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Review

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Epigenetic factors associated with peri-implantitis: a review

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Abstract: Peri-implant diseases are conditions characterized by the resorption of hard tissue and the inflammation of soft tissue. Epigenetics refers to alterations in the gene expression that are not encoded in the DNA sequence, influencing diverse physiological activities, including immune response, inflammation, and bone metabolism. Epigenetic modifications can lead to tissue-specific gene expression variations among individuals and may initiate or exacerbate inflammation and disease predisposition. However, the impact of these factors on peri-implantitis remains inconclusive. To address this gap, we conducted a comprehensive review to investigate the association between epigenetic mechanisms and peri-implantitis, specifically focusing on DNA methylation and microRNAs (miRNA, or miR). We searched for relevant literature on PubMed, Web of Science, Scopus, and Google Scholar with keywords including "epigenetics", "peri-implantitis", "DNA methylation" and "microRNA". DNA methylation and miRNAs present a dynamic epigenetic mechanism operating around implants. Epigenetic modifications of genes related to inflammation and osteogenesis provide a new perspective in understanding how local and environmental factors influence the pathogenesis of peri-implantitis. In addition, we assessed the potential application of DNA methylation and miRNAs in the prevention, diagnosis, and treatment of peri-implantitis, aiming to provide a foundation for future studies to explore potential therapeutic targets and develop more effective management strategies for this condition. The findings also have broader implications for understanding the pathogenesis of other inflammation-related oral diseases like periodontitis.

Key words: Peri-implantitis; Epigenetics; DNA methylation; MicroRNA

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1 Introduction

Peri-implantitis represents a complex inflammatory response, bone tissue remodeling, and immune reactions. Research indicates that about 28% to 51% of patients who undergo dental implant restoration suffer from this disease (Astolfi et al., 2022). Clinically, peri-implantitis initially manifests as inflammation of the soft tissues surrounding the implant, and as the condition progresses, it leads to progressive bone loss around the implant, ultimately resulting in implant failure (Berglundh et al., 2018). Compared to periodontitis affecting natural teeth, peri-implantitis tends to develop more rapidly and extensively (Schwarz et al., 2018). Without treatment, it appears to develop in a non-linear and accelerated pattern. Therefore, the early diagnosis of peri-implantitis is important. Peri-implantitis not only jeopardizes the long-term stability and functionality of dental implants but also impacts the overall health and quality of life of the patients.

Multiple factors influence an individual's susceptibility to peri-implantitis, including environmental, genetic, and epigenetic factors (Alves et al., 2022; Oh et al., 2024). Epigenetics serves as a crucial link between genetic and environmental factors. Epigenetic modifications can contribute to variation in tissue-specific gene expression, potentially inciting or intensifying inflammation, influencing disease susceptibility, and regulating

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bone regeneration (Michou, 2018; Farsetti et al., 2023). The most recognized epigenetic regulatory mechanisms involve DNA methylation, histone modifications, and non-coding RNAs (ncRNAs). Currently, the understanding of how epigenetics influences peri-implantitis is restricted to DNA methylation and microRNA (miRNA, or miR), a kind of ncRNA. DNA methylation controls gene expression by altering chromosomal structure, DNA conformation, DNA stability and the functional pathway between DNA and protein (Wang and Wu, 2018), while miRNAs function in post-transcriptional gene silencing (Hiers et al., 2024). Therefore, in this article, we present results from a comprehensive and systematic search of clinical studies and *in vitro* and *in vivo* research related to peri-implantitis and epigenetics and delve into high-quality articles that discuss related mechanisms. We introduce increasing evidence revealing the epigenetic modifications occurring in peri-implantitis, offering new insights into the mechanisms of its development and progression, and suggesting new directions for clinical diagnosis and management (Fig. 1).

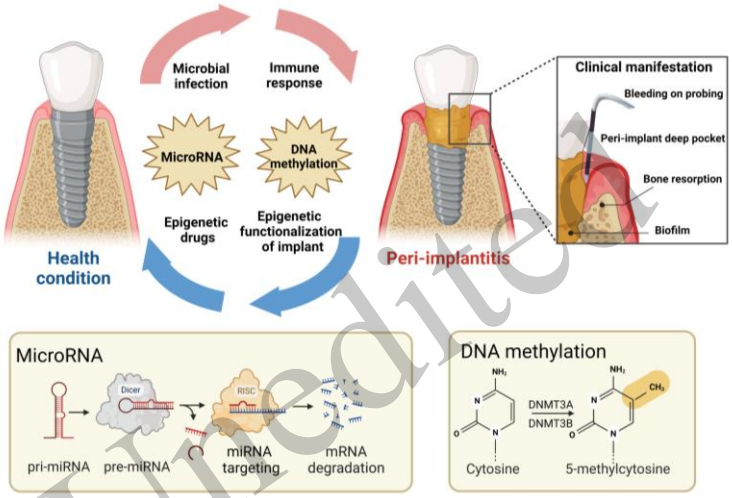

Fig. 1 Schematic of the relationship between epigenetics and peri-implantitis. Peri-implantitis is closely associated with microbial infections, host immune responses, and the tissue environment surrounding the implant. Epigenetic alterations in peri-implantitis may serve as a crucial link between environmental factors and host immune responses. Many investigators have begun to explore the use of epigenetically modified implant surfaces and epigenetic drugs for the diagnosis, prevention and treatment of peri-implantitis. (Created with bioRender.com).

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2 Epigenetics: general principles

The term epigenetics was coined by Conrad H. Waddington in the 1940s (Waddington, 2012). Epigenetics refers to changes in gene expression that are not encoded in the DNA sequence, including chemical alterations of the DNA and its associated proteins called histones, which result in remodeling of chromatin and activation or inactivation of a gene that contributes to the development of cancer, autoimmune diseases, and inflammatory diseases (Larsson, 2017).

