SMGroup

Tooth-Derived Stem Cells

Malgorzata Witkowska-Zimny*

Department of Biophysics and Human Physiology, Medical University of Warsaw, Poland

***Corresponding author:** Malgorzata Witkowska-Zimny, Department of Biophysics and Human Physiology, Medical University of Warsaw, Chalubinskiego 5, 02-004 Warsaw, Poland, Tel: +48 22 628 63 34; Fax: +48 22 628 78 46; E-mail: mwitkowska@wum.edu.pl

Published Date: August 10, 2015

ABSTRACT

Recently, stem cell biology has become an interesting topic, especially in the context of treating diseases and injuries using transplantation therapy. Several types of human stem cells have been isolated and identified *in vivo* and *in vitro.* Ideally, stem cells for regenerative medical application should be found in abundant quantities, harvestable in a minimally invasive procedure, and finally, safely and effectively transplanted to either an autologous or allogenic host. Adult stem cells have aroused great interest. Until the year 2000, the focus of publications in the field of adult stem cells was limited only to three types of cells, namely: Hematopoietic Stem Cells (**HSCs**), Bone Marrow Mesenchymal Stem Cells (**BM-MSCs**) and muscle satellite cells. The presence of stem cells within tooth was reported for the first time in 2000. To date, six different human dental stem/progenitor cells have been isolated. Stem cells-based tissue engineering is thought to be a promising way to replace the missing tooth and regenerate damaged tooth structures. This chapter highlights the characteristics and therapeutic potential of progenitor/stem cells isolated from dental tissues.

Keywords: Dental Tissue; Regenerative Medicine; Stem Cell

ABBREVIATIONS

APDCs–Apical Papilla-Derived Stem Cells; **BMSCS**–Bone Marrow Stromal/Stem Cells; **CD**–Cluster Of Differentiation; **DePDL**–Periodontal Ligament Of Deciduous Teeth Stem Cells; **DFPCS**–Dental Follicle Progenitor Cells; **DPSCS**–Dental Pulp Stem Cells; **IPSCS**–Induced Pluripotent Stem

Cells; **PDLSCS**–Periodontal Ligament Stem Cells; **SHED**–Stem Cells From Exfoliated Deciduous Teeth; **TEP**–Tissue Engineered Product.

INTRODUCTION

Tissue engineering is a relatively new branch of medicine that combines biology, engineering, and clinical science to be used for reconstruction or generation of new tissues and/or organs. Thus far, the developed strategies include the manipulation of the patient's own cells, the transplantation of stem cells, and the use of scaffold materials that trigger biological signals in order to accelerate the regenerative processes. The successful application of innovative therapies has been confirmed in clinical trials and experiments assessing the healing of broken bones, severe burns, blindness, deafness, damage of heart, blood vessel, nerve and muscles, and in the treatment of many others diseases. In the nearest future cell-based therapy will represent a new strategy to treat a wide array of clinical conditions. Changes observed in the body over time are due to the normal cell turnover. This sequential replacement of aged or damaged cells in tissues and organs suggested the existence of progenitor cells that replace mature, old, differentiated cells of complex tissues and organs. These progenitor cells are referred to as adult stem cells. Recent studies have revealed that adult/somatic stem cells may retain the potential to transdifferentiate from one phenotype to another, either *in vitro* or after transplantation *in vivo*.

The scope of current research includes the search for new sources of stem cells (dental pulp, hair follicles, amniotic fluid), and the investigation of their biology. Recently, the potential therapeutic applications of stem cells have aroused great interest. A few studies gave evidence that teeth contain special populations of adult stem cells (Figure 1)**.** Although the regeneration potential of mammalian teeth is limited, the presence of stem cells was demonstrated in dental pulp, periodontal ligament, dental papilla, and dental follicle. Therefore, many efforts have been made in the field of teeth engineering to explore these populations of stem cells. So far six different human dental-tissue-derived stem/progenitor cells have been isolated and characterized. They can be classified in two main groups: (i) connected with dental pulp and (ii) associated with the periodontium.

