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Review

Dental stem cells - characteristics and potential

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Summary. Soft dental tissues have been identified as easily accessible sources of multipotent postnatal stem cells. Dental stem cells are mesenchymal stem cells (MSC) capable of differentiating into at least three distinct cell lineages: osteo/odontogenic, adipogenic and neurogenic. They express various markers including those specific for MSC, embryonic stem cells and neural cells. Five different types of dental stem cells have been isolated from mature and immature teeth: dental pulp stem cells, stem cells from exfoliated deciduous teeth, periodontal ligament stem cells, stem cells from apical papilla and dental follicle progenitor cells. Dental stem cells may be used in dental tissue engineering including dental, enamel and periodontal tissue regeneration. They could also be used as a promising tool in potential treatment of neurodegenerative, ischemic and immune diseases.

Key words: Dental Pulp Stem Cells, Stem cells from Human Exfoliated Deciduous teeth, Periodontal Ligament Human Stem Cells, Stem Cells from Apical Papilla, Dental Follicle Progenitor Cells

Introduction

The hallmark feature of stem cells is their ability to self-renew and maintain multipotency (Mohyeldin et al., 2010). Stem cells are most commonly derived from two main sources: early embryos (embryonic stem cells, ESC) and adult tissue (adult stem cells, ASC) (Volarevic et al., 2011a). ESC play important roles in embryonic development and organogenesis (Escobedo-Lucea et al., 2010). The main advantages of ESC, compared to ASC, are their ability to proliferate for a long period *in vitro* and their potential for differentiation into a broad range of cell types (Stojkovic et al., 2004; Volarevic et al., 2013). However, the use of ESC is controversial and associated with ethical concerns (Cervera and Stojkovic, 2009), while the use of ASC in research and medical therapy does not bring up ethical and legal issues. Depending on their intended purpose, experimental or therapeutic use, ASC can be isolated from a variety of adult tissues including bone marrow (BM), liver, muscle, peripheral blood, adipose tissue, dental tissues, etc (Volarevic et al., 2010, 2011b).

Soft dental tissues such as dental pulp, dental follicle, dental papilla and periodontal ligament (PDL) have been identified as easily accessible sources of stem cells that could be cryopreserved and used for autogenic or allogenic cell therapy (Fig. 1).

Dental stem cells are mesenchymal stem cells (MSC) capable of both self-renewal and multi-lineage differentiation with the capacity to give rise to at least 3 distinct cell lineages: osteo/odontogenic, adipogenic and neurogenic (Miura et al., 2003; Zhang et al., 2006; Sonoyama et al., 2008; Morsczeck et al., 2008, 2009). Dental stem cells express various markers, including those specific for MSC and ESC (Ferro et al., 2012) (Table 1). Furthermore, they express neural cell markers indicating their neural crest origin (Morsczeck et al., 2008).

Up to now, five different types of dental stem cells have been isolated from mature and immature teeth: Dental Pulp Stem Cells (DPSC), Stem cells from

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Exfoliated Deciduous teeth (SHED), Periodontal Ligament Human Stem Cells (PDLSC), Stem Cells from Apical Papilla (SCAP) and Dental Follicle Progenitor Cells (DFPC) (Gronthos et al., 2000; Miura et al., 2003; Seo et al. 2004; Morsczeck et al. 2005; Sonoyama et al., 2006). All five sources have unique characteristics and therefore we summarize here the latest findings, including their differentiation potential.

Dental Pulp Stem Cells - DPSC

The first human dental stem cells, named as Dental Pulp Stem Cells (DPSC), were isolated from adult human dental pulp by enzymatic digestion of the impacted third molar tooth pulp tissue (Gronthos et al., 2000).

Within adult dental tissue, stem cells predominately reside in the perivascular regions of the pulpal cavity from where they migrate to the site of injury (Tecles et al., 2005).

