Stem Cells from Dental Pulp of Human Exfoliated Teeth: Current Understanding and Future Challenges in Dental Tissue Engineering

Hanan OUBENYAHYA[1]

To describe the current scientific knowledge concerning stem cells obtained from the pulp of discarded primary teeth and to discuss their contribution to dental tissue engineering, a narrative review of the relevant literature published in the past decade (2010–2019) in the PubMed database was conducted. The promise that stem cells from human exfoliated deciduous teeth (SHED) hold as a viable biological option to heal diseased dental organs has been the focus of research over the past decade. New ways of inducing higher levels of differentiation through various bioactive agents and scaffolds have been pursued. Attention has also been paid to the regeneration potential of the discarded pulp tissue that originates from high caries risk or inflamed teeth. In conclusion, the field of stem cell engineering is constantly evolving, and although there is still much to learn about the behaviour of SHED, there are endless opportunities for their exploitation in dental regeneration.

Key words: *deciduous tooth, dental pulp, stem cells, tissue engineering*
Chin J Dent Res 2021;24(1):9–20; doi: 10.3290/j.cjdr.b1105867

Stem cells are the most promising type of cells for tissue regeneration and engineering. They are unspecialised precursor cells capable of self-renewal and differentiation into a diverse range of mature cell types. Stem cells are vital to tissue development, maintenance and repair, and can be classified on the basis of their potency or their origins[1,2] (Tables 1 and 2).

As an alternative to the ethically controversial embryonic stem cell therapy, adult stem cells from various tissues have shown great promise in terms of plasticity and developmental potential in vivo[3]. Bone marrow mesenchymal stem cells (BMMSCs) are considered the standard for adult stem cells and have been most extensively studied. However, they present limitations relating to the invasive nature of their collection and possible adverse reactions concerning, for example, teratoma development[4].

Following the growing need for auxiliary resources of readily accessible and high quality human post-natal stem cells, scientists' attention has shifted to the dental organ and its surroundings.

The oral cavity is home to various stem cell niches that can be classified according to their harvest site[5,6]:

- dental pulp stem cells (DPSCs): from adult human dental pulp (teeth extracted for orthodontic reasons or extracted third molars);
- periodontal ligament stem cells (PDLSCs);
- gingiva-derived mesenchymal stem cells (GMSCs);
- oral mucosa stem cells (OMSCs) (found in the lamina propria of adult human gingiva);
- bone marrow mesenchymal stem cells (BMMSCs) (from orofacial bones);
- dental follicle stem cells (DFSCs);
- stem cells from the apical papilla (SCAP);
- periosteum-derived stem cells (PSCs);
- salivary gland–derived stem cells (SGSCs);
- stem cells from human exfoliated deciduous teeth (SHED) (pulp of exfoliated deciduous teeth).

These dental mesenchymal stem cells (MSCs) exhibit different characteristics but share the ability to adhere rapidly to plastic surfaces and the potential for multilineage differentiation towards various phenotypes: osteogenic, adipogenic, chondrogenic, neurogenic, angiogenic and myogenic[7].

1 Department of Dentistry, Military Hospital Agadir, Morocco.

Corresponding author: Dr Hanan OUBENYAHYA, Department of Dentistry, Military Hospital, 80000 Agadir, Morocco. Tel: 212 696060538; Fax: 212 0528272180. Email: hanan.kat@hotmail.fr

Table 1 Stem cell classification based on potency.

Type	Differentiation potency
Totipotent	Ability to generate all possible types of cells including extra embryonic cells (placenta)
Pluripotent	Ability to generate almost all types of cells except placenta
Multipotent	Ability to give rise to a limited number of cells of the same lineage
Oligopotent	A degree of potency giving rise to a few cell types
Unipotent	Capacity to differentiate into one cell type

Table 2 Stem cell classification based on origin.

Type	Characteristics
Embryonic stem cells	- Pluripotent - Derived from the inner cell mass of the blastocyst that forms 3 to 5 days after an egg cell is fertilised by the sperm - Ethically questionable - Can be stored in an undifferentiated state and stimulated to differentiate into any cell type - Risk of tumorigenicity and teratoma formation - Excellent platform for research but so far not used therapeutically
Adult stem cells	- Multipotent or totipotent - Found throughout the body after embryo development - Tissue-specific cells to the tissue or organ they live in - Include hematopoietic stem cells, mesenchymal stem cells, gut stem cells, liver stem cells, bone and cartilage stem cells, epidermal stem cells, neuronal stem cells, pancreatic stem cells, eye stem cells, and DPSCs
Induced pluripotent stem cells	- Pluripotent - Engineered in the laboratory by reprogramming somatic cells back to a pluripotent state - Share the same characteristics as embryonic stem cells but are not exactly the same

Different mechanisms are involved in the development processes, morphological features and functions of deciduous and permanent teeth. These differences translate to the stem cells isolated from each type of tooth. This has laid the foundation for further studies to determine the exact regenerative potential of each type of stem cell[3]. The present study aims to review the study of stem cells from the remnant pulp of exfoliated deciduous teeth and to provide a concise overview of their biological properties demonstrated thus far, and how they can be of use in dental tissue engineering.

SHED: history and characteristics

In 2003, Miura et al[3] identified a new population of highly proliferative and multipotent MSCs. These were isolated from normally exfoliated primary incisors of 7- to 8-year-old children and were termed stem cells from human exfoliated deciduous teeth (SHED). This newest source of progenitor stem cells is an exciting discovery since harvesting stem cells from easily available deciduous teeth is non-invasive and ethically non-controversial, and their banking is less expensive compared to more commonly studied adult stem cells. SHED express a various range of markers and lineage-specific genes[3,8] (Table 3).

Methods

Database and keywords

A search was conducted using the PubMed database (National Institutes of Health). This review only covers the literature on SHED and their applications in dental-related research in the past decade. The terms "stem cells" and "deciduous teeth", collected into medical subject headings, were used as primary keywords. The secondary keywords were "human exfoliated deciduous teeth", "regeneration", "cell therapy" and "tissue therapy".

