BRAZILIAN JOURNAL OF IMPLANTOLOGY AND HEALTH SCIENCES

ISSN 2674-8169

ORAL CAVITY MESENCHYMAL STEM CELLS: ORIGIN, SURFACE MARKERS, AND REGENERATIVE POTENTIAL

Marcos Fernando Xisto Braga Cavalcanti (1,2), Ícaro Gabriel Teles Pacheco De Matos (2,3), Ala Gustavo De Andrade Costa (2), Gianny Dominicci Batista Brito(2), Francesca Diomede, Durvanei Augusto Maria

https://doi.org/10.36557/2674-8169.2025v7n5p436-477
Artigo recebido em 30 de Março e publicado em 10 de Maio de 2025

ORIGINAL ARTICLE

ABSTRACT

Objective:

This review investigated the origin, surface markers, and the regenerative potential of mesenchymal stem cells from the oral cavity.

Background:

This review addresses the regenerative potential of mesenchymal stem cells (MSCs) from the oral cavity, including the formation of dental tissues, pulp tissue, and periodontal tissues, and a whole bio-hybrid tooth composed of dentin, cementum, periodontal ligament, and alveolar bone. Teeth sequelae can be caused by cavities, trauma, or infection, leading to endodontic problems, periodontal disease, and tooth loss.

Method:

This work selected 67 articles revising the literature using PubMed and Google Scholar, these stem cells were separated according to their origin, surface markers, and regenerative potential.

Conclusion:

Although a lot of research and clinical trials have to be done to validate clinical use of this knowledge in humans, this field is vast and promising, bringing light to new therapies that can solve clinical problems and reformulate dental treatments shortly.

Keywords: “Periodontitis WHO”; “Stem cells”; “Oral cavity stem cells”; “Periodontal Stem cells”; “Dental stem cells”; “Stem cell Surface Markers”; “ISCT Stem cells criteria”; “Stem cells morphology”; “Stem cells Scaffolds”; “Exosomes”; “hDFSCs”; “hDPSCs”; “SHEDS;

"SCAPs"; "hPDLSCs"; "hGMSCs".

Instituição afiliada – 1- PhD, Professor of Children and Adult Dental Clinics at Nove de Julho University, Postdoctoral position at the Development and Innovation Laboratory of Instituto Butantan.

2- Graduate students of Nove de Julho University.

3- Scientific position at the Development and Innovation Laboratory of Instituto Butantan.

4- PhD, Researcher of Università degli Studi, at Pescara-Chieti.

5- PhD, Head Professor of the Development and Innovation Laboratory of Instituto Butantan.

Autor correspondente*:* Prof. Dr. Marcos Fernando Xisto Braga Cavalcanti – mxistocavalcanti@gmail.com

INTRODUCTION

Teeth and periodontal tissues suffer from aging effects throughout life. Teeth can present cavities and other pathologies that start affecting dental enamel and then affect dentin. When this infection reaches the dental pulp, it develops an inflammation process that leads to endodontic pathologies, like pulpitis and necrosis. Dentin and enamel have been satisfactorily restored by composite, glass ionomer, and ceramic crowns. Dental pulp has been treated successfully by removing the inflamed or necrotic tissue and filling the root canals with a thermoplastic material. There is a new alternative, successfully for 60 percent of the cases, a new pulp tissue repair therapy, that, after cleaning and remodelling the root channels, they are filled with blood from the periapical region (revascularization process), when the tooth is ready to be restored using dental composite. This treatment is applied mostly to immature root apex teeth, but teeth with complete apices can also benefit from this therapy. Periodontitis is a common disease that affects the periodontal tissues of 45% to 50% of adults in its mildest form, and impacts 9 to 11% of the world's population in its most severe form. The etiopathogenesis of these pathologies consists of an accumulation of biofilm, which triggers an inflammatory response over dental tissues. In the tooth-supporting tissues, this response can happen in the presence of systemic diseases, increasing the irreversible destruction of the periodontal ligament and alveolar bone. Therefore, patients' risk factor management and systemic disease control can affect treatment. Periodontal treatments include coronal scaling, root planing, and surgical interventions such as flap surgery and guided tissue regeneration. Despite the huge evolution that dental treatments faced in this century, there is no effective repair after horizontal bone loss in periodontitis, but damage control. The use of mesenchymal stem cells (MSCs) from the oral cavity and their extracellular vesicles has been studied as an alternative for the repair of dental pulp, periodontal ligament, and alveolar bone tissues, showing promising results for repairing these damaged tissues in several degenerative diseases, both in animal models and in human clinical trials [01, 02, 03, 04, 05].

OBJECTIVE

This review investigated the origin, surface markers, and the regenerative potential of mesenchymal stem cells from the oral cavity.

LITERATURE REVIEW

STEM CELLS

Stem cells have two main characteristics: self-renewal, that is, the capacity for unlimited cell division, and the capacity for differentiation into other cell types, mainly into the three embryonic types (osteoblasts, adipocytes, and chondroblasts) [06].

There are four types of cells in terms of their differentiation potential: Totipotent, which can give rise to a new individual, including placental cells; Pluripotent, with the capacity to differentiate into any cell type, but without being able to give rise to a new individual; Multipotent, which can be differentiated into cells from the three tissue layers: endodermal, ectodermal and mesodermal; and unipotent cells, which can develop into just one cell type, such as somatic cells [06].

The potential of MSCs is linked to tissue regeneration. The injection of stem cells, which can differentiate into odontoblasts and fibroblasts, into the root canals can improve pulp regeneration. This new pulp formation is not identical to the dental pulp, since the dental pulp is made up of loose connective tissue, and the pulp resulting from this treatment is made up of dense connective tissue that may present spicules of bone and dentin inside [07].

In the oral cavity, stem cells can contribute to periodontal regeneration through their ability to undergo osteogenic differentiation. The alveolar bone plays a key role in supporting and anchoring teeth, and periodontal disease often results in bone loss, tooth mobility, and tooth loss. Advances in studies of stem cell differentiation into osteoblasts can contribute to the success of periodontal regenerative therapies [05].

CELL MARKERS

Regenerative cell therapy using stem cells has sparked interest in several areas. The International Society for Cellular Therapy (ISCT) has established criteria for standardizing and defining the identity of MSCs. First, under standard culture conditions in culture flasks, MSCs must adhere to plastic and differentiate into osteoblasts, adipocytes, and chondroblasts under standard in vitro differentiation conditions. (Dominici et al., 2006); (Kadkhoda et al., 2016). The MSCs population must express CD73, CD105, and CD90, as well as the Stromal Cell Surface Marker (STRO-1), and be negative for CD45, CD34, CD14, or CD11b, CD79a, or CD19 and HLA class II [08] (Figure 01).

Where is reference 9?

CD73 is a marker for lymphocyte differentiation that, in association with CD29, modulates (reduces) MSCs' migratory capacity. CD105 is a type I membrane glycoprotein, a component of the transforming growth factor receptor (TGF-ÿR) complex. CD90 is a marker that plays an important role in cell-cell and cell-matrix adhesion, which is important for tissue engineering. STRO-1 identifies the cell surface antigen produced by stromal cell types [10].

Stem cells from the oral cavity may present markers in addition to thess, which indicate specific regeneration potential and are called emerging markers, such as CD10 and CD92, found on osteogenic cells, CD13, a myeloid marker, and CD29, marker of late immunity, CD271, that identifies MSCs before culture, and stage-specific embryonic antigen 4 (SSEA-4), which is related to cancer progression [10, 11, 12, 13]. (Figure 01).

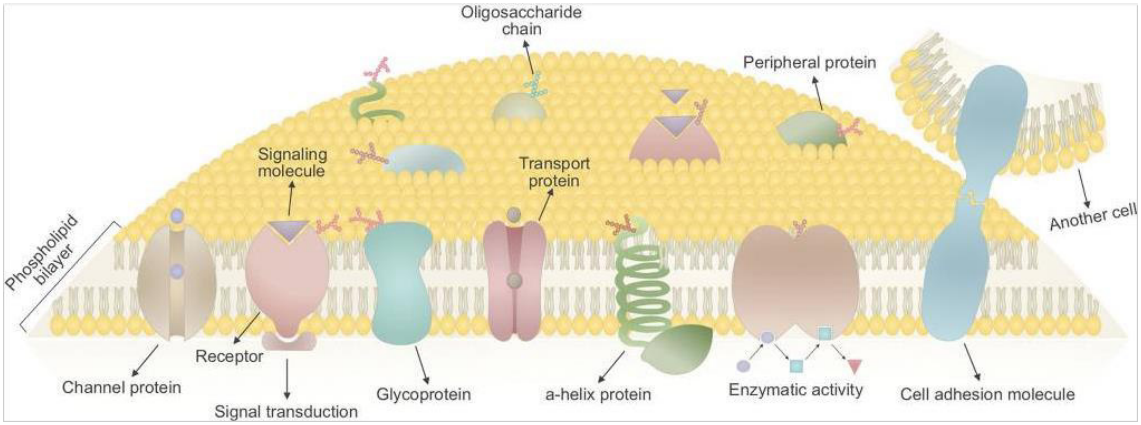


Figure 01: Clusters of cell markers. The plasma membrane is a lipid bilayer in which several proteins are associated. These proteins can perform functions, such as forming channels and transporters, acting as receptors, mediating cell adhesion, functioning as enzymes, and participating in signaling processes. https://www.ncbi.nlm.nih.gov/core/lw/2.0/html/tileshop_pmc/tileshop_pmc_inline.html?title=Click%20on%20image%20to%20zoom&p=PMC3&id=11073434_18_2020_3602_Fig1_HTML.jpg

MSCs present great proliferative capacity, and cell confluence can lead to differentiation after several passages, with changes in cell morphology (e.g., fibroblastic when flat and elongated) and cell behavior (e.g., secretory). These cells present “in vitro” a typical spindle-shaped fibroblast morphology[06, 08, 15, 16, 17] (Figure 02)

Where is reference 14?.

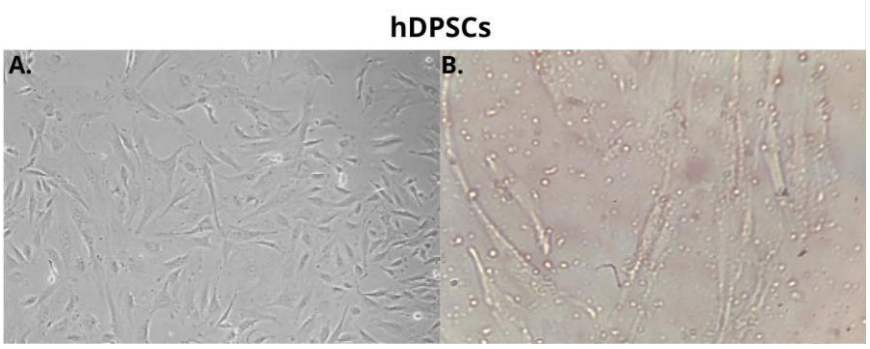


Figure 02: A comparison between the morphology of DPSCs cultivated by (A) Lee et al., 2019, after 3 days and (B) at the Development and Innovation Laboratory of Instituto Butantan after 7 days. https://pmc.ncbi.nlm.nih.gov/articles/PMC6834129/figure/ijms-20-05015-f001/

SCAFFOLDS

Scaffolds are structures colonized by cells to restore living tissues lost after trauma or infection. They can be made from natural materials such as collagens, chitosan, treated dentin matrix (TDM), and bovine pericardial membrane, which are used for soft tissue and muscle repair, in oral surgery, traumatology, implantology, and periodontology[18, 19].

In addition to these, there are biodegradable synthetic scaffolds such as polyglycolic acid (PGL), polylactic acid (PLA), and hydrogels such as Pura Matrix (PM). These structures offer regenerative and cell proliferation potential.

The criteria that define a suitable scaffold are: mechanical properties to resist to tissue pressures, surface properties to allow cells adherence and colonization, proliferation and formation of ECM, microarchitecture with the ideal size porosity to create new capillaries, providing blood supply to the repair, biocompatibility (host's immune response to the implant), biodegradability (the resorption needs to be compatible with the tissue regenerative potential), low cost and easy handling[07, 18].

Scaffolds can be used by mesenchymal stem cells, once attempt to specific details that should be provided to the biomaterial after implantation such as cell density, nutrients and oxygen diffusion inside the biomaterial (size of the repair), recent technologies, such as 3D bioprinting, allow the creation of personalized, high-precision grafts, which when combined with scaffolds promote promising results in the healing of complex tissues, such as the gingiva[18, 19, 20, 21].

