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REVIEW ARTICLE

Multi-lineage differentiation and clinical application of stem cells from exfoliated deciduous teeth

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Abstract

Stem cells from human exfoliated deciduous teeth (SHED) have now been considered one of the most promising sources of stem cells for tissue engineering and stem cell therapies due to their stemness and potential to differentiate into other cell lines. The high proliferation rate, the differentiation capacity, the easy access and less ethical concerns make SHED a brilliant solution for many diseases. The purpose of this review is to describe current knowledge of SHED's capability of differentiation, applications and immune status and to draw attention to further research on the mechanism and the dependability of stem cell therapy with SHED.

Keywords Stem cells from human exfoliated deciduous teeth · Phenotypic characteristics · Cell differentiation · Clinical application · Immune

Introduction

Stem cells from human exfoliated deciduous teeth (SHED) are a kind of mesenchymal stem cells which were first isolated by Miura et al. [1, 2]. SHED have the potential to differentiate into several cell lines, for example: osteo/odontogenic, adipogenic and neurogenic cells [1, 3, 4]. Besides, SHED are acquired from naturally "disposable" tissues without significant morbidity to host and with limited ethical concern [5, 6]. Due to their proliferative multipotency and easy acquisition, SHED have been considered to provide a new opportunity to tissue engineering-based and stem cell-based therapies [5].

Isolation of SHED was firstly described by Miura et al. [1] and was confirmed by later researches [7–9]. Extracted dental pulp from deciduous teeth is enzymatically treated with collagenase type I and dispase for 1 h at 37 °C to completely digest the pulp tissue. Following centrifugation, the cell pellet is obtained and single-cell suspensions are seeded in a Petri dish and cultivated in basal culture medium [1, 7–9].

The expression of MSC surface markers can be detected on SHED, for instance, STRO-1, CD10, CD13, CD29, CD44, CD73, CD90, CD105, CD117, CD166 and HLA-A, -B, -C [3, 9–13]. Meanwhile, SHED are negative for CD7, CD14, CD18, CD31, CD34, CD45, CD106, CD184, CD197 and HLA class II [9, 11]. Table1 shows a summary of the phenotypic characteristics of SHED. These results demonstrated that SHED originated from MSC since they fail to express CD34, CD45 (markers for early hematopoietic stem cells), CD7 (a T cell marker along with CD31) and CD106 (one of the endothelial cell markers) [13]. However, controversy persists regarding the positiveness of CD146. Most researches indicated that SHED are positive for CD146, while Suchánek et al. hold the opposite opinion. Due to different cultivation media used, it is not presently possible to explain this difference [7]. Moreover, Pivoriunas et al. mentioned that the expression of CD146 in clones was considerably weaker than in the parental cell line. Thus, they speculated that the proportion of CD146-positive cells may reflect the level of culture senescence [13].

As another significant type of human dental tissue-derived mesenchymal stem cells, dental pulp stem cells (DPSCs) also possess a capacity for self-renewal, multilineage differentiation potential and immunomodulatory

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Table 1 Phenotypic characteristics of SHED

Positive	Negative	Controversial
STRO-1, CD10, CD13, CD29, CD44, CD73, CD90, CD105, CD117, CD166, HLA-A, -B, -C	CD 7, CD14, CD18, CD 31, CD34, CD45, CD106, CD184, CD197, HLA class II	CD146

functions and have been compared with SHED in several studies [14, 15]. It has been demonstrated that SHED show a higher expression of CD117, making them more undifferentiated [7]. SHED also showed higher proliferation rate than DPSCs, since they presented a significantly higher percentage of cells in S + G2M phases compared to DPSCs [12, 16]. Furthermore, SHED have a higher number of population doublings [1]. These characters indicate that SHED have a stronger potential of self-renewal than DPSCs. As for the capabilities of multi-lineage differentiation, it has been shown that SHED have a higher osteogenic differentiation capability than DPSCs [14]. The capability of self-renewal, the expression of stem cell markers and the differentiation potential into other cell linages indicate that SHED comprise a type of stem cells and may provide a potential alternative source for regenerative medicine and therapeutic applications.

As a type of multipotent stem cells, the ability of differentiation into other kinds of cells is a significant character of SHED. Researches on applications taking advantage of this ability have been progressing over years. The immune status should be focused on when SHED are to be applied in treatments or even injected into human bodies. Thus, these aspects have been discussed in the following sections.

