International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01877.x

Mesenchymal stem cells derived from dental tissues

F. J. Rodríguez-Lozano[1], C. Bueno[2], C. L. Insausti[1], L. Meseguer[1], M. C. Ramírez[2], M. Blanquer[1], N. Marín[1], S. Martínez[2] & J. M. Moraleda[1]

[1]Cell Therapy Unit. Hospital Universitary Virgen de la Arrixaca, Faculty of Medicine, University of Murcia, Murcia; and [2]Institute of Neurosciences, University Miguel Hernández, Alicante, Spain

Abstract

Rodríguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramírez MC, Blanquer M, Marín N, Martínez S, Moraleda JM. Mesenchymal stem cells derived from dental tissues. *International Endodontic Journal*, **44**, 800–806, 2011.

Regeneration of tissues occurs naturally due to the existence of stem cells with the capacity to self-regenerate and differentiate; however, regenerative capacity decreases with age, and in many cases, regeneration is not sufficient to repair the damage produced by degenerative, ischaemic, inflammatory, or tumour-based diseases. In the last decade, advances have been made in the understanding of stem cells, the genes that control the alternative fates of quiescence and differentiation, and the niches that provide specific signals that modulate cell fate decisions. Embryonic stem-cell research is shedding light on the secrets of development. Adult stem cells (AS cells) are available from several sources. Bone marrow and connective tissue have been used in preliminary clinical trials for regenerative therapy. Recently, several types of AS cells have been isolated from teeth, including dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, dental follicle progenitor stem cells and stem cells from apical papilla. Preliminary data suggest that these cells have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes and neural cells. If confirmed, these data would support the use of these cells, which are easily obtained from extracted teeth, in dental therapies, including in regenerative endodontics, providing a new therapeutic modality.

Keywords: dental pulp stem cells, dental stem cells, mesenchymal stem cells, periodontal ligament stem cells, stem cells.

Received 18 July 2010; accepted 28 February 2011

Introduction

Cell therapy and tissue engineering have become a promising approach for dental pulp repair, especially in regenerative endodontics, where the regeneration of the dental-pulp complex with stem cells has been investigated (Ishimatsu *et al.* 2009, Kadar *et al.* 2009). However, the potential for pulp-tissue regeneration from implanted stem cells is yet to be tested in extensive clinical trials to evaluate their efficacy and safety (Murray *et al.* 2007). The use of stem cells in endodontic treatments such as apexification requires autogenous stem cells that revascularize dental pulp tissue, provide appropriate signalling molecule(s) as well as an ideal scaffold that will promote controlled cell growth and differentiation (Hargreaves *et al.* 2008). Although the challenges of introducing endodontic tissue engineering therapies are substantial, the potential benefits to patients and the profession are ground-breaking (Hans & Shetty 2009).

Stem cells are defined as clonogenic, self-renewing progenitor cells that can generate one or more specialized cell types (Moraleda *et al.* 2006). Based on their origin, there are two main types of stem cells: embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). ES cells are stem cells derived from the inner cell mass of an early,

Correspondence: Prof Jose M. Moraleda Jiménez, Servicio de Hematología y Hemoterapia, Unidad de Trasplante Hematopoyético y Terapia Celular, Hospital Universitario Virgen de la Arrixaca, Ctra. Madrid-Cartagena, El Palmar, 30120 Murcia, Spain (Tel.: 968 369532; fax: 968 369088; e-mail: jmoraled@um.es).

© 2011 International Endodontic Journal

pre-implantation stage embryo known as a blastocyst. AS cells are the self-renewable multipotential stem cells residing within most differentiated tissues and organs. AS cells are thought to migrate to the area of injury and differentiate into specific cell types to facilitate repair of the damaged tissues (McKay 2000, Leeb *et al.* 2010).

Stem cells can be classified according to their ability to differentiate as totipotent, pluripotent, or multipotent cells. Totipotent stem cells are those that can be implanted in the uterus of a living animal and give rise to a complete organism. Pluripotent stem cells are those that can give rise to every cell of an organism except its extraembryonic tissues, such as the placenta. This limitation restricts pluripotent stem cells from developing into a complete organism. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent stem cells. Multipotent stem cells are AS cells that only generate specific lineages of cells (McKay 2000, Leeb *et al.* 2010).

