Chapter

Prologue: Oro-Dental-Derived Stromal Cells for Cranio-Maxillo-Facial Tissue Engineering - *Past, Present and Future*

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1. Introduction

Stomal/Stem Cells (SCs) can be classified as either embryonic (ESCs) or adult stem cells (ASCs), depending on origin. Embryonic stem cells (ESCs) can be derived from the inner cell mass of blastocyst stage embryos [1–4] after fertilization. ESCs are potent and have un-limited self-renewal capacity, and can differentiate into cells of all three germinal layers of the organism; mesoderm, endoderm and ectoderm [2, 3]. While their characteristics are extraordinary and attractive for further investigation, the use of human ESCs is limited by an ethical controversy. Hence, ASCs have emerged, with no ethical debate (s) orbiting their study. ASCs can be harvested from many bodily tissues, and do fulfill all the criteria necessary to be considered as SCs, since they are long-lived, have a significant self-renewal capacity, can differentiate toward a set of various cellular types (such as chondrocytes, adipocytes, osteoblasts, among others) and have potential need/use in regenerative and reparative medicine [5, 6].

Mesenchymal Stem Cells (MSCs) are one of the most controversial groups, not because ethical concerns regarding their harvesting, but for the proper utilization of the term. MSCs were first described by Arnold Caplan in 1991, taking their name from the Greek terms "meso" (middle) and "enchyme" (infusion, related to cellular tissue) making a relation with the embryonic mesoderm layer, establishing their capacity to differentiate toward skeletal tissues (cartilage, bone, marrow stroma, connective tissue, etc) as one of their principal characteristics [7]. Despite the amount of evidence of their existence, characteristics and functionality, there is controversy around them with respect to the nuances of the term MSCs, and what specific characteristics do all MSCs share.

To solve this situation, the International Society for Cellular Therapy (ISCT) stated minimum criterions to classify a potential lineage as MSCs. The first criterion is to display plastic-adherence capacity. Second, they must express certain biomarkers, such as the surface membrane protein Thy-1, usually denominated CD90 (Cluster of Differentiation 90), or the membrane gluco-protein Endoglin (also called CD105). Besides from the presence of the already mentioned CD90 and CD105, MSCs must be at least CD73+, CD14-, CD34-, CD44- and HLA-DR-. Finally, should be capable to differentiate to chondrocytes, adipocytes and osteoblasts, to be classified as MSCs [8, 9].

Since the year 2000, several MSC types have been identified and isolated from oral tissues, including the teeth, dental pulp, gingival, periodontal and supporting structures (**Figure 1**) [10–12]. This has provoked cellular biologists and dentists to bridge and strengthen their relation, communication and collaboration even more,

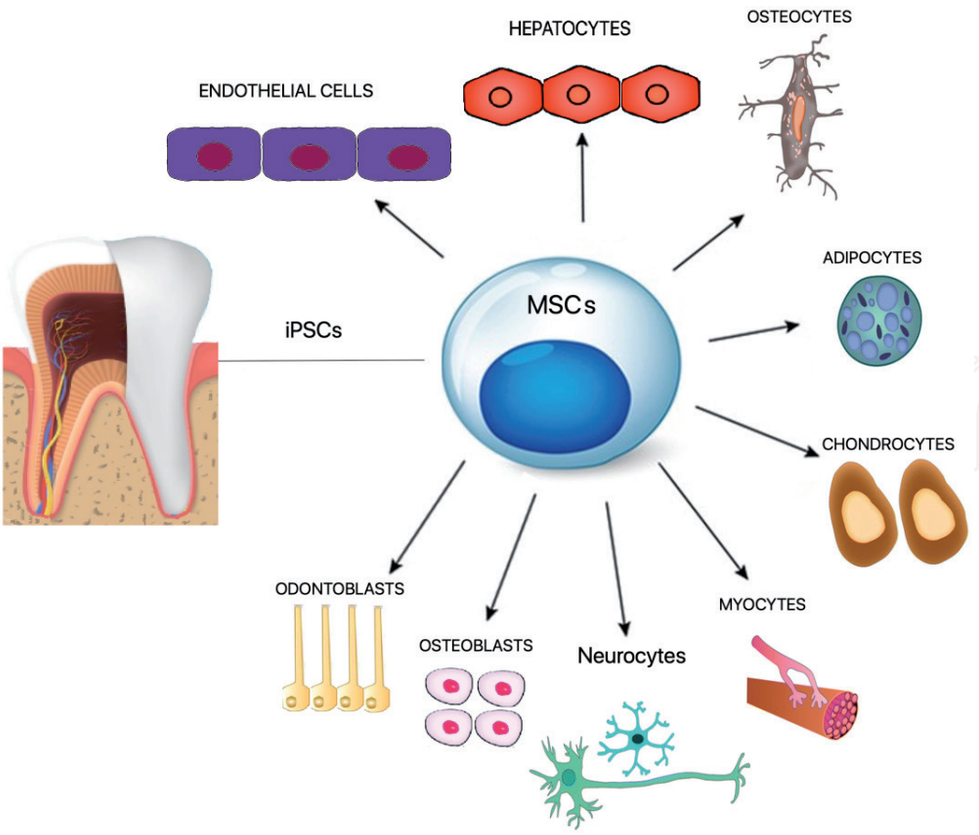

Figure 1.
Dental stem cells and their multi-lineage differentiation capability. Abbreviations; iPSCs: Induced pluripotent stem cells; MSCs: Mesenchymal stem cells.

as a thorough understanding of the cellular mechanisms underlying these oro-dental MSCs must come along or go in parallel with the expected development of their use in different and wide range of therapies and/or therapeutic strategies.

The aim of this introductory chapter is to provide a practically-comprehensive, systematic and updated SC overview (PRISMA flow-chart for the conducted literature e-search is illustrated in **Figure 2**) directed to general dentists, oral and maxillofacial surgeons and head and neck health students and professionals interested in oral cavity-derived MSCs, their reported characteristics and the possible uses/applications in oro-dental tissue engineering, regeneration and repair, and beyond.

1.1 Dental pulp stem cells

Dental Pulp Stem Cells (DPSCs) are one of the most attractive oro-dental derived stem cells, as they are highly clonogenic and rapidly proliferative, exhibit self-renewal, multiple differentiation capabilities, and have the potential for being used on tissue regeneration and immunotherapy [13]. DPSCs can differentiate into osteoblasts, chondrocytes, myocytes, cardiomyocytes, active neurons, Schwann's cells, melanocytes, and hepatocyte-like cells (**Table 1**) [12, 14].

Several studies have shown that DPSCs have immuno-modulatory capabilities, which include T cell-proliferation suppression [15], inhibition of the proliferation of peripheral blood mononuclear cells [16] and induction of T cell apoptosis *in vitro*, ameliorating inflammation-related tissue injuries in mice with colitis [17]. Regarding *in vivo* studies, there has been extensive research using DPSCs to promote tissue regeneration in animal models. A systematic review, (that focused in the use of DPSCs and SHED to repair non-dental tissues) concluded that the use of both of these MSCs on bone tissue repair/regeneration seems to be effective.

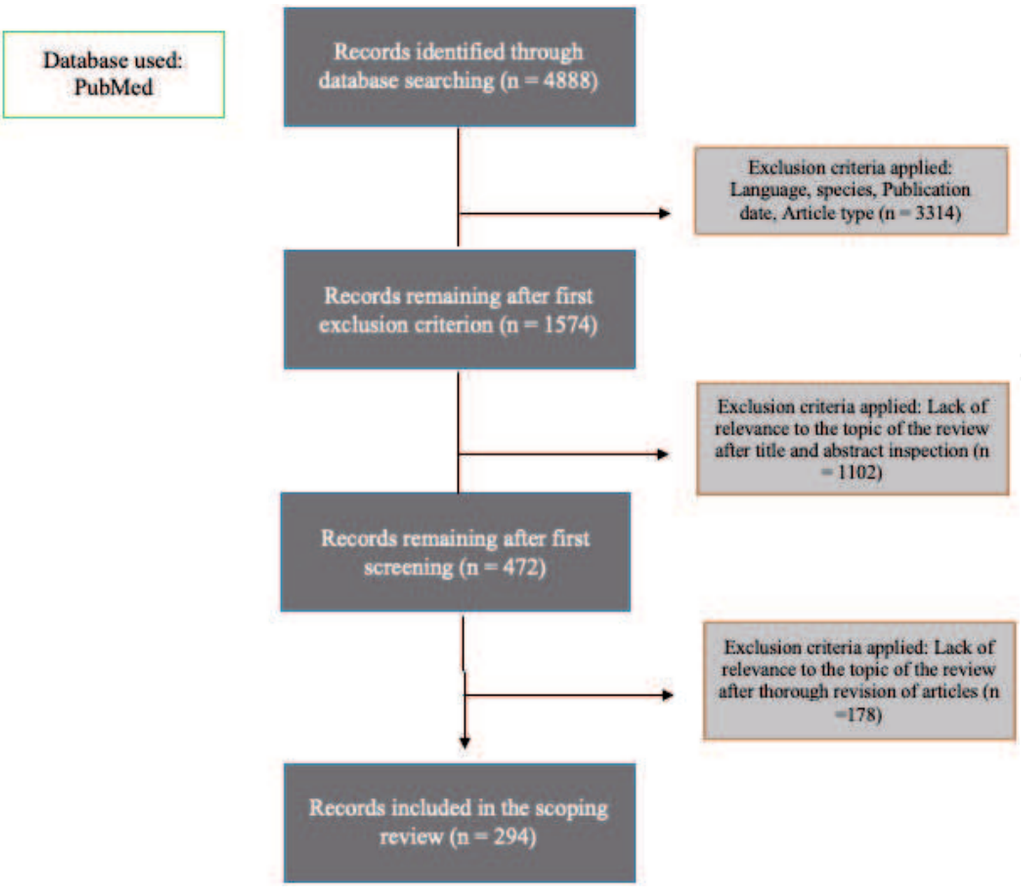

Figure 2.
PRISMA flow diagram for the bibliographic electronic search conducted on PubMed central.

