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Article

Study on cellular behavior and molecular mechanism of periodontal tissue in vitro

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Abstract: Periodontal regeneration is the ultimate goal of periodontal therapy. In the study of constructing periodontal tissue in vitro, attempts are made to simulate the regeneration process of periodontal tissue. The research and clinical application of periodontal ligament stem cells have made many breakthroughs, but they still face many challenges. To achieve true periodontal tissue regeneration, in-depth research on its molecular mechanism and signaling pathway is still needed. Combined with the research progress in recent years, this paper discusses the challenges and possible solutions faced in the process of periodontal tissue regeneration.

Keywords: periodontal regeneration; human periodontal ligament cells (PDLCs); human bone marrow derived mesenchymal stem cells (hMSCs); osteogenic differentiation; tissue engineering; Wnt/β-catenin signaling pathway; in vitro proliferation

1. Introduction

1.1. Research background

Periodontal disease is one of the most common diseases in the mouth and is also the main cause of tooth loss in adults [1]. Periodontal tissues play a crucial role in oral health, and the integrity of their structure and function directly affects the stability and function of teeth. Damage or diseases of periodontal tissues, such as periodontitis, often lead to tooth mobility and loss, imposing significant physical and psychological burdens on patients. Currently, conventional clinical treatments, such as basic periodontal therapy and flap inversion curettage surgery, although capable of removing plaque and controlling inflammation, struggle to achieve the regeneration of damaged periodontal tissues [2]. However, periodontal regenerative surgery, such as guided tissue regeneration and bone grafting, although has a certain clinical benefit rate, still has some problems such as limited clinical indications and poor predictability [3]. In recent years, with the rapid development of stem cell therapy and tissue engineering research, the study of in vitro construction of periodontal tissues has gradually become an important research field. It is planned to use methods such as cell membrane patch technology to attempt to simulate the regeneration process of periodontal tissues. By constructing single-layer or multi-layer cell membrane patches and combining them with scaffold materials, a certain mechanical performance and stability of tissue structures can be formed, thus providing new ideas and methods for the treatment of periodontal diseases.

In the process of constructing periodontal tissues in vitro, the study of cellular behavior and molecular mechanisms is particularly important. The proliferation, differentiation, migration, and interactions between cells are all key factors affecting the regeneration of periodontal tissues. Osteogenesis: One of the critical mechanisms by which stem cells promote periodontal regeneration is their osteogenic differentiation capability. Stem cells, especially MSCs, can differentiate into osteoblasts, which are specialized cells responsible for bone formation. This osteogenic potential is particularly crucial for regenerating alveolar bone, an important component of periodontal tissues. Alveolar bone plays a vital role in supporting and stabilizing teeth. Periodontal diseases often lead to bone loss, resulting in tooth mobility and loss. The differentiation of stem cells into osteoblasts aids in the repair of alveolar bone, contributing to the overall success of periodontal regeneration [4]. PDL cell differentiation: Another important aspect of stem cell-mediated periodontal regeneration is their potential to differentiate into PDL cells. PDL is a specialized connective tissue that fixes teeth in the alveolar bone and provides stability and shock absorption during chewing. Stem cells, particularly those with pluripotency, can differentiate into PDL cells. This differentiation process is crucial for restoring the periodontal ligament, ensuring that teeth are fixed and stable within the dental arch. By regenerating periodontal ligament cells, stem cell therapy aims to enhance tooth stability and prevent further tooth mobility, which is a common consequence of periodontal disease [5]. Studies have shown that the composition and structure of the extracellular matrix significantly influence cell behavior, providing crucial insights into the regeneration of periodontal tissues. Meanwhile, signaling pathways play a vital role in regulating cell behavior, with relevant research elucidating the functions of various signaling molecules in the regeneration of periodontal tissues [6].

With the in-depth study of the mechanisms of periodontal tissue regeneration, more and more biomaterials and biotechnologies are being applied in clinical practice. For example, injectable hydrogels have made significant progress in oral tissue regeneration, consisting of methyl methacrylate hyaluronic acid (me-HA) and platelet lysate (PL), which release growth factor proteins in situ. Multifunctional hydrogels provide ample space and stability for cell adhesion and proliferation, showing great potential in periodontal treatment. This offers new options for the repair of periodontal tissues [7]. By investigating these cellular behaviors and molecular mechanisms, we hope to develop more effective therapeutic strategies to improve the quality of life of patients with periodontal disease.