Embryonic stem cells have a high neoplastic potential. Moreover, their use is controversial and is surrounded by ethical and legal issues. iPS cells are a new inducible stem-cell type. The method for iPS cell induction is 'ground-breaking' because somatic cells are converted directly into pluripotent cells through introduction of four genes: Oct-4, Sox2, c-Myc and Klf4 (Takahashi & Yamanaka 2006). iPS cells have been shown to be similar to ES cells in morphology, proliferation and differentiation capacity and genomic and epigenomic states (Amabile & Meissner 2009). AS cells have generated great interest in the scientific community because of their high potential of expansion '*in vitro*', their self-renewal capacity and their immunomodulatory properties (Leeb *et al.* 2010). AS cells from autologous origin are an appealing, and practical source for cell-based regenerative therapies that hold realistic clinical potential. The identification of the regenerative potential of AS cells such as bone marrow haematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and dental MSCs has encouraged intense research during the last years (Cabanes *et al.* 2007, Huang *et al.* 2009, Jones *et al.* 2010).

To date, several types of AS cells have been isolated from teeth, including dental pulp stem cells (DPSCs) (Gronthos *et al.* 2000), stem cells from human exfoliated deciduous teeth (SHEDs) (Miura *et al.* 2003), periodontal ligament stem cells (PDLSCs) (Seo *et al.* 2004), dental follicle progenitor stem cells (DFPCs), (Morsczeck *et al.* 2005), and stem cells from apical papilla (SCAPs) (Sonoyama *et al.* 2006).

Review

Dental pulp stem cells

In the dental pulp of adult teeth, there is a population of clonogenic cells with a high proliferative capacity – the DPSCs. These cells were successfully isolated by enzymatic digestion of pulp tissue after separating the crown from the roots (Gronthos *et al.* 2000).

Dental pulp stem cells are multipotent cells that proliferate extensively (maintained for at least 25 passages), can be safely cryopreserved, possess immunosuppressive properties, and express markers such as CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146 and STRO-1, but do not express CD14, CD24, CD34, CD45, CD19 and HLA-DR (Lindroos *et al.* 2008, Huang *et al.* 2009, Karaöz *et al.* 2010).

The plasticity of DPSCs has been verified through *in vitro* and *in vivo* studies. DPSCs have the ability to differentiate into odontoblast-like cells, osteoblasts, adipocytes, neural cells, cardiomyocytes, myocytes and chondrocytes *in vitro* (Zhang *et al.* 2006, d'Aquino *et al.* 2007, Carinci *et al.* 2008, Armiñán *et al.* 2009). DPSCs can form mineralized nodules with a dentine-like structure under osteoinductive conditions *in vitro* and reparative dentine-like tissue on the surface of human dentine *in vivo*. DPSCs transplanted with the carrier hydroxyapatite/tricalcium phosphate (HA/TCP) produce a dentine-like structure lined with human odontoblast-like cells and surrounded by pulp-like interstitial tissue *in vivo* (Gronthos *et al.* 2000, Batouli *et al.* 2003). Thus, DPSCs have the capacity to differentiate into osteoblasts *in vivo* and produce a bone-like tissue (Carinci *et al.* 2008).

Stem cells from human exfoliated deciduous teeth

Miura *et al.* (2003) reported the potential to obtain stem cells from human deciduous teeth. As DPSCs, these multipotent cells are derived from dental pulp explants or by digestion of dental pulp tissue and have immunosuppressive properties (Miura *et al.* 2003).

The morphology of SHEDs, also termed immature (Kerkis *et al.* 2006), is similar to that of DPSCs, SCAPs and DFPSCs. SHEDs have a higher proliferation rate than bone marrow mesenchymal stem cells (BMMSCs) and DPSCs and express Oct4, CD13, CD29, CD44, CD73, CD90, CD105, CD146 and CD166, but do not express CD14, CD34, or CD45 (Huang *et al.* 2009, Pivoriuūnas *et al.* 2010).

© 2011 International Endodontic Journal

Stem cells isolated from the pulp tissue of exfoliated deciduous teeth are capable of differentiating into a variety of cells, such as neural cells, osteoblasts, chondrocytes, adipocytes and myocytes (Miura *et al.* 2003, Kerkis *et al.* 2006, Wang *et al.* 2010).

