# Stem cells from oral niches: a review

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**Summary**
**Stem cells from oral niches: a review.**

**Aim.** ***Stem cell research in recent years have been considered the most advanced sort of medical-scientific research and early results have aroused great expectations. Also in dentistry many studies were performed with the final aim of obtaining new bone and new teeth. In this work we describe the state of the art in dental science stem cell research.***
**Methods.** ***We have performed a web-based research on MEDLINE within (www.pubmed.gov). We have used "stem cells from human exfoliated deciduous teeth" (24 paper found), "periodontal ligament stem cells" (32 paper found), "stem cell apical papilla" (16 paper found), "dental pulp stem cells" (136 paper found) as keywords for research. For each keyword we have performed a complete review focusing on knowledge upgrade.***
**Results.** ***For each topic was created a selection of papers in chronological order of publication date so to give a timetable of the development of the research for each niche.***
**Conclusion.** ***Research about stem cell from oral niches began in 2000 and every year papers publicated were more than the precedent. This review analysed about 180 articles most of which in the last 5 years. Dentla pulp from adult as from deciduous teeth seems to be the most valuable font of stem cells due to the pluripotential type of cells.***

**Key words: stem cells, tissue engineering, osteogenesis, oral niches, review.**

## Introduction

Stem cells are cells with the ability to divide, generating a cell identical to itself plus another one capable of generate different kind of tissues (1). Stem cells can be recognized by potency: totipotent cells can differentiate into any type of progenitor cell; pluripotent cells can differentiate into any type of progenitor cell except those totipotent; multipotent cells can differentiate only into certain kinds of tissues; unipotent cells can differentiate only in one tissue type (Fig. 1). Stem cell research in recent years have been considered the most advanced sort of medical-scientific research and early results have aroused great expectations.
In dentistry the term "bone regeneration" is often improperly used by clinicians, since it doesn't distinguish the difference between the processes of "regeneration" and "repair". The first term shows the complete structural reconstruction of the tissue to rebuild with the same biological capacities and integration with surrounding tissues, while the term "repair " describes the process that is currently achieved with the reconstruction with biomaterials that never reaches complete biological and functional integration with surrounding tissues. It should be instead regarded as a "scar" to connect and fill the gap, with limited biofunctional capacities. Even in dentistry, authors have been carried out numerous studies on stem cells with the ultimate goal of obtaining bone regeneration (e.g. pre-implant) or new teeth. The enormous strides made by molecular biology research have made possible to speculate the creation of *in vitro* oral hard tissues. Currently, literature is divided between clinicians, which tests methods to form single tissues (bone, dentin, pulp, etc.) and biochemists that investigate the biochemical mechanisms that leads to cell differentiation. Therefore, this paper describes the state of the art of stem cell research in dentistry.

## Stem cells

Stem cells can be found in any region of human body, into three-dimensional anatomical regions called "niches" that contain elements that are involved in the proliferative regulation of stem cells, can control the destiny of the "daughter" cells and are concerned to prevent exhaustion and death of stem cells themselves (1,2). If it is easy to demonstrate their presence in tissues that obviously undergo reparation and regeneration as skin, bone and muscle, it is more difficult to think of their presence in other anatomical regions. In fact, only in the 90s stem cells niches were found in human brain (3,4,5). Earliest studies in dentistry were concentrated about finding niches in order to draw multi or unipotent stem cells. Currently, niches have been identified in the dental pulp of permanent teeth (Dental Pulp Stem Cells - DPSCs) (6), in naturally exfoliated deciduous teeth (Stem cells from Human Esfoliated Deci-

cuous teeth - SHED) (7), in the periodontal ligament (Periodontal Ligament Stem Cells - PDLSC) (8), in the apical papilla (Stem Cells from Apical Papilla)(9), in the dental follicle (Dental Follicular PCs) (10), and in the periosteum of the maxillary tuberosity (Oral Periostium Stem Cells) (11). In each of these studies a multipotent mesenchymal stem cell was isolated, capable of differentiating *in vitro* into at least three lines: osteo/odontogenic, adipogenic and neurogenic. For the evaluation of the potentiality of stem cells from oral niches, Bone Marrow Mesenchymal Stem Cells (BMMSCs) was used as a gold standard, as they are the most tested stem cells and the point of reference for each new line of stem cells.