Under the regulation of DNA methyltransferases (DNMTs), cytosine and guanine bases are separated by a phosphoric acid molecule called the CpG island, and a methyl group covalent addition occurs at the carbon-5 position of CG dinucleotides to form 5-methylcytosine (5-mC). This is DNA methylation (Choudhuri, 2011). Since 1975, DNA methylation has been considered a switch regulating gene activity during the development process (Holliday and Pugh, 1975). It plays an important role in regulating gene expression, maintaining

genome stability, and inactivating the X chromosome (Bommarito and Fry, 2019). 5-mC is not a continuously stable DNA modification and can undergo demethylation. Demethylation is the reverse process of methylation, in which methyl groups are removed. This process is catalyzed by several DNA demethylases, such as ten-eleven translocation 1 (TET1), growth arrest and DNA damage-inducible alpha (GADD45A), and octamer-binding transcription factor-4 (OCT4), leading to reduced methylation (Holliday and Pugh, 1975). DNA methylation can deactivate certain genes, while demethylation induces the reactivation and expression of genes. High levels of methylation can inhibit the activation of bone-related genes, thereby impairing osteogenic functions. Conversely, low methylation levels in promoter regions increase the expression of genes involved in osteogenic differentiation. Research has shown that DNA methylation in bones plays a crucial role in controlling genes related to the differentiation of osteoblasts and osteoclasts, including runt-related transcription factor 2 (RUNX2), osterix (Osx), osteocalcin (OCN), alkaline phosphatase (ALP), Wnt, receptor activator of nuclear factor-kB (RANK)/RANK Ligand (RANKL)/osteoprotegerin (OPG), and other important signaling pathways (Oton-Gonzalez et al., 2022).

MiRNAs are small ncRNAs that are about 22 nucleotides long. MiRNAs exist in various forms, with the most primitive being pri-miRNA, which is about 300 to 1000 nucleotides long. The pri-miRNA is cut by the Drosha/DGCR8 complex to pre-miRNA precursors that are transported into the cytoplasm by Exportin. Pre-miRNA is then cleaved by the Dicer enzyme, resulting in mature miRNA that is about 20 to 24 nucleotides long (Mohr and Mott, 2015). MiRNA selectively targets and regulates the expression of multiple genes through complementary pairing with the target mRNA. Interestingly, one miRNA can control the expression of multiple genes, and the expression of a gene can be controlled by multiple miRNAs (Selbach et al., 2008). Over 1000 miRNAs have been identified in the human genome. These miRNAs are involved in various physiological and pathological processes within the body, including the regulation of developmental processes, resistance to viral infections, modulation of immune functions in animals, maintenance of bone homeostasis, and the development of tumors (Shang et al., 2023).

MiRNAs participate in the body's inflammatory response by regulating various inflammatory and anti-inflammatory factors. On one hand, miRNAs mediate post-transcriptional gene expression regulation by binding to the 3'-untranslated region (3'-UTR) of cytokines and reducing their expression. A more common mechanism is achieved through targeting various molecular pathways, including nuclear factor NF-kappaB (NF-κB), Notch1, tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), interleukin-1 receptor-associated kinase 1 (IRAK1), and silent information regulator 1 (SIRT1) (Shen et al., 2020; Kaur et al., 2022). In addition, miRNAs are integral factors in osteogenesis, osteoclastogenesis, and the differentiation of osteoclasts/osteoblasts. They regulate the osteogenic lineage of various stem cells through a positive feedback loop, manage osteoclast activity and signaling intermediates, or influence osteoclast formation and differentiation through a negative feedback loop (Luan et al., 2017).

Unlike the human genome, epigenetic mechanisms are reversible and change with the environment. Epigenetics is a key mechanism linking environmental factors and changes in gene activity (Cavalli and Heard, 2019). Both infection and body immunity may lead to changes in epigenetic groups, which in turn affect the body's susceptibility to disease. Peri-implantitis is closely related to the interactions between microbial infection, host immune response, and the tissue environment at the implant site (Berglundh et al., 2024). The presence of pathogenic biofilms in peri-implantitis triggers inflammatory responses in the host, leading to the release of pro-inflammatory cytokines and mediators (Alves, et al., 2022). During this process, epigenetic modifications may occur. Epigenetics provides a bridge between genetic susceptibility and the environmental or external factors that lead to peri-implantitis. Changes in DNA methylation and miRNA expression levels in peri-implant tissues can regulate the expression of inflammation-related genes, thereby influencing peri-implantitis. By examining these alterations, we can identify specific epigenetic markers associated with peri-implantitis, which have the potential to predict an individual's susceptibility to the disease, its progression, or their response to treatment. Moreover, the epigenome exhibits tissue-specific and cell-specific characteristics. The risk of developing peri-implantitis also shows individual variability (Derks et al., 2023). It is reasonable to speculate

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that epigenetic changes in peri-implant tissues, triggered by local factors like plaque and systemic factors like diabetes, lead to individual differences in tissue-specific gene expression, thus inducing or enhancing inflammation and disease susceptibility. The reversibility and specificity of epigenetic changes open up new possibilities for developing diagnostic tools and personalized therapeutic approaches for peri-implantitis.

3 *In vitro* and *in vivo* studies focusing on miRNAs associated with peri-implantitis

3.1 Clinical observations: expression of miRNAs in samples from peri-implantitis patients

Altered expression of various miRNAs has been found in the blood, saliva, gingival crevicular fluid (GCF), and gingival tissues of patients with peri-implant diseases (Table 1). In 2013, a study in Iran collected blood samples from healthy individuals and patients with peri-implantitis to examine the expression levels of miR-146a and miR-499. The study identified two gene polymorphisms that may be genetic determinants of increased risk of peri-implantitis in Iranians (Kadkhodazadeh et al., 2013). Further research used saliva and crevicular fluid as clinical samples to investigate the characteristics of miRNAs in peri-implantitis. Urvasizoglu et al. (2021) found that miR-4484 in the saliva of peri-implantitis patients was significantly downregulated, and this downregulation began as early as one month after implant placement, suggesting that miR-4484 may serve as a potential early diagnostic biomarker for peri-implantitis. Subsequent research identified that in patients with peri-implantitis, the levels of miR-4484 in saliva were significantly correlated with C-X-C motif chemokine ligand 14 (CXCL14) (Urvasizoglu et al., 2023). However, whether CXCL14 levels are regulated by miR-4484 remains to be determined. Chaparro et al. (2021) quantified the expression of miR-21-3p, miR-150-5p, and miR-26a-5p in the GCF of healthy individuals, peri-implant mucositis patients, and peri-implantitis patients. They found that the levels of miR-150-5p were significantly downregulated in peri-implantitis patients compared to the other two groups. The levels of miR-21-3p were higher in peri-implantitis patients compared to peri-implant mucositis patients. MiR-150-5p is considered an osteogenic promoter, stimulating osteoblast proliferation and maturation by targeting and inhibiting matrix metalloproteinase 14 (MMP 14) expression and activating the Wnt/β-catenin pathway (Xu et al., 2023). MiR-21 plays a dual role in the process of bone formation, regulating osteoclastogenesis and osteoblastogenesis through complex signaling pathways (Subramaniam et al., 2023). While inhibition of OPG by miR-21 leads to the activation of RANKL, increasing osteoclastogenesis, miR-21 regulates the expression of osteogenic genes by inhibiting Dickkopf-1 (DKK1), glycogen synthase kinase-3β (GSK-3β), Sma- and Mad-related protein 7 (Smad7), Sprouty2 (SPRY2), and phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Numerous animal studies have confirmed its osteogenic properties. MiR-21-3p may exert protective and anti-inflammatory effects on periodontal tissues(Zhou et al., 2018). Mice with knocked-out miR-21-3p show exacerbated gingivitis and alveolar bone loss (Zhou, et al., 2018), as well as delayed healing of the alveolar socket post-extraction (Strauss et al., 2020). These findings suggest that miR-150-5p and miR-21-3p may influence peri-implantitis by regulating bone metabolism.