Nevertheless, much more tests are needed to assess whether this effectivity/efficiency extends to the promotion of functional recovery of other types of tissue(s), such as: neuronal tissue, blood vessels, muscle or cartilage [18].

DPSCs have been used in regenerative therapies in clinical trials. Nakashima *et al.* [18] disclosed an experiment performed on five patients, diagnosed with irreversible pulpitis, and no periapical radiolucency in the X-ray analysis. These pulps were isolated and then a fraction of them were separated through the addition of granulocyte colony-stimulating factor (G-CSF). This fraction called mobilized dental pulp stem cells (MDPSCs). These MDPSCs were transplanted with G-CSF in an atelocollagen scaffold into devitalized teeth. Patients were followed up at 1, 2, 4, 12 and 24/28/32 weeks after MDPSCs transplantation. No adverse events or toxicity were detected after clinical and laboratory evaluations. The electric pulp test (EPT) after 4 weeks demonstrated a positive response. The signal intensity of magnetic resonance imaging (MRI) of the regenerated tissue after 24 weeks was similar to those in control group. Finally, cone beam computed tomography demonstrated functional dentin formation in three of the five patients [19]. In another study, DPSCs were isolated from inflammatory dental pulp tissue (thus renaming them as DPSCs-IPs), loaded with a β-tricalcium phosphate scaffold, and engrafted into the periodontal intrabone defect area in the root furcation of two patients, with combined periodontal–endodontic lesions with pocket depth from 5 to 6 mm. Nine months after the surgical procedure, DPSCs-IPs engraftment and regenerative effect was detected on both patients [20]. In a different study, 17 systemically healthy patients (7 assisted to the 1-year follow-up) were subjected to extraction of their third molars for isolation of DPSCs. After this the cells were seeded onto a collagen sponge scaffold, which was then used to fill the injury site left by the

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
DPSCs	Dental pulp, can be harvested from wisdom teeth or other teeth removed from orthodontic reason	CD13, CD29, CD44, CD70, CD73, CD90, CD105, CD146 and CD166	CD14, CD31, CD34, CD35, CD45, CD117, CD133, CD144, CD271 and HLA-DR	Osteoblasts, chondrocytes, myocytes, cardiomyocytes, active neurons, melanocytes, and hepatocyte-like cells.	Transplantation of DPSCs seeded to fibroin scaffolds along with human amniotic fluid stem cells into rats to promote regeneration of critical-size calvarial defects showed bone-regeneration and higher expression of dental markers DPSCs cultured onto human treated dentin and transplanted into the dorsum of mice showed generation of dentin-like tissue and expression of dentin markers DSPP and BMP-1 Dentin/pulp-like structures are generated after transplantation of CD146-positive, CD146-negative and unsorted DPSCs into immunocompromised rats, with a higher degree of generation displayed by the first group.	Dentin formation after transplantation with an atelocollagen matrix and a G-CSF supplementation into a pulpectomized teeth.
						Engraftment and regenerative effect after transplantation of DPSCs isolated from inflammatory dental tissue mixed with a β-tricalcium phosphate matrix.
						Alveolar bone and periodontal tissue reparation after transplantation of autologous DPSCs into the injury site left by the extraction of mandibular third molars.
DFSCs	Dental follicle, ecto-mesenchymal tissue that surrounds the developing tooth germ prior to eruption	CD29, CD44, CD73, CD90, CD105, CD146, HLA-1, NOTCH-1, STRO-1 and Nestin	CD14, CD25, CD28, CD34, and CD45	Osteoblasts, chondrocytes, adipocytes, fibroblasts, cementoblasts and hepatocyte-like cells.	New bone formation when transplanted to a surgically-created cranial bone defect on immunosuppressed rats.	N/A
					Dentin-pulp like tissue and cementum-periodontal ligament complex formed after transplantation of DFSCs co-cultured with treated dental matrix.	
					New bone formation when a DFSCs – Demineralized bone matrix scaffold biocomplex was transplanted into mandibular defects of miniature pigs and subcutaneous tissue of mice.	

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
SHED	Living pulp obtained from the remaining crown of exfoliated deciduous teeth	CD29, CD44, CD73, CD90, CD105, CD146, STRO-1 and Nestin	CD34 and CD271	Osteoblasts, adipocytes, chondroblasts, angiogenic endothelial cells, hepatocyte-like cells and neuron-like cells.	Substantial bone formation after transplantation of SHED-HA/TCP biocomplex in critical-size calvarial defects in mice. Transplantation of SHED seeded in tooth slice/scaffolds differentiated into functional odontoblasts that generated tubular dentin.	An interventional clinical trial using scaffold-free SHED-derived pellets for the revitalization of young immature permanent teeth with necrotic pulps is yet to be published.

Abbreviations: ***DPSCs:*** *Dental Pulp Stem Cells;* ***DFSCs:*** *Dental Follicle Stem Cells;* ***SHED:*** *Stem Cells from Human Exfoliated Deciduous Teeth;* ***N/A:*** *not applicable or determined.*

Table 1.
Principal characteristics of Oro-dental-derived MSCs: DPSCs, DFSCs and SHED.

extraction of the mandibular third molars. The progression of the treatment was evaluated after three months, with a vertical alveolar bone repair and a complete reparation of the periodontal tissue. Furthermore, after histological analysis it was clear that complete regeneration of bone occurred at the injury site, and optimal bone regeneration was evident one year after the grafting [21]. Beyond the evident supporting the use of DPSCs on dental regeneration applications, it has been described that DPSCs could aid in the regeneration of tissues non-related with oro-dental structures, such as corneal epithelium, central nervous system tissues, craniofacial bone, among other examples [22–25].

1.2 Dental follicle stem cells

The dental follicle, has been identified as a source of stem cells and lineage-committed progenitor cells for cementoblasts, odontoblasts and periodontal ligament cells [12]. During cementogenesis, the inner and outer enamel epithelia fuse to form the bi-layered Hertwig's epithelial root sheath (HERS), which induces the differentiation of the dental follicle stem cells (DFSCs) into cementoblasts and odontoblasts [26]. DFSCs are thought to be the origin of the periodontum, (including cementum, periodontal ligament and alveolar bone) (**Table 1**). DFSCs are plastic adherent cells that are positive for the MSCs biomarkers CD29, CD44, CD73, CD90, CD105, CD146, HLA-1, NOTCH-1, STRO-1 and Nestin, while being negative for the hematopoietic markers CD14, CD25, CD28, CD34, and CD45 [27–29]. Yildrim *et al.* [26] proved the capacity of DFSCs to differentiate *in vitro* into osteoblasts, chondrocytes, adipocytes, and other cell types too, such as fibroblasts, cementoblasts and hepatocyte-like cells [27, 30, 31]. Regarding to *in vivo* experimentation, DFSCs have been reported to support new bone formation after the transplantation to a surgically cranial bone defect on immunosuppressed rats [26]. In another study, a treated-dentin matrix (TDM) was obtained from extracted premolars, and used as a scaffold for isolated and cultured DFSCs, from extracted wisdom teeth in humans. An *in vitro* experiment, showed an increase of the expression of DMP-1 and BSP the co-culture with TDM liquid extract in comparison with DFSCs co-cultured groups with HA/TCP liquid extract and DFSCs without co-culture, reflecting an up-regulation of formation and mineralization of dentin. Results have also been seen on a *in vivo* experiment, that showed the implantation of the TDM-DFSCs biocomplex resulted in the formation of dentin-pulp like tissue and cementum-periodontal ligament complex, with the expression of tooth root-related antibodies on the regenerated tissues.