**Dental pulp stem cells**

Dental Pulp Stem Cells (DPSCs) were isolated at first in 2000 by Gronthos et al (6). Cells can differentiate in pulp-like cells. Under appropriate growth factors, DPSCs also differentiated in adipocytes and neural-like cells. Subsequent studies (12,13,14) were made to understand the differentiative capacity of DPSCs, especially compared with BMMSC. Particularly, Gronthos et al in 2002 have established the ability of self-duplication in vivo. The same group in 2003 (15) showed that BMMSC and DPSCs can be traced in perivascular tissue of their respective origin tissue assumpting that this discovery could have implications for the tissue stem cells populations identification in other districts. Even in 2005, Shi et al (16) questioned whether stem cells could be helpful in dental tissue regeneration, raising doubts on the potential of stem cell research, since not much was achieved beyond the mere observation of their presence at five years from first observation. Moreover in 2005 Laino et al (17) showed how DPSCs are still detectable in patients over 30 and that there were no substantial differences with younger cells. In 2005 and 2006 the influence of various molecules in DPSCs differentiation as DMP1 and MEPE was deeply discussed (18, 19). Genetic map of DPSCs and BMSC was compared in order to better understanding of DPSCs differentiation mechanisms (20). A 2006 study compared different types of scaffolds (three-dimensional material) which are necessary to obtain bone tissue (21). The authors have tested a specially prepared collagen matrix based on bovine collagen, a matrix of hydroxyapatite/beta-tricalcium phosphate (HA/TCP) in 60/40 ratio and a web of titanium with different texture spaces between fibers (45 or 20 microns). Results showed bone generation though significantly inferior to the BMSC generated tissue. Of course DPSCs, since their discovery, have been studied from transcription factors, growth factors and molecular matrices that follows stem cell differentiation points of view (20, 22, 23, 24, 25,26, 27). However, international literature seems to have found since 2008 great interest in testing various types of scaffold used for new bone regeneration.
Graziano et al (28) have compared poly-lactide-co-glycoide (PLGA) (as concave substrate), HA 150 micron diameter (as convex substrate), and machined titanium disks (as flat substrate) and showed the best results data from the PLGA sample in terms of osteodifferentiation, including cellular maturation and the profile of specific proteins, resulting in a significant thickness of newly formed bone tissue.

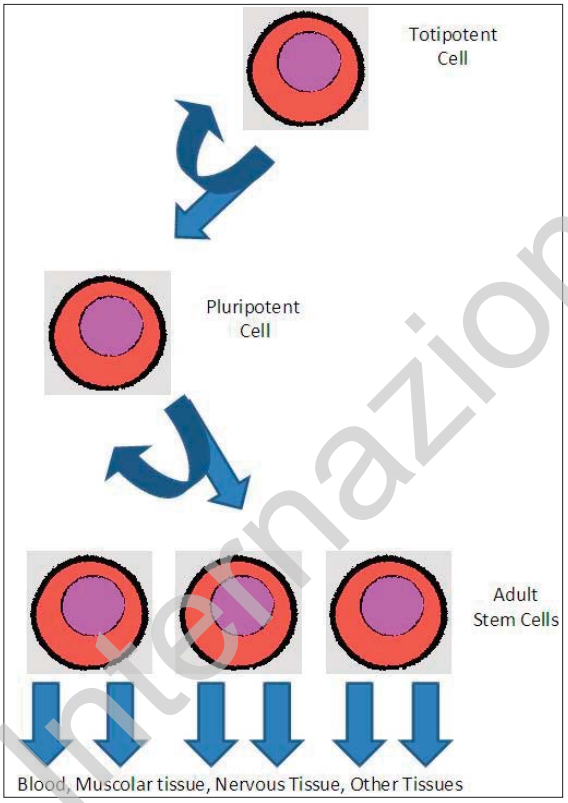


Figure 1 - The figure shows how totipotent cell can divide in totipotent cell and a pluripotent cell and following pluripotent cell can divide in a pluripotent cell and a unipotent cell. Finally, unipotent cell can generate only one kind of tissue.

Besides, PLGA is a well known osteoconductive biomaterial, but mechanisms that underlie its operation are still unknown.
Between 2008 and 2010, two main lines of research have been developed: (i) therapeutic applications of DPSCs and (ii) bone regeneration comparing different types of scaffold added with growth factors (as BMP).
Due to their proved versatility, DPSCs were used for the treatment of myocardial infarction (29), for the regeneration of nervous tissue, particularly in the treatment of muscular dystrophy (31), for the cerebral ischemia (32), exploiting the angiogenetic capabilities, and finally in corneal regeneration (33,34). As regard to scaffold a 2009 paper (35) studied polylactic acid, bovine collagen and calcium phosphate bioceramics. A higher number of survived cells was observed only for the first two materials, despite calcium phosphate bioceramics have proved to be an excellent scaffold for BMMSC. The reason why this type of scaffold did not have the same results with DPSCs as with BMMSC, is unknown. Authors have made assumptions about an inappropriate pH or surface characteristics and they suggest more specific studies. In mid-2009 (36) DPSCs were used with a different type of scaffold, poly (epsilon-caprolactone) (PCL) in association with nanohydroxyapatite (nHA) showing that nHA can promote DPCS differentiation in odontoblasts-like cells. Huang et al (37) in 2010 published a study on DPSCs cultured with low dose glucosamine (0.005 mg/ml) to obtain confirmation of their assumptions of better DPSCs osteodifferentiation.

