DOI: 10.7860/JCDR/2015/9871.5636

Review Article

Dentistry Section

SHED - Basic Structure for Stem Cell Research

RUCHA KASHYAP

ABSTRACT

The discovery that stem cells from dental pulp are capable of differentiating into endothelial cells raised the exciting possibility that these cells can be a single source of odontoblasts and vascular networks in dental tissue engineering. These so-called mesenchymal stem cell populations have been identified from human exfoliated deciduous teeth because of their ability to generate clonogenic adherent colonies when grown and expanded. In addition to these stem cells, other population of stem cells can be from adult human dental pulp and periodontal ligament. The identification and isolation of these stem cells in adult dental pulp was first reported by Gronthos and co-workers in 2000.These dental pulp stem cells have clonogenic abilities, rapid proliferative rates and the capacity to form mineralized tissues both in vitro and in vivo. The stem cells from human exfoliated deciduous teeth are distinct from dental pulp stem cells by virtue of their proliferation rate, increased cell population doublings and osteoinductive capacity in vivo. It is further demonstrated that human exfoliated deciduous teeth stem cells may not be a single-cell type, may well be a heterogenous population of cells from the pulp.

Keywords: Dental pulp stem cells, Stem cells from human exfoliated deciduous teeth, Stem cells

INTRODUCTION

Stem cells are defined as clonogenic cells capable of both self-renewal and multi lineage differentiation [1]. Stem cells have been differentiated from variety of body parts and have shown great promise in the management of variety of diseases in medicine. Recently, stem cells have been isolated and grown from –

- Human permanent dental pulp tissue stem cells (DPSCs),
- Stem cells from human exfoliated deciduous teeth (SHEDs), and
- Periodontal ligament apical papilla of immature teeth (PDLSCs).

If we have in hand population of stem cells that reproducibly reform bone, cementum, dentin, and perhaps even periodontal ligament; it is possible to envision complete restoration of the hard tissues in the oral cavity using the patient's own cells, thereby avoiding issues of histocompatability [2]. This would be a more biological approach rather mere mechanical one [3]. It has been reported that the stem cells obtained from the above said sources can generate dentin like tissue both in vitro and in vivo studies in animals [1,4,5]. In addition transplanted skeletal or dental stem cells have the potential to "rephrase" repair craniofacial defects and repair/regenerate teeth [4-7]. The purpose of this review is to discuss the human exfoliated deciduous teeth stem cells by further understanding and contemplating their differences with other stem cell populations. An attempt is also made to evaluate the variation in the number of cells isolated from different exfoliated deciduous teeth.

SHED MULTIPOTENTIALITY

The identification and isolation of stem cells in adult dental pulp was first reported by Gronthos and co-workers in 2000 [4]. These DPSCs have clonogenic abilities, rapid proliferative rates and the capacity to form mineralized tissues both in vitro and in vivo. Gronthos et al., demonstrated their multipotentiality; the ability to form adipocytes and neural precursors in vitro, in addition to dentin-like tissue following transplantation into immunocompromised mice [1]. In addition to DPSCs, other so-called mesenchymal stem cell populations have been identified from human exfoliated deciduous teeth [5] and adult periodontal ligament [8] because of their ability to generate clonogenic adherent colonies when grown and expanded [4,9].

It has been suggested that exfoliated teeth would be similar in some way to umbilical cord containing stem cells that may offer a unique stem cell resource for potential clinical applications. Gronthos et al., in his study found that the deciduous tooth had multipotent stem cells which were highly proliferative and clonogenic capable of differentiating into variety of cell types including neural cells, adipocytes, and odontoblasts. After in vivo transplantation, they were able to induce bone, dentin, and survive in mouse brain along with expression of neural markers. Currently there is a lot of evidence supporting Gronthos findings. These stem cells can be isolated and expanded ex vivo, thereby providing a unique and accessible population of stem cells from an unexpected tissue resource. SHED also demonstrates a strong capacity to induce recipient cell-mediated bone formation in vivo. According to investigations, SHED cannot differentiate directly into osteoblasts but do induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells. According to a study done by Fernando de sa silva et al., SHEDs induce an immune regulatory phenotype in moDCs cells, evidenced by changes in maturation and differentiation rates, inhibition of lymphocyte stimulation and ability to expand CD4+ T cells. Therefore, further can be established that SHEDs can be used for immune modulation in clinical practice [10]. These data imply that deciduous teeth may not only provide guidance for the eruption of permanent teeth, as generally assumed, but may also be involved in inducing bone formation during the eruption of permanent teeth.