Capability of differentiation

SHED have a strong capability to differentiate into other cell lineages. As mentioned before, three cell lines are the most typical: osteo/odontogenic, adipogenic and neurogenic cells.

Osteo/odontogenic differentiation

SHED can differentiate into osteoblasts, with an increase of the osteoblast marker gene expression, including osteopontin (OPN), osteocalcin (OCN), osteoprotegerin (OPG), osterix (OSX), ALP, alpha 1 type 1 collagen (Col1A1), runt-related transcription factor 2 (RUNX2) and CBFA1 [3, 12, 17]. Bone tissue can be observed after SHED are transmitted in vivo [8, 18], which also indicates the osteogenic differentiation potential of SHED. Several factors have been discovered to influence osteogenic differentiation. Sebastian et al. reported that interleukin-17A can induce osteogenic differentiation in SHED which is evidenced by higher ALP activity, increased matrix mineralization and upregulation of the mRNA expression of the osteogenic markers through downregulation of receptor activator of nuclear factor κB ligand (RANKL) as well as altering the OPG/RANKL ratio [19].

Telomerase reverse transcriptase (TERT)/Wnt/β-catenin cascade is also an important pathway through which osteogenic differentiation can be regulated. Liu et al. discovered that acetylsalicylic acid (ASA) treatment upregulates the TERT/Wnt/β-catenin cascade, leading to improvement of SHED-mediated bone regeneration. It was confirmed that ASA significantly improved SHED-mediated bone formation in vivo when SHED were implanted into immunocompromised mice [18]. Lithium chloride (LiCl), has been used as a canonical Wnt pathway activator. SHED treated with LiCl exhibited an accumulation of β-catenin, indicating the activation of the Wnt pathway. Yet, LiCl is not specific in activating the Wnt pathway. The effect of LiCl on cell proliferation is controversial and may depend on the sources and development stage of the cells. LiCl significantly decreased OSX and DMP1 mRNA expression levels while having scarcely any effect on RUNX2, ALP, OCN, or BMP2 mRNA expression in SHED. Furthermore, there was no significant difference in ALP activity and mineral deposition after treatment with LiCl [20].

Another significant pathway is bone morphogenetic protein (BMP) receptor signaling pathway, containing several cascades such as PKA, JNK and ASK1. Once stimulated by BMP-2, Smads-independent pathways were found to be accelerated, such as the p38, ERK and c-Jun N-terminal kinase (JNK) cascades, which play significant roles in osteoblast differentiation. Other genes were found to be upregulated as well, for instance, BMP-2, BMP-4, JNK (MAPK10) and PRKAR2B genes, among which the increase of BMP-4 is crucial to SHED's differentiation into mineralized-forming cells. This finding indicated that BMPs acted via activation of the mitogen-activated protein kinase (MAPK) or JNK pathways [21].

Other molecules or drugs such as Jagged-1 and Dll-1 (the surface immobilization of Notch ligands) [22], interleukin 6 (IL-6) [23], graphene oxide and graphene oxide quantum dot [24] and bezafibrate [25] have the ability to enhance the osteogenic differentiation through different pathways. The differentiation process of SHED might be induced by these pathways or molecules. This might be beneficial to stem cell therapy.

Apart from these, some molecules inhibit the mineralization process of SHED. Cobalt chloride ($CoCl_2$), a hypoxia-mimetic agent, was found to increase the expression of stem cell markers (OCT4, NANOG, and SOX2) as well as promoted the migration ability of SHED. But $CoCl_2$ has a negative effect on SHED osteogenic differentiation through reduction in alkaline phosphatase (ALP) activity and calcium deposition. The expression of other osteogenic-related genes was also suppressed [26].

Nowwarote et al. suggested that endogenous basic fibroblast growth factor (bFGF) induced colony formation and inhibited osteogenic differentiation ability [27]. Further researches indicated that bFGF resulted in the decrease of ALPL mRNA expression and ALP enzyme activity as well as the increase of ANKH mRNA. bFGF decreased both Pi/PPi ratio and mineral deposition. It was concluded that continuous bFGF treatment of SHED inhibited mineral deposition partly via regulation of genes controlling Pi/PPi metabolism [28]. Therefore, Nowwarote's work indicated that the inhibition of bFGF signaling may be useful to enhance osteogenic differentiation of stem cells.

In spite of the strong bone regeneration capability, the mechanisms of new bone formation after SHED implantation, unlike the bone/marrow organ-like structure generated by bone marrow mesenchymal stem cells, still need further researches [8, 18].