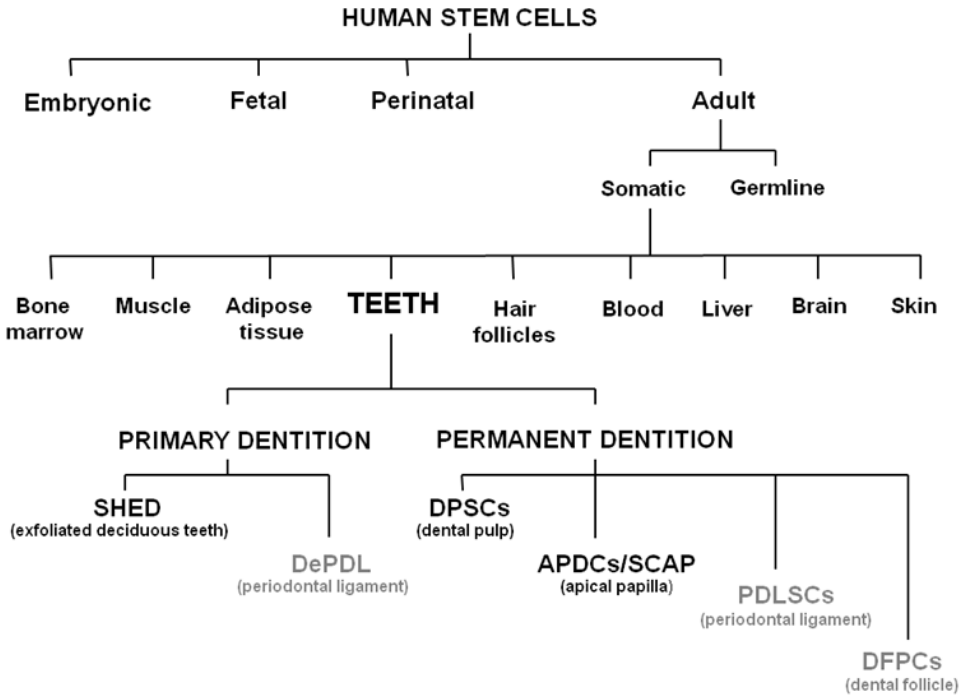

Figure 1: Diagram of human stem cells. Dental stem/progenitor cells associated with periodontium are colored in grey.

TOOTH-DERIVED STEM CELLS

Teeth develop from two interacting tissues: mesenchyme and ectodermal epithelium that initially forms lining of the mouth. The initiation of teeth formation starts around the 37th day of gestation. Teeth are complex organs containing dentine, enamel and dental follicle. The existence of stem cells within tooth was reported for the first time in 2000 [1]. Shi group isolated cells with high proliferative potential for self-renewal from adult human dental pulp that are capable to develop into multiple cell lineages *in vitro*. Since that time, various tooth-derived stem cells have been isolated and characterized, including: Dental Pulp Stem Cells **(DPSCs),** Apical Papilla Derived Stem Cells (**Apdcs/SCAP**), Dental Follicle Progenitor Cells (**Dfpcs**), Periodontal Ligament Stem Cells (**Pdlscs**) obtained from permanent dentition as well as Stem Cells Obtained from Exfoliated Deciduous Teeth (**SHED**) or Periodontal Ligament Of Deciduous Teeth Stem Cells (**DePDL**) typically isolated from primary dentition (Figure 2).

