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Dental Stem Cell: A Review

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ABSTRACT *Dentistry is likely to be revolutionized by biological therapies based on growth and differentiation factors that accelerate and/or induce a natural biological regeneration. Induced pluripotent stem cell-based therapy for treating genetic disorders has become an interesting field of research in recent years. However, there is a paucity of information regarding the applicability of induced pluripotent stem cells in dental research. It has been reported that postnatal stem cells are present in adult tissues such as bone marrow, liver, muscle, dental pulp, and periodontal ligament. Cell-based tissue repair of the tooth and tooth supporting periodontal ligament is a new attractive approach that complements traditional restorative or surgical techniques for replacement of injured or pathologically damaged tissues. Recent attention has been focused on tissue engineering and on the properties of these cells: Adult dental stem cells can differentiate into many dental components, such as dentin, periodontal ligament, cement and dental pulp tissue.*

1. Introduction

Stem cells, which are hallmarked by their ability to self-renew through replication as well as differentiation into a wide variety of cell types, can be broadly classified as ESCs or organ-derived stem cells. Totipotent stem cells are able to create an entire organism, a property retained by early progeny of the zygote up to the 8-cell stage of the morula [1]. Pluripotent cells are capable of forming tissues from all embryonic germ layers (endoderm, mesoderm, and ectoderm), and multipotent cells can produce a more restricted subset of cell lineages. However, some studies have suggested that adult stem cells can contribute to cell types of different tissue lineages giving rise to the concept of stem cell plasticity [2] It has been identified a putative post-natal stem cells in human dental pulp, dental pulp stem cells (DPSCs). The most striking feature of DPSCs is their ability to regenerate a dentin-pulp-like complex that is made of matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth [3]. Tooth regeneration is generally referred to as the regeneration of the whole tooth or root that can be integrated into the jaw bone. This technology is still at its infancy and when it fully developed, it may be used to restore missing teeth and replace artificial dental implants when the tooth is damaged but still in a reparable condition, regeneration of parts of the tooth structure can prevent or delay the loss of the whole tooth. Indeed, for example, to repair partly lost tooth tissues such as PDL, dentin, and pulp, one or two particular types of dental stem cells may be sufficient to fulfill the need. In light of such considerations, aim of the present chapter is to define the main strategies to isolate dental pulp stem cells, their characterization and differentiation, tissue engineering strategies and clinical applications for the creation of artificial tissue useful in odontoiatric field.

2. Source of stem cell-

The oral and maxillofacial region can be treated with stem cells from the following sources.

Bone marrow:
Bone marrow stem cells (BMSCs) can be harvested from sternum or iliac crest, composed of both hematopoietic stem cells and mesenchymal stem cells (MSCs). The majority of oro-maxillofacial oral structures are formed from MSCs. The advantage of bone marrow is that it has a larger volume of stem cells and can be differentiated in to wide variety of cells. Isolation of BMSCs can be carried out only under general anesthesia with possible post operative pain.

Adipose tissue:
They can be harvested from the lipectomy or liposuction aspirate. Adipose derived stem cells (ADSCs) contain a group of pluripotent mesenchymal stem cells that exhibit multilineage differentiation [4]. Advantage of adipose tissue is that it is easily accessible and abundant in many individuals.

Stem cells from the Oromaxillofacial Region:
Stem cells from oral and maxillofacial region predominantly contain mesenchymal stem cells. In oral and maxillofacial area different types of dental stem cells were isolated and characterized. They include

- Dental pulp stem cells (DPSCs)
- Stem cells from exfoliated deciduous teeth (SHED)
- Periodontal ligament stem cells (PDLSCs)
- Stem cells from apical papilla (SCAP)
- Dental follicle progenitor cells (DFPCs)

3. Dental Stem Cell -

A great challenge is the search for a source of human mesenchymal and epithelial stem cells which possess odontogenic potential, to enable the regeneration of a functional tooth. The human dental stem cells are classified in dental pulp stem cells (DPSCs), stem cells of human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle stem cells (DFSCs), and stem cells of the dental apical papilla (SCAPs). Classification of stem cell is shown in table 1.

