CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Mesenchymal Stem Cells Derived from Dental Tissues *vs.* Those from Other Sources: Their Biology and Role in Regenerative Medicine

ABSTRACT

To date, 5 different human dental stem/progenitor cells have been isolated and characterized: dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and dental follicle progenitor cells (DFPCs). These post-natal populations have mesenchymal-stem-cell-like (MSC) qualities, including the capacity for self-renewal and multilineage differentiation potential. MSCs derived from bone marrow (BMMSCs) are capable of giving rise to various lineages of cells, such as osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells. The dental-tissue-derived stem cells are isolated from specialized tissue with potent capacities to differentiate into odontogenic cells. However, they also have the ability to give rise to other cell lineages similar to, but different in potency from, that of BMMSCs. This article will review the isolation and characterization of the properties of different dental MSC-like populations in comparison with those of other MSCs, such as BMMSCs. Important issues in stem cell biology, such as stem cell niche, homing, and immunoregulation, will also be discussed.

KEY WORDS: MSCs, DPSCs, SHED, SCAP, PDLSCs, DFPCs, stem cell niche, apical papilla, stem cell homing, tissue regeneration.

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INTRODUCTION

Stem cell biology has become an important field for the understanding of tissue regeneration and implementation of regenerative medicine. Since the discovery and characterization of multipotent mesenchymal stem cells (MSCs) from bone marrow (BM), MSC-like populations from other tissues have now been characterized based on the 'gold standard' criteria established for BMMSCs (Friedenstein *et al.*, 1976; Caplan, 1991; Prockop, 1997; Pittenger *et al.*, 1999; Gronthos *et al.*, 2003). Of those, MSC-like populations from adipose tissues and umbilical cord blood have been shown to be promising alternative multipotent MSC sources (Mareschi *et al.*, 2001; Zuk *et al.*, 2001). These MSCs are capable of giving rise to at least 3 cell lineages: osteogenic, chondrogenic, and adipogenic. Other lineages, such as myogenic, neurogenic, and tenogenic, may also be derived from BMMSCs. The search for MSC-like cells in specific tissues has led to the discovery of a variety of stem cells in every organ and tissue in the body in the past decades (reviewed by Baksh *et al.*, 2004; Porada *et al.*, 2006; Kolf *et al.*, 2007). Dental-tissue-derived MSC-like populations are among many other stem cells residing in specialized tissues that have been isolated and characterized. The first type of dental stem cell was isolated from the human pulp tissue and termed 'post-natal dental pulp stem cells' (DPSCs) (Gronthos *et al.*, 2000). Subsequently, 3 more types of dental-MSC-like populations were isolated and characterized: stem cells from exfoliated deciduous teeth (SHED) (Miura *et al.*, 2003), periodontal ligament stem cells (PDLSCs) (Seo *et al.*, 2004), and stem cells from apical papilla (SCAP) (Sonoyama *et al.*, 2006, 2008). Recent studies have identified a fifth dental-tissue-derived progenitor cell population, referred to as 'dental follicle precursor cells' (DFPCs) (Morsczeck *et al.*, 2005). However, the precise relationship among these different stem cell populations remains unclear.

During the characterization of these newly identified dental stem cells, certain aspects of their properties have been compared with those of BMMSCs. Dental stem cells display multidifferentiation potential, with the capacity to give rise to at least 3 distinct cell lineages: osteo/odontogenic, adipogenic, and neurogenic. Differences have been noted between the dental stem cell populations and BMMSCs, where dental stem cells appear to be more committed to odontogenic rather than osteogenic development. To date, dental-tissue-derived stem/progenitor cells have been used for tissue-engineering studies in large animals to assess their potential in pre-clinical

applications (Sonoyama *et al.*, 2006; Liu *et al.*, 2008). A greater understanding of the biology of these dental stem cell populations is a prerequisite to understanding the extent of their efficacy for regenerative medicine. This article will review the current understanding of different dental stem cells in relation to MSC-like populations derived from other tissues.

CHARACTERISTICS OF MSCS

Among stem cells of mesenchymal origin, BMMSCs or BM-derived stromal stem cells (BMSSCs) are the most studied stromal stem cell populations (Caplan, 1991; Prockop, 1997; Pittenger *et al.*, 1999). In mice, MSC-like populations reside in all post-natal organs and tissues (Meirelles *et al.*, 2006). A rare population (0.02%) of very small embryonic-like (VSEL) Sca-1^+lin$^-$CD45$^-$CXCR4^+ SSEA-1^+Oct-4^+ stem cells has been identified in adult murine BM. It was hypothesized that VSEL stem cells are deposited early during development in BM (Kucia *et al.*, 2006; Ratajczak *et al.*, 2008). To relate the potency of dental stem cells, the characteristics and the multipotentiality of the better-known BMMSCs should be first reviewed.

BMMSCs

Bone-marrow-derived MSCs have been described as colony-forming unit-fibroblasts (CFU-Fs) *in vitro* which were able to commit to osteogenic differentiation (Cohnheim, 1867; Friedenstein *et al.*, 1976). Their capacity to form clonogenic colonies is characteristic of other somatic stem cells, such as hematopoietic stem cells, and defines their ability to undergo self-renewal. However, BMMSCs are limited to a growth potential of 30 to approximately 50 population-doublings (PDs) following *ex vivo* expansion (Bruder *et al.*, 1997; Bianco *et al.*, 2001).

Morphologically, BMMSCs are a heterogeneous population of cells (Colter *et al.*, 2000). Additionally, these expanded cells contain mixed populations of cells in terms of the stage of cell immaturity along differentiation pathways (Gronthos *et al.*, 1999; Stewart *et al.*, 1999). More recently, various cell-surface markers have been used in attempts to identify putative MSCs. Markers that are more consistently reported are STRO-1, CD73, CD90, CD105, CD146, Oct4, Nanog, beta2 integrin positive, and CD14, CD34, CD45, and HLA-DR negative expression (Gronthos *et al.*, 2003; Shi and Gronthos, 2003; Miura *et al.*, 2005; Dominici *et al.*, 2006; Battula *et al.*, 2007; Greco *et al.*, 2007; Gronthos and Zannettino, 2008). A purified population has gene and protein expression different from that of the cultured heterogeneous MSCs. At a clonal level, only a minor proportion of individual colonies can undergo extensive proliferation (> 20 PD) and completely regenerate a bone/marrow organ *in vivo*. This heterogeneity in colony morphology, growth, and function supports the stromal hierarchy of cellular differentiation (Owen and Friedenstein, 1988) and thus makes it difficult to identify the exact "phenotypic fingerprint" of a stromal stem cell (Bianco *et al.*, 2001).

BMMSCs are capable of differentiating into multiple cell lineages when grown in defined conditions *in vitro,* including osteogenic, chondrogenic, adipogenic, myelosupportive stroma, myogenic, and neurogenic lineages. The first 4 cell types are considered the essential lineages for defining multipotent MSCs (Tuli *et al.*, 2003; Baksh *et al.*, 2004). Some studies have reported the myogenic potential of MSCs, following the delivery of BMMSCs into muscle tissues, especially the myocardium, in animal models (Ferrari *et al.*, 1998; Orlic *et al.*, 2001; Barbash *et al.*, 2003; Gojo *et al.*, 2003). Numerous clinical trials with various human stem/progenitor cell delivery methods to the heart have reported various levels of success (Segers and Lee, 2008). Reported clinical trials with BMMSCs are limited, yet the intracoronary transplantation of autologous BMMSCs for ischemic cardiomyopathy has shown promising results (Chen *et al.*, 2006a). However, the benefits seen in the majority of cardiac studies are probably due to the paracrine effects of MSCs, rather than their capacity to develop into functional cardiomyocytes.

