

Oral Health: The Potential of Mesenchymal Stem Cells in Treating Various Oral Diseases and Promoting Tissue Regeneration

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Abstract. The WHO Global Oral Health Status Report (2022) estimates that over 3.5 billion individuals worldwide have an oral disease. Most of the cases are dental caries, periodontal disease, tooth loss etc. Tooth loss will not only affect the balance of the oral environment but also cause negative effects on facial development and the patient's psychological condition. At present, The implement of oral stem cells to encourage tissue regeneration has become a hot trend. Among them, Periodontal Ligament Stem Cells (PDLSCs) and Dental Follicle Stem Cells (DFSCs) have been discovered it's potential to promote tooth regeneration. Scientists proposed a variety of different treatment methods, including the use of advanced bioengineered technology to further target different oral diseases. So far, It is firmly established that oral stem cells have the potential to boost tissue regeneration.

Keywords: Tissue regeneration, Mesenchymal stem cells, Periodontal Ligament Stem Cells, Oral Diseases.

1. Introduction

When facing nerve damage, adult mammals lack the ability to regenerate neurons, resulting in limited repair function. The drugs used to treat nerve damage also have limitations, mainly due to the presence of many molecular growth inhibitors in the microenvironment of the injured site, which can prevent the damaged nerve from developing new synaptic connections, leading to the failure of regeneration function. However, researchers have discovered the potential of stem cells for treating neural reconnection. Transplanting stem cells can improve the microenvironment of nerve injury sites and help slow down and repair the deterioration of degenerative or traumatic neurological diseases. Mesenchymal stem cells (MSCs) significantly impact integrating neurons and updating neural function. They can not only release growth factors that promote cell survival but, most importantly, restore the secretion of synaptic neurotransmitters, which compensates for previous drugs' limitations. The oral cavity has a wide range of stem cells which provide rich sources of mesenchymal stem cells. These stem cells come with high differentiation ability and strong plasticity. Among all oral MSCs, periodontal ligament stem cells (PDLSCs), or periodontal ligament stem cells, have been shown to be a trustworthy source for the in vivo development of fresh cement-like structures. The regeneration experiment for treating periodontal tissue defects with PDLSCs is considered one of the most promising clinical trials. During the research process, scientists found that regulating the microenvironment of the periodontal region is the main breakthrough point in improving regeneration therapy. Most dental diseases are caused by inflammation, and periodontal disease is one of the diseases in which tissues get damaged due to chronic inflammation. However, the generation of inflammation does not necessarily only have negative effects. In fact, there is a specific connection between inflammation and stem cells, which can trigger tissue regeneration function.

2. Periodontal Ligament Stem Cells (PDLSCs)

2.1. Multipotency of PDLSCs

Mesenchymal stem cells mainly rely on paracrine pathways during the process of intervening in neural regeneration, specifically using cells to release a type of lipid-enclosed particles known as extracellular vesicles; In periodontal tissue, PDLSCs and DFSCs are the major types of mesenchymal stem cells for periodontal regeneration. In treating periodontitis, EVs boost the regeneration of periodontal nerves via regulating periodontal tissue cells functioning, immune cells, and the microenvironment of the injured site. MSCs do not necessarily adopt direct implantation in wound healing but rather using paracrine mechanisms, to release growth factors, cytokines, and chemokines, encouraging cell differentiation and repair of damaged cells. The key regulatory mechanism of MSCs is the release of EV, an important paracrine product that helps to connect cell communication. The molecules on the surface of EV can attach to the target cells, triggering endocytosis, and thereby transferring the expression into the receptor cells. By promoting cell proliferation, differentiation, and apoptosis in this way. MSC-EVs aim to enhance resistance to excessive reactive oxygen species (ROS) and alleviate oxidative stress on periodontal tissue damage. Dental plaque accumulating on teeth's surfaces and the migration to the surrounding periodontal pocket can lead to the recruitment of leukocytes to the infected area. As the first line of defense against bacterial pathogens, polymorphonuclear infiltrations, like leukocytes, will eliminate invaders by releasing reactive oxygen species. However, due to the excessive activity of ROS, the load of oxidants increases, leading to oxidative stress, tissue damage, and ultimately the formation of periodontitis. Huang and her colleges discovered that after using dental follicle stem cells-derived small extracellular vesicles (D-sEV) and LPS-preconditioned dental follicle stem cells-derived small extracellular vesicles (LD-sEVROS), the ROS level in PDLSC was significantly reduced. They speculate that this is because LD-sEV is rich in antioxidants GSR and SOD1, which exert antioxidant activity and improve the recovery of normal physiological function in damaged PDLSC [1].