1.2. Research objectives

In the study of periodontal tissues, it is crucial to clearly define the research objectives. This study aims to explore the cellular behavior and molecular mechanisms of in vitro constructed periodontal tissues, providing new ideas and methods for the treatment of periodontal diseases and tissue regeneration. By deeply analyzing the behavior of cells under different microenvironments, the study will reveal the specific mechanisms of cell proliferation, differentiation, migration, and their interactions. These changes in cellular behavior are closely related to the health status of periodontal tissues, and understanding these mechanisms will contribute to the development of new therapeutic strategies.

In addition, the study will focus on the role of signaling pathways and gene expression regulation in cell behavior [8]. Studies have shown that signaling pathways play a crucial role in cell proliferation and differentiation, especially in the regeneration of periodontal tissues, where the molecular mechanisms involved remain to be further explored. Research into these mechanisms can provide more effective therapeutic approaches for clinical use.

This study will combine the latest research findings from both domestic and international sources to analyze the current shortcomings in research and propose directions for future studies. Through a systematic literature review, the research will provide a foundation for academic exchanges in the field of periodontal tissue regeneration, promoting the advancement and application of related technologies. It is hoped that through in-depth exploration in this study, new theoretical foundations and practical guidance can be provided for the regeneration and repair of periodontal tissues.

1.3. Overview structure

In this review, a systematic discussion on the cellular behavior and molecular mechanisms of in vitro constructed periodontal tissues will be presented. Firstly, the basic composition of periodontal tissues and their importance in oral health will be introduced, emphasizing the potential impact of periodontal tissue regeneration on the treatment of periodontal diseases. Next, different aspects of cell behavior including cell proliferation, differentiation, migration, and adhesion will be analyzed, exploring how these behaviors are regulated by the microenvironment and signaling molecules. Relevant studies have shown that intercellular interactions play a crucial role in tissue reconstruction, involving the coordinated actions of various cell types [9].

In the molecular mechanisms section, the focus will be on signaling pathways and their role in cellular behavior. By analyzing major signaling pathways, the importance of their functions in cell proliferation and differentiation will be revealed, as well as how the interactions between different signaling pathways influence the regeneration of periodontal tissues [10]. In addition, gene expression regulation and the role of the extracellular matrix will be discussed in detail, emphasizing the importance of these factors in cell function and tissue structure [11].

The final part of the review will revisit the current status of related research both domestically and internationally analyze the shortcomings and challenges in current studies and propose directions and suggestions for future research Through a comprehensive analysis of existing literature it aims to provide a theoretical foundation for further research and further promote the development of periodontal tissue regeneration techniques.

2. Cellular behavior of periodontal tissues

2.1. Cell proliferation and differentiation

Cell proliferation and differentiation are key components in the regeneration process of periodontal tissues. The health status of periodontal tissues is closely related to cell proliferation capacity, especially in the repair and regeneration of periodontal diseases, where cell proliferation and differentiation capabilities directly affect the recovery outcomes of tissues. Studies have shown that fibroblasts and osteoblasts play crucial roles in the regeneration of periodontal tissues, and their proliferation and differentiation are regulated by multiple factors including growth factors, extracellular matrix components, and changes in the microenvironment.

In the periodontal tissues, fibroblast proliferation and differentiation are influenced by various growth factors. Growth factors and cytokines: Stem cells play a crucial role in promoting periodontal tissue regeneration by secreting growth factors and cytokines. These bioactive molecules include vascular endothelial growth factor (VEG), fibroblast growth factor (FbG), and TGF-β, among others. VEG stimulates the formation of new blood vessels (angiogenesis), which is essential for delivering nutrients and oxygen to regenerating tissues. FbG promotes cell proliferation and tissue repair, while TGF-β involves various cellular processes including tissue healing and immune regulation. By releasing these growth factors and cytokines, stem cells create a microenvironment conducive to tissue repair, angiogenesis, and overall regeneration within the periodontal region. Extracellular vesicles (EVs): Stem cells release EVs, which are small membrane-bound structures containing microRNA, proteins, and other bioactive molecules. These EVs play a key role in intercellular communication, regulating the behavior of neighboring cells in the periodontal microenvironment. The microRNA in EVs can influence gene expression in recipient cells, promoting various cellular processes including differentiation and proliferation. EVs from stem cells actively participate in supporting tissue regeneration and maintaining a balanced immune response. They play a crucial role in intercellular communication. The role of this helps to coordinate the regeneration process and make it an important part of periodontal regeneration stem cell therapy [12]. Like Transforming Growth Factor β (TGF-β) and Fibroblast Growth Factor (FGF) are among the most abundant cytokines in bone matrix. These factors can promote the proliferation of fibroblasts and induce their differentiation into osteoblasts thereby facilitating the regeneration of periodontal tissues [13]. The study found that TGF-β not only promotes cell proliferation, but also regulates the gene expression of cells and enhances the response ability of cells to external stimuli.

