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Dental Science

DENTAL TISSUE DERIVED MESENCHYMAL STEM CELLS AND THEIR CLINICAL IMPLICATIONS

Arati A. Inamdar* MD/PhD Ansicht Scidel Diagnostics and Therapeutics LLP, India. *Corresponding Author

Ajinkya C Inamdar Saiseva Biotech Pvt. Ltd. India

ABSTRACT Mesenchymal stem cells (MSCs) have long been explored for their potential regenerative properties and have shown promise in various acute and chronic human conditions. MSCs can be derived from various tissue sources, most commonly being bone marrow, cord blood, cord tissue, placenta, amnion, adipose tissues, menstrual blood and dental pulp. Among these sources, dental pulp derived MSCs support their use for not only orthodontic purpose but also for other non-orthodontic conditions for which these MSCs have been in tested through various clinical trials. The allogenic or autologous banking of dental pulp and their stem cells derived from both deciduous as well as permanent teeth present unique non-invasive modality to obtain MSCs. Such bio-banking also allows establishment of clinical grade cell storage banks for clinical uses for various human conditions. In this review article, we provide updates on the clinical conditions for which dental pulp derived stem cells have shown promise.

KEYWORDS : Bio-banking; Dental stem cells; Mesenchymal Stem cells; Regenerative medicine; Stem cells

Introduction

Regenerative Medicine deals with concept of replacement of adult cells or other cell types with adult or hematopoietic type of stem cells characteristically demonstrate self-renewal and ability to differentiate into various cell lineages and thus restores structure and function of damaged tissues and organs (Mao and Mooney, 2015). Mesenchymal stem cells (MSCs) derived from various tissues are especially being explored for their potential self-renewal and differentiation capability via clinical trials for various human conditions. MSCs, the term was first coined by Dr. Arnold Caplan in 1991 but these cells were initially described by Friedenstein in 1968 (Friedenstein et al., 1968). MSCs have been defined by the International Society for Cellular Therapy (ISCT) in 2006, as "the cells characterized by: a) their capacity to adhere to plastic; b) expression of specific surface markers, namely, CD73, CD90, and CD105, and no expression of CD14, CD19, CD34, CD45 and HLA-DR". Additionally, ISCT further defines that MSCs are able to undergo tri-lineage differentiation into adipocytes, chondrocytes and osteoblasts (Dominici et al., 2006). Among the several sources of MSCs, dental pulp derived MSCs provide an easy, non-invasive and straight forward way of procuring dental tissue derived MSCs. Dental MSCs can be obtained from different dental sources such as human permanent and primary teeth, human wisdom teeth, human exfoliated deciduous teeth, apical papilla, the periodontal ligament and the dental follicle (Bakopoulou and About, 2016; Ledesma-Martínez et al., 2016). Especially, exfoliated deciduous teeth bio-banking has been a great option for saving the allogenic and autologous form of MSCs for treatment of future disease conditions (Collart-Dutilleul et al., 2015).

Dental stem cells are of main five types: a) dental pulp stem cells (DPSC); b) Stem cells from human exfoliated deciduous teeth (SHED); c) Stem cells from apical papilla (SCAP); d) Periodontal ligament stem cells (PDLSCs) and dental follicle precursor cells (DFPCs) (Figure 1). DPSCs were the first to be cultured among these types (Gronthos et al., 2000) while others were isolated subsequently (Miura et al., 2003; Seo et al., 2004; Morsczeck et al., 2005; Sonoyama et al., 2008). Interestingly, dental tissue derived MSCs due to their close proximity to neural crest cells possess unique characteristics similar to those of neural crest cells (Huang et al., 2009).

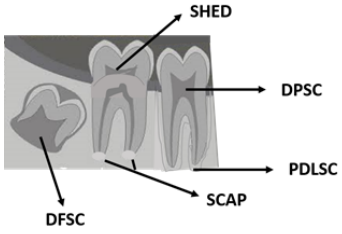

Figure 1: The location of various subtypes of dental stem cells giving rise to dental MSCs from human teeth.