In rodent systems, BMMSCs have been shown to differentiate into astrocytes and, in some instances, neurons after transplantation into the mouse brain (Kopen *et al.*, 1999), or to facilitate functional recovery of damaged brain or spinal cord in rats (Chen *et al.*, 2001; Hofstetter *et al.*, 2002). Clinical trials where granulocyte macrophage colony-stimulating factors (GM-CSF) were administered along with BMMSCs appeared to improve the conditions for acute and subacute spinal cord injuries, but not chronic cases (Yoon *et al.*, 2007). While BMMSCs from rats and humans can be induced to differentiate into neurons *in vitro* (Woodbury *et al.*, 2000), the neurogenic potential is weaker when compared with that of stem cells derived from neural tissues (Raedt *et al.*, 2007; Song *et al.*, 2007).

Potent MSCs from Other Sources

Due to certain shortcomings of obtaining the BMMSCs, including pain, morbidity, and low cell number upon harvest, alternate sources for MSCs have been sought. MSCs derived from adipose tissue obtained by suction-assisted lipectomy (liposuction) (Zuk *et al.*, 2001; Mizuno *et al.*, 2002) and MSC-like populations from umbilical cord blood (UCB) have been isolated and characterized (Mareschi *et al.*, 2001). MSCs from BM, adipose tissue, and umbilical cord blood are morphologically and immunophenotypically similar, but not identical (Kern *et al.*, 2006). UCB-derived MSCs form the fewest colonies and show the highest proliferative capacity, whereas adipose-tissue-derived MSCs form the greatest number of colonies, and BMMSCs have the lowest proliferative capacity. MSCs from adipose tissues and especially UCB (Ballen *et al.*, 2008) may gain more popularity, because these tissues are versatile and possess great potential for many clinical applications. Most importantly, they are discarded if not used.

Dental MSCs

Dental tissues are specialized tissues that do not undergo continuous remodeling as shown in bony tissue; therefore, dental-tissue-derived stem/progenitor cells may be more committed or restricted in their differentiation potency in comparison with BMMSCs. Additionally, dental mesenchyme is termed 'ectomesenchyme' due to its earlier interaction with the neural crest. From this perspective, ectomesenchyme-derived dental stem cells may possess different characteristics akin to those of neural crest cells.

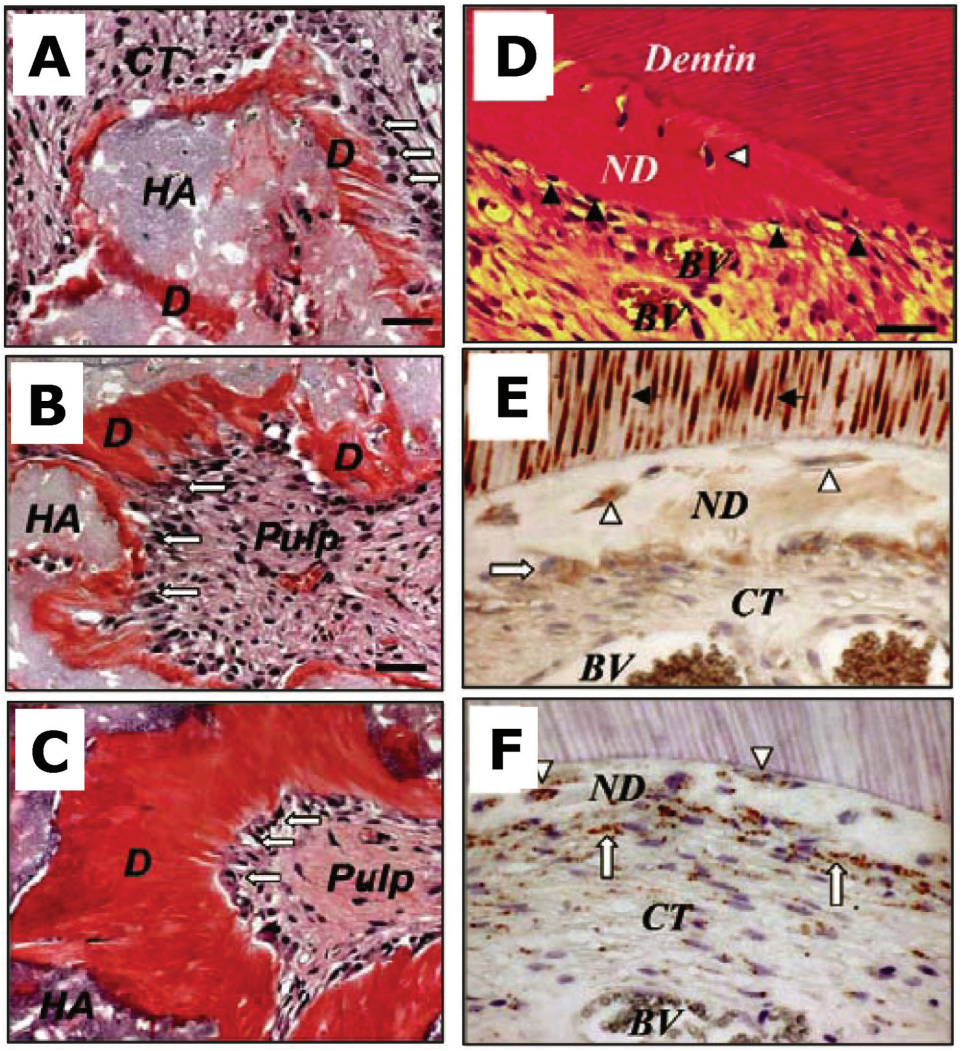

Figure 1. Subcutaneous DPSC transplants in immunocompromised mice **(A-C)** and characterization of DPSC-mediated dentinogenesis *in vivo* **(D-F)**. **(A)** Four wks after transplantation, DPSCs differentiated into odontoblasts (open arrows) responsible for new dentin (D) formation on the surface of the HA/TCP (HA). **(B,C)** At 8 and 16 wks post-transplantation, respectively. **(D)** Newly formed reparative dentin-like structure (ND) attached to the surfaces of human dentin in DPSC/dentin transplants. BV, blood vessels; CT, connective tissue; dentinogenic cells (black arrowheads). DPSCs formed reparative dentin-like structure containing entrapped cells (open arrowheads). **(E)** In DPSC/dentin transplants, dentinogenic cells (open arrows) and trapped cells (open arrowheads) within the newly formed reparative dentin-like structure (ND) were immunoreactive to human DSP antibody, as was the pre-existing dentin (black arrows). **(F)** Staining of human-specific anti-mitochondria antibody, showing the human origin of DPSCs (open arrows). Bar, 40 μm in A-C, 20 μm in D-F (adapted from Batouli *et al.*, 2003).

Dental Pulp Stem Cells (DPSCs)

Isolation of Heterogeneous Populations of DPSCs

One important feature of pulp cells is their odontoblastic differentiation potential. Human pulp cells can be induced *in vitro* to differentiate into cells of odontoblastic phenotype, characterized by polarized cell bodies and accumulation of mineralized nodules (Tsukamoto *et al.*, 1992; About *et al.*, 2000; Couble *et al.*, 2000). DPSCs isolated with enzyme treatment of pulp tissues form CFU-Fs with various characteristics (Gronthos *et al.*, 2000; Huang *et al.*, 2006a). There are different cell densities of the colonies, suggesting that each cell clone may have a different growth rate, as reported for BMMSCs (Gronthos *et al.*, 2002). Within the same colony, different cell morphologies and sizes may be observed. If seeded onto dentin, some DPSCs convert into odontoblast-like cells with a polarized cell body and a cell process extending into the existing dentinal tubules (Huang *et al.*, 2006a,b).

In vitro *Characterization of DPSCs—Multilineage Differentiation*

In addition to their dentinogenic potential, subpopulations of hDPSCs also possess adipogenic and neurogenic differentiation capacities by exhibiting adipocyte- and neuronal-like cell morphologies and expressing respective gene markers (Gronthos *et al.*, 2002). More recently, DPSCs were also found to undergo osteogenic, chondrogenic and myogenic differentiation *in vitro* (summarized in Table 1) (Laino *et al.*, 2005; Zhang *et al.*, 2006; d'Aquino *et al.*, 2007).