In addition, the extracellular matrix (Extracellular Matrix, ECM) plays a crucial role in cell proliferation and differentiation. Extracellular matrix proteins are a class of proteins found in the extracellular matrix of teeth, which are important for tooth growth, development, and maintaining dental health [14]. The composition and structure of the extracellular matrix can affect cell behavior, which in turn affects the regeneration of periodontal tissue. For example, collagen and glycosaminoglycans can provide attachment points for cells to promote cell proliferation and differentiation [15]. In different physiological and pathological states, the composition and function of the extracellular matrix may change, which will also affect the proliferation and differentiation process of cells.

To illustrate more clearly the mechanisms of cell proliferation and differentiation, the following **Figure 1** flow chart summarizes the key steps and relationships of this process:

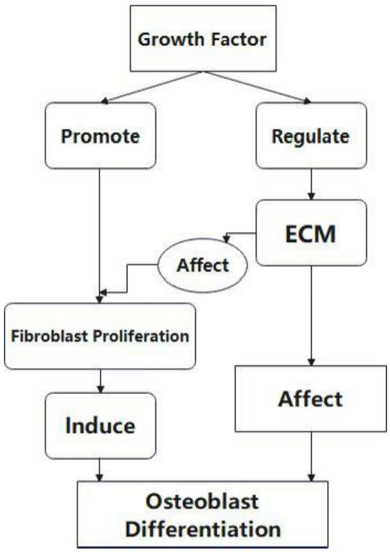

Figure 1. Flow chart of cell proliferation and differentiation mechanism.

In clinical research, different therapeutic approaches are adopted for various periodontal diseases to promote cell proliferation and differentiation, which has become a research hotspot. By deeply studying the mechanisms of cell proliferation and differentiation, new ideas and methods for the regeneration of periodontal tissues can be provided.

2.2. Cell migration and adhesion

Cell migration and adhesion are crucial biological behaviors in the regeneration of periodontal tissues. Cell migration involves the movement of cells within tissues, typically regulated by various biological signals. During migration, cells need to interact with the extracellular matrix (ECM), where adhesion molecules play a significant role. Cells bind to the ECM through integrins and other adhesion molecules, thereby achieving sensing and signal transduction of the matrix. This adhesion not only affects the cells migratory capacity but also has profound impacts on cell proliferation and differentiation.

In periodontal tissues, the process of cell migration is regulated by various factors including growth factors, cytokines, and mechanical forces. Studies have shown that periodontal auxiliary accelerated osseointegration orthodontics (Periodontally Accelerated Osteogenic orthodontics, PAOO) has a significant impact on the periodontal tissues of the dental areas of orthodontic patients, which is closely related to cell migration involving changes in cell proliferation and differentiation [16]. In addition, the changes in soft and hard tissues around the dental implants of patients with periodontitis are also related to the mechanism of cell migration and adhesion, which indicates that the changes in cell behavior may affect the health status of periodontal tissue under different pathological conditions [17].

In order to better understand the migration and adhesion behavior of cells in the construction of periodontal tissue, the following **Figure 2** flow chart shows the key links of this process:

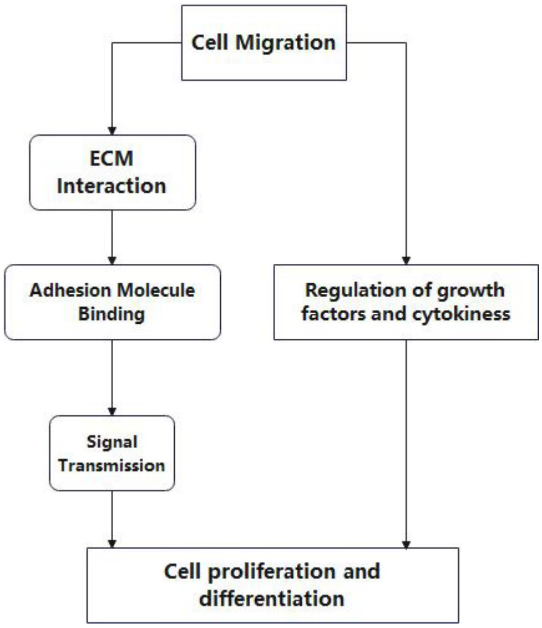

Figure 2. Flow chart of cell migration and adhesion behavior in periodontal tissue construction.