Comparative studies of Dental Pulp cells with other MSCs sources

Dental pulp derived Mesenchymal stem cells can be cultured using traditional culture methods.

When compared to bone marrow MSCs (BM-MSCs) and other tissue sources for MSCs, dental tissue have shown great advantages and uniqueness. It provides option to isolate and cryopreserve dental tissue derived MSC during any time of life non-invasively especially if one has missed to preserve cord blood and cord/placental tissues during the birth. DPSCs have predominantly osteogenic and chondrogenic potential *in vitro* and can differentiate into dentin, *in vivo* and also differentiate into dentin-pulp-like complex. When compared with BM-MSCs, DPSCs have demonstrated immune-phenotype, gene expression profile and multi-lineage differentiation potential similar to that of BM-MSCs (Huang et al., 2009). In terms of proliferative potential, BM-MSCs have limited proliferative capacity whereas umbilical cord blood (UCB)-derived MSCs show the highest proliferative capacity, while adipose-tissue-derived MSCs possess better proliferative potential than that of BM-MSCs (Ballen et al., 2009; Li et al., 2015). Recent studies have compared the gene expression profile of umbilical cord (UC) tissue with that of DPSCs where no significant difference in the genes associated with stem-ness of MSCs such as CD29, CD34, CD44, CD73, CD105, and CD106 was found (Kang et al., 2016). The same report also confirmed better osteogenic capacity by DPSCs while upregulation of immune response, cell proliferation and angiogenesis in the UC tissues than in Dental pulp. Similarly, adipogenic potential of DPSCs derived MSCs was also found to be superior to that of UC tissue and menstrual blood derived MSCs (Ren et al., 2016). The secretome or secreted factors in the conditioned media from MSCs derived from various subtypes of dental pulp and other tissue sources such as BM, umbilical cord tissue and cord blood, and adipose tissues have also been published by various groups (Balasubramanian et al., 2012; Balasubramanian et al., 2013; Amable et al., 2014). Detail description of the secretome derived from DPSCs, SHED and SCAP have been reported (Bakopoulou and About, 2016). Among them, factors involved with angiogenesis mainly include VEGF-A, VEGF-C, EG-VEGF (PK-1), HGF, IGF-1, FGF-2, SDF-1, SCF, EGF, TIMP-1, -2, MMP-2, -3, -9, MCP-1, ANG, TGF-b, ANGPT-1, ANG, DPPIV, EDN-1, PTX-3, PEDF (serpin F1), PDGF-AA and PDGF-AB/BB while those with neurogenic potential include BDNF, GDNF, GDF-15, NCAM-1, TACE, Nidogen-1, NRG-1, NGF, NT-3, CNTF, MDK, NEGF-1 (PTN) and NEGF-2 (Bakopoulou and About, 2016). Furthermore, dental tissue derived MSCs especially DPSCs, SHED and SCAP also reported to show increased though slightly variable expression of embryonic stem cell markers, such as Nanog, Oct3/4, SSEAs (-1, -3, -4, and -5), and to a less extent TRA-1-60 and TRA-1-81 as compared to other MSC types (Kerkis et al., 2006). This shows the embryonic potential of dental stem cells with immense differentiation capacity towards multi-lineage cells.

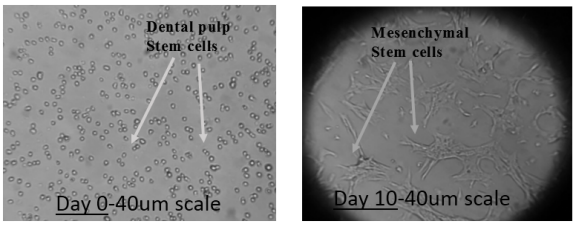

Figure 2:

Dental Pulp MSCs for Regenerative Therapy

As mentioned earlier, Dental pulp based MSCs are categorized into five different subtypes. The therapeutic potentials for these subtypes have been explored all around the world using *in vitro* and *in vivo* models by several research and clinical groups. In addition to their role in oral and dental engineering, dental stem cells possess tremendous regenerative potential for non-oral/dental applications which will be described in the subsequent sections in detail.

