Melian A, Vataman M, Andrian S. Dental pulp: correspondences and contradictions between clinical and histological diagnosis. Biomed Res Int 2015;2015:1-7.
2. Morotomi T, Washio A, Kitamura C. Current and future options for dental pulp therapy. Jpn Dent Sci Rev 2019;55:5–11.
3. Sonmez D, Duruturk L. Success rate of calcium hydroxide pulpotomy in primary molars restored with amalgam and stainless steel crowns. Br Dent J 2010;208:E18.
4. Chen X, Liu X, Zhong J. Clinical and radiographic evaluation of pulpectomy in primary teeth: a 18-months clinical randomized controlled trial. Head Face Med 2017;13:1-10.
5. Mathew A, Augustine R, Kalarikal N, Thomas S. Tissue engineering: principles, recent trends and the future. In: Nanomedicine and Tissue Engineering. New York: Apple Academic Press; 2016. p. 31-82.
6. Sukarawan W, Osathanon T. Stem cells from human exfoliated deciduous teeth: biology and therapeutic potential. In: Mesenchymal Stem Cells – Isolation, Characterization and Applications. InTech; 2017.
7. Li J, Xu SQ, Zhao YM, Yu S, Ge LH, Xu BH. Comparison of the biological characteristics of human mesenchymal stem cells derived from exfoliated deciduous teeth, bone marrow, gingival tissue, and umbilical cord. Mol Med Rep 2018;18:4969-77.

8. Hara K, Yamada Y, Nakamura S, Umemura E, Ito K, Ueda M. Potential characteristics of stem cells from human exfoliated deciduous teeth compared with bone marrow-derived mesenchymal stem cells for mineralized tissue-forming cell biology. J Endod 2011;37:1647-52.
9. Jeon M, Song JS, Choi BJ, Choi HJ, Shin DM, Jung HS, *et al.* In vitro and in vivo characteristics of stem cells from human exfoliated deciduous teeth obtained by enzymatic disaggregation and outgrowth. Arch Oral Biol 2014;59:1013-23.
10. Yu S, Diao S, Wang J, Ding G, Yang D, Fan Z. Comparative analysis of proliferation and differentiation potentials of stem cells from inflamed pulp of deciduous teeth and stem cells from exfoliated deciduous teeth. Biomed Res Int 2014;2014:1-13.
11. Shetty RM, Prasad P, Shetty S, Anushka R.V., Patil V, College D, *et al.* SHED (Stem Cells from Human Exfoliated Deciduous teeth) – A new source of stem cells in dentistry. Chhattisgarh J Helath Sci. 2013;1: 66-8.
12. Nakajima K, Kunimatsu R, Ando K, Ando T, Hayashi Y, Kihara T, *et al.* Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells. Biochem Biophys Res Commun 2018;497:876-82.
13. Vishwanath VR, Nadig RR, Nadig R, Prasanna JS, Karthik J, Pai VS. Differentiation of isolated and characterized human dental pulp stem cells and stem cells from human exfoliated deciduous teeth: an in vitro study. J Conserv Dent 2013;16:423-8.
14. Kunimatsu R, Nakajima K, Awada T, Tsuka Y, Abe T, Ando K, *et al.* Comparative characterization of stem cells from human exfoliated deciduous teeth, dental pulp, and bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun 2018;501193-8.
15. Wang X, Sha XJ, Li GH, Yang FS, Ji K, Wen LY, *et al.* Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Arch Oral Biol 2012;57:1231-40.
16. Yildirim S, Zibandeh N, Genc D, Ozcan EM, Goker K, Akkoc T. The comparison of the immunologic properties of stem cells isolated from human exfoliated deciduous teeth, dental pulp, and dental follicles. Stem Cells Int 2016;2016:1-16.
17. Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Cho A, Kohler T, *et al.* Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. Matrix Biol 2010;28:221-9.
18. Khazaei S, Khademi A, Torabinejad M, Nasr Esfahani MH, Khazaei M, Razavi SM. Improving pulp revascularization outcomes with buccal fat autotransplantation. J Tissue Eng Regen Med] 2020;14:1227-35.
19. Alkharobi HE, Al-Khafaji H, Beattie J, Devine DA, El-Gendy R. Insulin-like growth factor axis expression in dental pulp cells derived from carious teeth. Front Bioeng Biotechnol 2018;6:1-6.
20. Adel Ragab R, El Lattif AEA, El Wahab El Dokky NA. Comparative study between revitalization of necrotic immature permanent anterior teeth with and without platelet rich fibrin: a randomized controlled trial. J Clin Pediatr Dent 2019;43:78-85.
21. Lv H, Chen Y, Cai Z, Lei L, Zhang M, Zhou R, *et al.* The efficacy of platelet-rich fibrin as a scaffold in regenerative endodontic treatment: a retrospective controlled cohort study. BMC Oral Health 2018;18:1-8.
22. Zhang DD, Chen X, Bao ZF, Chen M, Ding ZJ, Zhong M. Histologic comparison between platelet-rich plasma and blood clot in regenerative endodontic treatment: an animal study. J Endod 2014;40:1388-93.
23. Wang QL i., Yang PP a., Ge LH on., Liu H. Preliminary evaluation of platelet rich fibrin-mediated tissue repair in immature canine pulpless teeth. Chinese J Dent Res 2016;19:49-54.
24. Palma PJ, Ramos JC, Martins JB, Diogenes A, Figueiredo MH, Ferreira P, *et al.* Histologic evaluation of regenerative endodontic procedures with the use of chitosan scaffolds in immature dog teeth with apical periodontitis. J Endod 2017;43:1279-87.
25. Farea M, Husein A, Halim AS, Abdullah NA, Mokhtar KI, Lim CK, *et al.* Synergistic effects of chitosan scaffold and TGFβ1 on the proliferation and osteogenic differentiation of dental pulp stem cells derived from human exfoliated deciduous teeth. Arch Oral Biol 2014;59:1400-11.