

Mesenchymal stem cells from dental tissues

Alfredo Salazar de Santiago¹, F. Javier Avelar González², Juan Manuel Diaz¹,
E. Eduardo Hernández Cuéllar¹, Adriana C. Moreno Flores¹,
Fabiola Galindo Guerrero¹ and Alma L. Guerrero Barrera^{1,*}

¹Laboratorio de Biología Celular y Tisular, Departamento de Morfología;

²Laboratorio de Ciencias Ambientales, Departamento de Fisiología y Farmacología,
Universidad Autónoma de Aguascalientes, CP 20131, Aguascalientes, Ags., Mexico.

ABSTRACT

Mesenchymal stem cells (MSCs) are non-specialized cells that have the property of self-renewal and differ in several ways from specialized cells. They are identified in a large number of adult tissues, such as skin, adipose tissue, peripheral blood, bone marrow, pancreas, intestine, brain, hair follicles and dental tissues, being an attractive source for tissue engineering. Currently, as MSCs constitute the means by which some tissues are generated during development, they have attracted interest to restore those tissues that are sick or damaged after birth. In recent years, important advances have been made in the research and knowledge of the MSCs and their potential to be used in cell therapies for a wide variety of medical treatments, such as spinal cord injuries, myocardial infarction, Parkinson's disease, diabetes, among others. Because of their location and given that isolating them is not an easy task as there could be clinical complications, it is necessary to identify accessible sources of MSCs in order to be less invasive and traumatic, trying at the same time to guarantee cell viability, and preserving their proliferation and differentiation capacity. Tissues of dental origin are considered an effective and easily accessible source for obtaining MSCs, collectively referred as Dental Stem Cells (DSC), which have been shown to have the ability to generate adherent and clonogenic cell clusters

in vitro. In addition, they proliferative and differentiate into several cell types, representing an alternative to be used in regenerative medicine to treat various types of diseases of immune, degenerative or traumatic origin. The aim of this review is to introduce the concept of mesenchymal stem cells derived from several dental tissues, describe their phenotype, differentiation capacity and their potential use in tissue engineering.

KEYWORDS: mesenchymal stem cells, dental pulp stem cells, gingival stem cells, stem cells of deciduous exfoliated teeth, periodontal ligament stem cells, dental follicle progenitor stem cells, apical papilla stem cells, stem cells of natal dental pulps, alveolar bone-derived mesenchymal stem cells.

1. Introduction

Stem cells (SC) are defined as those immature and non-specialized cells that have the ability to self-renew for long periods through cell division and give rise to many different cell lineages as a result of differentiation, even without having the morphology of a tissue-specific cell [1, 2]. This provides the opportunity to investigate the mechanisms that regulate embryonic development, cell differentiation and organ maintenance. Given their proliferation and differentiation capacity, these cells have great potential to develop alternatives based on cell therapy. The term stem cell was coined in 1868, from the work of the

*Corresponding author: alguerre@correo.uaa.mx

German Ernest Haeckel, one of the main supporters of Darwin's theory of evolution, who drew a series of phylogenetic trees to represent the evolution of descendants with common ancestors. He called these trees "Stammbäume" (from German to refer to genealogical trees or "stem trees"). In addition, he used the term "Stammzelle" (from German for stem cells) to describe that multicellular organisms evolved from unicellular ancestral organisms. Therefore, the term stem cell was first used at the end of the 19th century in the context of fundamental issues in embryology, such as the continuity of the germoplasm and the origin of the blood system. The demonstration of the existence of hematopoietic stem cells established these cells as prototypic stem cells, as cells capable to proliferate almost indefinitely (self-renewal) and giving rise to specialized cells (differentiation) [3, 4]. Currently, stem cells constitute a source that allows tissue to be generated during development and have attracted interest to restore those that are sick or damaged after birth. In recent years, important advances have been made in the knowledge of stem cell biology and its application in the medical field through cell therapy. Similarly, the field of stem cell research has grown exponentially, since it can be applied to a wide variety of medical treatments [5], among them those for spinal cord injuries, myocardial infarction, Parkinson's disease and diabetes, representing an opportunity to improve the quality of life of patients with such conditions [6]. The term "stem cell" was also proposed for scientific use by the Russian histologist Alexander Maximov in 1909, who suggested the existence of hematopoietic stem cells (HSC) with morphological appearance of a lymphocyte, capable of migrating through the blood to microecological niches, a situation that allow them to proliferate and differentiate.

Stem cells are classified according to their origin, into two main types: embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). ES cells are obtained from the internal cell mass of the blastocyst (early stage embryo, consisting of 50-150 cells), after the morula stage. These cells are responsible for forming the three embryonic layers (ectoderm, endoderm and mesoderm), and are capable of developing more than 200 types of

cells, showing a totipotential capacity. In 1998, the first line of human ES cells was established at the University of Wisconsin-Madison. However, obtaining and working with embryonic cells brings with them technical and ethical problems, since they can give rise to a human being due to their totipotential properties, implying the sacrifice of an embryo, and is technically difficult to control their growth, because tumors could arise after their inoculation. In spite of this, ES cells continue to be a theoretically potential source for regenerative medicine and have been used for tissue replacement after an injury or illness, given their capacity for expansion and pluripotentiality [7]. AS cells, mesoderm derivatives, have participation in symmetric and asymmetric cell divisions, maintain a pool of stem cells through cell renewal and differentiation, and provide cells for replacement and repair of normal or injured tissue [2, 8]. In summary, AS cells provide a promising tool for clinical application because of their easy accessibility, and although they have a limited ability to differentiate, they can still give rise to various cell lineages [6]. Thus, despite having a reduced plasticity, AS cells represent a promising source for application in the medical field. Their use can eliminate problems of autoimmune origin and the risk of transmission of pathogens, as they can be used as an autologous transplant [6].

Another group of generated stem cells that have interesting properties for clinical applications are the so-called induced pluripotent stem cells (iPS). Their generation process consists of the conversion of somatic cells through the induction of the transcription factors Oct-4, Sox2, c-Myc and Klf4. iPS cells are similar to ES cells in terms of morphology, proliferation and differentiation capacity, genomic and epigenomic states [9]. Furthermore, stem cells can also be classified according to their ability to differentiate into several tissues: (1) totipotent stem cell - these can give rise to a complete and viable organism, as well as cells of the three germ layers (endoderm, ectoderm and mesoderm). Only the fertilized oocyte and cells during the early stages of cell division after fertilization, up to the 8-cell stage, are considered totipotent, (2) pluripotent stem cells - these cells can give rise to all types of cells derived from the three germ layers, but do not

have the capacity to generate a complete and functional organism. ES and iPS cells are considered pluripotent and in the case of AS cells, scientific debate continues in this regard, (3) multipotent stem cells - these can only produce a limited number of cell types, typically a family of closely related cells. As an example, the hematopoietic stem cell can only give rise to cells of the hematopoietic system (mast cells, macrophages, neutrophils, eosinophils, platelets, erythrocytes, lymphocytes, etc.), (4) unipotent stem cells - these cannot produce any type of cells that are not of the same lineage, but have the capacity of self-renewal, for example, the muscle stem cell [10].