The presence of human mitochondria in a model mouse indicates that the presence of TDM-DFSCs biocomplex could participate in tooth regeneration [32]. Kang *et al.*, [32] transplanted a biocomplex of a demineralized bone matrix scaffold and human DFSCs, both isolated from fresh dental follicle and cryopreserved, into mandibular defects of miniature pigs and subcutaneous tissues of mice. Eight weeks after, the transplanted DFSCs generate bones when it was compared to the original size of the mandibular defects, with a high expression of osteocalcin and VEGF (Vascular endothelial growth factor). Furthermore, a decrease of CD4 expression was measured on the DFSCs-transplanted tissues compared to the control model, this could demonstrate the existence of an immunomodulatory capability [33].

1.3 Stem cells from human exfoliated deciduous teeth

In 2003, a team formed by Dr. Miura's, demonstrate the presence of stem cells population on an isolated deciduous tooth. These stem cells were named stem cells from human exfoliated deciduous teeth (SHED) [34].

As long as it exists the deciduous teeth, as biologically discarded tissues, their collection and isolation are far from ethical concerns around the scientific community. Miura *et al.* [33] discovered that SHED showed a significantly proliferation and number of populations compared to bone marrow mesenchymal stem cells (BMMSCs) and DPSCs.

SHED express biomarkers such as CD29, CD31, CD44, CD73, CD90, CD105, CD146, STRO-1 and Nestin [34–37]. A complete proteomic landscaping was conducted on these oro-dental derived stem cells, with the identification of 2032 proteins, with 1516 of them expressed also on periodontal ligament stem cells (PDLSCs). Furthermore, a more in-depth analysis of the proteomic profile on SHED, showed that they predominantly expressed molecules that are involved in organizing the cytoskeletal network, cellular migration and adhesion. This corresponds with more results presented on the same research, where SHED proved to have a strong migration capacity during wound-healing assays [38].

SHED can differentiate *in vitro* into osteoblasts, adipocytes, chondroblasts, angiogenic endothelial cells, hepatocyte-like cells and neuron-like cells [35, 36], and it has been proven that they can differentiate *in vivo* into adipogenic, chondrogenic and odontogenic lineages in mice models (**Table 1**) [39–41]. A large quantity of research has been conducted about the capacity displayed by SHED to aid on regenerative therapy. Seo *et al.* [41] performed an experiment to elucidate if SHED-mediated bone reparation could be used for therapeutic purposes, generating critical-size calvarial defects in immune-compromised mice and later transplanting a SHED-HA/TCP biocomplex into the defect areas. The defects were repaired after the treatment with bone formation [42]. This kind of research has been conducted in swine deciduous teeth, with similar results, after 6 months of the surgical procedure [43]. In another research, SHED was seeded on tooth slice/scaffolds and implanted into immunodeficient mice subcutaneously. The results showed the capacity of SHED to differentiate into functional odontoblasts, while *in vitro* tests studies, demonstrate, that they can organized into capillary-like sprouts and expressed endothelial markers such as CD31, vascular endothelial cadherin (VE-cadherin) and vascular endothelial growth factor receptor – 2 (VEGFR-2, when are inducted vascular endothelial growth factor (VEGF) [35]. Despite the lack of clinical trials; a recent study showed that the preparation of a biocomplex comprising SHED and a Polyhydroxybutyrate (PHB)/ Chitosan /Nano-bioglass (nBG) scaffold fabricated through electrospinning allowed SHED to differentiate into odontoblast-like cells after the induction with Bone Morphogenetic Protein – 2 (BMP-2), with a 6-fold increase in the expression of DSPP genes and collagen type-1 and a 2-fold expression of alkaline phosphatase (ALP) compared to the control [44]. This type of investigation shows promising results for the use of SHED in oral regenerative therapies, however, more studies are needed in this field [45].

1.4 Periodontal ligament stem cells

The periodontal ligament (PDL), a specialized connective tissue, is developed from dental follicle tissue during tooth formation and is the responsible for the regeneration of the adjacent periodontal structures. Although this regeneration process involves and depends of the recruitment of stem cells that differentiate into fibroblasts, cementoblasts or osteoblasts [46, 47]. Periodontal ligament stem cells (PDLSCs) were first isolated from third molars and since then, they have shown to be able to differentiate into periodontal cells, cementoblasts, adipocytes, collagen-producing cells and retinal ganglion-like cells [48–50]. PDLSCs express biomarkers such as CD13, CD29, CD44, CD73, CD90, CD105, CD166 and Nestin (**Table 2**). The expression of the MSCs biomarker CD146 is disputed, as some sources detected

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
PDLSCs	Periodontal ligament, soft connective tissue between the cementum and the inner wall of the alveolar bone socket	CD13, CD29, CD44, CD73, CD90, CD105, CD166 and Nestin. CD46 expression is disputed	CD14, CD19, CD34, CD45, CD117, CD133, CD144, CD271, STRO-1 and HLA-DR	Periodontal cells, cementoblasts, adipocytes, collagen-producing cells, retinal ganglion-like cells, cells of neurogenic, cardiomyogenic, chondrogenic and osteogenic lineages.	A cementum/PDL-like complex was generated after subcutaneous transplantation into the dorsal surface of mice.	The transplantation of a biocomplex comprising PDLSCs and the bone grafting material CALCITITE 4060–2 into deep intrabony defects generated through the removal of inflammatory periodontal tissues resulted in periodontal tissue regain, along with decrease in probing depth, increase in gingival recession and attachment gain.
					PDL-like tissue with PDLSCs closely associated to alveolar bone was observed 8 weeks after transplantation of a PDLSCs-HA/TCP biocomplex into two periodontal defects surgically created in the buccal cortex of rat's mandibular molar.	
					Osteogenic repair of calvarial defects on rats was detected after implantation of a collagen membrane with PDLSCs and conditioned medium.	
SCAP	Root apical papilla tissue on the exterior of the root foramen area	CD13, CD44, CD73, CD90, CD105, CD146, DSPP, Osteocalcin, Nestin, Neurofilament M, FGFR-1 and TGF-β-RI. STRO-1 expression is disputed	CD34, CD45 and HLA-DR	Odontoblast, adipocytes and hepatocyte-like cells.	A 3-D nerve-like tissue with axon and myelin structures can be obtained culturing SCAP using an integrated bioprocess composed of polyethylene glycol microwell-mediated cell spheroid formation and subsequent dynamic culture in a high aspect ratio vessel bioreactor.	N/A
					Hard tissue of uncertain characteristics is formed after transplantation of a SCAP/HA biocomplex into immuno-compromised mice.	
					A pulp-like tissue with well-established vascularity and a continuous layer of dentin-like tissue were induced after insertion of SCAP in tooth fragments and subsequent transplantation into immuno-deficient mice.	

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
TGPCs	Tooth germ of third molars	CD29, CD31, CD73, CD90, CD105, CD166, VEGFR2, VE-Cadherin, vWF, Cytokeratin-17, Cytokeratin-19 and STRO-1	CD14, CD34, CD45, CD133 and CD144	Osteoblasts, neural cells, adipocytes, chondrocytes and hepatocytes.	New bone matrix formation was observed, along with osteocytes and an active osteoblast lining in on the matrix surface, after transplantation of a TGPCs/HA biocomplex into immunocompromised mice	N/A

Abbreviations: ***TGPCs:*** *Tooth-Germ Progenitor Cells;* ***SCAP:*** *Stem Cells from the Apical Papilla;* ***PDLSCs:*** *Periodontal Ligament Stem Cells;* ***N/A:*** *not applicable or determined.*

Table 2.
Principal characteristics of Oro-dental-derived MSCs: PDLSCs, SCAP and TGPCs.

it, while other sources did not [46, 47]. In the previously mentioned proteomic landscape performed by Taraslia *et al.*, [37] 3235 proteins were identified on PDLSCs, where 1721 were found only in PDLSCs and 1516 were shared with SHED. It is interesting that researchers who performed this proteomic profile found that the recorded proteins found on PDLSCs, are tightly involved in cellular growth, proliferation and in the replication, recombination and repair of the DNA [38]. To explore the regenerative potential of these stem cells, PDLSCs were mixed with HA/TCP ceramic particles, and transplanted into two periodontal defects, that had been surgically created in the buccal cortex in rat's molar. In the first defect a cementum/PDL-like complex characterized by a layer of aligned cementum-like tissue and associated PDL-like tissues was generated. In the second defect, 6–8 weeks after transplantation a PDL-like tissue was observed, with PDLSCs associated with the alveolar bone. This may suggest a potential functional role in periodontal tissue regeneration [12, 48]. A recently study demonstrate an osteogenic capability when the collagen membrane is used in conjunction with human PDLSCs to repair a calvaria defect of a rat [51]. In a retrospective pilot study, three male patients between 25 and 42 years with periodontal disease were selected for transplantation of PDLSCs. The researchers found a decrease in probing depth during post-operatory controls with a follow-up of 72 months [52].

