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Review

Dental stem cells and their application in dentistry.

Las células madre dentales y su aplicación en odontología.

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Abstract: Recent advances in tissue engineering and regenerative medicine offer a long-term solution through biological repair, replacement of damaged teeth or maintenance and improvement of tissue and organ function through the use of stem cells. Stem cells or also called universal cells, progenitor cells or precursor cells; they are primitive, undifferentiated, clonogenic cells that are characterized by their self-renewal capabilities and that can be differentiated into more specialized cells with specific functions. Currently many sources are known from where you can obtain stem cells, one of which are those obtained from oral or dental tissues, called dental stem cells (DSC), from where it has been possible to identify, isolate and characterize around 8 unique populations: dental pulp stem cells (DPSC), human exfoliated deciduous tooth stem cells (SHED), periodontal ligament stem cells (PLDSC), dental follicle stem cells (DFSC), stem cells derived from bone alveolar (CMHA), the stem cells of the apical papilla (SCAP), the stem cells of the dental germ (DGSC) and the gingival stem cells (GSC). These DSC have attracted attention in recent years due to their accessibility, plasticity and high proliferation capacity. Currently, DSC have shown that they can be used in endodontic and periodontal regenerative therapy, in the regeneration of dentin and bone and in dental bioengineering. Tissue engineering methodologies combined with a greater understanding of the biology of DSCs will provide powerful tools for a broader spectrum of their application in various future therapeutic strategies.

Keywords: Stem cells; regenerative medicine; tissue engineering; cell differentiation; regeneration; cell proliferation.

Resumen: Los recientes avances en ingeniería de tejidos y medicina regenerativa ofrecen una solución a largo plazo mediante la reparación biológica, el reemplazo de dientes dañados o el mantenimiento y mejora de la función de los tejidos y órganos mediante el uso de las células madre (CM). Las CM o también llamadas células universales, células progenitoras o células precursoras; son células primitivas, indiferenciadas, clonógenas que se caracterizan por sus capacidades de autorenovación y que pueden diferenciarse en células más especializadas con funciones específicas. Actualmente se conoce muchas fuentes de donde se puede obtener las CM, una de ellas son las obtenidas de los tejidos orales o dentales, denominadas células madre dentales (CMD), de donde se ha podido identificar, aislar y caracterizar alrededor de 8 poblaciones únicas: las CM de la pulpa dental

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(CMPD), las CM de dientes deciduos exfoliados humanos (CMDDE), las CM del ligamento periodontal (CMLP), las CM del folículo dental (CMFD), las CM derivadas del hueso alveolar (CMHA), las CM de la papila apical (CMPA), las CM del germen dentario (CMGD) y las CM gingivales (CMG). Estas CMD han llamado la atención en los últimos años debido a su accesibilidad, plasticidad y alta capacidad de proliferación. Actualmente, las CMD han demostrado que se pueden usar en la terapia regenerativa endodóntica y periodontal, en la regeneración de dentina y de hueso y en la bioingeniería dental. Las metodologías de ingeniería de tejidos combinadas con una mayor comprensión de la biología de las CMD proporcionarán herramientas poderosas para un espectro más amplio de su aplicación en diversas estrategias terapéuticas futuras.

Palabra Clave: *Células madre; medicina regenerativa; ingeniería de tejidos; diferenciación celular; regeneración; proliferación celular.*

INTRODUCTION.

Teeth are highly differentiated organs and are composed of enamel, dentin, dental pulp, and root structures composed of dentin, cementum and periodontal ligament (PL), which stabilize the teeth to the underlying alveolar bone.[1-5]

Traumatic injuries, periodontal disease and dental caries are the main pathologies that affect teeth and their surrounding tissues. These pathologies continue to be a major clinical challenge, due to the limited self-healing capacity of dental tissues.[3,5-7]

The repair mechanisms that occur after dental or periodontal injury involve highly specialized genetic programs that are active during the development of embryonic teeth.

However, the reparative capacity of the dental pulp and the periodontium is often insufficient to restore all the damaged tissues and, if left untreated, these injuries compromise the integrity of the teeth, which can lead to more serious pathologies and the subsequent tooth loss.[3,6,8,9]

Knowledge about the repair events within dental tissues has contributed to the proposal of alternative methods for the treatment of dental pathologies.

However, traditional treatments, imperfect due to frequent failure, limited shelf life, or inability to fully restore dental function continue to be used in dental clinics. These treatments often use growth factors to improve the regenerative capacity of dental and periodontal tissues.[3,5,10-13]

Thus, recent advances in tissue engineering and regenerative medicine offer the possibility of a long-term solution through biological repair, replacement of damaged teeth or the maintenance and improvement of the function of tissues and organs through the use of stem cells (SC).[3,5,6,8,10-14]

The SCs or also called universal cells, progenitor cells or precursor cells; They are primitive, undifferentiated, clonogenic cells that are characterized by their self-renewal capacities and that can be differentiated into more specialized cells with specific functions.[4,8,15-17]

They are mainly divided into two groups: pluripotent SCs (PSC), cells that can differentiate into a variety of cells, and multipotent SCs, cells that can differentiate into a limited variety of cells. PSCs are further divided into embryonic SCs (ESC) and induced pluripotent SCs (IPSC), while multipotent SCs consist of adult SCs known as somatic or postnatal SCs.[7-10,13,15]

The potential of SCs is extensively investigated as a new therapeutic approach that shows great promise in regenerative medicine.[1,5,10,16-26]

Currently, many sources are known from which postnatal SCs can be obtained, including the bone marrow, brain, skin, hair follicles, skeletal muscle, adipose tissue, and they have also been obtained from various oral tissues such as the craniofacial bone, the dental pulp, the PL, the dental follicle, the germ of the teeth, the apical papilla, the oral mucosa, the gums and the periosteum.[1,2,5-10,17,18,27]

The SCs obtained from oral or dental tissues are called dental SCs (DSC) and have been extensively studied in recent years due to their great clinical potential, easy access, and less invasive collection. Several preclinical investigations carried out to date indicate the vast potential of these SCs in the repair and regeneration of dental tissues, as well as in other organs.[1,2,8-10,18]

For this reason, the objective of this article is to determine the therapeutic applications of DSCs in dentistry through a narrative review of the literature.