In vivo *Characterization of DPSCs—Ectopic Formation of Dentin-Pulp-like Complex*

Transplanted *ex vivo* expanded DPSCs mixed with hydroxyapatite/tricalcium phosphate (HA/TCP) form ectopic pulp-dentin-like tissue complexes in immunocompromised mice (Gronthos *et al.*, 2000; Batouli *et al.*, 2003) (Figs. 1A-1C). These pools of heterogeneous DPSCs form vascularized pulp-like tissue and are surrounded by a layer of odontoblast-like cells expressing dentin sialophosphoprotein (DSPP), which produces dentin containing dentinal tubules similar to those in natural dentin. Over time, the amount of dentin thickened (Batouli *et al.*, 2003). When DPSCs are seeded onto human dentin surfaces and implanted into immunocompromised mice, reparative dentin-like structure is deposited on the dentin surface (Batouli *et al.*, 2003) (Figs. 1D-1F).

While multiple-colony-derived DPSCs can have a PD of more than 120, single-colony-derived strains of DPSCs proliferate 10-20 PDs, and some are able to pass 20 PD to generate enough cells to form a dentin-pulp-like complex. Approximately two-thirds of the single-colony-derived hDPSCs were able to form

the same amount of dentin as multi-colony hDPSCs. The other one-third generated only a limited amount of dentin (Gronthos *et al.*, 2002).

Carinci and his colleagues identified a subpopulation of stem cells from human dental pulp with osteogenic potential forming bone-like tissue *in vivo*. They termed these cells 'osteoblasts derived from human pulpar stem cells' (ODHPSCs) and used microarrays to compare the genetic profiles of these cells with those of normal osteoblasts. They identified a long list of genes that are down-regulated in ODHPSCs *vs*. normal osteoblasts, which may explain the differences observed in the histological characteristics of the bone-like tissue formed by ODHPSCs compared with normal osteoblasts (Carinci *et al.*, 2008).

Table 1. Properties of Human Dental MSCs

	In vitro Analysis			*In vivo* Analysis
Cell Type	PD*	Multipotentiality		Ectopic tissue formation
DPSCs	60 - > 120	Osteo/Dentinogenic	+	Dentin-pulp-like complex
		Adipogenic	+	Odontoblast-like cells
		Chondrogenic	+	Bone-like tissue
		Myogenic	+	
		Neurogenic	+	
SHED	> 140	Dentinogenic	+	Dentin-pulp-like tissue
		Adipogenic	+	Odontoblast-like cells
		Chondrogenic	+	No dentin-pulp complex formation
		Myogenic	+	Bone formation
		Neurogenic	+	
		Osteo-inductive	+	
SCAP	> 70	Dentinogenic	+	Dentin-pulp-like complex
		Adipogenic	+	Odontoblast-like cells
		Chondrogenic	ND	
		Myogenic	ND	
		Neurogenic	+	
PDLSCs	ND	Osteo/Cementogenic	+	Cementum-like
		Adipogenic	+	PDL-like formation
		Chondrogenic	+	
		Myogenic	ND	
		Neurogenic	+	
DFPCs	ND	Cementogenic	+	PDL-like formation
		Odontogenic	+	Cementum matrix formation
		Adipogenic-Chondrogenic	+	
		Myogenic	+	
		Neurogenic	ND	
			ND	
BMMSCs	30 - > 50	Odontogenic	–	BMMSCs have shown both ectopic
		Osteogenic	+	and orthotopic tissue formation:
		Adipogenic-Chondrogenic	+	Bone and bone marrow-like,
		Myogenic	+	cartilage, muscle, and neuronal
		Neurogenic	+	cell/tissue formation
			+	

* PD, population doubling; ND, not determined.

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

In vitro *Characterization of SHED—Sphere-like Cluster Formation*

SHED proliferate faster with greater PDs than DPSCs and BMMSCs (SHED > DPSCs > BMMSCs). SHED form sphere-like clusters when cultured in neurogenic medium. This is due to the highly proliferative cells, which aggregate in clusters that either adhere to the culture dish or float freely in the culture medium. The sphere-like clusters can be dissociated by passage through needles and subsequently grown on 0.1% gelatin-coated dishes as individual fibroblastic cells. This phenomenon suggests a high proliferative capacity analogous to that of neural stem cells (Miura *et al.*, 2003).

Investigators subsequently also isolated SHED and termed the cells 'immature DPSCs' (IDPSCs) (Kerkis *et al*., 2006). Besides confirming the findings described above, they found that IDPSCs express the embryonic stem (ES) cell markers Oct4, Nanog, stage-specific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81) (Kerkis *et al.*, 2006).

In vitro *Multilineage Differentiation*

As reported for DPSCs, SHED showed the capacity to undergo osteogenic and adipogenic differentiation (Miura *et al.*, 2003). Furthermore, cultured SHED readily express a variety of neural cell markers (Table 2). If stimulated with neurogenic medium, expression of βIII-tubulin, GAD, and NeuN is increased, whereas the other neural markers remain unchanged. Under neurogenic conditions, SHED also exhibit multicytoplasmic processes instead of the typical fibroblastic morphology (Figs. 2A-2D) (Miura *et al.*, 2003). Myogenic and chondrogenic potentials have also been demonstrated (Kerkis *et al.*, 2006).

In vivo *characterization of SHED—Production of dentin-pulp-like structures but without a complex formation. Ex vivo*-expanded SHED transplanted into immunocompromised mice yield human-specific odontoblast-like cells directly associated with a dentin-like structure. The regenerated dentin expresses dentin-specific DSPP. However, unlike DPSCs, SHED are unable to regenerate a complete dentin-pulp-like complex *in vivo* (Figs. 2E-2H) (Miura *et al.*, 2003).

Osteo-inductive capacity. One striking feature of SHED is that they are capable of inducing recipient murine cells to

Table 2. *In vitro* Phenotypic Characteristics[#]

Gene	BMMSCs	SHED	DPSCs*	SCAP*	PDLSCs	DFPCs
STRO-1	+ (5-10%)	+ (9%)	+ (5-10%)	+ (> 18%)	+	+
CD + (selected)	CD44	CD146	CD13	CD13	CD13	CD13
	CD73		CD29	CD44	CD29	CD29
	CD105		CD44	CD24	CD44	CD44
	CD106		CD59	CD29	CD59	CD59
			CD73	CD73	CD90	CD73
			CD90	CD90	CD105	CD90
			CD105	CD105		CD105
			CD146	CD106		
				CD146		
CD- (selected)	CD14		CD14	CD18		CD45
	CD34		CD24	CD34		
	CD45		CD34	CD45		
			CD45	CD150		
Oct4	+	+	+			
Nanog	+	+				
Survivin			*–	*++		
hTERT	–		*+	*++		
Notch-1						+
TGFβRI		+			+	
TGFβRII		+	*+	*+/–		
Endostatin		+	*++	*++		
bFGF		+	*++	*++		
FGFR3		+	*+++	*+		+
Flt-1 (VEGF receptor 1),		+	*+	*+/–		
Flg (FGFR1)			*+++	*++		
MUC18 (melanoma-associated glycoprotein, CD146)	+	+	*+++	*++	+	
Collagen type I	+		+			+
Collagen type III	+		+			
Scleraxis	+		+		+	
Osteo/odontogenic						
ALP	+	+	*+++	*++	+	
BSP	+/–		*+	*+	+	+
DSP		+	*+++	*++		
MEPE (matrix extracellular phosphoglycoprotein)		+	*+++	*++	+	
Cbfa1/Runx2		+	*+	*+		
Osteocalcin (OCN)	+/–	+			+	+
Osteonectin	+/–		+			
Osterix	+	+				+
Neurogenic						
Nestin			*+	*+		+
βIII tubulin		+		+		
Tau		+				
MAP2 (microtubule-associated protein)				(+)		
GAD (glutamic acid decarboxylase),		+		(+)		
NeuN (Neuronal nuclear antigen),		+		(+)		
NFM (Neurofilament M)	–	+	*+/–	*+/– (+)		
NSE (neuron-specific enolase)				+		
CNPase (glial markers 2′,3′-cyclic nucleotide 3′-phosphodiesterase)		+		+		
GFAP (glial fibrillary acidic protein),		+	+	+		

* Comparison of relative expression levels of genes between DPSCs and SCAP. For other cells, + or—indicates only positive or negative expression; +, expression without stimulation; (+) expression after induction.

