**DENTAL PULP STEM CELLS**

**AUTHORS**

**Dr. GOPALAKRISHNAN THANGAVEL,** MDS
Reader,
Department of oral pathology and microbiology,
Sri Ramakrishna Dental College & Hospital,
Coimbatore, Tamil nadu.

 **Dr.UMA MURUGAIYAN,** M.Sc,Ph.D

Principal,

Professor, department of Biochemistry,

Prince sri venkateshwara arts and science college,

Gowrivakkam,

Chennai, Tamil nadu.

**Dr.P.E.CHANDRA MOULI**, MDS

Professor and head,

Department of oral medicine and radiology,

Sri Venkateswara Dental College and Hospital ,

Chennai, Tamilnadu.

**Dr. DEEPIKA EKAMBARAM** , MDS

Senior Lecturer

Department of oral pathology and microbiology,

Sri Venkateswara Dental College and Hospital ,

Chennai, Tamilnadu.

**INTRODUCTION**

 Stem cells are undifferentiated primitive cells that have the capacity to divide and differentiate into focussed cells, which have more importance in the field of medicine. Stem cells are derived from embryonic, foetal and adult tissues. In adults bone marrow, fat, brain tissue, human exfoliated deciduous teeth (SHED), dental pulp (DPSCS), periodontal ligament (PDLSCS) are the major sources. Researchers believe stem cells are capable of providing treatment options in a wide variety of the diseases. The regeneration of oral tissues that are injured by disease or trauma is now possible due the discovery of dental stem cells and recent advances in the cellular and molecular biology. These have led to the improvement of novel therapeutic approaches. The knowledge on the stem cell technology is increasing quickly in all the medical disciplines and it dictates the need for new protective approaches in all the fields, which include reparative dentistry. With the help of tissue engineering, the dream of repairing and regenerating defective tissues and organs will be a reality soon.

**HISTORY**

The term stem cell was projected by ALEXANDER MAKSIMOV a Russian histologist, during 1908 in congress of hematologic society at Berlin. During early 1960’s the Canadian scientists came out with the good results on stem cells. In 1998 at the University of Wisconsin-Madison the first human embryonic stem cell line was derived.

Stem cells are biological cells which are found in all multi cellular organisms that can divide and differentiate into diverse specialized cell types. They can self-renew to produce more cells. They are a promising tool for tissue repair. There are two types of stem cells, embryonic stem cells and adult stem cells. The embryonic stem cells are derived from the inner cell mass of the blastocyst- which is a thin-walled, hollow structure in the early embryonic development that contains a cluster of cells which is called the inner cell mass, from which the embryo arises. The outer layer of the cells gives rise to the placenta and other supporting tissues which are needed for the foetal development within the uterus, while the inner cell mass cells give rise to the tissues of the body. These cells have the capacity of forming the three germ layers and the ability to develop as many cell types. Adult stem cells are found in the blood from the umbilical cord, the bone marrow and the blood. The pluripotent stem cells that can be found in the blood from the umbilical cord are only few in numbers. These adult stem cells have been used for many years to treat leukaemia and bone\blood cancers through bone marrow transplantation and some haematopoietic diseases.

**STEM CELL BANKING**

Stem cell banks are progressively more seen as a fundamental resource of biological materials for both basic and translational research. Stem cell banks and registries maintain transnational access to qualitycontrolled and ethically sourced stem cell lines from different origins and of varying grades - for example, research versus clinical. They are furthermore the depositories of ‘biological standards’.Global initiatives are emerging to address synchronization and standardization processes for stem cell research and banking; these include the International Society for Stem Cell Research and the International Stem Cell Banking Initiative. Stem cell banks are on the edge to maintain domestic consistency with respect to policy frameworks relating to the acceptability of conducting stem cell research.The term ‘stem cell bank’ itself can refer to a number of altered levels and types of operations, as well as associations.

In India Life Cell International, India’s first stem cell banking services started in Bangalore (2009). The average amount they charge for collecting and saving the stem cells is around 3000 USD, yearly storage costs are separate. The problem with the stem cell banks is that the charges are not affordable by the normal economic group people.