Inclusion and exclusion parameters

The search filter was set to include studies published in English in the last ten years. The paper selection was performed by considering the keywords that appeared in the title and abstract, then each paper was selected for its content. Studies that focused on SHED and their use in dental-related therapy trials in both humans and experimental animal models were used. Initially, a total of 115 citations were included. Thirty-five abstracts were excluded as they were case reports, reviews or opinions, or were not in English. A further 49 studies were

Table 3 Immunophenotypical characterisation of SHED.

Positive markers	Function/differentiation capacity
Nanog, Oct4	Embryonic stem cell markers
SSEA-3, SSEA-4	Stage-specific embryonic antigens
TRA-1-60, TRA-1-81	Tumour recognition antigens
Stro-1 CD146, CD166, CD117, CD105, CD90, CD73, CD71, CD44, CD29, CD10	Mesenchymal stem cell markers
βIII tubulin GAD NeuN NFM Nestin GFAP CNPase	Neural lineage markers
CBFA1, ALP, MEPE, bone sialoprotein Osteocalcin and osterix	Osteoblast markers
DSPP, DMP-1, MEPE	Odontoblast differentiation
SOX 9, Col 2, Col X	Chondrogenic markers
PPAR-γ2, lipoprotein lipase	Adipogenic markers
Pax6	Retinal stem cell marker
Basic fibroblast growth factor	Cell growth, survival, migration and differentiation
Endostatin	Specific inhibitor of endothelial proliferation and angiogenesis

not considered because they were solely related to stem cells from permanent teeth, pertaining to animal stem cells, unrelated to dental regeneration, or repeat studies. A total of 28 studies were selected for the final review. Due to the heterogeneity of the included studies and the narrative nature of our work, no statistical analysis was performed.

Results

All the studies retained pertained to human SHED and their potential for dental tissue engineering. For clarity, the eligible information is presented in three sections in Table 4:

- Study design: 15 studies strictly concerned in vitro cell culture, 12 involved in vitro culture followed by in vivo tracking of transplanted cells in animal models, and one was a direct in vivo injection.
- Applications/objectives: These were diverse over the 10-year period, whether the study of molecular pathways, the comparison of SHED with different stem cells or their potential optimisation in various scaffolds.
- Results obtained and their clinical relevance to dental regeneration.

Discussion

The detailed analysis of the retained studies showed that the research focus in the last decade can be divided into four categories:

- mapping the potential differences in genetic expression and potential between SHED and other stem cells;
- investigating ways to induce higher levels of in vitro differentiation of SHED to regenerate dentine/pulp and bonelike tissues;
- conducting in vivo studies to support the in vitro findings using seeding in various scaffolds;
- exploring the potential of SHED extracted from inflamed tissues or teeth with high carries risk (teeth with two or more lesions).

Characterisation of SHED as opposed to other stem cells

To certify SHED as a key element in tissue engineering research and a suitable option for therapeutic applications, it is important to compare their characteristics with other stem cells that were discovered first and have a more extensive body of research behind them.

DPSCs and SHED are capable of extensive proliferation and multipotential differentiation as they possess osteogenic, dentinogenic, adipogenic and neurogenic capacities in vitro[37]. In vivo, SHED were capable of spontaneously generating robust amounts of bone, a dentine-like structure and express neural markers in mouse brains. In the same settings, DPSCs were capable of giving rise to osteoblasts and forming ectopic dentine and associated pulp tissue[37-39].

Unlike BMMSCs, DPSCs and SHED have limited bioethical issues; however, SHED have specific characteristics that differ from those of the aforemen-

Table 4 Studies from 2010–2019 describing the use of SHED in dental-related regeneration.

Study	Study design	Application/objective	Results/clinical relevance
Chadipiralla et al[9]	In vitro culture	To examine the effects of retinoic acid (RA) and dexamethasone (Dex) on the proliferation and osteogenic differentiation of SHED and PDLSCs, and to compare the osteogenic characteristics of SHED and PDLSCs under RA treatment	- RA can be an effective inducer of osteogenesis of SHED and PDLSCs - PDLSCs could be a better source of osteogenic stem cells than SHED - Treatments reported in this study may be employed to produce osteogenically differentiated SHED or PDLSCs efficiently for in vivo bone regeneration
Lee et al[10]	In vitro culture	To establish the effects of platelet-rich plasma (PRP) derived from human umbilical cord blood (UCB-PRP) on proliferation and osteogenic differentiation of SHED, DPSCs and PDLSCs	- Treatment with 1% and 2% UCB-PRP induces high levels of proliferation and osteogenic differentiation in SHED, DPSCs and PDLSCs
Kim et al[11]	In vitro culture	To understand the effects of signalling pathways on the differentiation and mineralisation of SHED	- Mammalian target of rapamycin (mTor) plays an important role in DPSC differentiation - Interesting target for dental pulp tissue engineering
Lee et al[12]	In vitro culture	To compare mesenchymal-like stem/progenitor cells in DPSCs from supernumerary teeth and SHED in three age- and sex-matched 6-year-old patients	- Supernumerary and deciduous teeth share many characteristics - Stem cells from supernumerary teeth are inferior to SHEDs for long-term banking - Supernumerary teeth might be an alternative source of stem cells for those who are short of deciduous teeth
Hara et al[13]	In vitro culture	To characterise SHED as compared with BMMSCs	- Osteogenic/odontogenic differentiation of SHED and BMMSCs is regulated by different mechanisms; BMP-4 might play a crucial role in SHED - Effective cell-based mineralised tissue regeneration, including that of bone, pulp and dentine, could be developed by applying the characteristics of SHED
Li et al[14]	In vitro culture and in vivo transplant	To understand the mechanism that controls SHED differentiation	- Basic fibroblast growth factor (bFGF) inhibits osteogenic differentiation of SHED via extracellular signal-regulated protein kinase (ERK1/2) pathway - Blockade of ERK1/2 signalling by small molecular inhibitor treatment improves bone formation of SHED after bFGF treatment
Wang et al[15]	In vitro culture and in vivo transplant	To characterise SHED in comparison with DPSCs	- SHED show a higher proliferation rate and differentiation capability in comparison with DPSCs in vitro - Results of in vivo transplantation suggest that SHED have a higher capability for mineralisation than DPSCs
Vakhrushev et al[16]	In vitro culture and in vivo transplant	To evaluate the osteogenic differentiation capacity of SHED in new-generation biodegradable polylactoglycolide scaffolds, and to undertake a preliminary evaluation of the possibility of using the prepared scaffolds as bone implants in in vivo experiments	- Considerable numbers of multipotent mesenchymal cells with high osteogenic differentiation potential can be isolated from exfoliated deciduous teeth - Polylactoglycolides are a promising material for scaffold fabrication - This approach could be successfully used for bone tissue engineering
Kim et al[17]	In vitro culture and in vivo transplant	To isolate and characterise stem cells from inflamed pulp tissue of human functional deciduous teeth (iSHFD), and to evaluate the influence of fibroblastic growth factor-2 (FGF-2) on their regenerative potential	- MSCs isolated from the inflamed pulp tissue of functional deciduous teeth potentially possess the qualities of SHED - FGF-2 applied to iSHFD during expansion enhanced the colony-forming efficiency of these cells, increased their proliferation and migration potential, and reduced their differentiation potential in vitro - Ectopic transplantation of iSHFD/FGF-2 in vivo increased the formation of dentine-like material