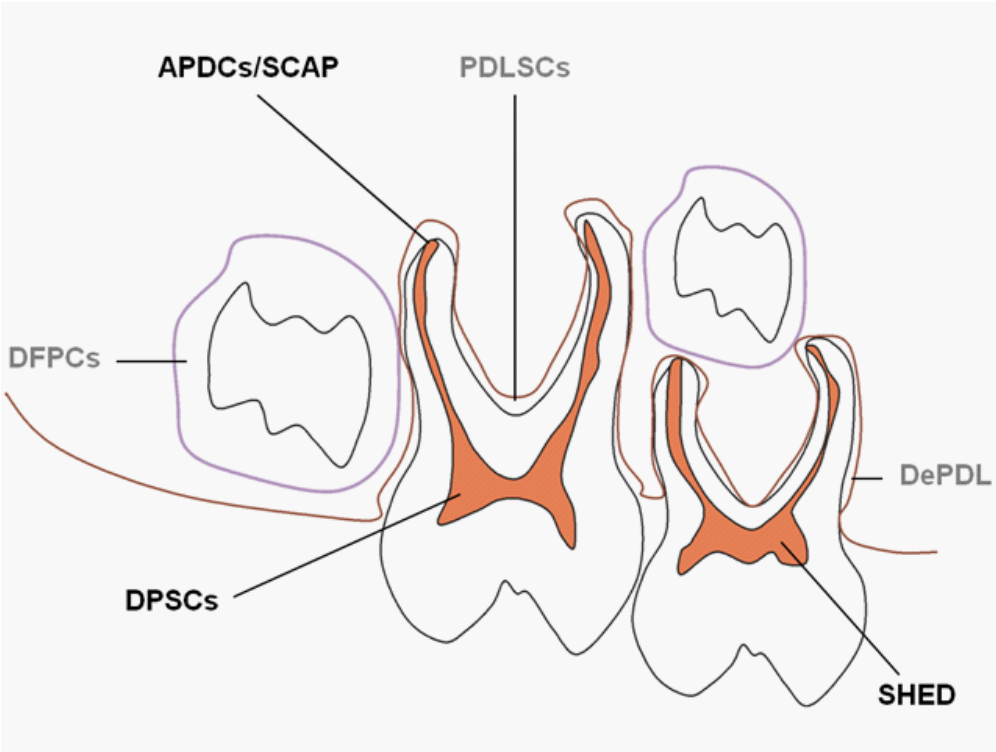

Figure 2: Sources of tooth-derived stem cells.

Mesenchymal stromal/stem cell characterization is based on the expression of cell-specific proteins and CD markers. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal set of four criteria for the identification of human mesenchymal stem cells [2], namely: (i) ability to adhere to plastic when maintained under standard culture conditions; (ii) the ability for osteogenic, adipogenic, and chondrogenic differentiation; (iii) expression of **CD73, CD90, CD105**; and (iv) lack of hematopoietic linage markers expression (**CD14, CD11b, CD34, CD45, CD19, CD79**).

The key characteristics of cells isolated from dental tissues as stem/stromal cells include: the ability to adhere to plastic to form fibroblast-like colonies; an extensive proliferative capacity; and the ability to express several common cell-surface antigens [3]. They also possess the capacity to differentiate into several mesodermal lineages, including bone, muscle, cartilage and epithelium, as well as neural progenitors [4]. However, heterogeneity has been observed within populations of stem cells isolated from dental tissues. Human deciduous and permanent teeth exhibit different developmental processes, morphologies, histological characteristics and life cycles. In addition, their pulp tissues react differently to external stimuli, such as pulp sensitivity test, dental trauma and pulp therapy materials. These suggest differences in gene expression and regulation, and many studies compared gene-expression profiles of the cells from different dental tissues for example

human dental pulp from deciduous and permanent teeth [5,6]. Deciduous and permanent human teeth represent an excellent model system to study aging of stromal populations. Aging is tightly connected to self-renewal and proliferation and thus, mapping potential molecular differences between populations constitutes an important task.

DENTAL PULP STEM CELLS (DPSCS)

DPSCs were the first cells isolated from dental pulp of third molars (wisdom teeth) from adults (19-29 years of age). The pulp tissue was gently separated from the crown and root and degradation using enzymatic digestion (collagenase and dispase) was performed. After filtration and washing in growth medium single-cell suspensions were seeded into culture plates. Thus isolated cells are able to adhere to plastic and form fibroblast-like colonies, extensively proliferate, form odontoblasts and possess the capacity to differentiate *in vitro* into various cell types e.g. osteoblast-, adipocytes-, neural-like cells under stimulation in culture medium [7,8].

The best known example of an adult stem cell is Bone Marrow Stromal/Stem Cell (**BMSCs**), considered to be the gold standard in the assessment of *in vitro* differentiation of mesenchymal stem cells. All newly discovered types of progenitor and stem cells are compared with them. DPSCs occurred at an apparently higher frequency in comparison to the BMSCs. The proliferation analysis revealed that human DPSCs have faster population doubling time than BMSCs *in vitro*. The studies performed by Shi group demonstrated that DPSCs and BMSCs share a similar pattern of protein expression [1,9]. DPSCs can be long-term cultured with no sign of senescence or changes in viability, phenotype, and genotype [10]. However, it was demonstrated that DPSCs telomere length decreases during extensive cell proliferation. In eukaryotes, the chronological cell ageing is studied through telomere shortening of their chromosomes. It is not clear if this observation can diminish transplantation capacity of these stem cells in therapeutic applications.