Potential relationships between clinical diagnostic parameters of peri-implantitis patients and miRNAs have been assessed in three studies by Menini et al. (2017; 2019; 2021). They extracted miRNAs from soft tissue samples and peri-implant crevicular fluid (PICF) and assessed gene expression at implant sites using microarray analysis. They collected PICF samples and soft tissue samples after three months of implant placement and found that several miRNAs were associated with clinical symptoms during the first year after implant insertion and at the five-year follow-up. In soft tissue samples, during the first year of follow-up, six miRNAs, including miR-182 and miR-495, were associated with bleeding on probing (BOP). At five years of follow-up, three miRNAs, including miR-425, were associated with BOP, while five miRNAs, including miR-30, were correlated with peri-implant pocket depth (PPD). Among them, miR-548 stood out as a key predictor of clinical outcomes, as it correlated with both PPD and BOP. Previous research has highlighted miR-548 as a regulatory factor balancing cell proliferation and apoptosis (Wang et al., 2019). In cases of

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peri-implant bone resorption (BR) > 1 mm, the expression of 14 miRNAs, including miR-34a, significantly changed in both PICF and soft tissue samples. The authors conducted a literature review on miRNAs associated with BR, finding that they mainly target genes related to inflammation, tissue growth, and wound healing. Furthermore, the study revealed an intriguing observation: soft tissue inflammation (as detected by BOP) appears to have a closer association with gene expression profiles, unaffected by plaque presence. However, implant sites with a high plaque index (PI) but normal BR exhibit a distinct miRNA expression profile compared to other sites. These results suggest that a specific miRNA spectrum may protect implant sites from BOP and BR damage. Thus, miRNAs may serve as predictive factors for clinical outcomes of dental implants and can be used as biomarkers for diagnosis and prognosis in the field of dental implants. However, experimental validation is necessary to confirm their clinical feasibility.

Competitive endogenous RNAs (ceRNAs) may serve as a potential mechanism for miRNA-mediated gene regulation. CeRNAs include mainly long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), which regulate gene expression by binding miRNAs through miRNA response elements (MREs) (Mazziotta et al., 2024). This interaction inhibits the function of miRNAs, thereby alleviating their suppressive effects on target genes. Extensive research indicates that circRNAs use the ceRNA mechanism to modulate osteogenesis in humans. By acting as molecular sponges, these circRNAs compete for miRNA binding, preventing miRNAs from inhibiting their downstream target genes. This process effectively shields target genes from miRNA-induced repression, facilitating gene expression patterns that promote bone formation (Mazziotta, et al., 2024). Zhou et al. (2020) collected gingival samples from healthy individuals and peri-implantitis patients and analyzed the expression matrix of mRNA, lncRNA, and miRNA based on whole-genome RNA-seq data from the samples. Compared to the healthy group, there were 160 significantly upregulated miRNAs and 44 downregulated miRNAs in the gingival samples of peri-implantitis patients. Among them, miR-338-5p, miR-9-5p, and 11 other miRNAs are involved exclusively in the regulation of miRNAs in peri-implantitis. These miRNAs affect the expression of genes such as FAM126B, CPEB2, RAP2C, and YOD1 by regulating the target protein-encoded RNA. These genes are highly related to Hippo signaling pathway, Toll-like receptor (TLR) signaling pathway, Wnt signaling pathway, NOD signaling pathway, oxidative stress, and innate immune processes. Li et al. (2020) explored the expression profiles of immune-related miRNAs and constructed immune-related ceRNA networks involved in the pathogenesis of peri-implantitis. Through cross-validation of two datasets, researchers have identified a signaling axis exclusively linking GSK-3β and miR-1297. Previous studies indicate that GSK-3β acts as a downstream target gene of miR-1297, and growth arrest specific-5 (GAS5) serves as a competitive endogenous RNA that mitigates the suppressive impact of miR-1297 on GSK-3β (Gao et al., 2019). GSK-3β is recognized as a negative regulator of bone metabolism, which modulates the differentiation of osteoblasts and osteoclasts through the activation of the Wnt/β-catenin signaling pathway (Amirhosseini et al., 2018). The potential role of miR-1297 and GSK-3β in bone loss associated with peri-implantitis warrants further experimental investigation. LncRNAs may regulate gingival tissue inflammation and oxidative stress by competitively binding with miRNAs (Cheng et al., 2022). Oxidative stress plays a crucial role in periodontitis and peri-implantitis, where bacteria within the periodontal and peri-implant tissues produce excessive reactive oxygen species (ROS), exacerbating tissue damage. Elevated levels of ROS activate the NF-κB signaling pathway, enhancing the expression of inflammatory genes and increasing the synthesis and release of pro-inflammatory cytokines, which trigger local inflammatory responses in periodontal tissues. Furthermore, ROS are involved in regulating the differentiation of osteoblasts and osteoclasts. They can inhibit osteoblasts involved in bone formation and enhance osteoclast activity through the activation of the OPG/RANK/RANKL signaling system, thereby promoting alveolar bone resorption and progressive tissue destruction (Mijiritsky et al., 2019; Sczepanik et al., 2020) The ceRNA network reveals a more detailed and complex disease regulatory network, offering new strategies for the treatment of peri-implantitis.