Study	Study design	Application/objective	Results/clinical relevance
Rosa et al[18]	In vitro culture and in vivo transplant	To investigate whether SHED can generate a functional dental pulp when injected into full-length root canals, using scaffolds PuraMatrix (3-D Matrix, Tokyo, Japan) or rhCollagen (CollPlant, Rehovot, Israel)	- SHED survive and differentiate into odontoblasts when transplanted into full-length human root canals with injectable scaffolds - The pulp tissue generated under these experimental conditions contains functional odontoblasts capable of regenerating tubular dentine - This might facilitate the completion of root formation in necrotic immature permanent teeth
Rajendran et al[19]	In vitro culture	To determine the regenerative potential of dental pulp MSCs harvested from teeth with high caries risk	Discarded teeth with high caries risk can be a good source for regenerative medicine and could be a potential source for MSCs and dental pulp MSC banking
Jeon et al[20]	In vitro culture and in vivo transplant	To establish the differences in the in vitro and in vivo characteristics between SHED isolated via enzymatic disaggregation (e-SHED) and outgrowth (o-SHED) primary culture methods	e-SHED exhibit stronger stemness characteristics, but o-SHED are more suitable for hard tissue regeneration therapy in teeth
Yu et al[21]	In vitro culture	To compare the proliferation and differentiation potential of stem cells from inflamed pulp of deciduous teeth (SCID) and SHED	- SCID have proliferation and differentiation potential similar to those of SHED - SCID represent a new potentially applicable source for MSC-mediated tissue regeneration
Farea et al[22]	In vitro culture	To test the inductive effect of chitosan and transforming growth factor-β1 (TGF-β1) as a scaffold/factor combination on SHED proliferation and osteogenic differentiation	- The combination of chitosan scaffolds and TGF-β1 enhanced proliferation and osteogenic differentiation of SHED - The combined application of chitosan scaffold and TGF-β1 in conjunction with SHED might be beneficial for in vivo bone regeneration
Behnia et al[23]	In vitro culture and in vivo transplant	To investigate the effect of SHED transplanted for bone regeneration in a dog mandibular defect	SHED which had been isolated and characterised 5 years previously and stored with cryopreservation banking were capable of proliferation and osteogenesis after 5 years, and no immune response was observed after three months of seeded SHED
Liu et al[24]	In vitro culture and un vivo transplant	To develop appropriate culture conditions to maintain SHED properties during ex vivo culture processes	- Ex vivo acetylsalicylic acid (ASA) treatment can significantly improve SHED-mediated osteogenic differentiation and immunomodulation - ASA treatment is a practical approach to improving SHED-based cell therapy
Werle et al[25]	In vitro culture	To isolate, cultivate and characterise stem cells from the pulp of carious deciduous teeth (SCCD) and compare them to SHED	- SCCD demonstrated a similar pattern of proliferation, immunophenotypical characteristics and differentiation ability to those obtained from sound deciduous teeth - SSCD can be an applicable source for cell-based therapies in tissue regeneration
Turrioni et al[26]	In vitro culture	To determine the effects of different energy densities of infrared light-emitting diodes (LEDs) on cell viability, number of cells and mineralised tissue production by SHED	Infrared LED irradiation can increase the viability and number of pulp cells as well as the formation of mineralised nodules, which play an important role in tertiary dentine formation (pulp healing)
Liu et al[27]	In vitro culture	To examine the effects of magnesium borate, zinc borate and boric acid blended into a chitosan scaffold for osteogenic differentiation of SHED	Divalent metal magnesium and zinc and nonmetal boron can be effective inducers of osteogenesis in SHED
Nakajima et al[28]	In vitro culture and in vivo transplant	To elucidate the nature of bone regeneration by SHED as compared to that of human DPSCs and BMMSCs	SHED may be one of the best cell source candidates for reconstructing an alveolar cleft due to less invasions during sampling of the cells