DENTAL STEM CELLS

Since the isolation of human dental pulp stem cells (hDPSCs) in 2000, as an alternative to the ethical and legal obstacles of using human embryonic stem cells (hESCs), various types of stem cells have been acquired from different dental tissues. To understand the regenerative potential of these cells, it is necessary to recall their origin in the oral cavity. During the bud stage of the tooth formation, the odontogenic mesenchyme gives rise to two distinct cell lineages: **Dental papilla cells (hSCAPs)**, which give rise to pulp tissue, odontoblasts and are related to the apical papilla stem cells (SCAPs), the dental pulp of permanent teeth and the dental pulp of exfoliated deciduous teeth (SHEDs); and **dental follicle cells (DFSCs)** that form the covering layers of the tooth germ, rising cementoblasts, periodontal tissue cells, and are related to periodontal ligament stem cells (PDLSCs) and gingival mesenchymal stem cells (GMSCs) [22, 23, 24] (Figure 03,04).

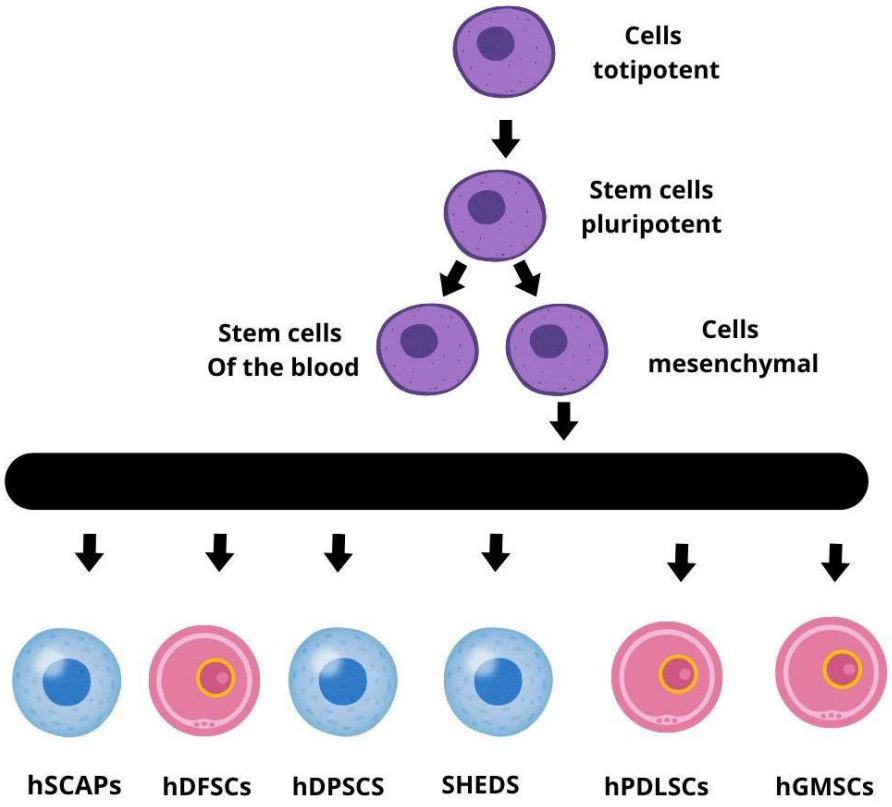


Figure 03 - Representative diagram of cell differentiation from totipotent to multipotent. Source: Author.

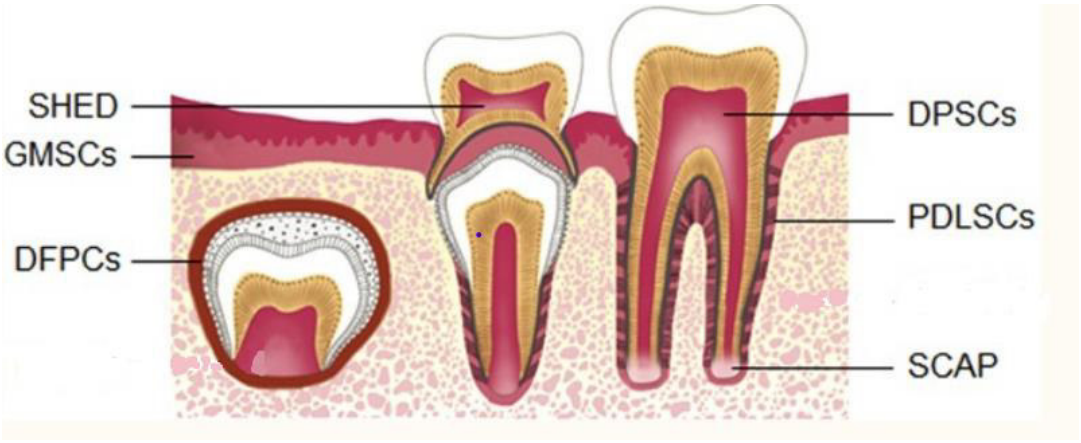


Figure 04 - Different origins of oral cavity stem cells. https://stemcellsjournals.onlinelibrary.wiley.com/cms/asset/fbf31104-25a2-4c03-9ac4-2f4df6baf24d/stem1909-fig-0001-m.jpg

STEM CELLS ORIGINATING FROM THE APICAL PAPILLA

SCAPS

The apical papilla refers to the loose connective tissue attached to the apices of immature permanent teeth and can be easily detached with tweezers. These cells can only be isolated at this stage of the tooth development. Cells from the apical papilla evolve into the dental pulp, therefore, they are essential for root maturation. They can be isolated from third molars and stored for future autologous use; they present

classic properties of MSCs, and maintain their differentiation capacity even after long periods of cryopreservation, preserving their typical morphology. SCAPs, compared to hDPSCs, show higher proliferation rates and greater expression of CD24, a mucin-type adhesion molecule with metastatic potential, which is lost as hSCAPs differentiate and increase the expression of alkaline phosphatase (ALP), an indicator of osteogenic potential; on the other hand, its proliferative potential is lower than DFSCs. SCAPs analyzed during inflammatory processes in the pulp remain histologically distinct from tissues that suffer from inflammation, which suggests that the stable environment provided by the apical papilla maintains the vitality and pluripotency of these cells. The proximity to the vascular-nervous axis gives SCAPs a survival advantage in a harmful environment, and the proliferation rate makes these cells good candidates for regenerative research[25, 26, 27, 28, 29] (Figure 07).

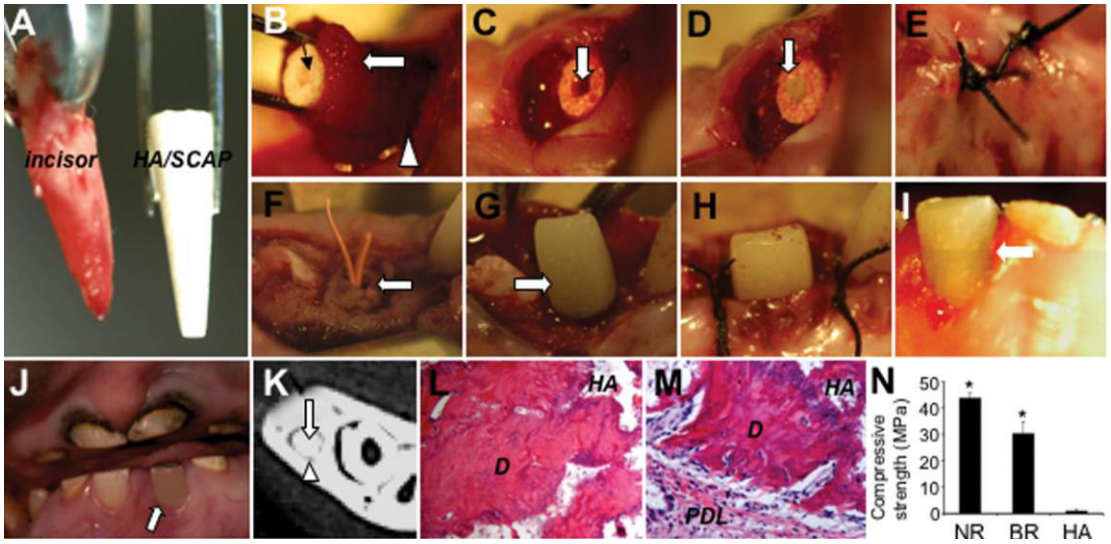
hSCAPs express STRO-1, STRO-3, CD13, CD29, CD44, CD90, CD106, CD105 and CD166 and are negative to hematopoietic surface marker CD45[30] (Table 1).

BIO-ROOT

The hSCAPs cultivated in hydroxyapatite/tricalcium phosphate (HA/TCP) and covered with gelfoam containing hPDLSCs, seeded in a bone socket, create a functional biological root in 3 months. After this period, this bio-root can support a porcelain crown in occlusion. This bio-root is surrounded by periodontal ligament tissue (PDL), presenting favorable biomechanical properties, like a tooth, becoming a possible alternative to titanium implants. hSCAPs transplanted into HA/TCP scaffolds secrete a layer of dentin tissue on the scaffold surface. When seeded onto synthetic poly-D, L-lactide/glycolide scaffolds, they secrete a continuous layer of dentin-like tissue and develop vascularized tissue similar to the pulp in the root canal [25, 27] (Figure 05).

Alveolar bone regeneration

The injection of $2x10^6$ SCAPs suspended in 0.2 ml of NaCl into a previously root-scaled bone defect promotes significant histopathological results in alveolar regeneration, returning the bone level and gingival margin at the cement-enamel junction (CEJ) after 12 weeks [27, 31](Figure 06).



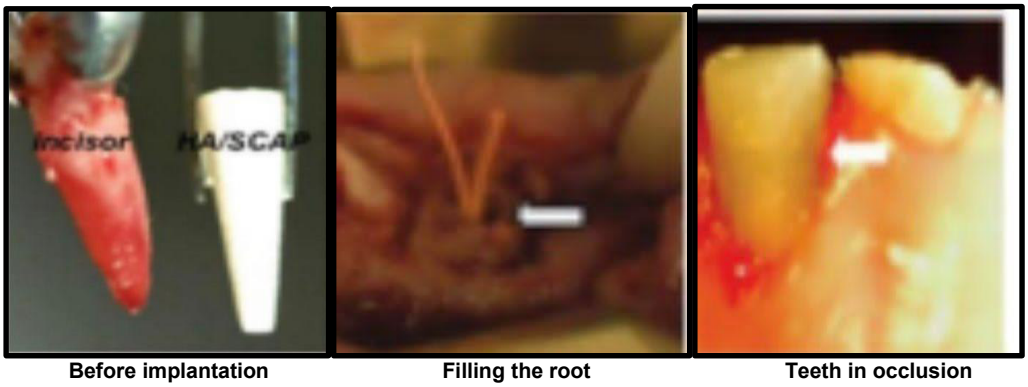


Figure 05 - Periodontal/root structure mediated by SCAP/PDLSC in pigs as an artificial crown support. (A) A Mini-pig's lower incisor was extracted and replaced by root-shaped HA/TCP colonized by SCAPs. (B) Gelfoam containing 106 PDLSCs (open arrow) was used to cover the HA/SCAP (black arrow) and implanted into the lower incisor socket (open triangle). (C) HA/SCAP-Gelfoam/PDLSCs were implanted into a freshly extracted incisor socket. A pin channel was pre-created within the root-shaped HA scaffold (arrow). (D) The pin canal was sealed with a temporary seal to bond a porcelain crown in the next step. (E) The HA/SCAP-Gelfoam/PDLSC implant was sutured for 3 months. (F). The HA/SCAP-Gelfoam/PDLSC implant (arrow) was re-exposed, and the temporary filling was removed to expose the post canal. (G) A prefabricated porcelain crown was cemented onto the HA/SCAP-Gelfoam/PDLSC framework. (H) The exposed section was sutured. (I and J) Four weeks after fixation, the porcelain crown was retained in the pig after normal use of the tooth, as shown by the open arrows (K)After 3 months of implantation, the HA/SCAP-Gelfoam/PDLSC implant formed a hard root structure (open arrows) in the mandibular incisor area, as shown by the CT image. A clear PDL space was found between the implant and the surrounding bone tissue (triangular arrows). (L and M) H&E staining showed that the implanted HA/SCAP-Gelfoam/PDLSC contains newly regenerated dentin (D) inside the implant (L) and PDL tissue (PDL) on the outside of the implant (M). (N) Compressive strength measurement showed that the newly formed biological roots have a compressive strength much higher than the original HA/TCP carrier (*P = 0.0002), but lower than that of natural dentin of porcine root (*P = 0.003) (NR: natural minipig root, BR: newly formed biological root, HA: original HA carrier). https://journals.plos.org/plosone/article/figure/image?size=large&id=10.1371/journal.pone.0000079.g005