After transplantation subcutaneously on the dorsum of immunocompromised mice, SHEDs form ectopic dentine-like tissue, but are unable to regenerate the dentine/pulp-like complex. These results suggest that SHEDs can differentiate into odontoblasts *in vivo*. Thus, the mineralized tissue generated by SHED in the pulp space of tooth-slice scaffolds had morphological features of dentine, including the presence of dentinal tubules and predentine, which distinguishes it from osteoid tissue (Sakai *et al.* 2010).

Stem cells from human exfoliated deciduous teeth are also capable of repairing critical-size parietal defects in immunocompromised mice; however, the bone generated by these cells lacks haematopoietic marrow elements (Miura *et al.* 2003).

In addition, neural developmental potential was studied by injecting SHEDs into the dentate gyrus of the hippocampus of immunocompromised mice (Miura *et al.* 2003). These studies showed that SHEDs can survive for more than 10 days inside the mouse brain microenvironment and express neural markers such as neurofilament M (NFM).

Stem cells from apical papilla

A potentially new type of stem cell has been discovered in the apical papilla of human immature permanent teeth (Sonoyama *et al.* 2006, 2008). The distinction between the dental pulp and the apical papilla is that the apical papilla represents a precursor tissue for the radicular pulp. SCAPs obtained by explant cultures or enzymatic digestion of apical pulp tissue, are derived from a developing tissue that may represent a population of early stem/progenitor cells. SCAPs may thus be a superior cell source for tissue regeneration. SCAPs express mesenchymal markers, such as CD13, CD24, CD29, CD44, CD73, CD90, CD105, CD106 and CD146 and do not express CD18, CD34, CD45, or CD150 (Huang *et al.* 2009, Ding *et al.* 2010).

Stem cells from apical papilla also have the capacity to undergo osteo/dentinogenic, neurogenic, and adipogenic differentiation. In fact, SCAPs display an expression pattern of osteo/dentinogenic markers and growth factor receptors similar to that observed in DPSCs, but these markers are expressed at lower levels in SCAPs than in DPSCs. Despite these findings, the myogenic and chondrogenic differentiation potential of SCAPs has not been determined (Sonoyama *et al.* 2006, Abe *et al.* 2007). Furthermore, in several cases of apexogenesis in an infected immature tooth with periradicular periodontitis or abscess, SCAPs had the ability to induce root formation (Huang *et al.* 2008, 2009, Friedlander *et al.* 2009).

When *ex vivo*-expanded human SCAPs were transplanted into immunocompromised mice with HA/TCP as a carrier, the typical dentine structure was regenerated. Dentine-forming cells were stained with anti-human-specific mitochondrial antibodies, suggesting that the donor-derived human SCAPs contributed to dentine formation (Sonoyama *et al.* 2006).

Dental follicle progenitor cells

The dental follicle is a mesenchymal tissue that surrounds the developing tooth germ. The dental follicle plays a crucial role in tooth development and contains precursors of the periodontium. Precursor cells have typically been isolated from human dental follicles of impacted third molars using explant cultures or enzymatic digestion of dental follicle tissue. Similar to other dental stem cells, these cells form low numbers of adherent clonogenic colonies when released from the tissue by enzymatic digestion and can be maintained in culture for at least 15 passages (Morsczeck *et al.* 2005, Yao *et al.* 2008).

Dental follicle progenitor cells (DFPCs) express CD10, CD13, CD29, CD44, CD53, CD59, CD73, CD90 and CD105, and do not express CD34, CD45, or HLA-DR (Lindroos *et al.* 2008, Huang *et al.* 2009, Yagyuu *et al.* 2010). DFPCs have the ability to differentiate into osteoblasts/cementoblasts, chondrocytes and adipocytes when grown in appropriate osteogenic, adipogenic or chondrogenic media (Yao *et al.* 2008).

In vitro findings suggest that DPSCs have greater hard tissue-forming potential than DFPCs. This might be explained by the developmental stage of the tooth germs from which these cells are derived. At the crown-forming stage, mineralization (dentinogenesis) can be detected in certain areas of the dental papilla, but not in the dental follicle (cementogenesis) (Yagyuu *et al.* 2010).