2. Mesenchymal stem cells (MSCs)

Alexander Friedenstein demonstrated in the 70s of the last century, the existence of a population of cells in the bone marrow that were not hematopoietic and that had the ability to self-renew and differentiate to bone tissue [11]. Subsequently, it was established that such cells could possess a great capacity for proliferation and differentiation to mesenchymal tissues that arise embryologically from the mesodermal layer and thus, Caplan in 1991 started to use the term "mesenchymal stem cells" (MSCs) or "mesenchymal Stromal Cells" [12]. MSCs are pluripotent cells that have the ability to differentiate into various cell types, both *in vivo* and *in vitro* [2], they are nonhematopoietic stromal cells that are found in a perivascular niche, adherent in culture, with a spindle or fibroblastoid morphology, besides, they have the potential for clonogenic proliferation and the ability to differentiate into cells of mesodermal origin [13, 14]. It is suggested that they are responsible for normal replacement and maintenance of adult mesenchymal tissues and with regard to tissue engineering, they are the most promising cells as they exhibit plasticity towards various cell lineages of skeletal and connective tissue origin, such as chondrocytes, osteocytes, adipocytes, among others [8, 15]. Due to the capacity of MSCs to differentiate themselves in all mesodermal cell lineages, their potential in mediating tissue regeneration has been investigated, a situation that has allowed us to visualize their capacity for differentiation and pluripotentiality, since they not only give rise to mesodermal tissues, but also

ectodermal and endodermal tissues [14]. MSCs, represent a promising therapeutic alternative and it has been observed that they can mediate paracrine mechanisms independent of cell differentiation. Currently there exists a large number of preclinical and clinical trials in which they are applied; however, the main obstacles that exist are the difficulties to perform the grafts and achieve cell survival, control of the fate of the stem cells and the compatibility between donors and patients in allogeneic applications. Current efforts search to promote the registration and creation stem of cell banks and the systematization of the data associated with the application of these biological materials [8]. The primary source of mesenchymal stem cells is the bone marrow (BM), which is considered to be the largest direct source of stem cells. However, it is possible to find other sources that are present within the human body (Figure 1) such as the pancreas, brain, dental pulp, placenta, adipose tissue, hair follicle, synovial membrane, periodontal ligament, peripheral blood, endometrium, umbilical cord and its blood, amniotic fluid, bone, cartilage, smooth, skeletal and cardiac muscle tissue, liver, spleen, testicles, menstrual blood, periosteum, dermis, pericytes, lung, trabecular bone, among others [13, 15-19].

3. Phenotype of MSCs

In order to determine that MSCs have the characteristics of cells derived from the mesenchyme, in 2006 the International Society of Cellular Therapy (ISCT) established the minimal criteria that differentiate the mesenchymal stem cells from hematopoietic cells: (1) adhesion to culture surface, (2) surface marker expression of endoglin CD105, ecto-5'-nucleotidase CD73 and Thy-1 CD90, (3) lack of hematopoietic markers as CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR; and (4) cell plasticity to differentiate into osteoblasts, chondroblasts and adipocytes [14, 20-22]. In addition to the ISCT proposal, a classification of the CDs expressed on the MSCs has been established, which defines that there are two types of antigens or surface markers expressed: (1) the most expressed markers (CD73, CD90 and CD105), and (2) markers that defines the ability of a cell to function as a stem cell; they are less

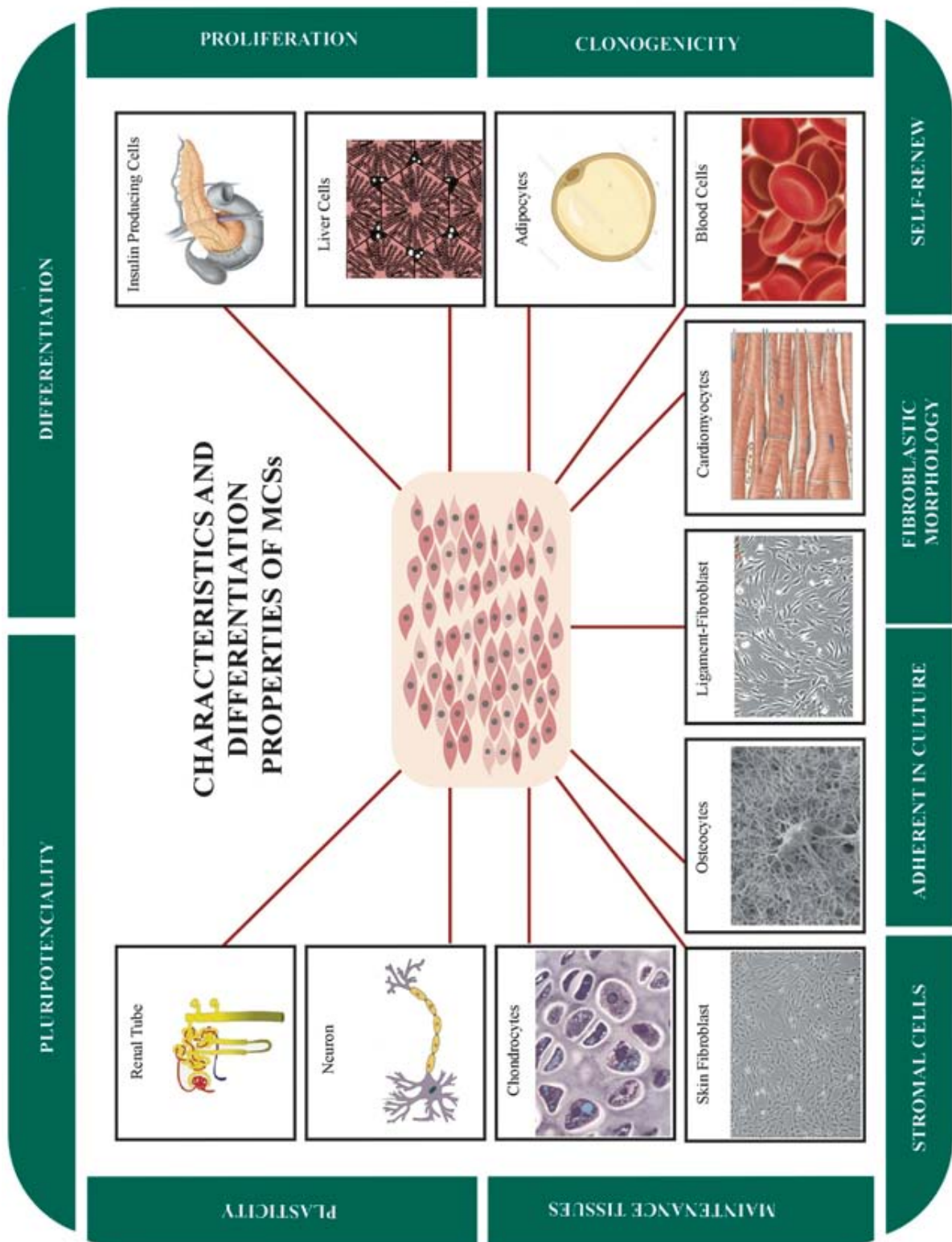


Figure 1. Multilineage differentiation properties and main characteristics of mesenchymal stem cells (MSCs) derived from human tissues.

expressed markers that allow a subset of MSCs to be identified with a great multipotentiality. Both types of markers are found not only in bone marrow-derived MSCs (BM-MSCs), but also in some other cells considered as stem cells [16]. Taking into account other criteria for phenotyping MSCs, it is stated that they must have the capacity to develop self-renewal processes, that is, during cell division only one of the daughter cells must initiate a cell differentiation program. In the same way, they must be able to develop clonogenic plasticity or differentiation towards tissues of different embryonic layers such as ectoderm (e.g., neurons) and endoderm (e.g., hepatocytes). Another condition to be MSCs, is their ability to “Homing” or nesting, that is, the possibility of migrating and accumulating in areas of the body where there is an injury and therefore, greater concentration of chemokines, since they have receptors for them [18]. Based on current knowledge about the wide distribution of MSCs in various tissues of the human body, it has been established that these cells come from blood vessels, a situation that makes them present in virtually all tissues of the human body. Therefore, the number of stem cells in each tissue is limited, and they do not divide under normal circumstances as they are inactive; however, when tissue damage or injury occurs, they act by dividing and differentiating, since their main function consists in repair [23]. A situation that must always be considered in the study of MSCs is that adult tissues and their regeneration capacity depend on their stem cell populations, which are stably renewed and provide a progeny with the ability to differentiate into specialized cells and, due to their location, MSCs have different names depending on the tissue of origin, such as hematopoietic, mesenchymal, endothelial, mammary, intestinal, neural, skin, muscle, dental pulp, hair follicle stem cells, etc. [24].

