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*Review*

# Potential clinical applications of dental stem cells

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**Complex human tissues harbor stem cells and/or precursor cells, which are responsible for tissue development or regeneration. Recently, dental tissues such as periodontal ligament (PDL), dental papilla or dental follicle have been identified as easily accessible sources of undifferentiated cells. Dental precursor cells are attractive for usage in regenerative dentistry, like for example regeneration of the dental pulp (biopulp), gingiva and periodontium, regeneration of osseous defects and complete reconstruction of the temporomandibular joint. Dental stem cells are widely used in the regenerative medicine, also. Diabetes, diseases of the bone, cartilage, fibrous tissue, muscular and adipose tissue, the neurological diseases and the spinal cord injures are all included in the future cell-based therapies with dental stem cells.**

**Key words:** Stem cells, dental pulp, cultivation, tissue engineering, clinical applications.

## INTRODUCTION

The current technology for preservation of stem cells enables keeping of these invaluable cells for the unforeseeable needs that may arise in the future. For years now, stem cell banks have preserved stem cells from the umbilical cord (Koliakos, Biohellenika laboratories, Athens, Personal Communication). The discovery of stem cells in the dental pulp of deciduous teeth and third permanent molars has provided a second chance for families who have missed their opportunity to preserve the umbilical cord (Koliakos, Biohellenika laboratories, Athens, Personal Communication). The preservation process is simple. Instead of discarding a deciduous tooth or third molar, the dentist can send it to special banks or laboratories that implement the technology of stem cell extraction and preservation. In 2003, Dr.Sangtao Chi from the NIH (National Institute of Health) made the discovery of dental stem cells. The first animal studies with dental stem cells for the treatment of bone defects and bone regeneration were carried out in 2007 (Graziano et al., 2008). In 2008, this was followed by initial studies on animals using dental stem cells to treat heart and nervous tissue diseases, as well as muscular dystrophy (Gandia et al., 2008; Kerkis et al., 2008).

The aim of this review was to describe the potential clinical applications of dental stem cells.

## IDENTIFICATION OF STEM CELLS IN OTHER MINERALIZED TISSUES IN THE ORAL CAVITY

### Isolation and identification of cementoblast-like cells

There are clear and precise differences in the organisation of the bone and the cementum, though it is still not clearly defined whether they develop from different cell types or from the same type of bone-forming cell present in a different environment (Grzesik et al., 1998). Human cementum-derived cells-HCDSs or cells that originate from the cementum are acquired from healthy teeth that are subjected to collagenase pretreatment. These cells have the capacity to differentiate and form tissues similar to the cementum (Grzesik et al., 1998).

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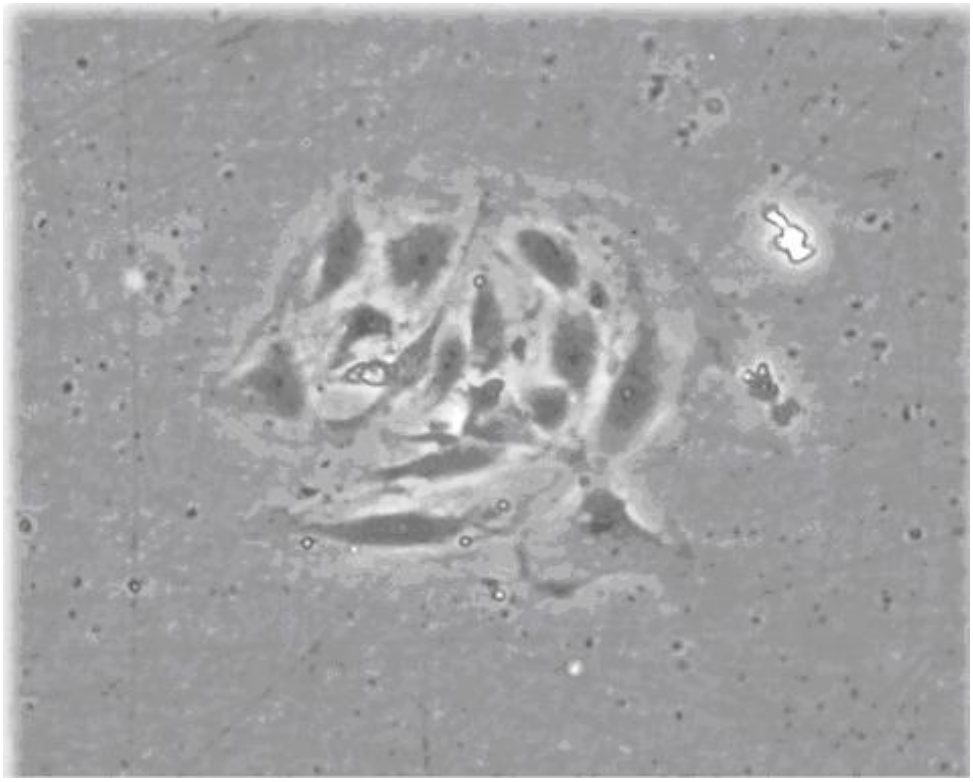


**Picture 1.** Small colony of DPSCs 24 h following inoculation. DPSCs are 12-18 µm in diameter. Phase contrast microscopy, direct magnification ×200.

