Dental pulp stem cells in regenerative medicine

P. Hollands,*[1] D. Aboyeji[1] and M. Orcharton[1]

Key points

- Highlights that dental pulp stem cells are mesenchymal stem cells capable of forming bone, nerves and connective tissue.
- Highlights that dental pulp stem cells have potential applications in dentistry as well as wider implications in regenerative medicine.
- Suggests that dentists should encourage the collection and storage of dental pulp stem cells by their patients to ensure that these clinically valuable cells are not discarded as medical waste.
- Highlights that dental pulp stem cells can be collected and cryogenically stored for later use from exfoliated deciduous teeth and healthy extracted adult teeth.

The mesenchymal stem cells (dental pulp stem cells; DPSC) found inside teeth represent a significant future source of stem cells for regenerative medicine procedures. This review describes the ontogeny of DPSC; the laboratory processing and collection of DPSC; the immuno-cytochemical characterisation of DPSC; the differentiation between adult DPSC and DPSC obtained from exfoliated deciduous teeth (SHED) and their potential use in regenerative medicine procedures in the future both in dental and general medical applications.

Introduction

'Every tooth in a man's head is more valuable than a diamond,' Miguel de Cervantes, Don Quixote, 1605.

Inside every tooth there are dental pulp mesenchymal stem cells which have the potential to treat a wide range of diseases when utilised in modern regenerative medicine protocols. This review describes the origin of these cells, the identification, harvesting and storage technology, the current clinical applications and the future clinical potential in regenerative medicine. Dental pulp mesenchymal stem cells have the ability to treat stomatognathic disorders such as periodontal disease and also a wide range of connective tissue, bone and neuronal diseases.

Ontogeny of dental pulp stem cells

The development of teeth involves interactions between oral ectodermal epithelial cells which form the enamel, papilla and dental follicle and mesenchymal stem cells which form dentine, pulp, cementum and the periodontal ligament.[1] Five subtypes of mesenchymal stem cells have been described, these are: dental pulp stem cells (DPSC), periodontal ligament stem cells (PDLSC), stem cells from apical papilla (SCAP), dental follicle stem cells (DFSC) and gingival mesenchymal stem cells (GMSC).[2] Teeth are therefore an excellent source of stem cells for therapeutic procedures in the future and can be easily harvested following tooth extraction or natural shedding of deciduous teeth.[3] Dental pulp stem cells (DPSC) were first isolated from third molars and were found to have high clonogenicity and the ability to produce densely calcified colonies.[4] DPSC have also been confirmed as mesenchymal stem cells by demonstrating their ability to form adipocytes, osteoblasts, odontoblasts, chondrocytes, neural ectodermal cells and myoblasts.[5] From a developmental view point the dental pulp is derived from ectomesenchyme arising in the periphery of the neural tube which migrates to the oral region where the cells differentiate into mesenchymal cells.[6]

In normal physiology the dental pulp cells maintain and repair the periodontal tissue and respond to damage. Deep caries result in the dental pulp cells migrating to the damaged area and the creation of odontoblasts and dentine in an attempt to repair the damaged tooth.[7,8] These observations led to the proposal that dental pulp stem cells could be active during reparative dentinogenesis.[9]

Laboratory processing of dental pulp stem cells

There are many approaches to dental pulp stem cell collection and processing, the key is to ensure the quality and safety of the end product. The following describes the process used in the WideCells Institute of Stem Cell Technology: once exfoliated, or extracted, teeth are sent to the processing laboratory within 72 hours of exfoliation or extraction. The tooth is transported in sterile phosphate buffered saline with calcium and magnesium inside a validated and monitored collection kit which keeps the tooth between 4 and 26 °C. On arrival at the laboratory the tooth is opened in a Grade A clean room environment using a medical circular saw, the pulp exposed to 10% DMSO and the whole tooth is then frozen in a controlled rate freezer and stored in the vapour phase of liquid nitrogen.[10] When the dental pulp stem cells are required then the tooth is thawed rapidly in a 37 °C waterbath and then processed using either one of two standard techniques: the explant method and the enzymatic digestion method. In the explant method the dental pulp is dissected from the tooth in a Grade A clean room environment and the cells are then grown *in vitro* from these tissue fragments.[11–13] In the enzymatic method the dental pulp tissue is digested in collagenase and dispase, in a Grade A clean room environment, and the resultant cells then grown *in vitro*.[14,15] Both

[1]WideCells Institute of Stem Cell Technology (ISCT), University of Manchester Innovation Centre, Core Technology Facility, 46 Grafton Street, Manchester, M13 9NT
*Correspondence to: Peter Hollands
Email: peter.hollands@widecellsgroup.com

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of these processing technologies yield good numbers of viable DPSC and future research will no doubt optimise these technologies to develop a gold standard.

