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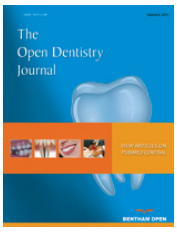

The Promising Applications of Stem Cells in the Oral Region: Literature Review

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Abstract:

Introduction:

For a long time researchers have tried to find out a way to grow tissues back to the human body in order to solve transplantation problems by offering the unique opportunity to have their organs back, working properly, in search of life dignity.

Literature Review:

Stem cells seem to be present in many other tissues than researchers had once thought; and in some specific sites they can be easily collected, without the need of expensive interventions. The oral cavity is one of these regions where their collection can be accomplished, with plenty of accessible sites enriched with these precious cells.

Aim:

The aim of this literature review is to research where in the mouth can scientists find stem cells to be used in the near future.

Key-message:

The aim of this literature review is to research where stem cells can be found and collected in the oral cavity.

Keywords: Bioengineering, cells, mouth, stem cells, transplantation.

INTRODUCTION

The appearance of the term stem cells in the scientific literature is not new and its story comes from nearly one and a half century ago. Their first mention was in 1868, brought about by Ernst Heinrich Philipp August Haeckel who used this expression to describe a fertilized ovule which would evolve into an organism [1]. Later on, in 1924 Alexander Maksimov identifies another kind of precursor mesenchymal cell within the bone marrow which would develop into many cell types [2], these cells promoted a revolution and opened the era of stem cells. However, in the end of the XIX century, the German scientists Hans Spemann and Jacques Loeb began to unlock the secrets of stem cells by using experiments with anfibios embryos, and since then up to now stem cells seem to be the solution of a number of

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problems that mankind has always wished to have solved, such as the replacement of damaged organs and neuron regeneration.

The oral cavity has been shown to have accessible sites available for stem cell collection and cultivation, to be used in many sorts and fields of research. Although stem cell applications for clinical dentists seem too far to come to daily routine, especially because of the financial and biological requirements for keeping these cells alive and in perfect conditions and in viable amount to be inserted in a human being, some steps have already been taken to fulfill this need mainly in the research field, specifically for what concerns Regenerative Endodontic Procedures (REPs) [57]. Therefore, the aim of this research is to accomplish a literature review of the kinds of stem cells in the oral cavity and the sites where they can be collected.

Literature Review

Stem cells can be defined, in terms of function, as cells that can self-renew and are able to be transformed into differentiated cells of many kinds [3, 4]. Self renewal could be described as the ability that a single mother cell has to generate daughter cells identical to it, while producing progeny with more restricted potential.

There are three kinds of stem cells reported in the literature and are divided in three main categories: embryonic stem cells, Induced pluripotent stem cells and adult stem cells. In the last decade there have been a number of stem cells isolated from a great variety of embryonic, fetal and adult tissues. The capacity of pluripotency and differentiation in the cells derived from all the germinal layers *in vitro,in vivo* and *ex vivo* have made the embryonic stem cells the main candidates for tissue regeneration and regenerative medicine in the treatment for Parkinson disease [5], medular lesion [6, 7], cardiac diseases [8] and diabetes [9]. Nevertheless, in spite of all the potential and promises of embryonic stem cells, their usages and applications became problematic all over the world because of ethical matters involving the use of human blastocytes as a biological research material, not to mention other problems such as purification techniques of isolated cells and their manipulation, proliferative and differentiation controls, as well as the possibilities of teratoma formation.

Induced pluripotent stem cells (iPS), produced by science with the aid of reprogrammation technology, which uses the insertion of the genes responsible for pluripotency, have also shown limitations such as low productive efficiency, difficulties concerning isolation and manipulation, and also the possibility of teratoma formation. Therefore, adult stem cells have been regarded as a viable alternative, especially the mesenchymal stem cells; which have shown extraordinary plasticity and possibilities to give rise to different mesodermal cells. Their main characteristic is the unequal cell division which leads to production of a stem cell and a multipotent precursor, which, on the other hand, produces another precursor with a more restricted potential, and so on until the formation of a specialized or differentiated cell type. The use of adult stem cells in the research field of tissue engineering and regenerative medicine is important and has some advantages since their differentiation process is more controlled, and for being more stable when introduced into a living organism, it is unlikely the possibility of tumor formation [10, 11]. Although adult stem cells can be isolated from many parts of the human body; this paper will only describe the cells that can be collected and isolated in the oral region, and the main reason for this is the many sites available and the low expenses for their collection, as well as the possibilities of future uses in odontology and medicine.