History o f the SCs

In 1868, the term "stem cell" first appeared in the works of the German biologist Ernst Haeckel, but it was Edmund B. Wilson who coined the word years later.

In 1908, the Russian histologist Alexander Maksimov postulated the existence of hematopoietic SC at the congress of the hematological society in Berlin, and it is there that the term *"stem cell"* was proposed for scientific use.[15]

Stem cells have multiple applications and have contributed to the creation of regenerative medicine. Regenerative medicine is the process of substitution or regeneration of cells, tissues or organs for therapeutic applications. The concept of regeneration in the medical field has advanced significantly after the discovery of SCs and in recent times its application in dentistry has been found after the identification of DSCs.[15,16]

Although the concept of dental regeneration was not initially accepted, the innovative work of the stomatologist Feldman (1932) showed evidence of regeneration of the dental pulp, under certain optimal biological conditions.

This work introduced the biological principles of dental therapies to achieve dental pulp regeneration using dentin fillings as a building material to stimulate pulp regeneration. However, later researchers further improved this work.

A breakthrough in dental history was made in 2000 when Gronthos et al. identified and isolated a population of odontogenic SC from the adult dental pulp. From this discovery, several researchers have reported varieties of DSCs located in their respective oral niches.[5,7,9,15,28]

The DSCs

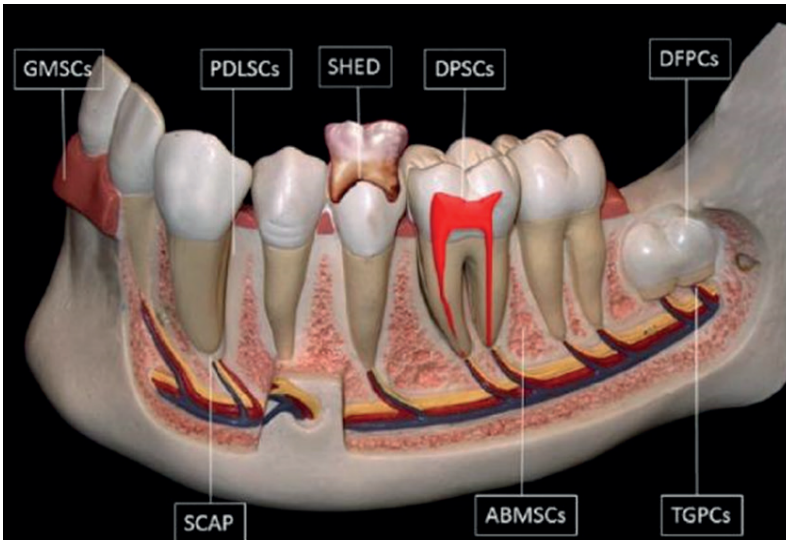
The DSCs are postnatal SCs populations that have characteristics similar to mesenchymal SCs (MSC), including the capacity of self-renewal and multilineage differentiation potential, but not all are equal in terms of their phenotypic and functional properties.[1,5,7,9,10,18,19,29,30]

These cells derive from the neural crest and therefore have a different origin from that of bone marrow-derived MSCs (BMC), which is derived from the mesoderm; playing a key role in homeostasis and tooth repair.[1,2,7,9,18,19,27]

The DSCs can be isolated from different areas of the oral cavity and the maxillofacial region. Currently, around eight unique populations of MSCs derived from oral tissues have been identified, isolated and characterized.[1,2,5,7,9,17,27]

Thus, we have: SCs of the dental pulp (DPSC), the SCs of deciduous human exfoliated teeth (SHED), the SCs of the periodontal ligament (PDLSC), the SCs of the dental follicle (DFPC), the SCs derived from the alveolar bone (ABMSC), the SCs of the apical part of the dental papilla or of the apical papilla (SCAP), the SCs of the dental germ (TGPC), and the gingival SCs (GMSC) (Figure 1).[1,2,5-10,17-19,27]

Figure 1. Schematic figure illustrating the sources of the DSCs. Chalisserry *et al.*[1]



SC: Stem Cells. **DSC:** Dental Stem Cells. **GMSC:** gingival SC. **SCAP:** SC of the apical part of the dental papilla or of the apical papilla. **PDLSC:** SC of the periodontal ligament. **SHED:** SC of deciduous human exfoliated teeth. **DPSC:** SC of the dental pulp; **ABMSC:** SC derived from alveolar bone. **DFPC:** SC of the dental follicle. **TGPC:** SC of the dental germ.

Each of these DSCs populations have their own characteristics, such as the expression of different differentiation clusters (CD), which are marker molecules on the cell surface and are of importance for the isolation and identification of SCs 31 (Table 1).