Study	Study design	Application/objective	Results/clinical relevance
Gao et al[29]	In vitro culture and in vivo transplant	To investigate the potential immunomodulatory effects of SHED on experimental periodontitis	Local delivery of SHED led to the induction of M2 macrophage polarisation, reduction of periodontal tissue inflammation and enhancement of periodontal regeneration
Kunimatsu et al[30]	In vitro culture	To compare the in vitro characteristics of SHED, human DPSCs and human BMMSCs	SHED exhibited higher proliferative activity and levels of basic fibroblast growth factor (bFGF) and bone morphogenetic protein-2 (BMP-2) gene expression compared with BMMSCs and DPSCs
Sebastian et al[31]	In vitro culture	To evaluate the effects of interleukin-17A (IL-17A) on the osteogenic differentiation of SHED	IL-17A enhances proliferation and osteogenic differentiation of SHED
Mohd Nor et al[32]	In vitro culture	To differentiate and characterise fibroblast-like cells from SHED	The fibroblast-like cells differentiated from SHED could be used in future in vitro and in vivo dental tissue regeneration studies as well as in clinical applications where these cells are needed
Yang et al[33]	In vitro culture and in vivo transplant	To evaluate SHED as an alternative seeding cell to dental follicle cells (DFCs) for the construction of bioroots in case of tooth loss	- SHED and DFCs possess a similar odontogenic differentiation capacity in vivo - SHED are regarded as a prospective seeding cell for use in bioroot regeneration in the future
Qiao et al[34]	In vivo transplant	To evaluate the therapeutic effect of local injection of SHED on periodontitis in mice	SHED administration suppresses expression of inflammatory factors, inhibits production of osteoclasts and promotes regeneration of periodontal tissues
Prahasanti et al[35]	In vitro culture and in vivo transplant	To analyse the osteoprotegerin (OPG) and receptor activator of NF-kB ligand (RANKL) expression after the application of hydroxyapatite scaffold and SHED	Hydroxyapatite scaffold and SHED increase osteoprotegerin and decrease receptor activator of NF-kB ligand expression with high potential as an effective agent in alveolar bone defect regeneration
Zhai et al[36]	In vitro culture	To identify expression of human β-defensin-4 (HBD-4) in SHED and characterise its effects on SHED	HBD-4 promoted osteogenic/odontogenic differentiation of SHED and may represent a suitable agent for vital pulp therapy in future clinical application

tioned stem cells. These characteristics, summarised in Table 5, make deciduous teeth a more advantageous and convenient stem cell source in terms of harvesting, culture or differentiation potential.

Another type of dental stem cell source routinely discarded as medical waste are supernumerary DPSCs. Lee et al[12] demonstrated that although they are equally accessible, supernumerary DPSCs still showed weaknesses in storage, making SHED a far better option for long-term banking, especially through cryopreservation[43]. Acceptance of tooth stem cell banking has particularly increased in developed countries[44].

Osteo-/odontogenic in vitro differentiation potential of SHED

One of the major biological properties of SHED that dental tissue engineering relies on is their osteo-/odontogenic differentiation potential. In the past decade, studies have shed some light on osteogenic-induced differentiation by diverse biologically active molecules, as well as the molecular pathways that affect this activity. Other studies have investigated ways of stimulating odontoblast differentiation.

Dexamethasone (Dex) has been traditionally used to promote osteogenic differentiation of adult stem cells[45-47]. Retinoic acid (RA) has been shown to induce osteogenic differentiation of stem cells from adipose tissues and to upregulate the activity of alkaline phosphatase, an osteogenic marker[48]. Chadipiralla et al[9] therefore focused on comparing the effects of RA and Dex on the proliferation and osteogenic differentiation of SHED, and concluded that RA was a stronger inducer of osteogenesis in SHED, which offers valuable information about in vivo bone regeneration.

The role of growth factors in enhancing bone regeneration is widely recognised. PRP is an excellent source of growth factors and its partnership with MSCs has been extensively documented and shown to activate proliferation and preserve stemness[49]. However, the effects of PRP on DPSCs have not been closely studied. Lee et al[10] showed that UCB-PRP contains similar levels of growth factors compared to the more commonly used peripheral blood–derived PRP. Various

Table 5 Advantages of SHED over BMMSCs and DPSCs.

Advantages of SHED over BMMSCs	Advantages of SHED over DPSCs
- Readily available and non-invasive source[39] - Limited ethical and legal concerns[39] - Higher growth potential[40] - Stronger tendency to induce odontoblasts[13]	- Higher proliferation rate and differentiation capability in vitro[15,41] - Higher capacity for osteogenic and adipogenic differentiation in vivo[15] - Abundance of extracellular matrix and growth factors[41] - Enhanced proliferation under adverse culture conditions (hypoxia, high glucose, low serum)[42]

concentrations were tested and the data found that treatment with 1% UCB-PRP induced the highest level of proliferation and osteogenic differentiation in SHED[10].

The molecular pool of PRP is very complex, however, and more in vitro studies are needed to isolate the exact PRP components that have beneficial effects on the proliferation or differentiation of SHED, as well as the optimal concentration for each cell type. These knowledge gaps should be filled in vitro before moving on to in vivo studies and transplantation.

One growth factor that has been incorporated in FDA cleared bone regeneration systems is basic fibroblast growth factor (bFGF)[50]. However, the signalling pathways that govern the fate of MSCs regarding osteogenesis are complex and the effect of bFGF on the osteogenic differentiation of MSCs has yielded contradicting results[51,52].

Li et al[14] proceeded to investigate the regulatory pathways whereby bFGF affects the osteogenic differentiation of SHED through signalling alteration. They found that a high dose of bFGF in vitro inhibited this process via extracellular signal–regulated protein kinases 1 and 2 (ERK1/2) pathway. The regulation of stem cell behaviour by bFGF may depend on several factors including cell type, dose and exposure time. Thus, more careful investigation of the effect of bFGF on SHED is needed before using these findings in stem cell–based therapies.

Bone regeneration remains a complex physiological process involving many cell types and molecular reactions. It starts with an inflammatory response that initiates healing. Successful bone regeneration rests on a balance in the immune response; this is why immunomodulation has emerged as a developing research field in bone repair. Interleukin 17A (IL-17A) is one such proinflammatory cytokine, increasingly recognised as a key player in immune responses. Sebastian et al[31] demonstrated that IL-17A–treated SHED are an excellent source for bone regeneration. The cytokine enhanced proliferation and osteogenic differentiation of SHED, thus providing promising entry into future studies on the intricate network of regulators governing the fate of stem cells.

Another inflammatory process that has benefitted from stem cell research is pulp inflammation. Moderate and deep caries lesions both attract cells to the injury site to repair the odontoblastic layer and secrete a reparative dentine matrix. Existing pulp capping methods have limitations, mainly that they are unable to operate on irreversible pulpitis[53]. The objective is to develop a bioactive protein that can activate stem cells contained in the pulp tissue and provide a suitable microenvironment. The latter would allow the replacement of damaged odontoblasts with new ones from differentiated stem cells and decrease the inflammatory response[54].