[#] Markers listed do not exclude others not listed herein.

differentiate into bone-forming cells, which is not a property attributed to DPSCs following transplantation *in vivo*. When single-colony-derived SHED clones were transplanted into immunocompromised mice, only one-fourth of the clones had the potential to generate ectopic dentin-like tissue equivalent to that generated by multicolony-derived SHED (Miura *et al.*,

2003). However, all single-colony-derived SHED clones tested are capable of inducing bone formation in immunocompromised mice. While SHED could not differentiate directly into osteoblasts, they appeared to induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells (Miura *et al.*, 2003). With the osteo-inductive potential, SHED can repair critical-sized calvarial defects in mice with substantial bone formation (Seo *et al.*, 2008). These findings 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.

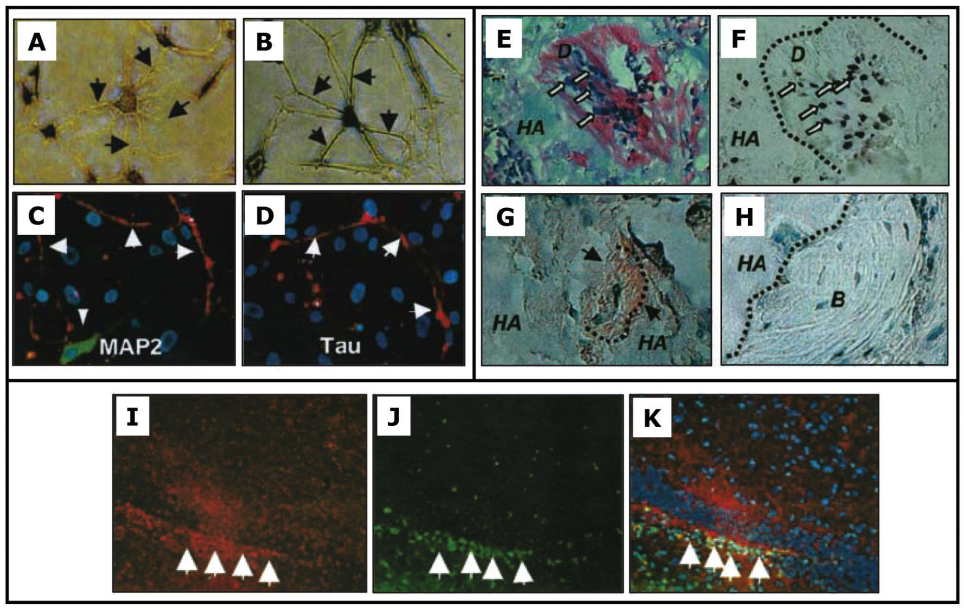

Figure 2. *In vitro* neurogenesis of SHED **(A-D)**, transplanted SHED into immunocompromised mice **(E-H)** and into the mouse brain **(I-K)**. **(A,B)** Toluidine blue staining of the altered morphology of SHED after neurogenic induction. **(C,D)** Immunopositive staining for MAP2 and Tau on dendrites and axons (arrows), respectively. **(E,F)** Eight wks after transplantation into the subcutaneous space, SHED differentiate into odontoblasts (open arrows) and form dentin-like structure (D) on the surfaces of HA. The same field is shown for human-specific alu *in situ* hybridization, indicating the human origin of odontoblasts (open arrows in **F**). **(G)** Immunohistochemical staining of DSPP on the regenerated dentin (black arrows). **(H)** Newly generated bone (B) by host cells in the same SHED transplant shows no reactivity to the DSPP antibody. **(I-K)** Neurogenically induced SHED injected into the dentate gyrus of the hippocampus of immunocompromised mice for 10 days. **(I)** NFM (red) and **(J)** human-specific anti-mitochondrial antibody (green) and **(K)** merged images showing co-localization of the two (adapted from Miura *et al.*, 2003, with permission).

In vivo *neurogenesis in mouse brain.* Neural developmental potential was studied by the injection of SHED into the dentate gyrus of the hippocampus of immunocompromised mice (Miura *et al.*, 2003). SHED can survive for more than 10 days inside the mouse brain microenvironment and express neural markers such as neurofilament M (NFM) (Figs. 2I-2K). This finding is similar to what was demonstrated for BMMSCs, which are capable of differentiating into neural-like cells after *in vivo* transplantation into the rat brain (Azizi *et al.*, 1998). SHED appear to represent a population of multipotent stem cells that are perhaps more immature than other post-natal stromal stem-cell populations. SHED express neuronal and glial cell markers, which may be related to the neural-crest-cell origin of the dental pulp (Chai *et al.*, 2000).

In vivo *engraftment into different tissues.* Three months following the injection of IDPSCs into the intraperitoneal space of nude mice, IDPSCs can be traced in various tissues and organs, including liver, spleen, and kidney, suggesting their potent differentiation plasticity (Kerkis *et al.*, 2006).

Stem Cells from Apical Papilla (SCAP)

Apical papilla refers to the soft tissue at the apices of developing permanent teeth (Sonoyama *et al.*, 2006, 2008). Apical papilla is more apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp (Fig. 3) (Rubio *et al.,* 2005).

In vitro *Characterization of SCAP—Multilineage Differentiation Potential*

Similar to DPSCs and SHED, *ex vivo* expanded SCAP can undergo odontogenic differentiation *in vitro*. SCAP express lower levels of DSP, matrix extracellular phosphoglycoprotein (MEPE), transforming growth factor β receptor II (TGFβRII), FGFR3, Flt-1 (VEGF receptor 1), Flg (FGFR1), and melanoma-associated glycoprotein (MUC18) in comparison with DPSCs. Significantly, CD24 is expressed by SCAP which is not detected on DPSCs or BMMSCs. The expression of CD24 by SCAP is down-regulated in response to osteogenic stimulation. However, the biological significance of this finding requires further investigation.

SCAP also demonstrate the capacity to undergo adipogenic differentiation following induction *in vitro* (Sonoyama *et al.*, 2006; Abe *et al.*, 2007). Interestingly, without neurogenic stimulation, cultured SCAP show positive staining for several neural markers (Abe *et al.*, 2007). After stimulation, additional neural markers are also expressed by SCAP, including glutamic acid decarboxylase (GAD), neuronal nuclear antigen (NeuN), neurofilament M (NFM), neuron-specific enolase (NSE), and glial markers 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPas) (Sonoyama *et al.*, 2008).

