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REVIEW ARTICLE

STEM CELLS-ISOLATION, CHARACTERIZATION AND BANKING-PART I

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ABSTRACT

Stem cells can self-renew and produce different cell types, thus providing new strategies to regenerate missing tissues and treat diseases. In the field of dentistry, adult mesenchymal stem/stromal cells (MSCs) have been identified in several oral and maxillofacial tissues, which suggest that the oral tissues are a rich source of stem cells, and oral stem and mucosal cells are expected to provide an ideal source for genetically reprogrammed cells such as induced pluripotent stem (iPS) cells. Furthermore, oral tissues are expected to be not only a source but also a therapeutic target for stem cells, as stem cell and tissue engineering therapies in dentistry continue to attract increasing clinical interest. Part I of this review outlines various types of intra- and extra-oral tissue-derived stem cells with regard to clinical availability and applications in dentistry. Additionally, appropriate sources of stem cells for regenerative dentistry are discussed with regard to differentiation capacity, accessibility and possible immunomodulatory properties.

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INTRODUCTION

Stem cells are uncommitted, immature, unspecialized cells entities capable of both self-renewal and differentiation into multiple cell lineages via differentiation. By definition, these cells can renew themselves indefinitely through ''self-renewal'', and they vary in terms of their location in the body and the type of cells that they can produce. (Slack, 2008) The two definite characteristics of the stem cells are (i) the ability for indefinite self-renewal to give rise to more stem cells; and (ii) the ability to differentiate into a number of specialized daughter cells to perform specific function. (National Institutes of Health (NIH), 2001) With their unique abilities, stem cells are particularly important for developing innovative technologies for tissue engineering strategies to regenerate or replace damaged, diseased or missing tissues and even organs by in vitro cell manipulation and design of the extracellular environment. (Langer and Vacanti, 1993) In dentistry, the tissues and organs targeted for such regenerative medicine strategies include the salivary gland, tongue and craniofacial skeletal muscles, as well as the condylar cartilage of the temporomandibular joint. (Izumi *et al*., 2011) Also, Stem cells are the foundation cells for every organ and tissue in the body, including the periodontium. Stem cell and tissue engineering therapies are expected to provide a novel capability to regenerate large defects in periodontal tissues and alveolar and to ultimately replace the lost tooth itself. Hence, with the development of improved methods, stem cell based regeneration, comprises a promising alternative for viable therapeutic modalities. (Bianco and Robey, 2001)

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Origin, Isolation and characterization of stem cells: There are two main types of stem cells – embryonic stem cells and adult stem cells – which are classified according to their origin and differentiation potential.

Embryonic stem cells: These types of stem cells are derived from the inner cell mass of blastocysts and are collectively referred to as pluripotent stem cells because they can develop into all types of cells from all three germinal layers of the adult body. They can be maintained in an undifferentiated state in vitro for an indefinite period of time, while retaining their ability to differentiate into all types of specialized cells in the body (Itskovitz-Eldor *et al*., 2000; Thomson *et al*., 1998). These cells have been demonstrated to produce approximately 200 different types of cell within the adult body.

Induced pluripotent stem (iPS): In addition to embryonic stem cells, which are naturally present in the human body, induced pluripotent stem (iPS) cells have been recently generated artificially via genetic manipulation of somatic cells. These induced pluripotent stem cells, were created from specialized somatic cells via the forced over-expression of four key factors Oct3 ⁄ 4, Sox2, cMyc and Klf4 genes (Takahashi *et al*., 2007; Takahashi and Yamanaka, 2006). ES cells and iPS cells are collectively referred to as pluripotent stem cells because they can develop into all types of cells from all three germinal layers. A pluripotent stem cell can differentiate into all cell types of the body, whereas a multipotent stem cell can differentiate along multilineages into many different cell types. The potential for self-renewal vs. differentiation is governed by extracellular signals coupled to intracellular signaling cascades (Watt and Hogan, 2000). Human embryonic stem cells, derived from the inner cell mass of blastocysts, are pluripotent stem cells capable of differentiating into cells of all three germ layers of the adult body. Human embryonic stem cell lines are unique in that they can be maintained in an undifferentiated state in vitro for an indefinite period of time, while retaining their ability to differentiate into all types of specialized cells in the body (Reubinoff *et al*., 2000). Adult, or tissue-specific, stem cells are found in the majority of fetal and adult tissues. Most adult stem cells are multipotent, i.e., they can only differentiate into a limited number of cell types. They are generally multipotent stem cells that can form a limited number of cell types corresponding with their tissues of origin, although some studies have suggested that they are more versatile and can develop into many other cell types than previously expected (Lagasse *et al*., 2000; Mezey *et al*., 2000).