**Adult human dental pulp-derived stem cells**

Another type of mineralized tissue with certain similarities to bone tissue is dentine. Dentine possesses limited reparative capacities. Dental pulp stem cells (DPSCs) are produced through enzyme degradation of adult human dental pulp. The DPSCs and BMSCs have similar genetic expressions for around 4,000 genes (Suchanek and Soukup, 2007). In general, DPSCs differentiate into odontoblasts, osteoblasts, chondrocytes, fibroblasts, adipocytes, myocytes, melanocytes and neurons.

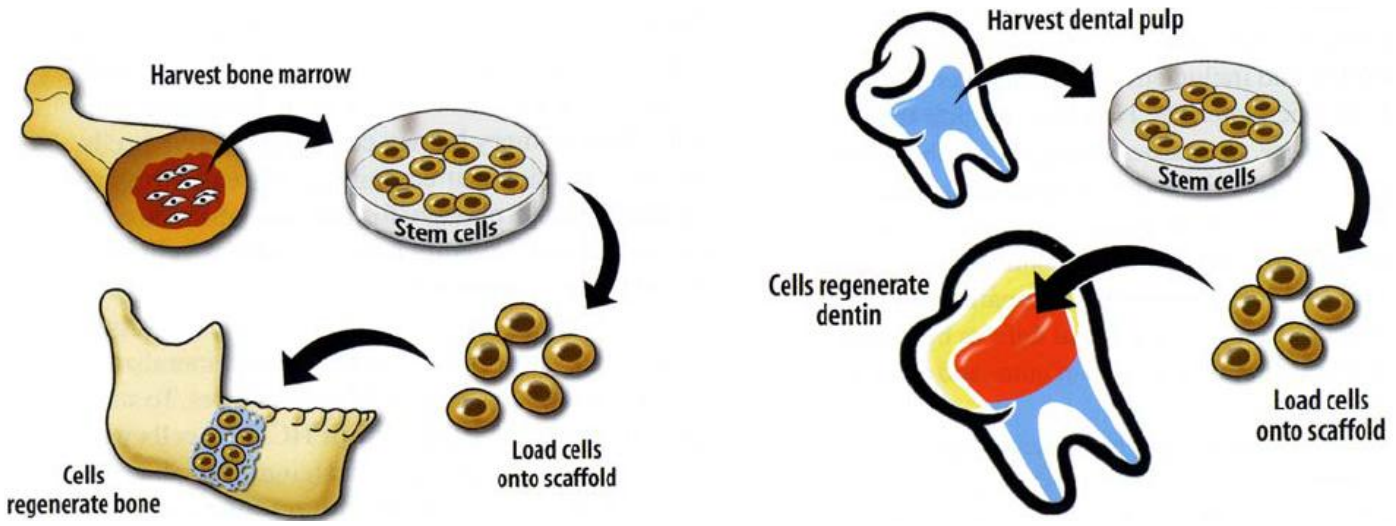
**Stem cell extraction and cultivation methods**

Stem cells are harvested from deciduous teeth (presence of vital pulp and physiological resorption of the root no bigger than two-thirds), third molars (age limit up to 28 years), extracted teeth for orthodontic reasons. mesiodens, trauma or parodontopathy (Anderson et al., 2008). According to a large number of analysed studies, it is very difficult to isolate DPSCs from teeth separated with a diamond dental drill or with separation instruments because of the high risk of thermal or mechanical damage to the pulp, as well as the high probability of its contamination (Suchanek and Soukup, 2007). In most cases the intact sample tooth should be transported to a laboratory in a safe manner placing it in special transport solution (Hank's balanced salted solution, HBSS). Afterwards, the pulp should be extracted in sterile conditions. The dental pulp is subjected to enzyme treatment with collagenase for 70 min. With the help of a centrifuge, two cell fractions are produced, pulp subodontoblastic compartment-derived cells and pulp perivascular compartment-derived cells (Suchanek and Soukup, 2007).