4. Mesenchymal stem cells derived from dental tissues

Due to their diverse locations within the human body, isolating MSCs is not an easy task, mainly because of the complications that can ensue. Hence it is necessary to identify those sources that allow a less invasive method without affecting their proliferation capacity. Therefore, currently

dental tissues are considered an effective and easily accessible source for obtaining MSCs [25], which have been collectively called Dental Stem Cells (DSCs) [26]. Dental organs can be lost by natural exfoliation [27], as occurs in temporary dentition after the age of 6 years and due to pathological reasons such as periodontal disease, trauma or surgically as in the case of third molars, which can be extracted by therapeutic indication of orthodontics or oral prostheses [28], being a recurring clinical-dental practice. Since they can be the cause of other affectations [29] and being the last dental organs to erupt, they are in a more advanced state of embryonic development [30]. Interestingly, DSCs can also be obtained from damaged dental tissues, and their properties are similar to those obtained from healthy tissues [26]. In recent years, various types of adult stem cells have been isolated from: (1) Dental pulp stem cells (DPSCs), (2) Gingival stem cells (GSCs), (3) Stem cells of deciduous exfoliated teeth (SHED), (4) Periodontal ligament stem cells (PDLSCs), (5) Dental follicle progenitor stem cells (DFPCs), (6) Apical papilla stem cells (SCAPs), (7) Stem cells of natal dental pulps (NDP-SCs), and (8) Alveolar bone-derived mesenchymal stem cells (ABMSCs) [15, 26, 28, 31-36] (Figure 2). Through the investigations carried out so far, it has been observed that DSCs are cell populations similar to MSCs, in addition to their ectomesenchymal origin, which means, they can show characteristics of both mesoderm and ectoderm. This fact is very important, since the association of mesenchymal and epithelial cells is necessary to regenerate or build a new tooth [26]. It is a relevant fact to mention that DSCs have a capacity for self-renewal and multidifferentiation potential, being able to differentiate into osteoblastic, odontogenic, dentinogenic, cementogenic, adipogenic, chondrogenic, myogenic and neurogenic tissues, and still maintain their multipotent properties after short-and long-term cryopreservation [36]. Some of their characteristics and similarities are shown in Table 1.

4.1. Human dental pulp stem cells (hDPSCs)

The search for alternative lineages to obtain MSCs has been part of the work in this field, since the bone marrow obtention shows limitations such as

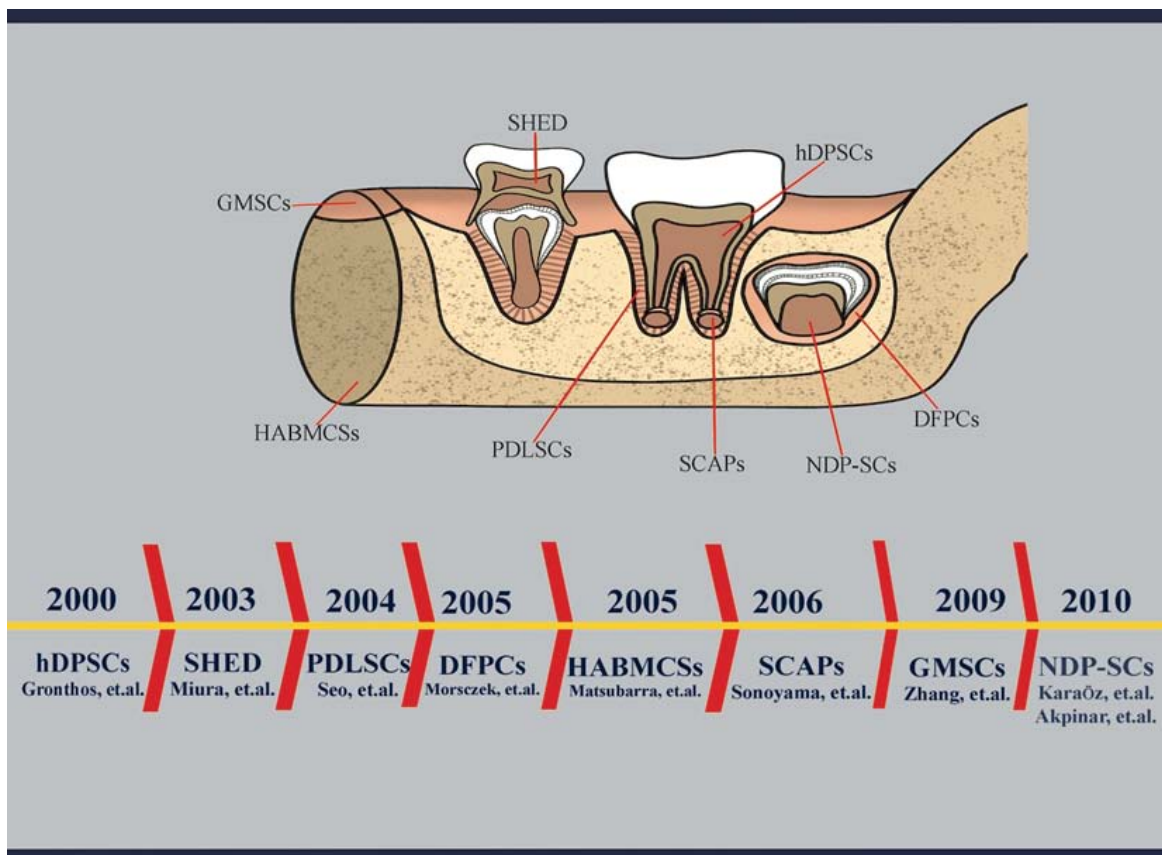


Figure 2. Schematic view illustrating the different sources of MSC derived from human dental tissue (DSCs) and the timeline about the historical aspects of their isolation and characterization. hDPSCs: human dental pulp stem cells, GMSCs: gingival stem cells, SHED: stem cells of human exfoliated deciduous teeth, PDLSCs: periodontal ligament stem cells, DFPCs: precursor cells of the dental follicle, SCAPs: stem cells of the dental apical papilla, NDP-SCs: stem cells of human natal dental pulps, HABMSCs: human alveolar bone-derived mesenchymal stem cells.

the risk of taking the sample and the donor's own health condition. Therefore, the use of the less-invasive MSC lineages such as hDPSCs, that have a greater proliferation capacity, is an alternative to obtain pluripotent cells. Human dental pulp stem cells have greater advantages over those derived from bone marrow, adipose tissue, peripheral blood or umbilical cord blood, since their sources are teeth extracted by therapeutic indication, with fewer post-surgical complications and involving few ethical implications [37]. Dental pulp is a soft connective tissue located inside the dental crown divided into four cell layers: (1) the outer layer composed of odontoblasts that produce dentin, (2) the second layer is poor in cells and rich in collagen fibers, (3) the third layer contains progenitor cells and undifferentiated cells, some

of which are considered stem cells. From the last layer, undifferentiated cells migrate to various places where they can differentiate under different stimuli and create new differentiated cells and tissues, (4) the innermost layer is the central pulp chamber of the dental pulp and comprises the vascular area containing nerves [8]. Human dental pulp stem cells were discovered by Gronthos *et.al.* in 2000, who described them as a new type of adult human dental pulp stem cells with the ability to differentiate into odontoblasts/osteoblasts, adipocytes and neuronal cells [38]. Since hDPSCs are considered as a population of MSCs, the same surface markers have been used for their characterization. In addition to CD146 and STRO-1, DPSCs are positive for other stromal-associated markers, such as CD9, CD10, CD13, CD29,

