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Applications of stem cells in dentistry: A review

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ABSTRACT

Stem cells have the capacity to replicate and produce cell lines that differentiate into multiple cell lineages. Stem cells may be harvested from various sites and are named adult stem cells or embryonic stem cells based on their origin. Owing to their self renewing capabilities, they are used to correct large defects caused by diseases, trauma or surgery. However, they are limited by ethical and moral considerations as well as difficulty in isolation, culturing and implantation. Dental stem cells retain the property of differentiation into neurogenic, adipogenic and odontogenic components and are used in the reconstruction of orofacial structures. Scaffolding impregnated with bone morphogenic proteins and growth factors is essential prior to stem cell implantation. This 3D scaffolding with biomatrix is then introduced into the clinical site to facilitate regeneration of tissues. In the maxillofacial region, stem cells may be derived from the pulp, apical papilla, dental follicle, periodontal ligament, deciduous teeth and mucosa. They can be used for bioengineering of pulp and periapical tissues, soft tissues, bone, temporomandibular joint and periodontium. A multi-speciality approach involving cell biologists, pharmacologists and bioengineers is required to harness the vast potential of stem cell therapy and to obtain reliable treatment outcomes in the future.

Key words: *Stem cell, tissue engineering, dental, bioengineering*

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Introduction

Certain cells in the body retain their capacity to divide and produce progeny owing to their undifferentiated state. These cells are capable of self-renewal and future differentiation into multiple functional cell types and are called stem cells(1,2). Stem cells maybe adult (post-natal) stem cells and foetal (embryonic) stem cells based on their origin. As the name suggests, embryonic stem cells are derived from embryos following in-vitro fertilization while adult stem cells are derived from various sources such as bone marrow, umbilical cord, cord blood, peripheral blood, muscles, skin, adipose tissue, dental pulp or organs such as lungs, liver, breasts, eyes and brain(2,3). The first instance of therapy using adult stem cells was in 1968 when allogenic bone marrow transplant was carried out in a patient with combined immunodeficiency(4).

Classification of stem cells

Stem cells may be classified(5) as:

1.Embryonic stem cells

2.Adult stem cells

a.Hematopoetic stem cells

b.Mesenchymal stem cells

3.Induced pluripotent stem cells

Types of stem cells

1.Embryonic stem cells

Embryonic stem cells are derived from the blastocyst containing 50 to 150 cells(6). They are pluripotent and versatile and have the plasticity needed to differentiate into cells of all three germ layers(6,7). With appropriate stimulation, a large quantity of any particular adult cell type can be produced(6). However, since the inner cell mass of a fertilized embryo is used to produce these cell lines, their use is restricted by ethical and legal considerations(8). They have the added disadvantage of increased tumorigenesis potential which makes their use less favourable(2,5).

2.Adult stem cells

Adult stem cells are also called somatic or postnatal stem cells. They are multipotent and differentiate into a limited number of cell lines(9,10). Adult stem cells are easier to isolate and are not bound by the same legal and ethical constraints as embryonic stem cells. This, along with their rarer incidences of immune rejection and teratoma formation makes them suitable for use in most clinical practices(2,5).

3.Mesenchymal stem cells

Mesenchymal stem cells (MSC) are a type of adult stem cells and are also called mesenchymal stromal cells. They are

multipotent and are found in bone marrow, skin, oral and maxillofacial structures and adipose tissue(10). They do not carry any rejection potential and can be used autologously(6). To be classified as MSC, they must satisfy the minimum criteria set by the International Society for Cellular Therapy that they must be adherent to tissue culture treated plastic under ideal conditions and must be able to differentiate into adipocytes, chondroblasts and osteoblasts in-vitro(11). Although systemic administration of MSC carries a significant risk of pulmonary embolism and accumulation in healthy organs, Kanawaza et al found that it has a higher efficacy when compared to local administration(12).

4.Adipose derived stem cells

These cells are extracted via liposuction, lipectomy or lipoaspiration and are subsequently isolated(10). They are abundant and accessible easily and are multipotent with a wide lineage after differentiation(5).