1.5 Stem cells from the apical papilla

In 2006, a research team led by Dr. Wataru Sonoyama found apical papilla tissue on the exterior of the root foramen area, contained a population of stem cells identified them as Stem Cells from the Apical Papilla (SCAP). The team generated single-cells suspension from third molars of 18–20 years old adult. They proved that a transplant of SCAP and PDLSCs could form a root/periodontal complex in a mini-pig model [53]. A Further study revealed, that this tissue contains less cellular and vascular components than dental pulp, although there is a cell-rich zone between the apical papilla and the dental pulp [54]. These cells proliferate faster than DPSCs, and can differentiate into odontoblast, adipocytes and hepatocyte-like cells. They express biomarkers, such as CD13, CD44, CD73, CD90, CD105 and CD146 (MSCs), DSPP and osteocalcin (dentinogenic), Nestin and neurofilament M (neurogenic) or FGFR-1 (Fibroblast growth factor receptor – 1) and TGF-β-RI (Transforming growth factor beta isotype, receptor 1) (**Table 2**).

The presence of the mesenchymal marker STRO-1 is in doubt, sources exposed that it is present in a portion of these stem cells, meanwhile other claims that it is not expressed. SCAP could form hard tissue *in vitro* and *in vivo* after subcutaneous transplantation of a SCAP/Hydroxyapatite biocomplex into immunocompromised mice, although the precise characteristics of this hard tissue could not be determined [55]. Another approach of SCAP on regenerative therapies was conducted by Bellamy *et al.* [55], when a bioactive chitosan-based scaffold with a sustained TGF-β-releasing nanoparticles system was prepared, to evaluate if it would promote migration and enhances differentiation of SCAP. The study showed that the scaffold was releasing TGF-β in a sustained manner thus facilitating delivery of a critical concentration of this molecule at the opportune time, demonstrating properties conducting to cellular activities and a bioactive time of up to 4 weeks. SCAP showed greater viability, migration and biomineralization when it is compared with the control group.

SCAP displays a promising battery of characteristics, that are useful for regenerative engineering purposes, but for stem cell population, there is a substantial lack of clinical studies that needs to be experienced sooner than later [56].

1.6 Tooth-germ progenitor cells

The tooth germ, is an aggregation of progenitor cells that forms a tooth, consisting of the dental papilla, the dental follicle, and the enamel organ [12]. It has been determined that a novel population of stem cells can be isolated from a third molar, called: tooth-germ progenitor cells (TGPCs). These cells have notorious proliferative and a multipotent nature, which could be explained due to the fact that tooth germ of third molars are reported to develop after the age of 6 years, but remain undifferentiated until this time. TGPCs are able to differentiate into osteoblasts, neural cells, adipocytes, chondrocytes and hepatocytes [57, 58]. This capacity, raises interesting possibilities, as it has been proven that these cells can engraft successfully in carbon tetrachloride (CCl4)-treated liver injured rats and help them to restore the liver function after 4 weeks [57]. TGPCs express the MSCs biomarkers CD29, CD73, CD90, CD105, CD166 and STRO-1 (at least in parts of their total population), along with CD31, VEGFR2, VE-Cadherin and vWF (endothelial cell markers) and Cytokeratin-17, Cytokeratin-19, Epithelial cell adhesion molecule and vimentin (epithelial cell markers). TGPCs respond well with different materials scaffolds, (Poly ε-caprolactone, poly L-lactide and a mix of both) (**Table 2**) [59]. TGPCs and TGPCs transfected with Venus (a variant of green fluorescent protein) were implanted with HA into immunocompromised rats. New bone formation in the presence of osteocytes was observed in the newly formed bone matrix and an active osteoblast lining on the matrix surface [57]. As it can be noted, TGPCs display characteristics that make them a suitable option to be used for regenerative therapies, but it is clear that more studies are needed, before their use on patients.

1.7 Gingiva-derived stem cells

The gingiva is an oral tissue overlaying the alveolar ridge and retromolar region that is recognized as a biological barrier and a fundamental component of the oral mucosal immunity [12]. The gingival tissue is constantly subjected to thermal, chemical, mechanical and bacterial aggression. However, it has the capacity to restore itself completely, if the destruction of its collagen network occurs. This unique healing capacity indicates that this tissue should have a significant amount of stem cells [60]. The isolation and characterizations of a subpopulation of gingival fibroblasts that possess stem cells characteristics, called Gingiva-derived Mesenchymal Stem cells or Gingiva-derived Stem cells (GMSCs), was described by many different research groups [60–63]. GMSCs are a multipotent type MSCs, that can differentiate into adipogenic, chondrogenic and osteogenic lines, expressing markers such as CD29, CD44, CD73, CD90 and CD105; (**Table 3**) Subpopulations of these cells can express the markers CD146 and STRO-1, that are widely present by other oro-dental derived MSCs [60, 62–64].

It is important to notice the immuno-modulatory capacity displayed by GMSCs. These cells are capable to: elicit a potent inhibitory effect on T-cell proliferation [12], generate distinct immune tolerance [61, 65], elicit M2 polarization of macrophages through cytokine modulation and an increase of the expression of a mannose receptor [66], and alleviate the sensitization and elicitation of contact hypersensitivity [67], displaying many other immuno-modulatory capabilities as well [12]. There have been different in vitro studies that evaluate the potential of GMSCs related to tissue generation. Zhang and colleagues [60] demonstrated that a GMSCs-HA/TCP biocomplex incubated in osteogenic supplemented with collagen gel, could raise bone-related proteins such as osteocalcin, osteopontin and collagen [61].

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
GMSCs	Gingival tissue overlaying the alveolar ridges and retromolar region	CD29, CD44, CD73, CD90 and CD105. CD146 and STRO-1 are expressed by fractions of their population	CD11b, CD19, CD34, CD45, CD117, CD200, CD271 and HLA-DR	Adipogenic, chondrogenic, neurogenic, entothelial and osteogenic lines.	A GMSCs-HA/TCP biocomplex incubated in osteogenic medium and supplemented with collagen gel raised the levels of bone-related proteins when transplanted subcutaneously into immunocompromised mice, suggesting new bone formation. This can also be observed after subcutaneous transplantation of a GMSCs/collagen gel biomatrix in the dorsal surface of immunocompromised mice.	N/A
					Newly formed bone with well-mineralized trabecular structure and an increase of bone-related proteins expression were found at the inner site of mandibular defects of Sprague Dawley rats after transplantation of a GMSCs/collagen gel biomatrix.	
					New bone formation and an increase of bone-related proteins expression was found in critical-size calvarial defects in rat model after transplantation of a GMSCs/collagen gel biomatrix.	
					Transplantation of a 3-D Poly-(lactide) scaffold enriched with GMSCs and GMSCs-CM on this same model showed a better osteogenic capacity compared to controls, repair of the calvarial defect, and upregulated genes involved in ossification and regulation of ossification.	

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
aBMMSCs	Bone marrow of the alveolar process	CD29, CD44, CD73, CD90, CD105 and CD146. A fraction of the population express STRO-1	CD11b, CD14, CD19, CD34, CD45, CD79α nor HLA-DR	Osteogenic, chondrogenic and adipogenic lines.	New bone formation along with cuboidal osteoblasts lining the surface of its margin was observed after transplantation of aBMMSCs mixed with HA/TCP ceramic powder into mice.	N/A
					Adipocityc fatty marrow support originated from aBMMSCs could also be observed around the site of the transplant.	
					Substantial new bone and osteocyte formation was found after transplantation of aBMMSCs mixed with matrixes with different ratio of HA/TCP into immunocompromised mice.	
					Newly formed cellular mixed fiber cementum, woven/lamellar bone and periodontal ligament was observed after transplantation of a biocomplex comprising aBMMSCs seeded into a chitosan/anorganic bovine bone (C/ABB) scaffold into beagle dogs.	

Abbreviations: aBMMSCs: *Alveolar Bone Marrow Mesenchymal Stem Cells;* ***GMSCs:*** *Gingiva-derived Mesenchymal Stem Cells;* ***N/A:*** *not applicable or determined.*

Table 3.
Principal characteristics of Oro-dental-derived MSCs: GMSCs and aBMMSCs.

GMSCs can generate tissues from neural crest cells, during embryonic development [65]. This data is supported on a study in which GMSCs were injected (subcutaneously) in four different immunodeficient Rag2 mice. Results showed no signs of tumors, into different organs after 6 months [64]. In another investigation, GMSCs were mixed with collagen gel matrix and transplanted (subcutaneously), into the dorsal surface of immunocompromised mice model. The results showed higher levels of osteopontin and collagen when it was compared with the control group, (supporting the findings of Zhang and colleagues in 2009). Also, this investigation proved a newly bone formation with mineralized trabecular tissue, after performing the transplantation of GMSCs, but this time into mandibular rat defects [68].