After transplantation into immunocompromised mice, DPSCs generated a dentine/pulp-like structure *in vivo* and expressed human-specific transcripts for dentin matrix component like osteocalcin or bone sialoprotein. Interestingly, DPSCs exist and can be isolated also from inflamed pulps, but there are some differences between DPSCs derived from inflamed and normal pulps [11].

DPSCs have a remarkable ability to survive in extremely adverse conditions what was observed by Thimios A. Mitsiadis and Anna Woloszyk in an opinion article [12]. It has been proposed that stem cells can adopt a reversible quiescence state characterized by reduced metabolic activity in hypoxic and low-nutrient conditions [13]. However, the mechanisms that allow quiescent stem cells to survive metabolic or environmental stress, to preserve their cellular and genomic integrity and to assure long-term survival are not yet elucidated. It has been suggested that quiescence is an actively maintained state regulated by intrinsic mechanisms to sustain metabolic function during persistent environmental stress, and thus ensuring stem cell survival [13].

Access to the DPSCs collection site is relatively easy. As they can be extracted with high efficiency, exert extensive differentiation ability, and demonstrate interactivity with biomaterials what DPSCs are considered ideal for tissue reconstruction.

APICAL PAPILLA-DERIVED STEM CELLS (APDCS, ALSO STEM CELLS FROM APICAL PAPILLA/SCAP)

Apical papilla is the soft tissue found at the apices of developing permanent teeth. Mesenchymal cells within the dental papilla are responsible for production of dentin and pulp. First report about new class of cells with high proliferation activity and multilineage differentiation potential obtained from immature tip of the apical papilla of human developing third molars was published by Abe et al. in 2007 [14,15]. Since then, only few information on human progenitor cells from apical papilla is available in the literature. APDCs show higher proliferation rate and mineralization potential than DPSCs, and express typical MSC markers, including **STRO-1**, **CD73**, **CD90**, and **CD105** [16]. Similarly to dental follicle progenitor cells, APDCs represent a population of cells from the developing tissue and might thus exhibit greater plasticity than other dental stem cells. The capacity of APDCs to differentiate into functional dentinogenic cells has been verified using animal models. They are capable to form odontoblast-like cells and produce dentin *in vivo*, and can serve as a cell source of primary odontoblasts for root dentin formation [17]. They are only slightly immunogenic and inhibit the proliferation of T cells and a one-way mixed lymphocyte reaction (MLR) *in vitro*. The mechanism of this immunomodulatory property and its function *in vivo* remains unknown [16].

DENTAL FOLLICLE PROGENITOR CELLS (DFPCS)

Dental follicle consists of various tooth germline tissues. Ectomesenchyme surrounds the dental/enamel organ and form a dental follicle which plays an important role in tooth development and will produce all the supporting structure of a tooth: cementum, alveolar bone, and periodontal ligaments. Such cells could therefore play a key role in periodontal regeneration. Dental follicle cells can be easily isolated after wisdom tooth extraction. Third molar is very often extracted during orthodontic therapy or to avoid inflammation, so dental follicle, like other parts of third molars, is commonly discarded as a medical waste. Thus third molars could be a practical source of cells for potential therapeutic applications. The existence of progenitor/stem cells within dental follicle was reported for the first time in 2002 [18]. DFPCs can differentiate toward a cementoblast, osteoblast, periodontal ligament, adipogenic, osteogenic, and neuronal lineage [19, 20]. Tsuchiya et al. results suggest that DFSCs potential for bone formation is similar to BMSCs [21]. Like PDLSCs, which originate from DFPCs, they are also a promising tool for periodontal tissue regeneration.

PERIODONTAL LIGAMENT STEM CELLS (PDLSCS)

The mesenchyme of the dental sac condenses to form the periodontal ligament (fiber). Periodontal ligament (PDL) is a tissue that connects cementum and alveolar bone to maintain and support teeth *in situ*. PDL performs supportive, sensory, nutritive, regenerative and homeostatic functions. After separation of PDL from the surface of the third molar root and enzymatic digestion, a unique stem-cell population can be isolated. These cells are plastic-adherent when maintained under standard culture conditions and have the ability to differentiate into cementoblast-like cells, adipocytes *in vitro* and cementum/PDL-like tissue *in vivo* [22]. Similar to the other dental stem cells described above, PDLSCs have the ability to differentiate into osteogenic, adipogenic, and chondrogenic cells under defined culture conditions [23].It is considered that PDLSCs have a potential for regeneration of periodontal tissues.

