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Dental stem cells and tissue regeneration in odontology: a brief systematic review

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Abstract

Introduction: New ideas for tooth and tissue regeneration began to appear with rapid developments in tissue engineering theories and technologies. Numerous types of stem cells have been isolated from dental tissue, such as dental pulp stem cells (DPSC), human pulp stem cells isolated from exfoliated primary teeth (SHED), periodontal ligament stem cells (PDLSC), apical papillary stem (SCAP) and dental follicular cells (DFC). All these cells can regenerate tooth tissue. **Objective:** It was to present the main considerations of bioengineering techniques and report the results obtained in experiments with dental stem cells, as well as their real trends in application in dentistry. **Methods:** The systematic review rules of the PRISMA Platform were followed. The search was carried out from October to December 2022 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases, using articles from 2001 to 2022. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed accordingly, according to the Cochrane instrument. **Results and Conclusion:** A total of 118 articles were found, 27 articles were evaluated and 16 were included and developed in this systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 29 studies with a high risk of bias and 42 studies that did not meet GRADE. It is concluded that the collection of mesenchymal stem cells from deciduous teeth should be widely disseminated, as it is of great importance and wide applicability, allowing the repair of the most diverse cell types. In addition, it has several advantages, such as a non-invasive technique, respecting the period of dentition change, there are more than 20 collection possibilities, it presents high compatibility with the donor and family members and storage for an indefinite period. The studies showed that implantation of stem cells from deciduous teeth led to the regeneration of three-dimensional pulp tissue equipped with blood vessels and sensory nerves up to 12 months after treatment, as well as increased root length and reduced apical foramen width, not showing adverse events over 24 months of follow-up.

Keywords: Dental pulp stem cells. Stem cells in deciduous teeth. Tissue regeneration. Dental application.

Introduction

New ideas for tooth and tissue regeneration began to appear with rapid developments in tissue engineering theories and technologies. Numerous types of stem cells have been isolated from dental tissue, such as dental pulp stem cells (DPSC), human pulp stem cells isolated from exfoliated primary teeth (SHED), periodontal ligament stem cells (PDLSC), apical papillary stem (SCAP) and dental follicular cells (DFC). All these cells can regenerate tooth tissue [1,2].

In this scenario, the use of stem cells (SC) in tissue regeneration constitutes a major advance in current medicine. However, since the 19th century, SC have been the subject of numerous studies, through which it was discovered that the pulp of deciduous teeth is a precious way of obtaining them, whose collection has several advantages, such as not being invasive and respecting the changeover period. teething. The collection of SC from deciduous teeth should be widespread, as it has great importance and wide

applicability, as it allows the repair of the most diverse cell types, in addition to 20 possibilities for collection and therapies for leukemia, corneal reconstruction, lung cancer, cirrhosis, among others, showing high compatibility with the donor and family, making it impossible to reject [3,4].

In this context, loss of teeth and periodontal tissues can result in movement of the remaining teeth, difficulty in chewing, phonation, muscle imbalance, and impairment of dental aesthetics and smile, compromising self-esteem [6,7]. Currently, there are several therapies for replacing dental organs, all of them based on non-biological techniques and subject to failure. Despite this condition being a common abnormality and not threatening the patient's life, efforts have been directed toward the development of mechanisms for the use of SC in the replacement of oral tissues [8,9]. For bioengineering, a triad composed of stem or progenitor cells, a matrix that works as a scaffold and signaling proteins, called growth factors, as a stimulus for cell differentiation is essential [10,11].

Still, it is necessary to guarantee the vitality of the dental pulp and prevent tooth loss in endodontic treatment. A tooth without viable pulp loses its defense mechanism and regenerative capacity, making it more vulnerable to severe damage and eventually requiring extraction. The cellular and molecular processes of tissue bioengineering can promote the regeneration of structures and functions of the dentin-pulp complex. SC, extracellular matrix, and signaling molecules (microRNAs and exosomes) are components of the triad of this approach. SHED is a promising and non-invasive source of stem cells for tissue regeneration. Not only can SHED regenerate pulp-dentin tissues (composed of fibroblasts, odontoblasts, endothelial cells, and nerve cells), but SHED also have immunomodulatory and immunosuppressive properties. The collagen matrix is a material of choice to provide structural and microenvironmental support for SHED-todentin pulp tissue differentiation. Growth factors regulate cell proliferation, migration, and differentiation into specific phenotypes through signal transduction pathways [12].

Given the above, the present study aimed to present the main considerations of bioengineering techniques and report the results obtained in experiments with dental stem cells, as well as their real trends in application in dentistry.

