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The Dental Pulp Stem Cell Clinical Applications

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Abstract

Dental pulp stem cells (DPSCs) are classified as adult stem cells from embryogenic origin, whose main advantage is their being easily collected in a relative cheap and easy-doing way. They are mentioned as possible producers of dentin-like tissues and bone-like tissues, opening new approaches and possibilities for their use in many fields of odontology and medicine as well. This article gives an overall view of the evolution of this cells in time, while explains how they are removed from the root canals and the way these cells are processed to be used in scientific studies. This article also describes how they can be used in clinical trials in the near future.
Keywords: Bioengineering; Cells; Mouth; Stem cells; Transplantation.

1. Introduction

The dental structure and its main components comes from the ectomesenchyme [1], and because of this many complex interactions take place during the embryonic development, mainly between a transitory population of embryonic cells which come from the neural crest, underneath the mesenchyme, and the ectoderm, in the process of odontogenesis [2]. The tooth then, after its formation, is protected externally by the enamel which comes from the ectoderm, while internally it is formed by dentin whose origins comes from the odontoblastic layer in direct contact with the vascularization of the root canal.

During the stages of differentiation and morphogenesis, there happens the organization of the pulp tissue and an aggregation of cells from the neural crest and the ectoderm, in the bud stage. Part of such cell will specialize to form the dental germ while the other will remain as a more immature subpopulation, which is likely the precussor of all the resident populations in the pulp tissue. Therefore, it is this embryonic feature that is responsible for the diversified source of stem cells to be found in the pulp tissue. In spite of the many stem cell types found in the teeth, the aim of this work was to make a literature review of the Dental Pulp Stem Cells (DPSCs) and their applications in medicine and Odontology.

1.1. The Dental Pulp Stem Cell

According to the chronology advent of the DPSCs, articles indicate that they were isolated for the first time by Gronthos, *et al.* [3], and were classified as Mesenchymal Stem Cells (MSCs) of dental origin, with the main advantage of being, at least theoretically, more easily obtained with lower costs from different clinical procedures. With the aid of techniques developed for the isolation and characterization of bone marrow stromal cells, the dental pulp showed populations of stem cells, later called DPSCs, with the ability to give rise to a great amount of primary dentin, as well as a pulp-like complex upon *in vivo* transplantation with hydroxyapatite/tricalcium phosphate ceramic particles.

Since then up to 2016, they have been exhaustively researched by a number of articles and different populations of stem cells and precussor cells have been isolated from many types of teeth (deciduous, permanent and supranumerary especially [4]. These populations have similar characteristics such as fibroblastoid morphology, efficient adherence, colony formation and high proliferative rate *in vitro*. Nevertheless, recent studies have shown that they differ in the expression of some stem cell and precussor markers from the pulp tissue [5-7]. The derivation

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from the neural crest make possible that stem cells from dental pulp be isolated and their differentiation in many other cell types, such as glia cells, neurons, osseous cells, tendon cells, melanocytes, chondrocytes, endocrine and adipose cells [8, 9].

Dental Pulp Stem Cells can be collected from teeth of adult patients as well as from children - Dental Stem cells from Human Exfoliated Deciduous teeth (SHEDs) – and display high efficiency *in vitro* for colony formation which leads to cell proliferation when compared to traditional MSCs from bone marrow.

DPSCs, according to some studies, do not react to hemotopoietic markers, such as CD14 (monocytes/macrophage), CD45 (pan leukocyte antigen) and CD34 (hematopoietic/progenitor/endothelial). However; they do express markers related to the endothelium (VCAM-1), muscle (α-SM actin), and also bone (type I collagen, osteonectin) and others, such as CD29 (integrin β1), a protein involved in cellular adhesion and also related to processes like embryogenesis and immune response [10, 11]. A study isolated SHEDs which demonstrated the very similar characteristics to DPSCs, while its proliferative potential and its clonogenic capacity were somehow higher probably due to the fact that SHEDs are more primitive and immature. For the characterization of such cells, antibodies anti-STRO-1 were used, which potentially defines a subpopulation of progenitor cells, and anti-CD146 (MUC18), which is a marker of progenitors belonging to MSCs. Nevertheless, after isolation and expansion *ex vivo*, only 9% of these cells showed positive reaction to anti-STRO-1 antibody.