The study of cell migration and adhesion not only helps to reveal the basic mechanism of periodontal tissue regeneration, but also provides new ideas for clinical treatment. By regulating cell migration and adhesion, it can promote the repair and regeneration of periodontal tissue, improve patients' oral health.

2.3. Cell interaction

Intercellular interactions play a crucial role in the development and maintenance of periodontal tissues. Cells communicate with each other through various mechanisms including cell signaling cell adhesion and material exchange between cells. These interactions not only influence cell growth and differentiation but also play a significant role in the remodeling and repair of tissues.

In the periodontal tissues, the interactions between fibroblasts, epithelial cells, and immune cells are particularly important. Fibroblasts regulate the behavior of surrounding cells by secreting cytokines and growth factors. For example, fibroblasts can promote osteoblast differentiation by secreting transforming growth factor-β (TGF-β), thereby enhancing bone tissue formation. Additionally, the interaction between fibroblasts and immune cells plays a crucial role in inflammatory responses, influencing the progression and treatment outcomes of periodontitis. Gingival fibroblasts (GFs) have been shown to differentiate into osteoblast-like cells that deposit calcium in nodular form. Their degree of differentiation reduces their ability to induce osteoclasts. This further suggests that through appropriate stimulation, GFs have the potential for use in regenerative periodontal therapy [18].

Cell adhesion molecules play a crucial bridging role in intercellular interactions. Cell adhesion molecules such as integrins and cadherins can promote cell-to-cell adhesion and enhance signal transduction between cells. These molecules not only influence cell migration and proliferation but also contribute to tissue structural stability. Studies have shown that the expression levels of cell adhesion molecules are closely related to the health status of periodontal tissues, and abnormal expression may lead to the occurrence of periodontal diseases.

The exchange of substances between cells cannot be overlooked. Cells transmit substances through cell junctions and exosomes, which include small molecules, proteins, and RNA. This exchange of substances can regulate cellular functions and influence the overall response of tissues. For example, miRNA in exosomes can modulate gene expression in neighboring cells, thereby affecting cell behavior and the repair process of tissues.

The complexity of intercellular interactions makes the study of periodontal tissues challenging. Future research needs to delve into the specific mechanisms of these interactions in order to provide new ideas and methods for the treatment of periodontal diseases. By understanding intercellular interactions, it is possible to better design therapeutic strategies for periodontal tissue regeneration, thereby improving patients oral health.

3. Molecular mechanism

3.1. Signal transduction pathway

In the cellular behavior of periodontal tissues, signaling pathways play a crucial role. These pathways regulate processes such as cell proliferation, differentiation, migration, and apoptosis by converting external signals into intracellular biological responses. The main signaling pathways include the Wnt/β-catenin pathway, MAPK pathway, PI3K/Akt pathway, and NF-κB pathway.

The Wnt/β-catenin pathway and its regulatory factors play an important role in the development and regeneration of periodontal tissues. This pathway regulates the stability of β-catenin, which further affects the proliferation and differentiation of fibroblasts and osteoblasts [19]. Studies have shown that the activation of Wnt signaling can promote osteoblast differentiation, thus enhancing the regenerative capacity of bone tissue [20].

The MAPK pathway plays a key role in cellular stress response and inflammatory response. This pathway regulates cell proliferation and apoptosis through different MAPK proteins such as ERK, JNK and p38 [21]. In periodontal inflammation, the activation of MAPK pathway is closely related to the release of inflammatory factors, affecting the damage and repair process of periodontal tissue.

The PI3K/Akt pathway plays an important role in cell survival and metabolism. Activation of this pathway can promote cell survival, inhibit apoptosis and promote cell proliferation [22]. In periodontal tissues, abnormal activation of PI3K/Akt pathway may be associated with the occurrence and development of periodontal disease [23].

The NF-κB pathway is one of the key pathways regulating inflammatory responses. In periodontal inflammation, the activation of NF-κB leads to increased expression of various inflammatory factors, thereby exacerbating tissue damage. Research has found that inhibiting the activity of the NF-κB pathway can alleviate periodontal inflammatory responses and promote tissue repair and regeneration [24].