Methods

Study Design

This was followed by a systematic literature review model on the main clinical findings of mandible fractures, according to the PRISMA rules.

Data sources and research strategy

The literary search process was carried out from October to December 2022 and was developed based on Scopus, PubMed, Science Direct, Scielo, and Google Scholar, using scientific articles from 2001 to 2022, using the descriptors (MeSH Terms): *Dental pulp stem cells. Stem cells in deciduous teeth. Tissue regeneration. Dental application*, and using the Booleans "and" between the descriptors (MeSH Terms) and "or" between the historical findings.

Study Quality and Risk of Bias

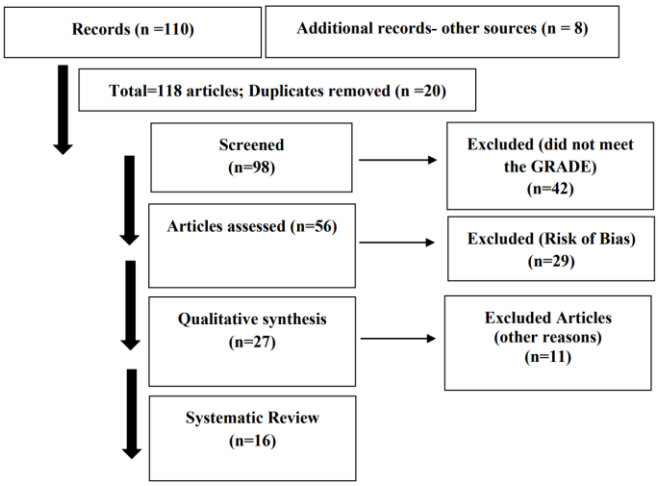
The quality of the studies was based on the GRADE instrument, with randomized controlled clinical studies, prospective controlled clinical studies, and studies of systematic review and meta-analysis listed as the studies with the greatest scientific evidence. The risk of bias was analyzed according to the Cochrane instrument.

Results and Discussion

Summary of Literary Findings

A total of 118 articles were found. Initially, duplication of articles was excluded. After this process, the abstracts were evaluated and a new exclusion was performed, removing the articles that did not include the theme of this article, resulting in 56 articles. A total of 27 articles were evaluated and 16 were included and developed in this systematic review study (Figure 1). Considering the Cochrane tool for risk of bias, the overall assessment resulted in 29 studies with a high risk of bias and 42 studies that did not meet GRADE.

Figure 1. Selection of studies.



Clinical Findings – Dental Stem Cells and Tissue Regeneration

For a collection to be possible, registration by the Dental Surgeon at a Cryogenic Center and authorization from those responsible for the donor child are required.

The procedure must be carried out in patients aged 6 to 12 years, during the tooth exfoliation phase, and the elements must not present carious lesions. Preceded by intraoral and extraoral antisepsis, the element(s) is extracted, in the shortest possible time and in contact with saliva, strictly respecting the aseptic chain. After removing any adhered soft tissues, the elements are immersed in a DMEM culture medium, kept between 4 and 8 °C, and sent to a laboratory for isolation and cultivation of SC. After being isolated and cultivated, as they are mesenchymal SCs, they can be used for the therapy of the most diverse pathologies of the nervous system, vascular system, heart, skin, pancreas, liver, eyes, muscles, lungs, kidneys, cartilage, intestines, bones, among others [1-3].

In this context, the use of SC in tissue regeneration constitutes a major advance in current medicine. However, since the 19th century, SC have been the subject of numerous studies, through which it was discovered that the pulp of deciduous teeth constitutes a precious way of obtaining them, whose collection has several advantages, such as not being invasive and respecting the storage period. dentition change [4]. In this sense, the viability of using adult stem cells in regeneration and reconstruction tissue repair has aroused great interest in the scientific community, given the increase in laws in several countries that prohibit the use of embryonic stem cells in research.

In that regard, adult stem cells, on the other hand, have the advantage of being autogenic, not incurring in moral limitations, and responsive to host-inherent growth factors [3-6]. Based on this, numerous studies have isolated highly proliferative cells derived from dental pulp. It was found that such cells are multipotent and have the capacity for self-renewal and differentiation into different cell types. Phenotypic conversion of these cells was observed, through the expression of adipose proteins (PPAR 2, peroxisome proliferator-activated receptor 2, and lipoprotein lipase), after stimulation by a culture medium with high adipogenic inductive potential [7].