Stem Cell Classification

As for plasticity; stem cells can be classified as *totipotent* (able to generate all kinds of cells); *pluripotent* (able to generate almost all kinds of cells – except embryonic membrane cells) and *multipotent* (able to differentiate into more than one kind of mature cell). As for growth stage, they can be classified as *Embryonic stem cells* and *Adult stem cells*. However; as for their source, they can be classified as *autologous stem cells* (cells obtained and used in the same individual); *Allogeneic stem cells* (cells obtained from a donor belonging to the same species) and *Xenogenic stem cells* (cells obtained from a donor belonging to the different species).

Adult Stem Cells in the Oral Region

The dental structures comes mainly from the ectomesenchyme [1, 12]. During the embryonic development, there happens in the oral region a complex interaction between a transitory population of embryonic cells (from the neural crest), underneath the mesenchyme, and the ectoderm, in the process denominated odontogenesis [13]. During the stages of differentiation and morphogenesis within the dental papilla, there is a cell aggregation from the neural crest and the ectoderm. One part of these cells will give rise to the dental germ and the other will remain as a subpopulation

of more immature cells which is more likely to be the precursor of the other resident populations in the pulp tissue. Adult stem cells of autologous origin are considered as promising and practical for regenerative clinical therapies and can be divided as follows: haemetopoietic stem cells and mesenchymal stem cells (MSCs); the latter is subdivided into MSCs of dental origin (DPSC; SHED, SCAP, DFSC) and non-dental origin (BMMSC and Adipose Tissue derived stem cells).

Human Pulp-derived Stem Cells (HPDSCs)

The main types of pulp derived stem cells in humans have common characteristics due to their origins namely: self-renewal, high proliferative activity associated with the ability of forming colony formation units (CFU). Multipotency is also a characteristic which allows HPDSCs to differentiate into osteoblasts, odontoblasts, chondrocytes and myocytes. Immunomodulation and regenerative capacity are also pertinent characteristics. These are applicable for Dental Pulp Stem Cells (DPSCs); Dental Stem cells from Human Exfoliated Deciduous teeth (SHEDs), Stem Cells from Apical Papilla (SCAPs) and Human Supernumerary Tooth-derived Stem Cells (SNTSCs). Their mesenchymal origin makes them multipotent, high proliferative and being able to be cryopreserved, also expressing mesenchymal markers.

Dental Pulp Stem Cells (DPSCs)

The dental pulp stem cells were firstly isolated by Gronthos *et al.* (2000) [14] and belong to the category of MSCs of dental origin. As they were the first kind of stem cells collected and cultivated from the oral region, there are numbers of articles describing their advantages and disadvantages, and the promising possibilities. They can be collected from teeth of adult patients and present *in vitro* high efficiency as for the colony formation and cell proliferation when compared to mesenchymal stem cell from bone marrow. Other studies have shown that these cells do not react to hemotopoietic markers, such as CD14 (monocytes/macrophage), CD45 (pan leukocyte antigen) and CD34 (hematopoietic/progenitor/endothelial), but do express many markers associated to the endothelium (VCAM-1), muscle (α-SM actin), bone (type I collagen, osteonectin) and others, such as CD29 (integrin β1), a protein involved in cellular adhesion and also related to other processes, such as embryogenesis, tissue repair and immune response [15, 16]. Another important characteristic is their potential of odontoblastic differentiation. These cells can be easily induced *in vitro* to differentiate into odontoblastic phenotype, characterized by polarized cells and mineralized nodules [17, 18]. However, the great advantage of DPSCs is their differentiation ability associated with the stability attributed to adult stem cells in general, making them less prone to teratoma formation, along with a high proliferative rate. There is also the simplicity concerning their collection, since dental pulp can be easily removed from many different teeth because of clinical needs, such as orthodontic therapy which many times requires dental extraction. Despite the fact of the open questions concerning how these cells would behave *in vivo* in individual patients, with individual genetic characteristics, DPSCs do seem to have interesting applications in odontology and medicine.