5.Umbilical cord derived stem cells

They are a type of adult stem cells that are derived from the umbilical cord blood(10).

6.Amniotic fluid derived stem cells

These cells are isolated either at the time of delivery or during a procedure of amniocentesis for genetic screening during gestation(10).

7.Bone marrow derived mesenchymal stem cells

They are a type of adult stem cells that are found abundantly in the marrow and are widely used in clinical practice due to their multipotency. Bone marrow from the iliac crest is one of the most common source for harvesting stem cells for regenerative medicine(10,13). They can also be derived from the periosteum or the synovial membrane and form cancellous bone(6,10). Their high potential for in-vivo bone regeneration makes them ideal for bone tissue engineering irrespective of the age of the patient(10,13). Since the marrow derived from bones of the orofacial region have a distinct mesenchymal stem cell marker expression pattern and differ in their differentiation, dental tissue derived stem cells are preferred for therapy involving the orofacial region(10).

8.Dental tissue derived stem cells

Dental tissue derived stem cells are one of the most accessible types of stem cells. They may be isolated from permanent or developing tooth, tooth follicle, extracted or exfoliated teeth, pulp and apical papilla, periodontal ligament and gingiva(1,10). They develop from the mesenchymal and neural crest cells during embryogenesis. The epithelial cell lines produce enamel through ameloblasts while the mesenchymal cell lines give rise to dentin through odontoblasts(10,14). However, with the use of dental tissue derived stem cells, an added procedural step of classification and purification of the cells exists since the cells are heterogenous and contain derivatives of fibroblasts and progenitor cells in addition to true stem cells(10). Other limitations for the use of dental tissue derived stem cells is the difficulty in obtaining sufficient quantity of cells, the need for professionals trained in the extraction, isolation and culture of these cells and the increased time required for culturing(6).

9.Induced pluripotent stem cells

Induced pluripotent stem cells mimic embryonic stem cells in their potential to divide but are exempt from the ethical restraints since they are produced by transfecting genes found in embryonic stem cells into a donor cell with the help of vectors(9). Here, autologous somatic cells produce a patient-specific embryonic stem cell equivalent and pave the way for treatments that are tailored to the needs of the individual(10).

Dental stem cells

Dental stem cells may be either dental mesenchymal stem cells derived from dental pulp, deciduous teeth, periodontal ligament and dental follicle or dental epithelial stem cells derived from the incisors and molars of mammals with the potential for continuous growth(1).

1.Dental pulp derived stem cells (DPSC)

These are the most common source of dental tissue derived stem cells and are obtained from the pulp of permanent teeth(15). They are multipotent and express STRO-1, CD 44 and CD 146 MSC markers(1,15). DPSC were first isolated from human teeth in 2000 and are capable of differentiating into osteogenic, odontogenic, myogenic, adipogenic and neurogenic components both in-vitro and in-vivo and can produce pulp-dentin complex in-vivo(1,15,16).

2.Stem cells from human exfoliated deciduous teeth (SHED)

They are derived from exfoliated teeth and are more proliferative than DPSC. They differentiate into neurogenic, adipogenic and odontogenic components and are used for tissue regeneration involving orofacial bony structures(1). They contain MSC markers such as STRO-1 and CD 146 and neuronal and glial markers such as Nestin and β III Tubulin. They have the capacity to produce bone and dentin in-vivo(15).

3.Periodontal ligament stem cells (PDLSC)

They are derived from separated periodontal ligaments of third molars in humans and contain progenitors for self-renewal of oral structures like cementum and bone(6). They were first isolated by Seo et al who found that they were multipotent(17). They contain MSC markers such as STRO-1, Muc 18, CD 44 and CD 146 and differentiate into adipogenic, chondrogenic and osteogenic components in-vitro and cementum and periodontal ligament in-vivo(1,6,15). A related entity known as alveolar periodontal ligament stem cells (aPDLSC) have increased affinity for the production of adiopocytes and osteocytes(15).