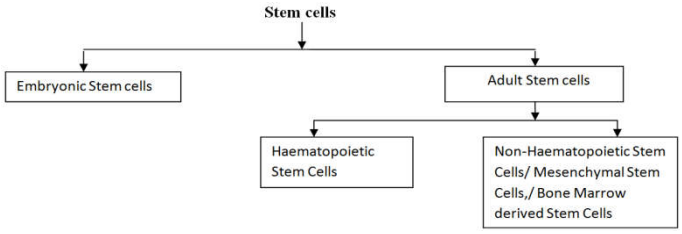
Source of adult stem cells is the bone marrow, which contains hematopoietic stem cells and bone marrow stromal cells or mesenchymal stromal ⁄ stem cells. Hematopoietic stem cells were the first stem cells to be successfully used in therapies, particularly in the treatment of blood malignancies and immunodeficiency syndromes, but they are not capable of giving rise to supporting connective tissues. Mesenchymal stem cells, by contrast, are a cell type of interest with the therapeutic potential to treat a range of musculoskeletal abnormalities, cardiac diseases and immune abnormalities (Till and McCulloch, 1961; Korbling and Estrov, 2003).

Advantages of adult stem cells versus embryonic stem cells: Adult stem cells are emerging as a major contender for use in regenerative therapies as their isolation and use is not subject to the same level of legal and ethical issues as embryonic stem cells.

1. A further advantage of adult stem cells is the greater capacity for their use in autologous transplantation, where adult stem cells can be extracted from a patient and then used to treat that patient, thereby decreasing the likelihood of complications arising from immune rejection.

Many mesenchymal stem cell-like populations derived from various tissues exhibit immunoprivileged properties with the capacity to inhibit immune responses, and thus could be used as a source of allogeneic stem cells. (Guhr *et al*., 2006)

Characterization of Stem cells



Characterization of stem cells based on their regenerative potential (Mao, 2008; Thomson *et al*., 1998)

Stem cell category	Definition	Derived from
Totipotent	The capacity to differentiate into all possible cell types,	Fertilized egg
Pluripotent	The ability to differentiate into almost all cell types. Pluripotent cells lack the capacity to contribute to extraembryonic tissue and therefore cannot develop into a fetal or an adult animal	Embryonic stem cells
Multipotent	The potential to give rise to cells from multiple, but a limited number of, lineages	Mesenchymal stem cells
Oligopotent	The capacity to differentiate into a few cell types	Myeloid stem cells
Unipotent	The ability to differentiate into only one type of cell	Skin

Types of stem cells	May differentiate to...
Embryonic	Any type of cell
Amniotic fluid-derived	Cartilage cells
	Fat cells
	Bone cells
	Muscle cells
	Endothelial cells
	Neuron-like cells
	Liver cells
Umbilical cord	Liver cells
	Skeletal muscle cells
	Neural tissue
	Immune cells
Bone-marrow-derivedmesenchymal	Bone cells
	Cartilage cells
	Muscle cells
	Fat cells
	Neuron-like cells
	Pancreatic islet beta cells
Tooth-derived	Neural cell lineages
	Bone cells
	Cartilage cells
	Muscle cells
	Fat cells
	Pancreatic islet beta cells
Adipose-derived	Fat cells
	Cartilage cells
	Muscle cells
	Neuronal cells
	Bone cells
Induced pluripotent stem cells	Any type of cell, potentially

Dental stem cells

Dental-derived SCs have been isolated and identified as the cell sources for tooth repair and regeneration. These cells are named according to their anatomical locations, and are characterized by their SC markers, colony-forming ability, and dental regenerative function.

Five different types of dental stem cells isolated from dental soft tissues are-

1. Dental pulp stem cells (DPSCs)
2. Stem Cells from Exfoliated Deciduous Teeth (SHED),
3. Periodontal Ligament Stem Cells (PdlSCs),
4. Stem Cells from Apical Papilla (SCAP)
5. Dental tissue from human third molar, apical papilla, dental follicle and periodontal ligament.