In order to help readers better understand the role of these signaling pathways in the construction of periodontal tissues, the **Figure 3** flow chart of major signaling pathways is shown below:

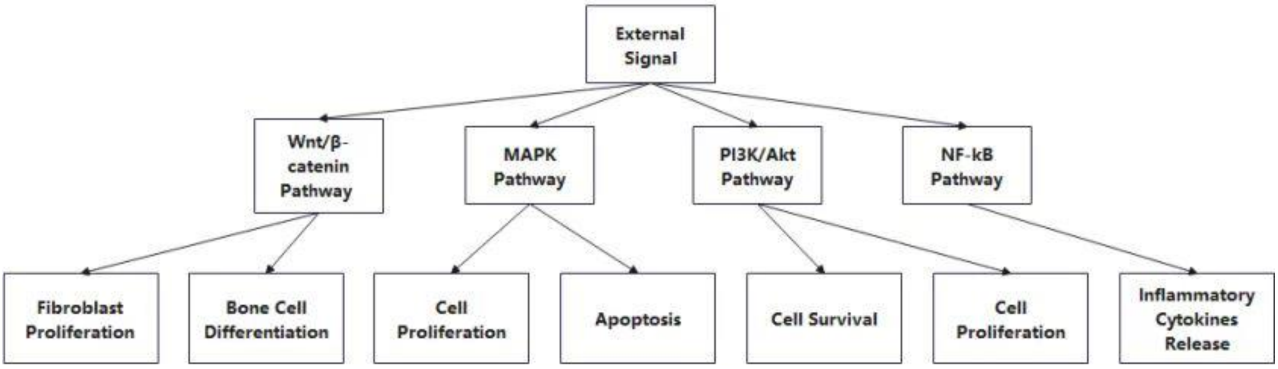

Figure 3. Flow chart of major signaling pathways.

Through in-depth research on these signaling pathways, we can better understand the cellular behavior and molecular mechanisms of periodontal tissues, providing new ideas and strategies for clinical treatment. Relevant studies have shown that the regulation of signaling pathways not only affects the biological characteristics of cells but may also provide new targets for the prevention and treatment of periodontal diseases.

3.2. Gene expression regulation

Gene expression regulation plays a crucial role in cell behavior [25]. Transcription factors are key proteins that regulate gene expression by binding to specific DNA sequences to initiate or inhibit gene transcription. Different transcription factors play roles in various cell types and physiological conditions affecting cell proliferation differentiation and migration behaviors. For example, certain transcription factors are found to be closely related to cell proliferation in fibroblasts enamel-forming cells dentin-forming cells pulp stem cells and odontoblasts in periodontal tissues these factors promote cell proliferation by regulating the expression of cell cycle-related genes [26].

Epigenetics also plays an important role in gene expression regulation. Epigenetic modifications such as DNA methylation and histone modification can alter the accessibility of genes, thus affecting the expression level of genes [27]. These modifications not only play a role in cell development but also significantly influence the cells responses to external stimuli. For example, in the inflammatory response of periodontal tissues, the expression of certain genes may be regulated by epigenetic modifications, thereby affecting cellular function and behavior.

The regulation of gene expression is not limited to the transcriptional level, but also includes post-transcriptional regulatory mechanisms, such as mRNA splicing, stability and translation efficiency. These regulatory mechanisms work together to ensure that cells can quickly adapt and respond to different environmental conditions [28]. In the process of regeneration of periodontal tissue, the precise regulation of gene expression is crucial for cell function recovery and tissue reconstruction.

3.3. The role of extracellular matrix

The extracellular matrix (ECM) plays a crucial role in the construction and regeneration of periodontal tissues. The ECM not only provides support and structure for cells but also regulates cell behavior through interactions with receptors on the cell surface. The main components of the ECM include collagen, glycosaminoglycans, and glycoproteins, which collectively form the microenvironment necessary for cell survival and function [29].

In the periodontal tissue, the composition and structure of ECM directly affect cell proliferation, migration and differentiation. Studies have shown that the type and arrangement of collagen can affect fibroblast behavior, which in turn affects the repair and regeneration process of periodontal tissue [30]. Cells interact with ECM through integrin receptors, activate downstream signaling pathways, regulate gene expression, and thus affect the physiological function and behavior of cells [31].