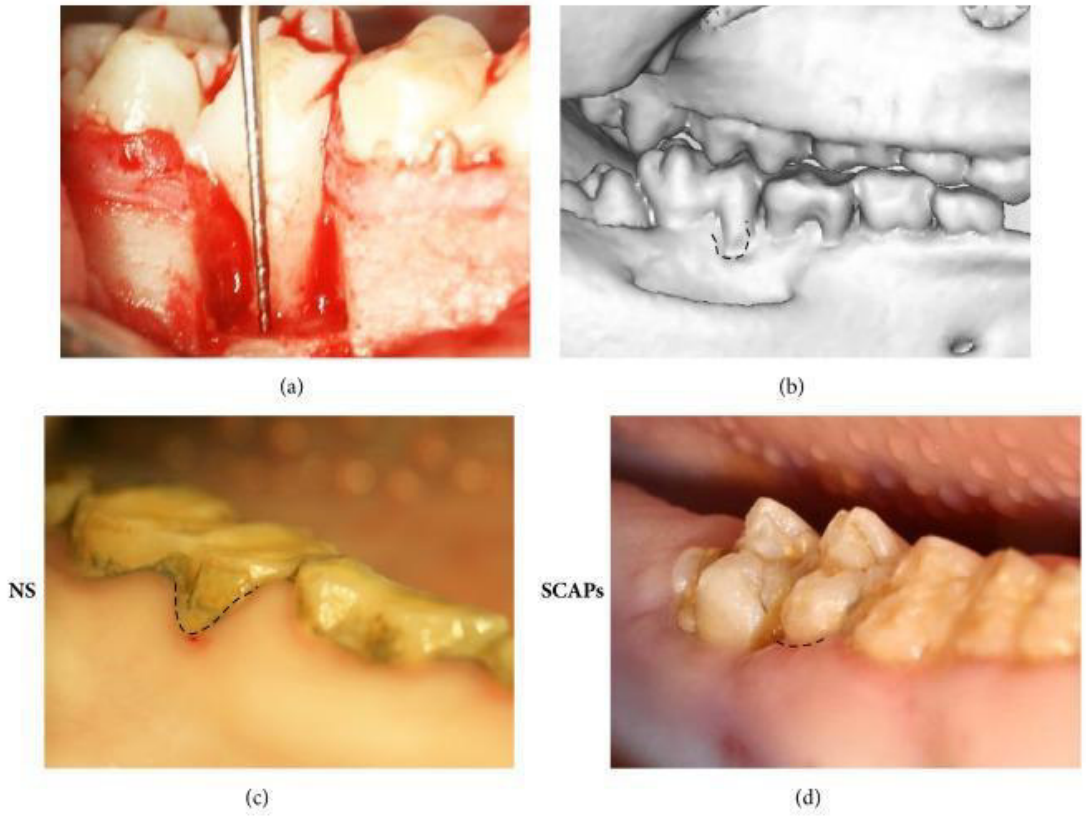


Figura 06 - Local application of SCAPs for regeneration in a case of periodontitis in a mini pig periodontal model. (ab) Bone defects measuring 3 mm × 5 mm × 7 mm were created in the mesial region of the bilateral lower first molars (a). Three-dimensional CT images showed a bone defect in the experimental region (b). (cd) Intraoral manifestations of tissue regeneration in the 0.9% NaCl group (c) and the SCAPs group 12 weeks after treatment (d). Regeneration of the gingival margin and bone defect with the use of SCAPS. https://cdn.ncbi.nlm.nih.gov/pmc/blobs/06a8/6077668/ac16da298e8c/BMRI2018-3960798.001.jpg.

The hSCAPs collected from just one tooth can provide enough stem cells for human transplantation. These cells can be cultivated in osteo/odontogenic, adipogenic, chondrogenic, hepatogenic, angiogenic, and neurogenic culture media, express specific cell markers, and differentiate into these cell types. The hSCAPs have low immunogenicity, which can contribute to the regeneration and repair of living tissues [27, 30, 32, 33, 34] (Figure 07).

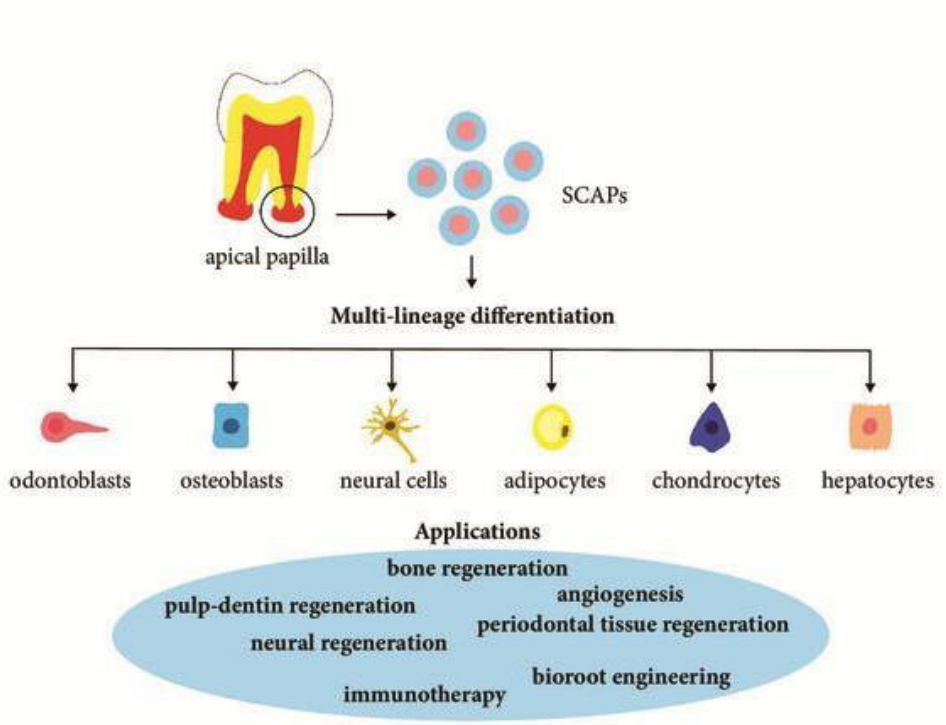


Figure 07 - Differentiation potential of SCAPs. Stem cells from the apical papilla can differentiate into multiple cell lineages such as: Odontoblasts, osteoblasts, neural cells, adipocytes, chondrocytes, and hepatocytes. https://onlinelibrary.wiley.com/cms/asset/9fac1a0c-3e4c-44c3-a24f-a275428a82dd/bmri6104738-fig-0001-m.jpg

hDPSCS

The hDPSCs are easy-to-harvest cells, with good potential for multilineage differentiation, which can be conveniently obtained from the pulp of extracted teeth by gently separating the pulp tissue from the crown and root or through pulpotomy. Third molars, which are often discarded, are good sources for cell isolation. However, hDPSCs in inflamed pulp tissues have reduced dentinogenic activity[35].

Cell surface markers that identify DPSCs are CD44, CD73, CD90, CD146, and CD166, as well as STRO-1 [36].

ROOT NEOFORMATION

The hDPSCs present proliferation and differentiation capacities influenced by growth factors, scaffolds, and microenvironments. They can differentiate into adipocytes, chondrocytes, osteocytes, and even promote a root neoformation [07, 35, 37, 38].

Scaffolds support proper cell attachment, migration, proliferation, differentiation, and function to produce specific tissue constructs [20, 21].

The hDPSCs transplanted into a three-dimensional rod-shaped construct, fabricated with a thermoresponsive hydrogel scaffold, can completely reconstruct the three-dimensional tissue pattern and recover the function based on neurovascular

restoration in empty root canals. The hDPSCs can regenerate the pulp and the PDL from avulsed immature permanent teeth. After curettage of the periodontal ligaments in the socket, and insertion of two aggregates of hDPSCs with a total of $2x10^7$ were implanted in the root canals and wrapped in each traumatized permanent incisor. After 12 months, pulp and periodontal ligaments regenerate without external root resorption. The tooth reimplanted with hDPSCs cells mimics the structures of normal teeth, with dentinal tubules, blood vessels, and periodontal fibers arranged in different ways in the cervical, middle, and apical parts of the root [20] (Figures 08, 09).

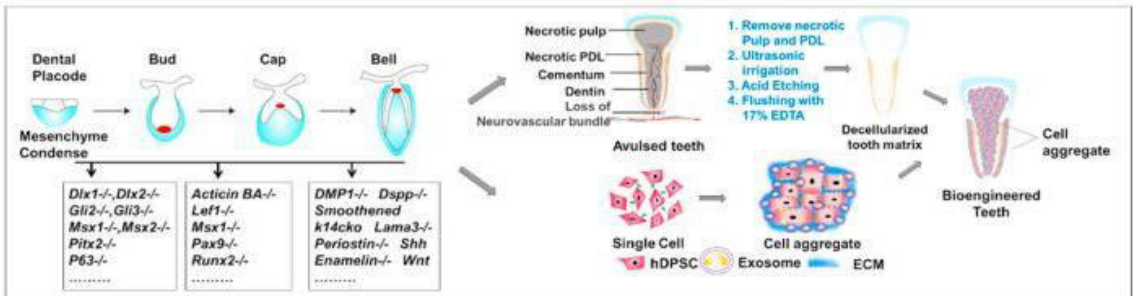


Figure 08 - Image illustrates tooth development and the process involved in bioengineering the reimplantation of the avulsed tooth. To use the TDM scaffold, the following steps are necessary: (1) Removal of the necrotic pulp and PDL; (2) Ultrasonic irrigation; (3) Acid attack; (4) Washing with 17% EDTA. Bioengineering of the avulsed tooth. https://ars.els-cdn.com/content/image/1-s2.0-S0142961221005809-gr1.jpg

