Journal of Science and Applications:
BioMedicine
Review

Odontogenic stem cells: biological characteristics and application in the dental tissue engineering

Guo Ye [1, 2], Xuesong Yu [2], Lei Dou [2], Yan Wu [2], Yan Zhang [2, 3*]

[1] *Chongqing Municipal Key Laboratory of Oral Biomedical Engineering of Higher Education, Chongqing, 401147, P.R. China;*
[2] *College of Stomatology, Chongqing Medical University, Chongqing, 401147, P.R .China;*
[3] *Chongqing Key Laboratory of Oral Diseases and Biomedical Sciences, Chongqing, 401147, P.R. China.*
[*] *Corresponding author: Tel: +86-23-88602353; Fax: +86-23-88860222; email: lexiner82@126.com.*

Abstract: The generation of dental structures depends upon the manipulation of stem cells and requires a synergy of all cellular and molecular events that finally lead to the formation of tooth-specific hard tissues, dentin and enamel. Five different types of dental stem cells have been isolated from dental soft tissues: dental pulp, apical papilla, dental follicle and periodontal ligament. The characteristic features of these cells have been explored. They express various arrays of biomarkers including those specific for mesenchymal and/or embryonic stem cells. In vitro and in vivo studies have revealed that these stem cells varied in their proliferation and differentiation potential. Recent studies have demonstrated their wide range of plasticity and their potential use for regenerative medicine and dentistry. This review focuses on the different sources of dental stem cells and discusses their potential use in regenerative medicine. Nevertheless, the development of biological approaches for dental reconstruction using stem cells is promising and remains one of the greatest challenges in the dental field for the years to come.

Published by www.inter-use.com. Available online Dec, 10 Vol. 4 Iss. 5, Page 50-54.

Keywords: Odontogenic stem cells, Differentiation, Regeneration, Dental tissue engineering

1. Introduction

Tooth development results from sequential and reciprocal interactions between the oral epithelium and the underlying neural crest-derived mesenchyme [1,2]. Compared with bone marrow mesenchymal stem cells (BMMSCs), odontogenic stem cells show more odontogenic differentiation than bone differentiation. Odontogenic stem cells are derived from the cranial neural crest, also known as ectomesenchymal stem cells, and have pluripotent differentiation potentials such as mineralization, cartilage, fatization, and neurotoxicity [3,4]. Therefore, odontogenic stem cells are an ideal seed cell for tooth regeneration, which has good research value and application prospect in dental tissue engineering.

At least five types of odontogenic stem cells have been isolated and identified [5], dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), Stem cells from human exfoliated deciduous teeth (SHED), stem cells from the apical papilla (SCAP), dental follicle stem cells (DFSCs), and so on. In this review, the biological characteristics of these five kinds of odontogenic stem cells and their applications in dental tissue engineering are summarized.

2. Dental Pulp Stem Cells (DPSCs)

In 2000, Gronthos et al. [6] isolated the first MSC like cells from the human dental pulp tissue of adult third molar. It is proved that dental pulp stem cells have good self-proliferation ability and multi-directional differentiation potential, and the most important characteristic is that they can differentiate into odontoblast and participate in the formation and repair of dentin. Yang et al. [7] suggested that stromal cell antigen (STRO-1) positive pulp cells differentiate into odontoblast cells, whereas STRO-1-negative dental pulp cell populations show fibroblast-like cells Phenotype. Abe et al [8] from the development of complete human apical root canal cells removed from the apical pulp cells, with the ability to induce hard tissue. Takeda et al. [9] isolated DPSCs from the tooth germs in the bell stage and found that these cells had a strong ability to proliferate and have the potential to form dentin-like matrices. Cavaleant [10] and other test DPSCs can express dentin saliva (DPSC), which can also form neurospheres and express a variety of neural stem cells in serum-free medium, indicating that DPSCs

contain the neural crest-derived primitive cells.