Immortalized dental follicle cells are able to re-create a new periodontal ligament (PDL) after *in vivo* implantation (Yokoi *et al.* 2007); however, hard tissues such as dentine, cementum, or bone have not been identified after transplantation of these cells into immunocompromised mice (Yagyuu *et al.* 2010). More *in vivo*

© 2011 International Endodontic Journal

studies are thus needed to confirm the potential for hard tissue regeneration.

Periodontal ligament stem cells

Seo *et al.* (2004) suggested that human PDL contains a population of postnatal multipotent stem cells that can be isolated using explant cultures or enzymatic digestion and expanded *in vitro*. PDLSCs express MSC markers such as CD10, CD13, CD29, CD44, CD59, CD73, CD90 and CD105, and do not express CD14, CD34, CD45, HLA-DR (Shi *et al.* 2005, Lindroos *et al.* 2008, Huang *et al.* 2009, Wada *et al.* 2009, Feng *et al.* 2010).

Periodontal ligament stem cells have the capacity to differentiate into cells similar to cementoblasts and collagen-forming cells. Formation of calcified nodules is less prominent than that observed with DPSCs and SHEDs. Furthermore, PDLSCs have the ability to differentiate *in vitro* into adipogenic, osteogenic and chondrogenic cells (Gay *et al.* 2007, Lindroos *et al.* 2008, Xu *et al.* 2009).

In vivo, PDLSCs have the capacity to differentiate into functional cementoblasts when transplanted subcutaneously on the dorsum of immunocompromised mice, and had the capacity to form collagen fibres embedded in the cementum-like tissue, suggesting the potential to regenerate the cementum/PDL-like tissue *in vivo* (Seo *et al.* 2004).

To determine whether the PDLSCs were able to contribute to the repair of periodontal tissue, they were transplanted into surgically created periodontal defects in the molar area of the mandible of immunocompromised rats (Seo *et al.* 2004). Transplanted PDLSCs were integrated in two of the six rats, and in some regions, they could be found connected to the surfaces of the alveolar bone and the tooth, suggesting that PDLSCs play a role in the repair of periodontal tissue and have the potential to regenerate damaged PDL.

Upon implantation into the tooth sockets of the mandible of a minipig, PDLSCs transplanted with HA/TCP as a carrier formed an artificial bio-root encircled with PDL tissue (Sonoyama *et al.* 2006).

Morphology and culture of stem cells derived from dental tissue

Pericytes are elongated contractile mesodermal cells that ensheath the walls of the small blood vessels with multipotent differentiation abilities that have recently been proposed as the common progenitor of the MSCs in multiple organs (Crisan *et al.* 2008). DPSCs and PDLSCs are located in contact with the blood vessels in the cellular area central to the dental pulp tissue and connective tissue of the PDL respectively. It has been speculated that they originate from pericytes (Lovschall *et al.* 2007).

Depending on the physiological requirements, PDSC and PDLSC can differentiate into osteoblasts, cementoblasts, fibroblasts, or macrophages. Their number decreases with age, implying a reduction in the regenerative potential of the dental pulp and the PDL (Gómez & Campos 2002).

PDSC and PDLSC can be cultured using two techniques. The first technique is the explant outgrowth method, in which the extruded pulp and periodontal tissues are cut into 2-mm^3 pieces and directly incubated in culture dishes containing essential growth medium, generally Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), L-glutamine and sometimes cytokines. During the culture period, stem cells migrate out of the tissues, and an optimal number of cells can be obtained after 2–3 weeks of culture (Groeneveld *et al.* 1994, Spath *et al.* 2010). The second technique for isolating dental stem cells is the enzymatic digestion method, in which the pulp and ligament tissue are digested with collagenase or a combination of collagenase and dispase, and the resulting cell suspensions are seeded in culture dishes containing DMEM supplemented with FBS and nutrient additives (Gronthos *et al.* 2000, Miura *et al.* 2003). After 15 days, colonies of cells proliferate, occupying the flask. In culture, the stem cells derived from teeth are large spindle-shaped cells with a large central nucleus abundant cytoplasm, and cytoplasmic extensions (Fig. 1). These adherent cells are morphologically identical to the mesenchymal stem cells obtained from bone marrow (BMMSCs) (Gronthos *et al.* 2000, Huang *et al.* 2006, Saber 2009).