The DSCs located in the PL play an important role in repair and are involved in the homeostatic turnover of the PL. But, not all dental tissue can be repaired or replaced; the cells that make up the enamel, the ameloblasts, are lost when the teeth erupt and therefore damage to the enamel cannot be repaired naturally.[2,16]

Dentin contains tubules that are produced by DSCs (odontoblasts) that persist in mature teeth and that have limited regenerative capacities to form restorative dentin in response to injury or disease. In the dental pulp, DSCs remain active throughout life and generate odontoblasts, which work to repair damaged dentin. That is, teeth develop through continuous and reciprocal interactions between the MSCs derived from the cranial neural crest and the SCs derived from the oral epithelium during early embryogenesis.[1,2,9,10,32]

Among DSCs, DPSCs were the first human dental MSCs that were identified from the pulp and have demonstrated great clinical potential, easy access, and less invasive harvesting. Interestingly, vascular endothelial cells and DPSCs were found to differentiate synergistically into osteoblasts and endothelial cells, respectively.[1,3-5,7-10,19,28]

The DPSCs and SHEDs are self-renewing MSCs that reside in the perivascular niche of the dental

Table 1. Characteristics of the DSCs.

DSC type	Isolation	Population doublings	Markers	CD antigen expression	
				Positive	Negative
DPSC[1, 2, 9, 10, 17, 19, 27, 36, 37]	++++	>120	CD29, CD34, CD44, CD45, CD73, CD105, CD106, CD117, CD146, 3G5, STRO-1, Oct4, Nanog, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4	CD9, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166, CD271	CD14, CD19, CD24, CD31, CD34, CD45, CD117, CD133
SHED[1,3-5, 8,17,19,27,42]	+++	>140	CD29, CD73, CD90, CD146, STRO-1, Nanog, Oct4, nestin, SSEA-3, SSEA-4, TRA-1-60, TRA1-81	CD13, CD29, CD44,CD56, CD73, CD90, CD105, CD146, CD166	CD11b, CD14, CD19, CD34, CD43, CD45
PDLSC[1,3,4,9,17,19,27]	++++	ND	CD44, CD90, CD105, CD146, STRO-1, escleraxis	CD9, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166	CD14, CD31, CD34, CD45
DFPC[1,3-5,9,17,19,27]	++	ND	CD29, CD44, CD105, Notch-1, nestin	CD9, CD10, CD13, CD29, CD44, CD53, CD59, CD73, CD90, CD105, CD106, CD166, CD271	CD31, CD34, CD45, CD133
SCAP[1,3,4,17,19,27]	+++	>70	CD24, CD73, CD90, CD105, CD146, STRO-1, nestin, survivin	CD13, CD24, CD29, CD44, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166	CD14, CD18, CD34, CD45, CD117, CD150
TGPC[1,17,19,27]	+	ND	CD29, CD44, CD73, CD90, CD105, CD106, CD166, STRO-1, Nanog, Oct4, Sox2, C-myc, Klf4	CD29, CD44, CD73, CD90, CD105, CD106, CD166	CD14, CD34, CD45, CD133
ABMSC[1,19,27,30,49]	++++	>30	CD73, CD90, CD105, STRO-1	CD13, CD29, CD44, CD71, CD73, CD90, CD105, CD146, CD166	CD11b, CD14, CD19, CD31, CD34, CD45
GMSC[1,17,19,27]	++++	>20	CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166, Nanog, nestin, Oct4, Sox2, SSEA-4, STRO-1	CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166	CD34, CD45, CD117

SC: Stem Cells. **DSC:** Dental Stem Cells. **GMSC:** gingival SC. **SCAP:** SC of the apical part of the dental papilla or of the apical papilla. **PDLSC:** SC of the periodontal ligament. **SHED:** SC of deciduous human exfoliated teeth. **DPSC:** SC of the dental pulp; **ABMSC:** SC derived from alveolar bone. **DFPC:** SC of the dental follicle. **TGPC:** SC of the dental germ.

pulp; they are believed to originate from the cranial neural crest, which expresses early markers for both MSCs and neuroectodermal SCs; and these cells demonstrate the ability to regenerate in various tissues (cartilage, bone, fat, etc).

Implantation of DPSCs or SHEDs has recently been shown to promote functional recovery after spinal cord injury. Furthermore, recent studies have shown that DPSCs also protect against ischemic brain injury in neonatal mice.[1,33,34]

In conclusion, DSCs have a therapeutic potential similar to that of BMCs and are a non-invasive source for future regenerative therapies.[1,4,7,8]

The DPSCs

The DPSCs were the first type of multipotent DSCs derived from dental pulp and were first isolated in 2000 by Gronthos *et al.*,[44] by enzymatic digestion of the pulp tissue of impacted third molars, exhibiting a morphology similar to fibroblasts and showing great capacity to proliferate and self-renew, and eventually differentiate into odontoblast and osteoblast-like cells to form dentin and bone.[1,3–10,13,15,19,27,28,35]

Furthermore, with the help of various differentiation means, the potential of these cells to undergo dentinogenic, osteogenic, adipogenic, neurogenic, chondrogenic, myogenic and hepatocyte-like differentiation has been demonstrated.[1,3–10,16,17,27,28,35,36]

Progenitors of dental pulp have not been clearly iden-2tified, but some data suggest that DPSCs are derived from pericytes, which are capable of differentiating into osteoblasts, and may also differentiate into odontoblasts.[1,2,9]

Table 2. Therapeutic potential of DSCs in Dentistry.