**CULTIVATION**

Stem cell suspensions (DPSCs) are cultivated in a special media for human mesenchymal progenitor cells (3 to 5 days (MPCs) composed of alpha-MEM (Gibco, Scotland), 2% FCS (PAA, USA), EGF (PeproTech, USA), PDGF (PeproTech, USA) and dexamethasone (Sigma, USA) and in some cases supplemented with ITS supplement (Sigma, USA) according to Suchanek and Soukup, 2007. The laboratory dishes holding the primary culture contain a so-called Cell+ surface. Finally, they are treated with trypsin - Ethylenediaminetetraacetic acid (EDTA) and divided into laboratory dishes with standard surfaces (Picture 1).

During their observation of the colonies, Gronthos et al. (2002); Muira et al. (2003) have identified morphological




**Picture 2.** Potential clinical applications.

differences between the two DPSCs types from the two different compartments. The DPSCs from the perivascular compartment (the inner side of the dental pulp) were more elongated with long processes compared to the DPSCs from the subodontoblastic compartment (the outer part of the dental pulp) which had a more rounded shape. Cytogenetic researches of DPSCs have shown a normal karyotype without differences in the compartments (Gronthos et al., 2002; Muira et al., 2003). There are two sources from which dental pulp elements are developed (dental mesenchyme from the neural crest and vascular mesenchyme) and it is presumed that there are two lines of DPSCs in the dental pulp. The pulp stem cell division time is as follows: 12 tp 50 h for the first forty divisions in the cell population, while after reaching 50 population divisions, the division time increases to 60-90 h (Gronthos et al., 2002; Muira et al., 2003). The dental pulp is an alternative and easily accessible source of tissue–specific stem cells which are histocompatible with the tissues of the patient from which they are isolated (Avery, 1994; Gronthos et al., 2004; Gronthos et al., 2000).

## POTENTIAL CLINICAL APPLICATIONS IN THE OROFACIAL SYSTEM

In the future, stem cells will be used to completely restore the hard and soft tissue in the patient's mouth cavity, thus by-passing problems with histocompatibility. Craniofacial skeletal defects are most often results of operative procedures to treat neoplasms, infections, congenital malformations and progressive diseases that lead to deformation of the craniofacial system. Transplantation of BMSCs cell populations containing mesenchymal stem cells can provide in the future an alternative approach to the reconstruction of craniofacial defects, thus avoiding the dangers of auto- and allotransplants. Picture 2.

There is a possibility for stem cells to be placed ("sown") in biocompatible molds in the shape of the anatomical structures that are to be repaired. So far, tissue engineering on animal models has been conducted for the purpose of repairing oral, dental and craniofacial structures -soft tissue grafts, dental tissues, temporomandibular joint, facial bones (Alhadlaq and Mao, 2005). The mandibular condyle has two casings: bone and cartilage. Alhadlaq and Mao (2005) first differentiated mesenchymal stem cells from chondrogenetic and osteogenetic cells, after which they encapsulated them in a biocompatible hydrogel with two stratification levels possessing the dimensions and form of an adult human mandibular condyle. Furthermore, they proceeded to conduct an *in vivo* implantation in an immunocompromised laboratory animal. After 12 weeks, mandibular condyles were created which had maintained the form and the dimensions of natural condyles (Alhadlaq and Mao, 2005; Krebsbach et al., 2002).

Biopulp represents a revolutionary novelty in endodontic treatment. It was patented by Mao Jeremy from Columbia University, New York, USA. In short, the biologically based endodontic therapy includes implementation of stem cells in previously endodontically treated pulp canal system (Mao, 2008; Moioli et al., 2007).

Certain success in endodontic biological treatments has also been achieved by Fu Susan. Using bioengineering technology in experimental studies, mammal pulp was successfully revascularised and repopulated *in-vivo* with cells -cytokine induced cell homing (Fu, 2009). Byoung-Mao et al. (2004) isolated stem cells directly from the periodontal ligament with excellent potential for regeneration. Lin and Menicahin (2008) consider that stem cells from the periodontal ligament are responsible for periodontal regeneration.

Periodontal stem cells successfully differentiate into fibroblasts which produce collagen fibers, as well as into cementoblasts and cementocytes. Shi et al. (2004) inform of certain successful implantations of periodontal stem cells during periodontological treatments.

According to Hirouki and Hidemi (2008), transplantation of BMSC from the iliac bone in cases of periodontal defects is a useful option for periodontal tissue regeneration. Gault et al. (2010) report of clinical implantations of metal cylinders (Ligaplant) covered with a mineral film with simultaneous autografting of dental pulp-derived stem cells. Radiological analyses after a period of 24 weeks have indicated the presence of alveolar bone formation around the implant and periodontal ligament attachment of the implant to the primary place of insertion.

