

Dental Stem Cells and Their Applications

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Stem cells are unspecialised cells that can divide, renew, and differentiate into more specialised cells. Due to their unique properties, stem cells are known for their use in therapies and treatments for missing tissues and damaged parts of the body. However, due to the invasive nature and other ethical issues with the retrieval process and usage of stem cells, stem cells are clinically being used in a limited manner. Furthermore, due to the invasive nature of the retrieval process elsewhere, dental tissues are one of the most preferred sources for stem cells. This review covers all of the characteristics of dental tissue-derived stem cells and their potential future uses.

Key words: application, dental stem cell, mesenchymal stem cell, regeneration
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Introduction

Stem cells are undifferentiated cells that are capable of differentiating into more specialised cells with specific functions¹. They mainly divide into two categories: pluripotent stem cells – cells that can differentiate into a variety of cells – and multipotent stem cells – cells that can differentiate into a limited variety of cells. Pluripotent stem cells further divide into embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSs), while multipotent stem cells consist of adult stem cells known as somatic or postnatal stem cells².

Embryonic stem cells

ESCs originate from the inner cell mass of a mammalian blastocyst, which is the early-stage of a preimplantation

embryo³. To be considered an ESC, the cell must satisfy the following properties: 1) form from the inner cell mass of the blastocyst; 2) be capable of an unlimited number of divisions; 3) be capable of continuous proliferation; 4) be clonogenic; 5) have the transcription factor Oct4; 6) be able to differentiate into cells that are from all three primary germ layers of the embryo (endoderm, mesoderm and ectoderm); and 7) not require a signal or G1 stage in the cell cycle, to initiate DNA replication. The size of human ESCs are around 14 μm while mouse ESCs are closer to 8 μm ⁴. ESCs are pluripotent and thought to have potential in cell replacement therapies, organ transplants and DNA restoration⁵. Despite its numerous beneficial characteristics and potential uses, today, ESCs are not being used for clinical use due to ethical issues that arise during its retrieval process. When isolating ESCs, the inner cell mass has to be isolated from the blastocyst and when the inner cell mass is isolated, the blastocyst or embryo destructs. In addition to the ethical issues, ESCs are not clinically being used due to the possibility of ESCs forming tumors⁶.

Induced pluripotent stem cells

When c-Myc, KLF4, Oct4 and Sox2 are imposed into somatic cells, the cells become, like ESCs, pluripotent⁷⁻⁹. These cells are then, called iPSs. iPSs are obtained from adult tissues. The derivation period of iPSs from somatic cells is around 3 to 4 weeks for human cells and 1 to 2 weeks for mouse cells. On top of having a long derivation period, the derivation process of iPS is inefficient.

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The derivation efficiencies are around 0.01% to 0.10%¹⁰. Despite the fact that iPSs were made to become the alternative of ESCs and were studied due to the invasive nature of retrieving ESCs, iPSs are very limited compared to ESCs, in terms of clinical use, due to the reprogramming process. On top of this, iPSs have a long and inefficient derivation process and can be reprogrammed in a detrimental manner. For example, an iPS can end up inducing growth of a damaged body part. This can eventually lead to tumours and cancer¹¹. Despite these issues, when fully understood, iPSs are believed to have potential in organ synthesis, tissue repair and formation of red blood cells¹².

Mesenchymal stem cells

Within adult stem cells, cells known as mesenchymal stem/stromal cells (MSCs), are considered to be special due to being immature and capable of a variety of differentiations. On top of being able to differentiate into more specialised cells with specific functions, MSCs have the ability to self-renew, grow fast, maintain their property after differentiation, and migrate to areas that need aid^{13,14}.

Positive markers that distinguish MSCs from other cells include CD13, CD73, CD105 and CD146. In addition to these positive markers, MSCs have negative markers: CD11b, CD14, CD19, CD34, CD45, CD79 α and HLA-DR. However, to be officially recognised as a human MSC, on top of having both the positive and negative markers stated above, the cells must be adherent to plastic and be able to differentiate into at least osteoblasts, adipocytes and chondroblasts^{2,15}.