STEM CELLS FROM EXFOLIATED DECIDUOUS TEETH (SHED)

At about 7 years of age the process of replacement of 20 deciduous teeth for 32 permanent teeth begins. Although exfoliated deciduous teeth seem dead, Miura et al. unexpectedly isolated a distinctive population of multipotent stem cells from the remnant pulp of exfoliated deciduous teeth [24]. The method of isolation was the same as for DPSCs. Stem Cells from Human Exfoliated Deciduous Teeth (SHED) are highly proliferative population of clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts. It has been shown that SHED have the highest proliferation rates *in vitro* compared to BMSCs or even DPSCs [25]. SHED transplanted onto scaffolds/tooth slices into immunodeficient mice differentiated into functional odontoblasts and generated dentine and angiogenic endothelium [26]. SHED also demonstrate a strong capacity to induce recipient cell mediated bone formation *in vivo*. According to many investigations, SHED cannot differentiate directly into osteoblasts but do induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells. SHED can be used for immune modulation in clinical practice since induce an immune regulatory phenotype, evidenced by changes in maturation and differentiation rates, inhibition of lymphocyte stimulation and ability to expand CD4+ T cells [27]. Because children naturally lose deciduous teeth, there are multiple opportunities to harvest this type of stem/progenitor cells in painless and minimally invasive procedure. Thus exfoliated teeth can be a unique resource for stem-cell transplantation in regenerative dentistry. Besides, in the future, the possibility to bank these cells may provide sources both for allogenic and autologous cell replacement in later life.

PERIODONTAL LIGAMENT OF DECIDUOUS TEETH STEM CELLS (DEPDL)

Literature data on the isolation and characterization of human Deciduous Periodontal Ligament Stem Cells (DePDL) are limited. DePDL show self-renewal ability and have the potential

to differentiate into osteoblasts-like, and chondrocytes-like cells, comparable with previously characterized dental stem cells, DPSCs, SCAP and PDLSCs. However, DePDLs lacked capacity to differentiate into adiopogenic-like cells [28]. This finding is contrary to results of Ji et al., who demonstrated adipogenic abilities in both periodontal ligament stem cells from deciduous and permanent teeth [29]. The biology and potential therapeutic applications of stem cells remains within the scope of current research interest. The successful use of any type of stem cells in regeneration therapy may be achieved only after we understand their extensive characteristics in detail.

INDUCED PLURIPOTENT STEM CELLS (IPSCS) FROM TOOTH-DERIVED STEM CELLS

Unlike adult stem cells, iPSCs have been successfully generated from mouse embryonic fibroblasts and human skin fibroblasts using retroviral transfection of the four Yamanaka factors (**Oct-4/Sox2/Klf4/c-Myc**) since 2007 [30].

The human SHED and DPSCs can undergo reprogramming to establish pluripotent stem cell lines without c-Myc expression. The work of Chang et al. has demonstrated that dental pulp stem cells from deciduous and permanent teeth can be reprogrammed into iPSCs without c-Myc and also had the capacity to differentiate into neuron-like cells [31]. In that study, the cells derived from iPSCs without c-Myc did not develop tumors during reprogramming. Ultimately, there results may prove to be of substantial value in assisting future investigations in the field of neuro-regenerative medicine.

PERSPECTIVES

Technologies implementing stem/progenitor cells might start the new era of personalized medicine. There is no doubt that the description of tissue engineering offers a new hope to both patient who suffer from tooth loss and the dentist as well. Although teeth are nonessential for life and thus they are not considered a major target for regenerative medicine research unlike bones or blood vessels; however, they are extremely desirable by patient mainly for aesthetic reasons. Dental stem/progenitor cells could be feasible tools for dental tissue engineering. Numerous attempts have been made to produce a tooth so far and the obtained results are very promising.

There is a growing number of preclinical studies examining the potential of dental-tissue-derived stem/progenitor cells. They have been used in tissue engineering studies in animals: (i) Sonoyama et al. using a minipig model, transplanted both human SCAP and Periodontal Ligament Stem Cells (PDLSCs) to generate a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function [32]; (ii) Liu et al. explored the potential of using autologous Periodontal Ligament Stem Cells (PDLSCs) to treat periodontal defects in a porcine model of periodontitis [33].