It is notable that SHED expresses neuronal and glial cell markers, which are related to the neural crest-cell origin of the dental pulp [11]. Neural crest cells play a pivotal role in embryonic development, giving rise to a variety of cell types such as neural cells, pigment cells, smooth muscle, craniofacial cartilage, and bone [12]. Dental pulp cells are also known to produce neurotrophic factors and even rescue motoneurons after spinal cord injury [13]. Moreover, neural progenitors were identified recently in mammalian dermal skin layers [14]. These evidences support the notion that stem cells of nonneural tissue may be capable of differentiating into neural cells.

Deciduous teeth may be an ideal resource of stem cells to repair damaged tooth structures, induce bone regeneration, cartilage

tissue engineering [15] and possibly to treat neural tissue injury or degenerative diseases. However, the biological significance of the existence of SHED remains to be determined. The use of multipotent postnatal stem cells from exfoliated deciduous teeth that can be readily isolated non-invasively and that retain their potentiality after in vitro expansion offers significant advantages. They have been the focus of commercial stem cell banks seeking autologous stem cell sources derived non-invasively for application in a variety of therapies [16] Studies show that the numbers of cells isolated from the deciduous canines are more in number compared to other type of teeth. This could be because of the longer unresorbed roots associated with canines which harbor more pulp tissue in comparison to other teeth. Most often these primary canines were extracted as a management of severe anterior crowding with unresorbed roots. In the case of incisors, the mandibular permanent incisors erupt lingual to the primary incisors resulting in partially resorbed roots. This is not the case with primary molars. They are extracted at various stages of resorption from partial to almost complete resorption of the roots resulting in less amount of pulp tissue obtained from these specimens. This factor results in less number of cells harvested from the primary incisors and primary molars. However, the association cannot be evaluated and interpreted effectively, owing to the small number of samples and an inequality in sample size amongst the groups. The limitations are the smaller number of samples in studies and the lack of standardization of the root length of exfoliated deciduous teeth. Each tooth exhibits root resorption at various levels and hence different root lengths. Since the quantity of pulp tissue present in each tooth varies with the difference in root lengths, this also influences the variation in number of stem cells isolated from different teeth.

Comparisons Amongst Dpscs, Sheds, Pdlscs and Bmscs

Comparisons amongst DPSCs, SHEDs, PDLSCs and BMSCs (bone marrow stromal cells) have demonstrated that DPSCs, SHEDs and PDLSCs maintain a higher growth potential in comparison to BMSCs [17]. Previous studies demonstrate that BMSCs are also capable of differentiating into neural-like cells after in vivo transplantation [18]. However, there are reported differences in their protein and gene expression profiles. Deciduous teeth are significantly different from permanent teeth with regards to their developmental processes, tissue structure, and function. SHED offers attractive advantages over other post natal stem cells, as they are derived from a source which is non-invasive, readily accessible, naturally being disposed and with very limited ethical or legal concerns [19]. It is not a surprise to find that SHED are distinct from DPSCs with respect to their higher proliferation rate, increased cell-population doublings, sphere-like cell-cluster formation, osteoinductive capacity in vivo, and failure to reconstitute a dentin–pulp-like complex. During long term cultivation, SHED does not show any signs of degeneration or spontaneous differentiation [20]. SHED apparently represent a population of multipotent stem cells that are perhaps more immature than previously examined postnatal stromal stem-cell populations.