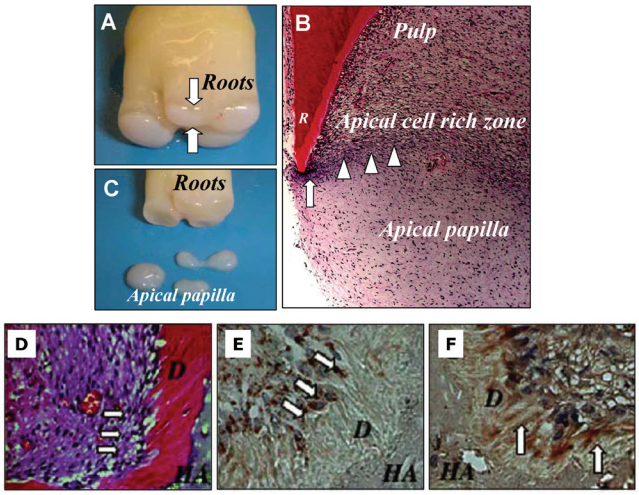

Figure 3. The anatomy of the human apical papilla **(A-C)** and dentinogenesis of SCAP in immunocompromised mice (**D-F**). **(A)** An extracted human third molar depicting root attached to the root apical papilla (open arrows) at the developmental stage. **(B)** Hematoxylin and eosin staining of human developing root (R) depicting epithelial diaphragm (open arrows) and apical cell-rich zone (open arrowheads). **(C)** Harvested root apical papilla for stem cell isolation. **(D)** Eight wks after transplantation, SCAP differentiated into odontoblasts (arrows) that formed dentin (D) on the surfaces of a HA carrier. **(E)** SCAP differentiated into odontoblasts (arrows) are positive for anti-human specific mitochondria antibody staining. **(F)** Immunohistochemical staining of SCAP-generated dentin (D) showing positive anti-DSP antibody staining (arrows) (adapted from Sonoyama *et al.*, 2006, 2008, with permission).

DPSCs vs. *SCAP*

The distinction between dental pulp and apical papilla is that apical papilla is the precursor tissue of the radicular pulp. From this perspective, it may be speculated that SCAP are similar to stem cells residing in the dental papilla that gives rise to the coronal dentin-producing odontoblasts. Once the apical papilla turns into pulp, whether the SCAP convert into DPSCs or the latter are derived from a different stem cell pool is currently unclear. Nonetheless, our previous studies showed that when SCAP and DPSCs are compared *in vitro*, there are some differences (Table 2). Overall, SCAP are derived from a developing tissue that may represent a population of early stem/progenitor cells which may be a superior cell source for tissue regeneration. Additionally, these cells also highlight an important fact that developing tissues may contain stem cells distinctive from those of mature tissues.

In vivo *Characterization of SCAP—Formation of Dentin-Pulp-like Complex*

The capacity of SCAP to differentiate into functional dentinogenic cells has been verified by the same approaches as for the above-mentioned dental stem cells. A typical dentin-pulp-like complex is generated when SCAP are transplanted into immunocompromised mice in an appropriate carrier matrix (Figs. 3D-3F) as described for DPSCs.

Potential Role of SCAP in Continued Root Formation

As described above, SCAP show characteristics similar to, but different from, those of DPSCs. SCAP appear to be the source of primary odontoblasts that are responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement odontoblasts that form reparative dentin. The role of apical papilla in root formation has been discussed previously (Huang *et al.*, 2008). In a pilot study with minipigs as a model, the surgical removal of the root apical papilla at an early developing stage halted root development, despite the pulp tissue being intact, whereas other roots of the tooth, containing apical papilla, maintained normal growth and development.

Periodontal Ligament Stem Cells (PDLSCs)

Earlier evidence has shown that PDL contains cell populations that can differentiate into either cementum-forming cells (cementoblasts) or bone-forming cells (osteoblasts) (McCulloch and Bordin, 1991; Isaka *et al.*, 2001). The presence of multiple cell types within PDL suggests that this tissue contains progenitor cells that maintain tissue homeostasis and regeneration of periodontal tissue. Enzyme digestion treatment of PDL releases a population of clonogenic cells with characteristics of postnatal stem cells (Seo *et al.*, 2004). The successful isolation and characterization of PDLSCs have led to the identification of tendon MSCs by the same approaches (Bi *et al.*, 2007).

In vitro *Characterization of PDLSCs—Multilineage Differentiation Potential*

PDLSCs express the MSC-associated markers STRO-1, CDs, and scleraxis (Table 2)—a tendon-specific transcription factor, which is expressed at higher levels in PDLSCs than in BMMSCs and DPSCs. Immunohistochemical staining and Western blot analysis showed that cultured PDLSCs expressed an array of cementoblastic/osteoblastic markers (Seo *et al.*, 2004). Similar to the other dental stem cells described above, PDLSCs exhibit osteogenic, adipogenic, and chondrogenic characteristics under defined culture conditions (Gay *et al.*, 2007; Lindroos *et al.*, 2008; Xu *et al.*, 2009).

In vivo *Characterization of PDLSCs—Formation of Cementum- and PDL-like Tissue*

Typical cementum/PDL-like structure can be regenerated after transplantation of *ex vivo*-expanded PDLSCs into immunocompromised mice. A thin layer of cementum-like tissue is formed along with condensed collagen fibers with sparse cells resembling PDL structures. The cementum/PDL-like structures are totally different from typical bone/marrow structures generated by BMMSCs and dentin/pulp-like structures generated by DPSCs.

Transplanted human PDLSCs form a dense type I collagen-positive PDL-like tissue within the transplants. More importantly, collagen fibers generated *in vivo* were able to connect with newly formed cementum-like structures that mimicked physiological attachment of Sharpey's fibers (Figs. 4A, 4B),

responsible for the functional attachment of cementum/PDL structures. From these findings, one can infer that PDLSCs may contain a subpopulation of cells capable of differentiating into cementoblasts/cementocytes and collagen-forming cells *in vivo*. After transplantation of hPDLSCs into the periodontal defects of immunocompromised mice, PDL-like tissue was regenerated, and these human stem cells were also identified to be closely associated with the trabecular bone next to the regenerated PDL, suggesting their involvement in alveolar bone regeneration (Figs. 4C-4E) (Seo *et al.*, 2004).

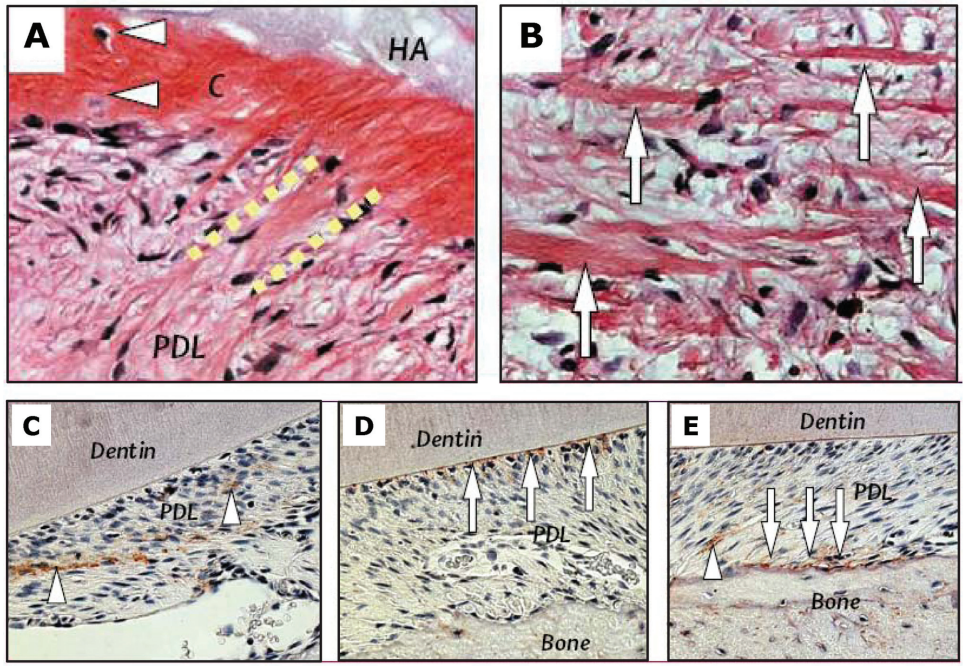

Figure 4. Generation of cementum-like structure and collagen fibers by PDLSCs in immunocompromised mice (**A,B**) and PDLSCs in periodontal tissue repair in immunocompromised rats (**C-E**). **(A,B)** Transplanted PDLSCs formed cementum-like structures (C) that connected to newly formed collagen fibers (dashed lines), similar to the structure of Sharpey's fibers, and generated a substantial amount of collagen fibers (arrows in **B**). **(C-E)** Staining of human-specific anti-mitochondria antibody showing human PDLSCs located in the PDL compartment (arrowheads in **C**), involved in the attachment of PDL to the tooth surface (arrows in **D**), and participating in the repair of alveolar bone (arrows in **E**) and PDL (arrowhead in **E**) (adapted from Seo *et al.*, 2004, with permission).