Mesenchymal immunophenotype of dental stem cells

Mesenchymal stem cells currently hold great promise for use in stem cell-based therapies due to their capacity for self-renewal and multilineage differentiation. Several studies have demonstrated that MSCs can be isolated from multiple tissues, such as bone marrow, peripheral blood, umbilical cord blood, adult connective tissue, placenta and amniotic membrane (Horwitz *et al.* 2002, Herzog *et al.* 2003, Moraleda *et al.* 2006, Changdong *et al.* 2007, Insausti *et al.* 2010).

© 2011 International Endodontic Journal

Table 1 Surface Marker expression in bone marrow and dental stem cells

	BMMSCs	DPSCs	SHED	SCAPs	DFPCs	PLSCs
CD (+) (Literature was quoted in specific paragraphs in the text.)					CD10	CD10
	CD 13	CD13	CD13	CD13	CD13	CD13
				CD24		
		CD29	CD29	CD29	CD29	CD29
	CD44	CD44	CD44	CD44	CD44	CD44
					CD53	
		CD59			CD59	CD59
	CD73	CD73	CD73	CD73	CD73	CD73
	CD90	CD90	CD90	CD90	CD90	CD90
	CD105	CD105	CD105	CD105	CD105	CD105
	CD106			CD106		
		CD146	CD146	CD146		
			CD166			
	HLA-A					
	HLA-B					
	HLA-C					
CD (–)	CD14	CD14	CD14			CD14
				CD18		
		CD19				
		CD24				
	CD34	CD34	CD34	CD34	CD34	CD34
	CD45	CD45	CD45	CD45	CD45	CD45
				CD150		
	HLA-DR	HLA-DR			HLA-DR	HLA-DR

BMMSCs, bone marrow mesenchymal stem cells; DPSCs, dental pulp stem cells; SHED, stem cells from human exfoliated deciduous teeth; SCAPS, stem cells from apical papilla; DFPCs, dental follicle progenitor cells; PLSCs, periodontal ligament stem cells.

In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy established minimal criteria to define human MSCs, which include the following: adhesion to plastic, expression of specific surface antigens and capacity for *in vitro* multipotential (chondrogenic, adipogenic and osteogenic) differentiation, which is demonstrated by staining for cell type-specific markers. The immunophenotypic definition of MSCs requires that more than 95% of the population expresses the CD105, CD73 and CD90 surface antigens by flow cytometry analysis and that <2% of the population expresses the pan-leucocyte marker CD45, the primitive haematopoietic progenitor and endothelial cell marker CD34, the monocyte and macrophage markers CD14 and CD11, the B cell markers CD79 and CD19, or HLA class II (Horwitz *et al.* 2005, Dominici *et al.* 2006).

Considering the published studies, DPSCs, SHED, DFPCs and PDLSCs fulfil all the requirements to be considered MSCs: adhere to plastic, express CD73, CD90 and CD105 and have the capacity to differentiate into osteoblasts, adipocytes and chondrocytes *in vitro* and *in vivo*. Nevertheless, in SCAPs, although mesenchymal markers are expressed, the chondrogenic differentiation has not yet been demonstrated (Huang *et al.* 2009). Table 1 presents an overview of surface markers characteristic for stem cells of dental origin assessed by flow cytometry.

Conclusions

Stem cells derived from teeth are easily accessible multipotent cells with the capacity to differentiate into distinct cell types. This new source of stem cells could be of benefit in cellular therapy and the eventual development of techniques for use in regenerative endodontics and degenerative diseases. Future studies will probably focus on subpopulations of stem cells derived from teeth characterized by specific surface markers, their distinctive properties and their use in clinical applications.

Acknowledgements

This work was supported by FIS EC07/90762, RETICS RD/0010/2012 grants from the ISCIII, the Junction Program of Biomedical Research in Advanced Therapies and Regenerative Medicine among the ISCIII and FFIS 2007, and by the Fundación Séneca (grant 08859/PI/08).

 © 2011 International Endodontic Journal

References

Abe S, Yamaguchi S, Amagasa T (2007) Multilineage cells from apical pulp of human tooth with immature apex. *Oral Science International* **4**, 45–58.

Amabile G, Meissner A (2009) Induced pluripotent stem cells: current progress and potential for regenerative medicine. *Trends in Molecular Medicine* **15**, 59–68.