Table 1. Profile of the surface markers and potential for differentiation of stem cells derived from dental tissues (DSCs). hDPSCs: human dental pulp stem cells, GMSCs: gingival stem cells, SHED: stem cells of human exfoliated deciduous teeth, PDLSCs: periodontal ligament stem cells, DFPCs: precursor cells of the dental follicle, SCAPs: stem cells of the dental apical papilla, NDP-SCs: stem cells of human natal dental pulps, HABMSCs: human alveolar bone-derived mesenchymal stem cells.

| Type of DSCs | Tissue origin | Markers | | Potential of differentiation | Reference |
|--------------|--------------------------------|---|-------------------------------|---|---|
| | | Positives | Negatives | | |
| hDPSCs | Dental pulp | CD146, STRO-1, CD9, CD10, CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD166, CD271, Oct-4, Nanog, Sox-2, Nestin, Vimentin ALP, COL1, ON, OPN, OCN, BSP, α -Actin, COL3, FGF2 | CD14, CD34, CD45, HLA-DR | Odontoblasts, Osteoblasts, Adipocytes, Neuronal Cells, Chondrocytes, Myocytes, Cardiomyocytes, Melanocytes, Hepatocytes, Epithelial Cells of the Cornea, Melanoma Cells, iPS, Insulin Producing Cells | [6] [13] [17] [25] [26] [35] [37] [38] [39] [40] [41] [42] [58] [77] [78] |
| GMSCs | Gum | CD13, CD29, CD44, CD73, CD90, CD105, CD146, STRO-1, Oct-4, SSEA-4 | CD14, CD34, CD45, HLA-DR | Osteoblasts, Adipocytes, Chondrocytes, Endothelial Cells, Neuronal Cells, Endodermal lineage cells | [25] [43] [44][45] [46] [76] |
| SHED | Dental pulp of deciduous teeth | STRO-1, CD146, CD13, CD29, CD44, CD73, CD90, CD105, CD166, SSEA4 Nestin, β III-tubulin, GAD, NeuN, GFAP, NFM CNPase, Sox-9, COL2, COL10 | CD14, CD34, CD45, HLA-DR | Odontoblasts, Osteoblasts, Adipocytes, Neuronal Cells, Endothelial Cells | [47] [48] [50] [51] [52] |
| PDLSCs | Periodontal ligament | STRO-1, CD146, Scleraxis, CD9, CD10, CD13, CD26, CD26, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166 | CD14 CD19, CD34, CD45, HLA-DR | Osteoblasts, Cementoblasts, Adipocytes, Chondrocytes, Collagen-forming Cells Insulin-producing Cells | [13] [25] [26] [42] [53] [54] [55] [56] [57] [58] |

Table 1 continued..

| Type of DSCs | Tissue origin | Markers | | Potential of differentiation | Reference |
|--------------|---|---|--|---|------------------------------------|
| | | Positives | Negatives | | |
| DFPCs | Dental Follicle of Teeth in formation | STRO-1, CD13, CD29, CD44, CD56, CD73, CD90, CD105, CD271, HLA-ABC, Oct3/4, Sox-2, Nanog, Notch1, β III-tubulin, P75, HnK1, GAPF, Nestin | CD34, CD45, CD117, CD133, HLA-DR | Periodontal Ligament Fibroblast, Enamel Matrix Derivates, Osteoblasts, Adipocytes, Cardiomyocytes, Chondrocytes, Neurons, Hepatocytes, Salivary Gland Cells, Ductal Cells | [25] [26] [60] [62] [63] |
| SCAPs | Apical Papilla of Teeth in formation | STRO-1, CD146, CD24, CD13, CD29, CD44, CD49, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166, Oct3/4, Sox-2, Nanog, Notch3, Vimentin, Survivin | CD14, CD18, CD34, CD45, CD117, CD150 | Osteoblasts, Odontoblasts, Adipocytes, Chondroblasts, Neurogenic Cells, Hepatocytes | [13] [25] [26] [65] [66] [67] |
| NDP-SCs | Dental Pulp of Natal and Neonatal Teeth | CD13, CD29, CD44, CD73, CD90, CD146, CD166, HLA-ABC, Nanog, Rex-1, Oct-4, Sox-2, FoxD3, COL1, COL2, OCN, ON, OPN, BMP-2, BMP-4, Desmin, Nestin, Vimentin, GFAP, Myogenin, β III-tubulin | CD14, CD34, CD45, CD106, CD117, HLA-DR | Adipocytes, Chondroblasts, Osteoblasts, Myocytes, Neurogenic cells, Pancreatic Islet Cells | [25] [68] [69] [70] [71] [72] [73] |

Table 1 continued..

| Type of DSCs | Tissue origin | Markers | | Potential of differentiation | Reference |
|--------------|---------------|---------------------------|------------------|--|----------------|
| | | Positives | Negatives | | |
| HABMCSs | Alveolar bone | CD73, CD90, CD105, STRO-1 | CD14, CD34, CD45 | Osteoblasts, Chondroblasts Adipocytes Muscle Neuronal Tissue | [74] [75] [76] |

CD44, CD73, CD90, CD105, CD106, CD146, CD166 and CD271 and are negative for hematopoietic markers, such as CD14, CD34 and CD45, and HLA-DR, although some works have reported a positive expression of CD34. The collection of dental pulp stem cells is a common non-invasive surgical practice that can be performed specially in adults, with the third molar pulp tissue being the most common source, as they are often discarded. Undifferentiated hDPSCs also express Oct-4, Nanog and Sox-2, besides the cytoskeletal proteins nestin and vimentin, all of which are characteristic of undifferentiated ES. In addition to the aforementioned stem cell markers, DPSCs show positive expression for a variety of markers associated with bone tissue, such as alkaline phosphatase (ALP), type I collagen (COL1), osteonectin (ON), osteopontin (OPN), osteocalcin (OCN) and bone sialoprotein (BSP), smooth muscle α -actin and from fibroblasts, the type III collagen (COL3) and fibroblast growth factor 2 (FGF2) [7, 18, 26, 39]. With the work of Shinya Yamanaka and John Gurdon in 2012 about iPSCs derived from adult somatic cells, it was proposed that stem cells derived from human dental pulp can develop iPSCs and be used in various therapies for human diseases [18]. There are very promising clinical trials on the treatment of diseases such as type 1 diabetes, neurological diseases, immunodeficiencies and bone and cartilage diseases through the use of hDPSCs [38, 40]. These cells have a rapid cell division and proliferation time, possess immunosuppressive properties and tend to form a pulp-dentine complex similar to the natural one. In terms of their ability to differentiate *in vitro*, it has been established that due to the acquisition of specific

genetic markers, they can be differentiated into osteoblasts, chondrocytes, myocytes, cardiomyocytes, melanocytes, hepatocyte-like cells, adipocytes, active neurons, epithelial cells of the cornea, melanoma cells and even induced pluripotent stem cells (iPS) [14, 36, 41]. Their differentiation to a specific cell lineage is mainly determined by the components of the local microenvironment, such as, growth factors, receptor molecules, signaling molecules, transcription factors and extracellular matrix proteins [27, 42-43].