The process of dentinogenesis might be similar in nature to osteogenesis, but unlike osteoblasts, it is still difficult to isolate a pure population of odontoblastic cell lines. The mechanism that modulates a progenitor cell's decision to differentiate into functional odontoblasts has still not been elucidated[55]. It involves a complex web of cell signalling molecule interactions. Rapid progress in cellular and molecular biology has led to the identification of many of these. Although bone morphogenic proteins have been the most extensively studied as strong mineralisation inducers, they cannot by themselves determine the direction of odontoblast differentiation. Other molecules seem to be involved, mainly transforming growth factor (TGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factor (IGF), cytokines and interleukins (IL)[56].

Zhai et al[36] focused on the gene expression of SHED when stimulated by proinflammatory cytokines and demonstrated that human beta-defensin 4 (HBD4) stimulated odontogenic differentiation; however, these results are yet to be tested in vivo to verify HBD4 as a potential pulp capping agent. Another study focused on the molecular mechanisms that control the protein translation in a cell's decision to differentiate. It showed that the mammalian target of rapamycin (mTor) signalling pathway could be an interesting target for dental pulp tissue engineering[11].

Following encouraging results involving cell biostimulation with phototherapy, Turrioni et al[26] evaluated the effects of in vitro 850-nm LED irradiation on

cultured stem cells from SHED. They reported that infrared phototherapy increased the viability and number of pulp cells as well as the formation of mineralised nodules that play an important role in tertiary dentine formation. In vitro studies on animal models are needed to determine optimal irradiation parameters before establishing the use of phototherapy as an adjuvant pulp healing treatment by transdentinal biostimulation of pulp cells.

In vivo studies involving SHED

In vivo studies are used to support in vitro findings through clonal culture. The potential to reconstitute bone and dental tissues is tested through stem cell seeding in biodegradable scaffolds, most often transplanted subcutaneously in immunocompromised mice. Scaffolds are typically made of biomaterials that can enable cell adhesion, migration, proliferation and differentiation, and can be natural or synthetic[57].Significant advances in tissue engineering in the last 10 years have made it possible for scaffolds to be pharmacologically modified to act as carriers of growth factors, medication or gene therapy[58].

Vakhrushev et al[16] tested a new generation of biodegradable synthetic polylactoglycolide scaffolds as subcutaneous bone implants. A typical disadvantage of synthetic polymers can be chronic or acute inflammatory host response[59]; in this particular study, however, no signs of inflammatory response were reported and the SHED responded positively to the induction of osteogenic differentiation[16]. These scaffolds can be further improved by incorporating osteoinductive substances, which makes polylactoglycolide a promising material for bone tissue engineering[16].

Synthetic polymers can also be fairly rigid, which might prove problematic within the 3D geometry of root canals. As such, the development of injectable scaffolds is a key step in pulp regeneration within the full length of the canal. SHED were previously shown to be capable of attaching to the dentinal walls and proliferating inside the root canals in vitro[60]. Rosa et al[18] tested two types of injectable scaffolds into the roots of human premolars: nanofibre hydrogel PuraMatrix (3-D Matrix) and human recombinant matrices (rhCollagen, CollPlant, Rehovot, Israel). Both have previously proved to support the odontoblastic differentiation of DPSCs[61,62]. The results showed that in these scaffolds, SHED survive and differentiate into odontoblasts in vitro. The pulp tissue generated under these conditions was capable of regenerating tubular dentine in vivo. However, these roots were transplanted into the subcutaneous tissue of mice and have yet to be tested in an oral environment containing the apical stem cells that are important for pulp regeneration in necrotic immature permanent teeth[18].

A polymer scaffold that has drawn considerable attention in tissue engineering in recent years is chitosan. Chitosan is especially attractive as a bone scaffold material because of its superior ability to promote adhesion and proliferation of osteoblast cells as well as the formation of mineralised bone matrix in vitro[63,64].

Farea et al[22] tested the combination of chitosan scaffolds and transforming growth factor beta 1 (TGF-β1) in conjunction with SHED. The latter were able to survive, attach, proliferate and differentiate into osteogenic lineages in vitro. Chitosan scaffolds supplemented with magnesium borate, zinc borate and boric acid elements also promoted osteogenic differentiation of SHED in vitro[27]. These cell-based seedings provide useful clues for developing combinations that can be translated into bone repair carriers.

Whole tooth root regeneration is still in its infancy, but dental follicle cells (DFCs) have proved to be suitable seeding cells for bioroot development. Since DFCs can only be obtained from unerupted tooth germs, their availability is restricted. Yang et al[33] confirmed that they can be replaced with SHED to generate dentine and periodontal tissue when combined with treated dental matrix in root-shaped scaffolds in vivo.

It must be noted that most of these studies involved subcutaneous implantations in immunocompromised mice. Although the results are certainly encouraging, implanting stem cells in subcutaneous settings does not recreate the mechanical and chemical stimulation that the bone or the dentine–pulp complex usually receives in oral clinical settings.

In recent years, some in vivo translational studies have attempted to address these shortcomings. Behnia et al[23] compared the effect of a SHED-seeded collagen scaffold and a cell-free collagen scaffold in mandibular defects created in dogs. While the main observation was the absence of an immune reaction, they still reported no significant difference in bone formation between the two study groups. Therefore, they could not demonstrate the ability of SHED to contribute to the regeneration of mandibular bone in this particular study.

Miura et al[3] and Seo et al[65] have previously shown the ability of SHED to produce lamellar bone when implanted into calvarial defects via an hydroxyapatite/tricalcium phosphate carrier as opposed to implantation of the carrier alone. Alkaisi et al[66] used SHED to enhance mandibular distraction osteogenesis in rabbits, and more mature bone was observed in the SHED-transplanted group as opposed to the cell-free group.

Though they might seem contradictory, these results still highlight the potential of SHED as a cell source for supporting osteogenesis in animal models without any immune rejection. However, further studies with longer follow-up periods need to be conducted to determine which inductive mixtures of SHED and biomaterials are best suited to produce optimal results.