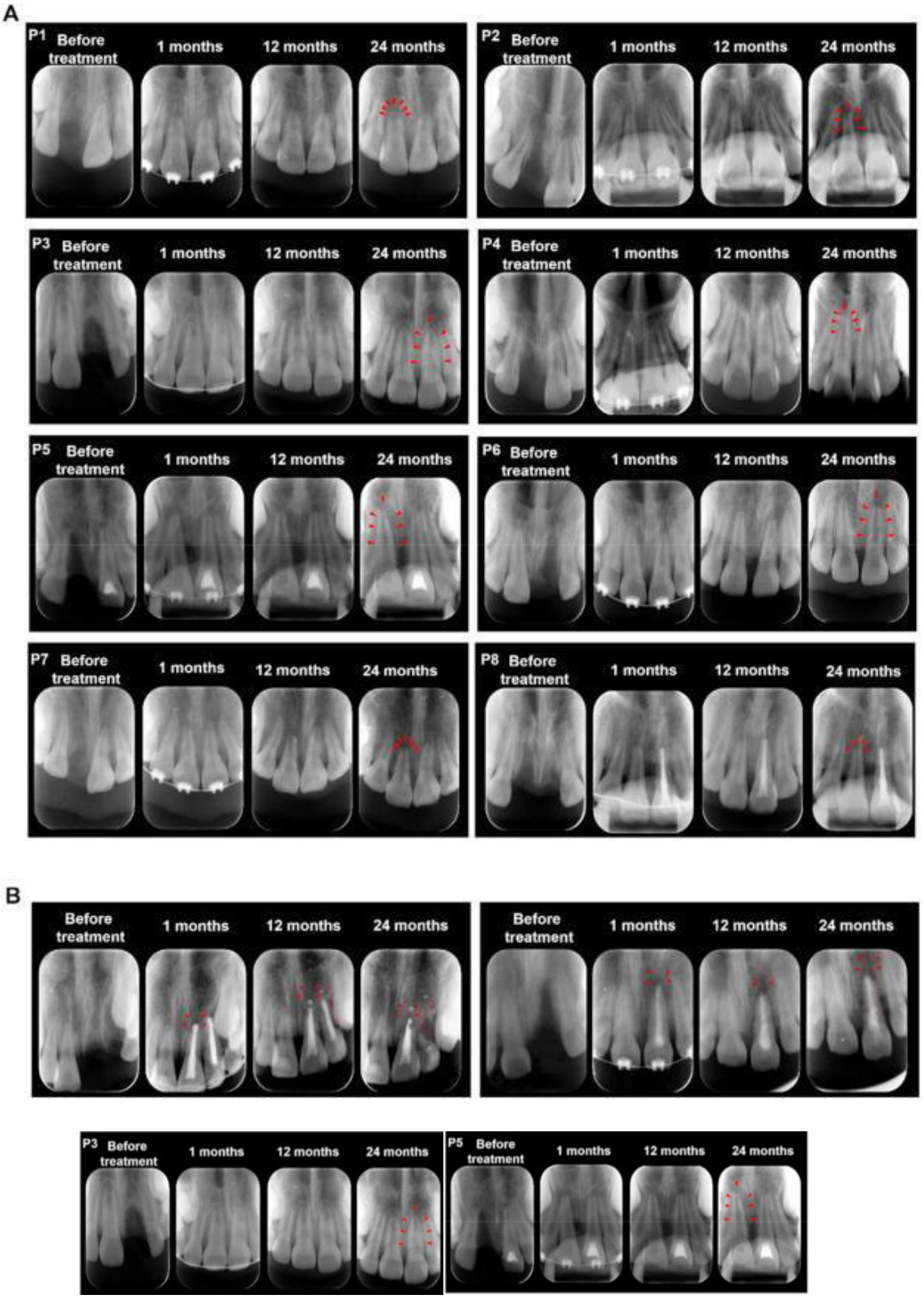


Figure 09 - Patient safety assessment after bioengineered reimplantation of avulsed teeth. (A) Periapical radiographs of 8 patients at intervals before treatment, 1 month, 12 months, and 24 months after reimplantation. (B) Control group, implantation of avulsed teeth from 2 patients without bioengineering. Red triangles indicate root absorption, and red dotted lines indicate ankylosis. The radiographic image of reimplantation of an avulsed tooth with bioengineering. (P3) and (P5) These image cutouts demonstrate little bone and root resorption in patients after stem cell-based tooth reimplantation. https://ars.els-cdn.com/content/image/1-s2.0-S0142961221005809-gr4.jpg

PULP NEOFORMATION

Collagen sponge biocomplex with micrografts of dental pulp cells hDPSCs applied to bone defects of patients without systemic involvement, presents an average reduction in probing depth, average gain in clinical adhesion level (CAL) and filling of bone defects of up to 4 .9 ± 1.4 mm in 12 months compared to the control group using only the collagen sponge [07, 39].

These cells stimulated with 100 ng/ml granulocyte colony-stimulating factor (G-CSF) for MDPSCs demonstrate the potential of blood vessel and dental pulp tissue formation, reversing "irreversible" pulpitis [21].

ANGIOGENESIS AND OSTEOGENESIS

hDPSCs demonstrate the ability to differentiate into neurons and repair injured neural systems. These cells, implanted on a spinal cord injury, with chitosan scaffolds, can recover the locomotor functions of the hind limbs. Another evidence of the differentiation potential of these cells is that their transplantation, associated with bone morphogenic protein (BMP 2) and Vascular Endothelial Growth Factor (VEGF) in a three-dimensional culture model, induces angiogenesis and osteogenesis [40] (Figure 10).

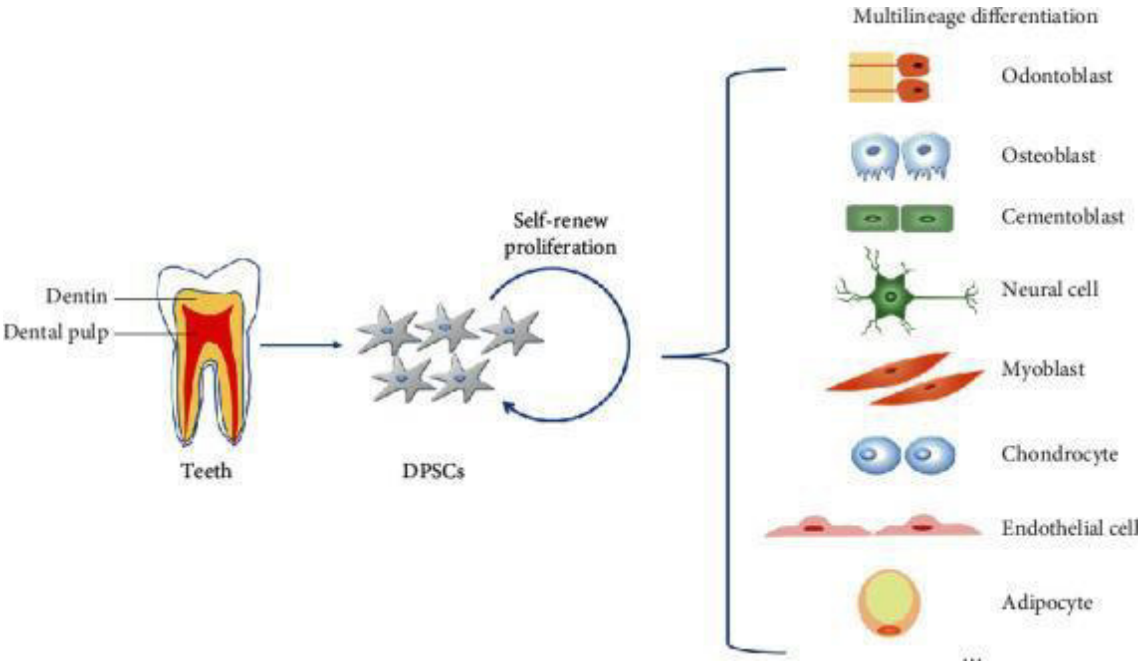


Figure 10 - DPSCs have the potential to differentiate into odontoblasts, osteoblasts, cementoblasts, neural cells, myoblasts, chondrocytes, endothelial cells, and adipocytes. https://cdn.ncbi.nlm.nih.gov/pmc/blobs/0a40/7603635/e0d7145249ff/SCI2020-8876265.001.jpg

SHEDs

SHEDs are cells isolated from the dental pulp of exfoliated primary teeth. They present regenerative potential similar to that of hDPSCs. SHEDs are easy to acquire; they can form mineralized dentin, structures similar to periodontal fibers, osteoblasts, neural

cells, and adipocytes [40, 41, 42].

SHEDs present cell surface markers CD31, CD34, CD45, CD73, CD90 and CD105 [43] (Table 01).

DENTAL PULP NEOFORMATION

These cells can regenerate the three-dimensional tissues of the dental pulp, like odontoblasts, connective tissues, blood vessels, and neural cells. SHEDs and human dermal microvascular endothelial cells (HDMEC), seeded at 0.8 to $2x10^5$ in a tooth slice, with poly-L-lactic acid (PLLA) gel scaffolds, implanted in a subcutaneous defect, regenerate the pulp and form active odontoblasts [21, 40, 44, 45] (Figure 11).

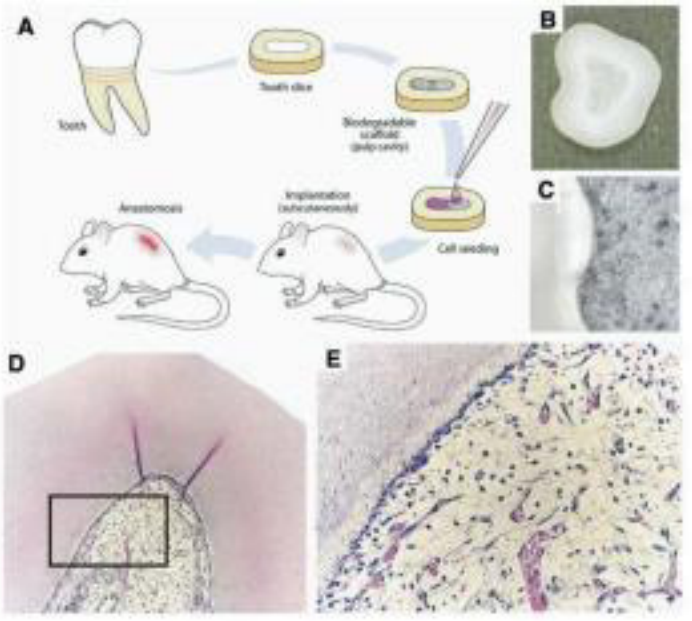


Figure 11 - Engineering a dental pulp tissue with pulp stem cells. (A) Schematic diagram of the strategy for pulp tissue engineering. (B) The biodegradable scaffold is prepared inside the root canal and then seeded with dental pulp stem cells alone or dental pulp stem cells mixed with endothelial cells. The tooth slice containing cells is then implanted into the subcutaneous tissue of immunodeficient mice. (C) High magnification of the tooth/scaffold slice showing the interface between the scaffold and predentin. (D) Low magnification (100) of a dental pulp engineered with SHED and primary HDMEC, 14 days after implantation into an immunodeficient mouse. (E) High magnification (400) of the boxed area of the projected dental pulp shown in (D). Schematic diagram of SHEDs isolation and microscopic image of the pulp. https://www.jendodon.com/cms/10.1016/j.joen.2008.04.009/asset/f2e1e0f9-bede-4397-a584-70a8ab3386bb/main.assets/gr1.sml

PERIODONTAL DEFECT REGENERATION

The bone repair capacity of SHEDs is demonstrated in osteoinductive media. A density of 10^6 fresh SHEDs resuspended in a solution of 15 µl thrombin, 15 µl fibrinogen, and Molday ION Rhodamine-BTM (MIRB), a fluorescent marker that influences the MRI tracer signal, injected into 5x4x1mm³ periodontal defects, regenerates in 9 weeks almost 100% of bone tissue [40].

STEM CELLS ORIGINATING FROM DENTAL FOLLICLE

hDFSCs

hDFSCs are separated from the dental follicle of human impacted third molars or immature permanent teeth [46].

They present the following cell surface markers: STRO-1, CD44, CD29, CD73, CD90, and CD105 [47] (Table 01).

They present immunomodulatory properties similar to those of mesenchymal stem cells. Their origin is linked to a developing tissue, they can differentiate into osteoblasts, cementoblasts, fibroblasts, odontoblasts, neural, and cardiac cells [46]. (Figure 12).

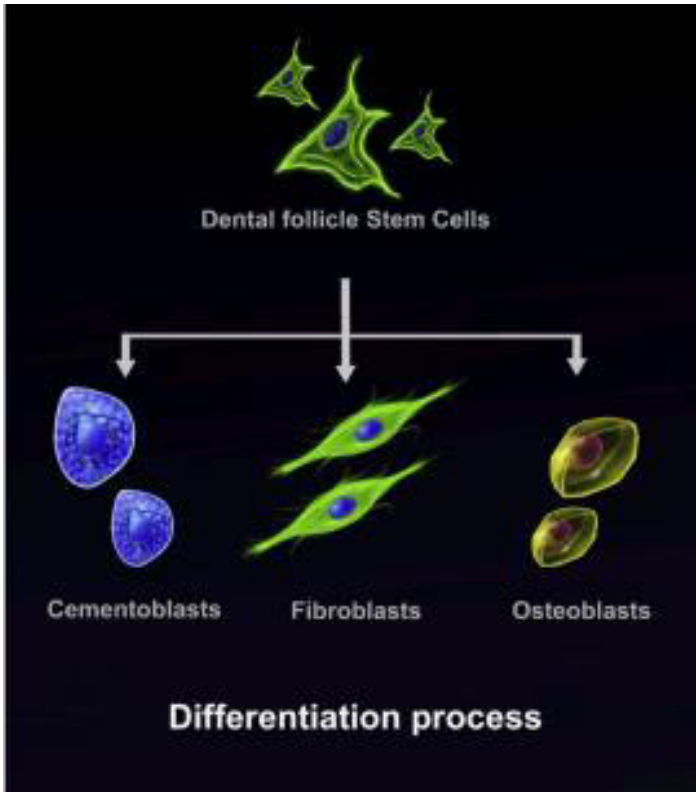


Figure 12 - Differentiation potential (DFSCs). Dental follicle stem cells have regenerative potential in Cementoblasts, Fibroblasts, and Osteoblasts. https://cdn.ncbi.nlm.nih.gov/pmc/blobs/9f94/6962327/b9eda8580dee/gr2.gif

OSTEOGENESIS

Osteogenic differentiation of hDFSCs can be induced by the microenvironment stimuli, such as growth factors. These cells do not mineralize or form bone without an inductive microenvironment. hDFSCs can form periodontal tissue inside scaffolds such as TDM and regenerate the periodontal ligament [48].

hDFSCs can differentiate into osteoblasts and repair bone. DFSCs at a density of 105, with osteogenic capacity confirmed by increased ALP, implanted in 100µl of stem cell growth medium seeded in a polycaprolactone (PCL) scaffold, promote a total thickness of 5mm in diameter of bone repair, after 8 weeks [49, 50]. (Figure 13).

