Stem Cell Transplantation/Cell-Free Treatment for Periodontal Regeneration

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Many in vivo and in vitro studies have demonstrated the efficacy of stem cell transplantation for the regeneration of periodontal tissues over the past 20 years. Although positive evidence has accumulated regarding periodontal regeneration using stem cells, the exact mechanism of tissue regeneration is still largely unknown.

periodontal disease regeneration cell transplantation conditioned medium exosome

1. Introduction

Periodontal disease is a chronic inflammatory state of periodontal tissues caused mainly by the colonization of Gram-negative bacteria [1][2]. In the local area affected by periodontitis, chronic inflammation over a long period of time leads to progressive destruction of the tooth-supporting tissues, and eventually, the affected teeth are extracted due to the loss of supporting tissues [3].

Periodontal disease is one of the primary causes of tooth extraction worldwide [4], and it is widely recognized that periodontal treatment is important in maintaining oral functions including mastication and pronunciation. In addition, recent studies have reported that periodontal disease is related to the pathogenesis of various systemic diseases such as diabetes, cardiovascular disease, low birth weight, osteoporosis, etc. [5][6]. The importance of periodontal treatment has been emphasized from the perspective of maintaining systemic health.

The ultimate treatment goal of periodontal disease is to regenerate lost periodontal tissues including cementum, periodontal ligament, and alveolar bone. However, there is still no complete periodontal regenerative treatment, and thus, research is being conducted to develop a new treatment for periodontal regeneration. In this context, attempts to regenerate periodontal tissues by stem cell transplantation have been made over the past 20 years. A number of studies have examined whether various stem cells can regenerate periodontal tissues in multiple animal models.

2. Periodontal Regeneration by Stem Cell Transplantation

Recent advances in cell biology and tissue engineering technologies have led to the development of therapeutic strategies to treat diseases by transplanting or administering cells cultured outside the human body [7][8]. In the field of regenerative medicine, the strategy of reshaping tissues that have lost their function by transplanting cells with the capacity to form these tissues is very reasonable. Of the many stem cell types, mesenchymal stem cells

(MSCs) are the most frequently tested for periodontal regeneration. MSCs were originally identified from bone-marrow aspirates as a plastic-adherent fibroblastic cell population with multi-differentiation potential mainly into mesenchymal linages [9].

3. Problems in Periodontal Regenerative Therapy Using Stem Cell Transplantation

3.1. Mechanism of Tissue Regeneration

In most stem cell-based tissue regeneration procedures, transplanted stem cells are expected to localize or migrate, proliferate, differentiate, and form new tissues at the transplanted site. However, many cell transplantation studies have reported that the actual engraftment of transplanted cells is very low [10][11][12][13]. In periodontal regeneration, very few studies have observed the long-term distribution of transplanted stem cells in regenerated periodontal tissues.

3.2. Limitations of Autologous Stem Cells

In animal experiments, allogenic and xenogeneic cells are often used to examine their regenerative potential. However, when considering cell therapy in humans, the first choice is to transplant autologous cells. Autologous stem cell transplantation in patients with periodontal disease requires several considerations. The first is the limitation of tissue retrieval for harvesting stem cells. In order to culture stem cells, tissues such as the periodontal ligament, dental pulp, gingiva, bone marrow, and fat need to be harvested; however, these tissues are sometimes difficult to collect due to their scarcity. Second, the majority of patients with periodontal disease are middle-aged or older, and the effects of aging on tissues and cells need to be taken into account when transplanting autologous cells. Aging is known to negatively affect the number and quality of tissue stem cells [14][15].

3.3. Safety Issues in Stem Cell Transplantation

The safety of stem cell transplantation for periodontal regeneration has been reported by many clinical studies [16][17][18][19][20][21]. Safety considerations regarding autologous stem cell transplantation include the transmission of infection during ex vivo expansion, and tumor or unintended tissue formation after transplantation. The number of cells required for cell transplantation may vary depending on the degree of periodontal tissue destruction; however, it is believed that approximately 10^6 to 10^7 cells are required per deep periodontal defect. Therefore, ex vivo expansion of cells is unavoidable in the stem cell treatment of periodontal disease. The contamination of pathogenic factors in the process of cell culture must be avoided.

In general, MSCs are thought to have very low tumorigenicity; however, it is assumed that, through cell culture and passaging, the cells are greatly affected by cellular stress, which increases the risk of tumorigenesis. Since most animal experiments were completed in a relatively short period of time, from several weeks to months, there is a paucity of data showing the long-term safety of MSC transplantation. Dannan et al. reported the development of

squamous epithelial-cell carcinoma after transplantation of human periodontal ligament-derived stem cells into periodontal defects in athymic rats [22].