Another study isolated Immature Stem Cells from the dental pulp (Human Immature Dental Pulp Stem cells – HIDPSCs). These cells are more immature and homogenous than DPSCs and SHEDs for expressing simultaneously Mesenchymal Stem Cell and Ectordermal Cell markers, especially Oct-4, Na-nog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. HIDPSCs show stable karyotype and proliferative rate of at least 25 passages, demonstrating high plasticity and differentiating into diversified tissues, such as muscular, neural, cartilaginous and osseous tissues [5, 9, 12].

A study [13], isolated a subpopulation of DPSC called Stromal Bone Producing Dental Pulp Stem Cells (SBP-DPSCs), multipotential cells that could give originate a range of cell types and tissues including adipocytes, neural cell progenitors and myotubes [14]. The woven bone tissue generated *in vitro* by these cells, denominated Living Autologous Bone (LAB), is gradually remodeled until they form a lamellar bone when transplanted *in vivo*. Surprisingly as it may seem, DPSCs still show plasticity and capacity for nodule formation and are capable of forming bone chips *in vitro* [15].

1.2. Processing of the Dental Pulp

For the obtention of DPSCs, the pulp must be removed from the root canal and prepared in explant culture, that means a technique used for the isolation of the cells from pieces of tissue after having been cut into small pieces and ready for the gathering the cells. Tissue harvested in this manner is called an explant. There are two traditional methods used for this purpose.

1.3. Enzimatic Methods

The aim is to promote proper processing for further culture of DPSCs with the aid of enzymes, in order to obtain the higher possible number of cells, assuring their viability and functional integrity.

In the laboratory, the tooth must be removed from the kit and must be washed with saline solution (PBS). After wash, the tooth must have its pulp removed with the aid of endodontic files which penetrate as close as possible to the dental foramen. After that, the pulp can be digested with a solution of colagenase type I (1-3mg/mL) and dispase (2,4-4 mg/mL) at 37ºC for 30-60 minutes. It can also be digested with trypsin (0,2%) for 5 minutes; or with colagenase type I (3%) at 37º for 60 minutes. The variation of enzymatic digestion time is dependent on the concentration of the enzymes chosen, therefore the higher the concentration the lower the time, but more risks involved for cell viability.

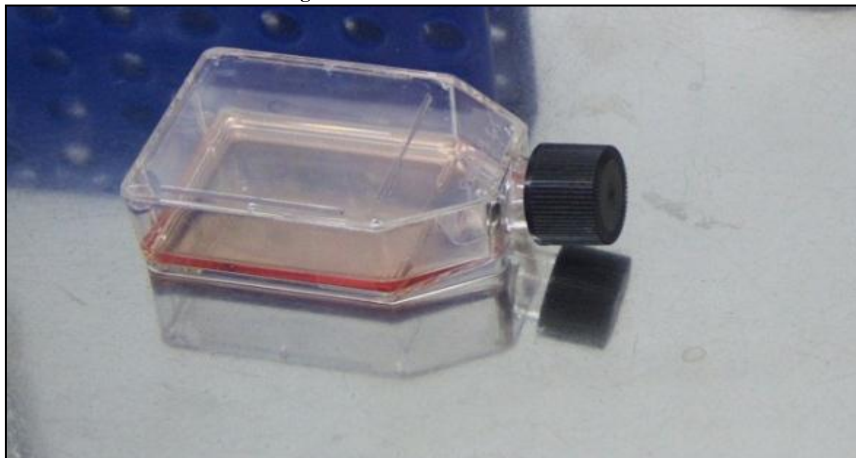
1.4. Non Enzymatic Methods

In the laboratory, the tooth must be removed from the kit and and must be washed with saline solution (PBS). After wash, the tooth must have its pulp removed with the aid of endodontic files. With the aid of tweezers and scissors, the pulp must be triturated for further placement of the fragments in cell culture, a procedure denominated cell explants.