A large number of studies in vivo on DPSCs have been carried out. The DPSCs have been expanded in vitro and then combined with hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolds for subcutaneous transplantation in nude mice. After a few weeks the pre-dentin, mineralized dentin, odontoblast layer and pulp tissue typical pulp-dentin complex-like structure were formed. Using the third molar of DPSCs as the seed cells, the problem of immune rejection can be avoided, and the third molar is the tooth with the latest eruption time in the oral cavity, and the pulp should contain more undifferentiated cells, which is more favorable for seed cells.

3. Periodontal Ligament Stem Cells (PDLSCs)

In 2004, Seo et al. [11] isolated PDLSCs from the human periodontal ligament. Stem cell niches were located around the blood vessels [12]. These cells expressed early markers of mesenchymal stem cells (STRO-1, CD106, CD146, etc.), bone markers (type Ⅰ collagen, alkaline phosphatase, osteocalcin, osteopontin) and specific tendon transcription factor Scleraxis [13]. Recent studies have shown that periodontal ligament cells containing multiple cell subtypes, can be divided into two phenotypes: osteoblastic phenotype (fibroblastic phenotype) and fibroblast phenotype (fibroblastic phenotype). It is a group of heterogeneity and differentiation potential of the cell population. These subtypes of cells can be in different stages of differentiation or have a different trend of differentiation, under certain conditions can be differentiated into osteoblasts, fibroblasts and cementoblasts [14].

Periodontal ligament stem cells have been regarded as ideal seed cells for periodontal defect repair and periodontal tissue regeneration. Seo attached PDLSCs to the scaffold material with subcutaneous transplantation in nude mice, and formed the cementum-like material on the surface of mineralized material, and a certain angle with the sand into the mineralized material. This structure was different from that of DPSCs or BMSCs. Kramer et al. [15] co-cultured PDLSCs with BMSC, and found that osteopontin and osteopontin expression of BMSC increased significantly, while the expression of bone sialoprotein was significantly reduced, reflecting the co-culture of BMSC could get the characteristics of periodontal ligament cells. Zhao et al. [16] found that the PDLSCs layer was wrapped into completely dislocated root and reset, the new periodontal tissue can be observed. PDLSCs were transplanted to the alveolar bone defect area, and promoted the periodontal tissue reconstruction and fiber attachment. PDLSCs can be isolated from cryopreserved periodontal ligament and maintain their stem cell characteristics. These studies established the experimental foundation for the clinical application of PDLSCs in periodontal tissue regeneration.

4. Stem Cells from Human Exfoliated Deciduous teeth (SHED)

In 2003, Miura et al. [17] isolated SHED in the active pulp of deciduous teeth. Compared with DPSCs, SHED showed a stronger proliferation rate and population doublings (PDs), but the proliferation rate was about 50 % [18]. Nakamura et al. [19] compared gene expression profiles of SHED and DPSCs, and found that 4386 genes were differentially expressed. SHED was particularly involved in cell proliferation and extracellular matrix-related genes, cytokines such as fibroblast growth factor and tumor growth Factor expression was significantly higher. SHED can differentiate into neural cells, adipocytes and odontoblasts in vitro. The cells not only express the markers of STRO-1 and CD146, but also express embryonic stem cell surface antigens such as Oct4, Nanog, SSEA-3/4, so sometimes the SHED was thought to the immature DPSCs [20].

Miura implanted the expanded SHED in vitro and hydroxyapatite / tricalcium phosphate scaffold material into nude mice subcutaneously, the formation of dentin-like structure, and the expression of dentin sialophosphoprotein (DSPP), dentin matrix protein DMP1 and MEPE were detected, but cannot form the dentinal-pulp complex structure like DPSCs. Casagrande et al. [21] implanted SHED and tooth slice into nude mice subcutaneously , and found that cells can be induced to express dentin markers, but with the deproteinized tooth slice or blocking the BMP2 signal pathway SHED cannot be induced differentiation, therefore it is inferred that dentin-derived BMP2 may be an important factor for the differentiation of SHED into odontoblast cells. Sakai et al [22] also found that in vivo experiments SHED addition to functional odontoblast cells can be induced into tubular dentin, but also differentiate into vascular endothelial cells to form

capillary-like bud structure. Because the deciduous deciduous tooth stem cells have the advantages of strong proliferative ability and multi-directional differentiation ability, and are simple and non-invasive, and have low immunological rejection in autologous transplantation, the developed countries such as North America, Scandinavia and Japan have built many "deciduous banks" regeneration therapy requires [23].