DPSC show similar characteristics to BM stem cells (BMSC) (Shi, 2001). Both populations express similar putative stem cell surface markers: CD44, CD106, CD146, 3G5 and Stro-1, bone-associated markers: alkaline phosphatase, osteocalcin and osteopontin (Shi et al., 2005) and ESC markers: Oct4 and Nanog (Ferro et al., 2012). A comparison of human DPSC and BMSC gene expression profiles has revealed similar gene expression for more than 4000 genes. Few genes such as *collagen type XVIII alpha 1, insulin-like growth factor-2 (IGF-2), discordin domain tyrosine kinase2, NAD(P)H menadione oxidoreductase, homolog 2* of *Drosophila large disk,* and *cyclin-dependent kinase 6* were highly expressed in DPSC, whereas *insulin-like growth factor binding protein-7* (*IGFBP-7*) and *collagen type I alpha 2* were more highly expressed in BMSC. Due to their strong expression of cell cycle activator, *cyclin-dependent kinase 6* and *insulin like growth factor,* DPSC show a 30% higher proliferation rate than BMSC (Shi, 2001). DPSC also express neuronal and glial cell markers, which may be related to their neural crest-cell origin (Chai et al., 2000).

DPSC have a typical fibroblast-like morphology. However, different cell morphologies can be observed in one DPSC colony indicating the presence of several progenitor stem cell populations within mature dental pulp (Gronthos et al., 2000). These cell populations differ in their embryonic status, neural crest origin (expressing the embryonic neural crest cell marker, low-affinity nerve growth factor receptor -LANGFR) versus mesenchymal origin (expressing high levels of beta 1 integrin).

Odontoblastic differentiation potential is an important feature of DPSC. Treatment with transforming growth factor beta1 (TGF beta1) alone or in combination with fibroblast growth factor (FGF2) induce differentiation of DPSC into odontoblasts (He et al., 2008). A non-collagen extracellular matrix protein extract from dentin, dentin matrix protein 1 (DMP1), promotes significantly the odontoblastic differentiation of DPSC and formation of reparative dentin over the exposed pulp tissue (Almushayt et al., 2006). DPSC are also capable of neurogenic, osteogenic, adipogenic, chondrogenic, and myogenic differentiaton (Zhang et al., 2006). More recently it has been shown that DPSC can also differentiate in melanoma cells (Stevens et al., 2008), endothelial cells (d'Aquino et al., 2007), hepatocytes (Ferro et al., 2012; Ishkitiev et al., 2010, 2012) and could be used for derivation of induced pluripotent stem cells (iPSC) (Yan et al., 2010). *In vivo*, they show potential to differentiate into dentin but also into bone or adipose tissue (Zhang et al., 2008). Importantly, DPSC retain their multipotential differentiation potential after cryopreservation (Papaccio et al., 2006; Zhang et al., 2006).

Ex vivo-expanded human adult DPSC responded to neuronal inductive conditions both *in vitro* and *in vivo*. When exposed to neuronal inductive media DPSC acquired a neuronal morphology, expressed neuronal specific markers at both the gene and protein levels and also exhibited the capacity to produce a sodium current consistent with functional neuronal cells. DPSC

Table 1. Different dental stem cell markers.

	DPSC*	SHED**	SCAP***	PDLSC****	DFPC*****
STRO-1	+	+	+	+	+
CD 146	+	+	+	+	+
OCT-4	+	+			
Nanog	+	+			
Nestin	+	+	+		+
Notch-1					+

*: Gronthos et al. 2000; **: Miura et al. 2003; ***: Sonoyama et al. 2006; ****: Seo et al. 2004; ***** Morsczeck et al. 2005

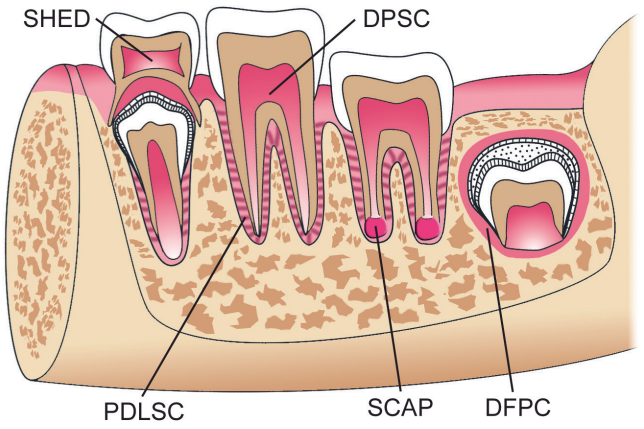

Fig. 1. Sources of dental stem cells. SHED: Stem cells from Human Exfoliated Deciduous teeth, DPSC: Dental Pulp Stem Cells, PDLSC: Periodontal Ligament Stem Cells, SCAP: Stem Cells of the Apical Papilla, DFSC: Dental Follicle Stem Cells.