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Table 1 MiRNAs changes in peri-implantitis tissues

MiRNAs	Species	Cell/ Tissue	Number of Ptients /animals	Number of Implants	Expression	Reference	Main outcomes
MiR-146a, miR-499	Human	Blood from the arm veins	38	*	*	(Kadkhodazadeh, et al., 2013)	MiR146a and MiR499 gene polymorphisms may be genetic determinants for increased risk of peri-implantitis in Iranians.
Let-7g, miR-27a MiR-145	Dog	Gingival tissues	6	24	Downregulate Up	(Wu et al., 2017)	MiRNAs play a complex and specialized role in inflammation and osteoclastogenesis, potentially in association with the MAPK signaling pathway.
MiR-517, miR-525,miR-624. et al.	Human	Gingival tissues	7	14	*	(Menini, et al., 2019)	MiRNAs may serve as a predictive factor for clinical outcomes in dental implants and can be utilized as a biomarker for diagnostic and prognostic purposes in the field of implant dentistry.
MiR34a, miR100, miR106a. et al.	Human	PICF	7	14	*	(Menini, et al., 2021)	MiR-100, miR-143, miR-146a, miR-181, miR-200, miR-223, miR-429, and miR-1248 are primarily involved in inflammatory responses, whereas miR-34, miR-106a, miR-126, miR-221, miR-375, and miR-378 mainly influence soft tissue growth and wound healing.

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MiR-21-3p, miR-150-5p	Human	PICF	18	18	Downregulate	(Chaparro, et al., 2021)	The down-expression of miR-21-3p and miR-150-5p may be related to the occurrence of peri-implantitis, which can be used as biomarkers for screening peri-implant health.
MiR-4484	Human	Saliva	33	33	Downregulate	(Urvasizoglu, et al., 2021)	Salivary miR-4484 is significantly downregulated in peri-implantitis patients, suggesting its potential as an early diagnostic biomarker for peri-implantitis.
MiR-375-3p, miR-375-3p, miR-544-3p. et al. miR-340-5p, let-7g-5p, miR-27a-3p. et al.	Rats	Gingival tissues	5	5	Up Downregulate	(Zhang et al., 2022)	Differences exist in the miRNA expression profiles between peri-implantitis and periodontitis, with many upregulated genes in peri-implantitis related to TLR signaling and RIG-I-like signaling pathways.

* means not mentioned in the references.

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3.2 *In vivo* studies of the expression of miRNAs in peri-implantitis

Animal models of peri-implantitis are an excellent tool for spatiotemporal analysis of changes observed in patient-derived samples. Using sequencing techniques, Wu et al. (2017) established a canine model for experimental peri-implantitis and examined miRNA expression. Their findings indicated that the expression of lethal-7g (let-7g), miR-27a, and miR-145 significantly varied between inflamed tissues and healthy control tissues. Levels of miR-145 in the soft tissue samples of patients with peri-implantitis were higher than in the healthy group, while the expression of let-7g and miR-27a was decreased in samples from patients with peri-implantitis. Besides, GO and KEGG analysis of changing miRNA target genes indicated significant enrichment of mitogen-activated protein kinase (MAPK) signaling. Studies have found that the let family is associated with bone development. Let-7g is downregulated in osteoporosis (Chen et al., 2018) and other members of the let family, such as let-7e, play a critical role in bone metabolism and inflammation regulation. Overexpression of let-7e can activate T helper 1 (Th1) and Th17 cell cytokines, exacerbating the immune response (Guan et al., 2013). Additionally, let-7e stimulates the nuclear translocation of NF-κB and activates the NF-κB pathway, promoting the differentiation of monocytes into osteoclasts (De La Rica et al., 2015). MiR-145 has a negative impact on bone formation. MiR-145-5p targets BTB Domain and CNC Homolog 2 (Bach2) and mediates TNF-induced apoptosis in human gingival epithelial cells (Liu et al., 2020). It also regulates the proliferation and chondrogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) by targeting the Smad4 pathway in osteoarthritis (Liu and Liu, 2021). MiR-145 targets and inhibits the osteogenic regulator semaphorin3A (SEMA3A), suppressing osteogenic differentiation in human jawbone marrow mesenchymal stem cells (MSCs) (Jin et al., 2020). MiR-27a may serve as a negative regulatory mechanism to prevent excessive inflammatory responses in peri-implantitis. Bacterial release of lipopolysaccharide (LPS) activates the TLR2 and TLR4 pathways, leading to the downregulation of miR-27a in macrophages. The downregulation of miR-27a activates the IL-10-dependent janus kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3) pathway, resulting in increased IL-10 expression, which in turn inhibits the exacerbation of gingival tissue inflammation (Xie et al., 2014). To clarify the exact role of miR-27a in peri-implantitis, Wu et al. (2019) induced overexpression of miR-27a in BMSCs using miRNA mimics and then treated these cells with TNF-α and bone-inducing mediators. MiR-27a counteracted the inhibitory effect of TNF-α on osteogenesis, promoting the expression of early markers, like Runx2, bone morphogenetic protein 2 (BMP-2) and late markers, like OCN, collagen type 1 alpha 1 (COLIA1). Moreover, it also heightened the levels of vascular endothelial growth factor (VEGF) and angiogenic factor 1, contributing to the formation of tubular structures and exhibiting significant angiogenic activity. This mechanism was attributed to the activation of the Wnt signaling pathway by directly targeting DKK2 and secreted frizzled-related protein 1 (SFRP1). Based on the anti-inflammatory effects and osteogenic effects of miR-27a, Wu et al. (2019) created a miRNA-enhanced delivery system, comprising Lenti-miR-27a, Lenti-miR-NC, canine BMSCs, and B-TCP, and implanted it into bone defects in canine peri-implantitis models. Remarkably, this miR-27a enhanced delivery system was found to significantly promote bone regeneration and integration around implant-bone defects.

Zhang et al.(2022) established a rat model of peri-implantitis using a ligature technique and after 6 weeks collected soft tissue samples for miRNA sequencing. The results showed that 105 miRNAs exhibited differential expression compared to normal tissues. Note that their study confirmed that miR-27a-3p was downregulated in peri-implantitis. Subsequently, they developed a micro-nanometer implant with responsive and sustained release of miR-27a, using the same method to construct a rat model of peri-implantitis and to test the role of the miR-27a-loaded implant in the prevention and treatment of peri-implantitis (Zhang et al., 2023). They found that the presence of miR-27a could mitigate bone resorption around the implant, a process likely mediated by targeting immune metabolism and mitochondrial function to regulate macrophage polarization.