1.5. Mesenchymal Stem Cell Culture From DPSCs

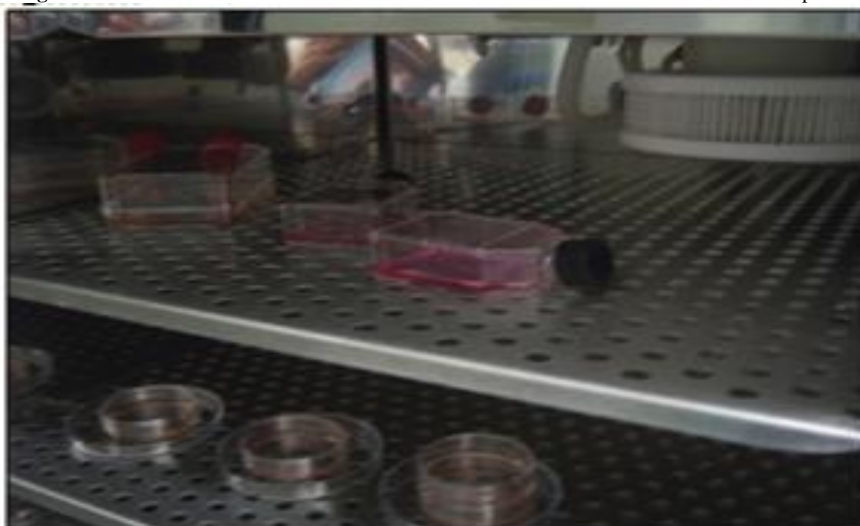
Briefly, the cells are put to grow with Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% antimycoticantibiotic solution (10.000 units of penicillin, 10 mg of streptomycin and 25 μg of amphotericin B per mL in 0.9% sodium chloride; Sigma). Figure 1.

Figure-1. DPSCs in culture medium



The cells are maintained in an incubator at 37ºC and a humidified 5% CO_2 atmosphere. Cultures are supplied with fresh medium every other day. Cells between the fifth and tenth passages can be used for experimental procedures. Figure 2.

Figure-2. The cells are maintained in an incubator at 37ºC and a humidified 5% CO_2 atmosphere.



1.6. Cell viability Analysis for Experiments

The cell viability is determined by the mitochondrial activity analysis. This analysis is carried out using the MTT-based cytotoxicity assay. The MTT assay involves the conversion of the water soluble MTT (3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble formazan salt. This process will occur only by viable cells. Then, the formazan is solubilized, and the concentration determined by optical density at ≈570 nm. A MTT reduction analysis kit (Vybrant MTT, Molecular Probes, Eugene, OR, USA) is used. Immediately after the end of the assay procedures the absorbance is read in a micro plate reader (Biotrak II, Biochrom Ltd, Eugendorf, Austria) using a 562 nm filter. The absorbance data is transformed into number of viable cells that is used to plot the cell growth curves.

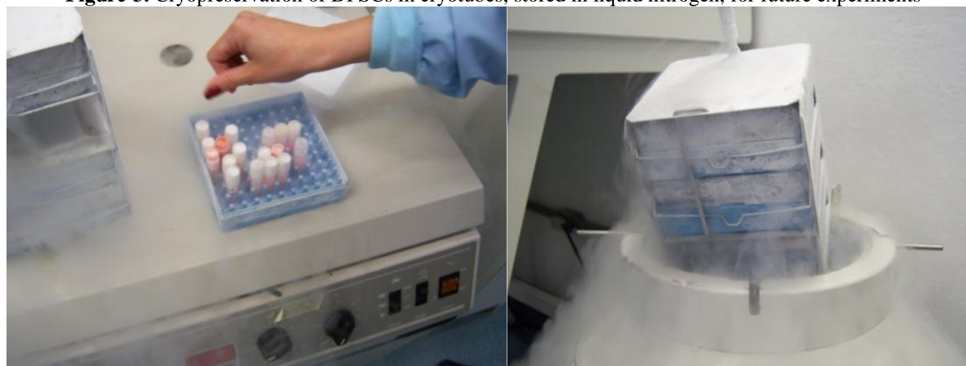
1.7. Morphological Analysis

The morphology and the distribution of cells are monitored throughout the experimental time. Using phase light microscopy, the relationships between the cells grown in the Petri dishes and the coverslips are studied. Additionally, the individual morphology of the cells, as well as the presence of both living cells and dead cells are analyzed. Phase photomicrographs can be obtained from a Zeiss Axiophot microscope (Carl Zeiss Inc., Oberköchen, Germany).