Stem cell mediated radicular regeneration provides possibilities for complete regeneration of the so-called dental bio-root and the associated periodontal tissue. Watary Sonoyama and Yi Liu have successfully reconstructed a functional tooth in animal models ("minipigs") with the help of stem cells (SCAP- stem cells from root apical papilla, PDLSC – periodontal ligament stem cells). 3 months after the implantation of a special hydroxyapatite implant (HA/SCAP) covered with a PDLSC stem cell casing. The authors noticed the forming of a solid radicular structure bound by a visible periodontal space separating it from the surrounding bone tissue. The overall study was conducted using a hybrid technique (Watary and Yi, 2006).

## POTENTIAL CLINICAL APPLICATIONS IN OTHER ORGANS AND TISSUES

Transforming dental stem cells into cardiomyocytes opens certain prospects regarding the modern treatment of certain cardiovascular diseases, such as repairing damage to the myocardium suffered in a state of acute infarction of the myocardium (Gandia and Armiñan, 2008). Furthermore, transformation has also been registered of DPSCs (D'Aquino and Graziano, 2007). This phenomenon can be utilized in regenerative therapy of bone tissue diseases. Transformation of the pulp-derived stem cells into chondrocytes could be used in cartilage tissue disease therapy, neurones (multiplex sclerosis, Alzheimer's and Parkinson's disease therapy), as well as transformation into adipocytes (fat tissue diseases therapy). There are certain prospects for treatment of diabetes and liver diseases with the help of dental stem cells (Bender and Bender, 2003). Gandia and Armiñan (2008) have identified certain improvements in the function of the myocardium in animal models following the application of DPSCs, such as reduction of the infarction zone, thickening of the frontal myocardium wall, increased ejection fraction and rising angiogenesis levels.

Nosrat and Widenfalk (2001) report of successful repairing of motoneurons following the application of dental stem cells in animal models with spinal injuries. Neurodegenerative diseases such as Parkinson's will be treated in the future with cell based therapies through injection of DPSCs directly into the CNS-basal ganglias (Nosrat et al., 2004). Great deal of research has been conducted along these lines which prove the creation of neurotrophic factors (glial cell line-derived neurotrophic factor (GDNF)-mRNA, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF)) *in vitro* by stem cells which support damaged neurons and lead to the replacement of avital cells with new vital cells. More specifically, they promote the survival of dopaminergic (DA) neurons and protect DA neurons against the harmful impact of the neurotoxin, 6-hydroxy-dopamine (6-DHDA) *in vitro*.

The study conducted by Apel and Forlenza (2009) also confirms the expression of neurotrophic factors by DPSCs which protect the primary motoneurons in the case of *in vitro* models of Alzheimer's disease. Experimental studies involving DPSCs, as well as stem cells derived from other sources, Yalvac and Rizvanov (2009), point to a certain degree of success in the attenuation of ischemic damage to the brain and contribute to a speedy, functional recovery. Stosich et al. (2007) emphasizes in their studies the advantages of using biologically compatible soft-tissue implants and fat tissue implants which comply 100% with the original dimensions and shape after an *in vivo* implantation. The authors describe the methods of de novo and *in vivo* synthesis of dental stem cell-derived fat tissue implants. The year 2008 saw the start of the first studies of muscular dystrophy therapy using dental stem cells in animal models (Kerkis and Ambrosio, 2008). The website of the International Society for Stem-cell Research: www.stemcells-research.org provides daily updates of the newest experimental and clinical experiences with dental stem cells and stem cells derived from other sources.

## CONCLUSION

A new era in dentistry is coming in which dental care is delivered increasingly by biologically based approaches. Dental professionals should be informed for the new opportunities given by dental stem cells and tissue engineering provided by continuing education courses. Dental schools should consider the addition of stem cells and tissue engineering courses to the existing curriculum.