expressed neuronal markers and acquired a neuronal morphology after transplantation into the mesencephalon of day-2 chicken embryo (Arthur et al., 2008). *In vivo* studies showed that rat DPSC and SHED after transplantion into the adult rodent brain managed to survive and express neuronal markers (Miura et al., 2003; Nosrat et al., 2004). In addition, Nosrat and collegues (Nosrat et al., 2001) showed that DPSC are able to produce neurotrophins and for that reason they can be used in the treatment of neurodegenerative diseases and the repair of injured motor neurons.

DPSC and other types of dental stem cells may be used for dental tissue engineering, including dental, enamel and periodontal tissue regeneration. Two different models were used for *de novo* regeneration of pulp, tooth slice model and tooth root fragment model. The implantation of human tooth slice, 1 mm thick, filled with DPSC combined with DMP1 seeded on a collagen scaffold in subcutaneous space of immunodeficient mice induce the formation of dental-pulp-like tissue after 6 weeks, but odontoblast-like cells were not observed on the dentinal wall (Prescott et al., 2008). Similarly, an *in vivo* study in which the canal of a section of human tooth root, 6-8 mm in length, was filled with DPSC and poly-DL-lactide and glycolide (PLG) scaffold showed that 4 months after transplantation the canal space was filled with regenerated well-vascularized pulp tissue. In this case, a layer of dentin-like mineral tissue was observed on the canal's dentin wall (Huang et al., 2010). Partial regeneration of pulp was demonstrated in a dog model by using two approaches. In both cases, after implantation of DPSC grown as a 3D pellet with bone morphogenetic protein 2 (BMP-2) or subfraction of pulp cells ($CD31^-/CD146^-$) seeded on collagen scaffold into the space of partially amputated pulp chamber reparative dentine formation by the newly differentiated odontoblasts was observed (Iohara et al., 2004, 2009). The fact that DPSC co-cultured with human fetal oral epithelial cells were amelogenin immunopositive after 3 days could imply the potential use of these cells in enamel-tissue engineering (Coppe et al., 2009).

DPSC are also a promising tool in potential treatment of ischemic diseases. When DPSC were transplanted to the border of the infarction zone, in an experimental model of acute myocardial infarction in nude rats, the cardiac repair occurred four weeks after transplantation in the absence of any evidence of DPSC differentiation into cardiac or smooth muscle cells. Cardiac repair was noted by improved cardiac function, increased number of vessels and a reduction in infarct size (Gandia et al., 2008). In models of mouse hind limb ischemia, local transplantation of subfraction of side population from dental pulp cells ($CD31^-$,$CD146^-$) resulted in successful engraftment and an increase in blood flow, including high density of capillary formation (Iohara et al., 2008).

Another important feature of DPSC is their immunomodulatory capacity. They produce transforming growth factor (TGF-beta) and suppress proliferation of peripheral blood mononuclear cells. The toll-like receptor 3 (TLR3) agonist augmented while TLR4 agonist abrogated the immunosuppressive effects of DPSC by inhibiting TGF-beta production and the expression of indolamine-2,3-dioxygenase-1 (Tomic et al., 2011) .

Immature Dental Pulp Stem Cells (IDPSC)

Recently, a subpopulation of DPSC has been described as human Immature Dental Pulp Stem Cells (IDPSC). IDPSC express ESC markers Oct-4, Nanog, SSEA-3, SSEA-4, TRA- 1-60 and TRA-1-81. It is assumed that these cells are precursors to the other two stem cell populations, known as DPSC and Stem cells from Human Exfoliated Deciduous teeth (SHED) (Kerkis et al., 2006).

Transplantation of a tissue-engineered cell sheet composed of human undifferentiated IDPSC in an animal model of total limbal stem cell deficiency led to the formation of functional corneal epithelium both in mild and severe chemical burn after a period of 3 months (Gomes et al., 2010).

They showed dense engraftment in various tissues after transplantation in immunocompromised mice. In addition, transplanted IDPSC significantly improved muscular dystrophy in golden retriever dogs without immunosuppression (Kerkis et al., 2008).