Armiñán A, Gandía C, Bartual M *et al.* (2009) Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. *Stem Cells and Development* **18**, 907–18.

Batouli S, Miura M, Brahim J *et al.* (2003) Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *Journal of Dental Research* **82**, 976–81.

Cabanes C, Bonilla S, Tabares L, Martínez S (2007) Neuroprotective effect of adult hematopoietic stem cells in a mouse model of motoneuron degeneration. *Neurobiology of Disease* **26**, 408–18.

Carinci F, Papaccio G, Laino G *et al.* (2008) Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulp stem cells. *Journal of Craniofacial Surgery* **19**, 616–25.

Changdong L, Weiyuan Z, Xiaoxia J (2007) Human–placenta derived mesenchymal stem cells inhibit proliferation and function of allogenic immune cell. *Cell Tissue Research* **330**, 437–46.

Crisan M, Yap S, Casteilla L *et al.* (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301–13.

d'Aquino R, Graziano A, Sampaolesi M *et al.* (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. *Cell Death and Differentiation* **14**, 1162–71.

Ding G, Wang W, Liu Y *et al.* (2010) Effect of cryopreservation on biological and immunological properties of stem cells from apical papilla. *Journal of Cellular Physiology* **223**, 415–22.

Dominici M, Le Blanc K, Mueller I *et al.* (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**, 315–7.

Feng F, Akiyama K, Liu Y *et al.* (2010) Utility of PDL progenitors for *in vivo* tissue regeneration: a report of 3 cases. *Oral Diseases* **16**, 20–8.

Friedlander LT, Cullinan MP, Love RM (2009) Dental stem cells and their potential role in apexogenesis and apexification. *International Endodontic Journal* **42**, 955–62.

Gay I, Chen S, MacDougall M (2007) Isolation and characterization of multipotent human periodontal ligament stem cells. *Orthodontic and Craniofacial Research* **10**, 149–60.

Gómez ME, Campos A (2002) *Histología y Embriología Bucodental*, 2nd edn. Madrid, Spain: Editorial Panamericana.

Groeneveld MC, Everts V, Beertsen W (1994) Formation of afibrillar acellular cementum-like layers induced by alkaline phosphatase activity from periodontal ligament explants maintained *in vitro*. *Journal of Dental Research* **73**, 1588–92.

Gronthos S, Mankani M, Brahim J, Gehron Robey P, Shi S (2000) 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* **97**, 13625–30.

Hans MK, Shetty S (2009) Regenerative endodontics: a paradigm shift in dental practice. *Indian Journal of Dental Research* **20**, 517–8.

Hargreaves KM, Giesler T, Henry M, Wang Y (2008) Regeneration potential of the young permanent tooth: what does the future hold? *Journal of Endodontics* **34**, S51–6.

Herzog E, Chai L, Krause DS (2003) Plasticity of marrow derived stem cells. *Blood* **102**, 3483–93.

Horwitz EM, Gordon PL, Koo WK *et al.* (2002) Isolated allogenic bone marrow-derived mesenchymal cell engraft and stimulate growth in children with imperfect osteogenesis: implications for cell therapy of bone. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 8932–7.

Horwitz E, Le Blanc K, Dominici M, Mueller I (2005) Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. *Cytotherapy* **7**, 393–5.

Huang GT, Sonoyama W, Chen J, Park SH (2006) *In vitro* characterization of human dental pulp cells: various isolation methods and culturing environments. *Cell Tissue Research* **324**, 225–36.

Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. *Journal of Endodontics* **34**, 645–51.

Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *Journal of Dental Research* **88**, 792–806.

Insausti CL, Blanquer M, Bleda P *et al.* (2010) The amniotic membrane as a source of stem cells. *Histology and Histopathology* **25**, 91–8.

Ishimatsu H, Kitamura C, Morotomi T *et al.* (2009) Formation of dentinal bridge on surface of regenerated dental pulp in dentin defects by controlled release of fibroblast growth factor-2 from gelatin hydrogels. *Journal of Endodontics* **35**, 858–65.

Jones J, Jaramillo-Merchán J, Bueno C, Pastor D, Viso-León M, Martínez S (2010) Mesenchymal stem cells rescue Purkinje cells and improve motor functions in a mouse model of cerebellar ataxia. *Neurobiology of Disease* **40**, 415–23.