Pan et al. (2020) focused on miR-146a. Previous research showed that miR-146a acts as a negative feedback regulator in periodontitis. Under the stimulation of LPS secreted by *Porphyromonas gingivalis* (*P.g*),

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miR-146a targets IRAK1 and inhibits the secretion of pro-inflammatory cytokines such as IL-6 by periodontal ligament stem cells (PDLSCs), thereby blocking TLR signaling pathways and suppressing periodontitis (Jiang et al., 2015; Jiang et al., 2018). Based on the role of miR-146a in periodontitis, Pan and colleagues (2020) hypothesized that miR-146a might play a similar function in peri-implantitis. Therefore, they established peri-implantitis models using ligatures in both normal mice and TLR2/4 knockout mice. On the 3rd, 6th, and 9th days after ligature placement, they injected anti-RANKL antibodies containing or lacking miR-146a into the palatal gingiva around the implants. When combined with anti-RANKL antibodies, miR-146a further reduced gingival mRNA expression of TNF-α and bone loss in normal mice but was ineffective in the absence of TLR2 and TLR4. This study indicated that miR-146a enhances peri-implant bone resorption through the regulation of TLR2/4 signaling and inhibition of TNF-α expression.

The aforementioned studies highlight the potential role of miRNAs, uniquely expressed in the peri-implant tissue or microenvironment, as critical determinants of peri-implantitis, particularly regarding bone homeostasis (Fig. 2). However, most studies have yet to elucidate how these miRNAs regulate target genes to affect the expression of inflammatory factors and osteogenic/osteoclastic factors. Consequently, further *in vitro* and *in vivo* experiments are imperative to validate the mechanistic roles of these miRNAs in peri-implantitis.

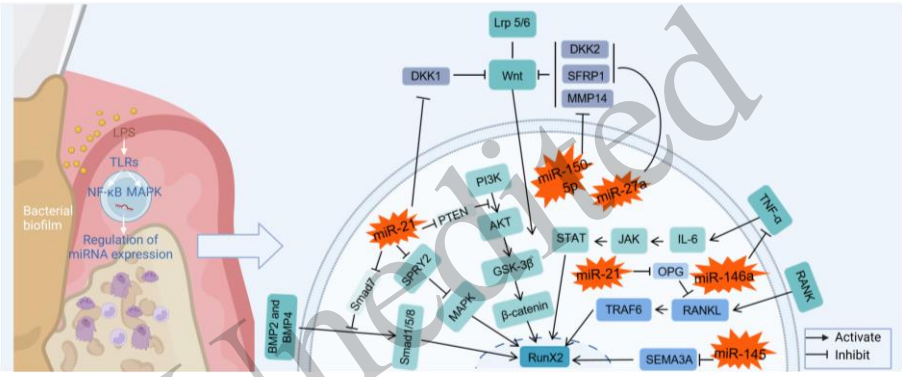

Fig. 2 MiRNAs specifically expressed in peri-implantitis are related to bone remodeling: when tissues are exposed to bacterial LPS, the expression of miRNAs can increase the sensitivity of TLRs, target the NF-kB signaling pathway, or regulate MAPK-mediated endotoxin tolerance. MiR21: Inhibition of DKK1, Smad 7, SPYR2, and PTEN by miR21 leads to activation of the Wnt and Smad pathways, increasing osteogenesis. Inhibition of PTEN can also result in osteogenesis by activating the STAT3 pathway. Inhibition of OPG by miR21 leads to the activation of RANKL, increasing osteoclastogenesis. MiR-150-5p: Inhibition of MMP14 by miR-150-5p leads to activation of Wnt pathways, increasing osteogenesis. MiR-27a: Inhibition of DKK2 and SFRP1 by miR-27a leads to activation of Wnt pathways, increasing osteogenesis. MiR-146a: Inhibition of TNF-α by miR-146a leads to suppression of osteoclastogenesis. MiR-145: Inhibition of SEMA3A by miR-145 leads to suppression of osteogenesis. (Created with bioRender.com).

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4 *In vitro* and *in vivo* studies focusing on DNA methylation associated with peri-implantitis

Recent research on DNA methylation changes in peri-implantitis has focused mainly on a few small-sample studies. Daubert et al. (2019) assessed the overall DNA methylation levels in the PICF of 21 patients with peri-implantitis and 24 healthy individuals. The levels of 5-mC in the crevicular fluid of patients with peri-implantitis were significantly higher than those in the control group. Khouly et al.(2022) collected gingival tissue and alveolar bone tissue and compared global DNA methylation in peri-implantitis patients and periodontally healthy patients. There were no significant differences between the two groups in global DNA methylation levels, but higher levels were found in gingival tissues than in bone tissue in the whole sample,

which may reflect different epigenetic responses among different tissues in the same microenvironment. However, this study had certain limitations, particularly concerning the appropriateness of the control group setup. For ethical reasons, the researchers chose healthy periodontal tissues around impacted teeth as the control group. Ideally, tissues surrounding healthy implants should have been selected for comparison. Moreover, further experiments are necessary to evaluate the DNA methylation of specific cytokines and inflammation-related genes to clarify which genes (such as those associated with pro-inflammatory/anti-inflammatory cells or osteogenic/osteoclastic activities) are activated or deactivated in peri-implantitis. Ganesan et al. (2022) collected peri-implant crevicular samples and microbiota for gene sequencing from both healthy and peri-implantitis sites of the same patients. The transcription factors related to DNA methylation, specifically zinc finger proteins (ZFPs), were upregulated in the sites with peri-implantitis and positively correlated with numerous bacterial virulence factors. Previous studies had indicated that ZFPs can suppress gene transcription by binding methylated DNA through their zinc finger domains (Hodges et al., 2020).