**THE DENTAL PULP STEM CELLS**

A pedodontist, Dr. Songtao Shi, discovered baby tooth stem cells while he used the deciduous teeth of his six year old daughter 2003 and he named the cells as stem cells from the human exfoliated deciduous teeth (SHED). Dental Pulp Stem Cells (DPSCs) can be found within the ‘‘cell rich zone’’ of the dental pulp. Their embryonic origin from neural crests, explains their multipotency. These stem cells, under specific stimuli, differentiate into many cell types which include adipocytes, neurons, chondrocytes and mesenchymal stem cells. These are the most potential stem cells which have wide therapeutic applications. Dental pulp stem cells can be found both in adults and children. The stem cells of dental origin can certainly generate the dental tissues. The SHED and DPSCs are capable of generating a tissue that has morphological and functional characteristics that closely resemble those of the human dental pulp.

**ADVANTAGES OF DENTAL PULP STEM CELLS**

Unlike the umbilical cord blood cells which have to be collected immediately at birth, the dental stem cells are derived from the deciduous and permanent teeth. There are 20 viable deciduous teeth and 32 permanent teeth which can be used for collecting the stem cells. This is non-controversial and the stem cells can be collected without the involvement of any ethical issues. The viable dental stem cells are very simple to collect, without any mortality and morbidity.

 **Sample selection criteria**

**Deciduous teeth**

(1) Pulp should be vital.

(2) Deciduous teeth with two third of root is preferred.

(3) Extracted teeth are preferred than loose teeth.

 **Adult teeth**

 (1) Only vital teeth should be harvested.

 (2) Teeth with infection and any pathology are avoided.

 (3) Mobile teeth with lack of blood supply can’t be harvested.

(4) Teeth should have sufficient amount of pulp.

**Steps in the dental clinic**

• Examine the tooth and rule out any infection. The tooth has to be removed

 • Rinse the tooth.

 • Transfer to transportation tube.

• Add saline solution. • Wait for five minutes.

• Seal the tube.

• Transport under room temperature before 48 hrs.

**Steps in the laboratory**

• Identification of stem cells with markers.

• Separation of viable cells by centrifuge.

• Cryopreservation.

• Retrieval.

**THE ISOLATION OF THE DENTAL PULP STEM CELLS**

The stem cells are identified by various techniques like flow cytometry, fluorescence-activated cell sorting and magnetic-activated cell sorting and by using biomarkers (surface markers and side populations). Magnetic-Activated Cell Sorting (MACS) is a method which is used for the separation of various cell populations, depending on their surface antigens. This method allows the cells to be separated, by allowing their incubation with magnetic nanoparticles which are layered with antibodies against a particular surface antigen. This causes the cells which express this antigen, to join the magnetic nanoparticles. Afterwards, they are placed in a strong magnetic field. During this process, the cells attached to the nanoparticles which stay on the column, while the other cells flow through. With this method, the cells can be separated with respect to the particular antigen. Fluorescence-Activated Cell Sorting (FACS) is a specific type of flow cytometry. It provides a way for sorting a heterogeneous mixture of cells into two or more containers, one cell at a time, based upon the specific light scattering and the fluorescent characteristics of each cell. It provides a fast, objective and a quantitative recording of the fluorescent signals from individual cells, as well as the physical separation of the cells which are of particular interest. The cell surface markers are useful for classifying and isolating stem cells and for monitoring their states of differentiation, because they can be visualized directly with the intact cells.

**CRYOPRESERVATION**

 The haematopoietic stem cells have been cryopreserved and successfully utilized for transplantation. The dental pulp can be easily cryostored for long periods and it can be used to form a acryobank for adult tissue regeneration. The dental pulp stem cells retain their potential after cryopreservation. In a study which was performed on the cryopreserved tissue samples of periodontal ligaments. The cryopreservation of the whole dental pulp leads to a safe recovery. Different cryopreservation techniques are required for the whole pulp. These features make these cells for a therapeutic three-dimensional tissue reconstruction, with the potential of storage and recovery as per the needs of the patient. Dental pulp stem cells can also be obtained from the patient’s vital pulp, since we have 20 deciduous and 32 permanent teeth. This can be done with the help of stem cell markers, which help in the identification of stem cells.