5. Stem Cells from the Apical Papilla (SCAP)

In 2006, Sonoyama et al. [24] isolated a group of mesenchymal stem cells from the immature human permanent apical papilla, which had less cell mass and vascular composition than the pulp tissue-derived cells, 3 times the proliferation rate [25], was named apical papilla stem cells (SCAP). Similar to DPSCs, SCAP also showed fibroblast-like cell growth and expression of early mesenchymal cell markers STRO-1 and CD146, but specific expression of CD24 and high expression of apoptosis suppressor gene (survivin), telomerase reverse transcriptase Human telomerase reverse transcriptase (hTERT) may be involved in the development of tissue cell source. It was confirmed that SCAP could be induced into osteogenic, chondrogenic, adipogenic, neurotoxic and odontogenic differentiation, which had higher plasticity and self-renewal ability than other odontogenic stem cells. It is a promising seed cells for tissue regeneration [26].

Huang et al. [27] found in the 9 month-old minipigs the distal apical papilla of first mandibular first molar was resected and retained the integrity of the pulp, and then the root would stop development, but the other roots were normal. It indicated that SCAP play an important role in the formation and development of the root, but whether related to the presence of Hertwig's epithelial root sheath (HERS) need to be further confirmed. Sonoyama et al. implanted the HA/ SCAP-Gelfoam/PDLSCs (with SCAP and root-shaped HA/TCP scaffolds and PDLSCs-coated gelatin sponges) into the lower incisors of the minipigs. After three months, the stent surface could be formed with the dentin-like structure of the connective tissue, CT and histological analysis confirmed that the root periodontal structure was regenerated, but the biological root compression strength than the natural root weaker. Huang et al [28] also filled SCAP and PLG scaffold composite material into one end closed and one end of the human root canal, nude mice after three months of subcutaneous transplantation, showing the root canal is full of associated with angiogenesis of pulp-like tissue . The dentin-like cells in the root canal showed uniform dentin-like structure, and the odontoblast-like cells were layered and formed a small tube-like structure. Therefore, the retention of SCAP cells is necessary for the survival of clinically undeveloped autologous dental implants and has important biological significance.

6. Dental Follicle Stem Cells (DFSCs)

Dental follicle derived from the ectoderm mesenchyme and wrapped around the enamel and dental papilla around the loose connective tissue, plays an important role in the eruption of teeth and development of periodontal ligament tissue. The dental follicle contains stem cells and progenitor cells or precursors (PCs), which are believed to be the origin of periodontal ligament, cementum and alveolar bone. In 2005, Morsczeck et al. [29] successfully isolated a group of fibroblast-like dental follicle precursors from the human dental follicle of an unerupted 3rd molar, expressing stem cell markers (Notch-1, Nestin), insulin-like growth factor-2 (IGF-2) and bone sialoprotein (BSP) [30]. Luan et al. [31] isolated and immortalized three major cell lines from follicle of the 8-day-old Swiss Webster rat, one type showed high proliferative activity but no biomineralization ability, possibly as a periodontal ligament cell line; one type showed high proliferative and alkaline phosphatase activity, but in a highly undifferentiated state, may be a pluripotent stem cell line; the other type showed high mineralization ability, probably for the cementum and osteoblast cell lines, these indicated that DFSCs have the cell heterogeneity. DFSCs have the ability of multi-lineage differentiation potential, such as BMP, Wnt, IGF, NOTCH signaling pathways play an important role [32-35], and interestingly the gene expression patterns is different between before and after differentiation [36].