Furthermore, dental pulp stem cells expressed nestin and glial fibrillar acid protein (GFAP), which are markers of neural precursors and glial cells, respectively. There is evidence that stems cells from deciduous teeth are similar to those found in the umbilical cord. When compared to stem cells from bone marrow and pulp of permanent teeth, it was noted that SHED (stem cells from human exfoliated deciduous teeth) have a higher proliferation rate [8-10].

In addition, data from this study indicate that SHED can differentiate into functional odontoblastic cells, adipocytes, and neural cells, in addition to stimulating osteogenesis after *in vivo* transplantation. Research has shown that pulpal stem cells require an appropriate inducer medium and a hydroxyapatite/tricalcium phosphate scaffold to induce bone, cementum, and dentin formation *in vivo* [1].

Some authors have demonstrated the formation of autologous fibrous bone tissue from of stem cells from the pulps of individuals aged over 30 years, as well as the differentiation of these cells into odontoblasts [1-4]. Markers for stem cells are of utmost importance as these cells reside in different locations within the tissue. More studies are needed on specific markers that can identify niches of stem cells present in the dental pulp in situ and how these niches develop. Stem cells from the human pulp and periodontal ligament may be associated with the microvasculature. Currently, the following microvascular markers are used to locate such cells: STRO-1 (stromal cell marker), Von Willebrand Factor, and CD146 (endothelial cell surface molecule). The expression of cellular telomerase in normal tissues seems to be associated with the presence of stem cells [13].

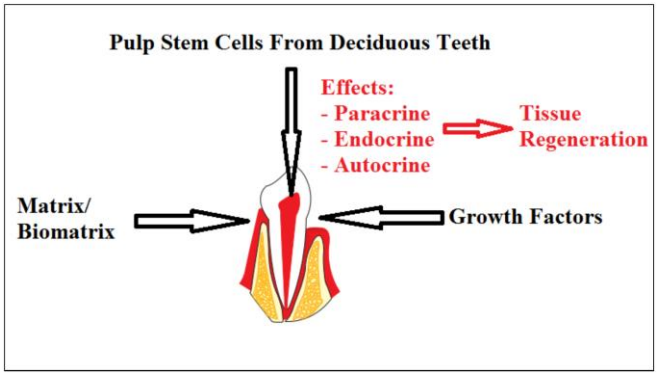
In this scenario, tooth morphogenesis involves a series of dynamic and reciprocal interactions between ectoderm and mesenchyme. Growth factors are extracellularly secreted proteins that govern morphogenesis during such interactions and comprise five protein families: bone morphogenetic proteins (BMP); fibroblast growth factors (FGF); Hedgehog proteins (Hhs), wingless and int-related proteins (Wnts) and tumor necrosis factor (TNF) [13].

Although these distinct families are involved in tooth development, BMP are sufficient for tertiary dentin formation. Some authors have also shown that the calcium hydroxide-induced pulp regeneration is mediated by cell-cell Notch signaling. The results were consistent to state that this signaling controls the fate of SC from the pulp, during its regeneration. The BMP family is part of the super-family TGF is composed of 25 molecular factors. BMPs can be divided into 4 distinct sub-families. The first BMP-2 and 4, the second BMP-3 and BMP-3B, the latter also known as growth/differentiation factor (GDF-10); the third BMP and fourth GDF, also known as morphogenetic proteins 1, 2, and 3 are derived from cartilage [2].

Early in tooth morphogenesis, BMP-2, BMP-4, and BMP-7 act as important epithelial signals that regulate the differentiation of neural crest-derived mesenchyme into an odontogenic lineage. Such flags further determine the number and position of tooth cusps. Among the three members of the Hedgehog (Hh) family present in vertebrates, Shh (Sonic Hedgehog) is the only Hh ligand expressed in teeth, being expressed during the initial development of the tooth germ. To investigate the function of the Shh protein, researchers blocked its

signaling through neutralizing antibodies and observed that Shh has two functions at the beginning of odontogenesis. The first is during tooth bud formation by stimulating epithelial proliferation, and the second is increased epithelial cell survival during the cap stage [13]. Figure 2 presents the adequate biological niche of the tooth and the main cellular and molecular elements and processes for tissue regeneration.

Figure 2. Ilustração do nicho biológico adequado do dente e os principais elementos e processos celulares e moleculares para a regeneração tecidual.



Source: Own authorship.