Tissue engineering is defined as "…an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ..." [34]. There are several potential aspects of using cells in reparation and reconstruction of dental tissues. Tissue Engineered Products (TEPs) consisting of scaffold and cells will become an alternative to the currently used methods of substitution and regeneration of damaged or removed tissues. Despite the hope evoked by regenerative dentistry, patients and dentists eagerly anticipate TEPs. Nevertheless, we still need a lot of patience until TEPs become easily accessible on the medical market. At the moment there is no product approved in reparative and reconstructive dentistry, but there are some extensively studied on the way. It is certain that the field has the explosive potency.

Based on the promising results of animal experiments with dental stem/progenitor cells, a few clinical trials have also been initiated. However, compared with the more conventional sources of stem cells i.e. bone marrow or adipose tissue, the number of reports describing the results of clinical investigations using stem cells derived from dental tissues is still limited.

Since the discovery of the dental pulp stem cells approx. 10 years ago, several studies have reported various types of stem/progenitor cells in mature permanent teeth, developing teeth, and tooth germs [1, 35-39]. However, to date, there are few published papers concerning their in-depth characterization. Despite the fact that dental tissue-derived stem/progenitor cells share several common characteristics (high proliferation rate, multidifferentiation potential, high viability), they present significant heterogeneity that may be enhanced with their different function in tissue microenvironment.

There is little information about the immunological features of dental-related stem/progenitor cells in the literature. They are available only in specific points of life, some of them only during wisdom tooth eruption. Their quantities are debatable. Ideally, as mentioned above, stem cells for regenerative medical applications should be found in abundant quantities, harvestable in a minimally invasive procedure, then safely and effectively transplanted to either an autologus or allogenic host. Today, the practical use of dental stem/progenitor cells is still problematic. Although tooth banking is not currently a popular practice, the trend is catching up mainly in developed countries. Banking tooth-derived stem cells is a reasonable and simple alternative to harvesting stem cells from other tissues. Current licensed tooth banks are summarized in Table 1.

Table 1: List of licensed tooth banks.

Name of bank	Country	Website
Store-A-Tooth	USA/ Littleton, MA	www.store-a-tooth.com
StemSave	USA/New York	www.stemsave.com
BioEden	USA/ Austin, TX	http://us.bioeden.com/
Tooth Bank	USA/ Brownsburg, IN	http://www.toothbank.com
National Dental Pulp Laboratory	USA/ Newton, MA	http://www.ndpl.net/
Advanced Center for Tissue Engineering,	Japan	www.acte-group.com
Teeth Bank, Co.	Japan	www.teethbank.jp
Stemade Biotech Pvt.	India	www.stemade.com
The Norwegian Tooth Bank	Norway	www.fhi.no/morogbarn
BioBank	United Kingdom	http://www.futurehealthbiobank.com/int/en/tooth-stem-cells
Korea Tooth Bank	Republic of Korea	http://www.brts.kr

Without fail, the dental stem cells biology might provide meaningful insights into the development of dental tissue and cellular differentiation processes. There are also some safety issues inherent in stem cell therapy. Like any other new technology, it is completely unknown what are the long-term effects of such interference with nature. Before the development of effective cellular-based therapies it is necessary to better understand the mechanisms of self-renewal (allow us to regulate stem cells growth, generate sufficient cell number and decreased risk of unlimited malignant transformation), regulation of differentiation and specific tissue production, and interaction stem cells with immune system.

References

1. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000; 97: 13625-13630.
2. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315-317.
3. Saito MT, Silvério KG, Casati MZ, Sallum EA, Nociti FH Jr. Tooth-derived stem cells: Update and perspectives. World J Stem Cells. 2015; 7: 399-407.
4. Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res. 2014; 320: 92-107.
5. Kim JH, Jeon M, Song JS, Lee JH, Choi BJ. Distinctive genetic activity pattern of the human dental pulp between deciduous and permanent teeth. PLoS One. 2014; 9: e102893.
6. Kaukua N, Chen M, Guarnieri P, Dahl M. Molecular differences between stromal cell populations from deciduous and permanent human teeth. Stem Cell Res Ther. 2015; 6: 59.
7. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N. Stem cell properties of human dental pulp stem cells. J Dent Res. 2002; 81: 531-535.
8. Morsczeck C. Gene expression of runx2, Osterix, c-fos, DLX-3, DLX-5, and MSX-2 in dental follicle cells during osteogenic differentiation in vitro. Calcif Tissue Int. 2006; 78: 98-102.
9. Liu H, Gronthos S, Shi S. Dental pulp stem cells. Methods Enzymol. 2006; 419: 99-113.
10. Mokry J, Soukup T, Micuda S, Karbanova J, Visek B. Telomere attrition occurs during ex vivo expansion of human dental pulp stem cells. J Biomed Biotechnol. 2010; 2010: 673513.