Author(es)	DSC type	Oral site	Results
Yu *et al.*[56]	GMSC	Periodontal regeneration	The GMSCs significantly improved regeneration of damaged periodontal tissue, including alveolar bone, cementum, and functional PL.
Li *et al.*[57]	DPSC	Periodontal regeneration	The DPSCs had a positive effect on the regeneration of new bone to repair periodontal defects.
Zhu *et al.*[58]	PDLSC + ABMSC	Periodontal regeneration	The PDLSCs and ABMSCs regenerated the PL (fibers and mineralized matrix) on the surface of the Titanium scaffold.
Nagata *et al.*[59]	PDLSC	Periodontal regeneration	The PDLSCs improved periodontal regeneration by suppressing the inflammatory response through TNF-α production in mice.
Lucaciu *et al.*[60]	DFPC	Osseointegration	The DFPCs have a spontaneous tendency to osteogenic differentiation and can be used to improve bone regeneration on the surfaces of titanium implants.
Zhang *et al.*[61]	GMSC	Oral mucositis	Preconditioned GMSCs improve oral mucositis mitigation.
Gao *et al.*[62]	GMSC	Odontogenic regeneration	The GMSCs showed a greater potential for odontogenic differentiation when induced with germ cell embryonic cells.
Fawzy El-Sayed *et al.*[63]	GMSC	Periodontal regeneration	The GMSCs show significant periodontal regenerative potential.
Xuan *et al.*[64]	SHED	Pulp regeneration	The SHEDs can regenerate the entire dental pulp and can be useful in treating dental injuries due to trauma.
Ferrarotti *et al.*[65]	DPSC	Periodontal regeneration	The application of DPSCs significantly improved the clinical parameters of periodontal regeneration 1 year after treatment.
Chen *et al.*[66]	PDLSC	Periodontal regeneration	The use of autologous PDLSCs decreased the depth of periodontal intra-osseous defects, so treating these defects with PDLSCs is safe and does not produce significant adverse effects.
d'Aquino *et al.*[67]	DPSC	Regeneration in mandibular defects	A collagen / DPSCs sponge biocomplex can completely restore human jaw bone defects.
Giuliani *et al.*[68]	DPSC	Regeneration in mandibular defects	At 3 years of follow-up the collagen sponge/DPSCs biocomplex restores bone defects of the human jaw.
Gault *et al.*[69]	PDLSC	Osseointegration	The PDLSCs radiographically demonstrated osteogenic differentiation around titanium implants.
Feng *et al.*[70]	PDLSC	Periodontal regeneration	There is clinical and experimental evidence supporting the possible efficacy and safety of using autologous PDLSCs in the treatment of human periodontitis.

SC: Stem Cells. **DSC:** Dental Stem Cells. **GMSC:** gingival SC. **SCAP:** SC of the apical part of the dental papilla or of the apical papilla. **PDLSC:** SC of the periodontal ligament. **SHED:** SC of deciduous human exfoliated teeth. **DPSC:** SC of the dental pulp; **ABMSC:** SC derived from alveolar bone. **DFPC:** SC of the dental follicle. **TGPC:** SC of the dental germ.

Although there is no specific biomarker available for the identification of DPSC, these cells express several markers. DPSCs express epithelial markers and share common characteristics with neural stem cells; they are also able to differentiate in vitro into neural or vascular endothelial cells.[1,5,7,9,10,17,19,27,35,37,38]

The DPSCs and endothelial cells have a synergistic effect on co-cultures, which improves the differentiation of osteogenic, odontogenic and angiogenic phenotypes.[1,4,5,16,19,39] Yasui *et al.*,[40] demonstrated that DPSCs can generate bone-like tissues *in vivo*.

The DPSCs grown by the explant culture method have better proliferation capacity and also differentiate into various types of cells of osteogenic, adipogenic and myogenic lineages.[1,5,7,40]

DPSC cultures contain neural crest multipotent SCs that can differentiate into a series of cells derived from the neural crest lineage including melanocytes.[1,19,27] Paino *et al.*,[41] demonstrated that DPSCs sponta-neously differentiate *in vitro* into the melanocytic lineage.

One of the interesting characteristics of DPSCs is their immunosuppressive capacity since they suppress the proliferation of peripheral blood mononuclear cells (PBMC) through the production of transforming growth factor beta (FCT-β) and interferon gamma (IF- γ).[19, 27]

Furthermore, it has been shown that DPSCs can suppress T-cell proliferation and, therefore, may be suitable for preventing or treating T-cell alloreactivity associated with allogeneic hematopoietic or solid organ transplantation. In another study, Toll-like receptors (TR), key molecules that bind innate and adaptive immune responses, were shown to trigger DPSC immunosuppression by regulating expression of FCT-β and interleukin 6 (IL-6).[7, 27]

The DPSCs have shown the greatest potential to produce a high volume of mineralized matrix, suggesting that these cells also hold promise for use in regenerative dental therapies. Furthermore, these cells remain with their differentiation potential even after cryopreservation.[1,2,7–10,17,19,27,42]

The SHEDs

Stem cells isolated from dental pulp of deciduous exfoliated teeth revealed a high proliferative and clonogenic nature.[1,3–5,7–10,17–19,27,43]

Miura *et al.*,[44] isolated MSCs from deciduous exfoliated teeth; these cells were called SHED and showed high plasticity, since they could differentiate into neurons, adipocytes, osteoblasts, and odontoblasts. They differ from DPSCs in that they were isolated from the pulp tissue of the crown of exfoliated deciduous teeth, and these SCs did not grow fully.[1,3-5,8,19,27]