Stem cells from Human Exfoliated Deciduous teeth - SHED

In 2003, Miura and coworkers isolated a population of multipotent stem cells from the remnant pulp of exfoliated deciduous teeth and showed for the first time that one naturally occurring exfoliated organ contains stem cells.

SHED have been identified as a population of clonogenic, highly proliferative postnatal stem cells capable of differentiating into osteogenic and odontogenic cells, adipogenic, neural, endothelial cells and hepatocytes (Miura et al., 2003; Ishkitiev et al., 2010; Sakai et al., 2010; Yamaza et al., 2010). They can also be reprogrammed into iPSC (Yan et al., 2010).

It has been shown that, *in vivo*, transplanted SHED were able to induce bone formation, generate dentin, and survive in mouse brain along with expression of neural markers (Miura et al., 2003). Due to their osteoinductive potential, SHED can repair critical sized calvarial defects in mice with substantial bone formation (Seo et al., 2008). However, unlike DPCS, they are unable to regenerate complete dentin/pulp-like complexes *in vivo* (Gronthos et al., 2000). In comparison with BMSC and DPSC, SHED show a higher proliferation rate and a higher number of population doublings. This elevated proliferative capacity may be associated with the significantly increased telomerase activity (Yamaza et al., 2010). When cultured in neurogenic medium SHED

do not grow as individual cells but in sphere-like clusters, either adhering to the culture dish or floating freely in the culture medium.

SHED were found to express ECS 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). Cultured SHED also express the cell surface molecules STRO-1 and CD146, early mesenchymal stem-cell markers previously found to be present in BMSC and DPSC (Miura et al., 2003) and several stromal- and vascular-related markers: antialkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), basic fibroblast growth factor (bFGF), and endostatin (Seo et al., 2004). As neural crest cell-associated postnatal stem cells, SHED express a variety of neural cell markers, including nestin, beta III tubulin, GAD, NeuN, GFAP, NFM, and CNPase (Miura et al., 2003). Pax6, a marker of retinal stem cells, was also expressed in SHED indicating a good neural cell differentiation potential (Morsczeck et al., 2008). Taghipour and coworkers showed that transplantation of SHED and neural induced SHED (iSHED) in a rat model of acute contused spinal cord injury (SCI) led to significant functional recovery and neural and glial differentiation, as well as limited proliferation 5 weeks after transplantation. However, an open-field locomotor functional test showed that the group that received iSHED were in better state compared with the group that received SHED (Taghipour et al., 2012).

An *in vivo* study of *de novo* regeneration of pulp using tooth slice model and SHED seeded onto the biosynthetic scaffold, poly-l-lactic acid (PLLA), showed that after transplantation in subcutaneous space of immunocompromised mice pulp-like tissue was formed in the pulp chamber space after 4-6 weeks of implantation. Odontoblast like cells but no new dentin was observed to form on the existing dentin surface (Cordeiro et al., 2008).

Similar to BMSC, SHED have immunomodulatory characteristics. They inhibit T helper 17 (Th17) cell function and reduce their number in peripheral blood. They also elevate the ratio of regulatory T cells (Tregs). SHED transplantation showed a more significant effect in up-regulating the ratio of Tregs/Th17 ratio in comparison to BMMSC transplantation in Systemic lupus erithematosus (SLE)-like MRL/*lpr* mice. For that reason, further experiments should be conducted in order to determine SHED as potentially new therapeutic agents for the treatment of SLE (Yamaza et al., 2010).

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). SCAP can only be isolated at a certain stage of tooth development, because during maturation and formation of the crown, dental papilla becomes the dental pulp. The apical portion of the dental papilla is loosely attached to the apex of the developing root and it is separated from the differentiated pulp tissue by a cell rich zone (Huang et al., 2008). The dental papilla contains a higher number of ASC compared to the mature dental pulp (Sonoyama et al., 2006). SCAP are capable of forming odontoblast-like cells and produce dentin *in vivo* and are likely to be the cell source of primary odontoblasts for the root dentin formation, whereas DPSC are likely the source of replacement odontoblasts that form reparative dentin (Sonoyama et al., 2008).

Due to their proximity to the periapical tissues, SCAP residing in the apical papilla are able to survive during the process of pulp necrosis.