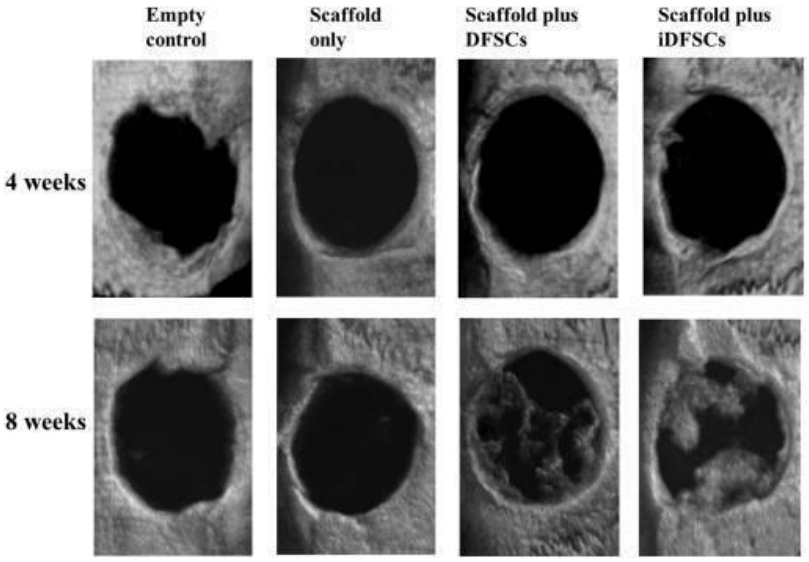


Figure 13 - Microcomputed tomography (micro-CT) scan to evaluate bone regeneration after 4 and 8 weeks of DFSC transplantation (N=4). Note the lack of bone regeneration in the defects of all treatment groups at 4 weeks (top panel). In contrast, bone regeneration appeared within 8 weeks (bottom panel). No bone formation was seen in controls without DFSCs, whereas in defects treated with PCL plus DFSCs or PCL plus iDFSCs, the new bone formation filled almost half of the defects. Image Computed microtomography of bone regeneration. https://www.isct-cytotherapy.org/cms/10.1016/j.jcyt.2015.07.013/asset/5a46ffb2-5a00-4539-8148-fae37c9048b9/main.assets/gr5.sml

ROOT NEOFORMATION

These cells, seeded in soft tissue scaffolds such as the electrofiltered gelatin sheet and the native dental pulp extracellular matrix (APES and DPEM), combined with TDM similar to the structure of tooth roots, are capable of forming roots with characteristics close to those of a natural root. The two soft tissue scaffolds (APES and DPEM), combined with TDM, generate a sandwich composite with APES/TDM/DPEM that is seeded with dental follicle stem cells (DFSCs) and transplanted into a mandibular socket, demonstrating, in 12 weeks, the proliferation of periodontal fibers and cementum/bone-like cellular tissues generated on the surface of the TDM [51].

DFSCs can differentiate into adipocytes in a medium containing dexamethasone, insulin, isobutyl-methyl-xanthine, and indomethacin. They can also differentiate into neural cells in a medium containing dimethyl sulfoxide, butylated hydroxyanisole, potassium chloride, valproic acid, forskolin, hydrocortisone, insulin, and L-glutamine [48].

BIO-HYBRID IMPLANT

Dental follicle stem cells can form a complete periodontal tissue, with cementum, PDL, and alveolar bone on the HA surface. hDFCs seeded in a 2mm HA scaffold associated with a titanium dental implant (1.7 in length and 0.6 in diameter, with shaved sides in a conical shape) implanted in the region of the first molar forms, in 50 days, a bio-hybrid tooth equipped with proprioceptive and nervous function, periodontal space similar to that of a natural tooth, cementum on the implant surface and is capable of reacting to orthodontic movements physiologically [23, 52] (Figure 14).

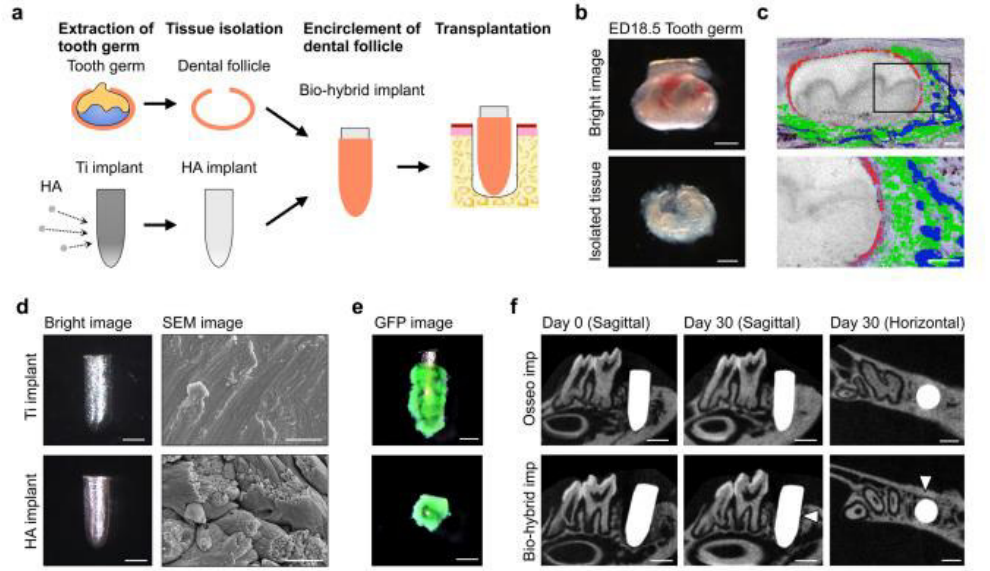


Figure 14 - Bio-hybrid implant grafting in a tooth loss model. (a) Schematic representation of the generative technology of the biohybrid implant. (Drawings by C.T.). (b) Photographs of ED18.5 tooth germ (top) and isolated tooth follicular tissue (bottom). Scale bar, 100 mm. (c) Layer arrangement indicated by in situ hybridization analysis for the expression patterns of F-spondin (red), Periostin (green), and Osteocalcin (blue) in ED18.5 dental follicle tissue. Scale bar, 100 mm. (d) Photographs (left) and surface analysis (right) of the titanium implant and HA implant using SEM. Scale bar, 500 mm and 1.0 mm in photographs and SEM images, respectively. (e) Merged images of a biohybrid dental implant using ED18.5 dental follicles isolated from GFP transgenic mice (top view, sagittal; bottom view, horizontal). Scale bar, 500 mm. (f) Micro-CT images of a bone-integrated implant and a bio-hybrid implant in the sagittal section (left, center) and horizontal section (right) in the transplantation period of day 0 and day 30. Images of bio-hybrid implants were observed in the periodontal ligament space (arrowhead). Scale bar, 500 mm. Bio-hybrid tooth. https://media.springernature.com/lw685/springer-static/image/art%3A10.1038%2Fsrep06044/MediaObjects/41598_2014_Article_BFsrep06044_Fig1_HTML.jpg?as=webp

IMMUNOMODULATORY POTENTIAL

Another important function of DFSCs currently explored is their immunomodulatory capacity. DFSCs, even cryopreserved, can inhibit the acquired immune system through a response mediated by CD4 cells, Major Histocompatibility complex class II (MHC II), and T helper lymphocytes (TH). These cells inhibit the function of TH1 proinflammatory lymphocytes, transform T lymphocytes into TH2 anti-inflammatory cells, regulate them, and can lead to mast cells degranulation, and eosinophils to release histamine, resulting in allergic diseases. These cells can be used to treat diseases such as rhinitis and asthma [48].

OTHER APPLICATIONS WITHOUT SCAFFOLDS

Finally, DFSCs have differential potential beyond dental support tissues, being candidates for differentiation into salivary gland cells and cardiac cells, since, when injected without scaffolds, they can lodge in the myocardium, suggesting regeneration potential for treatment of heart disease [48].

hPDLSCs

Human periodontal ligament stem cells (hPDLSCs), obtained from curettage of the

periodontal ligament and root surface of extracted teeth, which express CD271, a marker for neural crest cells (NCCs) and MSCs, highlighted in osteogenic potential cells. The environment where periodontal ligament stem cells are isolated directly influences their differentiation capacity, since they can be found both in the root (r-PDLSCs) and the alveolar bone (a-PDLSCs). The latter have greater differentiation potential in osteogenic and adipogenic media, in addition to expressing higher markers associated with mineralization, greater alkaline phosphatase activity, and more complete alveolar bone reconstruction in specific culture media when compared to r-PDLSCs [09].

Like hDPSCs, hPDLSCs express mesenchymal stem cell markers, CD44, CD73, CD90, CD146, and CD166, in the initial passage [53] (Table 1).

The hPDLSCs are capable of generating structures similar to the periodontal ligament [25].

ALVEOLAR BONE NEOFORMATION

The differentiation potential of this cell type is also dependent on the microenvironment, growth factors, and scaffolds, moreover, this potential is inhibited in the presence of inflammation.

hPDLSCs seeded in 96-well plates at a proportion of $2x10^4$ cells/ml associated with platelet-derived growth factor BB (PDGF-BB) and implanted in alveolar defects measuring $7x4x3mm^3$ show, in 8 weeks, 40% greater volume of alveolar regeneration than in individuals who do not receive hPDLSCs, or 10% higher compared to individuals who receive them associated with fluorescent protein green (GFP) [54].

PERIODONTAL POCKET REDUCTION AND IMMUNOMODULATION

Another application of PDLSCs is in reducing periodontal pockets. The implantation in 5-8 mm periodontal pockets, in patients without systemic complications, of PDLSCs seeded in condensed gelatin sponge, promotes, within one year, a reduction in pocket depth, gain in clinical attachment level, and resolution of the bone defect [07, 55].

Although the osteogenic differentiation capacity of hPDLSCs is inhibited in periodontitis, they induce the anti-inflammatory action of macrophages, regulating the polarization of macrophages to M2, making these cells agents in the regeneration of inflamed tissues, and regulating the function of leukocytes [25, 56].

OTHER APPLICATIONS

hPDLSCs can differentiate into cells of the mesenchymal and the ectodermal lineages. For example, neural cells, oligodendrocytes, astrocytes, and Schwann cells. hPDLSCs exposed to short-term mechanical strain can differentiate into cardiomyocytes

expressing cardiac cell markers, sarcomeric actin, cardiac troponin T proteins, and retinal ganglion cells [57,58].

This cell type can also be used in vascular tissue repair. hPDLSCs isolated from premolars extracted for orthodontic reasons, cultured ex-vivo and seeded on a natural bovine pericardial membrane scaffold with 1cm² microporosities (BioR-Ps) demonstrate, compared to the application of these cells on the membrane without porosities, present a significant increase in VEGF levels, the main indicator of angiogenic potential, due to its ability to initiate the formation of vascular networks. The holes in this scaffold allow cellular infiltration and the deposition of neotissues, and promote the selective release of biomolecules essential for clinical regeneration [19] (Figure 15).