This literature review also sheds some light on a novel approach to the treatment of periodontitis: in vivo cell-based allogeneic transplantation. It is widely known that periodontitis is a disease affecting the tooth's supporting tissues whereby dysregulation of inflammatory and immune pathways leads to chronic inflammation and tissue destruction. Conventional surgical and non-surgical therapies have shown limitations in that they do not stimulate the host's innate capacity for regeneration. However, to explore the allogeneic usefulness of direct MSC injection in periodontal defects, it is fundamental to study these cells' immunomodulatory properties. In this regard, multiple MSCs have demonstrated favourable periodontal regenerative potential. Ding et al[67] demonstrated that PDLSCs can repair allogeneic bone defects in an experimental swine model of periodontitis without detected immunological rejections. Likewise, Du et al[68] found that local administration of BMMSCs can repair defects due to periodontitis, exerting anti-inflammatory and immunomodulatory functions in rat models.

In later studies, and using the known immunomodulatory effects of SHED, Gao et al[29] and Qiao et al[34] attempted a direct stem cell injection in periodontal defect sites in rat and mice models. They found that SHED contributed to macrophage conversion into the M2 anti-inflammatory phenotype, decreased proinflammatory cytokines, increased new periodontal ligament attachment, reduced osteoclast formation and promoted bone regeneration.

Multiple key parameters still need to be addressed before clinical grade applications are possible, such as optimisation of culture conditions, biosecurity concerns, stringent quality protocols and scaffolding that can truly mimic the natural extracellular matrix. Reproducibility of results from preclinical research cannot be achieved without standardising in vivo protocols in animal models and experimental defects to avoid heterogeneity and reduce bias. Appropriate double-blind randomised clinical trials are necessary to confirm the true regenerative power of these stem cells.

Use of SHED from inflamed tissues/teeth with high caries risk

One of the main advantages of deciduous teeth as a source of stem cells is their accessibility. However, due to the high prevalence of caries in children and the fact that the natural exfoliation process does not necessarily happen in a clinical visit, healthy SHED are not always available. Researchers have thus sought to investigate a new potential source of MSCs: the inflamed pulp tissue from deciduous teeth.

The retrieval of stem cells from damaged pulp started with DPSCs. Alongi et al[69] successfully isolated a population of MSCs from inflamed dental pulp tissue. Although they retained their regenerative potential in vivo, these DPSCs exhibited diminished stem cell properties including a reduction in osteo-/dentinogenic differentiation. However, Wang et al[70] determined the existence of functional DPSCs in clinically compromised dental pulp with irreversible pulpitis, despite low colony formation and low proliferation rate. Pereira et al[71] found no differences regarding the presence of DPSCs, their proliferation and differentiation potential between healthy and inflamed pulp. While contradictory, these findings still reinforce the hypothesis that the population of functional stem cells is not necessarily depleted post-inflammation.

In light of these results, studies emerged about the potential use of stem cells from deciduous teeth with inflamed pulp. Yazid et al[72] found that SHED isolated from inflamed tissues exhibited highly dysfunctional MSC characteristics, stemness and immunomodulatory properties. However, Yu et al[21] showed that there were no significant differences between SHED in sound and damaged pulp in their in vitro proliferation and multidifferentiation potential. Multilineage differentiation (osteogenic, adipogenic and chondrogenic) is proposed as a criteria for defining multipotent MSCs[73]. For Werle et al[25], this cell capacity was similar when comparing stem cells from deciduous teeth with inflamed and normal pulp, suggesting that the carious process did not impair the stem cells' capacity to differentiate into different cell lineages.

Different theories can be pursued to explain the conflicting results about the regenerative potential of stem cells under damaged pulp conditions. The volume of tissue obtained from the inflamed pulp is usually lower and could explain the low colony formation due to decreased stem cell number[70]. Age differences between sound and inflamed samples have also been suggested as a hypothesis in stem cell function decline[21]. Inflammation is a complex molecular phenomenon that

can happen with different intensities and has been suggested as a variable to look out for regarding stem cell viability and differentiation potential[71].

Seeking to remedy the potentially reduced regenerative potential of MSCs from inflamed tissue, Kim et al[17] proposed exposing the inflamed pulp of deciduous teeth to fibroblast growth factor-2 (FGF-2) during expansion and cellular passage. In vitro, this method enhanced colony forming, increased proliferation and migration and reduced differentiation potential. Ectopic transplantation in vivo increased dentinogenesis.

The usually discarded inflamed pulp tissue from deciduous teeth might represent a new, viable source of cells for MSC-mediated tissue regeneration applications. However, further studies are needed to better understand the molecular mechanisms behind altering the limits to which these stem cells can differentiate in a similar way to those from sound SHED. Future studies with larger sample sizes are also required to correlate the degree of inflammation with cell proliferation and/or differentiation competence.

Conclusion

This review sought to illustrate the progress made in using readily available SHED in various strategies for dental tissue engineering. Although there is an extensive body of evidence to support the notion that SHED can be used for dentine/pulp/bone regeneration, there is still a need to deepen understanding of the mechanisms behind the differentiation process and the key elements controlling the fate of stem cells after transplantation (growth factors, nutrients and immunoreactivity). Preventing loss of stem cell properties during ex vivo culture processes is also of utmost importance. Further efforts need to be made in scaffold development and protein delivery strategies before moving to clinical implementation. The potential is certainly encouraging, but recognising the challenges is key to delivering safe and effective stem cell–based therapies to patients in the future.

Conflicts of interest

The author declares no conflicts of interest related to this study.