Applications of Mesenchymal Stem Cells, Exosomes and microRNAs in Dentistry

Evidence has shown that mesenchymal stem cells (MSC) exert biological functions primarily through the secretion of exosomes. Exosomes, which contain RNA, proteins, lipids, and metabolites, play important roles in regenerative medicine. Exosomes not only mimic the effects of their parent cells, but also have many advantages, such as high drug-carrying capacity, low immunogenicity, excellent biocompatibility, and low side effects. Currently, a total of 6 different dental stem cells (DSC), including dental pulp stem cells (DPSC), exfoliated primary tooth stem cells (SHED), periodontal ligament stem cells (PDLSC), progenitor cells of the dental follicle (DFPC), apical papillary stem cells (SCAP) and gingival mesenchymal stem cells (GMSC) were isolated and identified. DSC-derived exosomes (DSC-Exos) are actively involved in intercellular communication, anti-inflammation, osteogenesis, angiogenesis, immunomodulation, neuron nutrition, and the promotion of tumor cell apoptosis [14].

Still, a study carried out by the authors Liu et al. 2022 demonstrated the role of exosomes derived from human exfoliated primary teeth stem cells (SHED-Exos) in the regulation of angiogenesis and the underlying molecular mechanism. The results showed that SHED-Exos inhibit cell proliferation and migration and induce apoptosis in human umbilical vein endothelial cells. SHED-Exos suppress the formation of tubular structures from human umbilical vein endothelial cells *in vitro*. SHED-Exos downregulates several factors related to angiogenesis, including VEGFA, MMP-9, and ANGPT1. The data suggested that SHED-Exos are enriched with miR-100-5p and miR-1246 and are transferred into endothelial cells, which results in decreased tube formation through down-regulation of VEGFA expression. Therefore, SHED-Exos inhibit angiogenesis both *in vitro* and *in vivo*, which suggests that SHED-Exos could potentially serve as a new and effective therapeutic approach for antiangiogenic treatment [15].

Added to this, the authors Xuan et al. 2018 showed that the implantation of autologous dental stem cells from primary teeth regenerated the dental pulp with a layer of odontoblasts, blood vessels, and nerves in two animal models. These results prompted these authors to carry out a translational study on 40 patients with pulp necrosis after traumatic dental injuries in a randomized and controlled clinical trial. Thirty patients were randomly allocated to the human deciduous pulp stem cell implantation (hDPSC) group and 10 patients to the group that received traditional apexification treatment. Four patients were excluded from the implant group due to loss of followup (three patients) and trauma of the treated tooth (one patient). We examined 26 patients (26 teeth) after hDPSC implantation and 10 patients (10 teeth) after apexification treatment. Implantation of hDPSC, but not apexification treatment, led to the regeneration of threedimensional pulp tissue equipped with blood vessels and sensory nerves 12 months after treatment. Implantation of hDPSC increased root length ($p<0.0001$) and reduced apical foramen width ($p<0.0001$) compared to the apexification group. Furthermore, the implantation of hDPSC led to the regeneration of dental pulp tissue containing sensory nerves. To assess the safety of hDPSC implantation, we followed 20 patients implanted with hDPSCs for 24 months and no adverse events were observed [16].

In this sense, adult stem cell therapy generally it is preceded by the understanding of all its properties, the control of its proliferation, and the factors that determine its differentiation. The regeneration of a dental organ is not simple, since its development is determined by complex interactions and numerous growth factors, and also, cell differentiation is linked to morphological changes during the formation of the tooth germ. The use of adult stem cells has been proposed in several areas of Dentistry [2-4].

There is a great advance in experiments with adult stem cells from oral tissues. Their easy access and the fact that they are not vital organs are attractive for testing the practicality and viability of bioengineering techniques. It is possible that, soon, bioengineering will be used in endodontic and periodontal therapy,

although, currently, science is far from developing complete dental organs from stem cells, due to the complex mechanisms of tooth formation [1-5].

Conclusion

It is concluded that the collection of mesenchymal stem cells from deciduous teeth should be widely disseminated, as it is of great importance and wide applicability, allowing the repair of the most diverse cell types. In addition, it has several advantages, such as a non-invasive technique, respecting the period of dentition change, there are more than 20 collection possibilities, it presents high compatibility with the donor and family members and storage for an indefinite period. The studies showed that implantation of stem cells from deciduous teeth led to the regeneration of three-dimensional pulp tissue equipped with blood vessels and sensory nerves up to 12 months after treatment, as well as increased root length and reduced apical foramen width, not showing adverse events over 24 months of follow-up.

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Ethical Approval

Not applicable.

Informed consent

Not applicable.

Data sharing statement

No additional data are available.

Conflict of interest

The authors declare no conflict of interest.

Similarity check

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