a. Adult DPSCs and clinical uses

Adult DPSCs derived MSCs are isolated from dental pulp of permanent tooth. It contains the progenitors that are responsible for formation of dentin and vascularized pulp-like tissue and thus have been successfully used for orthodontic purpose especially dentin-pulp regeneration (Huang et al., 2010). Similar positive results have been seen with animal models of oro-facial conditions such as repair of calvarial defects (Petridis et al., 2015), segmental alveolar defects (Liu et al., 2011) and mandibular bone defects (Ito et al., 2011). Non oral/dental application for DPSCs include their utility for several human conditions. The unique ability of DPSCs to differentiate into cardiomyocytes (Armiñán et al., 2009) as well ability to secrete pro-angiogenic factors imply their potential role in myocardial infarction cases (Gandia et al., 2008). DPSCs have been used for reconstruction of corneal epithelium with the help of human amniotic membrane (Gomes et al., 2010). Due to high proliferative capacity along with ability to differentiate into blood vessels and associated structures, DPSCs have been successful engraftment and capillary formation in a rat model of hind limb ischemia (Iohara et al, 2008). The myogenic potential of DPSCs has been explored in the dog model of muscular dystrophy where histochemical analysis for dystrophin protein was successfully visualized thus confirming the differentiation of DPSCs into dystrophin producing multinucleated muscle cells (Yang et al., 2010). Similarly, neurotrophic ability of DPSCs has been assessed in various neurological conditions. Especially, DPSCs led production of NGF, GDNF, BDNF and BMP2 demonstrated neuroprotective effect in Parkinson's and Alzheimer's diseases (Apel et al., 2009). In compression spinal cord injury (SCI), DPSCs derived cells caused myelination and improvement in the locomotor score in the mouse model of compressive SCI (de Almeida et al., 2011). Peripheral neuron injury was also reversed by DPSCs expressing STRO-1+ /c-Kit+ /CD34+ cells when implanted in the animal model of sciatic nerve defect (Carnevale et al., 2016). Interestingly, DPSCs have also been differentiated into islet -like aggregates indicating their potential use for Diabetes Type 2 treatment (Govindasamy et al., 2011). Due to inherit osteogenic potential of DPSCs, DPSCs demonstrated efficient bone healing along with lower incidence of non-bridging and fibrous tissue in the rat model of osteotomy model (Kitraki et al., 2014). Such osteogenic potential of DPSCs could be enhanced with addition of calcium hydroxide and/or with biological scaffolds (Chen et al., 2016; Paduano et al., 2016).

b. SHED and their clinical application

The regenerative potential of SHED derived MSCs which are isolated from the pulp of deciduous teeth have been assessed via several independent study groups. Similar to DPSCs, SHED also possess osteoblastic capacity and induce dentin formation (Govindasamy et al., 2010). In fact, SHED derived cells shown to produce extensive mineralized matrix but with lower crystallinity and carbonate content in comparison with DPSCs (Volponi et al., 2015). Such osteogenic capacity have been explored in both *in vitro* and *in vivo* models with or without scaffolds (Zheng et al., 2009; Jiao et al., 2014). Angiogenic capability due to expression of pro-angiogenic factors through VEGF/VEGFR and Angiopoietins/Tie pathways demonstrated differentiation of endothelial cells both in *in vivo* and *in vitro* models supported cell survival, migration, and capillary network formation (Bento et al., 2013). SHED is known to express multiple neurogenic proteins such as nestin, glial fibrillary acidic protein (GFAP), doublecortin and neuronal nuclei (NeuN) which when injected into the dentate gyrus of the hippocampus of mice showed improved cell survival and expression of neurofilament M expression (Miura et al., 2003) and further being evaluated for other neurological conditions like focal cerebral ischemia, spinal cord injuries, Alzheimer's disease, and others. Recently, the differential neuronal plasticity towards dopaminergic neurons has been recently reported from SHED derived stem cells (Majumdar et al., 2016). Inoue et al. (2013) and Mita et al. (2015) used SHED derived serum free conditioned media to determine the neuroprotective effect of neurogenic secretome where such media enhanced the recovery of focal cerebral ischemia and improved the cognitive function in mice model of Alzheimer's disease, respectively (Inoue et al, 2013; Mita et al., 2015). Such neurotrophic rich secretome from SHED derived MSCs also led to regeneration of injured peripheral neurons (Sugimura-Wakayama et al., 2015). Similarly, SHED have been demonstrated to differentiate into hepatic lineage cells with appropriate induction (Ishkitiev et al., 2015). Furthermore, hepatic differentiation as well as regeneration has also been confirmed in carbon tetrachloride (CCl4)-induced liver fibrosis model of mice (Yamaza et al., 2015). Immunologically, SHED have been reported to cause significant effects on T helper 17 (Th17) cells *in vitro* where SHED transplantation elevated the ratio of regulatory T cells (Tregs) leading to reversing autoimmune responses in SLE-associated disorders in MRL/lpr mice (Yamaza et al., 2010). The comparative study for SHED, DPSCs and DFSCs were compared for various immunomodulatory parameters where SHED were found to capable of inducing IL-10 and inhibit lymphocyte induced IL-4 and INF-gamma (Yildirim et al., 2015).