### REFFERENCES

Alhadlaq A, Mao J (2005). Tissue-engineered osteohondral constructs in the shape of an articular condyle. J. Bone Joint Surg., 87: 936-944.
Anderson HH, Yuk-Kwan C , Lin-Min L, Tien-Yue S, Anthony WC (2008). Isolation and characterization of dental pulp stem cells from a

supernumerary tooth. J. Oral Pathol. Med., 37(9): 571-574.
Apel C, Forlenza OV (2009). The neuroprotective effect of DSC in models of Alzheimer and Parkinson disease. J. Neural Transm., 116(1): 71-78.
Avery JK (1994). Oral development and histology. 2nd Edition. New York: Theme Medical Publishers Inc., pp. 71-79.
Bender IB, Bender AB (2003). Diabetes Mellitus and the Dental Pulp. J. Endodont., 29(6): 383-389.
Byoung-Mao S, Miura M, Granthos S (2004). Multipotent postnatal stem cells from human periodontal ligament. Lancet, 364(9429): 149-155.
D'Aquino R, Graziano A (2007). Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: A pivotal synergy leading to adult bone tissue formation. Cell Death Differentiation, 14: 1162-1171.
Gandia C, Armiñan A (2008). Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells, 26(3): 638-645.
Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD, Sanchez-Torrijos J, Payá R (2008). Human Dental Pulp Stem Cells Improve Left Ventricular Function, Induce Angiogenesis, and Reduce Infarct Size in Rats with Acute Myocardial Infarction. Stem Cells, 26(3): 638-645.
Gault P, Black A, Romette JL, Fuente F, Schroeder K, Thillou F, Thierry, Brune AB, Wurtz T (2010). Tissue-engineered ligament: Implant constructs for tooth replacement. J. Clin. Periodontol., 37(8): 750-758.
Graziano A, D'aquino R, Angelis M, De Francesco F, Giordano A, Laino G, Piattelli A, Traini T (2008). Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. J Cell. Physiol., 214(1): 166-172.
Gronthos S, Brahim J, Li W (2002). Stem cell properties of human dental pulp stem cells. J. Dent. Res., 81(8): 531-535.
Gronthos S, Cherman N, Robery P, Shi S (2004). Human dental pulp stem cells. Adult stem cells. Totowa, New Jersey: Humana Press, pp. 37-51, 101-149.
Gronthos S, Mankom M, Brahim J (2000). Postanatl human dental pulp stem cells *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. USA., 97: 13625-13630.
Grzesik WJ, Kuzentsov SA, Uzawa K, Mankani M, Robey PG, Yamauchi M (1998). Normal human cementum-derived cells: Isolation, clonal expansion and *in vitro* and *in vivo* characterization. J Bone Miner. Res., 13(10): 1547-1554.
Hirouki K, Hidemi K (2008). Clinical trial of periodontal tissue regeneration. Nippon Rinsho, 66(5): 948-954.
Kerkis I, Ambrosio CE (2008). Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? J. Trans. Med., 3(6): 35.
Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca AS, Cabral RM, Maranduba MC (2008). Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic?. J. Transl. Med., 6: 35.
Krebsbach PH, Robery GP (2002). Dental and skeletal stem cells: Potential cellular therapeutics for craniofacial regenerations. J. Dent. Educ. 66(6): 776-773.
Lin N-H, Menicahin D (2008). Putative stem cell in regenerating human periodontium. J. Periodont. Res., 43: 514-523.
Mao J (2008). Stem Cells and the Future of Dental Care. NYSDJ, 74(2): 20-24.
Moioli E, Clark P, Xin X, Lal S , Mao J (2007). Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering. Adv. Drug Deliv. Rev., 59: 308-324.
Muira M, Gronthos S, Zhao M, Lu B, Fisher LW (2003). Stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad. Sci. USA., 100: 5807-5812.
Nosrat CA, Nosrat IV, Smith CA (2004). Dental pulp stem cells provide neurothrophic support for dopaminergie neurons and differentiate into neurons *in vitro*: Implications for tissue engeering and repair in the nervous system. Eur. J. Neurosci., 19(9): 2388-2398.
Nosrat IV, Widenfalk J (2001). Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons *in vitro* and rescue motoneurons after spinal cord injury. Dev. Biol., 1238(1): 120-132.
Shi S, Seo BM, Miura M (2004). Multipotent postnatal stem cells from human periodontal ligament. Lancet, 364(9429): 149-155.
Stosich M, Bastian B, Marion N, Clark P, Reilly G, Mao J (2007). Vascularized Adipose Tissue Grafts from Human Mesenchymal Stem Cells with Bioactive Cues and Microchannel Conduits. Tiss. Eng., 13: 2881-2890.
Suchanek J, Soukup T (2007). Human dental pulp stem cells-isolation and long term cultivation. Acta Medica, 50(3): 195-201.
Watary S, Yi L (2006). Mesenhimal stem cell-mediated functional tooth regeneration in swine. Plos One, 1(1): 79.
Yalvac M, Rizvanov A (2009). Potential role of DPSC in the cellular therapy of cerebral ischemia. Curr. Pharmaceut. Des., 15(33): 3908-3916.