Properties	DPSC	SCAP	SHED	PDLSC	DFSC
Full name	Dental Pulp Stem Cells	Stem Cells of the (Dental) Apical Papilla	Stem Cells of Human Exfoliated Deciduous Teeth	Periodontal Ligament Stem Cells	Dental Follicle Stem Cells
isolation	From Dental Pulp	Impacted third molars	Exfoliated Deciduous Teeth and Coronal pulp	Root from Extracted teeh	from the follicles of impacted third molars

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Location	Permanent Tooth Pulp	Apical papilla of developing root	Exfoliated deciduous tooth pulp	Periodontal ligament	Dental follicle of developing tooth
Proliferative rate	Moderate	High	High	High	High
Heterogeneity	Yes	Yes	Yes	Yes	Yes
Multi-potentialy	Odontoblast Osteoblast Chondrocytes Myocytes neurocytes adipocutes, cornial epithelial cell, melanomna cell, iPS	Odontoblast Osteoblast neurocytes adipocutes, iPS	Odontoblast Osteoblast Chondrocytes Myocytes neurocytes adipocutes, iPS	Odontoblast Osteoblast Chondrocytes, cementoblast, neurocytes	Odontoblast Osteoblast neurocytes
Tissue repair	Bone regeneration, neuroregeneration, Myogenic regeneration, Dentin pulp regeneration	Bone regeneration, neuroregeneration, Dentin pulp regeneration, root formation	Bone regeneration, neuroregenerati-on, tubuler dentin	Bone regeneration, root formation, periodontal regenreration	Bone regeneration, periodontal regenreration
Population Doubling	60- > 120	>140	> 70	Not determined	Not determined
Stro-1 Gene Expression	+ (5-10%)	+(>18%)	+ (9%)	+	+
CD(+) Selected	CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146	CD13,CD44, CD24, CD29, CD44, CD59, CD73, CD90, CD105, CD146	CD146	CD13, CD29, CD44, CD59, CD90, CD105,	CD13, D29, CD44, D59, CD73, D90, CD105, CD146
CD(-) Selected	CD14, CD24, CD34, CD45	CD18, CD34, CD45, CD150	Not determined	Not determined	CD45

Table 1- Classification and Characterization of Dental Stem Cell

3.1. Non-dental stem cells

Dental tissue can also be regenerated from non-dental adult multipotent stem cells [5,6]. Embryonic oral epithelium can stimulate an odontogenic response in mesenchyme which does not have a dental origin [7]. It would be desirable to have an extraoral, easily accessible source of stem cells, in order to make odontogenesis possible in a minimally invasive manner.

4. Dental stem cell markers

Stem cell markers help to identify, characterize, and isolate stem cells. Some examples are described below as an overview (In table 2). A trypsin-resistant cell-surface antigen, is known as STRO-1, commonly used as dental stem cell marker. [8,9,10]. STRO-1 is one of the early surface markers of mesenchymal stem cells. Stro-4, is another stem cell marker, binds to heat shock protein–90 beta of multipotent MSCs and is also accessible to identifying stem cells [11]. For instance, the osteoblast marker osteocalcin is also a stem cell marker of DPSCs [3]. The mesenchymal stem cell, immature dental pulp stem cells also express markers of embryonic stem cells. These markers are Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 [12]. The neural marker on dental stem cells indicates that dental mesenchymal stem cells originate in progenitor cells of the neural crest, which can also differentiate into neural tissue [13].

Marker	Expression Observed in	Function
Stro-1	DPSC, SCAP, SHED, PDLSC, DFSC	Mesenchymal Precursor cell marker
CD-146/ MUC18	DPSC,SCAP, SHED, DFSC	Mesenchymal stem cell and endothelial cell marker
CD-105	DPSC,SCAP, ,PDLSC,DFSC	Vascular endothelial Marker
CD-44	DPSC,SCAP, PDLSC,DFSC	Mesenchymal cell marker
3G5	DPSC	Mesenchymal stem cell/ Marker
C-kit(CD-117)	DPSC	Mesenchymal cell and Hepatopoitic stem cell Marker
CD-29	DPSC,SCAP, PDLSC,DFSC	Mesenchymal stem cell and endothelial cell marker
Oct4	DPSC, SHED	Embryonic stem cell Marker
E2F2, PTTG1, TWST-1	DPSC	Mesenchymal transcription factor
Nanog	DPSC, SHED	Embryonic stem cell Marker, regulate embriyonic development and determine stem cell fate
SSEA-4	DPSC, SHED	Embryonic stem cell Marker
Sox2	SHED	regulate embriyonic development and determine stem cell fate
Flk1	SHED	Vascular endothelial growth factor receptor expressed by endothelial cells and neural progenitor cells
c-myc	SHED	Proto-oncogene, Stem cell differentiation
Klf4	SHED	Transcription factor expressed by embryonic stem cells and Mesenchymal stem cells
EphB	DPSC	Transmembrane receptor responsible for cell-cell interaction in neural tissue embryogenesis
Nestin	DPSC, SHED	Intermediate filament protein , central nervous system progenitor cell marker