SCAP, like other dental stem cells, express STRO-1 and CD146 but they also express CD24, which could be used as a marker for this stem cell population (Sonoyama et al., 2006, 2008). Although SCAP express many osteo/dentinogenic markers following *ex vivo* expansion, compared to DPSC they express lower levels of dentin sialoprotein (DSP), matrix extracellular phosphoglycoprotein (MEPE), transforming growth factor beta receptor II (TGF beta RII), fibroblast growth factor receptor 3 (FGFR3), vascular endothelial growth factor receptor 1 (Flt-1), fibroblast growth factor receptor 1 (Flg) and melanoma-associated glycoprotein (MUC18) (Sonoyama et al., 2008). In addition, SCAP express a higher level of anti-apoptotic protein survivin than DPSC and are positive for hTERT (human telomerase reverse transcriptase) that maintains the telomere length activity (Sonoyama et al., 2006). As neural crest associated cells, SCAP express a variety of neural cell markers including ßIII tubulin, GAD, NeuN, nestin, GFAP, neurofilament M, NSE, and CNPase (Sonoyama et al., 2008).

In vitro, SCAP have the capacity for osteo/dentinogenic, adipogenic and neurogenic differentiation (Sonoyama et al., 2008). Like DPSC and SHED, SCAP can be reprogrammed into iPSC (Yan et al., 2010). Similar to DPSC, SCAP have a similar potential for osteo/dentinogenic differentiation to BMMSC. After transplantation of *ex vivo* expanded SCAP into immunocompromised mice, using hydroxyapatite/tricalcium phosphate (HA/TCP) particles as a carrier, a typical dentin-pulp-like complex was generated. A layer of dentin tissue was formed on the surface of the HA/TCP along with connective tissue (Sonoyama et al., 2006).

After the BrdU incorporation, cells in apical papilla show two to three fold higher proliferation rate *in vitro* than those in pulp. The number of population doublings of SCAP was measured to be up to 80 (Sonoyama et al., 2006). It is possible that SCAP are less differentiated than DPSC, due to the fact that they originate from an embryonic-like tissue.

Periodontal Ligament Stem Cells (PDLSC)

Periodontal tissue is composed of periodontal

ligament, gingiva, alveolar bone and cemetum covering the tooth root. PDLSC can be isolated from cryo-preserved periodontal ligaments (Seo et al., 2005).

PDLSC show similar characteristic to DPSC and BMSC. PDLSC express the MSC-associated markers such as STRO-1 and CD146 and cementoblastic/osteoblastic markers: alkaline phosphatase, bone sialoprotein, osteocalcin and TGF-beta receptor type I. A tendon-specific transcription factor, scleraxis, is expressed at higher levels in PDLSC than in BMMSC and DPSC. PDLSC are multipotent cells that can differentiate into osteogenic, adipogenic, neurogenic and chondrogenic cells *in vitro* (Gay et al., 2007; Lindroos et al., 2008; Xu et al., 2009; Li et al., 2010). Most importantly, PDLSC are capable of differentiation into cells similar to periodontium (Seo et al., 2004). After transplantation of *ex vivo*-expanded PDLSC into immunocompromised mice, cementum/PDL-like structures were formed, suggesting PDLSC as new therapeutic agents in reconstructive dentistry. In addition, PDLSC can be successfully re-implanted into periodontal defects where they promote periodontal regeneration (Hasegawa et al., 2005).

PDLSC, similar to DPSC, have immuno-suppressive characteristics. These cells are able to produce indoleamine 2, 3-dioxygenase (IDO), TGF-beta 1 and hepatocyte growth factor (HGF). However, it is interesting to note that secretion of all these factors is mediated by IFN gamma produced by activated peripheral blood mononuclear cell during inflammation (Wada et al., 2009).