Dental Follicle Precursor Cells (DFPCs)

Dental follicle is an ectomesenchymal tissue surrounding the enamel organ and the dental papilla of the developing tooth germ prior to eruption. This tissue contains progenitor cells that form the periodontium, *i.e.,* cementum, PDL, and alveolar bone. Precursor cells have been isolated from human dental follicles of impacted third molars. Similar to other dental stem cells, these cells form low numbers of adherent clonogenic colonies when released from the tissue following enzymatic digestion (Morsczeck *et al.*, 2005).

In vitro *Characterization*

Cells in dental follicles express markers such as Notch-1 and Nestin, suggesting the presence of undifferentiated cells. After cells are released from the tissue, only a small number of single dental follicle cells are attached onto the plastic surface and form CFU-F. DFPCs show a typical fibroblast-like morphology and express Nestin, Notch-1, collagen type I, bone sialoprotein (BSP), osteocalcin (OCN), and fibroblast growth factor receptor (FGFR)1-IIIC (Morsczeck *et al.*, 2005). DFPCs demonstrate osteogenic differentiation capacity *in vitro* after induction. A membrane-like structure forms in DFPC cultures after 5 wks of stimulation with dexamethasone. It has been reported that STRO-1 and BMP receptors (BMPR) are expressed in dental follicles *in vivo* (Kemoun *et al.,* 2007). Incubation with rhBMP-2 and rhBMP-7 or enamel matrix derivatives (EMD) for 24 hrs increases the expression of BMP-2 and BMP-7 by DFPCs. Expression of cementum attachment protein and cementum protein-23 (CP-23), two putative cementoblast markers, has been detected in EMD-stimulated whole dental follicle and in cultured DFPCs stimulated with EMD or BMP-2 and BMP-7 (Kémoun *et al.*, 2007).

In vivo *Characterization*

Transplantation of DFPCs by the same methods as described for other dental stem cells generates a structure comprised of fibrous or rigid tissue. These transplants expressed human-specific transcripts for BSP, OCN, and collagen type I. Gene expression was increased more than 100 times for BSP and OCN and was decreased for collagen type I transcripts after transplantation into immunocompromised mice. However, there was no dentin, cementum, or bone formation observed in the transplant *in vivo*. The authors explained that it could be due to the low number of cells in the original cultures (Morsczeck *et al.*, 2005, 2008).

Dental MSCs *vs.* BMMSCs

Immunophenotype

Although there has been no systematic comparison between dental stem cells and BMMSCs, certain differences between some dental MSCs and BMMSCs have been compared. Immunophenotypic analysis is presented in Table 2. Collectively, current evidence suggests that biochemical pathways involved in the differentiation of DPSCs into functional odontoblasts are similar to differentiation pathways of BMMSCs into osteoblasts (Shi *et al.*, 2001). DPSCs do share a similar pattern of protein expression with BMMSCs *in vitro*.

Gene Expression Profile

A similar level of gene expression between DPSCs and BMMSCs was found for more than 4000 known human genes, except a few differentially expressed genes, including collagen type XVIII alpha1, insulin-like growth factor-2 (IGF-2), discordin domain tyrosine kinase 2, NAD(P)H menadione oxidoreductase, homolog 2 of Drosophila large disk, and cyclin-dependent kinase 6, which are highly expressed in DPSCs, whereas insulin-like growth factor binding protein-7 (IGFBP-7) and collagen type I α2 are more highly expressed in BMMSCs (Shi *et al.*, 2001).

In a cDNA microarray system consisting of 12,814 genes, a clustering algorithm was applied to characterize and compare the expression profiles and functional classifications of odontogenic and osteogenic human stem cell populations between DPSCs and BMMSCs (Yamada *et al.,* 2006a). These investigators discovered the high expression levels of the ALP gene, DSPP, and DMP-1 in DPSCs after osteoinduction compared with levels in BMMSCs. When they focused on differences between induced DPSCs and BMMSCs on a cluster that contains genes which are up-regulated in DPSCs and down-regulated in MSCs after induction, they found a notable feature of this cluster to be the cooperative regulation of genes for cell signaling, cell communication, or metabolism (Yamada *et al.*, 2006a).

Multilineage Differentiation Potential

Although DPSCs and BMMSCs are regulated by similar factors, and share a common protein expression profile, these populations differ significantly in their proliferative ability and developmental potentials *in vitro*, and, more importantly, in their ability to develop into distinct tissues representative of the micro-environments from which they were derived *in vivo*. BMMSCs formed only bone tissue in the mouse model when treated in the same manner (Gronthos *et al.*, 2000; Batouli *et al.*, 2003). The chondrogenic potential of DPSCs appears weak, and both DPSCs and SCAP are weaker in adipogenesis in comparison with BMMSCs (Zhang *et al.*, 2006; Sonoyama *et al.*, 2008). Conversely, the neurogenicity of dental stem cells may be more potent than that of BMMSCs, most probably due to their neural crest origin.

MSC NICHE

The stem cell niche concept was first proposed as a specialized micro-environment needed for cells to retain their 'stemness' (Schofield, 1978). The niche is considered a fixed compartment of a three-dimensional structure containing elements that participate in the regulation of stem cell proliferation, control the fate of stem cell progeny, and prevent the stem cells from exhaustion or death (Scadden, 2006; Jones and Wagers, 2008). The bone marrow micro-environment is a major site of MSC niche in the body, in which a complex cellular and non-cellular interaction occurs among hematopoietic stem cells (HSCs). HSCs are known to reside in two different niches: endosteal and perivascular niches. The endosteal niche is thought to maintain HSC quiescence over the long term, whereas the perivascular niche is to maintain HSC proliferation and mediate circulation (Mitsiadis *et al.*, 2007). Little is known regarding the niche for the BMMSCs. Findings from previous studies suggested that BMMSCs and adipose-derived MSC 'harbor' in the perivascular areas of BM (Shi and Gronthos, 2003; Zannettino *et al.*, 2008).

The DPSC niche in human dental pulp was identified by antibodies against STRO-1, CD146, and pericyte-associated antigen (3G5) and was found to be localized in the perivascular and perineural sheath regions (Shi and Gronthos, 2003). These STRO-1^+/$CD146^+$ DPSCs form a dentin-pulp-like complex *in vivo*, similar to the multiple-colony-derived DPSCs. The STRO-1-positive region in the pulp of deciduous teeth is similar to that of permanent teeth, also in the perivascular regions. STRO-1/CD146/CD44 staining of the PDL has shown that it is located mainly in the perivascular region, with small clusters of cells in the extravascular region (Chen *et al.*, 2006b), suggesting that these are the niches of PDLSCs. STRO-1 staining of apical papilla has shown that the positive stain is located in the perivascular region as well as other regions scattered in the tissue (Sonoyama *et al.*, 2006). Thus, it appears that dental stem cells and BMMSCs secure at least one niche in the perivascular region. It is speculated that the MSC compartment extends through the whole post-natal organism as a result of its perivascular location (Meirelles *et al.*, 2006). Currently, it is not known if tissue-specific MSCs originate from the local mesenchymal tissues and later migrate toward the ingrown vasculature, or if they are derived from the vasculature and then influenced by the local signals to acquire their tissue specificity.