Odontogenic differentiation is another important capability of SHED. Markers such as dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP-1) and matrix extracellular phosphoglycoprotein (MEPE) [5, 29] are shown to be upregulated in SHED-derived odontoblasts. According to Casagrande et al., BMP-2 is required for odontoblastic differentiation, both in vivo and in vitro. When seeded in human tooth slice/scaffolds and cultured in vitro, or implanted subcutaneously into immunodeficient mice, SHED express the BMP receptors, BMPR-IA, BMPR-IB and BMPR-II. Moreover, Blockade of BMP-2 signaling inhibits the expression of markers of odontoblastic differentiation by SHED cultured in tooth slice/scaffolds [30]. This conclusion was further evidenced by Khoroushi et al. [31]. A sixfold increase in the expression of DSPP genes and collagen type-I, and a twofold increase in the expression of ALP were discovered in the scaffold of the BMP2 group compared to the scaffold of the control group.

Scaffold is another fundamental element of the mineralization process of SHED [32]. Ideally, a scaffold must allow cell attachment and migration, permit the localized and sustained delivery of growth factors and enable the influx of oxygen to maintain the high metabolic demands of cells engaged in tissue regeneration [33–36]. The result of miura et al. illustrated that SHED are capable of growing on the surface of the HA/TCP scaffold [1]. β-TCP is still a kind of scaffold which is widely used for bone reconstruction [37].

Adipogenic differentiation

When adipogenic differentiation is induced, the increase in the expression of a few adipose cell markers can be detected: peroxisome proliferator-activated receptors-γ2 (PPARγ2) and lipoprotein lipase (LPL) [1, 3]. The presence of lipid vacuoles can be revealed by Oil Red O staining after adipogenic differentiation reduction [3, 38]. Yamaza et al. discovered that SHED suffered remarkable impairment of adipogenic differentiation and reduced expression of adipocyte-specific molecules, PPARγ2 and LPL, when compared to human bone marrow mesenchymal stem cells (BMMSCs) [38]. This result demonstrated that the adipogenic differentiation capacity of SHED may not be as strong as BMMSCs. Furthermore, some researchers were even not able to induce in vitro adipogenic differentiation of the SHED and clonal cell strains, which was attributed to the pre-commitment of SHED toward the osteogenic lineage [13].

Neurogenic differentiation

Tissue engineering of artificial nerve is now a suitable and promising treatment available for nerve injuries [39, 40]. SHED have always been considered as an alternative source of stem cells compared to adult human stem cells, due to their stemness and potential to differentiate into other cell lines [39]. The neurogenic differentiation ability of SHED has already been confirmed by the upregulation of neuronal markers including βIII-tubulin, glutamic acid decarboxylase (GAD), nestin, polysialylated-neural cell adhesion molecule (PSA-NCAM), tau, tyrosine-hydroxylase (TH), glial fibrillary acidic protein (GFAP), 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) and neuronal nuclei (NeuN) when cultured under proper conditions [5, 41–45].

Cytokines including fibroblast growth factor8 (FGF8), sonic hedgehog (SHH), basic fibroblast growth factor (bFGF) and glial cell line-derived neurotrophic factor (GDNF) are considered to play a fundamental role in the neurogenesis of SHED [41, 42, 46]. Wang et al. mentioned that SHED are even able to be induced to form neural-like spheres and to further differentiate into a cell population that contains specific dopaminergic neurons, which, after transplantion into the striatum of parkinsonian rats, could partially improve the apomorphine-evoked rotation of behavioral disorders. This indicated that SHED could be a new promising treatment for Parkinson's disease (PD) [41].

Neurogenic differentiation is modulated by complicated pathways. Liu et al. explored a mechanism involved in the differentiation of SHED into neurons. They discovered that long noncoding RNA C21orf121/bone morphogenetic protein 2/microRNA-140-5p gene network promoted direct differentiation of SHED to neuronal cells. lncRNA C21orf121 is a competing endogenous RNA, which competes with

BMP2 binding to miR-140-5p, thereby promoting SHED to differentiate into neuronal cells via upregulating BMP2 expression. Upregulation of lncRNA C21orf121 and downregulation of miR-140-5p enhance the differentiation of SHED to neuronal cells. Also, this study might provide a possible molecular mechanism as well as a novel target for the treatment of autism through the observation of SHED [47].