Dental Follicle Progenitor Cells – DFPC

Dental Follicle Progenitor Cells (DFPC) are isolated from the dental follicle or dental sac. As dental follicle is present in impacted teeth which are commonly extracted and disposed of as medical waste, there are no ethical issues regarding DFPC isolation. Some studies showed the presence of three morphologically different

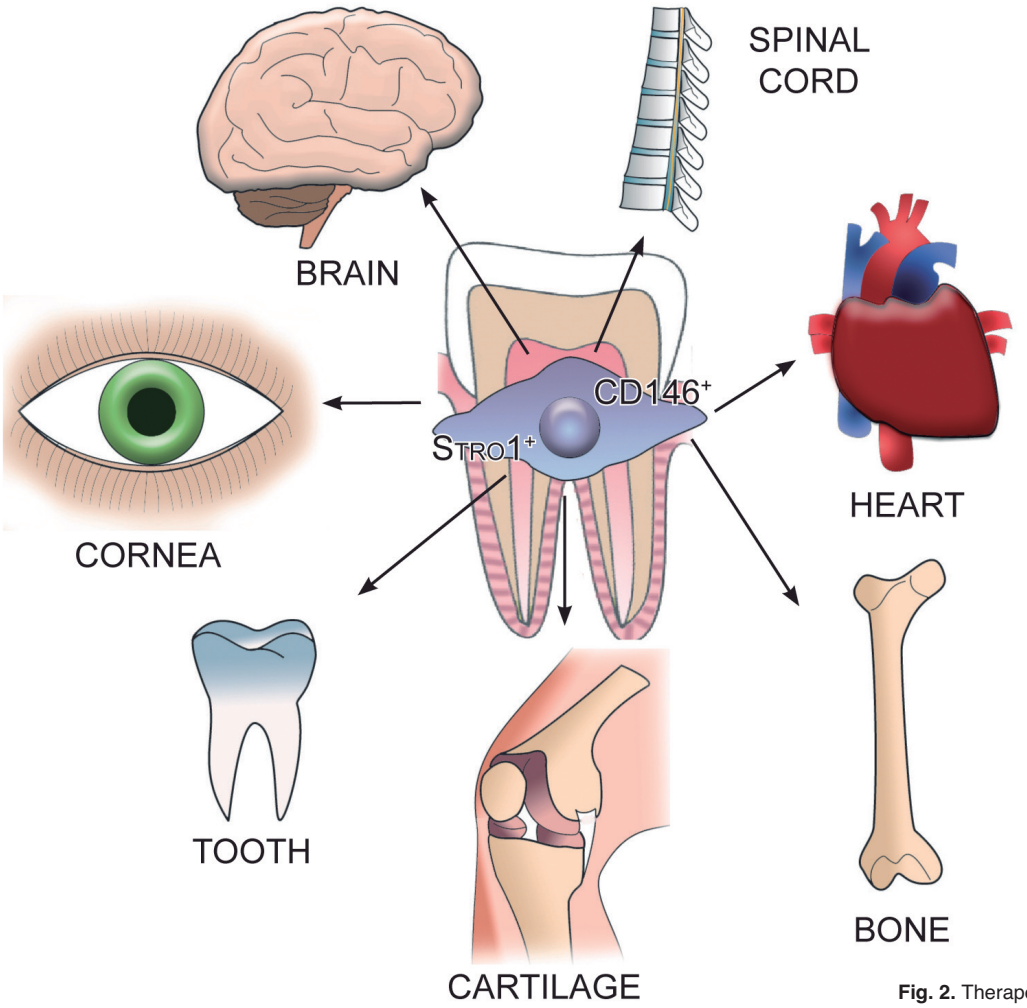

Fig. 2. Therapeutic potential of dental stem cells.

populations of DFPC in human dental sac: HDF1, HDF2 and HDF3. Only HDF2 cells are spindle-shaped while other cell types have a polygonal morphology (Honda et al., 2010).

DFPC have the potential to differentiate into cells of the periodontium such as alveolar osteoblasts, periodontal ligament (PDL), fibroblasts and cementoblasts (Morsczeck et al., 2005; Kemoun et al., 2007). Also, they are capable of differentiating into adipocytes, chondrocytes and neuronal-like cells (Morsczeck et al., 2008, 2009; Yao et al., 2008). In addition, DFPC express neural progenitor cell markers such as Notch-1 and nestin (Morsczeck et al., 2005).

Immunomodulatory properties of DFPC depend on activation of toll-like receptors: the treatment with TLR3 and TLR4 agonist augmented the immunosuppressive potential of these cells by increasing TGF-beta production (Tomic et al., 2011).

Conclusion

Dental stem cells are a powerful tool to study early human developmental biology (normal and abnormal), to improve tissue engineering and the potentials of regenerative medicine. They open new perspectives in therapy of neurodegenerative, ischemic and immune diseases (Fig. 2).

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