DPSCs

DPSCs are SCs derived from dental pulp. These cells are quiescent and reside in a specific perivascular microenvironment where they maintain their SC characteristics. DPSCs show a multipotential differentiation ability, which is similar to that of MSCs. These DPSCs express MSC markers, including Stro-1 and CD146, and undergo colony forming *in vitro* and can regenerate the dentin/pulp complex *in vivo*. (Harada *et al*., 1999)

SHED

SHEDs are multiple SCs found in the pulp tissue of human exfoliated deciduous teeth. They were originally identified as a population of extensively proliferative clonogenic cells, and can differentiate plastically into neuronal cells, adipocytes and odontoblasts. In addition, SHEDs show higher proliferation rates than DPSCs, and can form significant amounts of alveolar and orofacial bone for tissue regeneration. (Morotomi *et al*., 2005)

PDLSCs

As periodontal tissues are able to regenerate after mild trauma, researchers in the early 1970s postulated that PDLSCs might play an important role in periodontal repair. PDLSCs were first isolated by Seo *et al*. and were found to be capable of differentiating into cementoblast-like cells, adipocytes and collagenforming cells.13 Cell-surface markers of PDLSCs include Stro-1 and CD146/Muc18.Moreover, PDLSCs have been used to generate a root/periodontal complex to support normal tooth function in animal model. (Melcher, 1970; Gronthos *et al*., 2006)

DFSCs

Dental follicles comprise the neural crest, which is derived from ectomesenchymal tissue surrounding the developing tooth germ. Human dental follicles can be isolated after wisdom tooth extraction, and they play an important role in tooth eruption by regulating osteoclastogenesis and osteogenesis. After tooth eruption, the dental follicle differentiates into cells of the periodontium, including alveolar osteoblasts, the PDL, fibroblasts and cementoblasts. The pluripotency of DFSCs has also been demonstrated. For example, the neuronal-differentiation ability of DFSCs was documented using the neural progenitor cell markers Notch-1 and Nestin. Mean while, the adipocyte differentiation capability of DFSCs was demonstrated by cultivating dental follicle cells with an adipogenesis medium.18 These observations suggest the presence of pluripotent SCs in human dental follicles. In addition to human wisdom teeth, SCs have been isolated from mouse or bovinedental follicles. (Luan *et al*., 2006; Saito *et al*., 2005)

Dental epithelial SCs

Tooth enamel, the most mineralized tissue of the body, is first formed in the crown stage of dental development. 35 Before the tooth erupts into the mouth, the ameloblasts are broken down. Consequently, human enamel, unlike continuously growing mouse incisors and some mammalian molars, is unable to regenerate itself. (Ross *et al*., 2003; Smith, 1980) Dental epithelial SCs in the mouse cervical loop form a unique structure, the apical bud. The apical bud is a condensed SC compartment responsible for replenishing the growing dentition when it interacts with mesenchymal cells. (Ohshima *et al*., 2003)

Stem Cell Banks

Stem cell Isolation: "Dental stem cells are a valuable source of stem cells and are found in teeth with healthy pulp," Stem Save's Chotkowski explained. "These teeth could be deciduous teeth, wisdom teeth and other permanent teeth.

The BD Stemflow hMSC Analysis Kit is a proven tool for rapidly evaluating the purity of BM-MSC cultures, between cell culture expansion passages, prior to storage, and before use in their final research applications (eg, in vitro differentiation). The BD Stemflow™ hMSC Analysis Kit provides a comprehensive system based on the phenotypic signature described by The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT). (Dominici *et al*., 2006)

The ISCT has proposed a minimal set of three standard criteria to be used as the uniform definition of multipotent MSCs: 1) adherence to plastic, 2) specific surface antigen expression, and 3) multipotent differentiation potential. The phenotype of multipotent MSCs is defined to be, at a minimum, the cell surface co-expression of the antigens CD105, CD73, and CD90 (≥95% positive) and the absence of hematopoietic lineage markers CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR (≤2% positive). The ISCT has emphasized that the optimal flow cytometric assay uses multicolor analysis to demonstrate that individual cells co-express unique MSC markers and lack hematopoietic antigen expression. (Dominici *et al*., 2006)

2. Stem cell processing and preservation- after isolation of stem cells they are processed and preserved in the following manner-

a) Cryopreservation- During cryopreservation, the cells are put to sleep through a process called vitrification, in which the tissue is placed in liquid nitrogen at a temperature of –196 degrees Celsius. The cryopreservation process stops all cellular metabolism involving both cell growth and cell death. The cells preserved today can be applied to future regenerative therapies. (Pegg, 2002)

b) When needed, the cryopreserved sample will be removed from the liquid nitrogen and the tissue will go through a thawing process. This tissue can either be digested enzymatically and placed into a cell sorter or, depending on the size and quantity of tissue, placed into a growth medium where the stem cells are allowed to grow into colonies. These colonies are characterized, and specific colonies are separated and allowed to continue to expand to the required number of cells indicated by the planned regenerative therapy protocol. (Karlsson, 2002; Huang *et al*., 2008)

REFERENCES

Bianco P, Robey PG. 2001. Stem cells in tissue engineering. *Nature*, 414: 118–121.