Recently, one study has shown that DPSCs may be induced for osteogenic differentiation simply by the addition of ascorbic acid (Asc), dexametasone (Dexa) and b- glycerophosphate (b-gly) during cultivation, applied in the culture medium, with the use of fetal bovine serum [19]. Nevertheless, DPSCs can also express similarities with neural cells when they are cultivated with lack of fetal serum, giving rise to spheroid structures similar to neurospheres that stain positively to stem marker Nestin, differentiating into fibroblast-like cells or neuroblast-like cells [20, 21].

Pancreatic β-cells which come from the ectoderm share common characteristics with ectodermal neurons and also with neurons from the neural crest [31 - 34]. This data suggest that the pulp tissue may be a promising source of insulin-producing-cells. Because of the fact that DPSCs have been described as being able to differentiate into bone tissue, one study tested this capacity by using different types of three dimenstional scaffolds [22] designed to enhance cell growth, and the results showed that, when compared to Bone Marrow Stem Cells (BMSCs), bone regeneration was significantly inferior. There is also evidence that DPSCs can be used for myocardial infaction [29], cerebral ischemia [23], and corneal regeneration [24].

As for what concerns the paracrine effects of DPSCs, many studies revealed the expression of angiogenic factors such as VEGF (Vascular Endothelial Growth Factor), PDGF (Platelet-Derived Growth Factor), MMP9 (Matrix metallopeptidase 9), IGF-1 (Insuline-like Growth Factor-1) and TGF-β (Transforming Growth Factor- β) [25 - 28]. Therefore, DPSCs seem to have a paracrine pro-angiogenic effect *in vivo* for myocardial infarction. In order to assure this possibility, DPSCs were injected seven days after infarct induction in mice. Four weeks later the animals treated with them showed improvement in the cardiac functions, checked by the reduction of the infarct size [29]. All these data suggest that DPSCs could be an alternative for cardiac tissue repair, as well as for other cardiovascular diseases.

Periodontal Ligament Stem Cells (PDLSCs)

The teeth suffer constant stress because of their shape and function in the oral cavity, which implies in supporting and distributing pressure. The periodontal ligament (PDL) is part of the periodontum apparatus and anchors the teeth to their respective alveolar bone. This intermediary position, between the cementum and the alveolar bone, makes this specific site very populated by many cells types, establishing an intense metabolism. The periodontal ligament is also a source of stem cells designated as Periodontal Ligament Stem Cells (PDLSCs), which share this microenvironment with immunocompetent cells, fibroblasts and epithelial cell rests of Malassez (ERM). The researches concerning the relationship between PDL and stem cells were firstly investigated [30] in 2004, when the possibility that the PDL might contain stem cells that could be used to regenerate the periodontal tissue. Their isolation, from a single colony, showed that they had a capacity for tissue regeneration, by differentiating into cementoblast-like cells and adipocytes-like cells. PDLSCs are usually collected from mature periodontal ligaments and are more similar, as for what concerns cell properties, to mesenchymal stem cells (MSCs) [31], because of the expression of surface markers such as CD105, CD90 and CD73 [32 - 34].

Dental Stem cells from Human Exfoliated Deciduous Teeth (SHED)

Exfoliated deciduous teeth have been described as donors of a specific group of stem cells with interesting potentials, denominated Stem Cells from Human Exfoliated Deciduous Teeth (SHED), which were firstly reported by Miura *et al*, in 2003, and ever since became progressively used in *in vivo* and *in vitro* studies. SHEDs, in general, show characteristics similar to DPSCs; however, their proliferative potential and clonogenic capacity are higher. For the characterization of these cells, the antibodies anti-STRO-1, which potentially defines a subpopulation of progenitor cells, was used along with anti-CD146 (MUC 18), which is a progenitor marker of mesenchymal stem cells (MSCs). Nevertheless, after the isolation and expansion *ex vivo*, only 9% of these cells showed positive reaction to the antibody anti-STRO-1 [35].