Pulp tissues from deciduous and permanent teeth of patients below the age of 25 y after flow cytometry analysis using a panel of cell surface markers revealed expression pattern for a variety of markers for both DPSCs and SHED [21]. The results show that these cells are not hematopoietic in origin and that they are pure mesenchymal stem cells, similar to the study of Gronthos, who showed that profiles for both cell populations of DPSC and BMSC failed to react with the hematopoietic markers CD14, CD45, and CD34. In general, DPSCs and BMSCs exhibited a similar expression pattern for a variety of markers associated with endothelium (vascular cell adhesion molecule), smooth muscle (smooth muscle actin), bone (alkaline phosphatase), type I collagen, osteonectin, osteopontin, and osteocalcin, and fibroblasts (type III collagen and fibroblast growth factor 2). A study by Miura et al., [5] showed that ex vivo-expanded SHED were found to express the cell surface molecules STRO-1 and CD146, the two early mesenchymal stem-cell markers previously found to be present in BMSCs and DPSCs [22]. Kerkis showed in her study that immature dental pulp stem cells are positive for CD13 and CD31 and negative for CD34, CD43, and CD45 [23] According to Sonoyama, stem cells from the apical papilla (SCAP) expressed many surface markers including STRO-1, ALP, CD24, CD29, CD73, CD90, CD105, CD106, CD146, CD166, and ALP; but were negative for CD34, CD45, CD18, and CD150. According to the researcher, CD24 appears to be a specific marker for SCAP, not detectable in other mesenchymal stem cells including DPSCs and BMSCs [24]. Cheng did experiments on chimpanzee teeth and found that both chimpanzee DPSCs and human BMSCs share identical expression profiles on common cell surface antigens. They were all negative for hematopoietic cell surface markers: CD14, CD18, CD24, CD34, and CD45; and positive for CD29, CD44, CD59, CD73, CD90, CD105, CD150, and CD166 [25]. Both SHED and DPSCs indicate that they might have the capacity to differentiate into ectoderm and mesodermal organs [26]. These cells do not express endodermal markers. However, the expression pattern needs to be confirmed at a protein level to emphasize if there is any physical significance. Extensive studies in vivo, on animals with suitable combination of growth factors and scaffold materials [27], is essential before resorting to human trails. Studies indicate the potential for using SHED as a source of pluripotent stem cells for future cellular-based therapies in medicine and dentistry [28].

CONCLUSION

There is a need to gain clarity and further insight into specific properties of stem cells, derived from deciduous tooth pulp. Studies regarding their proliferation abilities, differentiation potential, and immunoreactivity profiles do open up new horizons to make this concept a reality. Therefore, methods to isolate and characterize stem cell population are a preliminary step of any such research, and are crucial for the development of novel therapies based on stem cell regeneration and the data obtained aids in conducting further research on efficacy of ex vivo expanded stem cells for various cell-based therapies in dentistry. Studies demonstrate that stem cells exist in human deciduous and permanent pulp tissue that can be isolated, cultivated, and expanded in vitro. These stem cells can be successfully differentiated into adipocytes and osteoblasts. It is still necessary to gain further insight into the characteristics of these stem cells and examine their full developmental potential.

REFERENCES

[1] Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. *J Dent Res*. 2002;81:531-35.
[2] Sedgley CM, Botero TM. Dental stem cells and their sources. *Dent Clin North Am*. 2012; 56:549-61.
[3] Shetty S, Farooq U, Chandra SB, Nanjappa S. Molecular biology and preservation of tooth vitality current implication. *Endodontol.* 2006;2:12.
[4] Gronthos S, Mankani M, Brahmin J, Robey PG, Shi S. Postnatal human dental pulp cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA*. 2000;97:13625-30.
[5] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA*. 2003;100:5807-12.
[6] Robey PG. Stem cells near the century mark. J *Clin Invest*. 2000;105:1489-91.
[7] Wang X, Thibodeau B, Trope M, Lin LM, Huang GT. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *J Endod*. 2010;36:56-63.
[8] Seo BM, Miura M, Gronthos S. Investigation of multipotent post natal stem cells from human periodontal ligament. *Lancet*. 2004;364;149-55.
[9] Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod Craniofac Res*. 2005;8:191-99.
[10] Silva FdS, Ramos RN, Almeida DCd, Bassi EJ, Gonzales RP, et al. Mesenchymal stem cells derived from human exfoliated deciduous teeth (SHEDs) induce immune modulatory profile in monocyte-derived dendritic cells. PLoS ONE. 2014;9(5):e98050. doi:10.1371/journal. pone. 0098050