Dissolved titanium particles in the peri-implant tissue might be one of the significant local environmental factors affecting the epigenetic patterns around implants. Daubert et al. (2019) also assessed the titanium particle content in the GCF of peri-implantitis patients, finding that PICF with higher Ti content showed significantly higher overall DNA methylation levels. Previous research found that titanium ions could increase the expression of chemokine (C–C motif) ligand 2 (CCL2, a type of inflammatory cytokine) and elevate the ratio of RANKL to osteoprotegerin in rat gingival tissue with *P.g*, potentially leading to peri-implant bone destruction (Wachi et al., 2015). Wang et al. (2020) suggested that this process might be due to titanium particle-induced methylation of the OPG and RANKL genes. The researchers established a mouse skull osteolysis model induced by titanium particles and observed significant bone loss in the calvaria, with increased expression of RANKL and decreased expression of OPG, leading to a higher RANKL/OPG ratio. Examination of the methylation of CpG islands near the transcriptional regulatory regions of OPG and RANKL genes revealed hypermethylation of OPG and hypomethylation of RANKL. However, after treatment with the DNA methyltransferase inhibitor 5-Aza-2-deoxycytidine (Azad C), both RANKL and OPG expression increased to various extents, the RANKL/OPG ratio decreased, and OPG underwent significant demethylation, inhibiting bone resorption. Although these findings suggest that high methylation may be a potential mechanism of peri-implantitis, and dissolved titanium particles might be a local environmental factor inducing epigenetic changes, further research is needed to determine whether these associations are causal.

Systemic factors and lifestyle habits of patients with peri-implantitis also influence their DNA methylation levels. Exposure to tobacco smoke can lead to DNA methylation modifications in multiple genes. In one study, gingival tissues were collected from patients with periodontitis and peri-implantitis and DNA methylation analysis was performed to assess the impact of smoking on gene pattern alterations in both diseases. The findings indicated that peri-implantitis is more sensitive to smoking than periodontitis (Cho et al., 2020). Diabetes is also closely related to peri-implantitis (Nibali et al., 2022) and the oxidative stress and epigenetic changes induced by hyperglycemia may be reasons for the increased susceptibility to periodontitis and peri-implantitis in diabetic patients. In periodontitis, a hyperglycemic state can induce the overexpression of cytokines, which in turn form ROS, with or without enzyme involvement. In the gingival tissues of diabetic states, 1163 genes underwent epigenetic modifications leading to keratinization of squamous and junctional epithelia. Hypomethylation of TNF-α and IL-6 genes induces their overexpression in the junctional epithelium area, triggering the onset of periodontitis (Li et al., 2021b). Furthermore, prolonged high blood sugar causes non-enzymatic glycosylation of various proteins, ultimately forming advanced glycation end products (AGEs) (Khalid et al., 2022). Increased AGEs upregulate DNMT1 expression, enhance the methylation level of the calcitonin-related polypeptide α (CALCA) promoter, downregulate CALCA expression, and exacerbate inflammatory conditions in periodontal tissues (Wang et al., 2022a).

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5 Further approaches to epigenetics in the clinical management of peri-implantitis

5.1 *In vitro* and *in vivo* studies of surface-modified titanium implants

Dental implants are anchored directly within the bone through a process known as osseointegration (Guglielmotti et al., 2019). Using epigenetics tools to coat implant surfaces to achieve better osseointegration between the implant and the alveolar bone is very promising. Many studies are attempting to construct surface-functionalized implants with miRNAs or anti-miRNAs to modulate the osteogenic gene network, thereby enhancing osseointegration and preventing the occurrence of peri-implantitis (Table 2).

Some studies have encapsulated miRNAs in nanoparticles and incorporated them into the surface coatings of titanium implants. One of the most extensively studied miRNAs is miR-21, which has been used to modify the surface of titanium implants to test its potential to enhance osseointegration. For example, Wang et al. (2015) developed a biocompatible chitosan (CS) and hyaluronic acid (HA) nanoparticle loaded with miR-21 to create a miR-21 functionalized micro-arc oxidation (MAO) titanium surface. *In vitro* results showed upregulation of early osteogenic gene markers COL1, COL3, RUNX2, OPN, and OCN in the Ti surface-coated group, indicating its potential to promote hBMSC osteogenic differentiation. Geng et al.(2018) modified Ti implants sequentially through acid treatment, SrHA deposition, and fixation of miR-21 nanocapsules. *In vitro* results demonstrated that this coating promoted osteoblast proliferation, differentiation, and mineralization. Furthermore, results from *in vivo* implantation in rabbits showed that this coating increased the expression of the vascular growth factor CD31, enhanced the expression of osteoblast-related proteins, and significantly improved bone-implant contact and bond strength between bone and implant. The same group subsequently conducted more detailed *in vivo* assessments of titanium implants surface-treated with miR-21 nanocapsules, involving X-ray, micro-CT, histological, immunohistochemical, biomechanical, Raman, and SEM-EDS testing. These studies revealed that miR-21 nanocapsule coatings significantly promoted vascularization, bone remodeling, and bone maturation, leading to a significant improvement in bone-implant contact and increased bond strength (twice that of Ti at 1 month) (Geng et al., 2020). Other miRNA candidates from classical osteogenic gene networks have also been used in studies on implant coatings. For example, miR-335-5p lipid nanoparticles coated on implants may promote osteoblast lineage differentiation and maintain the long-term stability of the implant (Wang et al., 2021). Meng et al. (2016) used biodegradable coating to modify the titanium surfaces with the osteogenic-related gene miR-29b. This functional coating not only enhanced osteogenic differentiation of hUMSCs *in vitro* but also promoted the bone regenerative capacity of titanium alloy implants *in vivo*. Shao et al. (2018) prepared miR-122-modified cell sheets and complexed them onto MAO titanium implants. The miR-122-coated group promoted the expression of RUNX2, OSX, OCN, COL1, ALP, and BMP-2, facilitating MSC osteogenic differentiation. Anti-miRNAs have also been used in the surface modification of titanium implants. Yan et al. (2018) delivered antimiR-138 to MSCs cultured on Ti implants and conducted *in vitro* and *in vivo* experiments. The implants promoted osteogenesis and angiogenesis through the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling and downstream effectors RUNX2 and Osx. It has been suggested that MiR-132-3p inhibits the osteogenic differentiation of BMSCs by targeting BMP-2. Therefore, Li et al. (2021a) used a gene carrier system based on selenium-modified methionine PEI-PEG (SeNP) to deliver inhibitors of miR-132-3p to titanium implants. This modification significantly enhanced the osteogenic differentiation and bone regeneration of BMSCs.