Dominici M, Le Blanc K, Mueller I, *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315-317.

Frandson RD, Spurgeon TL. *Anatomy and Physiology of Farm Animals*. Ames: Wiley-Blackwell, 2007:340.

Gronthos S, Mrozik K, Shi S, Bartold PM. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. *Calcif Tissue Int* 2006;79:310–7.

Guhr A, Kurtz A, Friedgen K, Loser P. 2006. Current state of human embryonic stem cell research: an overview of cell lines and their use in experimental work. *Stem Cells*, 24: 2187–2191.

Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. *J Cell Biol* 1999;147:105–20.

Huang AH, Chen YK, Lin LM, Shieh TY, Chan AW. Isolation and characterization of dental pulp stem cells from a supernumerary tooth. *J Oral Pathol Med* 2008;3

Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N. 2000. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med*., 6: 88–95.

Izumi Y, Aoki A, Yamada Y, Kobayashi H, Iwata T, Akizuki T, *et al*. 2000. Current and future periodontal tissue engineering. *Periodontology*, 2011;56:166–87.

Karlsson JO, 2002. Cryopreservation: freezing and vitrification. Science. 296 (5568): 655-656

Korbling M. and Estrov Z. 2003. Adult stem cells for tissue repair – a new therapeutic concept? *N Engl J Med*., 349: 570– 582.

Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. 2000. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med*., 6: 1229–1234.

Langer R, Vacanti JP. 1993. Tissue engineering. *Science,* 260:920–6.

Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium.*Stem Cells Dev* 2006;15:595–608.

Mao JJ. 2008. Stem cells and the future of dental care. *NY State Dent J*., 74(2): 20-24.17,18

Melcher AH. Repair of wounds in the periodontium of the rat. Influence of periodontal ligament on osteogenesis. *Arch Oral Biol* 1970;15:1183–204.

Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. 2000. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science,* 290:1779–1782.

Morotomi T, Kawano S, Toyono T, Kitamura C, Terashita M, Uchida T, Toyoshima K, *et al*. In vitro differentiation of dental epithelial progenitor cells through epithelial-mesenchymal interactions.*ArchOralBiol.*2005;50:695–705.

National Institutes of Health (NIH). 2001. Stem cells: scientific progress and future research directions. Bethesda: NIH.

Ohshima H, Kenmotsu S, Harada H. Use of the term apical bud to refer to the apical end of the continuously growing tooth. *Arch Comp Biol Tooth Enamel* 2003;8:45–9.

Pegg DE. The history and principles of cryopreservation. *Semin Reprod Med* 2002;20:5–13.

Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. 2000. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol*., 18:399–404.

Ross MH, Kaye GI, Pawlina W. *Histology: A Text and Atlas*. Philadelphia: Lippincott Williams & Wilkins, 2003:412.

Saito M, Handa K, Kiyono T, Hattori S, Yokoi T, Tsubakimoto T, Harada H, *et al*. Immortalization of cementoblast progenitor cells with Bmi-1 and TERT. *J Bone Miner Res* 2005;20:50–7.

Slack JM. 2008. Origin of stem cells in organogenesis. *Science,* 322: 1498–501.

Smith CE. Cell turnover in the odontogenic organ of the rat incisor as visualized by graphic reconstructions following a single injection of 3H-thymidine. *Am J Anat* 1980;158:321–43.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131: 861–872.

Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126: 663–676.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. 1998. Embryonic stem cell lines derived from human blastocysts. *Science,* 282:1145–1147.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. 1998. Embryonic stem cell lines derived from human blastocysts. *Science*, 282: 1145–1147.

Till JE. and McCulloch EA. 1961. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*., 14: 213–222.

Watt FM, Hogan BL. 2000. Out of Eden: stem cells and their niches. *Science*, 287: 1427–1430.

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