4.Dental follicle stem cells (DFSC)

They are derived from the follicle surrounding human third molars and are pluripotent. They are made up of ectomesenchyme and contain markers such as Notch1, STRO-1 and Nestin. They have the potential to differentiate into osteoblasts, adipocytes and neuroblasts in-vitro and periodontal ligament in-vivo(1,6).

5.Stem Cells from Apical Part of Papilla (SCAP)

They are derived from the apical part of a developing tooth and have high proliferation, migration and regeneration capabilities(15). They contain fibroblast-like and odontoblast-like cells with MSC markers such as STRO-1, CD 24, CD 44 and CD 146. They differentiate into pulp-dentin complex in-vivo(6,15).

6.Oral mucosa derived stem cells

It may either be oral epithelial stem cells or gingival stem cells. The oral epithelial stem cells are unipotent and develop only into epithelial cells in-vivo. However, when used ex-vivo,

they develop a well stratified oral mucosal graft and are used for grafting procedures involving the oral structures. Gingival stem cells are multipotent, have reprogramming capabilities, are more abundant, are easy to isolate and have a rapid ex-vivo proliferation that makes their use clinically viable(10).

7.Human dental epithelial stem cells (hDESC)

They are derived either from third molars or the epithelial sheaths that disintegrate into rests of Malasez. They express epithelial stem cell markers such as p75, E-CAM and Bmi-1 along with embryonic stem cell markers like Nanog and Oct-4(15).

8.Periosteum derived stem cells

Human periosteum derived stem cells are multipotent with odontogenic, chondrogenic, adipogenic and myogenic potential both in-vitro and in-vivo. They produce cortical bone and are therefore used for regeneration of large defects in the orofacial region(10).

9.Salivary gland derived stem cells

Cells derived from salivary gland have the potential to form duct cells and acinar cells in-vitro and retain the capacity to produce both mucin and amylase. They can therefore be used in rehabilitation of patients with reduced salivary gland function following irradiation. However, an added difficulty in isolating the cells exists since the harvested cells contain cells from the parenchymal, stromal and blood vessel cells(10).

Dental stem cell therapy

Stem cell based therapy aims to restore the anatomy and function of a damaged tissue through regeneration(15). Goals of regenerative dentistry include continuation of root formation, regeneration of pulpal tissues, reconstruction of periodontium, aiding in transplantation and replantation, root bio-engineering and engineering of pulp-dentin complex(2). In order to obtain stem cells, an appropriate method of collection, isolation, culture and replication has to be established. SHED is one of the most commonly banked stem cells. Banking of SHED provides various advantages. It is easily available and is analogous to cord blood cells, it provides an autologous source of stem cells for the individual as well as their close relatives and it does not have the same ethical constraints as embryonic stem cells(6,18).

Collection and isolation of dental stem cells

The source of stem cell such as tooth with a healthy and vital pulp with adequate blood supply, follicle, periodontal ligament, apical tissue etc should be placed in a vial with hypotonic phosphate buffered saline solution and should be transferred to the processing facility within 40 hours of removal. The tissue is then disinfected, isolated and cultured in a specific MSC culture medium based on the cell lines that are required. Storage can be done with the help of cryopreservation or magnetic freezing using Cell Alive System (CAS)(6,19). The cultured stem cells should be tested for endotoxin and for markers like TRA-1-81, TRA-1-60, Nanog, Oct4 and SSEA4 to know the cell lineage. They are then seeded on appropriate scaffolds and inserted into place to regenerate the required tissues(5).

Principles of tissue engineering include three aspects- inducing signals, responding cells and scaffolding. Tooth regeneration can be achieved either by utilizing these tissue engineering approaches or by simulating the process of embryonic tooth formation(6). The goal of treatment is to achieve regeneration of diseased or damaged structures such as dental pulp, periodontal ligament or other oro-facial structures.