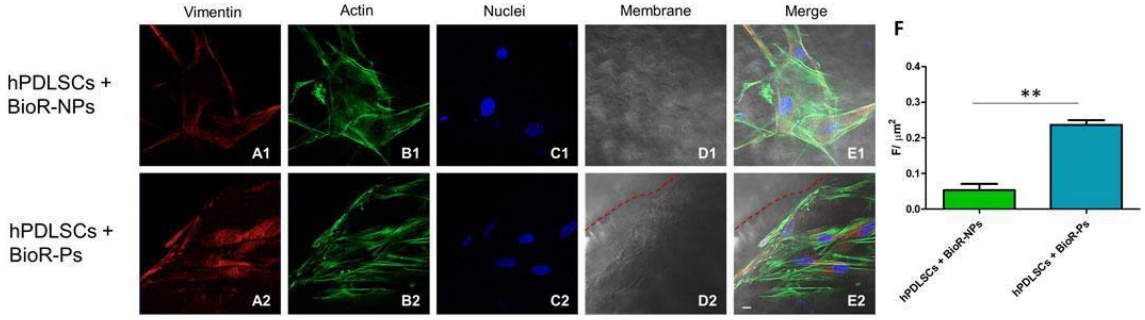


Figure 15 - VEGF expression analyzed by confocal microscopy. VEGF (A1-E2) expression was evaluated in hPDLSCs + BioR-NPs and hPDLSCs + BioR-Ps after 1 week of culture. Red fluorescence: VEGF (A1-A2); Green fluorescence: cytoskeletal actin (B1-B2); Blue fluorescence: cell nuclei (C1-C2), BioR membranes (D1-D2); Merge (E1-E2). (F) Histograms represent positive cells for the analyzed marker.***p < 0.001. Scale bar: 20 µm.

https://www.frontiersin.org/files/Articles/1455215/fbioe-12-1455215-HTML/image_m/fbioe-12-1455215-g007.jpg

hGMSCs

The hGMSCs are taken from the palatal and buccal gingival connective tissue[59].

These cells express CD73, CD105, CD90, STRO-1, and CD34, the last in a low percentage [60] (Table 1).

The hGMSCs are 90% derived from NCC, 10% originate from mesoderm, and can be easily extracted from gingival tissue. Due to their origin, they have differentiation potential into neural cells. Furthermore, more than 85% of the connective cells of the palate, palatal gingiva, and buccal gingiva are also derived from the neural crest, compared to 65% of those in the oral mucosa. This correlation may contribute to the healing process of scarless oral wounds and supports the notion that gingival tissue is an easily accessible reservoir of NCC-derived MSCs. These cells are capable of multiple differentiation into both adipocytes and osteoblasts. Cells isolated from inflamed tissue keep their ability to differentiate into p adipocytes, osteocytes, and chondrocytes [59, 61] (Figure 18).

hGMSCs accelerate the closure of skin wounds with minimal scarring, promote re-

epithelialization, angiogenesis, and suppress local inflammatory responses. 2x106 hGMSCs injected systemically after full-thickness skin excision show complete wound closure within 10 days. Local application of 3D-printed medical-grade polycaprolactone (mPCL) dressings notably reduces wound contracture and facilitates granulation and re-epithelialization of skin tissue. The 3D printed biomimetic dressings and GMSCs improve physiological wound closure with reduced scar tissue formation, supporting the idea of healing with minimal scars [48, 59].

BONE NEOFORMATION

hGMSCs demonstrate potential for regeneration of bone defects, bone formation, and reduction of inflammatory responses around implants in peri-implantitis after local injection encapsulated by adhesive hydrogel. Osteogenic and pre-differentiated GMSCs (dGMSCs) promote new bone formation in 7x1x1mm³ mandibular defects. $1x10^6$ PM/dGMSCs/BMP2 cells, suspended into 15ml of serum-free medium, and mixed with the self-assembling nanofiber hydrogel scaffold, injected into the alveolar defect, achieves neovascularization, and mature bone formation with presence of osteocytes and firm integration of the new bone [59, 62, 63] (Figure 16, 17).

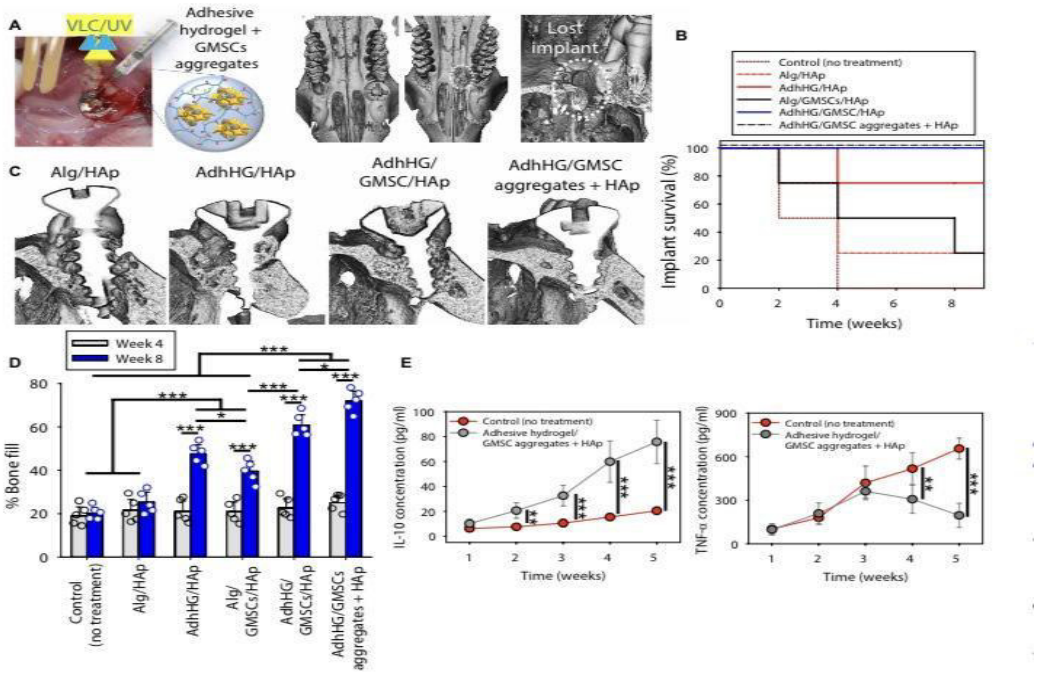


Figure 16 - (A) Photograph, schematic, and 3D micro-CT images of the development of the peri-implantitis model induced by A. actinomycetemcomitans treated with an adhesive compound associated with hygrodel and GMSC aggregates. (B) Survival assessment of titanium dental implants after 8 weeks, dashed circles indicate lost implants. (C) 3D Micro-CT of animals from the control group and the group that received GMSCs. (D) Analysis of bone volume filling at the implant site 4 and 8 weeks after implantation. (E) Quantification of anti-inflammatory and inflammatory cytokines at different time intervals (1, 2, 3, 4, and 5 weeks) after implantation.GMSCs in the treatment of peri-implantitis. https://www.science.org/doi/10.1126/scitranslmed.aay6853#F5

POTENTIAL OF REEPITHELIALIZATION

The local transplantation of $2x10^6$ of human fetal GMSCs cells around a 5mm gingival defect significantly facilitates the closure of the gingival wound and re-epithelialization. Three weeks after transplantation, the morphology, height, and color of the gingival tissues formed are similar to normal gingival tissues from the control group [59, 64].

IMMUNOMODULATORY POTENTIAL

The hGMSCs elicit a potent inhibitory effect on T lymphocyte proliferation in response to mitogenic stimulation. The application of GMSCs, as well as PDLSCs, demonstrates immunomodulatory potential by controlling the innate immune responses of mast cells by significantly suppressing their degranulation, production of pro-inflammatory cytokines, and attenuating chronic hypersensitivity (CHS). At the site of injury, these cells stimulate macrophage polarization and promote the M2 anti-inflammatory phenotype by increasing the secretion of anti-inflammatory cytokines such as interleukin 10 (IL-10), and decreasing the expression of IL-6 and necrosis tumor factor alpha (TNF-ά). They also reduce peripheral blood mononuclear cell proliferation and B lymphocyte differentiation (part of the adaptive immune response) [47, 59].

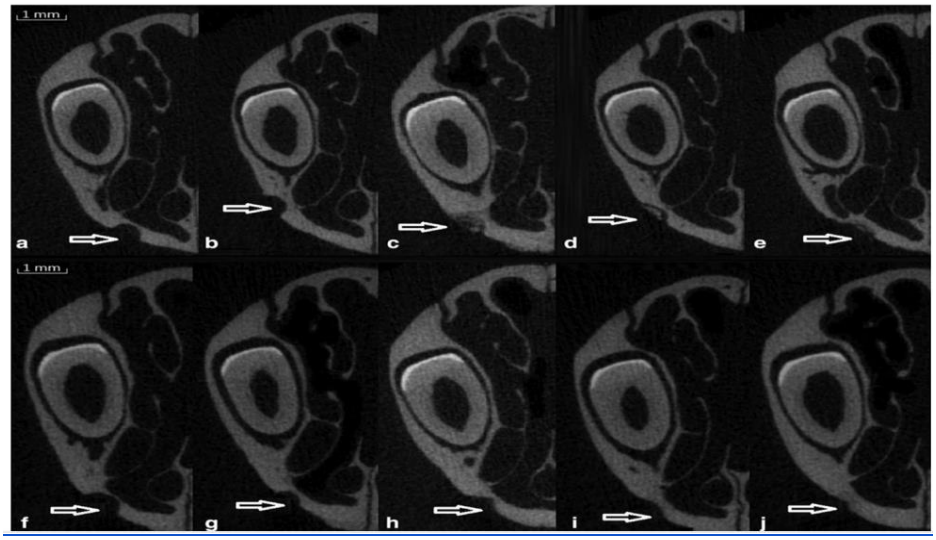


Figure 17 CT showing the bone regeneration capacity of various groups. (a–e) Four weeks after surgery, (f–j) 8 weeks after surgery; arrows represent the defect area, (a) defect, (b) PM, (c) PM/BMP2, (d) PM/GMSCs, (e) PM/dGMSCs/BMP2, (f) defect, (g) PM, (h) PM/BMP2, (i) PM/dGMSCs and (j) PM/dGMSCS/BMP2. There was no spontaneous cure in the defect group (a) at 4 or 8 weeks. All other groups showed bone healing. The PM/dGMSCs/BMP2 group (j) filled the bone volume within 8 weeks. PM, Puramatrix; BMP2, bone morphogenetic protein 2; dGMSC, pre-differentiated GMSC. Bone regeneration with a combination of PM/dGMSCs/BMP2 and injected into the alveolar defect. https://cdn.ncbi.nlm.nih.gov/pmc/blobs/bafe/8098763/7f0b66fe5d25/ten.tea.2020.0052_figure3.gif

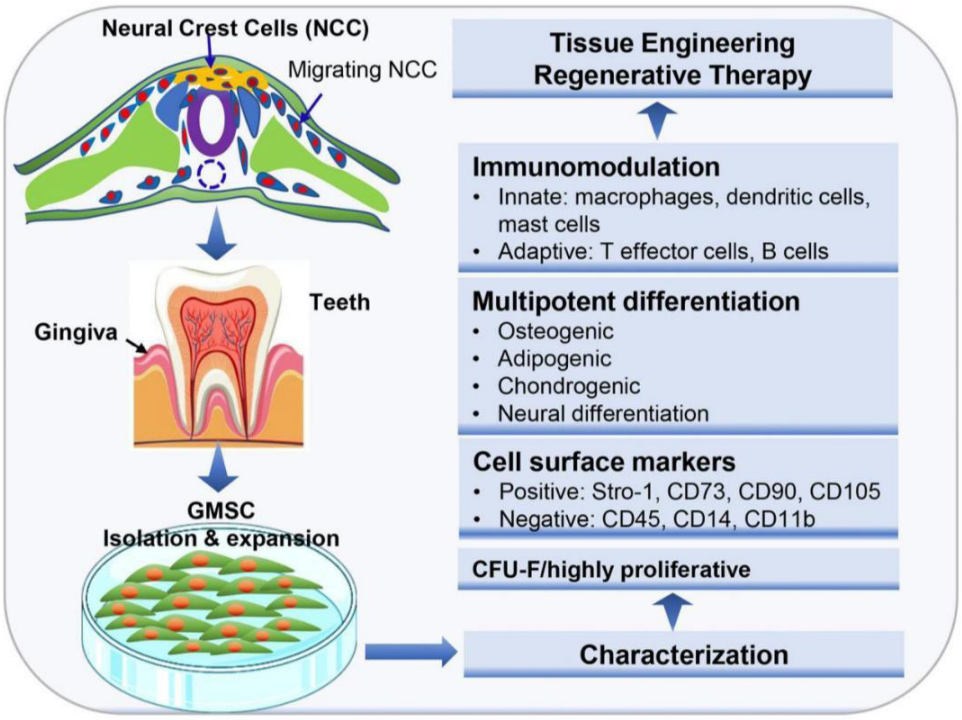


Figure 18 - Isolation and characterization of mesenchymal stem cells derived from the gingival tissues of neural crest origin. A unique subpopulation of mesenchymal stem cells can be isolated from neural crest-derived gingival tissues (GMSC), thus representing a reservoir of neural crest-derived MSCs.

https://images-provider.frontiersin.org/api/ipx/w=290&f=webp/https://www.frontiersin.org/files/Articles/667221/fimmu-12-667221-HTML/image_m/fimmu-12-667221-g001.jpg

The GMSCs have also demonstrated therapeutic potential in several preclinical models of autoimmune and inflammatory diseases such as colitis, arthritis, atherosclerosis, osteoporosis, hyperlipidemia, and systemic inflammatory responses [59].