In addition, ECM plays a crucial role in cell-to-cell interactions. Cells communicate with surrounding cells through the ECM, promoting intercellular coordination. This interaction not only helps maintain tissue homeostasis but also plays a key role in the repair process following tissue injury. Research has found that the degradation and remodeling of ECM are essential steps in tissue regeneration, during which cells regulate the composition and structure of the ECM by secreting enzymes such as matrix metalloproteinases (MMPs).

In the research on periodontal tissue regeneration, utilizing biomaterials to mimic the properties of ECM can effectively promote cell adhesion, proliferation, and differentiation. These biomaterials not only provide physical support but also enhance cell function by releasing growth factors and other bioactive substances. Therefore, in-depth studies on the role of the extracellular matrix and its interaction with cell behavior are of great significance for advancing periodontal tissue regeneration technology.

4. Literature review at home and abroad

4.1. Domestic research status

In recent years, significant progress has been made in the research of constructing periodontal tissues in vitro domestically. Many research teams have dedicated themselves to exploring the cellular behavior and molecular mechanisms of periodontal tissues, promoting the development of related technologies. Researchers have investigated the roles of these cells in periodontal tissue regeneration through various cell sources, such as fibroblasts, bone marrow mesenchymal stem cells, and deciduous tooth regenerating stem cells. Studies have shown that fibroblasts play a crucial role in the repair and regeneration of periodontal tissues, with their proliferation and differentiation regulated by multiple growth factors, including transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF).

In terms of cell migration, research has found that the composition of the extracellular matrix significantly affects cell migration capability. By modulating the components of the extracellular matrix, researchers can effectively promote cell migration, thereby accelerating the regeneration process of periodontal tissues.

Additionally, the interactions between cells are considered a crucial factor influencing the construction of periodontal tissues. The signaling mechanisms between cells, such as the expression of intercellular adhesion molecules, directly impact cell behavior and tissue reconstruction.

Domestic research also focuses on the regulation of gene expression, where researchers use gene editing techniques to explore the role of specific genes in periodontal tissue regeneration. These studies provide new insights into the development and regeneration of periodontal tissues. Meanwhile, with the advancement of biomaterials technology, researchers are beginning to attempt combining biomaterials with cells to construct more ideal periodontal tissue models. These studies not only provide crucial data for basic science but also lay the foundation for clinical applications.

4.2. International research trends

In recent years, significant progress has been made internationally in the research of constructing periodontal tissues ex vivo. Many research teams are dedicated to exploring the role of different cell types in periodontal tissue regeneration. For example, some studies have shown that fibroblasts play a crucial role in the repair process of periodontal tissues, as these cells can promote tissue regeneration and reconstruction by secreting extracellular matrix components. In addition, the application of stem cells has also become a hot topic of research, especially the potential of dental pulp stem cells and mesenchymal stem cells in periodontal tissue regeneration has been widely discussed.

In terms of molecular mechanisms researchers have discovered that multiple signaling pathways play crucial roles in cell behavior regulation. For example, the Wnt/β-catenin signaling pathway is considered to play a significant role in the proliferation and differentiation of fibroblasts and related studies have revealed its potential application in periodontal tissue regeneration. Meanwhile the TGF-β signaling pathway has been proven to be important in regulating the synthesis and degradation of the extracellular matrix providing new insights for periodontal tissue regeneration.

International cooperation in this field is also increasing, with many research institutions promoting the progress of studies through cross-border collaboration by sharing resources and data. For example, certain international projects focus on developing novel biomaterials to support the regeneration of periodontal tissues, and the biocompatibility and functionality of these materials have been widely validated. These studies not only provide new perspectives for fundamental science but also lay the groundwork for clinical applications, driving innovations in the treatment strategies for periodontal diseases.

4.3. Research gaps and future directions

In the current research on periodontal tissue regeneration, there are some significant gaps and future directions. Firstly, although existing studies have revealed the relationship between cellular behavior and molecular mechanisms, there is still a lack of in-depth understanding of the specific roles of particular cell types in periodontal tissue regeneration. For example, the interactions between fibroblasts, osteoblasts, and epithelial cells under different microenvironments and their impact on tissue reconstruction have not been adequately explored. These cells play crucial roles in the regeneration of periodontal tissues, and future research should focus on the functional characteristics and regulatory mechanisms of these cells to better understand their role in tissue regeneration.