**Stem cells from human esfoliated deciduous**

In 2003, Miura et al (7) published an article announcing the detection of multipotent stem cells among physiologically exfoliated deciduous teeth pulp cells (SHED). Comparing SHED with BMSCs, they show a higher degree of proliferation and a higher number of population doubling. The cells ex-vivo studies have shown markers STRO-1 and CD146 that have been demonstrated even in BMSSCs and DPSCs. These two markers were located around blood vessels of the pulp, suggesting that SHED may originate from the perivascular microenvironment. To study a cell ability to differentiate into new line rather than into another one author examined the expression of surface markers, and in particular have been found bone markers CBFA1, ALP, MEPE, and bone sialoprotein.
It has also been demonstrated, both *in vitro* and *ex vivo,* SHED capacity to differentiate into odontoblasts-like cells, producing dentin reactive antibody DSPP after growing on a scaffold of HA / TCP. Finally, SHED also showed markers related to neural cells such as nestin, III-tubulin, GAD, NeuN, GFAP, NFM, and the possibility of developing fat cells full of lipids and Oil-red-O+. In this study, SHED have not been shown to be able to differentiate *(in vivo)* in osteoblasts, but to induce the new bone establishment composing a supporting osteoinductive network to murine osteogenic cells. In a 2005 article the same researchers focuses on the higher growth potential of stem cell niches of the oral cavity, compared to those derived from bone marrow (38). In fact, they report CFU-F referred to 10-12 days are 14 for the BMSC, 400 for DPSCs, 200 and 170 for the SHED for PLDSC each $10^5$ cells plated. Even in the cloning experiments, the population doubling of BMSC hardly exceeds 50, while DPSCs, SHED and PDLSC can reach a value of 100, but the mechanism of these differences is not yet known.
A practical application of SHEDs was published on JADA in 2008 (39). Authors tested on 105 premolar with a single root canal the possibility of pulp tissue regeneration by implanting SHED seeded on two different scaffold (open-cell polylactic acid and collagen from cow hide), added with different types of growth factors (BMP2, TGF beta 1, beta-Glycerol-phosphate). They thus demonstrated that the scaffold is essential for the generation of pulp-like tissue, while growth factors did not improve the amount of newly formed tissue. Also in 2008, Seo et al (40) studied the use of SHED in bone regeneration associating them with HA/TCP and obtaining good results in filling critical size defects in mice calvaria. These results are in contrast with those of Miura in 2003 (7) but agree with Koyamain 2009 (41), emphasizing that SHED are capable of osteoblast-like cells differentiatiation, thus having an active role in new bone development and not just a passive role, as stated previously. In 2008, Cordeiro et al (42) studied SHED in order to get more results and better understanding of the mechanisms underlying the formation of the pulp to obtain a tissue architecture and cellularity similar to those of physiologic dental pulp. At well as studies on cellularity, studies on scaffolds were also carried out, as Galler et al in 2008 that used an hydrogel scaffold for SHED and DPSCs proliferation, resulting in resorption of the scaffold and a collagen production by SHED and phenotypically osteoblastic cells from DPSCs (43). Zheng et al in 2009 (44) reconstructed critical-size defects (2,5 x1, 5x1, 5cm) in minipig mandibles using SHED / ß-TCP and obtaining a good bone regeneration at 24 weeks and showing how it is possible to obtain bone regeneration in an animal model of large size. It was also proposed by Arora et al in 2009 to create a SHED bank for future use by the same donors.
In 2009, several reviews have been published (45, 46) . Particularly, it is to quote the work of Nakamura et al who have compared SHED, BMSC and DPSCs (47). He emphasize SHED having a greater proliferative capacity by assigning this characteristics to higher expression of FGF2 and TGF-ß2 and presenting SHED as the best candidate to play a leading role in tissue engineering and regenerative medicine.
In 2010 was published the first clinical application of SHED in the treatment of Parkinson's disease, and preliminary results were very encouraging (48).