11. Alongi DJ, Yamaza T, Song Y, Fouad AF, Romberg EE. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regen Med. 2010; 5: 617-631.

12. Mitsiadis TA, Woloszyk A. Odyssey of human dental pulp stem cells and their remarkable ability to survive in extremely adverse conditions. Front Physiol. 2015; 6: 99.

13. Cheung TH, Rando TA. Molecular regulation of stem cell quiescence. Nat Rev Mol Cell Biol. 2013; 14: 329-340.

14. Abe S, Yamaguchi S, Watanabe A, Hamada K, Amagasa T. Hard tissue regeneration capacity of apical pulp derived cells (APDCs) from human tooth with immature apex. Biochem Biophys Res Commun. 2008; 371: 90-93.

15. Abe S, Yamaguchi S, Amagasa T. Multilineage cells from apical pulp of human tooth with immature apex. Oral Science International. 2007; 1: 45-58.

16. Ding G, Liu Y, An Y, Zhang C, Shi S. Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs. 2010; 191: 357-364.

17. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod. 2008; 34: 166-171.

18. Handa K, Saito M, Yamauchi M, Kiyono T, Sato S. Cementum matrix formation in vivo by cultured dental follicle cells. Bone. 2002; 31: 606-611.

19. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 2005; 24: 155-165.

20. Yao S, Pan F, Prpic V, Wise GE. Differentiation of stem cells in the dental follicle. J Dent Res. 2008; 87: 767-771.

21. Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ. Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connect Tissue Res. 2010; 51: 197-207.

22. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004; 364: 149-155.

23. Xu J, Wang W, Kapila Y, Lotz J, Kapila S. Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev. 2009; 18: 487-496.

24. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003; 100: 5807-5812.

25. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod. 2009; 35: 1536-1542.

26. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA. SHED differentiate into functional odontoblasts and endothelium. J Dent Res. 2010; 89: 791-796.

27. Silva Fde S, Ramos RN, de Almeida DC, Bassi EJ, Gonzales RP. Mesenchymal stem cells derived from human exfoliated deciduous teeth (SHEDs) induce immune modulatory profile in monocyte-derived dendritic cells. PLoS One. 2014; 9: e98050.

28. Owusu Y. Isolation and Characterization of Periodontal Ligament Stem Cells of Human Deciduous Teeth- project. 2014.

29. Ji K, Liu Y, Lu W, Yang F, Yu J. Periodontal tissue engineering with stem cells from the periodontal ligament of human retained deciduous teeth. J Periodontal Res. 2013; 48: 105-116.

30. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.

31. Chang YC, Li WC, Twu NF, Li HY, Lo WL. Induction of dental pulp-derived induced pluripotent stem cells in the absence of c-Myc for differentiation into neuron-like cells. J Chin Med Assoc. 2014; 77: 618-625.

32. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One. 2006; 1: e79.

33. Liu Y, Zheng Y, Ding G, Fang D, Zhang C. Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells. 2008; 26: 1065-1073.

34. Langer R, Vacanti JP. Tissue engineering. Science. 1993; 260: 920-926.

35. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res. 2009; 88: 792-806.

36. Nishino Y, Yamada Y, Ebisawa K, Nakamura S, Okabe K. Stem cells from human exfoliated deciduous teeth (SHED) enhance wound healing and the possibility of novel cell therapy. Cytotherapy. 2011; 13: 598-605.

37. Ikeda E, Yagi K, Kojima M, Yagyuu T, Ohshima A. Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation. 2008; 76: 495-505.

38. Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z. The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials. 2006; 27: 5658-5668.

39. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A. 2010; 16: 605-615.