MSCs can be retrieved from many locations: bone marrow, human endometrium, adipose tissue, amniotic fluid, the human amnion membrane, the chorion membrane, the placenta, cord blood, the umbilical cord, exfoliated deciduous teeth etc¹⁶. From these sources, dental tissues are considered a suitable source for future clinical stem cell usage, due to being a rich source and having less invasive stem cell-retrieval processes².

Dental stem cells

There are eight major populations of dental tissue-derived MSCs: dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone-derived mesenchymal stem cells (ABSMCs), stem cells from apical papilla (SCAPs), tooth germ progenitor cells (TGPCs) and gingival mesenchymal stem cells (GMSCs)^{16,17}.

Dental pulp stem cells (DPSCs)

DPSCs were the first adult human dental stem cells to be identified. They can be recognised through the following markers: CD9, CD10, CD13, CD14, CD19, CD24, CD29, CD31, CD34, CD44, CD45, CD59, CD73, CD90, CD105, CD106, CD117, CD133, CD146, CD166, CD271, dentin sialophosphoprotein during odontoblast differentiation, dentin matrix protein 1 during odontoblast differentiation, alkaline phosphatase during calcified and mineralized tissue differentiation, osteopontin during early stages of osteogenic differentiation and bone sialoprotein during late mineralized tissue differentiation¹⁸. Furthermore, STRO-1 and telomerase activities show the differentiation availability of each DPSC. Low telomerase activity indicates that the DPSC was differentiated. On the other hand, when the telomerase activity is high, the DPSC is undifferentiated. DPSCs are easily extractable and revivable¹⁹. Although DPSCs are inactive in their dental pulp form, when an injury occurs, DPSCs become active. Furthermore, similar to other dental tissue-derived MSCs, DPSCs do undergo multi-differentiation and self-renewal. DPSCs, along with SHEDs, are also known to take a long time in terms of initial colonisation periods⁴. However, unlike many other dental tissue-derived MSCs, DPSCs are known to be affected by their retrieval location. For example, MSCs from the inner dental pulp chamber have a higher growth rate compared to those from the outer pulp chamber²⁰. DPSCs were also observed to react differently *in vitro* and *in vivo*. *In vitro*, DPSCs are able to differentiate into adipocytes, osteoblasts, odontoblasts, chondrocytes, myocytes, cardiomyocytes, active neurons, melanocytes and hepatocyte-like cells²¹. While *in vivo*, DPSCs can only differentiate into adipocytes, endotheliocytes and myofibers²²⁻²⁵.

Stem cells from human exfoliated deciduous teeth (SHEDs)

SHEDs are tooth-derived MSCs that are commonly compared with DPSCs. They are more proliferative compared to DPSCs and bone marrow derived mesenchymal stem cells (BMSCs)^{26,27}. In addition, they have a higher cell population doubling rate than DPSCs. The markers that distinguish SHEDs are CD11b, CD13, CD14, CD19, CD29, CD34, CD43, CD44, CD45, CD56, CD73, CD90, CD105, CD146 and CD166. SHEDs also contain embryonic stem cell markers, Oct4 and Nanog, neural stem cell markers, Nestin, and stage-specific embryonic antigens, SSEA-3 and SSEA-4²⁸. In addition to being able to differentiate into osteogenic and adipogenic

cells, SHEDs encourage its host cells to form bone²⁹. Although SHED's quality is not heavily affected from its extraction location, SHED was observed to differentiate differently *in vitro* and *vivo*. *In vitro*, SHED was observed to differentiate into osteocyte, odontocyte, adipocyte, chondrocyte, myocyte, neuronal cells, endothelial cells and other specific hepatic proteins. *In vivo*, SHEDs do not directly differentiate into osteogenic cells, instead they induce new bone formation, and when injected into an existing tooth, they are known to aid the creation of functional blood vessels²⁸. Furthermore, *in vivo*, when injected along PEGylated fibrin, SHEDs can induce vascularised soft connective tissue^{30,31}.