MSC HOMING

Circulating, adherent clonogenic cells in post-natal human blood are very rare—found in three of 66 individuals (Kuznetsov *et al*., 2007). The blood from the three donors yielded only 1-2 colony-forming cells each, and these cells displayed immunophenotype characteristics of fibroblastic/smooth muscle/weakly osteogenic cells and adipogenic conversion *in vitro*. Chondrogenic potential could not be tested due to insufficient cell numbers (Kuznetsov *et al.*, 2007). Other investigators applied a two-step method that included an enrichment of mononuclear cells followed by depletion of unwanted cells, and were able to obtain 4-6 CFU-Fs from peripheral blood of all 14 donors, showing osteogenic and adipogenic potential (Valenti *et al.*, 2008). It seems that the number of circulating MSCs in human blood is low under steady-state conditions. However, if *ex vivo*-expanded MSCs are injected into the blood stream, they show some limited capacity to home into various tissues and organs. Systemic delivery of *ex vivo*-expanded BMMSCs through intravenous infusion led to lodging of these MSCs mainly in the lungs, with significantly smaller amounts in the liver, heart, and spleen (Barbash *et al.*, 2003). Intravenous injection of BMMSCs to rats significantly improved functional neurologic recovery (Li *et al.*, 2005). These injected BMMSCs showed some capacity to migrate into damaged areas of brain tissue when administered at an early stage after the onset of ischemia. The signals that guided BMMSCs to the sites of injury may have been the stromal-cell-derived factor-1 (SDF-1), since its expression was up-regulated in the ischemic boundary zone of the brain. Additionally, these BMMSCs expressed CXCR4,

the specific receptor of SDF-1, suggesting that the interaction of SDF-1 with CXCR4 mediated the trafficking of these stem cells to the impaired site (Kortesidis *et al.*, 2005)**.** However, BMMSCs improving cerebral recovery by becoming brain cells is highly unlikely because of the small number of donor cells found in the brain (Shen *et al.*, 2006). Therefore, the beneficial effect of these stem cells on cerebral recovery is likely to be their ability to induce neurogenesis (Chopp and Li, 2002). Currently, no evidence has been shown that BMMSCs administered intravenously migrate to orofacial or dental organs.

IMMUNOMODULATION OF MSCs

The immunoregulation of MSCs can be viewed from two perspectives: (i) immunosuppressive effects of allogeneic MSCs, and (ii) the effects of inflammatory cytokines on MSC activity and differentiation. Due to interest in the allogeneic or xenogeneic MSC supply to compensate for the paucity and time constraint of autogeneic MSC sources, there has been considerable progress in the understanding of the MSC immunosuppressive effect. While xenogeneic MSCs are rejected by the host after transplantation (Grinnemo *et al.*, 2004), allogeneic MSCs are well-tolerated by the recipient hosts. Many *in vivo* studies have confirmed the immunosuppressive effects of MSC (Chen *et al.*, 2006). The potential mechanisms underlying this immunosuppression are the capacity of MSCs to down-modulate immune reactions executed by T-, dendritic, NK, and B-cells. MSCs may potentially be used *in vivo* for enhancing the engraftment of other tissues (*e.g.,* hematopoietic stem cells), for prophylactic prevention, and even possibly as a treatment of graft-*vs*.-host-disease or autoimmune diseases such as rheumatoid arthritis (Jorgensen *et al.*, 2003a,b), to prevent rejection, and to promote transplant tolerance and patient survival. DPSCs immunosuppression has also been shown (Pierdomenico *et al.*, 2005). Other dental stem cells, including SCAP and PDLSCs, also showed immunosuppressive properties *in vitro* (our unpublished observations).

Relatively limited information is available on the effects of pro-inflammatory cytokines on MSCs. A preliminary study in porcine MSCs showed that interferon may act to differentiate MSCs into osteoblasts (Abukawa *et al.*, 2006). In the context of autologous implantation for chondrogenesis, a study has shown that inflammatory reactions against scaffold materials and serum components led to the production of cytokines such as IL-1α that may inhibit cartilage tissue formation (Rotter *et al.*, 2005).

DENTAL MSC-BASED THERAPY FOR REGENERATIVE MEDICINE

Stem-cell-based tooth tissue engineering has been a much-discussed subject, because cell-based therapy for the regeneration of tissue is considered a promising mode of future medicine (Morsczeck *et al.*, 2008; Yen and Sharpe, 2008). To engineer and regenerate a whole tooth, the cell source may have to come from tooth buds in which all the needed cell types are retained. To repair partly lost tooth tissues such as PDL, dentin, and pulp, one or two particular types of dental stem cells may be sufficient to fulfill the need.

SCAP and PDLSCs for Bio-root Engineering

In the past decade, dental implants have become more practical and reliable for restoring the dentition. However, the fundamental pitfalls of dental implants are the lack of a natural contour and structural relationship with the alveolar bone, *i.e*., the artificial cylindrical shape and absence of the PDL. These shortcomings have led to the search for other alternatives. Experimental models of tooth regeneration have been tested on the ectopic formation of tooth-like structures *in vivo,* with cells isolated from tooth buds and seeded onto scaffolds (Young *et al.*, 2002; Duailibi *et al.*, 2004; Nakao *et al.*, 2007). A few reports (discussed below) have demonstrated the orthotopic regeneration of engineered teeth. In larger animal studies, single cells from dog tooth buds at the bell stage were directly seeded onto scaffolds and transplanted back to the original tooth sockets. Dentin structure regeneration was observed, but not enamel or root formation (Honda *et al.*, 2006). In a swine model, *ex vivo*-expanded tooth bud cells (from bell stage) were cultured onto cylindrical scaffolds and autografted back to the original alveolar sockets (Kuo *et al.,* 2007). This group was able to observe tooth formation with root structures along with periodontium (Kuo *et al.*, 2007). Overall, tooth regeneration still faces many obstacles: (i) lack of formation of normal tooth size, (ii) lack of consistent root formation, and (iii) no evidence of complete eruption into functional occlusion.

Instead of attempting to form an entire tooth, Sonoyama *et al*. demonstrated that by utilizing SCAP along with the PDLSCs, they were able to generate a bio-root with periodontal ligament tissues. A mini-swine model was used, and the autologous SCAP and PDLSCs were then loaded onto HA/TCP and gel-foam scaffolds, respectively, and implanted into sockets of the lower jaw. Three months later, the bio-root was formed in the porcine jaw and was exposed for the insertion of post and a porcelain crown (Fig. 5) (Sonoyama *et al.*, 2006). The bio-root structure was comprised of dentin randomly deposited by the SCAP. The bio-root was encircled with periodontal ligament tissue and appeared to have a natural relationship with the surrounding bone. However, the presence of residual HA in the newly generated dentin formed a structure different from that of the naturally formed dentin. This led to a reduced mechanical strength of the bio-root (approximately two-thirds of a natural tooth), where the integrity of this tissue remains to be assessed in long-term studies.

Regeneration of Periodontal Defects with PDLSCs

The use of processed acellular allogenic bone grafts to facilitate the repair of periodontal defects has been a common clinical practice. The beneficial effects of PDGF (platelet-derived growth factor) and IGF (insulin-derived growth factor) on periodontal repair were evidenced from animal studies as well as in human clinical trials (Lynch *et al.*, 1989; Giannobile *et al.*, 1994; Howell *et al.*, 1997; Camelo *et al.*, 2003; Nevins *et al.*, 2003, 2005; McGuire *et al.*, 2006). Platelet-rich plasma (PRP) has also been shown to improve periodontal healing and promote bone regeneration, and the preparation of PRP from