Aside from factors mentioned above, some researchers have shown that SHED aggregated into a neurosphere-like clusters and differentiated into neural and glial cells more rapidly in dynamic culture than in static culture, which can be explained by the generation of fluid shear stress and enhanced mass transport to the interior of the scaffold, mimicking the natural microenvironment of nerve cells [39, 43, 48]. Table 2 shows a summary of the markers SHED express when differentiated into other cell lines.

Apart from these cell lines, SHED are also able to differentiate into other types of cells. Endothelial cell differentiation, for instance, is another significant potential of SHED. This progress in regulated by several pathways including VEGF/Wnt/β-catenin pathway, VEGF/MEK1/ErK pathway as well as VEGF-DLL4/Notch-EphrinB2 signaling [49–52]. With the stimulation of vascular endothelial growth factor (VEGF), the expression of VEGFR2, CD31 and VE-Cadherin (markers of endothelium) is significantly upregulated [53].

Furthermore, SHED can express HLA-A, -B, -C, human hepatocyte-specific antigen hepatocyte paraffin 1 and human albumin. Transplanted SHED improved hepatic dysfunction and directly transformed into hepatocytes without cell fusion [54]. Human-specific hepatic markers could be found in the blood examination of the animals into which hepatically differentiated SHED were transplanted. The production of these markers might be the key factor that the animal could survive the acute phase of hepatic injury [55]. Several researches indicated that SHED may provide a feasible cell source for liver regeneration [54–57], thus providing an effective treatment for patients with acute or chronic liver failure [58].

Applications of SHED

The existing applications of SHED can be divided into two aspects. The first is tissue engineering with SHED as a source of stem cell, which depends on the differentiation potential of SHED that we have discussed before. The other one is making use of the various growth factors and cytokines secreted by SHED which are known as secretomes and can be detected in stem cell-cultured conditional medium [59].

Regeneration induction after injury

Nerve injury which is often cause by trauma may lead to persistent functional deficits [60]. The ability of SHED to induce neural cell regeneration and minimize the expansion of secondary injury has been demonstrated by several studies [59–62]. Wakayama et al. indicated that the concentrations of growth factors, such as NGF, BDNF, NT-3, GDNF, CNTF, VEGF, and HGF could be detected in SHED-cultured conditioned medium. These factors significantly increased the proliferation, migration, and the expression of neuron-related, extracellular matrix (ECM)-related, and angiogenesis-related genes of Schwann cells [59]. Sakai et al. transplanted SHED into the completely transected adult rat spinal cord and observed recovery of hindlimb locomotor functions. SHED exhibited three major neuroregenerative activities in this progress: the inhibition of injury-induced apoptosis of neurons, astrocytes and oligodendrocytes, the promotion of the regeneration of transected axons and the replacement of lost cells by differentiating into mature oligodendrocytes under extreme conditions. SHED showed multifaceted neuroregenerative activities that fulfill many requirements for functional recovery of neural cells, so that they can be an excellent and practical cellular resource for the treatment of spinal cord injury [61].

The reconstruction induction ability of SHED was confirmed by a couple of researches, though the mechanisms differ from one another [59–62]. Through the modulation of cytokines secreted after the injury, the adjustment of the material transportation of cell membrane, the inhibition of apoptotic of neural cells, the balancing of relative cells as well as the promotion of the migration and differentiation of endogenous neuronal progenitor cells, SHED showed strong therapeutic effect after neural cell injury [60, 62–64].

Table 2 Markers SHED expressed when differentiated into other cell lines

Cell lines	Osteogenic	Odontogenic	Adipogenic	Neurogenic
Markers	OPN, OCN, OPG, OSX, ALP, Col1A1, RUNX2, CBFA1	DSPP, DMP-1, MEPE	PPARγ2, LPL	βIII-tubulin, nestin, GAD, PSA-NCAM, Tau, TH, GFAP, NeuN, CNPase

Apart from inducing neural cell regeneration, SHED are also discovered to have the ability to repair bone defect. Seo et al. have proved that SHED are capable of repairing critical-size parietal defects in immunocompromised mice. Proliferation and differentiation of SHED which participated in the process of bone repairing may be governed by the signaling cascade triggered by TGFβ, FGF and VEGF, whose receptors are also expressed by SHED [8].

Lee et al. [17] manufactured cell sheets for bone repair of cleft palates derived from SHED. The cell sheet could be introduced into the palatal shelf, and when in place become an osteogenic prelude to mature bone formation. Their work provided a new way of designing a proper therapeutic model for palatal bone regeneration and might be an alternative choice to treat cleft palate.