The SHEDs demonstrated a higher proliferation rate and a higher number of colony forming cells compared to DPSCs with early expression of MSC markers (STRO-1 and CD146).[1,3-5,8,17,19,27,43]

The SHEDs developed multiple cytoplasmic processes and expressed different markers of glial and neuronal cells, such as nestin when cultured with neurogenic inducing media, suggesting its origin in the neural crest.[1,3,4,7,10,19,27,37,38]

It has been possible to demonstrate that the SHEDs have myogenic and chondrogenic properties. Reports also support the observation that SHEDs can differentiate into endothelial cells when grown in a portion of dentin *in vitro*.[5,7,27]

Transplantation of SHEDs into immunocompromised mice showed the formation of dentin-like tissues. This regenerated dentin was formed due to odontoblast-like cells that indicate the odontogenic differentiation potential of SHEDs.[1,4,8,27]

An *in vitro* study on SHEDs showed that this population has a higher proliferation rate, adipogenic potential, and osteogenic potential than DPSCs.

In addition, SHEDs were reported to inhibit performance and decrease the number of T helper 17 (Th17) lymphocytes in the peripheral blood, and increase the proportion of regulatory T lymphocytes (Treg).[7,19,27,45]

Furthermore, systemic inoculation of SHEDs was able to effectively reverse disorders associated with systemic lupus erythematosus, possibly due to its superior immunomodulatory effects that promote recovery of the relationship between Treg cells and Th17 cells; suggesting then that these may be an accessible and feasible source of cells for the treatment of immune disorders.[7,27,46]

The SHEDs, unlike DPSCs, induce host cells to undergo osteogenic differentiation.SHEDs have a higher proliferation rate, as well as a high potential for odontogenic and osteogenic differentiation, which that differentiates them from the DPSCs and they represent the most immature form than the DPSCs.[1-8,17,18,27,40,43]

The PDLSCs

The PL is a specialized connective tissue located between the cementum and the alveolar bone, it is surrounded by connective tissue and has the function

of maintaining and supporting the teeth. It contains different types of cells, which are responsible for maintaining homeostasis and building periodontal tissue, which can be differentiated into cementoblasts and osteoblasts. Its regeneration is believed to involve MSCs arising from the dental follicle.[1,4,5,8,9,19,27]

Multipotent PDLSCs, isolated from teeth for the first time in 2004 by Songtao Shi, extracted by enzymatic digestion or *in vitro* culture, demonstrated a fibroblast-like morphology and exhibited a clonogenic nature.

It has also been shown that PDLSCs are capable of differentiating into adipocytes, chondrocytes, osteoblasts, neural cells, and cementoblast-like cells both *in vitro* and *in vivo* when cultured with the appropriate inductive medium.[1-5,8,16,17,19,27]

The PDLSCs have been used successfully for regeneration of the periodontium in areas with periodontal defects. Furthermore, they have low immunogenic effects and have immunomodulatory properties.[7-9,18,27] Therefore, it is obvious that the PL contains progenitor cells, which can be activated to self-renew and regenerate other tissues apart from the PL, such as cementum and alveolar bone.[1,3-6,8,9,19,27]

Studies have shown that activated human PBMCs induce PDLSCs to secrete soluble factors, such as TGF-β for example. Furthermore, PDLSCs were found to possess low immunogenicity and marked immunosuppressive activity through prostaglandin E2 (PGE2)-induced T-cell anergy.[5,7,27]

The PDLSCs isolated from the inflamed periodontium were also reported to show significantly reduced inhibitory effects on the proliferation rate of T cells compared to healthy cells. In co-cultures, the stimulated PBMCs showed a significant decrease in Treg induction, suppression of Th17 differentiation, and IL-10 and IL-17 secretion in the presence of inflamed PDLSCs compared to healthy PDLSCs, demonstrating that inflamed PDLSCs had markedly dysfunctional immunomodulatory properties, which may explain the pathogenesis of periodontitis and facilitate the development of therapies for this condition.[5,7,9,27,47]

The DFPCs

The dental follicle is of ectomesenchymal origin and surrounds the unerupted tooth as a protective sac; controls the processes of osteoclastogenesis and osteogenesis during dental eruption and differs in the periodontium.[1,4,8-10,19,27]

In vitro studies demonstrated the multilineage potential of DFPCs to differentiate into chondrocytes, adipocytes, neuronal cells, PL, osteoblasts, cementoblasts, and fibroblasts.[1,3-5,9,17,19,27,48] STRO-1 positive DFPCs can differentiate into cementoblasts *in vitro* and can form cementum *in vivo*.