[11] Chai Y, Jiang X, Ito Y, Bringas PJr, Han J, Rowitch DH, et al. Fate of the mammalan cranial neural crest during tooth and mandibular morphogenesis. *Development Cambridge, UK.* 2000;127:1671–79.
[12] LaBonne C, Bronner, Fraser M. Molecular mechanisms of neural crest formation. *Annu. Rev. Cell Dev Biol*. 1999;15:81–112.
[13] Nosrat, IV, Widenfalk J, Olson L, Nosrat CA. Dental pulp cells produce neurotrophic factors,interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. *Dev. Biol.* 2001;238:120–32.
[14] Toma JG, Akhavan M, Fernandes K J, Barnabe, Heider F, Sadikot A, et al.Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol.* 2001;3:778–84.
[15] Chen K, Xiong H, Xu N, Shen Y, Huang Y, Liu C. Chondrogenic potential of stem cells from human exfoliated deciduous teeth in vitro and in vivo. Informa Healthcare. 2014 [cited 2014 March 3]; (doi:10.3109/00016357.2014.888756). Available from: http://informahealthcare.com/doi/abs/10.3109/00016357.2014.888756.
[16] Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood:a hematopoeitic fate adopted by adult neural stem cells in vivo. *Science.* 1999; 283:534–37.
[17] Alastair J, Sloan, Rachel J, Waddington. Dental pulp stem cells: what, where, how? *Int J Pediatr Dent.* 2009;19:61-70.
[18] Azizi SA, Stokes D, Augelli BJ, Di Girolamo C, Prockop DJ.Engarftment and migration of human bone marrow stromal cells implanted in the brains of albino rats - similarities to astrocyte grafts. *Proc Natl Acad Sci USA.* 1998; 95:3908–13.
[19] Brar GS, Toor RS. Dental stem cells: Dentinogenic, osteogenic, and neurogenic differentiation and its clinical cell based therapies. *Indian J Dent Res.* 2012;23:393-97.
[20] S Jakub, et al. Stem cells from human exfoliated deciduous teeth – isolation, long term cultivation and phenotypical analysis. *Acta medica.* 2010; 53(2):93-99.
[21] Vishwanath VR. 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;16:423-28.
[22] Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation.* 2005; 80:836-42.
[23] Kerkis I, Kerkis A, Dozortsev D, Stukart, Parsons GC, Massironi SMG, 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.
[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] Cheng PH, Snyder B, Fillos D, Ibegbu CC, Huang AH, Chan AW. Postnatal stem/progenitor cells derived from the dental pulp of adult chimpanzee. *BMC Cell Biol.* 2008;9:20.
[26] Shekar R, Ranganathan K. Phenotypic and growth characterization of human mesenchymal stem cells cultured from permanent and deciduous teeth. *Indian J Dent Res*. 2012; 23:838-39.
[27] Su Wt, Wu PS, Ko CS, Huang TY. Osteogenic differentiation and mineralization of human exfoliated deciduous teeth stem cells on modified chitosan scaffold. *Mater Sci Eng C Mater Biol Appl.* 2014;41:152-60.
[28] Brar GS, Toor RS. Dental stem cells: dentinogenic, osteogenic, and neurogenic differentiation and its clinical cell based therapies. *Indian J Dent Res.* 2012;23:393-97.

PARTICULARS OF CONTRIBUTORS:
1. Senior Lecturer, Department of Prosthodontics, People's College of Dental Sciences and Research Centre, Bhopal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rucha Kashyap,
Department of Prosthodontics, People's College of Dental Sciences and Research Centre, Bhopal, India.
E-mail : dr.rk4u@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 06, 2014**
Date of Peer Review: **Jul 14, 2014**
Date of Acceptance: **Jul 18, 2014**
Date of Publishing: **Mar 01, 2015**