4.2. Gingival stem cells (GMSCs)

The gum is the band of tissue of the masticatory mucosa that surrounds the necks of the erupted teeth and is attached to the alveolar bone of the dental cavities. It is recognized as an immunological barrier of the oral mucosa and as a component of the periodontium. Embryologically, the gum connective tissue is derived from both the neural crest and the mesenchyme. Histologically, the gum is composed of three layers: an epithelial layer, a basal layer and a lower spinous layer that is similar to the dermis and anatomically divided into: (1) free gum, (2) gum inserted and (3) interdental gum. Zhang and his collaborators in 2009 were the first to report the isolation of GMSCs from the spinous layer of the human gum. The gum represents the most accessible, abundant, conservative and minimally invasive source for the isolation of stem cells from the oral cavity, which can be isolated by enzymatic digestion or by culture of explants from normal gum or with gingivitis or hyperplasia, and from the attached and free gum. Periodontal lesions have a potential for regeneration given the presence of cells similar to MSCs, which have immunophenotypic

characteristics similar to those found in healthy periodontal tissue [26, 44]. The GMSCs have the characteristics that the International Society of Cellular Therapy (ISCT) suggest for multipotent mesenchymal cells; they attach onto the culture surface when they are maintained in standard culture, proliferate *in vitro*, show fibroblastic morphology, form colonies and have the capacity to differentiate into adipocytes or osteocytes. Additionally, the GMSCs derived from individual colonies have *in vivo* self-renewal and differentiation capabilities, high proliferation and duplication rates [45]. They show a stable phenotype and maintain a normal karyotype and telomerase activity in long-term cultures. With regard to the expression of surface markers, GMSCs are positive for CD13, CD29, CD44, CD73, CD90, CD105, CD146, STRO-1, Oct-4 and SSEA-4. They lack expression of the hematopoietic markers CD14, CD34 and CD45, and HLA-DR. Gingival tissues can be obtained with minimally invasive procedures at any time in life [26, 46-47]. Therapeutically, these cells have been used to repair skin wounds, periodontal lesions and regeneration of bone defects. They are also known to have antitumor effects [44].

4.3. Stem cells of exfoliated deciduous teeth (SHED)

During the change of temporary teeth to adult permanent pieces, their development and eruption is coordinated by the resorption of the roots of the temporary teeth [14]. Thus, after the physiological detachment of the temporary pieces the pulp tissue remnants in the site represent an alternative and easily accessible MSC source.

Stem cells of exfoliated deciduous teeth (SHED) were first identified by Miura *et al.* in 2003 [48] and it has been suggested that exfoliated teeth have similarity to the umbilical cord because they contain stem cells that can offer a unique resource with possible clinical applications due to their pluripotent capacity and regenerative potential, with SHED being considered a unique population of human postnatal MSCs [49-53]. SHED have a high proliferation rate, even more than those of dental pulp, periodontal ligament and bone marrow; they also have a clonogenic and self-renewal capacity and, similar to the MSCs, exhibit

a fibroblast-like morphology, and adhere to the surface of tissue culture plastic. They can be isolated and expanded in *ex vivo* conditions, and also they have high biosecurity because they are autologous and non-immunogenic, with fewer controversies associated with morality and ethics. SHED express ES markers, in addition to multipotential differentiation capacity towards odontoblasts, osteoblasts, adipocytes, neuronal and endothelial cells [48-49, 51-53]. SHED express the mesenchymal STRO-1 and CD146, in addition to CD13, CD29, CD44, CD73, CD90, CD105, CD166 and SSEA4 and are negative for CD14, CD34, CD45 and HLA-DR. They are also positive for several neuronal and glial markers, such as nestin, β III-tubulin, glutamic acid decarboxylase (GAD), neuronal nuclei (NeuN), fibrillar glial acid protein (GFAP), neuro filament M (NFM) and 2', 3'- Cyclic nucleotide-3'-phosphodiesterase (CNPase), possibly due to the origin of the neural crest of the dental pulp. Likewise, they have been shown to express the chondrogenic markers Sox-9, type II collagen (COL2) and type X collagen (COL10) [26-27, 48].

4.4. Periodontal ligament stem cells (PDLSCs)

The periodontal ligament is one of the highly specialized and complex connective tissues of the human body; it is located between the cement and the inner wall of the alveolar bone cavity. The periodontal ligament not only plays an important role in the support of the teeth, but also contributes to dental nutrition, homeostasis and repair of damaged tissue and contains a heterogeneous cell population, including the epithelial Malassez' rests [14, 54]. Periodontal tissues arise from cells that migrate from the neural crest during tooth development; however, stem cells derived from the periodontal ligament (PDLSCs) obtained from mature periodontal ligaments possess properties of stem cells similar to MSCs instead of the neural crest cells. PDLSCs were first isolated in 2004 by Seo [55] and by Trubiani in 2005 [54]. These cells can be obtained from periodontal ligament tissue after their separation from the root surface of the extracted third molars [26], which can be expanded *ex vivo* and provides an exceptional reservoir of autologous stem cells with phenotype similar to BM-MCSs, but with greater cell proliferation, and a capacity for self-renewal.

It has been shown that when cultured, they reach up to fifteen passages without showing signs of senescence. Primary cultures of PDLSCs present colonies of bipolar fibroblastic cells with oval nuclei that contain two or three nucleoli and through ultrastructural analysis, they show a large cytoplasm, large amounts of rough endoplasmic reticulum, abundant mitochondria, lysosomal bodies and a dispersed chromatin nuclei, indicating a state of active genetic transcription. Its cytoplasmic membrane makes contact with neighboring cells and has numerous filopodia; desmosome junctions can also be detected [54, 56]. The pluripotent capacity of PDLSCs as well as MSCs is proved by the expression of the STRO-1 and CD146 markers, which means that these cells might even be derived from a population. They presented specific high levels of scleraxis, a selected transcription factor of the sinew. In addition to the above, they express the surface markers CD9, CD10, CD13, CD26, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146 and CD166, and are negative for CD14, CD19, CD34, CD45 and HLA-DR. They also express the following proteins: CLPP, NQO1, SCOT1, a new isoform of TBB5 and DDAH1 that are not present in BM, involved in cell cycle regulation and that react to stress, orientation and detoxification. It has been shown that PDLSCs are multipotent and have great plasticity, as they have the ability to differentiate into osteoblasts, cementoblasts, adipocytes, chondrocytes and collagen-forming cells, in addition to insulin-producing cells, which implies that these cells could also generate cells of the endodermal lineage. Finally, they have the capacity of pluripotentiality being able to differentiate into cells of the three germ layers [14, 26-27, 43, 54-59].

4.5. Dental follicle progenitor stem cells (DFPCs)

The dental eruption through osteoclastogenesis and osteogenesis is regulated by the dental follicle which is an ectomesodermic coat of fibrous connective tissue that surrounds the enamel organ and the dental papilla of the developing dental germ before the eruption. Cementoblast, periodontal ligament and osteoblast are derived from dental follicles that regulate the dental eruption and have progenitor clonogenic and fibroblastoid cells that allow the periodontium formation [26, 60-63].