The DFPCs showed their potential to differentiate and express cementoblast markers under stimulation with bone morphogenetic proteins-2 (BMP-2) and BMP-7 and matrix derived from dental enamel. Immortalized DFPCs are able to recreate a new PL after in vivo implantation.[1,3,4,9,27]

Furthermore, some studies have shown that DFPCs produce FCT-β and suppress PBMCs proliferation. Treatment of TR-3 and TR-4 agonists increased the suppressive potential of DFPCs and potentiated secretions of FCT-β and IL-6. These properties of DFPCs are desirable for the treatment of diseases caused by chronic inflammation accompanied by tissue injury.[7,27]

The ABMSCs

The alveolar bone is a tissue with dental embryonic follicular origin, which holds the teeth in place with the help of the PL.[19,27]

Matsubara *et al.*,[49] performed successful isolation and cultivation of ABMSCs; These isolated cells had a spindle-like fibroblast-like morphology, plastic adherence, and colony formation. ABMSCs can differentiate into osteoblastic lineages, with high expression of alkaline phosphatase, chondrocytes, and adipocytes.[1,19,27]

Studies have shown that treatment of ABMSCs with a dichloromethane fraction of Dipsaci Radix, IF-1 induced transmembrane protein, nicotine, low frequency electromagnetic pulse fields, low intensity ultrasound pulses, low dynamic fluid shear stress , and orbital shear stress; could improve osteogenesis in these cells.[1,27,50,51]

They have chondrogenic and adipogenic differentiation potentials similar to those of other stem cell populations.[3,17,27] Bioceramics can provide a good scaffold for the binding, proliferation, migration and differentiation of ABMSCs for use in bone tissue engineering applications.

The SCAPs

The apical papilla is a loose connective tissue located at the apex of the root of developing permanent teeth and is a rich source of MSCs compared to dental pulp. During tooth development, the dental papilla contributes to tooth formation and subsequently becomes the dental pulp and contributes to root development.[1,4,5,8,9,19,27]

SCAPs have been isolated in the apical papilla of immature permanent teeth, comparing their potential to differentiate in odontoblasts with that of DPSCs, obtaining that they have a higher proliferation rate, mineralization potential and seem more effective than DPSCs for tooth formation. since they are easily accessible because they can be isolated from third molars.[1,4,5,8-10,17,27]

The SCAPs express early mesenchymal surface markers, especially CD24, which may be a unique marker for your population. These cells demonstrate their ability to undergo osteogenic, adipogenic, chondrogenic, and neurogenic differentiation when cultured in the appropriate inductive media.[1,3-5,17,19,27]

Expanded SCAPs *ex vivo* shows positive staining for various neuronal markers without neurogenic stimulation. After stimulation, they also express additional neuronal markers, including neuronal nuclear antigen, neurofilament M, and neuron-specific enolase.[10,27,36-38]

Following SCAPs transplantation into immuno-compromised mice in an appropriate carrier matrix, a typical dentin-like pulp-like structure was formed due to the presence of odontoblast-like cells.[1,19,27] In addition, SCAPs can generate bone-like and root-cementum-like tissue with embedded cells similar to cementum and osteocytes *in vivo*.

However, it could not be identified if the material is dentin, cementum or bone.[5,9,27] The SCAPs have low immunogenicity, can inhibit T-cell proliferation *in vitro* through an apoptosis-independent mechanism, and can suppress lymphocyte reaction.

Certain soluble factors may be involved in SCAPs-mediated immune suppression, but the exact mechanisms require further study. Furthermore, cryopreservation did not affect the immune properties of SCAPs.[7,27]

The TGPCs

The TGPCs are newer multipotent stem cell populations that were identified in the dental mesenchyme of the third molar germ during the final bell phase. They can be expanded and maintained for almost 60 population doublings, during which they retain their spindle-shaped morphology and high proliferation rate.[1, 19, 27]

The TGPCs show multilineage differentiation ability similar to that of other DSCs, including the ability to differentiate into adipocytes, osteoblasts, odontoblasts, chondrocytes, and neurons. Hydroxyapatite (HA)/GPC implants showed new bone formation in the presence of osteocytes in the newly formed bone matrix and a bucket-shaped active osteoblast coating on the surface of the matrix.[1,17,19,27]

The GMSCs

The gingiva is an oral tissue that covers the alveolar ridges and the retromolar region, it is recognized as a biological mucosal barrier. GMSCs can be obtained from gingival tissue that is easily accessible from the oral cavity with minimal discomfort.[1, 19, 27] GMSCs have clonogenicity, self-renewal and multi-potent differentiation capacity and also possess immunomodulatory and BMCs-like properties, but show a faster proliferation rate.[1,17,19,27,52]

Gingival tissues also exhibit wound healing properties and regenerative capacity with a rapid constitution of tissue architecture using the production of IL-6 and 10. Interestingly, GMSCs show stable phenotypic and telomerase activity in cultures. long-term and are not tumorigenic.[1,17,19,27] In particular, GMSCs have demonstrated the formation of connective tissue-like structures *in vivo*.

The GMSCs have been demonstrated to have osteogenic potential *in vivo* after incubation in vitro in osteoinductive medium. These properties indicate that the clinical use of GMSCs is an attractive therapeutic option for tissue regeneration and repair.[1,17,19, 27]

The GMSCs are capable of causing a potent inhibitory effect on T-cell proliferation in response to mitogenic stimulation and mechanically exert an anti-inflammatory effect. In addition, GMSCs can cause a marked acceleration of wound healing. In summary, GMSCs can function as an immunomodulatory and anti-inflammatory component of the immune system in vivo and represent a source of promising and easily accessible cells to treat inflammatory and allergic diseases.[7,27]

Therapeutic applications in Dentistry

The DSCs can be used in the repair of damaged dentin, in the revascularization and regeneration of the pulp, and in periodontal diseases. The combination of DSCs with new scaffolding materials could allow us to reach the goal of designing oral tissues in the near future.