2.2. Immunomodulatory Effects of PDLSCs

Treatment of periodontal disease by recruiting and differentiating localized stem cells to aggregate damaged tissue is widespread and, although still in the research process, is believed to hold therapeutic promise. Since PDLSCs were isolated from human periodontal ligament in 2004, researchers have discovered that it has great potential different from other stem cells, and they exhibit more intense self-renewal and differentiation abilities. Bone marrow stromal stem cells (BMSSCs) have been shown to maintain immunomodulatory functions before, inhibiting allogeneic mixed lymphocyte reactions and the proliferation of T cells after mitotic stimulation in vitro, but the potential immune Regulatory capacity is unclear. Subsequently, in 2009, Wada et al first disclosed the experimental data related to the regulation of immune function by PDLSCs [2].

Collecting dental pulp from normal adult patients and co-culturing cells with a mitogen stimulator, Con A and activated peripheral blood mononuclear cells (PBNC), to examine whether PDLSCs can affect PBNC proliferation. Meanwhile, by co-culturing PDLSCs with PBMNCs using Transwell, tested whether PDLSCs can affect PBNC through direct contact or not. The final experimental results examined that PDLSCs from different donors showed inhibition of PBMNC proliferation in response to Con A stimulation. However, PDLSC after transwell did not display inhibition of PBMNC, so it is confirmed that the effect on PBMNC needs to be mediated by MSCs or other soluble factors. In addition, this experimental observation mentions that PDLSC, as well as other allogeneic stem cells, without immune co-stimulatory factors, such as MHC class II antigens, CD40, CD80, and CD86 [3].

2.3. Macrophage polarization in PDLSCs enhanced periodontal regeneration

The true cause of this connection is suspected to be due to the interactions among immune cells, including monocytes and macrophages, and local stem cells that ultimately affect regenerative function after sensing adverse reactions caused by inflammation. There are many different

experiments that have tested the connection between macrophages and stem cells, such as through macrophage polarization, testing deproteinized bovine bone matrix promotes osteoblast development. It was found that when macrophages and myoblasts were co-injected into injured skeletal muscle, it would lead to satellite cells proliferating and differentiating in muscle tissue. Moreover, after practicing experiments, Németh et al. discovered that the prostaglandin E2 released by bone marrow stromal cells (BMSCs) can act on the EP2 and EP4 receptors of macrophages, thereby enhancing the production and releasing anti-inflammatory IL-10 [4].

In summary, it was found that macrophages have the potential to affect MSC immunomodulatory regulation. So, in 2019, after isolating and culturing PDLSCs while polarizing bone marrow-derived macrophages (BMDMs), researchers ultimately found that PDLSCs can induce macrophage polarization, which is surprising because in previous studies, after deproteinized bovine bone matrix (DBBMs) triggers macrophage polarization, resulting in wound healing and facilitating the microenvironment of bone formation induced by biomaterials [5]. Furthermore, in the 2018 experiment, it was found that Human deciduous tooth stem cells (SHED) can induce M2 macrophage polarization, reduced inflammatory responses in the periodontal tissue, and enhanced periodontal regeneration in a rat model of periodontitis. These examples all support the positive impact of macrophage polarization will enhance periodontal tissue regeneration ability [6].

3. PDLSCs tissue regeneration engineering

3.1. Pulp Regeneration

Dental pulp is often difficult to self-heal and eradicate infected areas due to its intricate structure, tiny size, and vulnerability to inadequate blood flow. Patients with pulpitis will generally receive root canal treatment to completely eradicate the infected area and fill it with inorganic substances. However, the side effect of this treatment method is that pulp extraction can cause the teeth to become fragile and easy to break. Therefore, restoring normal tooth function through regenerated pulp is a field worth exploring. There are two ways to achieve dental pulp regeneration: through cell transplantation to complete dental pulp regeneration, or by recruiting donor cells to repair or regenerate damaged tissue.