Stem cells from apical papilla (SCAPs)

Like DFSCs, SCAPs also have a higher mineralization potential and proliferation rate compared to DPSCs. The positive and negative markers that distinguish SCAPs are the following: CD13, CD14, CD18, CD24, CD29, CD34, CD44, CD45, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD117, CD146, CD150 and CD166^{18,32}. *In vitro*, SCAPs were observed to have additional neural markers such as neurofilament M, neuronal nuclear antigen, and neuron-specific enolase, and they differentiated into odontogenic, osteoblastic, neuronal cells, and hepatocyte-like cells. *In vivo*, SCAPs were observed to produce dentin-pulp like complexes and cement/woven bone-like tissue³³.

Periodontal ligament stem cells (PDLSCs)

Like SHEDs, PDLSCs are also more proliferative than BMSCs. On top of this, they are more clonogenic than BMSCs. PDLSCs contain the following markers: CD9, CD10, CD13, CD14, CD29, CD31, CD34, CD44, CD45, CD59, CD73, CD90, CD105, CD106, CD146 and CD166. The quality of PDLSCs, like DPSCs, depends on their harvest location. Compared to PDLSCs, which are harvested around the alveolar bone, those from the root surface are inferior. PDLSCs can restore periodontal tissues such as alveolar bone, cementum and periodontal ligaments¹⁷. *In vitro*, PDLSCs were observed to differentiate into cementoblasts, osteoblasts, adipocytes, neuronal cells and chondrogenic cells. While *in vivo*, they were observed to, as stated above, regenerate periodontal tissue³⁴.

Dental follicle progenitor stem cells (DFSCs)

From the dental tissue-derived MSCs, DFSCs have the best plasticity. DFSCs have a higher proliferation rate

compared to DPSCs and SCAPs. They can produce cementum and bone, such as the root surface of teeth and are known to be used in periodontal and bone regeneration therapies³⁵. DFSCs have CD9, CD10, CD13, CD29, CD31, CD34, CD44, CD45, CD53, CD59, CD73, CD90, CD105, CD106, CD133, CD166, CD271, Aggrecan, type 1 collagen, type 3 collagen, Notch-1 and Nestin as their markers³⁶. DFSCs have heterogeneity: each DFSC harvested from different teeth have different mineralization and proliferation rates². *In vitro*, DFSCs differentiate into calcified nodules, cementoblasts, chondrocytes, adipocytes and osteogenic cells. While *in vivo*, DFSCs were observed to produce woven bone-like tissue; however, DFSCs were not observed to form hard tissue³⁷.

Others

Unlike the other dental tissue-derived MSCs stated above, ABSCs do not have all the positive and negative markers as stated in the human MSC criteria: ABSC does not have CD14, CD34 and CD45 but additionally has CD90 and STRO-1. More specifically, ABSCs have CD11b, CD13, CD14, CD19, CD29, CD31, CD34, CD44, CD45, CD71, CD73, CD90, CD105, CD146, CD166 and STRO-1³⁸⁻⁴⁰. *In vitro*, ABSCs were observed to differentiate into osteoblastic, chondrogenic and adipogenic cells. In addition to its own differentiation, ABSCs can induce osteogenesis in its host. *In vivo*, ABSCs were observed to form new bone⁴¹.

Like SCAPs, TGPCs have a high proliferation rate. TGPCs have the following markers: CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105, CD106, CD133, CD166 and STRO-1⁴²⁻⁴⁴. *In vitro*, they differentiate into adipocytes, osteoblasts, odontoblasts, chondrocytes, endothelial cells and neuronal cells. While *in vivo*, they were observed to suppress liver inflammation⁴².

Compared to BMSCs, GMSCs have a high proliferation and population doubling rate. The positive and negative markers GMSCs contain are CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105, CD106, CD117, CD146 and CD166. Furthermore, GMSCs have several additional positive markers on top of those included in the human MSC criteria: Oct4, Sox2, Nanog, Nestin, SSEA-4 and STRO-1⁴⁵⁻⁴⁷. *In vitro*, GMSCs differentiate into adipocytes, chondrocytes, osteoblasts, neuronal cells and endoderm-like cells. While *in vivo*, GMSCs were observed to produce tissues in fibrin gels and induce bone regeneration when injected into an osteogenic medium with collagen gel².

