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 characterizedly 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 lineage cells. Stem cells with immense differentiation capacity towards multi-lineage (Kerkis et al., 2006). This shows the embryonic potential of dental stem cells (Bakopoulou and About, 2016). Furthermore, dental tissue derived MSCs (Ren et al., 2016). The secretome or secreted factors in 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, PFG-2, SDF-1, SCF, EGF, TIMP-1, MMP-2, MMP-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 cell types.
Bento et al., 2013). SHED is known to express multiple neurogenic supported cell survival, migration, and capillary network formation due to expression of pro-angiogenic factors through VEGF/VEGFR and Angiopoietins/Tie pathways demonstrated capability without scaffolds (Zheng et al., 2009; Jiao et al., 2014). Angiogenic in comparison with DPSCs (Volponi et al., 2015). Such osteogenic potential of DPSCs could be enhanced with addition of osteogenic media (Chen et al., 2016; 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 (Armitage 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., 2011). 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 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, showed a significantly greater bromodeoxyuridine uptake rate, number of population doublings, tissue regeneration capacity, and number of STRO-1 (stromal progenitor antigen 1) –positive cells when compared with other dental stem cells (Ledesma-Martinez et al., 2016). Like other subtypes of dental stem cells, SCAP also expresses neurogenic, angiogenic 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, IGFGBP-3, and VEGF) factors and less of anti-angiogenic (Serpin-E1, TIMP-1, and TSP-1) factors using serum, conditioned media (SCAP) and oxygen deprivation (Yildirim 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 cardiomyocytes, adipocytes, osteocytes, neurons 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., 2015). SCAP cells with appropriate induction (Ishkitiev 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.

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 vitro 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 dorsa of gryus of the hippocampus of mice showed 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 show 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 perineuronal net (Wakayama et al., 2015). Similar positive results have been reported from SHED have been demonstrated to differentiate into hepatic lineage cells with appropriate induction (Ishikiev 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).
periodontal ligament stem cells (PDLCs) The stem cells isolated from the cultured periodontal ligament stem cells (PDLCs), 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 PDLCs (Mrozik et al., 2017). In addition, angiogenesis, immunomodulatory, angiogenic and neurogenic potential as seen with other subtypes of dental stem cells as described above, PDLCs 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, PDLC-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 PDLCs into retinal ganglion cells like cells with expression of retinal ganoglion cell markers namely MITF, POLG, β-III tubulin, MAP2, TAT1, NEUROD1 and SIX6 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 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 secretive associated properties, but only few studies have compared various aspects of regenerative parameters for dental tissue subtypes, DPSCs, SHED, SCAP, PDLCs 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 high 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 tumorigenesis 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-nvasive 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 other subtypes of dental MSCs. Considering the angiogenic, neurogenic and immunomodulatory properties of dental derived MTGF, POLG, β-III tubulin of 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 allogeneic use.

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