(Received Mar 09, 2020; accepted Jun 09, 2020)

References

1. Chagastelles PC, Nardi NB. Biology of stem cells: an overview. Kidney Int Suppl (2011) 2011;1:63–67.
2. Nadig RR. Stem cell therapy - Hype or hope? A review. J Conserv Dent 2009;12:131–138.
3. Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807–5812.
4. Kerkis I, Kerkis A, Dozortsev D, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 2006;184:105–116.
5. Lymperi S, Ligoudistianou C, Taraslia V, Kontakiotis E, Anastasiadou E. Dental stem cells and their applications in dental tissue engineering. Open Dent J 2013;7:76–81.
6. Popuri SK. Concerns of a pediatric dentist in dental stem cells: An overview. Open Dent J 2018;12:596–604.
7. Bakopoulou A, About I. Stem cells of dental origin: Current research trends and key milestones towards clinical application. Stem Cells Int 2016;2016:4209891.
8. Khazaei M, Bozorgi A, Khazaei S, Khademi A. Stem cells in dentistry, sources, and applications. Dent Hypotheses 2016;7:42–52.
9. Chadipiralla K, Yochim JM, Bahuleyan B, et al. Osteogenic differentiation of stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth. Cell Tissue Res 2010;340:323–333.
10. Lee JY, Nam H, Park YJ, et al. The effects of platelet-rich plasma derived from human umbilical cord blood on the osteogenic differentiation of human dental stem cells. In Vitro Cell Dev Biol Anim 2011;47:157–164.
11. Kim JK, Baker J, Nor JE, Hill EE. mTor plays an important role in odontoblast differentiation. J Endod 2011;37:1081–1085.
12. Lee S, An S, Kang TH, et al. Comparison of mesenchymal-like stem/progenitor cells derived from supernumerary teeth with stem cells from human exfoliated deciduous teeth. Regen Med 2011;6:689–699.
13. Hara K, Yamada Y, Nakamura S, Umemura E, Ito K, Ueda M. Potential characteristics of stem cells from human exfoliated deciduous teeth compared with bone marrow-derived mesenchymal stem cells for mineralized tissue-forming cell biology. J Endod 2011;37:1647–1652.
14. Li B, Qu C, Chen C, et al. Basic fibroblast growth factor inhibits osteogenic differentiation of stem cells from human exfoliated deciduous teeth through ERK signaling. Oral Dis 2012;18:285–292.
15. Wang X, Sha XJ, Li GH, et al. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Arch Oral Biol 2012;57:1231–1240.
16. Vakhrushev IV, Antonov EN, Popova AV, et al. Design of tissue engineering implants for bone tissue regeneration of the basis of new generation polylactoglycolide scaffolds and multipotent mesenchymal stem cells from human exfoliated deciduous teeth (SHED cells). Bull Exp Biol Med 2012;153:143–147.
17. Kim J, Park JC, Kim SH, et al. Treatment of FGF-2 on stem cells from inflamed dental pulp tissue from human deciduous teeth. Oral Dis 2014;20:191–204.
18. Rosa V, Zhang Z, Grande RHM, Nör JE. Dental pulp tissue engineering in full-length human root canals. J Dent Res 2013;92:970–975.
19. Rajendran R, Gopal S, Masood H, Vivek P, Deb K. Regenerative potential of dental pulp mesenchymal stem cells harvested from high caries patient's teeth. J Stem Cells 2013;8:25–41.
20. Jeon M, Song JS, Choi BJ, et al. In vitro and in vivo characteristics of stem cells from human exfoliated deciduous teeth obtained by enzymatic disaggregation and outgrowth. Arch Oral Biol 2014;59:1013–1023.
21. Yu S, Diao S, Wang J, Ding G, Yang D, Fan Z. Comparative analysis of proliferation and differentiation potentials of stem cells from inflamed pulp of deciduous teeth and stem cells from exfoliated deciduous teeth. Biomed Res Int 2014;2014:930907.