4. Strategies for Solving Problems

4.1. Allogeneic Cell Transplantation

The problems of qualitative and quantitative limitations of stem cells and the cost of treatment considered in autologous cell transplantation may be solved to some extent by allogeneic cell transplantation. The use of cells from young donors allow for the transplantation of a large number of cells with a high therapeutic efficacy. In the case of using stem cells from dental tissues, tissue donations are readily available because most patients with premolar or wisdom teeth extraction are young, and these teeth are often discarded as medical waste. The safety issues associated with allogeneic cell transplantation need to be confirmed in clinical studies in humans. Dhote et al. transplanted allogenic umbilical cord-derived MSCs into human periodontal defects and reported improved periodontal tissue formation and safety after 6 months [16].

4.2. Cell-Free Treatment

The basic idea of tissue regeneration by MSC transplantation is to utilize the differentiation ability of stem cells and to expect that the transplanted stem cells will grow and differentiate locally to regenerate the tissues. However, in many stem cell transplantation studies, the local retention of transplanted cells has been much lower than expected, and it is clear that tissue regeneration and wound healing promotion are mediated by factors secreted from the cells [10][11][12][13]. Secreted factors produced by MSCs are known to have anti-inflammatory, immunoregulatory, angiogenic, and anti-apoptotic functions [23][24]. Based on the potential of secreted factors, tissue regeneration using secreted factors/substances from stem cells is being investigated as a cell-free treatment.

4.2.1. Conditioned Medium

A method has been reported in which secreted factors released from stem cells are collected in the form of culture supernatant and transplanted instead of the cells themselves. Nagata et al. showed that the transplantation of a conditioned medium (CM) obtained from PDLSCs resulted in the regeneration of periodontal tissues. This study demonstrated that PDLSC-CM contained various growth factors and angiogenic factors and that the gene expression of tumor necrosis factor (TNF)-alpha was suppressed at the CM implantation site [25].

4.2.2. Exosomes/Extracellular Vesicles

Exosomes/extracellular vesicles are small particles (<100 nm) from cells with a lipid bilayer structure. Exosomes contain various cellular components, such as proteins, mRNAs, DNA, and miRNAs. Exosomes released from cells have recently been recognized as new tools for cell–cell communication, as exosomes can be taken up by distant cells and can exert their functions within those cells [26][27]. MSC-derived exosomes contain molecules that

modulate wound healing and are believed to be responsible for many of the actions of MSC humoral factors, including angiogenesis, anti-inflammation, anti-apoptosis, and immune regulation [28][29].

4.2.3. Features of Cell-Free Treatment

Cell-free treatment, consisting of a CM and exosomes, has features that cover some of the disadvantages of cell transplantation. In CM and exosome transplantation, the possibility of tumor formation and immune rejection is considered to below. In addition, because cells are not transplanted, problems with donor aging and cell sources can be avoided. CMs and exosomes can be stored in freezers, which makes them more practical than cells, as they are inexpensive and easy to manage and use. However, since tissue regeneration with cell-free treatment improves the patient's wound healing process, the amount of tissue regeneration may be lower than that with cell transplantation.

5. Conclusions

Recently, clinical studies on periodontal tissue regeneration by stem cell transplantation in humans have been reported. In three of these studies, there was a trend toward improvement of clinical parameters in the stem cell transplantation group; however, no statistically significant differences were observed [17][19][20]. Autologous cells were used in these studies, and the results of the studies differed from the results of previous animal studies. The quality of transplanted stem cells due to patient aging and chronic inflammation or changes in the transplantation site may have affected the results of human clinical studies. The efficacy of stem cell transplantation for periodontal regeneration has been shown mainly in animal experiments, and we need to find a way to solve the remaining problems to establish the stem cell therapy as a new periodontal regenerative strategy in human patients. Considering the cost and safety issues, much more research needs to be conducted to make stem cell therapy practical for periodontal regeneration. To achieve this, it is necessary to optimize the technique, including the appropriate cell type, number of cells, and graft carrier, and to verify the therapeutic effect and safety through rigorous clinical studies with appropriate controls. Furthermore, the mechanism of periodontal tissue regeneration by stem cell transplantation, including the fate of transplanted cells, needs to be elucidated. Cell-free treatment also requires detailed mechanisms, verification of therapeutic effects, and clinical studies in humans.

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