Second, existing research is mostly focused on the exploration of single signaling pathways while there is less attention paid to the interactions between signaling pathways and their comprehensive impact on cellular behavior. The interactions between different signaling pathways may affect cellular proliferation migration and differentiation behaviors and future research should adopt a systems biology approach to comprehensively consider the interactions of multiple signaling pathways to reveal their complex mechanisms in periodontal tissue regeneration.

In addition, existing in vitro experimental models often fail to fully simulate the in vivo environment, limiting the translatability of research results. Dental tissue-derived stem cells are easily obtained and can serve as an alternative cell type to bone marrow mesenchymal stem cells for clinical therapy. However, dental tissue-derived mesenchymal stem cells have limited proliferation capacity, and their differentiation ability gradually diminishes with increasing in vitro expansion cycles. Therefore, finding a method to maintain the biological characteristics of dental tissue-derived mesenchymal stem cells while preserving their viability and differentiation capacity requires further research. Future studies could consider developing more complex three-dimensional culture models or organ-on-a-chip technologies to more realistically replicate the microenvironment of periodontal tissues, thereby enhancing the clinical relevance of research. Through the application of these new technologies, researchers can better understand the relationship between cellular behavior and molecular mechanisms, providing more effective strategies for periodontal tissue regeneration.

5. Conclusions and outlook

5.1. Main findings

In this study, the relationship between cell behavior and molecular mechanisms has been thoroughly explored. The research found that cell proliferation and differentiation in periodontal tissues are regulated by multiple signaling pathways, particularly the Wnt/β-catenin and MAPK pathways which play a crucial role in cell proliferation and osteoblast differentiation. These signaling pathways influence gene expression by modulating the activity of transcription factors, thereby promoting cell function and growth. Additionally, the composition of the extracellular matrix has been confirmed to impact cell behavior, with the interaction between cells and the matrix significantly altering cell migration and adhesion capabilities.

In terms of cell migration, studies have shown that intercellular interactions regulate cell migration behavior through cell adhesion molecules (such as integrins) and cytokines (such as chemokines). These molecules play a crucial role in the regeneration of periodontal tissues, promoting directed cell migration and tissue reconstruction. The signaling mechanisms between cells have also been found to have a profound impact on cell behavior, particularly in inflammatory responses and tissue repair processes.

In terms of gene expression regulation, transcription factors such as Runx2 and Osterix play a crucial role in osteoblast differentiation. The study also highlights the importance of epigenetics in gene regulation, where mechanisms such as DNA methylation and histone modifications significantly influence gene expression. These findings provide new insights into understanding cellular behavior in periodontal tissues and lay the groundwork for future regenerative medicine research.

For the convenience of readers, the **Table 1** is a summary of the main findings of this study:

Table 1. Summary table of main findings of this study.

Main findings	**concrete content**
Regulation of signal pathways	The Wnt/β-catenin and MAPK pathways play a key role in cell proliferation and osteoblast differentiation
The influence of the extracellular matrix	The interaction between cells and matrix significantly changes the migration ability and adhesion of cells
Regulation of cell migration	Cell adhesion molecules and chemokines regulate cell migration behavior
Gene expression regulation	Runx2 and Osterix are crucial in osteoblast differentiation, and epigenetic mechanisms significantly influence gene expression

The main findings of this study provide theoretical support for the clinical application of periodontal tissue regeneration emphasizing the complex relationship between cellular behavior and molecular mechanisms promoting further research in this field. By gaining a deeper understanding of these mechanisms future more effective therapeutic strategies are expected to be developed to improve the prognosis of patients with periodontal disease.

5.2. Limitations of the study

In this study despite the in-depth exploration of cellular behavior and molecular mechanisms of in vitro constructed periodontal tissues there are still some limitations. Firstly, the choice of experimental models may affect the generalizability of the results. The current research mainly relies on two-dimensional cell culture systems which cannot fully simulate the complex microenvironment in vivo. The behavior of cells on three-dimensional cultures or biomimetic scaffolds may differ significantly from that in two-dimensional cultures leading to an incomplete understanding of cellular behavior.

Second, the limitation of sample size may also affect the reliability of research results. Although multiple cell types and experimental conditions were used in this study, the sample size was relatively small and may not adequately represent the biological differences between individuals. Such differences may lead to different treatment outcomes in clinical applications; thus, it is necessary to increase the sample size in future studies to improve the reproducibility and reliability of the results.

In addition, research on molecular mechanisms mainly focuses on known signaling pathways and gene expression regulation while paying insufficient attention to other potential regulatory mechanisms. Many emerging regulatory factors and signaling pathways have not been fully explored which may lead to an incomplete understanding of cellular behavior. Future studies should consider integrating multi-omics data to reveal more complex regulatory networks.