The dental follicle progenitor stem cells (DFPCs) are the most commonly isolated cells of the follicular sac of third molars removed, and unlike those derived from BM can be easily collected, in a less invasive way and without ethical problems, presenting great capacity to adhere to the plastic surface and self-renewal in cell culture. Comparing them with other stem cells derived from dental tissues, the DFPCs have great proliferative capacity, superior pluripotentiality and a high immunosuppressive effect, and, like those derived from the apical papilla, represent a group of cells derived from developing tissue, a situation that gives them greater plasticity and favors their use in tissue engineering [60, 61-62, 64]. Due to their pluripotentiality, DFPCs can differentiate into periodontal ligament fibroblasts [27], enamel matrix derivatives, osteoblasts, adipocytes, cardiomyocytes, chondrocytes, neurons, hepatocytes, salivary gland cells and ductal cells under appropriate induction conditions. Likewise, combining different scaffolds in suitable microenvironments, they can form different tissues. Recently, it has been discovered that DFPCs perform immunosuppressive functions for innate and acquired immunity systems and have been used in the treatment of autoimmune diseases, allergic diseases and inflammatory diseases in animal models [14, 60, 61]. Regarding the expression of markers, DFPCs express STRO-1, Oct3/4, Sox-2, Nanog, Notch1, beta-III-tubulin (an early neuronal marker), markers of neural crest (P75 and HnK1) and glial stem cells (GFAP), as well as nestin, both in the nucleus and in the cytoplasm and are also positive for CD13, CD29, CD44, CD56, CD73, CD90, CD105, CD271 and HLA-ABC which indicates that these cells are mesenchymal and pluripotential. Likewise, they are negative for markers of hematopoietic lineage CD34, CD45, CD117, CD133 and HLA-DR [26, 61, 63, 64].

4.6. Apical papilla stem cells (SCAPs)

In 2006, Sonoyama and collaborators were the first to describe the presence of stem cells derived from the apical papilla of permanent teeth and human exfoliated deciduous teeth (SCAPs) [65]. The apical papilla is the tissue of the apical part of what during the embryological development of the tooth is known as the dental papilla, involved in the interactive process between the mesenchyme

and buccal epithelium that leads to the development of the tooth. To refer specifically to the apical part of the papilla, at least two thirds of the root of the developing dental organ must have been formed, since the formation of the root begins as the cervical loop epithelial cells proliferate apically and influence the differentiation of odontoblasts from undifferentiated mesenchymal cells and cementoblasts from follicular mesenchyme. Histologically, there is an area rich in cells that lies between the apical papilla and the pulp. The apical papilla is different from the pulp in terms of containing fewer cellular and vascular components; however, the apical papilla contains a greater number of MSCs than the pulp tissue. Although both the dental pulp and the apical papilla are basically a continuity with each other, when the pulp is affected by an injury, the apical papilla responds trying to control the damage and tends to survive due to its accessibility to an apical collateral circulation. The apical papilla and therefore the SCAPs can be easily isolated after a dental extraction, although they can only be isolated at a specific stage of dental development, since the apical papilla evolves into dental pulp during the formation of the crown and the root. These types of cells are characterized by their great plasticity, high proliferative potential, capacity for self-renewal, pluripotentiality, low immunogenicity, clonogenicity and have a fibroblastic form. SCAPs have shown better proliferation properties than DPSCs and it has been confirmed that cryopreservation does not affect their biological and immunological properties nor does it affect its cell regeneration potential [26, 65-67]. The SCAPs differentiate into different cell types such as osteoblast, odontoblasts, adipocytes, chondroblast, neurogenic cells and hepatocytes; the differentiation process depends on the media used. The SCAPs develop near highly vascularized and innervated locations; thus it can be a good therapeutic alternative for the regeneration of damaged tissues caused by several diseases.

SCAPs could be the source of primary odontoblasts involved in the development of root dentin, in contrast to DPSCs, which are probably involved in the formation of restorative dentin [66-68]. Due to their perivascular location of the SCAPs, they

show positive expression of the STCS-1 and CD146 MSCs markers, although some differences have been found in the amount of expression due to the different positions in the forming dental root, which also defines the orientation towards a specific lineage at the time of differentiation. It has been found that the CD24 pluripotency marker is directly related to this type of cells, and research of SCAPs' differentiation to osteoblasts showed that an increase in the level of alkaline phosphatase (ALP) and a decrease in CD24 indicate that the cells have started to leave their undifferentiated state. These cells positively express CD13, CD29, CD44, CD49, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166, Oct 3/4, Sox-2, Nanog, Notch 3, vimentin and survivin. In addition to these superficial and intracellular molecules, the secretome of SCAPs have also been well described, since they have been shown to release a total of 2046 proteins, including chemokines, angiogenic factors, immuno modulators, antiapoptotics, neuroprotectors and extracellular matrix proteins. Significantly, they secrete more chemokines, neurotrophins and proteins that participate in metabolic processes and transcription compared to BM-MCSs. SCAPs are negative for CD14, CD18, CD34, CD45, CD117 and CD150 markers [14, 26-27, 66-68].

4.7. Stem cells of human natal dental pulps (NDP-SC)

Natal teeth are deciduous teeth that are present in the newborn and erupt in the first 30 days after birth. They are smaller than primary teeth and have little or no root development. Its incidence is very rare; however it is estimated that they occur in 1 of every 2000 or up to 3500 births. They are considered to be supernumerary pre-deciduous teeth (<10%) or series of deciduous teeth of premature eruption (> 90%), which tend to appear instead of lower primary central incisors (85%). For these types of teeth a tooth extraction is indicated, as they can induce deformity or mutilation of the tongue, which leads to dehydration, inadequate absorption of nutrients by the infant, followed by stunted growth and risks to the mother during lactation. The extracted teeth are normally considered a biological waste; however, the dental pulp present within the tooth is a

rich source of stem cells (NDP-SCs), which can potentially be used for regenerative medicine [26, 69-71]. hNDP-SCs were characterized by Karaöz *et al.* in 2010, who described them as a population similar to stem cells, metabolically active and at the peak of their activity, expressed markers of embryonic MSCs but did not exhibit that of hematopoietic markers. Research has shown that NDP-SCs show greater proliferation activity than SHED and DPSCs. From the work carried out to analyze the differentiation potential of NDP-SCs, their ability to move towards adipogenic, chondrogenic, osteogenic, myogenic and neurogenic lineages and similarly, a successful *in vitro* differentiation of NDPSCs into cells that were morphologically similar to pancreatic islet cells have been demonstrated [71-73]. They express the surface markers CD13, CD29, CD44, CD73, CD90, CD146, CD166 and HLA-ABC, while the CD14, CD34, CD45, CD106, CD117 and HLA-DR markers are negative. In addition, they also express detectable levels of the embryonic stem cells markers Nanog, Rex-1 and Oct-4, as well as the transcription factors Sox-2 and FoxD3, suggesting that these cells show some of the characteristics of pluripotentiality. The NDP-SC expresses a positive reaction for the osteogenic markers COL1, OCN, ON, OPN, BMP-2 and BMP-4, for some myogenic markers, such as desmin and myogenin, the chondrogenic marker COL2, as well as for the nestin neural markers, vimentin, GFAP and β III-tubulin [26, 74].

4.8. Human alveolar bone-derived mesenchymal stem cells (HABMSCs)

Bone marrow stromal cells (BMSCs) can be differentiated into a variety of tissues such as bone, cartilage, tendon, muscle, adipose tissue and neuronal tissue, and their transplantation promotes the regeneration of various tissues. BMSCs have been isolated from several bones, including iliac crest, femur, tibia and spine, but it is unknown if their proliferation and differentiation potentials depend on their location *in vivo*. The use of bone marrow cells is common in regenerative medicine. However, their obtention has several complications as bone marrow aspiration is an invasive and traumatic procedure.