**Periodontal ligament stem cell**

Seo et al. in 2004 published *"Investigation of multipotent postnatal stem cells from human Periodontal ligament"* (8) announcing the isolation of stem cells from the periodontal ligament, funding confirmed by Gay et al. in 2007 (49). Despite the identification of this new oral cavity niche, other authors have grappled with the isolation of stem cells from this niche (50, 51, 52) but until 2007 there has been no new addition to the simple cell type isolation.
In the same year Leon et al. (53) and Gay et al.(54) developed various topics adding news to the research on PDLSCs. The work of Leon et al. focuses on a cytokine, interleukin-11 (IL-11), currently used by patients receiving chemotherapy for its ability to stimulate the production of platelets. Authors use the IL-11 following some new scientific findings that demonstrate its osteogenic activity and then adding the drug to the culture of PDLSC resulting in a osteoblast differentiation demonstrated through the production of collagen type 1.
Gay et al. instead developed an interesting study that compares PDLSC and BMSC in terms of the ability of differentiation in osteogenic cultures, emphasizing that the expression of alkaline phosphatase (ALP) can be noted in the first case after 14 days and after 7 days in the second, while the bone sialoprotein (BSP) are visible after 7 days in both cell types. Authors also make condrogenetic adipogenetic crops, noting that the surface antigens can be found in both PDLSC than in BMSC after the same number of days, showing that PDLSC are cells with the same differentiation potential of BMSC.
The following year Lindroos et al (55) with a study focused on surface antigens related to the bone - alkaline phosphatase, Runx2, type I collagen, osteocalcin and osteopontin - stress that dental stem cells shows the same surface antigens of stem cells derived from bone marrow and then are as valid to continue the search for bone regeneration.
In 2008 an article was published that tries to take a step forward in PDLSC research (56) demonstrating the regeneration of the periodontal ligament on pig model and laying the foundation for stem cell therapy of periodontitis. Even Ma and colleagues presented a paper on regeneration of the periodontal ligament demonstrating formation of cement-like cells from PDLSC cells stimulated with Non-collagenous Dentin Proteins (DNCPs) (57).
Thus in 2007 and in 2008 research on PDLSC has found

a new impetus thanks to the fact that these cells were used in the regeneration of the periodontal ligament, a cellular type so difficult to regain once lost. Some authors tried other less traditional ways for PDLSC cultivation in order to have a greater resemblance to the situation *in vivo* using three-dimensional methods, such as pellets on crops,or in three-dimensional rotational systems, the results are controversial (58, 59).

### Stem cells from apical papilla

The isolation of stem cells from apical papilla (Stem Cell from Apical Papilla - SCAP) took place in 2006 (9,60) with the demonstration of many stem cells surface markers as STRO-1, ALP, CD24, and CD29, and established their ability to differentiate into adipocytes and odontoblasts-like cells. A study by the same author in 2008 (61) adds new details, showing SCAP good ability to differentiate in osteo/dentinogenic way (equal to that of DPSCs and confirmed by a similar immunophenotype) but a lower adipogenic potential. Experiments showed that STRO-1 is present together with dentinogenic markers such as osteocalcin, growth factors FGFR1 and TGF RI. In addition, SCAP have demonstrated some of neurogenic markers.

### Oral periostium stem cell

Periosteal origin of stem cells to intraoral are mentioned for the first time in 2006 (62) with a work that compares the properties of osteogenic stem cells of different origin such as bone marrow, alveolar process and, indeed, the periosteum, showing that best results are obtained from periosteum cells with a percentage of mineralization at 12 weeks equal to 58.2 % versus 26.9% of BMSC cell and 41.1% of those in origin from the alveolar process. In 2007 (63) it was confirmed the possibility of growing stem cells from the maxillary tuberosity and jaw periosteum. The research about stem cell from periosteal origin, however, is stopped despite the ease of finding this type of tissue, the good results in the production of new bone, and then in clinical phase of pre-implant GBR.

### Conclusions

Ten years have passed since the early work on stem cells derived from the niches of the oral cavity, but authors still have doubts.
Cells with the best results in terms of potentiality and proliferative capacity differentiation seem to be the SHEDs, but more and more studies must be made to explain the reason of these differences.
As for bone regeneration, the protocols described in the literature are very controversial. It 's still impossible to define the ideal breeding ground for cellular differentiation as well as identify the best scaffold. Many scaffolds were proposed but one of the more tested material as HA / TCP is currently out of market. Also on how scaffold seeding methods there are different and dissenting views.
Almost every author used a method of its own production, making impossible a true meta-analysis of several experimental works, so research and methodologies have still many aspects to be explored in this field.

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