Finally, the time frame of the study may also affect the interpretation of the results. Cell behavior and molecular mechanisms may exhibit different characteristics at different time points, and the observation period of this study was relatively short, which may have failed to capture the dynamic changes in cell behavior. Therefore, extending the observation time and conducting long-term tracking studies will help to gain a more comprehensive understanding of cell behavior and mechanisms in the construction of periodontal tissues.

To help readers better understand the limitations of current research and their implications, the following **Table 2** summarizes the main limitations and their possible consequences:

Table 2. Limitations and implications of the current study.

Boundedness	**Influence**
Selection of experimental model	The universality of the results is limited and may not reflect the true situation in the body
Limitations on sample size	The reliability of the results decreases and may not represent individual differences
Insufficient attention to potential regulatory mechanisms	A lack of comprehensive understanding of cell behavior may miss important factors
The observation time is short	It may not capture the dynamic changes in cell behavior

5.3. Future research suggestions

In future research, it is recommended to strengthen interdisciplinary collaboration to promote progress in the field of periodontal tissue regeneration. The integration of biomaterials science, cell biology, molecular biology, and bioengineering will contribute to the development of more effective regenerative strategies. By integrating knowledge from different disciplines, a more comprehensive understanding of the complex relationship between cellular behavior and molecular mechanisms can be achieved, thereby providing a more solid theoretical foundation for clinical applications.

At the same time, developing new technologies to improve the accuracy and reliability of research is also an important direction for future studies. The application of emerging technologies such as high-throughput sequencing technology, single-cell analysis technology, and three-dimensional culture models will provide richer data support for research. Three-dimensional culture technology: a special method of cell cultivation that can simulate the spatial interaction between cells, partially restoring the physiological environment of cells in the body, maintaining the dryness of stem cells and enhancing their osteogenic differentiation potential, is one of the current research trends in tissue engineering. Compared with traditional two-dimensional (two-dimensional, 2D) culture, three-dimensional (three-dimensional, 3D) cell culture technology can better simulate the interaction between cells and establish a beneficial microenvironment for the establishment of mesenchymal stem cells for exogenous transplantation [32]. In recent years, increasing evidence suggests that 3D spherical cell culture is considered more physiological compared to traditional 2D culture. Compared with 2D cultured MSCs-Exo, the yield of MSCs-Exo cultured in 3D is significantly higher. Meanwhile, 3D cultured MSCs-Exo exhibit stronger and more effective anti-inflammatory, antioxidant, anti-apoptotic, and immunosuppressive effects by secreting more anti-inflammatory and immunosuppressive factors [33]. The study also showed that 3D culture technology is more conducive to maintaining the dryness of mesenchymal stem cells and improving the osteogenic differentiation potential than 2D culture [34]. Currently 3D cell culture devices can be divided into static and dynamic culture devices. Static culture devices are simple they promote aggregate formation through static physical forces such as droplet technology and liquid overlay technology while dynamic culture systems induce cell aggregation by applying external forces such as centripetal force magnetic fields etc. [35]. These techniques can help researchers explore the interactions between cells and their specific mechanisms of action in periodontal tissue regeneration, thus providing more precise targets for clinical treatment.

Encouraging the integration of clinical and basic research is also an important recommendation for future research. The findings from basic research should be promptly translated into clinical applications to address practical clinical issues. At the same time, challenges and problems encountered in clinical practice should be fed back into basic research to promote continuous research advancement. We need to enhance communication between basic research and clinical application to effectively apply innovative outcomes to patient treatment. Through this two-way interaction, the development of new technologies and therapies can be accelerated, thereby improving the treatment outcomes for periodontal diseases.

During the research process, paying attention to ethical issues and patient informed consent is also an aspect that cannot be ignored. As the research progresses, related ethical issues may become increasingly prominent, therefore, when designing the research protocol, these factors should be fully considered to ensure the compliance and sustainability of the research. By comprehensively considering these recommendations, future research will be able to more effectively promote the development of periodontal tissue regeneration, providing patients with better treatment options and opportunities for improved quality of life.

Author contributions: Conceptualization, ZT; investigation, ZT; data curation, ZT; writing—original draft preparation, ZT and TC; writing—review and editing, ZT and TC; visualization, ZT and TC; supervision, TC; project administration, ZT and TC. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare no conflict of interest.

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