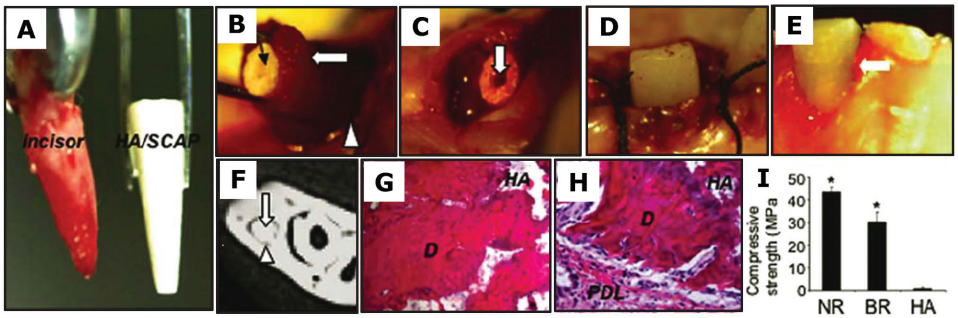

Figure 5. Swine SCAP/PDLSC-mediated bio-root engineering**. (A)** Extracted minipig lower incisor and root-shaped HA/TCP carrier loaded with SCAP. **(B)** Gelfoam containing PDLSCs (open arrow) to cover the HA/SCAP (black arrow) and implanted into the lower incisor socket (open arrowhead). **(C)** HA/SCAP-Gelfoam/PDLSCs were implanted into a newly extracted incisor socket. A post channel was pre-created inside the root-shaped HA carrier (arrow). **(D)** Three months after implantation, the bio-root was exposed and a porcelain crown inserted. **(E)** Four wks after fixation, the porcelain crown was retained after normal tooth use. **(F)** After 3 months' implantation, the HA/SCAP-Gelfoam/PDLSC implant formed a hard root structure (open arrows) in the mandibular incisor area, as shown by CT scan image. A clear PDL space was found between the implant and surrounding bony tissue (arrowhead). **(G, H)** H&E staining showed that implanted HA/SCAP-Gelfoam/PDLSC contains newly regenerated dentin (D) and PDL tissue (PDL) on the outside of the implant. **(I)** Compressive strength measurement showed that newly formed bio-roots have compressive strength much higher than that of the original HA/TCP carrier (*P = 0.0002), but lower than that in natural swine root dentin (*P = 0.003) (NR, natural minipig root; BR, newly formed bio-root; HA, original HA carrier) (adapted from Sonoyama *et al.*, 2006, with permission).

patients has become part of the periodontal practice (Tozum and Demiralp, 2003).

Cell-based regenerative periodontal therapy has gained attention since the isolation of mesenchymal stem cells from various tissues. A clinical case report has demonstrated that using *ex vivo*-expanded autologous BMMSCs facilitated the repair of periodontal defects (Yamada *et al.*, 2006b). To repair periodontal defects, it has been considered that regenerating the PDL is as important as repairing the bone. Focusing only on bone regeneration using the recombinant human bone morphogenetic protein-2 (rhBMP-2) can stimulate clinically significant regeneration of alveolar bone and cementum, but not a functionally oriented PDL, which frequently results in ankylosis between the teeth and the newly formed bone in the coronal aspect of the supra-alveolar defect (Selvig *et al.*, 2002). PDLSCs may be an ideal cellular source for regeneration of the PDL. A recent report on a minipig model has shown that periodontal defects may be repaired by the application of PDLSCs (Liu *et al.*, 2008). This PDSC-mediated treatment resulted in a regeneration of PDL and the recovery of the heights of alveolar bone. This is the first report demonstrating the application of autologous PDLSCs to regenerate PDL and alveolar bone heights in a large animal model.

Stem Cells for Pulp Tissue Engineering and Regeneration

Attempts to induce tissue regeneration in the pulp space have been a long quest. It has previously been proposed to induce hemorrhage and form blood clots in the canal space of mature teeth in the hope of guiding tissue repair in the canal (Ostby, 1961). As a result, attempts were made to regenerate dental pulp with a blood clot filling the canal (Myers and Fountain, 1974). However, the connective tissue that grew into the canal space was limited (0.1-1.0 mm from the foramen) and was not pulp tissue. More recently, with the emergence of tissue-engineering sciences, dental pulp tissue regeneration has been explored with the use of various biomaterials, where pulp cells grown on polyglycolic acid (PGA) formed pulp-like tissue in both *in vitro* and *in vivo* models (Gu *et al.*, 1996; Mooney *et al.*, 1996; Buurma *et al.*, 1999).

Since the isolation and characterization of DPSCs, SHED, and SCAP, the use of these stem cells for dentin/pulp tissue regeneration has been investigated (Huang *et al.*, 2006a, 2008; Murray *et al.*, 2007; Prescott *et al.*, 2008). In a tooth slice model (horizontal section, 1 mm thick), it was shown that SHED seeded onto synthetic scaffolds seated into the pulp chamber space formed odontoblast-like cells that localized against the existing dentin surface (Cordeiro *et al.*, 2008). However, no orthotopic regeneration of pulp-like tissues in the pulp space has been reported with this approach. One concern is that implanting stem cells/scaffolds into root canals that have a blood supply only from the apical end may compromise vascularization to support the vitality of the implanted cells in the scaffolds. It has been proposed that, because of the concern over vascularization, a stepwise insertion of engineered pulp may have to be implemented clinically to achieve the desired pulp tissue regeneration (Huang *et al.*, 2008).

There has been speculation that the undifferentiated mesenchymal cells residing in the periapical tissue or the BMMSCs in the alveolar bone of the jaws can be introduced into the root canal space *via* the formation of blood clots, to allow for pulp-like tissue regeneration and the formation of new odontoblasts (Myers and Fountain, 1974). From our understanding of the characteristics of PDLSCs, DPSCs, and SCAP, it is unlikely that odontoblasts can be derived from PDL or periapical bone. As described above, when BMMSCs and DPSCs are transplanted into the subcutaneous space of immunocompromised mice, the two types of stem cells form BM-like and dentin-pulp-like complexes, respectively (Gronthos *et al.*, 2000). Although some reports showed that DPSCs have osteogenic potential and may form bone-like structure *in vitro* and *in vivo* (Laino *et al.*, 2005, 2006; d'Aquino *et al.*, 2007), there has been no evidence demonstrating that BMMSCs can give rise to functional odontoblasts and generate dentin. One report showed that crude bone marrow cells rarely give rise to dental cells, and only c-kit+-enriched bone marrow cells can acquire the characteristics of odontoblasts. Nonetheless, this phenomenon requires interactions between oral epithelial cells and enriched BM cells (Hu *et al.*, 2006).

FUTURE PROSPECTS

There are several main objectives that need to be addressed before the development of effective cellular-based therapies for regenerative medicine:

(i) Understanding the mechanisms of self-renewal will allow us to regulate adult stem cell growth *in vitro* to generate sufficient cell numbers needed for different applications. One alternative is embryonic stem cells (ESCs) by way of nuclear transfer technologies. However, this process involves the use of unfertilized donor eggs and discarded embryos. Another approach is the *in vitro* manipulation of stem cells to allow for the maintenance of their 'stemness'. Recent demonstrations of the reprogramming of somatic cells to revert to ES-like cells by introducing only 3-4 factors shed light on the possibility of manipulating cells into pluripotent stem cells for a wide variety of applications (Takahashi *et al.*, 2007; Yu *et al.*, 2007; Nakagawa *et al.*, 2008).

(ii) Understanding the regulation of stem cells during differentiation and specific tissue production. Certain tissues require the production of specialized extracellular materials such as bone, dentin, cartilage, and tendon. The production of the extracellular matrix and its maturation into specialized tissues involve a sequential activation of cascades of signals. Controlling and providing these signals artificially at a particular step may facilitate the desired tissue regeneration (Kolf *et al.*, 2007).

(iii) Understanding the interactions between stem cells and the immune system. Immunosuppressive allogenic MSCs may present an abundant cell source for clinical applications. However, immune responses should be noted, as shown by some *in vivo* studies (Poncelet *et al.*, 2007). Further research is needed to determine whether allogenic dental MSCs may suppress recipient host short- and long-term immunorejection.

(iv) Controlling and preventing *ex vivo*-expanded MSCs from transformation. Careful monitoring and observation of this possibility are of paramount important, since evidence has shown that adipose-derived MSCs lost genetic stability over time and are prone to tumor formation (Rubio *et al.*, 2005).

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