**REGENERATION OF THE TOOTH TISSUE AND BLOOD VESSELS**

It has been observed that SHED has the potential to differentiate into functional vascular endothelial cells by a process that resembles that of vasculogenesis. These findings raise the hope that the stem cells of dental pulp origin may be useful in treating severe ischaemic conditions of the heart, brain, or the limbs. Specifically, one of the challenges of dental pulp tissue engineering is the production of a functional vascular network, considering the fact that all vascularizations must access the root canal through the apical foramen. Hence, more research is needed for the induction of vasculogenesis accompanying efforts for dental pulp tissue engineering.

**WHOLE TOOTH REGENERATION**

By placing the stem cells on biodegradable scaffolds, tooth-like tissues have been generated. Ikeda et al reported a fully functioning tooth replacement in an adult mouse, which was achieved by the transplantation of a bioengineered tooth germ into the alveolar bone in the lost tooth region. Xu et al., seeded a tooth bud from the rat on scaffolds which were fabricated from silk fibroin, with 2 pore sizes that were either used as fabricated or treated, with the Arg-Gly-Asp attachment site binding peptide. Although dental tissues are regenerated, the success rate for the correct arrangement of a natural tooth is only 15-20%. So, further studies are required to achieve structurally sound teeth.

**BONE TISSUE REGENERATION**

DPSCs, when they undergo differentiation into pre-osteoblasts, form an extracellular matrix that becomes a calcified woven bone tissue. Other than this, it has been demonstrated that such tissues undergo remodelling, when they were transplanted in immunocompromised rats, and that they form a lamellar bone with entrapped osteocytes.

**IN TREATING VARIOUS DISEASES**

Stem cells play a vital role in treating various lives threatening diseases. Other than forming the bone, the blood vessels, the whole tooth and the dental tissues, the dental pulp stem cells can also be used to treat myocardial infarction, Parkinson’s disease, Diabetes mellitus and certain forms of cancer.

**CONCLUSION**

The identification of several types of epithelial and mesenchymal stem cells in the tooth is a significant achievement. But still, the control of morphogenesis and cyto differentiation is a challenge that necessitates a thorough understanding of the cellular and the molecular events which are involved in the development, repair and the regeneration of teeth. The current research which is being carried out on stem cells also helps us in understanding the cancer stem cells and in turn, in developing novel therapies to eliminate cancer. But still, the need persists to find out new and easily accessible sources of both epithelial and mesenchymal stem cells that can be reprogrammed for an odontogenic potential and which can be then associated to form a fully functional tooth. We must use technology, drive and dedication to solve these problems. This will require continued interaction between different disciplines medicine, biotechnology, bio engineering and bio materials etc. major support by governments and international agencies, as well as an understanding and supportive public.

**REFERENCES**

[1] Amit Gandhi, Taru Gandhi, Natasha Madan. Dental pulp stem cells in endodontic research: a promising tool for tooth tissue engineering. RSBO. 2011.Jul-Sep;8(3):335-40.

 [2] Bianco P, Robey PG. Stem cells in tissue engineering. Macmillan Magazines Ltd. 2001;414: 118–21.

[3] Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. Nov 1998; 282(5391):1145-47.

[4] KMK Masthan, N Aravindha Babu, S Leena Sankari, T Gopala Krishnan. Teeth – as a life bank (stem cells in dentistry), reviewarticle. Journal of Medicine and Medical Sciences. 2012; 3(7): 456-58.

[5] Srisawasdi S, Pavasant P. Different roles of dexamethasone on transforming growth factor-beta1-induced fibronectin and nerve growth factor expression in dental pulp cells. Journal of Endodontics. 2007; 33: 1057–60.

[6] Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. Stem Cells. 2006;24: 2493–503.

 [7] Jo YY, Lee HJ, Kook SY. Isolation and characterization of postnatal Stem cells from human dental tissues. Tissue Engineering. 2007;13: 67–73.

[8] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007. Nov;25(11):2739-49. Epub 2007 Jul 26.

[9] JJiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortizgonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002. Jul 4;418(6893):41-49. Epub 2002 Jun 20.

[10] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci. USA. 2000;97:13625–30.

 [11] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci. USA. 2003;100:5807–12.

[12] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell mediated tooth regeneration in swine. PLoS One. 2006; 1:79.