Another interesting capacity of GMSCs in regenerative therapies, is related to the use of the secretome (SM) in their cultures It has been proven, that implantation of a three-dimensional Poly- (lactide) scaffold enriched with GMSCs-SM on rat calvaria defect model, showed a better osteogenic capacity compared to group controls [69]. Although further studies are needed to prove that GMSCs can be useful in pre-clinical and clinical trials too, it is clear that these MSCs have great potential in orofacial tissue engineering, regeneration and repair.

1.8 Alveolar bone marrow mesenchymal stem cells

Bone Marrow Mesenchymal Stem Cells, are the predominant MSCs used in clinical studies for craniofacial tissue regeneration, with a high osteogenic ability [5]. Clinical trials regarding in the use of BMMSCs obtained from iliac bone, have shown promissory results, other evidence suggests that for craniofacial regeneration, it may be a better option to use craniofacial tissues as a cell source [70].

It has been shown that contrary to what happens with iliac bone marrow extraction, alveolar bone marrow MSCs (aBMMSCs) can be easily obtained from alveolar bone, during a dental surgery as: wisdom tooth extraction, crown lengthening surgery, and other examples. 0.5 cc of bone marrow are needed to predictably isolate these cells [71, 72], emerging as an interesting alternative in the craniofacial field.

BMMSCs and aBMMSCs have no significant differences on their osteogenic potential, as measured mRNA levels of osteocalcin, osteopontin, and bone sialoprotein are similar in both populations, but there are differences on their chondrogenic and adipogenic potentials. aBMMSCs are reported to differentiate with more difficulties toward these lines than BMMSCs [73]. aBMMSCs express the markers CD29, CD44, CD73, CD90, CD105 and CD146 while do not express the markers CD11b, CD14, CD19, CD34, CD45, CD79α nor HLA-DR, consistently showing the classic MSCs marker pattern (**Table 3**). A fraction of the cell population (approximately 3%) also expresses the marker STRO-1, a trend that can also be observed in other oro-dental derived MSCs [70, 74–76].

Several *in vitro* and *in vivo* studies have been performed with these stem cells. aBMMSCs mixed with HA/TCP ceramic powder were transplanted into immunodeficient mice. 8 weeks after transplantation a significant new bone formation could be observed around the material, and cuboidal osteoblasts were seen lining the surface of the margin of formed bone. In a different experience, aBMMSCs were transplanted into the left and right dorsal surfaces of 24 immunocompromised mice. The experimental groups consisted in: aBMMSCs mixed with a 60%HA/40%TCP (MBCP) matrix, aBMMSCs mixed with a 20% HA/80% TCP (MBCP plus) matrix and aBMMSCs mixed with deproteinized sterilized bovine bone (Bio-Oss). Substantial new bone and osteocyte formation as noted in the first two groups, while in the third case the new bone formation was poor. Similarly, positive immunostaining for alkaline-phosphatase, RUNX-2, osteocalcin and osteopontin was evidenced in the first two groups but only limited expression was

found on the third group. It is important to notice that although Bio-Oss gave rise to the lower quantity of new bone, it also was associated with low osteoclasts formation, while both MBCP and MBCP plus induced a higher formation of these cells, so Bio-Oss cannot be discarded as a potential material for bone regeneration [77].

The potential of this stem cells in the periodontal regeneration field, was explored by seeding them into a chitosan/anorganic bovine bone (C/ABB) scaffold, and transplanting this biocomplex into six male beagle dogs with one-wall critical size periodontal defects. The experimental group that had the C/ABB seeded with aBMMSCs exhibited newly formed cellular mixed cementum, woven/lamellar bone and periodontal ligament in higher levels than the different controls used in the experiment [71].

1.9 Salivary gland stem cells

The use of salivary gland stem cells (SGSCs) in regenerative therapy is widely inclined toward the restoration of the function of salivary glands. When their function is impaired and saliva production decreases, current therapies are limited to bring secretagogues and artificial saliva, to restore salivary gland function.

The search of stem cells that can restore the lost function, has come to the exploration of salivary gland stem cells (SGSCs) as a novel resource [78, 79]. SGSCs express the markers CD24, CD29, CD34, CD44, CD49f, CD73, CD81, CD90, CD105, CD133, CD146, CD166, STRO-1, Nestin and aldehyde dehydrogenase (ALDH) (**Table 4**). There is a controversy if they express CD117 marker or not, as it was reported that SGSCs that were isolated from minor salivary glands do not express this marker [78, 80, 81]. These cells are capable of differentiate toward the classic MSCs lines; osteogenic, adipogenic and chondrogenic, and into amylase-expressing cells [82].

A study demonstrates the ability of SGSCs to restore the function of a damage salivary gland in irradiated rats. After one day, SGSCs were transplanted to each gland through intra-glandular injection, measuring their body weight and saliva flow rate during 60 days. The body weight of the rats decreased the first week, but after that, consistently increased until day 60, with significant difference respecting to the control group. Furthermore, the acinar and duct structures were evaluated, along with the composition of their mucosubstances. The parenchyma of the salivary glands of un-treated rats were intact, as well the parenchyma of the damaged-rats that were treated with SGSCs, while the parenchyma of the radiated but un-treated group showed vacuoles and a disrupted acinar structure. One week after the treatment, apoptotic cells could be observed in the un-treated damaged-rats, while in the treated group they were not present [82]. In another study, with the same protocol but differences irradiated dose and transplantation time, the results showed that SGSCs can restore homeostasis of salivary glands by a combination of engraftment, proliferation, differentiation and potential stimulation of recipient cells [80].

It is apparent that SGSCs are a potential useful solution for patients with xerostomia, thus requiring further studies to prove if these promissory results are replicated in pre-clinical or clinical interventions.

1.10 Periosteum-derived mesenchymal stem cells

The periosteum is in direct contact with the bone surface and contains a mixed cell population that includes fibroblasts, osteoblasts, pericytes and a subpopulation of MSCs known as Periosteum-derived stem cells (PDSCs) [83]. PDSCs (**Table 4**) express the biomarkers CD9, CD13, CD29, CD49e, CD44, CD54, CD73, CD 90, CD105, CD166 and HLA-ABC, while not expressing the markers CD14, CD31, CD33, CD34, CD38,

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
SGSCs	Major, parotid, submandibular, sublingual and minor salivary glands	CD24, CD29, CD34, CD44, CD49f, CD73, CD81, CD90, CD105, CD133, CD146, CD166, STRO-1, Nestin and ALDH. CD117 expression is disputed	CD45 and CD271	Osteogenic, adipogenic, chondrogenic, and amylase-expressing cells.	In several different studies it has been shown that the transplantation of SGSCs toward previously irradiated salivary glands restore their function (measured by saliva flow rate), and promote a decrease of their degeneration by a combination of engraftment, proliferation, differentiation and potential stimulation of recipient cells. This suggests that they have potential to differentiate toward cell lines that conform the acinar and/or ductal structures of salivary glands.	N/A
PDSCs	Inner cell-rich cambium layer of the outer lining of long bones. Can be found on the upper vestibule, lower vestibule or hard palate	CD9, CD13, CD29, CD49e, CD44, CD54, CD73, CD 90, CD105, CD166 and HLA-ABC	CD14, CD31, CD33, CD34, CD38, CD45, CD106, CD117, CD133 and HLA-DR	Osteogenic, adipogenic and chondrogenic lines.	iPSCs (induced pluripotent stem cells) derived from PDSCs and un-differentiated PDSCs were transplanted under the kidney capsules of diabetic mice. Hyperglycemia and glucose tolerance improved, and human insulin was detected on their serum and kidney sections for both groups. These results suggest that these stem cells could engraft, proliferate and differentiate when subjected to these conditions. Autologous PDSCs in a Collagen I/Collagen II matrix were transplanted to Beagle dogs. Bone fill within the limits of implant threads and bone-implant contact was observed after transplantation. These results were not statistically similar that the ones yielded with the transplantation of BMMSCs using the same methodology.	N/A

Name	Niche	Biomarkers expressed	Biomarkers not expressed	Differentiation *in vitro*	Tissue Formation *in vivo*	Clinical Trials *human*
OESCs	Basal layer of the oral mucosal epithelium	CD29, CD44, CD73 and CD90. Subsets of their population are positives for CD105, CD146 and STRO-1	CD34 and CD45	Osteogenic, chondrogenic, adipogenic and neurogenic lines.	in vivo differentiation potential toward corneal tissues has been thoroughly researched. Cultivated oral mucosal epithelial cell sheets (COMECS) have shown that OESCs can differentiate into stratified epithelial cells upon transplantation into rabbit corneal surface using this methodology, being well-attached to the host corneal stroma and able to survive up to two weeks after transplantation.	The integrity of the ocular surface of eyes with total bilateral limbal stem cell deficiency was restored for at least 2 years after transplantation of OESCs cultured with human amniotic membrane as biological substrate.
IPAPCs	Inflamed peri-apical tissues after endodontic infection	CD73, CD90 and CD105	CD45	Adipogenic and osteogenic lines.	Mineralized tissue was observed 8 weeks after transplantation of a IPAPCs-HA/TCP biocomplex into mice.	N/A
*LESCs**	Second and third layer of the epithelium cell layer at the base of the inter-papillary pit	Bmi-1	N/A	Keratinized epithelial cells.	N/A	N/A

Abbreviations: ***SGSCs:*** *Salivary Gland Stem Cells;* ***PDSCs:*** *Periosteum-derived Mesenchymal Stem Cells;* ***OESCs:*** *Oral Epithelial Stem Cells;* ***IPAPCs:*** *Inflamed Periapical Progenitory Cells;* ***LESCs:*** *Lingual Epithelial Stem Cells;* ***N/A:*** *not applicable or determined;* * *status as MSCs not yet conclusive.*

Table 4.
Principal characteristics of Oro-dental-derived MSCs: SGSCs, PDSCs, OESCs, IPAPCs and LESCs.