Applications of dental stem cells and their immunomodulatory properties

Today, dental tissues, salivary glands and orofacial bone marrow are known to be the safest and most accessible stem cell source. Applications of stem cells, which are an exciting prospect include bone regeneration, tooth regeneration, dentin-pulp formation, periodontal regeneration and neural tissue regeneration^{21,48}.

With regard to bone and tooth regeneration, DPSCs can form fibrous bone tissue, and those from human third molars were observed to restore human mandible defects⁴⁹. SHEDs repair calvarial defects, while DFPCs induce new bone formation²⁹. SCAPs can make bone-like mineralized tissue⁵⁰. Not only do SCAPs make bone-like mineralized tissue but they also, along with PDLSCs, can form root periodontal complexes and support a dental crown on top⁵¹. GMSCs, like DPSCs and SHEDs, can repair mandibular and calvarial defects².

Not only can DPSCs form fibrous bone tissue but can also form dentin pulp-like structures along with odontoblast cells²². While SCAPs and SHEDs do not make dentin structures like DPSCs, SCAPs and SHEDs can make a layer of dentin-like tissue. Furthermore, SHEDs can differentiate into odontoblast cells during this process⁵².

For periodontal regeneration, PDLSCs are known to differentiate into osteoblast and cementoblasts and induce new tissue formation around the surface of dental implants²⁸.

For neural tissue regeneration, SHEDs have shown the most promising results. When SHEDs were injected into Parkinsonian rats, the rats recovered from their behavioural disorder¹⁷. Along with SHEDs, dental pulp cells also demonstrated a positive effect on the tissue organisation of axons and neurons⁵³.

Although the most popular uses of dental tissue-derived stem cells are bone regeneration, tooth regeneration, dentin-pulp formation, periodontal regeneration and neural tissue regeneration, there are also other potential uses for these dental tissue-derived stem cells. PDLSCs can improve facial wrinkles, while SHEDs induce smooth skeletal muscle cells^{54,55}. Furthermore, SHEDs are known to be capable of corneal reconstruction⁵⁶. DPSCs improve ischemic diseases and could potentially offer a treatment alternative for strokes⁵⁷. TGPCs can deter liver injuries from worsening⁴². GMSCs cannot only be used for skin injuries and inflammatory diseases but also reverse body weight loss in chemotherapy⁵⁸.

In general, dental tissue-derived MSCs play a unique role in the inhibition and rise of T cell proliferation. For

example, DPSCs suppress T cell proliferation⁵⁹. This infers that DPSCs can be used as an anti-inflammatory treatment. SHEDs help the growth of regulatory T cells (Tregs), while inhibiting the proliferation of T helper 17 (Th17). This specific type of inhibition and growth allows SHEDs to restore the balance between Th17 and Tregs, which induces the reversal of systemic lupus erythematosus-associated disorders². PDLSCs, on the other hand, inhibit the induction of Tregs⁶⁰. DFSC and ABSC's relationship with T cell proliferation is not clear up until today. However, the pattern between DFSCs and the T cell proliferation infers that DFSCs can potentially become a treatment for tissue injuries. GMSCs, like DPSCs, inhibit T cell proliferation and show similar results to current anti-inflammatory therapies and medications². In addition, by inducing M2 polarization, GMSCs accelerate wound healing, decrease oral mucositis caused by chemotherapy and treat allergic diseases⁵⁸.

Conclusion

With further research on the immunology of dental tissue-derived MSCs, it is believed that dental tissue-derived MSCs can treat parts of the body that currently have no therapies or treatments when damaged, such as the heart and brain. Furthermore, it is known that MSCs can potentially replace organ transplants and become a more personalised cure-all drug that does not cause side effects. Once there has been enough research on these MSCs, complete tooth regeneration will become possible and the supply of dental tissue-derived MSCs, whose retrieval process is not invasive, will become limitless and enough to be clinically used for numerous cases. Today, it is known that DPSCs, along with scaffold technology, can be used to solve the majority of dental-related problems. Numerous outcomes and research indicate that, once completed and mastered, dental tissue-derived MSC therapies will have a huge impact in the medical field.

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