They promote the regeneration of periodontal tissue, with height increases of the alveolar bone, reaching the same bone level as before the injury. Administration of these cells demonstrates differentiation potential into osteoblasts, cementoblasts and PDL fibroblasts in a class III furcation defect model in beagle dogs, in addition to notably increasing the regeneration of damaged periodontal tissue, including alveolar bone, cementum, PDL and also from noble tissues such as: facial nerve, sciatic nerve and salivary glands [59, 65].

Finally, all these stem cells secrete 90 to 1200 nm extracellular vesicles of lipoprotein membrane, which contain fragments of DNA, RNA messenger (mRNA), and microRNA (miRNA), however, the exosome content of each stem cell is slightly different depending on their origin. Some specific markers of these exosomes are CD67, CD81, and CD9. These exosomes are absorbed into other cells, altering the cellular microenvironment through cell signaling. The application of these exosomes in drug delivery and as alternatives to scaffolds is being explored. [07, 21, 43, 65, 66, 67].

Table 01 - Oral cavity Stem Cells

Stem Cells	Cell Markers (CD)	Origin	Regenerative Potential in the Oral Cavity	References
hSCAPS	CD13, CD29, CD44, CD90, CD106, CD105, CD166, STRO-1, STRO-3,	Obtained from the apical papilla of the immature root of permanent teeth	Root maturation: functional biological root formation in empty sockets in 3 months.	25, 26, 27,28, 29, 30, 31, 32, 33, 34.
hDPSCS	CD44, CD73, CD90, CD146, CD166, STRO-1.	Obtained from the pulp tissue of permanent teeth extracted or subjected to pulpotomy.	Root maturation, periodontal pocket closure; alveolar bone formation, dentin formation, treatment of pulpitis, pulp-like tissues formation with nerve vascular bundle, reimplantation of avulsed teeth without root resorption.	20, 31, 35, 36, 37, 38, 40.
SHEDS	CD31, CD34, CD45, CD73, CD90, CD105.	Obtained from the pulp of exfoliated deciduous teeth.	Periodontal ligament formation; odontoblasts generation; dentin formation, bone tissue formation, chondroblasts formation, dental pulp formation.	21, 40, 41, 42, 43, 44, 45.
hDFSCS	CD44, CD29, CD73, CD90, CD105. STRO-1.	Separated from the dental follicle of developing teeth, such as impacted human third molars.	Complete periodontal tissue regeneration, formation of a root-like structure.	08, 23, 46, 47, 48, 49, 50, 51, 52.
				The table continues on next page.
hPDLSCS	CD44, CD73, CD90, CD146, CD166	Obtained from the periodontal ligament of extracted teeth.	Periodontal tissue formation, alveolar bone formation, and immunomodulatory effects.	24, 25, 53, 54, 57, 58.
hGMSCS	CD34, CD73, CD90, CD105, STRO-1.	Obtained from the gingival, palatal, and buccal connective tissue.	Bone formation, reepithelialization; wound closure with minimal scarring, immunomodulatory	47, 59, 60, 61, 62, 64, 65.

			effects.	

DISCUSSION

The oral cavity is a great reservoir of stem cells that can regenerate damaged tissues of the body.

Third molars, erupted and unerupted, teeth extracted for orthodontic purposes, primary teeth, and gingival tissues are a source of mesenchymal stem cells that can be collected, cultured, expanded, cryopreserved, and used for autologous or allogeneic purposes.

The hDFSCs, hDPSCs, SHEDs, hSCAPs, hPDLSCs, and hGMSCs can be collected from the patient for expansion before autologous implantation, eliminating the ethical conflict associated with their use. Moreover, their immunomodulatory properties allow the allogeneic use of these cells [22].

Stem cells originating from the oral cavity exhibit differentiation potential into tissues of all the embryonic folicles (endoderm, mesoderm, and ectoderm) [22].

Stem cells derived from the apical papilla — hSCAPs, SHEDs, and hDPSCs — are three cell types with similar origins that present slightly different differentiation potentials, and survival rate, they can differentiate into tooth-supporting and pulp tissues, enabling periodontal and pulp tissue regeneration, like apexogenesis, and even reversing "irreversible" pulpitis. Moreover, hSCAPs maintain their viability and stemness even when isolated from inflamed tissues [21, 22, 23, 29, 35].

The hDFSCs, hGMSCs, and hPDLSCs are three cell types originating from the neural crest (NCCs) that exhibit similar differentiation potential in periodontal tissues, immunomodulatory and re-epithelialization properties. hDFSCs can be cryopreserved without losing their immunomodulatory capacity. hPDLSCs demonstrate a strong ability to differentiate into neural cells but are limited when extracted from an inflamed periodontal ligament (PDL). Lastly, hGMSCs present strong immunomodulatory properties and promote epithelial tissue regeneration with minimal scarring [21, 25, 43, 46, 57, 59].

A comparison between hSCAPs and hDFSCs, the two most promising cell types, reveal that the hSCAPs can provide a large number of stem cells, that are enough for human transplantation, on the other hand hDFSCs exhibit a high differentiation potential, restoring the natural functionality of critical periodontal tissues in 8 weeks, and the cementum and periodontal ligament in 5 weeks [50].

For periodontal therapy, both cell types exhibit promising regenerative potential. SCAPs can form periodontal tissues and a functional bio-root capable of withstanding masticatory forces. hDFSCs, on the other hand, seeded into proper scaffolds can generate a root structure similar to a natural one, creating a bio-hybrid tooth with an outer dentin layer, periodontal ligament, and blood vessels [25, 51, 52].

Moreover, regenerative periodontal therapy is gaining interest over hGMSCs and hDPSCs due to their differential potential. The hGMSCs can be used for bone neoformation, and periodontitis/peri-implantitis control, and hDPSCs can promote periodontal ligament and dental pulp neoformation of avulsed teeth, and for apexogenesis of immature teeth, without long-term external resorption [40, 59, 62].

The effects of stem cells have been attributed to their differentiation potential into determined cell lineages and their immunomodulatory effects. Most of these effects are due to the cell-to-cell interactions and the extracellular signalling to the regional microambience caused by their extracellular vesicles.

FINAL CONSIDERATIONS

The oral cavity is a great source of mesenchymal stem cells. The hDFSCs, hDPSCs, SHEDs, hSCAPs, hPDLSCs, and hGMSCs are versatile stem cells collected in the oral cavity that can be used in regenerative dentistry, including specialities like periodontology, endodontics, surgery, and implantology. Moreover, these cells can be stimulated to form a new biotooth or a new bioroot, opening space for advances in oral rehabilitation.

Although a lot of research and clinical trials have to be done to validate clinical use of this knowledge in humans, this field is vast and promising, bringing light to new therapies that can solve clinical problems and reformulate dental treatments shortly.

AUTHORS DISCLOSURE

All the authors disclose financial interests.

ACKNOWLEDGEMENTS

We would like to acknowledge Professor Durvanei Augusto Maria for the opportunity given to Icaro de Matos, and Professor Marcos Cavalcanti for his co-supervision in Icaro's scientific initiation in the Laboratory of Development and Innovation, of Instituto Butantan, São Paulo, Brazil.

WORK MANAGEMENT

Ícaro Gabriel Teles Pacheco De Matos, Alan Gustavo De Andrade Costa, and Gianny Dominicci Batista Brito wrote the final course essay, as a requirement for their Dental graduation, under Professor Marcos Fernando Xisto Braga Cavalcanti's supervision, who wrote the manuscript. Francesca Diomede and Durvanei Augusto Maria revised the manuscript.

REFERENCES

[01] Schmalz, G., Widbiller, M., & Galler, K. M. (2020). Clinical perspectives of pulp regeneration. Journal of endodontics, 46(9), S161-S174.

[02] Saad, W., Almaslamani, M., & Saleh, A. R. (2024). Revascularization of a Permanent Tooth with Necrotic Pulp and Apical Periodontitis. Clinical, Cosmetic and Investigational Dentistry, 16, 227–235. https://doi.org/10.2147/CCIDE.S464214

[03] Darby, Ivan. “Risk factors for periodontitis & peri-implantitis.” Periodontology 2000 vol. 90,1 (2022): 9-12. doi:10.1111/prd.12447.

[04] LADEIRA, Lorena Lúcia Costa et al. Cárie, periodontite e outras doenças não transmissíveis: abordagem transdisciplinar para o cirurgião-dentista. São Luís: EDUFMA, 2023.

[05] Bharuka, Tanvi, and Amit Reche. “Advancements in Periodontal Regeneration: A Comprehensive Review of Stem Cell Therapy.” Cureus vol. 16,2 e54115. 13 Feb. 2024, doi:10.7759/cureus.54115.

[06] Dziubiÿsk, P et al. “Stem cells in dentistry--review of literature.” Polish journal of veterinary sciences vol. 16,1 (2013): 135-40. doi:10.2478/pjvs-2013-0021.

[07] 0394 Celulas-tronco na Odontologia regenerative / André Antonio Pelegrine . et al.] Campinas: Faculdade São Leopoldo Mandic, 2022. 29p: il.ISBN: 978-65-86718-17-1 Catalogação na publicação: Somanta Capeletto - CRB 8/8458.

[08] Dominici, M et al. “Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.” Cytotherapy vol. 8,4 (2006): 315-7. doi:10.1080/14653240600855905.

[09] Kadkhoda, Zainab et al. “Assessment of Surface Markers Derived from Human Periodontal Ligament Stem Cells: An In Vitro Study.” Journal of dentistry (Tehran, Iran) vol. 13,5 (2016): 325-332.

[10] Kavyasudha, Chavali et al. “Conventional and Emerging Markers in Stem Cell Isolation and Characterization.” Advances in experimental medicine and biology vol. 1341 (2021): 1-14. doi:10.1007/5584_2019_475.

[11] Mak, T. W., & Saunders, M. E. (2006). Cells and Tissues of the Immune Response. The Immune Response, 35–67. doi:10.1016/b978-012088451-3.50005-3.10.4252/wjsc.v7.i2.470, 10.1093/glycob/cwv032.

[12] Sivasubramaniyan, Kavitha et al. “Expression of stage-specific embryonic antigen-4 (SSEA-4) defines spontaneous loss of epithelial phenotype in human solid tumor cells.” Glycobiology vol. 25,8 (2015): 902-17. doi:10.1093/glycob/cwv032.

[13] Álvarez-Viejo, María et al. "CD271 as a marker to identify mesenchymal stem cells from diverse sources before culture." World journal of stem cells vol. 7,2 (2015): 470-6. doi:10.4252/wjsc.v7.i2.470.

[14] Meyfour, Anna et al. "The quest of cell surface markers for stem cell therapy." Cellular and molecular life sciences: CMLS vol. 78,2 (2021): 469-495. doi:10.1007/s00018-020-03602-y.

[15] Lee, Yu-Chieh et al. "Comparing the Osteogenic Potentials and Bone Regeneration Capacities of Bone Marrow and Dental Pulp Mesenchymal Stem Cells in a Rabbit Calvarial Bone Defect Model." International journal of molecular sciences Vol. 20, 20 5015. 10 Oct. 2019, doi:10.3390/ijms20205015

[16] Basabrain, Mohammed S et al. "Formation of Three-Dimensional Spheres Enhances the Neurogenic Potential of Stem Cells from Apical Papilla." Bioengineering (Basel, Switzerland) vol. 9,11 604. 22 Oct. 2022, doi:10.3390/bioengineering9110604

[17] Agha-Hosseini, Farzaneh et al. "In vitro isolation of stem cells derived from human dental pulp." Clinical transplantation vol. 24,2 (2010): E23-8. doi:10.1111/j.1399-0012.2009.01137.x

[18] Grelewski, Piotr Grzegorz et al. "Properties of scaffolds as carriers of mesenchymal stem cells for use in bone engineering." Polimery w medycynie vol. 53,2 (2023): 129-139. doi:10.17219/pim/166471.