CD45, CD106, CD117, CD133 and HLA-DR. PDSCs can differentiate toward to: osteogenic, adipogenic and chondrogenic line [84]. It has been proven that PDSCs express clonogenical and proliferative activity independent of the age and the donor site [85].

In 2015, a three-dimensional culture system designed for mass production of PDSCs. The cells formed spheres by spontaneous aggregation, thus passing from a two-dimensional culture to a three-dimensional system. The spheres retained their viability and proliferation ability, even when the culture was scaled-up to 125 mL Erlenmeyer flasks. These results open up the possibility of developing a secure, fast and economic method to achieve high MSCs biomass for future clinical applications [86]. In another study, PDSCs were isolated, and then subjected to a three-step differentiation process to become: Induced Pluripotent Stem Cells (iPSCs; pluripotent stem cells generated artificially via genetic manipulation of somatic cells). Insulin release by iPSCs was confirmed in the immunocytochemical analysis. Hyperglycaemia and glucose tolerance of these mice were improved and human insulin was detected on their serum and kidney sections. Transplantation of undifferentiated PDSCs also improved blood glucose levels and increased serum human insulin levels [87]. In another interesting study PDSCs and BMMSCs were harvested from seven Beagle dogs. After this, the animals were subjected to teeth extraction, and after three months, implants were mixed with a Collagen I/Collagen II sponge as a scaffold and transplanted to a bone dehiscence created for this purpose. Both stem cells populations showed osteogenic potential *in vitro* evidenced by mineral nodule formation and expression of bone markers, and after transplantation both had similar bone fill within the limits of implant threads and bone-implant contact. There was no significant difference between both MSCs, thus presenting a similar potential for bone reconstruction [88].

1.11 Oral epithelial stem cells

The oral mucosal epithelium (OME) is a stratified tissue that posse tight junction proteins in its supra-basal layer and hemidesmosomes in its basal layer. These characteristics, which are similar to the characteristics of corneal epithelium, define OME as a potential source of material for cross-therapies in the reparation of damaged corneal surfaces [89]. Furthermore, the cultivated oral mucosal epithelial transplantation (COMET), a technique that uses OME to repair other tissues, has been used to repair intraoral mucosal defects and esophageal mucosa during endoscopic mucosal resection procedures, thus suggesting a wide variety of potential applications [90, 91]. The potential for regenerative therapies displayed by OME is related to the presence of a cell population with stemness potential, called Oral epithelial stem cells (OESCs), located in the basal layer of the OME [92].

OESCs are undifferentiated cells, ultra-structurally and biochemically, that retain a high capacity of long-term self-renewal and proliferative potential, that response to injury and certain growth stimuli [93]. These stem cells are capable to differentiate toward osteogenic, chondrogenic, adipogenic and neurogenic lines [94]. Regarding their surface markers, there are positive for CD105, CD146 and STRO-1, while they are consistently positive for CD29, CD44, CD73 and CD90 and negative for CD34 and CD45 (**Table 4**) [95]. Furthermore, the p75-positive subset of the population displayed higher *in vitro* proliferative capacity and clonal growth potential [96].

The use of COMECS for ocular transplantation has given good results, as this tissue has showed to be able to survive two weeks after being attached to rabbit corneal surfaces, being well-attached to the host corneal stroma, and involving differentiated stratified epithelial cells [95]. Nonetheless, alternative treatments

that do not rely on cell sheets have also been studied. A clinical grade fibrin gel for the culture of OESCs was prepared utilizing fibrinogen and thrombin that served as base for the culture of OESCs previously harvested from oral mucosa. Tranexamic acid was used to prevent the digestion of the fibrin gel during the culture. A clinical trial was conducted to prove if cultivated oral mucosa epithelium expanded, without depending of cell sheets, could achieve improvement in two patients with histologically confirmed bilateral total limbal stem cell deficiency (LSCD). The use of OESCs for the regeneration of other tissues should be explored further, but these stem cells definitely have the potential to cause an impact regarding regeneration of damaged tissues.

1.12 Inflamed periapical progenitory cells and lingual epithelial stem cells

Inflammation of the periapical progenitory cells (IPAPCs), are MSCs that can be found in inflamed periapical tissues. It was explored that these cells can differentiate into adipogenic and osteogenic lines. Also, they proved that after mixing an IPAPCs culture with an HA/TCP matrix resulted in the appearance of mineralized tissue [97]. Regarding their surface marker expression, IPAPCs are positive for the classical MSCs markers CD73, CD90 and CD105, but only 66.3% of their population co-expressed the three of them at the same time (**Table 4**). Nevertheless, IPAPCs are consistently negative for CD45 [98]. Even though IPAPCs are difficult to harvest than the previously described MSCs, because they inhabit exclusively on inflamed periapical tissue, while the majority of the aforementioned oro-dental derived MSCs can be harvested from healthy patients, more studies should be conducted to definitely assess their characteristics and potential use on regenerative therapies.

The surface of the tongue is covered with stratified squamous cell layers, and the lingual epithelium is continuously replaced throughout the life of mammals, which suggest the presence of stem cells [99].

Lingual epithelial stem cells (LESCs), are located in the basal layer of the lingual epithelium. A population of cells that were harvested from the second and third layer of the epithelium cell layer at the base of the interpapillary pit, and are positive for the marker Bmi-1 (B cell-specific Moloney murine leukemia virus integration site 1), that has a role in cell cycle, self-renewal and maintenance of hemopoietic and neural stem cells [100].

The Bmi-1 stem cells, replace keratinized epithelial cells but no the taste bud cells. Studies have shown the presence of two types of stem cells (slow-cycling, long term stem cells and rapidly proliferating, short term stem cells), with different role in tissue maintenance and regeneration. There still unidentified the specific biomarkers for short term stem cells, this put in doubt if the mechanism of maintenance exists [100].

2. Combinatorial technology: nano-based stem cell therapy

For example, when dental pulpitis is diagnosed, the indicated treatment is to remove the pulp by an endodontic treatment. One of the most promising/emerging treatment approach is to regenerate this lost tissue. A study tried to replicate vital pulp tissue, with seeding SC from the dental tissue, with promising result. It is yet to be developed, an ideal cell-seeding system, due to the nature of the root and root canal system [101].

There is a need of regenerative therapies, capable to recover the function of the lost tissue due to diseases or trauma. To be a possible option, three elements are needed: cells, scaffolds / extracellular matrix and growing factors.

Tissue engineering it is bringing significant changes in clinical results. Nanomaterials were first explored/used in 2002, for dental reconstruction. The income of new membranes in the guided tissue regeneration was the beginning of techniques considering three principal elements: SC, scaffolds and molecules signals. The combination of stem cells with nano-structured materials and scaffolds is a promising research area [102, 103].

2.1 Growth factors

These proteins generate a stimulus that induce cell growth (regulate, proliferation and migration) and the receptors binding in cell membrane. In tissue engineering the most proteins used includes: Hedgehog proteins (HHS), morphogenetic proteins (BMPs), interleukins, fibroblast growth factor (FGF) vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF) [104].

BPM have been studied and extensively applied for dental regeneration. These proteins can divide into four families (BMP-2/BMP-4, BMP-3/BMP-3B, BMP-5/BMP-6/BMP-7/BMP-8 and GDF-5/GDF-6/GDF-7), that shows an important function in the differentiation of dental biological cells (odontoblast and ameloblast) [104].

2.2 Scaffolds

Scaffolds are biomaterials that provide an optimal environment to cells allowing to: migrate, proliferate and differentiate. Biocompatibility is the main characteristic (among others as: mechanical strength, surface, pore size, biodegradable), that prevents cytotoxicity and inflammatory reactions, allowing an optimal regenerative functioning of the biomaterial. Materials with these properties are challenge in the tissue engineering field (**Figure 3**) [102–105].