Immunomodulatory function

Studies have shown that SHED have the ability to correct immune imbalance, thus becoming a promising cellular therapy in autoimmune diseases. Several researchers demonstrated that SHED have the ability to regulate the number of $CD4^+$ T cells and/or Treg cells [65–67]. Rossato et al. indicated that SHED could reduce the number of infiltrating IFN-γ^+CD8^+, IL-4^+CD8^+, IFN-γ^+CD4^+ and IL-4^+CD4^+ T cells into the central nervous system, while promoting a significant increase in $CD4^+FOXP3^+$ T cell population in the spleen of experimental autoimmune encephalomyelitis-affected animals [66].

Dai et al. [67] proved that SHED could correct the $CD4^+$ T cell immune imbalance via Treg cells. After injecting SHED to ovalbumin (OVA)-sensitized mice, they discovered a significant decrease of OVA-specific IgE and IgG1 levels in serum and an increase of IL-4, IL-5, IL-13 and IL-17A levels in the spleen. The level of IFN-γ was upregulated by SHED administration. Their research may provide potential therapeutic agents for the treatment of allergic diseases, such as allergic rhinitis.

Yamaza et al. [38] found that SHED had significant effects on inhibiting T helper 17 (Th17) cells in vitro and elevated the ratio of regulatory T cells (Tregs) via Th17 cells after transplantation. Systemic SHED transplantation is capable of offering at least similar therapeutic effect on SLE murine model compared with BMMSC, which indicated that SHED may be an ideal choice for stem cell treatment.

As for the pathway through which SHED modulate immune cells, Liu et al. indicated that SHED mediated T-cell apoptosis and amelioration of disease phenotypes in dextran sodium sulfate-induced colitis mice through upregulation of TERT/FASL signaling, which could be influenced by treatment with acetylsalicylic acid (ASA) [18].

In addition, the potential immunomodulatory effects of SHED on experimental periodontitis were investigated. When cocultured with monocytes/macrophages in a transwell system, SHED were found to be capable of promoting monocyte/macrophage conversion to $CD206^+$ M2-like phenotype. Further in vivo experiment showed an increase in the number of $CD206^+$ M2 macrophages in periodontal tissues following the delivery of SHED. These results demonstrated that SHED altered the cytokine expression profile in gingival crevicular fluid, reduced gum bleeding, increased new attachment of periodontal ligament and decreased osteoclast differentiation. The application of SHED modulated the immune response and inhibited bone destruction as well, which showed great therapeutic benefits for periodontitis [68].

Immune status

Stem cell-based therapy has now been considered to have a good future. Although the number of studies on the application of SHED continues to grow, research on their immune status is nearly blank. However, studies on the immunomodulatory properties of mesenchymal stem/stromal cells (MSCs) have been progressing for many years. MSCs have long been reported to be hypoimmunogenic or 'immune privileged' [69, 70]. Researchers have found that survival of human embryonic stem cells (hESCs) after transplantation into immunocompetent mice are significantly limited. Their data demonstrated that transplanted hESCs trigger robust cellular and humoral immune responses, resulting in intragraft infiltration of inflammatory cells and subsequent hESC rejection [71].

Although preclinical studies and complete clinical trials have shown that treatment with autologous or allogeneic MSCs do not carry any significant adverse effects [72], treatment by MSC injection still needs caution. As a member of MSC, SHED have a great value in clinical application; however, their immune characteristics need further study. It has been reported that apoptosis could be detected when SHED were cocultured with activated immune cells such as natural killer cells (NK cells) and peripheral blood mononuclear cells (PBMCs). Yet, the mechanism and detailed process had not been fully explained in the research [73]. Given the fact that SHED may migrate to sites of inflammation, it is of great significance to know the role they play in immune response: to exacerbate or decrease the inflammatory process.

Conclusion

As a type of MSC, SHED have great capacity of proliferation, potential of multi-lineage differentiation, easy access as well as immunomodulatory function. SHED can be easily

Table 3 Properties of SHED

Capability of differentiation	Osteo/odontogenic differentiation Adipogenic differentiation Neurogenic differentiation Endothelial cell differentiation
Clinical application	Regeneration induction after injury Immunomodulatory function

acquired and the biological, immunological and therapeutic functions of SHED can be retained after a long period of cryopreservation. Table 3 provided a conclusion of the properties of SHED. In conclusion, SHED can be reckoned as a hopeful solution for many diseases and an ideal source of stem cells for tissue engineering.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was disclosed.

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