Regeneration of pulp

Post-natal stem cells as well as stem cells grown on external scaffolding can also be used for regeneration of pulpal tissue. The patient's own cells can be used for regeneration of pulp thereby reducing the risk of immune rejection. Bleeding and clot formation in the root canals is facilitated via over-instrumentation and the canal is irrigated with antimicrobials. However, care should be taken with this approach since the composition and concentration of cells trapped in the clot cannot be predicted. The scaffolding should contain bone morphogenic protein, growth factors, nutrients and antimicrobials to aid in cell regeneration(6,20). Three-dimensional cell printing for precise orientation of cells and gene therapy that introduces therapeutic protein into the canal are newer approaches that require further research(6). A successful treatment is one where revascularization and reinnervation of pulp is achieved for the production of new dentin(15).

Regeneration of periodontium

Periodontal plastic surgery incorporates tissue engineering using a scaffolding and a matrix that grows over it(10). Various growth factors are used to facilitate the production of sufficient number of cells to close the defect. Bone marrow stem cells show the capacity to differentiate into cementum, periodontal ligament and alveolar bone. Platelet rich plasma is mixed with MSC derived from the iliac crest to fill periodontal defects(5). A biocompatible scaffolding is created and seeded with periodontal ligament stem cells to create a 3D matrix which is then incubated with growth factors and signalling molecules to obtain a transportable construct before being transplanted into the area requiring regeneration(5,10,21). The use of temperature responsive dishes to engineer cell sheets ensures delivery of extracellular matrix proteins and intact cell to cell interactions. Kim et al used nanopatterned surfaces for the delivery of human periodontal ligament stem cells extracted from premolars and found that the cells were capable of differentiation into multiple lineages when incubated in adipogenic and osteogenic media and formed strong colonies(22).

Regeneration of orofacial tissues

Prosthetic and functional correction of the soft and hard tissues of the orofacial region becomes a necessity following large space occupying lesions, trauma or surgery(21,23). Oral keratinocytes can be seeded onto 3D scaffolding to create tissue engineered oral mucosa. Collagen seeded with myoblasts can be used for reconstruction of orofacial muscles along with growth factors and platelet rich plasma(10). Iliac bone graft with adipose derived cells are used for the correction of defects of the calvarium. These adipose derived cells can also be used as scaffolds for soft tissue repair. Bone marrow derived stem cells along with DPSC that differentiate into odontoblasts in a collagen sponge scaffold are used for correction of large defects(5,21).

Regeneration of teeth

Regeneration of a tooth requires interaction between epithelial and mesenchymal signals. Therefore, any scaffolding that hopes to achieve odontogenesis should contain both epithelial and mesenchymal stem cells or cells that are capable of diffe-

rentiating into these germ layers(21). While both prenatal and postnatal tooth germs can be used for regeneration, the use of prenatal tooth germ is preferred since it has higher propensity towards development of normal tooth structure(10).

Regeneration of bone and temporomandibular joint

Injectable composites or polymer with periosteal stem cells are used for the correction of bone defects. Inorganic bone with bone factor rich plasma can also be used for closure of defects. Inorganic bone can be shaped into the form of a condyle to act as a scaffolding. This, along with autogenic stem cells in an injectable smart hydrogel can be used for the regeneration of the temporomandibular joint (TMJ)(10,21). Adult mesenchymal stem cells in a biomimetic scaffold is used for production of bioengineered TMJ(21). Chang et al found that among the various micro RNAs (MiRNAs), Mi-R-222 and Mi-R-423 significantly modulated osteoblastogenesis. Scaffolds reinforced with mi-RNA showed enhanced functionality and osteoinductive properties(24).

Stem cell therapy aims to limit or reverse the morbidity associated with disease, trauma or treatment. Minimal donor site complications make it one of the ideal methods of reconstruction of both soft and hard tissues. However, the dental stem cells differentiate into multiple lineages and the regenerated tissues may not mimic the original morphology. At present there is no embryonic environment that converts BMSC into tooth germ. There is an added disadvantage of immune rejection and major ethical considerations. Therefore, a multilevel approach that involves matrix biologists, cell biologists, pharmacologists, bioengineers and nanotechnologists is required to harness the vast capabilities of stem cell therapy and to obtain predictable and reliable treatment outcomes.

Conflict of Interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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