Hydrogels are highly hydrophilic (due to the presence of carboxyl, amide, amino, hydroxyl groups) polymers commonly used as scaffolds, with the ability to support cell proliferation, migration and differentiation, letting a correct transport if oxygen and nutrient (**Figure 3**). Their preparation depends on the designed and the application, including physical, chemical, irradiation crosslink and free radical polymerization. In addition, chitosan and alginate-based hydrogels, demonstrate desirable biocompatibility [105, 106].

A common approach to developed a tissue, involves isolation of tissue-specific cells, from the patient and harvested in vitro. Cells are expanded and seeded into a scaffold of the targeted tissue then, the cell-loaded scaffold is transplanted into the patient, by a direct injection or trough implantation of the fabricated tissue at the desired site [106].

Porosity (total surface of the structure for incoming cells) is the key to provide space for cell migrate and vascularization of the tissue. The minimum pore size required is 100 μm, due to the cell size, transport and migration conditions. The degradation rate is intimately related to the porosity, high porosity can reduce the accumulation of acidic products.

These scaffolds can mimic the extracellular matrices, providing an integral structure and giving an optimal guidance to cellular organization. A successful approach to improve the cellular attachment, is the combination of a peptide sequence; Arginine-glycine-aspartic acid (RGD). This incorporation has shown a better binding between cells-RGD hydrogel scaffolds on different cells (fibroblast, osteoblast, muscle cells) [107]. Naturally polymer materials (collagen, fibrin, glycosaminoglycans, chitosan, alginates, starch, agarose, silk fibroin), are biocompatible, low cytotoxicity and inflammatory response. Collagen is a widely natural polymers, that provide mechanical support to the connective tissue [103].

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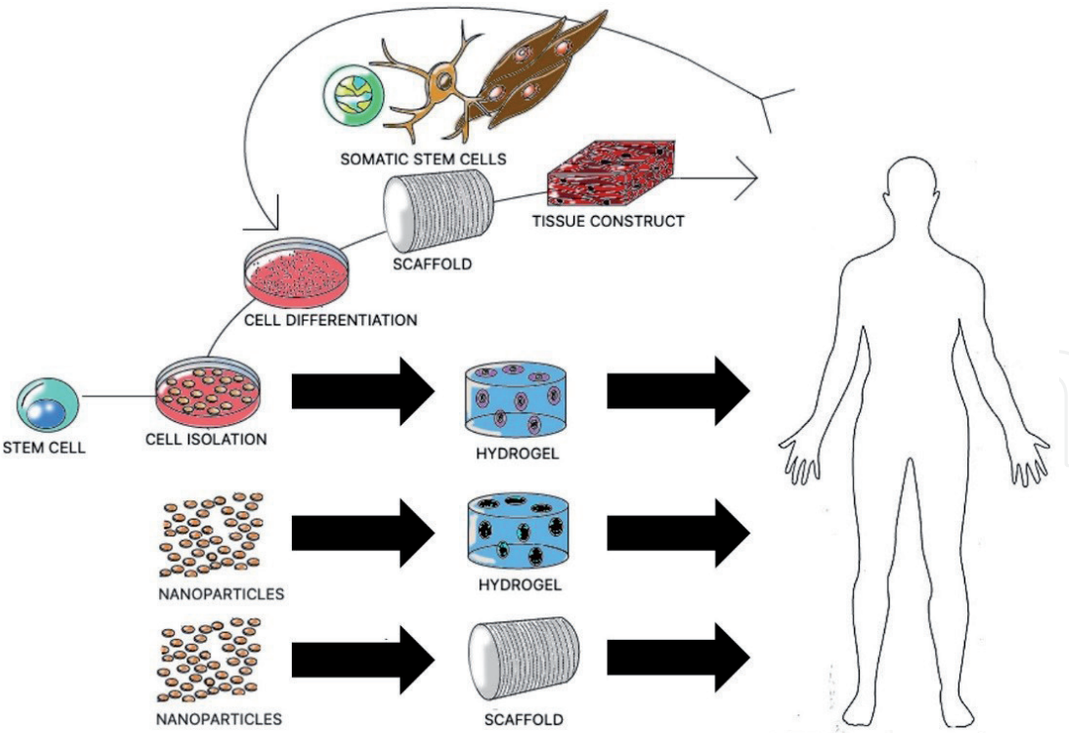

Figure 3.
Dental stem cells and their potential clinical applications in regenerative medicine.

Synthetic Polymer (poly-lactic acid, poly-glycolic acid, copolymers, poly-e-caprolacton, polyurethanes, poly-ortho ester, poly-anhydrides), are used because their high versatility, properties and reproducibility. A disadvantage of this polymers, is the less biocompatibility and not bioactive. The most used scaffold is poly-glycolic & poly-lactic acid [104].

Different techniques to fabricate scaffolds have been used to produce random structures with different pore size and reduced pore interconnections that facilitate cell support, adhesion, proliferation and differentiation. Among these techniques, we can find: solvent casting, phase inversion, freeze drying, melt-based technology, fiber bonding and high pressure-based. Lately, electrospinning has been studied for engineering applications [108].

3. Nanotechnology

The nanotechnology is the use of atoms, molecules or supra molecules structures for diverse purpose. Nanomaterials are made up of units of a size between 1 and 100 nm. Nanoparticles with sizes from 10 to 1000 nm, can provide high control of scaffolds properties, such as mechanical strength, improve biodegradability, corrosion rates, and a control of release bioactive agents [102], additionally, low solubility, short circulation life of bioactive molecules (growing factors and cytokines) [103–105].

The preparation methods, are based in the rupture of the material block (top-down), or the fabrication through the addition of components (atoms or molecules).

The objective of encapsulate the substance, is to preserve the function, and the possibility to have a controlled rate, of the substance.

Nanoparticles include nanospheres and nanocapsules, which vary in their morphology and architecture. Nanospheres are composed by a polymeric matrix, with the ability of a substance could join to its surface or disperses on it. On the other hand, nanocapsules are vesicular structures, made of synthetic or natural

polymeric membrane, with the drug inside [101–103]. These substances are released by diffusion or degradation of the polymer. Nanoparticles, can be created from different types of materials (ceramic, metals, polymers).

3.1 Metallic nanoparticles

3.1.1 Gold nanoparticles

Using as a drug delivery, and in different size (20-50 nm), these metallic nanoparticles have antifungal action, in candida species, performing an interaction with cell membrane, that leads to the lysis of the cell. Golden nanoparticles offer antibacterial action over, gram-positive and gram-negative, due to the alteration in the biological process (ribosome for t-RNA binding) [109]. The increase surface to volume ratio, enhance the antibacterial and antifungal properties Additionally it changes the membrane potential and disrupt the ATP synthase, decreasing the metabolism of the microorganism.

In tissue engineering, has promising results, in new bone formation, when is used in a photocurable and biodegradable gelatin hydrogel. Also, can enhance osteogenic differentiation of stem cells, when is used coated over TiO2 surfaces and even as an injectable hydrogel scaffold. The objective of adding these nanoparticles, is to increase scaffolds structures and to guide cell behavior.

Gold nanoparticles, also can be applied for enhance MSCs properties. Used with VEGF, enhance cell migration on the scaffold of FN-Au nanoparticles.

3.1.2 Silver nanoparticles

Described as a colloid, this widely used metal, has antimicrobials properties. These particles can be formed by chemical or physical process. The physical methods used are, evaporation-condensation process, laser ablation of metallic bulk material, gamma irradiation or ultrasonic irradiation. The chemical methods consist in the use of sodium borohydrine or polyol as reducing agents. The size of the nanoparticles, could be controlled with the use of surfactants, this also depends on the obtention method and the reducing agents [105].

Silver ions, have been used for their antimicrobial effects, due to the possibility to block the respiratory system, and precipitate bacterial cellular protein. Depends on the size of the particles (1 to 10 nm), could have different potential against gram-negatives [110].

The particles also have been studied in tissue engineering. These particles have been incorporated into scaffolds, with diverse materials (gelatin, chitosan-alginate and cellulose acetate) with promising results on the antimicrobial activity with accelerated healing, in diabetic wound treatments. This result could be better with the use of a hydrogel, producing a contraction of the injury, due to the water content. In the same field, silver nanoparticles, have been employed to create chitin/nanosilver antimicrobial composite scaffold. This results in a better blood clotting, due to the effect of the silver to affect the coagulation path, by denaturing the anticoagulant proteins [111]. It is important to mention that in a porous chitosan-alginate, with biosynthesized silver particles, shown cytotoxic results in breast cancer cells [112].