Understanding the molecular mechanisms of tooth development and repair, using emerging technologies in tissue and biomaterial engineering, and harnessing the potential of DSCs to form complex dental tissues are the foundation of regenerative dentistry. Thanks to tissue engineering, complete dental regeneration would reduce the difficulties associated with currently offered dental treatments, such as prosthetics, implants and tooth

transplantation.[1,2,7,9,10,19,27,53-56]

Table 2 lists some studies[57-70] that demonstrate the therapeutic potential of dental stem cells in dentistry.

Dental bioengineering

The biological replacement of a tooth should include the generation of a root and PL with nerve and blood supplies. The bioengineering of a non-embryonic cell tooth, one of the cell populations, either epithelial or mesenchymal, must be able to provide inductive signals to the other.[1,5]

Complete dental regeneration by tissue engineering currently uses two methods: scaffolding method and cell aggregate method.

In the scaffolding dental regeneration method, the SCs are organized in a suitable spatial orientation using a biodegradable polymer membrane or collagen sponge scaffolds to generate an artificial dental germ.[1,5,7]

The cell aggregate method, dental epithelial tissue, and mesenchymal cell granules are dispersed in a well-controlled culture condition to create an artificial tooth germ. The dental germ formed in this method mimics a dental germ from the early inductive stage of tooth development where cell-to-cell and epithelial-mesenchymal interactions predominate.[1,5,71]

Hung *et al.*[72] they were able to use DPSCs to form tooth-like structures in the alveolar cavities of rabbits, but there was no visible tooth eruption at any of the graft sites. The *in vivo* study showed DPSCs-derived crown-like structures associated with adult rat SCAPs with distinct regions of enamel, dentin, pre-dentin, and ameloblast and odontoblast layers.

IPSCs could generate epithelial cells, with properties similar to embryonic ones, that could be recombined with autologous DPSCs and transplanted to form a tooth. Using IPSCs of DPSCs may provide a future solution to this problem.[1,5,73]

Yang *et al.*[74] showed the positive role of DSCs in the regeneration and formation of the dental root; reported that transplanted DFPCs, seeded in dentin matrix treated as biological scaffolds, form dental structures (root, dentin, and pulp), indicating their successful rate of dental regeneration.

The combination of SCAPs and PDLSCs have recently been used to form a tooth with an artificial crown and the use of this combination has been found to be a suitable option to provide functional dental regeneration 7, 19, 27.

Currently, a bioengineered primary tooth germ can be obtained, which could be transplanted into an alveolus after tooth extraction and could become a new tooth with a typical spatial orientation of structures, such as enamel, dentin, the dental pulp, the root, the blood vessels, the PL and the alveolar bone. These findings suggest the future possibility of a successful generation of whole teeth by transplanting bioengineered dental germs into the adult oral environment 1, 5, 7.

Endodontic regenerative therapy

The current treatment of an infected root pulp is the removal of necrotic tissue and its replacement with bioinert cements to fill its canals. Although this treatment is effective in fighting infection, it does not restore the loss of pulp tissue or the vitality of the tooth; Thus, the use of DPSCs to regenerate healthy pulp tissue represents a simple and very effective biological treatment. DPSCs can be easily expanded in vitro and have been shown to reconstitute pulp-like tissue *ex vivo* and *in vivo*.[1-6,9,10, 16,19,27]

Complete regeneration of the pulp with neurogenesis and vasculogenesis occurred in an adult canine model with pulpectomy and with autogenous DSCs transplantation. Another preclinical trial using "mobilized" autologous DPSCs for teeth of dogs with pulpectomy, showed regeneration and recovery of pulp tissue without adverse effects.[1,4,9,27,75,76]

A dentin-pulp-like complex associated with vascularized tissue and surrounded by a layer of odontoblast-like cells can be generated by *ex vivo* expanded DPSCs when transplanted into immunocompromised mice with HA/tricalcium phosphate (HA/TP) as a vehicle.[5,7,27]

Rosa *et al.*[77] they found that SHEDs survive and differentiate into odontoblasts when transplanted into full-length human root canals with scaffolding. The pulp tissue generated in these experimental conditions contains functional odontoblasts capable of regenerating tubular dentin.

Xuan *et al.*[64] conducted a clinical trial in 40 patients finding that SHEDs (pulp) can regenerate an entire dental pulp and can be useful in treating dental injuries due to trauma. The future regenerative endodontic protocol could be a combination of disinfection or debridement of infected root canal systems along with the use of SCs, scaffolding, and growth factors to allow revascularization of this pulp.

The results of ongoing studies suggest that it might be feasible to restore viability in a young necrotic permanent tooth by engineering a new dental pulp. The potential impact of such therapy is immense and may allow the completion and strengthening of the dental structure through biological regeneration in the near future.[1,2,6, 7,78]

Dentin regeneration

The regenerative property of the dentin-pulp complex depends mainly on the formation of tertiary dentin, reactionary dentin and restorative dentin. DPSCs, a unique group of cells, are extremely crucial elements for tertiary dentinogenesis.[1,5,9,27,79]

The DPSCs migrate, proliferate, and differentiate into odontoblasts, which then synthesize the matrix to form tertiary dentin at damaged sites. There are two different approaches implemented in the regeneration of dentin through the use of tissue engineering techniques.[1,5,80,81]

The first approach includes a device that can be used as a filling material in a deep cavity of the tooth with a partial layer of dentin on the pulp. In this process, some growth factors or molecules that can form restorative dentin are used.[1,5,82,83]

The second approach is to place scaffolds on the open pulp along with odontoblast-like cells to grow into it and synthesize restorative dentin.[1,5,84]

The DPSCs have been grown on a variety of scaffolds to design dentin tissues. However, the signaling pathways underlying DPSCs regulation in dentin regeneration remain unknown, limiting its effective application in dentin tissue engineering.