Handa et al. [37] transplanted DFSCs isolated from bovine tooth germs into subcutaneous nude mice. After 4 weeks, ligamentous fibrous tissue and cementum-like matrix were formed, expressing collagen type I, osteopontin, osteocalcin, Bone sialoprotein and cementum attachment protein (CAP). Tsuchiya et al. [38] filled calvarial bone defects of the nude mice with the DFSCs and extracellular matrix of pig, and achieved the same bone repair effect with bone marrow mesenchymal stem cells. Guo et al. [39] transplanted DFSCs with treated dentin matrix (TDM) into nude mice subcutaneously, after a few weeks found the

integrated dentin-like structure around TDM and expressed DSP and DMP1. Liu et al [40] co-cultured normal human DFSCs with the periodontitis derived PDLSCs, and then transplanted the cell/ scaffold complex into the subcutaneous nude mice, after 8 weeks found the formation of compact collagen bundles. It suggested that the microenvironment provided by DFSCs could reverse the regeneration function of periodontal ligament tissue in some periodontitis patients.

7. Perspectives

The successful separation of odontogenic stem cells is a milestone in the field of tooth regenerative medicine. However, this technology is not only applied to clinical tissue engineering, but also has many bottlenecks to be solved urgently. 1) The five types of odontogenic stem cells have their own advantages, but lack of specific surface markers, no special selective culture mode, easy to aging, difficult to mass amplification; 2) Current the engineering tooth are small, how to control such as size, shape, development time are still exist many problems; 3) To ensure the reliability and safety of stem cell application, carcinogenicity test need to long-term monitor. However, with the rapid development of stem cell research and the continuous improvement of biological materials, we believe a new era of tissue engineering teeth will launch in the future.

Acknowledgements

This work was supported by Chongqing Research Program of Basic Research and Frontier Technology (No.cstc2012jjA10156 and cstc2014jcyjA10055), Scientific and Technological Research Program of Chongqing Municipal Education Commission （No.KJ1600212）and Program for Innovation Team Building at Institutions of Higher Education in Chongqing in 2016.

References

[1] Huang J, Lin X, Shi Y, Liu W. Tissue Engineering and Regenerative Medicine in Basic Research: A Year in Review of 2014. Tissue engineering Part B, Reviews. 2015;21(2):167-76.

[2] Dassule HR, McMahon AP. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Developmental biology. 1998;202:215-27.

[3] Brar GS, Toor RS. Dental stem cells: dentinogenic, osteogenic, and neurogenic differentiation and its clinical cell based therapies. Indian journal of dental research: official publication of Indian Society for Dental Research. 2012;23:393-7.

[4] Rodriguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramirez MC, Blanquer M, et al. Mesenchymal stem cells derived from dental tissues. International endodontic journal. 2011; 44:800-6.

[5] Bojic S, Volarevic V, Ljujic B, Stojkovic M. Dental stem cells--characteristics and potential. Histology and histopathology. 2014;29:699-706.

[6] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2000;97:13625-30.

[7] Yang X, van den Dolder J, Walboomers XF, Zhang W, Bian Z, Fan M, et al. The odontogenic potential of STRO-1 sorted rat dental pulp stem cells in vitro. Journal of tissue engineering and regenerative medicine. 2007;1:66-73.

[8] 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. Biochemical and biophysical research communications. 2008;371:90-3.

[9] Takeda T, Tezuka Y, Horiuchi M, Hosono K, Iida K, Hatakeyama D, et al. Characterization of dental pulp stem cells of human tooth germs. Journal of dental research. 2008;87:676-81.

[10] Cavalcanti BN, Zeitlin BD, Nor JE. A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. Dental materials: official publication of the Academy of Dental Materials. 2013;29:97-102.

[11] Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364:149-55.

[12] Chen SC, Marino V, Gronthos S, Bartold PM. Location of putative stem cells in human periodontal ligament. Journal of periodontal research. 2006;41:547-53.

[13] Gronthos S, Mrozik K, Shi S, Bartold PM. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcified tissue international. 2006;79:310-7.