Immunocytochemical identification of dental pulp mesenchymal stem cells

The International Society for Cellular Therapy (ISCT) state that mesenchymal stem cells express the following surface antigens: CD105 (endoglin: a putative novel endothelial cell specification gene), CD73 (5' ectonucleotidase: an enzyme which metabolises nucleotides to nucleosides) and CD90/Thy-1 (glycosylphosphatidylinositol-anchored glycoprotein) and a negative for CD11b, CD14, CD19, CD34, CD45, CD79a surface antigens and HLA-DR. These are assessed by the use of flow cytometry.

Other workers propose that mesenchymal stem cells express STRO-1 (stromal precursor antigen 1), VCAM-1 (vascular cell adhesion molecule 1), SH2 (Src homology 2), SH3/SH4, CD271, GD2 (ganglioside 2), and SSEA-4 (stage-specific embryonic antigen-4).[16–20] Some workers even suggest that DPSC may have a different immunophenotype to those traditionally thought to be MSC.[21–22] This variation in surface antigen expression may reflect the proliferative potential of DPSC.[23] STRO-1 positive DPSC have been shown to have odonto-osteogenic characteristics whereas CD34+, CD117+ and CD45- DPSC have a greater capacity for cell renewal and osteogenic differentiation.[24] Other authors have referred to DPSC MSC expressing CD29+, CD44+ and CD73+.[25–27] The expression of transcription factor genes Oct-4 and Nanog have also been used to identify DPSC MSC.[28] The identification of DPSC mesenchymal stem cells is clearly a developing science which will no doubt be refined in the future to clearly describe each sub-population of DPSC.

The fact that DPSC have low expression of Class II HLA-DR (MHC)[29] molecules means that they are immunologically privileged and it may be possible to transplant these cells from one person to another without the need for tissue matching. This raises the possibility of a public DPSC bank, perhaps in collaboration with key dental hospitals, to provide DPSC to anyone in need. Such donated DPSC could be extremely useful when using artificial bone to provide new bone for dental implants where the artificial bone could be used along with donated DPSC to enhance bone formation.[30]

Sources and characteristics of dental pulp stem cells (DPSC) and stem cells from human exfoliated deciduous teeth (SHED)

DPSC have been isolated from exfoliated deciduous teeth (SHED: stem cells from human exfoliated deciduous teeth), from permanent secondary dentition, from teeth extracted due to impaction or periodontitis and from inflamed pulp tissue.[31] SHED cells have been shown to have a high proliferative rate and are capable of producing osteoblasts, adipocytes, neuronal cells and odontoblasts.[32] Some workers suggest that SHED cells have a greater proliferative capacity than DPSC obtained from adult third molars, incisors or supernumerary teeth on the basis that SHED cells represent a more immature type of stem cell.[33–34] This is a similar hypothesis to that put forward for the observed differences between cord blood stem cells and adult haemopoietic stem cells such as bone marrow. It has been shown that the properties of DPSC are directly related to the physical age of the tooth from which they are obtained.[34] It is interesting to note that in terms of cell cycle 69.8% of SHED cells were found to be in the S and G2 stage, but only 56% of the DPSC were in those phases indicating increased proliferative capacity in SHED cells.[35] The surface antigen expression of SHED cells also differs from that seen in DPSC. This is reflected in the fact that proliferation related and extracellular matrix (ECM) formation genes, for example genes encoding transforming growth factor (TGF) and fibroblast growth factor 2 (FGF), are expressed in SHED cells. Genes coding for collagen I and collagen III and pluripotency markers, such as Pou5f1, Oct3/Oct4, Sox2, and Nanog are also expressed higher in SHED cells.[36] The expression of Nestin (a marker of neuroepithelial stem cells)[37] is reduced in SHED resulting in their reduced ability to form neurospheres in comparison to DPSC.[37]

Permanent teeth, impacted third molars and supernumerary teeth are an excellent source of DPSC which have the following mesenchymal stem cell surface antigens: CD90+, CD146+, CD105+ and CD45−; and also express Oct4 and Nanog[38–40] but lower expression than that seen in SHED cells.