c. Stem cells from the apical part of the papilla (SCAP) and clinical application

Stem cells isolated from apical part of the dental papilla, commonly known as SCAP, are capable of differentiating into odontoblastic-like cells and osteogenic cells *in vitro* and into vascularized dentin/pulp-like complexes (Bakopoulou et al., 2011). Among DPSCs, SHED and SCAP, SCAP showed a significantly greater bromodeoxyuridine uptake rate, number of population doublings, tissue regeneration capacity, and number of STRO-1 (stromal precursor antigen 1) –positive cells when compared with other dental stem cells (Ledesma-Martínez et al., 2016). Like other subtypes of dental stem cells, SCAP also expresses neurogenic, angigeogenic and immunomodulatory factors thus harboring inherit potential for neurological, vascular and immunological conditions (Bakopoulou and About, 2016). Studies have elaborated the optimization protocol for producing more of proangiogenic (Angiogenin, IGFBP-3, and VEGF) factors and less of anti-angiogenic (Serpin-E1, TIMP-1, and TSP-1) factors using serum, glucose, and oxygen deprivation (SGOD) conditions (Bakopoulou et al., 2015). SCAP cells are also known to release about 2,046 proteins in conditioned media which include various types of angiogenic, chemokines, immunomodulatory and neuroprotective factors, and ECM proteins (Yu et al., 2016). Studies have presented evidence that SCAP may impart various beneficial effects via both paracrine effect of several secreted proteins as well as *in vivo* differentiation into tissues where they home.

d. Stem cells from the dental follicle (DFSC)

Stem cells isolated from the dental follicle from teeth is commonly known as DFSC. DFSCs are characterized by small colonies which exhibit fibroblast like morphology in later passaging stages have shown to differentiate into osteocytes, adipocytes, and chondrocytes (Yildirim et al., 2015). Improved growth of DFSCs has been reported when DFSCs are cultured at 1% or 5% hypoxic conditions (Dai et al., 2011). DFSCs have been reported to possess high proliferation potential, colony-forming ability, and differentiation potential compared to other cell lines, in particular compared with SHEDs and DPSCs. DFSCs have not been explored in in vivo models for their neuroprotective, angiogenic or immunomodulatory potential although such potential has been confirmed through in vitro or culture based studies by few research group. Of note is study performed by Yildirim et al (2015) where immunomodulatory capacity of DFSCs were found to be superior than that of SHED and DPSCs. Lymphocyte proliferation assay performed with lymphocytes co-cultured with SHED, DFSC and DPSC were quantified using carboxyfluorescein succinimidyl ester (CFSE). DFSCs significantly suppressed the proliferation of lymphocytes as well as Fas (CD95) and Fas ligand (CD178) rate of the lymphocytes. Furthermore, DFSCs suppressed L-4 and INF-gamma levels while possessed anti-inflammatory

properties as evaluated via increased levels of IL-10 (Yildirim et al., 2015). In another study, Vollner et al. (2009) reported formation of neurosphere-like structures from DFSCs when cells were plated onto low-attachment cell culture dishes in serum-free medium containing EGF and FGF-2 indicating neurogenic potential of DFSCs. More studies are needed to completely understand the utility of DFSCs for clinical purposes.