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Low affinity nerve growth Factor	DPSC	Receptor for neurotrophins responsible for neural development and survival

Table 2- Dental Stem cell marker and their Function (14-29)

6. Cell therapy for repair and regeneration of tooth

6.1. Early tooth regeneration research

In vitro organ cultures of mouse tooth germs, grafts on chick chorioallantois [30] and recombination experiments in ocular grafts [31] were carried out and demonstrated the possibility of growing teeth in an appropriate environment. Further tests disclose that dental epithelium could be produced from non-teeth bearing tissues [32] and non-dental mesenchyme can become competent for odontogenesis when interacting with inductive dental epithelium [33].

6.2. Current tooth regeneration research

Tooth regeneration from presumptive dental stem cells such as DPSCs exhibit partial teeth structure formation, but not a whole biological tooth germ with equivalent function. An approach using a biodegradable polymer scaffold seeded with cells dissociated from the third molar tooth germs of 6-month-old pigs or cultured 3- to 7-day postnatal rat tooth bud cells has been proposed. Although most of the bioengineered teeth exhibit disorganised heterogeneous morphologies, 15% of tooth structures shown spatial organised pulp, dental and enamel [34]. However, the bioengineered teeth had not the expected size or shape of the scaffold, suggesting that these tiny toothlets were formed by reaggregation of small fragments of epithelium and mesenchyme cells. Further explanation of the progression of reaggregation comes from experiments where dissociated, cap-stage mouse molar epithelium was reassociated with dental mesenchyme with or without dissociation and cultured in vitro [35]. The use of non-dental stem cells as possible replacements for embryonic cells for tooth regeneration has been demonstrated for ES cells, neural stem cells and bone marrow-derived cells by responding to the inductive signals from dental epithelium, expression of odontogenic genes and, in some cases, in tooth formation [7, 36]. Recombinants between odontogenic-inducing epithelium and non-dental stem cells (i.e., neural stem cells, mouse ES cells and mouse bone marrow-derived cells) were shown to express early mesenchymal odontogenic markers, such as Pax9, Msx1 and Lhx7 [7]. The ability of non-dental mesenchymal cells to fully develop into teeth was exhibited in those recombinants where teeth with organised enamel, dentin and pulp formation surrounded by bone were formed. The results reinforce the idea that odontogenic signals can instruct tooth crown formation in embryonic or adult stem cells without a scaffold. These teeth were of the appropriate size and shape for mouse molars. An essential prerequisite of all the approaches being developed to produce teeth for replacement (Bioteeth) is that these must be able to form teeth in the adult mouth. In many respects this is a tall order, as the environme2t of the adult mouse is very different to that of an early embryo. However, remarkably, it appears that embryonic tooth primordia can develop normally and form teeth when transplanted into the adult mouth [7].

7. Approaches in periodontal regeneration

Periodontal disease is the most common infectious disease in human beings caused by bacteria present in the oral cavity, which attach on the teeth and cause inflammation of the periodontal tissues. Epidemiological survey exhibits that approximately 7–13% of the global population is at high risk of developing severe forms of destructive periodontitis [37] posing a tremendous burden to health care. Periodontal regeneration is defined as reproduction or reconstruction of a functional attachment apparatus consisting of new cementum, alveolar bone and PDL, on a root surface that was previously exposed due to progression of periodontitis. Early regeneration approaches have focused on providing appropriate conditions for wound healing. These included a range of surgical procedures along with use of bone grafts as tissue substitutes, barrier membranes to prevent the unfavourable tissues from the healing area [38,39] and more lately, growth factors as means to induce the wound healing capacity of the remaining tissue. One of the very early approaches for regeneration of the lost periodontal tissues was mechanical or surgical removal of the diseased tissues [40,41] allowing establishment of health via reduction of inflammation. Histological studies in both human beings and animals exhibited that these approaches do not ensure a predictable outcome of periodontal regeneration and often result in healing with epithelial lining rather than new tissue formation [42,43]. At a later stage the surgical therapy was combined with the placement of bone grafts in the defect, aiming to stimulating periodontal regeneration. A wide range of bone grafting materials has been applied including autografts (such as intra-oral or iliac crest), and commercially available allografts (i.e. freezedried bone), xenografts and alloplasts (i.e. hydroxyapatite, .-tricalcium phosphate) [38,44,45]. These materials were expected to fill the space and to either contain the appropriate cell source, or provide the mechanical scaffold for the surrounding cells to repopulate the healing area. To date, periodontal regeneration is considered to be biologically possible but clinically unpredictable. As earlier suggested, the cell type that repopulates the root surface after the therapy determines the nature of healing. In this respect, PDLSCs may hold a strong promise for improvement of periodontal regeneration approaches. Recent studies have applied populations of PDLSCs in animal models with improved success outcome forperiodontal regeneration [46,47]. Although similar human studies are not yet available, such cell-based therapy approaches may become visible in the future of periodontal regeneration.