Functionalized dental implants with miRNAs are not only used to enhance osseointegration but can also promote soft tissue healing around the implants. For example, smooth titanium surfaces loaded with miR-21 nanoparticles can promote the adhesion and proliferation of human gingival fibroblasts, aiding in the formation of a tight soft tissue seal between the gingiva and the implant interface(Wang et al., 2022b). The underlying mechanism may involve miR-21 downregulating PTEN expression through AKT signaling pathway, influencing fibroblast proliferation and apoptosis (Liu et al., 2014).

Research on implant modification from the perspective of DNA methylation is scarce, and we found only one related publication. It is well known that sandblasted/large-grit/acid-etched (SLA) surfaces of Ti have

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higher osteogenic potential than mechanically treated surfaces. Cho et al. (2021) proposed that this might be related to DNA methylation. They found that the degree of DNA methylation of the ALP gene on SLA surfaces was lower than on mechanically treated Ti surfaces. Moreover, stimulating mechanically treated Ti surfaces with DNA methyltransferase inhibitors can achieve ALP gene expression similar to that on SLA surfaces. Therefore, it might be possible to use external pharmacological modifications on dental implant surfaces to create an environment more conducive to osteogenesis.

These research findings suggest that epigenetic functionalization of implant surfaces may be important for promoting osseointegration, which is crucial for bone repair in both healthy individuals and patients with implant-associated inflammation.

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Table 2 Summary of studies on functionalized implant surfaces with miRNAs or anti-miRNAs

MiRNA /Anti-miRNA	Study Type	Implant Surface Feature	Functionalized Coating Method	Purpose	Reference	Main outcomes
MiR-21	*In vitro*	MAO processed pure titanium surface	CS/HA/miRNA nanoparticles crosslinked with 0.2 % gel solution	Implant osseointegration	(Wang, et al., 2015)	*In vitro* results indicate that the Ti surface-coated group showed early upregulation of osteogenesis-related gene markers COL1, COL3, RUNX-2, OPN, and OCN, indicating its potential to promote hBMSC differentiation into osteoblasts.
MiR-29b	*In vitro* and *in vivo* (rat)	Titanium alloy surface	MiR-29b nanocapsules encapsulated in O-carboxymethyl chitosan coating	Implant osseointegration	(Meng, et al., 2016)	Expression of OCN and RUNX2 were Increased *in vitro* and the bone regeneration ability of the titanium alloy was promoted *in vivo* And *in vitro*.
MiR-21	*In vitro* and *in vivo* (rabbit)	SrHA coated pure titanium surface	MiR-21 nanocapsules encapsulated in strontium/hydroxy apatite coating	Implant osseointegration	(Geng, et al., 2018)	MiR-21 coating promotes angiogenesis and osteogenesis and can synergize with strontium/hydroxyapatite to enhance angiogenesis, osteogenesis, anti-resorptive activity, bone integration, and mineralization, thereby promoting the strength of bone-implant bonding.
AntimiR-138	*In vitro* and *in vivo* (rat)	MAO processed pure titanium surface	Anti-miR-138 delivered MSC sheet to the titanium surface, forming MSC sheet–implant complex (MSIC)	Implant osseointegration	(Yan, et al., 2018)	MSIC delivered anti-miR-138 showed significant promotion of osteogenesis and angiogenesis coupling both *in vitro* and *in vivo*.

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MiR-122	*In vitro*	MAO-treated titanium surfaces	miR-122-modified cell sheets complex	Implant osseointegration	(Shao, et al., 2018)	MiR-122 cell-promoted experiments demonstrated increased expression of RUNX2, OSX, OCN, COL1, ALP, and BMP-2 in the experimental group, promoting osteogenic differentiation of BMSCs.
MiR-21	*In vitro* and *in vivo* (rabbit)	Acid processed pure titanium surface	miR-21 nanocapsules incorporated CMC	Implant osseointegration	(Geng, et al., 2020)	*In vitro* studies indicate that miR-21 can promote vascularization and osteogenic differentiation of MSCs and enhance osteoclast activity. *In vivo* studies demonstrate that miR-21 nanoparticle-coated surfaces accelerate vascular formation (high expression of CD31), bone remodeling (high expression of osteogenic and osteoclastic proteins), and bone maturation (a high ratio of apatite).
MiR-335-5p	*In vitro*	SLA and SLActive titanium surface	Lipid/miR-335-5p nanoparticles	Implant osseointegration	(Wang, et al., 2021)	Lipid/miR-335-5p nanoparticles adhere well to SLActive titanium surfaces and upregulate osteogenic activity of transfected BMSCs, increasing the expression of ALP, collagen I, OCN, and BSP.
AntimiR-132-3p	*In vitro* and *in vivo* (rat)	TA processed pure titanium surface	SEMET-modified PEG/PEI/antimiR-132-3p nanoparticles crosslinked with 0.2% gelatin solutions	Implant osseointegration	(Li, et al., 2021a)	SeNP successfully deliver miR-132-3p inhibitors, promoting osseointegration on the implant surface by upregulating BMSC osteogenic differentiation.
MiR-21	*In vitro*	SiC sandpapers polished pure titanium surface	Chitosan/ tripolyphosphate/hyaluronic acid (CTH) /miR-21 nanoparticles	Biological seal at percutaneous areas of implants	(Wang, et al., 2022b)	CTH/miR-21 nanoparticle coating promotes adhesion and proliferation of gingival fibroblasts on smooth titanium surfaces, facilitating the formation of a stable biological seal in the transgingival area of Ti implants.

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5.2 *In vitro* and *in vivo* studies of potential diagnostic and therapeutic targets in peri-implantitis

In recent years, specific DNA methylation patterns and miRNAs expressed in blood samples have been extensively studied as biomarkers for various diseases, with their clinical detection methods continuously evolving (Dumitrescu, 2018; Ho et al., 2022). Biomarker levels in the blood can also be used to assess the long-term therapeutic effects of disease treatment (Isola et al., 2023). Specific changes in miRNAs have been observed in the soft tissues around implants, GCF, and saliva of patients with peri-implantitis, increasing the possibility of using these miRNAs for diagnosing peri-implantitis in larger patient cohorts after further validation. A meta-analysis of six peri-implantitis datasets was conducted using the interactive online tool GEO2R to identify differentially expressed genes from high-throughput gene expression databases. This analysis identified miR-31-5p as a potential biomarker for diagnosing peri-implantitis (Yadalam and Thiyagaraj, 2020). Additionally, some miRNAs are associated with clinical symptoms of peri-implantitis (Menini, et al., 2017; 2019; 2021), suggesting that these miRNAs could potentially serve as predictive biomarkers for the condition. However, using miRNAs as a diagnostic tool for peri-implantitis involves several considerations. For instance, there are variations in miRNA expression across different samples, and it is currently unclear whether these differences are related to the pathophysiology of the disease. Saliva samples are easily obtainable, but they are farther from the disease site compared to PICF, and thus cannot provide relevant information at the site of the disease. The secretion and composition of saliva are also influenced by various factors including diet and oral hygiene. The low flow and protein content of GCF samples requires detection methods with higher amplification sensitivity and lower detection limits. Moreover, systemic factors in patients, such as smoking, diabetes, and age, might also cause local epigenetic changes in the oral cavity. How to eliminate these confounding factors warrants further investigation.