Despite the fact that SHEDs and DPSCs are both from pulp origin, SHEDs differ from those found in permanent teeth. One of the reasons is due to their cell division being more quickly and able to generate osseous tissues, while DPSCs seem to be more prone to form tooth-like complexs of dentin and pulp. The fact that SHEDs are more primitive seem to play an important role in their being less committed to forming one specific type of cell than do older post-natal stem cells [35]. Their high proliferative capacity associated with the collection facility, because temporary teeth are easily available, make them attractive for dental researchers, along with the ability to form dentin and other cell types like bone or neural cells, most probably due to the neuroectodermal backgrounds of dental pulps. The possibility to secrete neurotropic factors makes them an alternative for researches involving neuro-degenerative diseases, such as Parkinson´s disease and also researches concerning the recovery of neuron injuries [36].

Human Supernumerary Tooth-derived Stem Cells (SNTSCs)

In 2008, a new population of MSC dental pulp stem cells was identified [37]. They were designated as supranumerary teeth stem cells (SNTSCs). For the fact that supranumerary teeth are not so commonly found, such cells are more difficult to be acquired, and therefore there are many unanswered questions and lack of articles when compared to those of more studied cells like DPSCs and SHEDs. One work, however, evaluated whether SNTSCs had immunomodulatory capacity and whether they offered therapeutic efficacy for immune diseases. They concluded that they offer an accessible and axecutable MSC source for cell-based immune therapies for human autoimmune diseases, especially as for what concerns systemic lupus-erythematosus (SLE) [38].

Stem Cells from the Apical Papilla (SCAP)

In the up to date scientific literature, there is little information concerning the apical papilla; a source of stem cells located in the root apex. However, the region is rich in undifferentiated cell types, and one of the reasons seems to be the fact that during the development of the teeth, the apical proliferation of epithelial cells from the cervical loop stimulates undifferentiated mesenchymal cells to differentiate into odontoblasts. They are named Stem Cells from the Apical Papilla (SCAP) and have been shown to proliferate *in vitro* in higher rates than do DPSCs. Among all the stem cells involved in the root development process, the cells from the apical papilla are the most undifferentiated ones [39]. The stability and low rate of teratoma formation is common to all HPDSCs, and it is particularly true when involves SCAPs. One of their main characteristics is the expression of neuronal markers even when they are in the undifferentiated state [40], making them an attractive alternative for researches involving neuronal regeneration, for

they also secrete growth factors [41]. Cultivating neurons and applying them over damaged nerves, or using them to repopulate vast damaged neural system areas would enhance lives of many people suffering from sicknesses such as Parkinson disease or even those who have suffered traumatic spinal cord injuries. Although bone marrow tissues have been implanted into the spinal cord with outstanding results, dental stem cells show superior neural properties than do the bone marrow´s, especially SCAPs [42], which have also been studied to engineer bio-root models using minipigs, opening the possibility of new parameters for regenerative guided therapy into the search of tissue regeneration [43].

Dental Follicle Stem Cells

The dental follicle can be described as a sac which contains and involves the tooth in development and is considered the odontogenic producing organ, as the changes and cell interactions and differentiation occur inside it, constituting an intense metabolic site in the maxillaries. It is known to be the precursor of many traditional and well described cell types of the periodontum, including their main cells such as alveolar osteoblasts, cementoblasts and fibroblasts, and under this scope, the dental follicle can be considered as a multipotent tissue. When the dental follicle is surgically removed, especially from wisdom teeth, and is seeded in dentin matrix sheets, multiple odontogenesis and osteogenesis genes are quickly expressed [44], and they are usually extracted from impacted third molars [45] mainly because of orthodontic therapy or because of malocclusions worsened by their presence. A study using immunodeficient mice and immortalized dental follicle stem cells transplanted into them showed that a new periodontal-like tissue was formed in a four week period [46]. Under specific conditions and given the appropriate inductions, DFSCs are also able to demonstrate odontogenic, osteogenic and cementogenic differentiation capacity [47].