[14] Nagatomo K, Komaki M, Sekiya I, Sakaguchi Y, Noguchi K, Oda S, et al. Stem cell properties of human periodontal ligament cells. Journal of periodontal research. 2006;41:303-10.

[15] Kramer PR, Nares S, Kramer SF, Grogan D, Kaiser M. Mesenchymal stem cells acquire characteristics of cells in the periodontal ligament in vitro. Journal of dental research. 2004; 83:27-34.

[16] Zhao YH, Zhang M, Liu NX, Lv X, Zhang J, Chen FM, et al. The combined use of cell sheet fragments of periodontal ligament stem cells and platelet-rich fibrin granules for avulsed tooth reimplantation. Biomaterials. 2013;34:5506-20.

[17] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences of the United States of America. 2003;100:5807-12.

[18] Suchanek J, Visek B, Soukup T, El-Din Mohamed SK, Ivancakova R, Mokry J, et al. Stem cells from human exfoliated deciduous teeth--isolation, long term cultivation and phenotypical analysis. Acta medica. 2010;53:93-9.

[19] Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene

expression profile from promising dental pulp. Journal of endodontics. 2009;35:1536-42.
[20] Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells, tissues, organs. 2006;184:105-16.
[21] Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nor JE. Dentin-derived BMP-2 and odontoblast differentiation. Journal of dental research. 2010;89:603-8.
[22] Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S, et al. SHED differentiate into functional odontoblasts and endothelium. Journal of dental research. 2010;89:791-6.
[23] Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. The Journal of clinical pediatric dentistry. 2009;33:289-94.
[24] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PloS one. 2006;1:e79.
[25] Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. Journal of endodontics. 2008;34:166-71.
[26] Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, et al. Characteristics and Potential Applications of Human Dental Tissue-Derived Mesenchymal Stem Cells. Stem cells. 2015;33(3):627-38..
[27] Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. Journal of endodontics. 2008; 34:645-51.
[28] Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, et al. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue engineering Part A. 2010;16:605-15.
[29] Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, et al. Isolation of precursocells (PCs) from human dental follicle of wisdom teeth. Matrix biology: journal of the International Society for Matrix Biology. 2005;24:155-65.
[30] Morsczeck C, Moehl C, Gotz W, Heredia A, Schaffer TE, Eckstein N, et al. In vitro differentiation of human dental follicle cells with dexamethasone and insulin. Cell biology international. 2005;29: 567-75.
[31] Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. Stem cells and development. 2006;15:595-608.
[32] Pan K, Sun Q, Zhang J, Ge S, Li S, Zhao Y, et al. Multilineage differentiation of dental follicle cells and the roles of Runx2 over-expression in enhancing osteoblast/cementoblast-related gene expression in dental follicle cells. Cell proliferation. 2010;43:219-28.
[33] Morsczeck C, Vollner F, Saugspier M, Brandl C, Reichert TE, Driemel O, et al. Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clinical oral investigations. 2010;14:433-40.
[34] Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C. Dental follicle stem cells and tissue engineering. Journal of oral science. 2010;52:541-52.
[35] Yao S, Pan F, Prpic V, Wise GE. Differentiation of stem cells in the dental follicle. Journal of dental research. 2008;87:767-71.
[36] Morsczeck C, Schmalz G. Transcriptomes and proteomes of dental follicle cells. Journal of dental research. 2010;89:445-56.
[37] Handa K, Saito M, Yamauchi M, Kiyono T, Sato S, Teranaka T, et al. Cementum matrix formation in vivo by cultured dental follicle cells. Bone. 2002;31:606-11.
[38] Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ. Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connective tissue research. 2010;51:197-207.
[39] Guo W, He Y, Zhang X, Lu W, Wang C, Yu H, et al. The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. Biomaterials. 2009;30:6708-23.
[40] Liu J, Wang L, Liu W, Li Q, Jin Z, Jin Y. Dental follicle cells rescue the regenerative capacity of periodontal ligament stem cells in an inflammatory microenvironment. PloS one. 2014;9:e108752.