3.1.1 Cell-transplanted approach

Cell transplanted approach separates dentin cells and cultivates them in vitro to rapidly proliferate cell numbers. Multiple experiments demonstrate the potential of dentin cell transplantation. In 2000, Gronthos et al. first cultured the progenitor cell group DPSC from adult dental pulp and attempted to compare it with BMSCs, which can develop into several stromal cell lineages and exhibit great repeatability [7]. To test whether DPSC has the same tissue specificity as BMSC, in order to study the potential of cell regeneration in the dental pulp area. Gronthos et al. mixed DPSC and BMSCs with hydroxyapatite/tricalcium phosphate (HA/TCP) and subcutaneously transplanted them into 10-week-old mice with immune dysfunction. The final experimental data showed that DPSC even exhibited higher proliferation rates in vitro cultivation. And the cell population derived from a single progenitor cell exhibits a typical fibroblast-like morphology, which confirms the clonal characteristics of DPSC. These two points demonstrate the identity of DPSC as a stem cell, and DPSC shares a similar protein expression pattern with known stem cell BMSCs, demonstrating the ability of cell regeneration in the dental pulp. This discovery was further confirmed in subsequent clinical studies. Khorsand et al. found in the experimental data 2013 that the combination of implanted DPSC and Bio-Oss particles not only demonstrated the ability of alveolar bone regeneration but also led to the reconstruction of cementum and periodontal ligament (PDL) [8]. This experiment shows that Additionally, DPSC offers enormous potential for the treatment of periodontitis. However, in George's experimental data, it was found in the dog model that dentin cell transplantation seemed to only work in most cases where the pulp was not completely lost. The pulp system would not be able to replenish itself if more than half of the pulp was lost [9].

3.1.2 Cell homing approach

Despite the astonishing potential of cell transplantation technology, there are always risks associated with this therapy. Many obstacles hinder the cultivation and transformation of cell transplantation, such as the high cost of operation and transformation, difficult to determine which part of the cell to use, the rejection reactions generated after transplantation, and the risk of pathogen invasion. However, as another type of dentin regeneration therapy, cell homing approach mainly relies on recruiting autologous endogenous cells to the defect site, utilizing the innate healing potential of endogenous cells, and stimulating tissue regeneration through biological signaling molecules as induction. Lee et.al injected transforming growth factor β 3 (TGF β 3) into a complex of poly- ϵ -caprolactone and hydroxyapatite to regenerate the articular surface of rabbit synovial joints. After four months, TGF β 3 mediated biological scaffold was covered by chondrocytes. The experimenter indicates that complex tissue regeneration is possible through combined transforming growth factors and endogenous cells [10]. In the same year, Kim et al. used cell homing to demonstrate tooth like structure regeneration and periodontal integration in vivo. They used 3D layer-by-layer apposition structure of mandibular central incisor and human mandibular first molar through a composite material created by 80 wt% polycaprolactone (PCL) and 20 wt% of hydroxyapatites (HA). At the same time, 3D micro-strands and interconnected microchannels were constructed for cell migration. Subsequently, The SDF1, which is suitable for CXCR4 receptor binding in multiple cell lineages, is mixed with BMP7. The mixed solution was injected into the microchannel and placed in experimental mice for observation. After nine weeks, researchers observed that more cells were detected in the scaffold microchannels with or without growth factor intervention, While with growth factor intervention, greater angiogenesis was seen in the microchannels. Therefore, this experiment not only proves that cells can recruit into the microchannels of the scaffold but also speculates on the possibility of periodontal regeneration and the generation of new alveolar bone [11].

3.2. Whole Tooth Regeneration

Severe periodontal disease, dental caries, or traumatic oral diseases may lead to tooth loss. This not only seriously affects the daily life of patients, but also causes significant psychological harm. In 2000, the Director of the United States Department of Health stated that "multiple facial and oral deformities can negatively impact one's self-esteem, inhibit social contact as a whole, cause chronic stress and melancholy, and result in significant financial costs." Subsequently, Whole Tooth Regeneration therapy began to receive attention.

3.2.1 Epithelial- Mesenchymal-based Whole Tooth Regeneration

As we introduced earlier, inducing tissue regeneration using cell transplantation technology is considered a highly promising technique in regenerative studies. However, obstacles remain in converting tooth regeneration based on cell delivery into treatment methods. Autologous human embryonic tooth germ cells are unable to undergo tissue regeneration in adults, which can easily lead to rejection reactions and pathogen transmission. Xenogeneic nonhuman embryonic tooth germ cells, due to different patterns and changes in shape and size, can result in immune rejection and facial deformities in the shape of the dental crown and root. Postnatal autologous tooth germ cells were judged to lack normal tooth function, which led to the limitations of cell transplantation. Subsequently, the application of autologous stem cells for cell recruitment gradually became a more popular treatment method. Although stem cell transplantation is known as a highly promising treatment option, it is more commonly used to target individual damaged areas. Modern technology is still unable to accurately utilize stem cells to achieve the ability to reconstruct complex organs. Nowadays, a new biotechnology utilizes the natural mechanism of tooth formation to mimic the regulation of epithelial and interstitial interactions as a breakthrough point, to achieve tooth regeneration ability. This field is divided into two categories: Epithelial-independent and dependent mesenchymal.