Gingiva-derived Mesenchimal Stem Cells

A new population of MSCs derived from human gingival has been reported [48], exhibiting clonogenicity, self renewal and multipotent differentiation characteristics. One of its main advantages is the fact that gingival is a tissue easily accessible, with stem-cell like along with immunomodulatory functions, such as peripheral lymphocyte proliferation and the induced expression of immunosuppressive factors like Interleukin-10 (IL-10), inducible nitric oxide (iNO) and cyclooxygenase 2 (COX-2). The authors concluded that GMSCs are capable of suppressing Peripheral Blood Mononuclear cells (PBMC) in the presence of Polyhydroxyalkanoates (PHA) for 72 hours, indicating that direct cell-cell contact contributes in part, to the mechanisms of GMSC-mediated immunosupression by suppressing PBMC proliferation.

Oral Mucosa CTS Cells

The oral mucosa is formed by the oral epithelium and by connective tissue underneath, named lamina propria. A group of adherent highly proliferative stem cells has been isolated from the postnatal lamina propria and are called Oral Mucosa CTS cells [49]. Up to now very little information is available concerning these cells and more studies are necessary to understand their potentials.

Perspectives for Regenerative Procedures

In the last few years researchers have focused on the possibility to use the properties of stem cells to regenerate human body structures [50 - 52]. This *era* of Regenerative Procedures (REPs) would link researches to clinical applications in Medicine as well as in Odontology, and would be possible mainly due to the characteristics of oral stem cells to differentiate into different cell types in culture [53]. There have been several approaches in oral surgery, especially is situations where bone repair is necessary, such as autologous grafts, allogenic and xenogenic grafts, osteoinductive biomaterials and synthetic materials able to regenerate bone [54, 55].

As for what concerns Odontology, Regenerative Endodontic Procedures (REPs) have evolved in the last decade with the prerogative of being able to allow immature teeth to have their root formation completed, simply by giving the infected root canal conditions to have the root canal space free of contamination associated with a new stimulated blood supply. Immature teeth that had blood circulation impeded due to trauma or infections are unable to finish root formation originating teeth with open apexes. The gap between the research world and the clinical one has been narrowed by the clinical applications of stem cell therapy; particularly for Endodontics, whose conventional treatment consists basically in the application of techniques designed to remove the content of a root canal whose tissues may be free of microorganisms or contaminated by them, a task which is many times difficult to accomplish due to the complexity of the root canal system, complemented by the obturation of the root canal with gutapercha. In the case of

immature teeth with open apexes, the root walls are fragile due to the thin thickness of the root canal dentin, making it difficult to accomplish the obturation of the canal, and with the real risk of solid and plastic material overflow into the periapex. The incomplete root development may be caused by trauma or infections powerful enough to halt mineral deposition by the destruction of blood flow, impeding the root to complete its formation [56].

The periapex is a region with plenty of different kinds of traditional cells populating an area along with stem cells correspondent to the varied tissues present therein, such as DPSCs, SHEDs, DFSCs and SCAPs. Stem cell–mediated regeneration and repair of the damaged pulp-dentin complex is the wished final outcome of this therapy, which promote healing of apical periodontitis, continued radiographic root development, and, in some cases, vitality responses [57]. In short, according to the authors [58], REPs appear to promote a guided endodontic repair process rather than a true regeneration of physiological-like tissue.

A study accomplished in 2012 described the treatment of two non-vital central incisors with incomplete rhyzogenesis due to trauma [59]. The 8-year-old male patient showed coronary fracture in tooth 11 and 21. After coronary opening, rubber dam placement and determination of the working length, copious irrigation was firstly accomplished with 2.5% sodium hypochlorite, and then a new irrigation took place by using antibiotic paste diluted in saline solution. The roots were dried with paper points and filled with the antibiotic past, followed by coronary sealing with glass ionomer. After 35 days the teeth were anesthetized, and the canals were accessed and irrigated with sterile saline solution, and a small bleeding was stimulated. After the clot formation, MTA barrier was made and the tooth was sealed with composite resin. Root formation was observed as well as the apex closure.

CONCLUSION

Adult stem cells in the oral cavity can be collected by relatively simple processes with low costs and their stability. Their many clinical potentials make them a promising alternative in the near future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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