Furthermore, transplantation of SCAPs into hydroxyapatite scaffolds in immunodeficient mice managed to form a continuous layer of dentin-like deposits.[5,9,19,27]

Bone regeneration of craniofacial defects

Different types of DSCs have been used for bone regeneration, therefore, DPSCs are capable of generating a structure similar to autologous fibrous bone tissue *in vitro* and transplantation of this tissue leads to lamellar bone forms with the presence of osteocytes *in vivo*. In addition, implantation of DPSCs seeded onto HA/TP or polylactic and polyglycolic acid scaffolds in animal models generates bone-like tissue.[8,16,19,27,85]

The DPSCs loaded onto a collagen sponge scaffold were used to restore defects of human mandibular bone.[67,68,86] Implanted SHEDs can regenerate critically sized cranial defects with strong evidence of bone formation.[8,9,19,27]

Honda *et al.*,[87] demonstrated that transplantation of DFPCs in rats managed to form bone in surgically generated critically-sized bone defects. Implantation of SCAPs seeded on hydroxyapatite scaffolds in immunodeficient rats showed the formation of mineralized structures similar to bone tissue. In addition, the local implantation of GMSCs in animal models can improve baldness defects and mandibular injuries.[9,19]

The ABMSCs induced significant new bone formation after subcutaneous transplantation in immunocompromised mice, and osteoblasts and cuboid osteocytes covering the surface along the newly formed bone margin were observed. These data support the feasibility of using these cells as a source of SCs to treat bone defects.[27]

Recent research compared, in vitro the properties of PDLSCs with other SCs of non-dental origin, finding that PDLSCs are probably the most appropriate for the generation of vascularized bone. Furthermore, DFPCs and PDLSCs have been shown to have a spontaneous tendency to osteogenic differentiation, and were suggested for bone regeneration.[8,16,60,69,86,88]

Regenerative periodontal therapy

The challenges in regenerative periodontal therapy lie in the ability to induce regeneration of a complex apparatus made up of different tissues, such as bone, cement, and PL. Despite recent advances in periodontal therapy, a complete regeneration of the damaged periodontium is still unattainable. Research results have established that PDLSCs can differentiate into osteoblasts or cementum blasts to contribute to periodontal regeneration.

The multipotent differentiation properties of PDLSCs to generate both hard and soft tissues were further demonstrated by constructing multi-layered cell sheets supported by polyglycolic acid tissue. The polyglycolic acid sheets seeded with transplanted cells regenerated the new bone, cementum and well-oriented collagen fibers when inserted into the root surfaces. Efforts are underway to find a suitable delivery system to design an efficient cell-based therapeutic tool for regeneration of periodontal tissue.[1,4,5,7,9,19,27]

Flores *et al.*,[88] they have found that fibrin gels that carry multiple layers of PDLSCs can be successfully used as a delivery system for these cells. PDLSCs-seeded collagen sponge scaffolds were successfully tested to determine regeneration of periodontal fenestration defects in Beagle dogs.[1,4]

Another PDLSCs delivery system based on a combination of bovine bone with human dentin was shown to be effective because these SCs formed a cementum-like complex in the subcutaneous dorsal pouches of immunocompromised mice 1.

Furthermore, Fawzy El-Sayed *et al.*,[63] found that GMSCs showed significant regenerative potential in periodontal defects.[86]

A clinical trial demonstrated the regeneration potential

of PDLSCs in three patients with severe periodontal disease: two patients recovered healthy periodontal tissue with reasonable clinical regeneration; and the degree of mobility and probing depth of another patient was reduced. This study summarizes the significant effects of autologous PDLSCs on periodontal disease.

Furthermore, other recent clinical studies have reported the use of autologous PDLSCs to repair periodontal defects, suggesting that autologous PDLSCs therapy is safe and effective in curing periodontal defects.[4,9,27,65,70,86,90,91] Therefore, successful therapies for tissue regeneration with PDLSCs will not only facilitate the treatment of periodontal diseases, but can also be used to improve current dental implant therapies, however further studies are needed to obtain a protocol, successful with this group of cells.[1,4,7,27]

Recently, scaffold-free tissue engineering has been of increasing interest to researchers. There are two strategies without scaffolding: cell injection and cell sheets. Cell injection has been the common treatment in SCs-based tissue engineering, so local injection of DPSCs or PDLSCs has also been shown to be effective in the treatment of periodontal diseases.[5,65,92]

Cell sheet engineering has been developed as a unique, scaffolding-free method of cell processing by acid culture or by using temperature sensitive cell culture vessels.[5,93,94] Cellular sheets manage to maintain the extracellular matrix and cell-cell junctions, which would be degraded by proteolytic enzymes such as trypsin and/or dispase, showing positive results in the treatment of many diseases.

Transplantation of PDLSCs with the cell sheet method in a rat mesial dehiscence model led to identifiable PL-like tissues that included an acellular cement-like layer and collagen fibers embedded in this layer.

In addition to PDLSCs, DPSCs and SHEDs also have the potential for periodontal regeneration.[5,95,96]

CONCLUSION.

The DSCs have shown that they can be used in endodontic and periodontal regenerative therapy, in the regeneration of dentin and bone, and in dental bioengineering.

Tissue engineering methodologies combined with a greater understanding of the biology of DSCs will provide powerful tools for a broader spectrum of their application in various future therapeutic strategies.

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