Epigenetics is a reversible process, meaning that disease-associated epigenetic modifications can potentially be repaired or reversed. Currently, several epigenetic drugs have been approved, including mainly DNA methylation inhibitors and histone deacetylase inhibitors (HDACi) for cancer treatment (Hogg et al., 2020). Clinical studies have also identified significant roles for epigenetic drugs in inflammatory diseases related to autoimmunity, such as acute graft-versus-host disease (Choi et al., 2017) and systemic juvenile idiopathic arthritis (Vojinovic et al., 2011). *In vitro* and *in vivo* studies suggest that the anti-inflammatory properties of these drugs may also be applicable in treating periodontitis. Tanaka et al. (2021) treated periodontitis in mice with the DNA methyltransferase inhibitor decitabine and found that it could upregulate the expression of the anti-inflammatory cytokines IL-10 and transforming growth factor beta-1 (TGF-β) through a kruppel-like factor-2 (KLF2)-dependent mechanism. These anti-inflammatory factors can inhibit the formation of osteoclasts in periodontitis, thereby reducing bone loss in the mouse model of periodontitis (Matarese et al., 2013; Sun et al., 2020). However, reversible hematological dose-limiting toxicities, including thrombocytopenia, neutropenia, fatigue, and diarrhea, are commonly observed in patients treated with epigenetic therapies (Meteran et al., 2024). Therefore, the use of epigenetic drugs in treating local oral inflammations such as periodontitis and peri-implantitis must be approached with caution. On one hand, it is essential to determine whether these drugs might disrupt the balance between the host and oral microbiota; on the other hand, attention must be paid to the mode of administration, including dosages and methods of delivery. Local and sustained delivery of epigenetic drugs for the treatment of peri-implantitis has been explored in *in vivo* studies. As previously mentioned, three studies tested specific miRNA deliveries in different animal models of peri-implantitis. Two of these studies developed miR-27a delivery systems on the surfaces of titanium implants (Wu, et al., 2019; Zhang, et al., 2023), finding that implants loaded with miR-27a could modulate immune metabolism around the implant and enhance bone regeneration, presenting a potential therapeutic approach for peri-implantitis. Another study used direct peri-implant gingival injections, finding that miR-146a effectively reduced bone resorption around implants (Pan, et al., 2020).

Epigenetics offers efficiency, precision, and personalization, which hold substantial potential for the

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diagnosis and treatment of diseases. However, it is still in the developmental phase. To enhance its clinical application accuracy and reliability, more research is needed to clarify the pathological roles of epigenetic factors in the development of peri-implantitis. Additionally, it is essential to establish standardized protocols for clinical sample collection and to further advance sequencing technologies for broader applications.

6 Discussion and conclusion

Exploration of the relationship between peri-implantitis and epigenetics is relatively new, having gained attention only in the past decade. Current insights reveal that dynamic epigenetic mechanisms, such as DNA methylation and miRNAs, play a crucial role around implant sites. However, to translate these findings into clinical applications, significant gaps in our understanding of epigenetic regulation need to be addressed. Currently, our knowledge revolves mainly around miRNAs and DNA methylation. Histone modifications are also recognized as vital epigenetic regulators that can drive genomic responses by inducing local chromatin structural changes (Millan-Zambrano et al., 2022). Infections by periodontopathic bacteria like *P.g* or *Treponema denticola* can alter the expression and activity of chromatin-modifying enzymes in gingival cells, leading to site-specific and global modifications in histone acetylation and methylation. Although HDACi have shown potential in improving periodontitis outcomes by reducing inflammation and bone resorption (Jurdzinski et al., 2020), similar studies in peri-implantitis are notably lacking. Furthermore, the relationship between DNA methylation changes induced by Ti particles around implant tissues and their pathological or physiological impacts remains unclear. Studies have been conducted on overall DNA methylation levels in peri-implantitis without focusing on specific genes, indicating a need for well-designed high-throughput epigenomic and transcriptomic studies to clarify the disrupted signaling pathways in peri-implantitis.

MiRNAs are promising as potential biomarkers for the prediction and diagnosis of peri-implantitis, potentially impacting the course of the disease by targeting genes involved in immune responses and cell differentiation processes related to osteoblasts and osteoclasts. Current research on miRNAs in peri-implantitis has focused mainly on cytokines and chemokines, with insufficient examination of the coding genes for receptors, signaling molecules, and transcription factors that undergo specific changes in the condition. Therefore, comprehensive studies using genomic, epigenomic, and transcriptomic methods are crucial to establishing a comprehensive map of epigenetic changes in peri-implantitis. Such efforts should aim to clarify the specific gene networks and early signaling events distorted in the disease, necessitating larger research cohorts to mitigate the influence of individual variability and other confounding factors. Currently, information on epigenetics and peri-implantitis is limited, and there is a lack of consensus. However, epigenetic modifications of genes related to inflammation and osteogenesis provide a new perspective in understanding how local and environmental factors influence the pathogenesis of peri-implantitis. At the same time, epigenetic testing is a promising diagnostic and therapeutic tool. Therefore, further research is needed to establish the connection between such epigenetic modifications and peri-implantitis, to develop diagnostic and therapeutic methods for peri-implantitis based on epigenetics.

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Author contributions

Writing - original draft and Conceptualization: Qianhui Li. Writing - review & editing: Hongye Lu, Mengyuan Zhang and Yuting Ye. Project administration and supervision: Ping Sun and Qianming Chen. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Qianhui Li, Hongye Lu, Mengyuan Zhang, Yuting Ye, Qianming Chen, and Ping Sun declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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