Application of Dental Pulp Stem Cell

1- The most striking feature of DPSCs is their ability to regenerate a dentin-pulp-like complex that is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth [3].
2- DPSCs possess stem-cell-like qualities, including self-renewal capability and multi-lineage differentiation. DPSCs were capable of forming ectopic dentin and associated pulp tissue in vivo [48].
3- Stem Cell was isolated from Porcine Dental pulp exhibited Self-Renewal and Multipotency for Dentinogenesis, Chondrogenesis, Adipogenesis, and Neurogenesis [49].
4- Human Dental Pulp Stem Cells Improve Left Ventricular Function, Induce Angiogenesis [50].
5- DPSCs and osteoblasts seeded on Biocoral showed a diffuse bone formation within this scaffold, although it was impossible to establish the exact amount of new bone deposition, because it was disseminated within the internal cavities [51]. In the future, DPSCs could also be used to treat perforated furcations.
6- Alveolar bone regeneration: Defects of at least 1.5 cm in the alveolar ridge of humans were filled with a construct of stem cells collected from third molars and seeded onto a collagen matrix. One year later in many cases, the gap was filled with bone [52].
7- Pulp tissue regeneration involves either delivery of autologous/allogenic stem cells into the root canals or implantation of pulp that is grown in the lab, using stem cells [53].
8- Human immature dental pulp stem cells (hIDPSC) would present similar key characteristics as LSC (Limbal Stem Cell) and whether they could be used for corneal surface reconstruction in a rabbit total limbal stem cells deficiency (TLSCD) model. [54]
9- Human tooth germ progenitor cells (TGPCs), from discarded third molar, commonly called as wisdom teeth TGPCs to diferentiate into hepatocytes and their potential efectiveness in suppressing liver inflammation and preventing liver fibrosis in carbon tetrachloride (CCl 4)-treated rats[55].

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10- Dental pulp is an easily accessible and efficient source of MSCs, with different kinetics and differentiation potentialities from MSCs asisolated from the bone marrow. The rapid proliferative capacity together with the immunoregulatory characteristics of DP-MSCs may prompt future studies aimed at using these cells in the treatment or prevention of T-cell alloreactivity in hematopoietic or solid organ allogeneic transplantation [56].
11- Stem cells from human exfoliated deciduous teeth (SHED) have been identified as a population of postnatal stem cells capable of differentiating into osteogenic and odontogenic cells, adipogenic cells, and neural cells. These stem cell play the important role for treating immune disorders like SLE.[57]
12- Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system a population of neural crest-derived dental pulp cells acquire clear neuronal morphology and protein expression profile in vitro, indicating the presence of a cell population in the dental pulp with neuronal differentiation capacity that might provide additional benefits when grafted into the CNS [58].
13- Adult human DPSCs provide a readily accessible source of exogenous stem/precursor cells that have the potential for use in celltherapeutic paradigms to treat neurological disease [59].
14- PDLSCs- PDLSCs were implemented to treat periodontal lesions [60]
15- (DFSCs) plays a major role in the generation of cementum, periodontal ligament, and alveolar bone [61].

9. Conclusions

Taken together these recent findings clearly indicate that the control of morphogenesis and cyto differentiation is a challenge that necessitates a thorough understanding of the cellular and molecular events involved in development, repair and regeneration of teeth. Although the prospective of tooth tissue engineering is very attractive. Despite the large amount of interest in this field, no clinical trials have been performed for dentine repair and very limited clinical applications are available in periodontal disease treatment. In addition, cell therapies are in their infancy and many issues need to be taking into account. The use of culture expanded cell populations needs to take into account the possibility of genetic and epigenetic instability, which could possibly result in malignant transformation. In the case of tooth engineering, the possibility of autologous cell replacement and the usage of cells naturally occurring in the site of injury may minimize the risk of side effects. In addition, a better understanding of the biology of tooth repair opens the exciting prospect to develop cell free approaches. The utilization of bioactive factors and biomaterials will support and enhance the intrinsic mechanisms of tooth repair. For dentistry, stem cell biology and tissue engineering are of great interest.

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