1.8. Cryopreservation of the Mesenchymal Stem Cells in Liquid Nitrogen

After the growth of the cells and their use in experiments, the surplus must be cryopreserved in liquid nitrogen (-196ºC) in order to preserve this cells for future experiments. Figure 3. Cryopreservation of cells and also tissues have been improved recently, which have been cryopreserved and then successfully utilized for transplantation, mainly because DPSCs seem to be able to survive for longer periods than once thought. In laboratorial analysis this can be easily observed for they can be passaged several times. A study showed that they withstand more than 80 passages without clear signs of senescence[13], [14].

Figure-3. Cryopreservation of DPSCs in cryotubes, stored in liquid nitrogen, for future experiments



1.9. Clinical Application of Dental Pulp Stem Cells

Stem cells from the dental pulp is considered a promising source of these precious cells, because their development results in a multipotent cell types that can be used for the regeneration of many kinds of tissues. They can be banked easily from many kinds of teeth, including deciduous and permanent teeth, the former from naturally exfoliation process and the latter from orthodontic or surgical indications, such as impacted or supranumerary teeth; not to mention the ethical considerations that sometimes make the use of stem cells problematic, such as the matter of embryonic stem cells.

2. Neurology

Neuroregeneration has ever been a challenge for medicine, due to the difficulty to replace demaged neurons and the clinical consequences of this fact. In the near past many researchers believed in the cure of many diseases but not for neural ones. Bone Marrow Mesenchymal Stem Cells (BMMSCs) have been the pointed as the best candidates, although limitations such as the accessibility the quality of their differentiation may jeopardize their choice [16]. In this context, DPSCs may be an alternative, since they express markers of embryonic stem cells (ESC) pluripotency, such as Oct-4, Nanog, SSEA-4 and TRA-1-60 [17]. They also express multipotency markers which indicate that they enhance chondrogenic and osteogenic tissue formation, as well as neural differentiation [18].

According to their derivation from the neural crest, DPSCs express a variety of neural cell markers, such as nestin, βIII-tubulin, microtubule-associated protein), glutamic acid decarboxilylase (GAD), CNPase (glial markers 2'3'-cyclic nucleotide 3'-phosphodiesterase), glial fibrillary acidic protein (GFAP) and, if stimulated with neurogenic medium, the expression of these neural markers increases.

The potential of neural development was studied by injecting SHEDs in the dentate gyrus of the hippocampus in immunocompromised mice [19]. The cells managed to survive for over 10 days within the brain of the mice and expressed neural markers. The immature Dental Pulp Stem Cells (IDPSCs) were also studied to evaluate their potential for bone marrow lesions induced in murine models described by Almeida et al. [20].

They were transplanted in two different moments, in order to compare the healing between one subacute lesion and another chronic one. The subacute lesion group, 28 days after the lesion, both with the same cell concentration and volume, and also the same injection site in the epicenter or the lesion. Analysis showed better conservation of the white matter areas in the groups that received IDPSCs when compared to the control groups, which used Dulbecco's Modified Eagle Medium (DMEM). The electronic microscopy of the animals that received DMEM revealed tissue disorganization with many cavities and strong astrocytosis. Both subacute and chronic lesion groups which received the IDPSC transplant showed better conservation of the tissues, with significant number of preserved fibers within the white matter, and a remarkable number of macrophage with many cytoplasmic inclusions, traces of myelin and lipids in its core. Intact Schwann cells were also observed and oligodendrocytes for axon remyelination. Some of these neurons displayed contacts and preserved synapses. The behavioral analysis revealed that the group which received subacute IDPSCs showed improvement in comparison to the group that receive DMEM, and the betterment in locomotion took place seven days after cell transplantation and kept steady along the week following the study. The group of animals of the chronic lesions showed a slight improvement seven days after the treatment. Fourteen days after the transplantation, the animals showed greater velocity of locomotion. In both groups that received IDPSCs, the animal did not achieve normal rates, but showed better exploratory patterns, with much more movements and crossing open areas many times during the tests. The functional betterment of these animals may also be attributed to the release of neurotrophic factors by the transplanted cells which may act stimulating the establishment of new synapses.