3.2.2 Epithelial-independent mesenchymal-based Whole Tooth Regeneration

During tooth development, the initial signal transmission comes from the induction of mesenchymal cells by epithelial cells, then triggering the stimulation of tooth development. The cell aggregation method grasps this characteristic, and through the dissociation–reassociation experiments, it is necessary to achieve the correct crown shape in tooth tissue engineering to reconstruct the occlusal relationship, as well as the formation of tooth roots and periodontal ligaments, in order to achieve normal tooth function. Hu et al reported that after the initial crown formation, odontoblasts and ameloblasts undergo finer cell differentiation by culturing coronaviruses in vitro. The implementation of this method involves temporary ectopic implantation under the patient's skin to reduce rejection reactions while observing the development of the crown and surrounding tissues [12]. Another related experiment triggered tooth development by using induced pluripotent stem cells (iPSCs) to differentiate into epithelial slices and reconstitute them with mouse tooth mesenchyme. The final results demonstrated that the eight different pluripotent cell lines used all successfully responded to dental mesenchyme cells, and initiated odontogenic signaling. The success rate is as high as 30% [13].

3.2.3 Epithelial-dependent mesenchymal-based Whole Tooth Regeneration

Donor organ transplantation is currently the most effective method for treating diseases caused by organ dysfunction, but its development is limited due to the insufficient number of donor organs. The current research goal of regenerative therapy is to compensate for this deficiency, attempting to replace damaged or aging organs with fully functional bioengineered replacements. One concept of bioengineering regeneration is to use three-dimensional tissue engineering technology to construct fully functional artificial organs, which are interconnected with natural cell molecules using biodegradable materials. In 2009, scientists attempted to construct three-dimensional engineered organ germs. Ohshima et al. conducted bioengineered dental germ into the alveolar bone of the missing tooth area in adult mice, they found that nerve fibers in the pulp of bioengineered teeth and reacted with nerve fibers in PDL tissue. As a representative technology of three-dimensional tissue engineering, scaffolding technology mainly achieves tissue regeneration by transplanting target cells into biodegradable materials such as cytokines and synthetic polymers. Honda et al. examined the proliferation of chondrocytes on various rib cartilage resting scaffolds in experimental rats, and subsequently, the proliferation of chondrocytes was detected [14]. A similar experiment was conducted by Ohshima et al. in 2011. They found that after implanting bioengineered teeth, not only did it compensate for missing teeth, but it also triggered the regeneration of surrounding alveolar bone. In mice, 14 days after the transplantation of regenerated teeth, a significant increase in regenerated bone mass was observed [15]. These all demonstrate the enormous potential of scaffold technology for bone regeneration.

3.3. Periodontal Tissue Regeneration

One of the key factors in the loss of adult teeth is periodontitis, an infectious condition of the periodontal tissue. Utilizing human stem cells to achieve periodontal regeneration has become one of the most promising therapeutic methods. Among different mesenchymal stem cells, PDLSCs are the major candidate for periodontal regeneration. In a 2009 experiment, Yi Liu and his colleagues tested PDLSCs-mediated treatment of small pig periodontitis. Since human PDLSCs have been successfully cultured from human periodontal tissue, the objective of this experiment is to test whether PDLSCs can complete tissue regeneration. Experiments on 14 self-bred miniature pigs ultimately found that the initial efforts to directly transplant PDLSCs to the periodontal injury site to promote tissue regeneration were not optimistic. However, at 12 weeks after transplantation, new periodontal bone formed and differentiated into osteoblasts, indicating that the transplanted PDLSCs contribute to in vivo periodontal tissue regeneration. But bone regeneration is limited, so researchers hypothesized that: “the connections among transplanted stem cells, the residual precursor cells, and the local periodontal microenvironment could be critical player.” Newly transplanted PDLSCs have the

function of stimulating progenitor cells and secreting cytokines, but at the same time, they need to rely on the local periodontal microenvironment to help stabilize.

4. Conclusion

In conclusion, MSCs have shown amazing potential in the treatment of different oral diseases. Severe dental disease will directly lead to tooth loss, which will bring irreversible harm to oral health. The development of tooth regeneration technology fully compensated for this risk. From the initial cell-transplanted approach, people tried to cultivate cells in vitro and transplant them back to the infected site to promote tissue regeneration. However, the immune rejection led to a high failure rate. Subsequently, researchers discovered a way to recruit cells in vivo, making up for the shortcomings of the cell-transplanted approach. The in-depth study of cell homing therapy has also enabled scientists to focus more on the treatment method of autologous cells to heal the infection site and boost tissue regeneration. Meanwhile, combined with previous knowledge of human stem cells, researchers started using stem cells in clinical treatment. In previous content, we explore the treatment of PDLSCs. In a large number of clinical trials, PDLSCs have shown incredible healing and tissue regeneration capabilities for periodontal diseases, and this discovery further confirms the great potential of stem cells in promoting the regeneration of oral tissue.

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