22. Farea M, Husein A, Halim AS, et al. Synergistic effects of chitosan scaffold and TGFβ1 on the proliferation and osteogenic differentiation of dental pulp stem cells derived from human exfoliated deciduous teeth. Arch Oral Biol 2014;59:1400–1411.
23. Behnia A, Haghighat A, Talebi A, Nourbakhsh N, Heidari F. Transplantation of stem cells from human exfoliated deciduous teeth for bone regeneration in the dog mandibular defect. World J Stem Cells 2014;6:505–510.
24. Liu Y, Chen C, Liu S, et al. Acetylsalicylic acid treatment improves differentiation and immunomodulation of SHED. J Dent Res 2015;94:209–218.
25. Werle SB, Lindemann D, Steffens D, et al. Carious deciduous teeth are a potential source for dental pulp stem cells. Clin Oral Investig 2016;20:75–81.
26. Turrioni AP, Montoro LA, Basso FG, de Almeida Lde F, Costa CA, Hebling J. Dose-responses of stem cells from human exfoliated teeth to infrared LED irradiation. Braz Dent J 2015;26:409–415.
27. Liu YJ, Su WT, Chen PH. Magnesium and zinc borate enhance osteoblastic differentiation of stem cells from human exfoliated deciduous teeth in vitro. J Biomater Appl 2018;32:765–774.
28. Nakajima K, Kunimatsu R, Ando K, et al. Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells. Biochem Biophys Res Commun 2018;497:876–882.
29. Gao X, Shen Z, Guan M, et al. Immunomodulatory role of stem cells from human exfoliated deciduous teeth on periodontal regeneration. Tissue Eng Part A 2018;24:1341–1353.
30. Kunimatsu R, Nakajima K, Awada T, et al. Comparative characterization of stem cells from human exfoliated deciduous teeth, dental pulp, and bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun 2018;501:193–198.
31. Sebastian AA, Kannan TP, Norazmi MN, Nurul AA. Interleukin-17A promotes osteogenic differentiation by increasing OPG/RANKL ratio in stem cells from human exfoliated deciduous teeth (SHED). J Tissue Eng Regen Med 2018;12:1856–1866.
32. Mohd Nor NH, Berahim Z, Azlina A, Kannan TP. Identification of novel fibroblast-like cells from stem cells from human exfoliated deciduous teeth. Clin Oral Investig 2019;23:3959–3966.
33. Yang X, Ma Y, Guo W, Yang B, Tian W. Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. Theranostics 2019;9:2694–2711.
34. Qiao YQ, Zhu LS, Cui SJ, Zhang T, Yang RL, Zhou YH. Local administration of stem cells from human exfoliated primary teeth attenuate experimental periodontitis in mice. Chin J Dent Res 2019;22: 157–163.
35. Prahasanti C, Subrata LH, Saskianti T, Suardita K, Ernawati DS. Combined hydroxyapatite scaffold and stem cell from human exfoliated deciduous teeth modulating alveolar bone regeneration via regulating receptor activator of nuclear factor-Kb and osteoprotegerin system. Iran J Med Sci 2019;44:415-421.
36. Zhai Y, Wang Y, Rao N, et al. Activation and biological properties of human β defensin 4 in stem cells derived from human exfoliated deciduous teeth. Front Physiol 2019;10:1304.
37. Cristaldi M, Mauceri R, Tomasello L, et al. Dental pulp stem cells for bone tissue engineering: a review of the current literature and a look to the future. Regen Med 2018;13:207–218.
38. Gronthos S, Brahim J, Li W, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531–535.
39. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.
40. Kashyap R. SHED - Basic structure for stem cell research. J Clin Diagn Res 2015;9:ZE07–ZE09.
41. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod 2009;35:1536–1542.
42. Kanafi MM, Ramesh A, Gupta PK, Bhonde RR. Influence of hypoxia, high glucose, and low serum on the growth kinetics of mesenchymal stem cells from deciduous and permanent teeth. Cells Tissues Organs 2013;198:198–208.
43. Ma L, Makino Y, Yamaza H, et al. Cryopreserved dental pulp tissues of exfoliated deciduous teeth is a feasible stem cell resource for regenerative medicine. PLoS One 2012;7:e51777.
44. Bansal R, Jain A. Current overview on dental stem cells applications in regenerative dentistry. J Nat Sci Biol Med 2015;6:29–34.
45. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.
46. Young HE, Steele TA, Bray RA, et al. Human reserve pluripotent mesenchymal stem cells are present in the connective tissues of skeletal muscle and dermis derived from fetal, adult, and geriatric donors. Anat Rec 2001;264:51–62.
47. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211–228.
48. Malladi P, Xu Y, Yang GP, Longaker MT. Functions of vitamin D, retinoic acid, and dexamethasone in mouse adipose-derived mesenchymal cells. Tissue Eng 2006;12:2031–2040.
49. Rubio-Azpeitia E, Andia I. Partnership between platelet-rich plasma and mesenchymal stem cells: in vitro experience. Muscles Ligaments Tendons J 2014;4:52–62.
50. Farokhi M, Mottaghitalab F, Shokrgozar MA, Ou KL, Mao C, Hosseinkhani H. Importance of dual delivery systems for bone tissue engineering. J Control Release 2016;225:152–169.
51. Pitaru S, Kotev-Emeth S, Noff D, Kaffuler S, Savion N. Effect of basic fibroblast growth factor on the growth and differentiation of adult stromal bone marrow cells: enhanced development of mineralized bone-like tissue in culture. J Bone Miner Res 1993;8:919–929.
52. Majors A, Ehrhart L, Muschler GF. Basic FGF enhances proliferation and reversibly inhibits differentiation of osteoblastic progenitors. Trans Orthop Res Soc 1996;21:113.
53. Cohenca N, Paranjpe A, Berg J. Vital pulp therapy. Dent Clin North Am 2013;57:59–73.
54. Janebodin K, Horst OV, Osathanon T. Dental pulp responses to pulp capping materials and bioactive molecules. CU Dent J 2010;33: 229–248.
55. Bleicher F, Couble ML, Buchaille R, Farges JC, Magloire H. New genes involved in odontoblast differentiation. Adv Dent Res 2001;15:30–33.
56. Kawashima N, Okiji T. Odontoblasts: Specialized hard-tissue-forming cells in the dentin-pulp complex. Congenit Anom (Kyoto) 2016;56:144–153.
57. O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Mater Today 2011:14:88–95.
58. De Witte TM, Fratila-Apachitei LE, Zadpoor AA, Peppas NA. Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. Regen Biomater 2018;5:197–211.
59. Vacanti NM, Cheng H, Hill PS, et al. Localized delivery of dexamethasone from electrospun fibers reduces the foreign body response. Biomacromolecules 2012;13:3031–3038.
60. Gotlieb EL, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. J Am Dent Assoc 2008;139:457–465.
61. Cavalcanti BN, Zeitlin BD, Nör JE. A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. Dent Mater 2013;29:97–102.

62. Gebhardt M, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. Cell survival within pulp and periodontal constructs. J Endod 2009;35:63–66.
63. Seol YJ, Lee JY, Park YJ, et al. Chitosan sponges as tissue engineering scaffolds for bone formation. Biotechnol Lett 2004;26:1037–1041.
64. Levengood SL, Zhang M. Chitosan-based scaffolds for bone tissue engineering. J Mater Chem B 2014;2:3161–3184.
65. Seo BM, Sonoyama W, Yamaza T, et al. SHED repair critical-size calvarial defects in mice. Oral Dis 2008;14:428–434.
66. Alkaisi A, Ismail AR, Mutum SS, Ahmad ZA, Masudi S, Abd Razak NH. Transplantation of human dental pulp stem cells: enhance bone consolidation in mandibular distraction osteogenesis. J Oral Maxillofac Surg 2013;71:1758.e1–e13.
67. Ding G, Liu Y, Wang W, et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells 2010;28: 1829–1838.
68. Du J, Shan Z, Ma P, Wang S, Fan Z. Allogeneic bone marrow mesenchymal stem cell transplantation for periodontal regeneration. J Dent Res 2014;93:183–188.
69. Alongi DJ, Yamaza T, Song Y, et al. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regen Med 2010;5:617–631.
70. Wang Z, Pan J, Wright JT, et al. Putative stem cells in human dental pulp with irreversible pulpitis: an exploratory study. J Endod 2010;36:820–825.
71. Pereira LO, Rubini MR, Silva JR, et al. Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. Int Endod J 2012;45:1080–1090.
72. Yazid FB, Gnanasegaran N, Kunasekaran W, Govindasamy V, Musa S. Comparison of immunodulatory properties of dental pulp stem cells derived from healthy and inflamed teeth. Clin Oral Investig 2014;18:2103–2112.
73. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8: 315–317.