Kadar K, Kiraly M, Porcsalmy B *et al.* (2009) Differentiation potential of stem cells from human dental origin - promise for tissue engineering. *Journal of Phisiology and Pharmacology* **60**, 167–75.

Karaöz E, Doğan BN, Aksoy A *et al.* (2010) Isolation and *in vitro* characterisation of dental pulp stem cells from natal teeth. *Histochemistry and Cell Biology* **133**, 95–112.

© 2011 International Endodontic Journal

Kerkis I, Kerkis A, Dozortsev D *et al.* (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. *Cells Tissues Organs* **184**, 105–16.

Leeb C, Jurga M, McGuckin C, Moriggl R, Kenner L (2010) Promising new sources for pluripotent stem cells. *Stem Cells Reviews* **6**, 15–26.

Lindroos B, Mäenpää K, Ylikomi T, Oja H, Suuronen R, Miettinen S (2008) Characterisation of human dental stem cells and buccal mucosa fibroblasts. *Biochemical Biophysical Research Communications* **368**, 329–35.

Lovschall H, Mitsiadis TA, Poulsen K, Jensen KH, Kjeldsen AL (2007) Coexpression of Notch3 and Rgs5 in the pericyte-vascular smooth muscle cell axis in response to pulp injury. *The International Journal of Deveploment Biology* **51**, 715–21.

McKay R (2000) Stem cells – hype and hope. *Nature* **406**, 361–4.

Miura M, Gronthos S, Zhao M *et al.* (2003) SHED: stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 5807–12.

Moraleda JM, Blanquer M, Bleda P *et al.* (2006) Adult stem cell therapy: dream or reality? *Transplant Immunology* **17**, 74–7.

Morsczeck C, Gotz W, Schierholz J *et al.* (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biology* **24**, 155–65.

Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. *Journal of Endodontics* **33**, 377–90.

Pivoriuūnas A, Surovas A, Borutinskaitė V *et al.* (2010) Proteomic analysis of stromal cells derived from the dental pulp of human exfoliated deciduous teeth. *Stem Cells and Development* **19**, 1081–93.

Saber SE (2009) Tissue engineering in endodontics. *Journal of Oral Science* **51**, 495–507.

Sakai VT, Zhang Z, Dong Z *et al.* (2010) SHED differentiate into functional odontoblasts and endothelium. *Journal of Dental Research* **89**, 791–6.

Seo BM, Miura M, Gronthos S *et al.* (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* **364**, 149–55.

Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S (2005) The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthodontics and Craniofacial Research* **8**, 191–9.

Sonoyama W, Liu Y, Fang D *et al.* (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS ONE* **1**, e79. 1–8.

Sonoyama W, Liu Y, Yamaza T *et al.* (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *Journal of Endodontics* **34**, 166–71.

Spath L, Rotilio V, Alessandrini M *et al.* (2010) Explant-derived human dental pulp stem cells enhance differentiation and proliferation potentials. *Journal of Cellular and Molecular Medicine* **14**, 1635–44.

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–76.

Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S (2009) Immunomodulatory properties of human periodontal ligament stem cells. *Journal of Cellular Physiology* **219**, 667–76.

Wang J, Wang X, Sun Z *et al.* (2010) Stem cells from human exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. *Stem Cells and Development* **4**, 1375–83.

Xu J, Wang W, Kapila Y, Lotz J, Kapila S (2009) Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. *Stem Cells and Development* **18**, 487–96.

Yagyuu T, Ikeda E, Ohgushi H *et al.* (2010) Hard tissue-forming potential of stem/progenitor cells in human dental follicle and dental papilla. *Archives of Oral Biology* **55**, 68–76.

Yao S, Pan F, Prpic V, Wise GE (2008) Differentiation of stem cells in the dental follicle. *Journal of Dental Research* **87**, 767–71.

Yokoi T, Saito M, Kiyono T *et al.* (2007) Establishment of immortalized dental follicle cells for generating periodontal ligament *in vivo*. *Cell Tissue Research* **327**, 301–11.

Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA (2006) Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. *Tissue Engineering* **12**, 2813–23.

© 2011 International Endodontic Journal

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy.
Users should refer to the original published version of the material.