3.2 Ceramic nanoparticles

Inorganics combinations, also metals, metal sulfides and oxides, used in the fabrication of materials with different porosity, shapes and forms. These nanoparticles, are classified as inert, bioactive or resorbable ceramics [103].

3.2.1 Bioactive nanoceramics

Bioglasses are materials, formed from different elements (sodium, silicone, magnesium, oxygen), that can be absorbed by the cells. These are promising materials due to the possibility to control con stimulate new tissue formation. There are different techniques to generate nanoscale bioactive glasses as: laser spinning, sol–gel (most common), micro-emulsion or gas-phase synthesis [113]. These nanoceramics, can bring a faster ion release, when compared with the bulk bioactive glasses, due to their better specific surface area, boosting the bioactivity and the protein adsorption. Bioactive glass nanoparticles, have interesting antibacterial and angiogenic properties. The use of boron in a cellulose construct, can bring promising results on dental tissue regeneration, that could increase cellular viability [114].

Bioactive glass nanoparticles, are also attractive for bone tissue engineering. In vivo experiments have exhibited new bone formation, in chitosan-gelatin hydrogels with 5% bioactive glass nanoparticles, after 8 weeks, compared to control groups.

3.2.2 Bioinert nanoceramics

As their positive interaction on the body tissue, bioinert nanoceramics, are used for different medical applications. Titanium dioxide, are commonly used, because of their exceptional biocompatibility (due to the oxide layer formed, on the surface). Their mechanical stability, corrosion resistance, high biocompatibility, are considered as highly recommended materials for biomedical applications. As one of the most utilized materials. These nanoparticles can be synthesized by hydrothermal, solvothermal, sol–gel and emulsion precipitation methods [115]. Nowadays, these nanostructured materials have been useful as bone scaffolds, bringing acceleration to the rate of apatite construction and increase the osteoblast adhesion, proliferation and differentiation [113]. The roughness of the dental implant, enhanced the adhesion and proliferation of osteoblast [114].

3.3 Polymeric nanoparticles

Compounds, containing predominantly carbon, hydrogen, oxygen and nitrogen in a monomeric chemical structure. With a size of 40–400 nm, and produced from synthetically polymers as poly- D,L.lactic-co-glycolic acid (PLGA) and from biopolymers (chitosan, alginate), these nanoparticles are attractive for drug delivery [115]. Using PLGA, has become interesting due to the biodegradation, biocompatibility, formulation techniques, on dental and periodontal treatments. The use of PLGA nanoparticles include effect on the oral biofilm, disrupting the plaque structure and direct impact on antibiotic resistance [116].

Classification of these nanoparticles, could be based on diverse measures such as: structure, structure and manufacturing method. Design, dimension, peripheral chemistry, porosity, mechanical strength, solubility, degradation rate, can be modified for dentistry purpose. Polymeric nanoparticles can be created, in different forms as: nanospheres, polymersomes, polymeric micelles, nanogels or even nanocapsules. For preparing these nanoparticles there are, diverse systems of emulsifications, supercritical fluid, nanoprecipitation, self-assembly [115].

Nowadays to enhanced the delivery of bioactive agents, nanoparticles changed from a simple delivery to multifunctional responsive systems, even the possibility to has a controlled release.

3.4 Iron oxide, zirconia and silver

The superparamagnetic iron oxide nanoparticles, with a controllable size and nontoxicity, are used as antimicrobial agent. With the use of an external magnetic field, these nanoparticles can be guided to the local infection [117].

Nano zirconia-alumina materials, are new implants materials, that avoid the biofilm formation. Also, they can be used as polish substance [118].

Silver nanoparticles have interesting properties as biocompatibility, low toxicity, low bacterial resistance, and antimicrobial. These particles can infiltrate and disrupts the bacteria wall and interact with de DNA. The tooth discoloration is a disadvantage to consider, thinking about esthetics treatments [108–118].

A collagen scaffold/silver nanoparticles/BMP-2 composite, presented antimicrobial activity and no adverse effects over adherence and proliferation of BMMSCs after preparation [108]. On other study, an injectable chitosan-based thermosensible hydrogel scaffold loaded with BMP-2-plasmid DNA-charged nanoparticles yielded good results on bony defects regeneration, in rat and dog models [106].

Nanostructured biomaterials have a modifiable nature, as they can be personalized by engineering their structure, shape, size, and surface properties in order to be applied in precise anatomical sites, allowing them to be used on many different contexts. Nanoparticles can be used to overcome some of the limitations of scaffold materials in bone regeneration, such as insufficient mechanical strength, issues related to cell growth and differentiation [108].

Nanotechnology has come, to revolutionize the biomedical field, with a reformed on the traditional approaches on tissue engineering and regenerative medicine. Using different types of nanoparticles (ceramics, metals, synthetic or natural polymeric), for various applications, including: bioactive agent delivery, tissue targeting and imaging, modulated scaffolds [118].

3.5 Nanotechnology and dental caries

The key to prevent dental caries is the remineralization of the enamel surface and to avoid the production of acid substance from the dental plaque. Unfortunately, saliva function, makes preventive products (toothpaste, mouthwash, fluor varnish), decrease their effectiveness due to the salivary flow rate (unstimulated: 0.3–0.4 mL/min, stimulated: 1–3 mL/min in adults) [119]. The creation of nanoparticles to enhance the fluoride concentration on dental surface, would allow increase effectiveness in the prevention of dental caries. The hydroxyapatite nanoceramic particles, have a promising future. A structure comparable to tooth and bone structure, biocompatible, and a stable form of phosphate salts, have been applied as bone substitutes. These particles, can join to dental tube and even seal them, reducing the sensibility [120, 121]. Various techniques have been established for the fabrication of hydroxyapatite nanoparticles with particular control over the nanostructure [120].

3.6 Nanotechnology and periodontal diseases

The loss of the surrounded structures of the tooth is a consequence of a disequilibrium of the oral microbiota, triggering a periodontal disease. The treatment objective consists in localized therapeutic substance. The inefficiency to reach an adequate penetration (periodontal pocket) and the inadequate period of contact of the substance, are points to consider for an alternative treatment [29–48].

Numerous nanoparticles into dental composites and adhesives, (zinc oxide, and silver), are used to impede the bacterial progression. Due to the large

surface-to volume ratio, they are effective in the lysis of the bacterial membrane. Also, they are efficient obstructing the sugar metabolism, producing reactive oxygen species [122].

Tetracycline nanoparticles in calcium sulfate, are used in periodontal treatment as a matrix, in which drugs are dissolved. Because of their size, these particles could enter more deeper, in the infectivity pocket. Polymersomes are amphiphilic vesicles, that could enclose a substance. This vehicle, is used has a vehicle for antimicrobials (metronidazole), as an alternative to periodontal treatments [119].

3.7 Innovation in nano-based stem cell therapeutic systems

Stem cells with nanocarriers, has increasing as an interesting field, with favorable results. These mesenchymal stem cells could act as a reservoir for delivering nanoparticles [123, 124].

Innovative nanostructured materials could be useful in manipulating stem cells. DPSCs and Human umbilical vein endothelial cells (HUVECs) combined with an injectable, self-assembling peptide hydrogel scaffold exhibited vascularized pulp-like tissue with patches of osteodentin after transplantation [108]. The use of carbon nanomaterial's, such as carbon nanotubes, carbon nanohorns and/or graphene on bone tissue engineering, shows that these biocompatible materials can promote new bone formation and could even make possible the enhancement of their biocompatibility or induce new characteristics [118]. These examples test the capability of nanomaterials to offer versatile and relatively simple solutions to classical tissue engineering problems.

3.8 Perspective

There are promising results with the use of nanoparticles in tissue engineering, regeneration and repair; enhancing mechanical and biological characteristics, and/or even involving an anti-microbial effect. Though, there are still challenges ahead, to develop and advance in the wide medical and dental fields. Risk assessments (including data requirements and testing strategies) would be useful for better knowledge accumulation and technology advancement. While some nanomaterials might enjoy potential applications in the engineering area, additional research is needed to establish their therapeutic efficacy and safety [52].

Currently there is an emerging field, based on the dental delivery systems that could bring promising results in the therapeutic dental diseases. But there is insufficient knowledge, to let the expansion for such technologies in dental field [24–125].

New challenges will continue to emerge after more research is conducted regarding the possibilities of mixing nanotechnology with oral MSCs, but the promising results already obtained, and the extraordinary potential of both of them, mark this union as an important and interesting target for more investigation, research and innovation.

4. Conclusions

Tissue engineering is a promising and rapidly-evolving field in the clinical dental practice. Stem cells demonstrated the capability to produce dental tissues, bringing solutions to the healthcare services. Ongoing research efforts continue to establish their therapeutic efficacy and replication. Nanomaterials are essential, for the proper or "ïdeal" innovation, development and introduction (clinical translation) of novel, safe, non-invasive and effective therapies, in oro-dental tissue repair and beyond.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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