 [13] Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J Dent Res. 2002;81:695–700.

 [14] Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. J Dent Res. 2004;83:518–22.

 [15] Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod. 2008;34:962–69.

 [16] Sakai VT, Zhang Z, Dong Z, Neiva K, Machado M, Shi S, Santos C, et al. differentiate into functional odontoblasts and endothelium. J Dent Res. 2010;89:791-96.

 [17] Demarco FF, Casagrande L, Zhang Z, Dong Z, Tarquinio SB, Zeitlin BD, et al. Effects of morphogen and scaffold porogen on the differentiation of dental pulp stem cells. J Endo. 36:1805–11.

 [18] Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nör JE. Dentin-derived BMP-2 and odontoblast differentiation. J Dent Res. 2010;89:603–08.

 [19] Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med. 2001;12:425–37.

 [20] Smith AJ, Murray PE, Sloan AJ, Matthews JB, Zhao S. Transdentinal stimulation of tertiary dentinogenesis. Adv Dent Res. 2001;.15: 51–4.

[21] Tziafas D. Basic mechanisms of cytodifferentiation and dentinogenesis during dental pulp repair. Int J DevBiol. 1995;39:281–90.

[22] Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. Biomaterials. 2006;27:2865–73.

23] Fitzgerald M, Chiego DJ Jr, Heys DR. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. Arch Oral Biol. 1990;35:707–15.

 [24] Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H. Reactionary dentinogenesis. Int J DevBiol. 1995;39:273–80.

[25] Murray PE, Smith AJ. Saving pulps: a biological basis. An overview. Prim Dent Care. 2002;9:21–26.

[26] Thomas B.Brunner, LeoniA.Kunz-Schughart, Philipp Grosse Gehling, Michael Baumann. Cancer Stem Cells as a Predictive Factor in Radiotherapy. Seminars in Radiation Oncology, April 2012;22(2): 151–74.

[27] Kohji Nagano, Yoko Yoshida, Toshiaki Isobe. Cell surface biomarkers of embryonic stem cells, Proteomics. 2008; 8: 4025–35.

 [28] Papaccio G, Graziano A, d’Aquino R, et al. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: A cell source for tissue repair. Journal of Cellular Physiology. 2006; 208: 319–25.

[29] Graziano A, Biunno I, De Blasio P, Giordano A. The tissue banking in cancer and stem cell research. Journal of Cellular Physiology. 2007;212: 345–47. [30] Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derivedfrom human dental pulp after cryopreservation. Tissue Engineering. 2006; 12: 2813–23.

[31] Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreservedperiodontal ligament. Journal of Dental Research. 2005; 84: 907–12.

[32] Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod. 2008;34:962–69.

[33] Sakai VT, Zhang Z, Dong Z, Neiva K, Machado M, Shi S, et al. SHED differentiate into functional odontoblasts and endothelium. J Dent Res. 2010; 89:791–96.

[34] Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, TakanoYamamoto T et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci USA. 2009. Aug;106(32):13475–80.

[35] Xu WP, Zhang W, Asrican R, Kim HJ, Kaplan DL, Yelick PC. Accurately shaped toothbud cell-derived mineralized tissue formation on silk scaffolds. Tissue Eng Part A. 2008 Apr;14(4):549–57.

 [36] Laino G, d’Aquino R, Graziano A, Lanza V, Carinci F, Pirozzi G, Naro F, Papaccio G. Dental pulp stem cells can be detected in aged humans: an useful source for living autologous fibrous bone tissue (LAB). J Bone Miner Res. 2005; 20:1394–1402.

[37] Laino G, Graziano A, d’Aquino R, Pirozzi G, Lanza V, Valiante S, et al. An approachable human adult stem cell source for hard-tissue engineering. J Cell Physiol. 2006;206:693–701.

 [38] Yapeng Hu, Liwu Fu Targeting cancer stem cells: a new therapy to cure cancer Patients. Am J Cancer Res. 2012; 2(3):340-56.

[39] KMK Masthan, SL Sankari, NA Babu, T**.**Gopalakrishnan**.** Mystery inside the tooth: the dental pulp stem cells.Journal of clinical and diagnostic research: JCDR 7 (5), 945-47