[19] Morgante, Olga et al. "Geometrically modified bovine pericardium membrane promotes the expression of molecules targeted for a faster integration and vascularization process." Frontiers in bioengineering and biotechnology vol. 12 1455215. 13 Nov. 2024, doi:10.3389/fbioe.2024.1455215

[20] Sui, Bingdong et al. "Dental Pulp Stem Cells: From Discovery to Clinical Application." Journal of endodontics vol. 46, 9S (2020): S46-S55. doi:10.1016/j.joen.2020.06.027.

[21] Li, Xin-Lu et al. "Dental pulp regeneration strategies: A review of status quo and recent advances." Bioactive materials vol. 38 258-275. 7 May. 2024, doi:10.1016/j.bioactmat.2024.04.031.

[22] Cui, Dixin et al. "The Origin and Identification of Mesenchymal Stem Cells in Teeth: from Odontogenic to Non-odontogenic." Current stem cell research & therapy vol. 13,1 (2018): 39-45. doi:10.2174/1574888X12666170913150403.

[23] Ouchi, Takehito, and Taneaki Nakagawa. "Mesenchymal stem cell-based tissue regeneration therapies for periodontitis." Regenerative therapy vol. 14 72-78. 15 Jan. 2020, doi:10.1016/j.reth.2019.12.011.

[24] Liu, Junjun et al. "Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells." Stem cells (Dayton, Ohio) vol. 33,3 (2015): 627-38. doi:10.1002/stem.1909.

[25] Sonoyama, Wataru et al. "Mesenchymal stem cell-mediated functional tooth regeneration in swine." PloS one vol. 1,1 e79. 20 Dec. 2006, doi:10.1371/journal.pone.0000079.

[26] Klemba, Aleksandra et al. "Surface markers of cancer stem-like cells of ovarian cancer and their clinical relevance." Contemporary oncology (Poznan, Poland) vol. 22, 1A (2018): 48-55. doi:10.5114/wo.2018.73885.

[27] Kang, Jun et al. "Stem Cells from the Apical Papilla: A Promising Source for Stem Cell-Based

Therapy." BioMed Research International vol. 2019 6104738. 29 Jan. 2019, doi:10.1155/2019/6104738.

[28] Lin, Zhengjun et al. "Functions and mechanisms of circular RNAs in regulating stem cell differentiation." RNA biology vol. 18,12 (2021): 2136-2149. doi:10.1080/15476286.2021.1913551.

[29] Liu, Qi et al. "Stem Cells from the Apical Papilla (SCAPs): Past, Present, Prospects, and Challenges." Biomedicines vol. 11,7 2047. 20 Jul. 2023, doi:10.3390/biomedicines11072047.

[30] Damrongsri, Damrong et al. "Differential expression of Notch-related genes in dental pulp stem cells and stem cells isolated from apical papilla." Journal of oral biology and craniofacial research vol. 11,3 (2021): 379-385. doi:10.1016/j.jobcr.2021.04.004.

[31] Li, Guoqing et al. "Local Injection of Allogeneic Stem Cells from Apical Papilla Enhanced Periodontal Tissue Regeneration in Minipig Model of Periodontitis." BioMed Research International vol. 2018 3960798. 12 Jul. 2018, doi:10.1155/2018/3960798.

[32] Bakopoulou, Athina et al. "Angiogenic Potential and Secretome of Human Apical Papilla Mesenchymal Stem Cells in Various Stress Microenvironments." Stem cells and development vol. 24,21 (2015): 2496-512. doi:10.1089/scd.2015.0197.

[33] Sowmya, S et al. "Tri-Layered Nanocomposite Hydrogel Scaffold for the Concurrent Regeneration of Cementum, Periodontal Ligament, and Alveolar Bone." Advanced healthcare materials vol. 6,7 (2017): 10.1002/adhm.201601251. doi:10.1002/adhm.201601251.

[34] Digka, Anna et al. "Effect of Long-Term Cryopreservation on the Stemness of Stem Cells of Apical Papilla." International Journal of Dentistry vol. 2022 6004350. 27 Dec. 2022, doi:10.1155/2022/6004350.

[35] Zhang, Weibo et al. "The performance of human dental pulp stem cells on different three-dimensional scaffold materials." Biomaterials vol. 27,33 (2006): 5658-68. doi:10.1016/j.biomaterials.2006.07.013.

[36] Hwang, Hyo-In et al. "Immunomic Screening of Cell Surface Molecules on Undifferentiated Human Dental Pulp Stem Cells." Stem cells and development vol. 24,16 (2015): 1934-45. doi:10.1089/scd.2014.0493.

[37] Ito, Kenji et al. "Osteogenic potential of effective bone engineering using dental pulp stem cells, bone marrow stem cells, and periosteal cells for osseointegration of dental implants." The International Journal of Oral & Maxillofacial Implants vol. 26,5 (2011): 947-54.

[38] Guimarães, Elisalva Teixeira et al. "Mesenchymal and embryonic characteristics of stem cells obtained from mouse dental pulp." Archives of oral biology vol. 56,11 (2011): 1247-55. doi:10.1016/j.archoralbio.2011.05.008.

[39] Ferrarotti, Francesco et al. "Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial." Journal of clinical periodontology vol. 45,7 (2018): 841-850. doi:10.1111/jcpe.12931.

[40] Zhang, Nan et al. "Tracking of Stem Cells from Human Exfoliated Deciduous Teeth Labeled with Molday ION Rhodamine-B during Periodontal Bone Regeneration in Rats." International journal of stem cells vol. 16,1 (2023): 93-107. doi:10.15283/ijsc21204. .

[41] Miura, Masako et al. "SHED: stem cells from human exfoliated deciduous teeth." Proceedings of the National Academy of Sciences of the United States of America vol. 100,10 (2003): 5807-12. doi:10.1073/pnas.0937635100.

[42] Sakai, V T et al. "SHED differentiate into functional odontoblasts and endothelium." Journal of dental research vol. 89,8 (2010): 791-6. doi:10.1177/0022034510368647.

[43] Liu, Fei et al. "Preliminary study on the mechanism by which exosomes derived from human exfoliated deciduous teeth improve the proliferation and osteogenic inhibitory effect of glucocorticoid-induced BMSCs." Gene vol. 923 (2024): 148575. doi:10.1016/j.gene.2024.148575.

[44] Cordeiro, Mabel M et al. "Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth." Journal of endodontics vol. 34,8 (2008): 962-9. doi:10.1016/j.joen.2008.04.009.

[45] Shi, Xin et al. "Pulp stem cells derived from human permanent and deciduous teeth: Biological characteristics and therapeutic applications." Stem cells translational medicine vol. 9,4 (2020): 445-464. doi:10.1002/sctm.19-0398.

[46] Kémoun, Philippe et al. "Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro." Cell and tissue research vol. 329,2 (2007): 283-94. doi:10.1007/s00441-007-0397-3.

[47] Fonticoli, Luigia et al. "A Narrative Review: Gingival Stem Cells as a Limitless Reservoir for Regenerative Medicine." International journal of molecular sciences Vol. 23, 8 4135. 8 Apr. 2022, doi:10.3390/ijms23084135.

[48] Zhang, Jie et al. "Dental Follicle Stem Cells: Tissue Engineering and Immunomodulation." Stem cells and development vol. 28,15 (2019): 986-994. doi:10.1089/scd.2019.0012.

[49] Tsuchiya, Shuhei et al. "Osteogenic differentiation capacity of porcine dental follicle progenitor cells." Connective tissue research vol. 51,3 (2010): 197-207. doi:10.3109/03008200903267542.

[50] Rezai-Rad, Maryam et al. "Evaluation of bone regeneration potential of dental follicle stem cells for treatment of craniofacial defects." Cytotherapy vol. 17,11 (2015): 1572-81. doi:10.1016/j.jcyt.2015.07.013.

[51] Chen, Gang et al. "Combination of aligned PLGA/Gelatin electrospun sheets, native dental pulp extracellular matrix and treated dentin matrix as substrates for tooth root regeneration." Biomaterials vol. 52 (2015): 56-70. doi:10.1016/j.biomaterials.2015.02.011.

[52] Oshima, Masamitsu et al. "Functional tooth restoration by next-generation bio-hybrid implant as a bio-hybrid artificial organ replacement therapy." Scientific Reports vol. 4 6044. 13 Aug. 2014, doi:10.1038/srep06044.

[53] Choi, Jung-Kwon et al. "The efficiency of the in vitro osteo/dentinogenic differentiation of human dental pulp cells, periodontal ligament cells and gingival fibroblasts." International journal of molecular medicine vol. 35,1 (2015): 161-8. doi:10.3892/ijmm.2014.1986.

[54] Pan, Jie et al. "Thermosensitive Hydrogel Delivery of Human Periodontal Stem Cells Overexpressing Platelet-Derived Growth Factor-BB Enhances Alveolar Bone Defect Repair." Stem cells and development vol. 28,24 (2019): 1620-1631. doi:10.1089/scd.2019.0184.

[55] Shalini, Halappa Sasvehalli, and Kharidhi Laxman Vandana. "Direct application of autologous

periodontal ligament stem cell niche in treatment of periodontal osseous defects: A randomized controlled trial." Journal of Indian Society of Periodontology vol. 22,6 (2018): 503-512. doi:10.4103/jisp.jisp_92_18

[56] Liu, Jiaying et al. "Macrophage polarization in periodontal ligament stem cells enhanced periodontal regeneration." Stem cell research & therapy vol. 10,1 320. 15 Nov. 2019, doi:10.1186/s13287-019-1409-4.

[57] Kadar, K et al. "Differentiation potential of stem cells from human dental origin - promise for tissue engineering." Journal of physiology and pharmacology: an official journal of the Polish Physiological Society vol. 60 Suppl 7 (2009): 167-75.

[58] Pelaez, Daniel et al. "Cardiomyogenesis of periodontal ligament-derived stem cells by dynamic tensile strain." Cell and tissue research vol. 367,2 (2017): 229-241. doi:10.1007/s00441-016-2503-x.

[59] Kim, Dane et al. "Gingiva-Derived Mesenchymal Stem Cells: Potential Application in Tissue Engineering and Regenerative Medicine - A Comprehensive Review." Frontiers in immunology vol. 12 667221. 16 Apr. 2021, doi:10.3389/fimmu.2021.667221.

[60] Subbarayan, Rajasekaran et al. "Gingival spheroids possess multilineage differentiation potential." Journal of cellular physiology vol. 233,3 (2018): 1952-1958. doi:10.1002/jcp.25894.

[61] Xu, X et al. "Gingivae contain neural-crest- and mesoderm-derived mesenchymal stem cells." Journal of dental research vol. 92,9 (2013): 825-32. doi:10.1177/0022034513497961.

[62] Kandalam, Umadevi et al. "Predifferentiated Gingival Stem Cell-Induced Bone Regeneration in Rat Alveolar Bone Defect Model." Tissue engineering. Part A vol. 27,5-6 (2021): 424-436. doi:10.1089/ten.TEA.2020.0052.

[63] Hasani-Sadrabadi, Mohammad Mahdi et al. "An engineered cell-laden adhesive hydrogel promotes craniofacial bone tissue regeneration in rats." Science translational medicine vol. 12,534 (2020): eaay6853. doi:10.1126/scitranslmed.aay6853.

[64] Li, Jing et al. "Treatment of gingival defects with gingival mesenchymal stem cells derived from human fetal gingival tissue in a rat model." Stem cell research & therapy vol. 9,1 27. 5 Feb. 2018, doi:10.1186/s13287-017-0751-7.

[65] Tomasello, Laura et al. "Mesenchymal stem cells derived from inflamed dental pulpal and gingival tissue: a potential application for bone formation." Stem cell research & therapy vol. 8,1 179. 1 Aug. 2017, doi:10.1186/s13287-017-0633-z.

[66] Melzer, Catharina et al. "Taxol-Loaded MSC-Derived Exosomes Provide a Therapeutic Vehicle to Target Metastatic Breast Cancer and Other Carcinoma Cells." *Cancers* vol. 11,6 798. 9 Jun. 2019, doi:10.3390/cancers11060798.

[67] Tatullo, Marco et al. "Promising Scaffold-Free Approaches in Translational Dentistry." *International journal of environmental research and public health* vol. 17,9 3001. 26 Apr. 2020, doi:10.3390/ijerph17093001.