Researches on DPSCs have shown that they do express various neural markers, and many studies evaluating effective culture strategies to promote neurosphere formation and neural induction have been made [21-25].

A study conducted by Mead, *et al.* [26], assessed implanted rat DPSCs into the vitreous body of the eye after an induced crush injury surgically made in the optic nerve. The results showed that DPSCs promoted the survival of the rat ganglion through axon regeneration. In the *in vitro* study of Apel, *et al.* [27], DPSCs showed neuroprotective effects in models of Alzheimer's and Parkinson's disease. It has also been shown that the transplantation of differentiated stem cells from dental pulp caused an improvement of motor disability and reduced the infarct volume [28], [29].

3. Vasculogenesis and Angiogenesis

Although similar in concepts, vasculogenesis and angiogenesis differ basically on the grounds of embryogenesis. Vasculogenesis is a process that gives rise to the heart and the first primitive vascular plexus in the embryo, including their membranes, in the yolk sac circulation, through in situ differentiation and growth of blood vessels from mesodermal derived hemangioblasts. This process is fundamental and critical at the early stages of embryonic development. The remodeling and the expansion of this blood supply network concerns angiogenesis, which can be defined as the process of new blood vessel formation from preexisting vasculature, and so it differs from vascularization, and comprises basically two different mechanisms: endothelial sprouting, based on endothelial cell migration, proliferation and tube formation; and intussusceptive microvascular growth (IMG), which divides existing vessel lumens by formation and insertion of tissue folds and columns of interstitial tissue into the vessel lumen [30], It is the angiogenesis that remodels the preexisting vascular network for a number of physiological and pathological situations, particularly those where appropriate blood supply is needed to nourish growing cells to regenerate tissues, like healing after wounds. In the recent literature, there have been growing evidence that human derived dental pulp stem cells have a significant likelihood to offer a chance for treatment for diseases that need blood vessels neoformation.

The study of Cordeiro, *et al.* [31], shows that SHEDs have the potential to differentiate into functional vascular endothelial cells via a process that is similar to vasculogenesis, probably due to the more primitive characteristics of these cells. There are also reports that DPSCs may differentiate into endothelial cells able to give rise to perfect functional blood carrying vessels [32].

Dental pulp stem cells have also been reported to give rise to blood vessels after injection in damaged tissues. Human Pulp-derived Stem Cells (HPDSCs) display high proliferative activity and have high rates of colony formation units (CFU). Based on that, some studies assessed the potential of DPSCs to treat ischemic areas and myocardium infarction. [33], in 2008, used HPDSCs as a treatment for induced myocardium infarction in a rat model. They concluded that Human DPSCs could repair infracted areas by increasing the number of newly formed blood vessels, and thus reducing the infarct size, probably due to the secretions of paracrine factors, such as vascular endothelial growth factor (VEGF) and interleukins.

Nakashima, *et al.* [34] in 2009 demonstrated that a successful engraftment and the increase of capillary formation could be achieved after local transplantation of DPSCs. They concluded that DPSCs have high angiogenic and neurogenic potential for pulp regeneration.

Bronckaers, *et al.* [35] in 2013, showed that hPDSCs were able to induce paracrine-mediated angiogenesis. The cells produced a considerable high amount of angiogenic molecules, while stimulated endothelial cell migration by activation of the P13l– AKT and MEK–ERK pathways, inducing blood vessel formation in their study accomplished in a chicken chorioallantoic membrane model; promoting evidence for the treatment of pathologies that involves inadequate or insufficient blood flow due to angiogenesis.

4. Conclusions

Dental Pulp Stem Cells have shown promising results for many fields in odontology and medicine. Their easy collecting and storage, and no need of polemical ethical matters make them one of the best types of adult stem cells available in the human body for near and long term researches.

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