Both DPSC and SHED cells are an excellent source of mesenchymal stem cells for regenerative medicine procedures,[41] in addition, SHED cells have recently been proposed as potential immuno-modulators in the treatment of autoimmune encephalomyelitis and other autoimmune pathologies of the central nervous system.[42]

Clinical applications of DPSC and SHED cells in regenerative medicine

The basic cell biology described above illustrates the enormous potential of DPSC and SHED cells in regenerative medicine procedures in orthopaedics, oral and maxillofacial applications. Studies using canine DPSC have shown that the cells are capable of bone formation when grafted into the jaw.[43] In human studies DPSC have been transplanted along with some sort of scaffold or porous biomaterial to enable the cells to develop to facilitate bone formation.[44–45] There is clearly potential in the use of DPSC in bone formation and such an approach is the subject of current research in our WideCells ISCT using our artificial bone product Indus as the scaffold for DPSC.

The DPSC mesenchymal stem cells which are found in teeth have been shown to be capable of repairing periodontal tissue, diabetic critical limb ischaemic tissue, bone damage caused by osteonecrosis, skin lesions caused by burns, liver, neuronal tissue, skeletal muscle tissue and blood vessels.[46–50] For these reasons DPSC are now considered to be one of the best future sources of mesenchymal stem cells for use in regenerative medicine[51] including treatment for diseases of oro-facial, neurological, corneal, cardiovascular, hepatic, pancreatic and renal tissues and in muscular dystrophy.[52] Medical laser activation of DPSC has been shown to enhance osteogenic differentiation[53] and this is a subject of ongoing research in the WideCells ISCT.

Treatment of periodontal disease using DPSC

Periodontal disease (PD) is estimated to be present in 90% of the population of the world, making it the most common known chronic infectious disease.[54] PD is also the most common cause of tooth loss in adults.[55] The current standard treatment of PD often involves the use of autologous bone grafts, allografts or alloplastic materials but these types of intervention, at best, result in tissue repair rather than regeneration.[56] These types of intervention for PD may also be unsuitable for many patients.[57] It is possible to add differentiation factors or anti-inflammatory molecules

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to these treatments, which do not have a cellular base, but the short half-life of these molecules results in generally poor clinical ourcomes.[58–60] Xenografts of human third molar DPSC into immunodeficient athymic mice have shown that the donor human cells can produce adipocytes and collagen forming cells with the potential to produce material similar to periodontal tissue cement.[56] It appears that the expression of STRO-1, CD146, and CD44 along with the presence of stromal cell-derived factor-1 (SCD-1) is important in the development of periodontal tissue regeneration.[60–61] SHED cells have been shown to be capable of stimulating bone formation making them a possible route to treatment for disease requiring craniofacial bone regeneration.[62] It has also been shown that the treatment of DPSC with valproic acid can improve mineralised matrix formation and increase the expression of bone glycoproteins.[63] It has been proposed that in the future the use of epigenetic regulators such as HDAC inhibitors[64] could be useful in regenerative medicine procedures[63] using DPSC. Researchers support the concept of the use of DPSC and related stem cells as a source of stem cells in the treatment of periodontal disease.[65]

Neural regeneration using DPSC

Neural crest stem cells have been isolated from dental pulp,[66] periodontal ligaments[67] and salivary glands.[68] Animal studies have indicated that DPSC could be useful in the regeneration of neural tissue.[69] The neural crest ontogeny of DPSC is also reflected in their ability to produce neurotrophic factors which promote neuronal survival and axonal guidance.[70–71] DPSC have been used as a graft into hemisected spinal cords in animal models resulting in an increased number of surviving motor neurons[70] illustrating the promise of this technology in future clinical trials. DPSC have also been shown to promote neuritogenesis when co-cultured with rat retinal cells, this is thought to be related to the ability of DPSC to induce the expression of neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3).[71] The ultimate application in ophthalmology is to develop cell based regenerative technology which could repair or replace a damaged retina and therefore restore sight. In this context DPSC have been shown to be able to express markers found on mature photoreceptors such as BDNF and rhodopsin. This raises the possibility that DPSC could differentiate into functional photoreceptors which could in turn be used to restore sight in patients suffering from a damaged retina.[72] The current opinion, supported by the literature, is that DPSC are an excellent potential source of stem cells for neural tissue engineering and for use in neural induction protocols.[73]

Conclusion

Dental pulp stem cells can be collected, processed and cryogenically stored each time a deciduous tooth is exfoliated or a healthy adult tooth is extracted. This represents a considerable source of mesenchymal stem cells which at present are being discarded as medical waste. These mesenchymal stem cells have enormous potential in future stem cell-based regenerative medicine procedures. Patients, dentists and physicians need to work together to ensure that this valuable resource is not wasted and that every possible dental pulp stem cell is available to use in the future.

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