BMSCs derived from the alveolar bone have been obtained as an alternative and less invasive source

of MSCs and by virtue of the fact that it is feasible to obtain them during the surgical extraction of third molars [75]. Matsubara *et al.* reported in 2005 the successful isolation and culture of mesenchymal stem cells derived from human alveolar bone (HABMSC). This type of cells, like other DSCs, show a fibroblast-like morphology, in the form of a spindle, with clonogenic capacity and adhesion to plastic and a greater osteogenic proliferation and differentiation than those obtained from this same source of long bones and iliac crest. This could provide an advantage for bone regeneration at the orofacial level; however, since the amount of these cells could be lower compared to that from other bones, a reliable and safe cell expansion protocol should be established when ABMSCs are used in clinical trials. These cells express the surface markers CD73, CD90, CD105 and STRO-1 but do not express the hematopoietic markers CD14, CD34 and CD45 [76, 77].

5. Conclusions

Nowadays, during the treatment of several diseases that provoke tissue losses or cause irreversible tissue damage, stem cell obtention and its use is one of the main goals to be achieved. Thus, several research groups are seeking methods to obtain these cells using less-invasive and -traumatic procedures that guarantee the viability, proliferation and pluripotentiality of the cells obtained. As was explored in this review, several lineages of dental stem cells can be obtained from patients after dental extraction surgery, such as DPSCs, GSCs, SHED, PDLSCs, DFPCs, SCAPs, hNDP-SCs and HABMSCs.

The advantages of the stem cells obtained from dental tissues are among others, their pluripotent phenotype, the proliferation characteristics during several culture passages and the possibility to obtain specific cell types depending on the culture conditions that induce their differentiation. Thus, these tissues represent a promising autologous source for tissue regeneration. Autologous cells have been privileged to bypass immunological barriers, diminishing the immunological reject risk of the differentiated cells obtained. More multidisciplinary studies are necessary in order to advance in this matter.

ACKNOWLEDGEMENTS

The present work was supported by PRODEP Folio No. UAZ-399, Conventional Support for Doctoral Studies, letter number 511-6/17-688. The project was also supported by Special Resource UAA for Research 2017 and 2018. It was also supported by Social Impact Projects of the UAA Special Resource.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Egusa, H., Sonoyama, W., Nishimura, M., Atsuta, I. and Akiyama, K. 2012, *J. Prosthodont. Res.*, 56, 151.
2. Oláñez, D., Salmen, S., Padrón, K., Lobo, C., Díaz, N., Berrueta, L. and Solórzano, E. 2014, *Univ. Odontol.*, 33, 187.
3. Ramalho-Santos, M. and Willenbring, H. 2007, *Cell Stem Cell*, 1, 35.
4. Rodríguez-Hernández, C., Torres-García, S., Olvera-Sandoval, C., Ramírez-Castillo, F., Loera-Muro, A., Avelar-González, F. and Guerrero-Barrera, A. 2014, *Int. J. Curr. Res. Aca. Rev.*, 2, 188.
5. Sun, H., Chen, B., Zhu, Q., Kong, H., Li, Q., Gao, L., Xiao, M., Chen, F. and Yu, Q. 2014, *Biomaterials*, 35, 9459.
6. Fortier, L. A. 2005, *Vet. Surg.*, 34, 415.
7. Rodríguez-Lozano, F. J., Insausti, C. L., Iniesta, F., Blanquer, M., Ramírez, M. C., Meseguer, L., Meseguer-Henarejos, A. B., Marín, N., Martínez, S. and Moraleda, J. M. 2012, *Med. Oral Patol. Oral Cir. Bucal.*, 1, e1062-7.
8. Collart, P., Chaubron, F., De Vos, J. and Cuisinier, F. 2015, *World Journal of Stem Cells*, 7, 1010.
9. Amabile, G. and Meissner, A. 2009, *Trends in Molecular Medicine*, 15, 59.
10. Leeb, C., Jurga, M., McGuckin, C., Moriggl, R. and Kenner, L. 2010, *Stem Cell Rev.*, 3, 15.
11. Friednestein, A., Chailakhyan, R. and Gerasimov, U. 1987, *Cell Tissue Kinet.*, 20, 263.
12. Caplan, A. I. 1991, *J. Orthop. Res.*, 9, 641.
13. Arévalo, J., Páez, D. and Rodríguez, V. 2007, *Nova, Pub. Cient. en Ciencias Biom.*, 5, 177.
14. Liu, J., Yu, F., Sun, Y., Jiang, B., Zhang, W., Yang, J., Xu, G., Liang, A. and Liu, S. 2015, *Stem Cells*, 33, 627.
15. Brizuela, C., Galleguillos, S., Carrión, F., Cabrera, C., Luz, P. and Inostroza, C. 2013, *Int. J. Morphol.*, 31, 739.
16. Lv, F., Tuan, R. S., Cheung, K. M. C. and Leung, V. Y. L. 2014, *Stem Cells*, 6, 1408.
17. Camejo, M. V., Merentes, E., Payares, G., Márquez, M. L. and Espinoza, M. 2015, *Acta Odontológica Venezolana*, 53, 1.
18. Potdar, P. D. and Jethmalani, Y. D. 2015, *World J. Stem Cells*, 7, 839.
19. Guadix, J. A., Zugaza, J. L. and Gálvez, P. 2017, *Medicina Clínica (Barc.)*, 3896.
20. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R. J., Keating, A., Prockop, D. J. and Horwitz, E. M. 2006, *Cytotherapy*, 8, 315.
21. Otsuru, S., Hofmann, T. J., Olson, T. S., Dominici, M. and Horwitz, E. M. 2013, *Cytotherapy*, 2, 146.
22. Cortes, Y., Ojeda, M., Araya, D., Dueñas, F., Fernández, M. S. and Peralta, O. A. 2013, *BMC Vet. Res.*, 9, 133.
23. Agha-Hosseini, F., Jahani, M.-A., Jahani, M., Mirzaii-Dizgah, I. and Ali-Moghaddam, K. 2010, *Clin. Transplant.*, 24, E23.
24. Ledesma-Martinez, E., Mendoza-Nunez, V. M. and Santiago-Osorio, E. 2016, *Stem Cells International*, 2016, 4709572.
25. Peng, L., Ye, L. and Zhou, X. D. 2009, *Int. J. Oral. Sci.*, 1, 6.
26. Zavan, B. and Bressan, E. 2016, *Dental Stem Cells: Regenerative Potential*, Humana Press, Switzerland.
27. Estrela, C., Alencar, A. H., Kitten, G. T., Vencio, E. and Gava, E. 2011, *Braz. Dent. J.*, 22, 91.
28. Seedgley, C. and Botero, T. 2012, *Dental Clinics of North America*, 56, 549.
29. Olate, S., Alistar, J. P., Alveal, R., Thomas, D., Soto, M., Mancilla, P. and Ceballos, M. 2007, *Int. J. Odontomat.*, 1, 29.