e. Periodontal ligament stem cells (PDLSC)

The stem cells isolated from the cultured periodontal ligament stem cells (PDLSCs), the tooth-supporting tissue, have long been assessed for the regenerative purposes for oral and non-oral tissues. Several variation in the culture methodology has been reported where addition of factor/factors and separation mechanisms have led to isolate, harvest and culture pure population of PDLSCs (Mrozik et al., 2017). In addition to immunomodulatory, angiogenic and neurogenic potential as seen with other subtypes of dental stem cells as described above, PDLSCs have been extensively studied for their novel anti-inflammatory effects in various *in vitro* and *in vivo* models (Wada et al., 2009; Liu et al., 2013; Trubiani et al., 2016). Interestingly, PDLSC-Conditioned Media also shown to enhance periodontal regeneration by suppressing the inflammatory response through TNF-α production and thus present alternative source for periodontal regeneration (Nagata et al., 2017). Recently, these cells also have shown propensity to differentiate into cardiomyocytes as evident via expression of miRNA related with heart development upon dynamic tensile strain (Pelaez et al., 2017). Similarly, differentiation of PDLSCs into retinal ganglion like cells with expression of retinal ganglion cell markers namely ATOH7, POU4F2, β-III tubulin, MAP2, TAU, NEUROD1 and SIX3 along with formation of synapses showing glutamate-induced calcium responses electrical activities indicate their potential use for ocular disorders (Ng et al., 2015).

Clinical grade Dental stem cells and Clinical trials status

The promising condition based regenerative results evident from various *in vitro* and *in vivo* studies with DPSCs, SHED and SCAP and other subtypes of dental cells have indeed indicating them as the alternative sources for MSCs. Dental MSCs have not been used extensively for clinical trials most likely due to lack of consensus regarding the protocol for large scale production of clinical grade of dental tissue derived MSCs. Although, various subtypes have been shown to induce almost similar kind of self-renewal, differentiation and secretome associated properties, but only few studies have compared various aspects of regenerative parameters for dental tissue subtypes, DPSCs, SHED, SCAP, PDLSC and DFSC. The production of clinical grade dental cells requires cGMP (clinical grade manufacturing practices) facility where cells should be produced using standard operating procedures (SOPs) formulated towards highest level of quality of the *ex-vivo* expanded cells. The donor candidates should be screened for the infectious diseases and the cell production from dental cells should be subjected to appropriate SOPs to confirm the phenotypical nature and genetic stability of cultured dental MSCs; ability to efficiently regenerate target tissues; screening for the lack of microbial, viral, fungal, mycoplasma and endotoxin in cultured cells, and absence of tumorigenicity and mutational changes before they could be used for clinical applications. Due to all these reasons, clinical trials have been only undertaken for Phase I/II stages and mainly for the oral or dental purposes (Bakopoulou and About, 2016).

Conclusion

The regenerative potential of dental derived tissues is still in infancy in terms of clinical applications. Due to non-invasive nature, easy accessibility and huge proliferative capabilities as compared to BM-MSCs, dental tissue offers unique source for MSCs. The isolated MSCs could be further characterized using specific markers associated with different subtypes of dental MSCs. Considering the angiogenic, neurogenic and immunomodulatory properties of dental derived MSCs as evident through various *in vitro* and *in vivo* studies, dental tissues present alternative source for vascular, neurological and immunological conditions. More research is advocated towards the large scale production of dental derived MSCs using optimized cost-efficient and universal protocol with applicability for both autologous and allogenic use.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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