30. Mori, G., Brunetti, G., Oranger, A., Carbone, C., Ballini, A., Lo Muzio, L., Colucci, S., Mori, C., Grassi, F. R. and Grano, M. 2011, *Ann. N.Y. Acad. Sci.*, 1237, 47.
31. Honda, M. J., Imaizumi, M., Tsuchiya, S. and Morsczek, Ch. 2010, *J. Oral. Sci.*, 52, 541.
32. Volponi, A., Pang, Y. and Sharpe, P. 2010, *Trends Cell Biol.*, 20,715.
33. Rodríguez, F. J., Bueno, C., Insausti, C. L., Meseguer, L., Ramírez, M. C., Blaquer, M., Marín, N., Martínez, S. and Moraleda, J. M. 2011, *Int. Endod. J.*, 44, 800.
34. Karamzadeh, R. and Baghaban, M. 2013, *IntechOpen*, London.
35. Junjun, L. and Shangfeng, L. 2017, *Stem Cells in Toxicology and Medicine*, John Wiley&Sons, U.S.
36. Botelho, J., Cavacas M. A., Machado, V. and Mendes, J. J. 2017, *Annals of Medicine*, 49, 644.
37. Nakashima, M., Iohara, K. and Sugiyama, M. 2009, *Cytokine&Growth Factor Reviews*, 20, 435.
38. Gronthos, S., Mankani, M., Brahimi, J., Robey, P. G. and Shi, S. 2000, *Proc. Natl. Acad. Sci. USA*, 97, 13625.
39. Kawashima, N. 2012, *Archives of Oral Biology*, 57, 1439.
40. Kanafi, M., Rajeshwari, Y., Gupta, S., Dadheech, N., Nair, P., Gupta, P. and Bhonde, R. 2013, *Cytotherapy*, 15.
41. Nuti, N., Corallo, C., Chan, B., Ferrari, M. and Gerami-Naini, B. 2016, *Stem Cell Rev. and Rep.*, 12, 511.
42. Munévar, J. C., Becerra, A. and Bermúdez, C. 2008, *Acta Odont. Ven.*, 46, 1.
43. Yu, J., He, H., Tang, Ch., Zhang, G., Li, Y., Wang, R., Shi, J. and Jin, Y. 2010, *BMC Cell Biol.*, 11, 32.
44. Grawish, M. 2018, *World Journal of Stem Cells*, 10, 116.
45. Venkatesh, D., Mohan, K. K. and Alur, J. 2017, *J. Oral and Maxillofacial Path*, 21, 296.
46. Padrón, K., Salmen, S., Berrueta, L., González, E., Dávila, L., Rojas, J., Sosa, L., Oláñez, D. and Solórzano, E. 2012, *Avances en Biomedicina*, 1, 4.
47. Brizuela, C., Saint, N. and Inostroza, C., 2014, *Int. J. Morphol.*, 32, 767.
48. Sukarawan W. and Osanthanon, T. Karamzadeh, 2017, *IntechOpen*, London.
49. Kashyap R. 2015, *J. Clin. and Diagn. Res.*, 9, ZEO7.
50. Gazarian, K. and Ramírez-García, L. 2017, *PLoS One*, 12, e0170321.
51. Yang, X., Ma, Y., Guo, W., Yang, B. and Tian, W. 2019, *Theranostics*, 9, 2694.
52. Tangjit, N., Dechkunakorn, S., Anuwongnukroh, N., Khaneungthong, A. and Sritanaudomchai, H. 2018, *Int. J. Stem Cells*, 11, 96.
53. Fujiyoshi, J., Yamaza, H., Sonoda, S., Yuniartha, R., Ihara, K., Nonaka, K., Taguchi, T., Ohga, S. and Yamaza, T. 2019, *Sci. Rep.*, 9, 1535.
54. Trubiani, O., Pizzicannella, J., Caputi, S., Marchisio, M., Mazzon, E., Paganelli, R., Paganelli, A. and Diomedede, F. 2019, *Stem Cells and Develop.*, 28, 995.
55. Zhu, W. and Liang, M. 2015, *Stem Cells Int.*, 2015, 1.
56. Maeda, H., Tomokiyo, A., Fujii, S., Wada, N. and Akamine, A. 2011, *Stem Cell Res. & Therap.*, 2, 33.
57. Wang, Y., Papagerakis, S., Faulk, D., Badylak, S., Zhao, Y., Ge, L., Qin, M. and Papagerakis, P. 2018, *Front. Physiol.*, 2018, 942.
58. Seok, J., Yeong, S., Kwon, I.K. and Sun, J. 2014, *Cell Biochem & Fun.*, 32, 605.
59. Sawangmake C., Nowwarote, N., Pavasant, P., Chansiripornchai, P. and Osathanon, T. 2014, *Bioch. Biophys. Res. Com.*, 452, 581.
60. Zhang, J., Ding, H., Liu, X., Sheng, Y., Liu, X. and Jiang, C. 2019, *Stem Cells and Develop.*, 28, 986.
61. Zhou, T., Pan, J., Wu, P., Huang, R., Du, W., Zhou, Y., Wan, M., Fan, Y., Xu, X., Zhou, X., Zheng, L. and Zhou, X. 2019, *Stem Cells Int.*, 2019, 1.
62. Zheng, C., Chen, J., Liu, S. and Jin, Y. 2019, *Int. J. Oral Sci.*, 11, <https://doi.org/10.1038/s41368-019-0060-3>.
63. Lucaciu, O., Sorițău, O., Gheban, D., Ciuca, D., Virtic, O., Vulpoi, A., Dirzu, N., Câmpian, R., Băciuț, G., Popa, C., Simon, S., Berce, P., Băciuț, M. and Crisan, B. 2015, *BMC Biotech.*, 15, 114.

64. Lopes, R., Carvalho, R., Moura-Neto, V., Bolognese, A. M., DosSantos, M. F. and Souza, M. M., 2017, *Arch. Oral Biol.*, 73, 12.
65. Sonoyama, W., Liu, Y., Yamaza, T., Tuan, R., Wang, S., Shi, S. and Huang, G. 2008, *J. Endodon.*, 34, 166.
66. Nada, O. and El Backly, R. 2018, *Front. Bioengin. and Biotech.* 2018, doi:10.3389/fbioe.2018.00103.
67. Kang, J., Fan, W., Deng, Q., He, H. and Huang, F. 2019, *BioMed. Res. Int.*, 2019, 1.
68. Friedlander, L., Cullinan, M. and Love, R. 2009, *Int. Endodon. J.*, 42, 955.
69. Pisal, R., Suchanek, J., Siller, R., Soukup, T., Hrebikova, H., Bezrouk, A., Kunke, D., Micuda, S., Filip, S., Sullivan, G. and Mokry, J. 2018, *Sci. Rep.*, 8, 6168.
70. Karaöz, E., Doğan, B., Aksoy, A., Gacar, G., Akyüz, S., Ayhan, S., Genç, Z., Yürüker, S., Duruksu, G., Demircan, P. and Sarıboyacı, A. 2009, *Histoch. and Cell Biol.*, 133, 95.
71. Suchánek, J., Browne, K., Nasry, S., Kleplová, T., Pilbauerová, N., Schmidt, J. and Soukup, T. 2018, *Braz. Dent. J.*, 29, 427.
72. Shetty, H., Kakade, A., Shetty, S., Neelakantan, P., Nagar, S., Desai, R. and Beri, K. 2018, *Fut. Sci. OA.*, 4, FSO342.
73. Suchánek, J., Nasry, S. and Soukup, T. 2017, *Folia Biologica (Praha)*, 63, 132.
74. Akpınar, G., Kasap, M., Aksoy, A., Duruksu, G., Gacar, G. and Karaoz, E. 2014, *Stem Cells Int.*, 2014, 1.
75. Matsubara, T., Suardita, K., Ishii, M., Sugiyama, M., Igarashi, A., Oda, R., Nishimura, M., Saito, M., Nakagawa, K., Yamanaka, K., Miyazaki, K., Shimizu, M., Bhawal, U., Tsuji, K., Nakamura, K. and Kato, Y. 2005, *J. Bone and Mineral Res.*, 20, 399.
76. Chalisserry, E., Nam, S., Park, S. and Anil, S. 2017, *J. Tis. Eng.*, 8, 1.
77. Egusa, H., Sonoyama, W., Nishimura, M., Atsuta, I. and Akiyama, K. 2012, *J. Prosthodont. Res.*, 56, 229.