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Influence of epigenetics on periodontitis and peri-implantitis pathogenesis

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1 | INTRODUCTION

Periodontitis is a widespread disease that has recently been shown to be the sixth-most prevalent condition worldwide; its severe forms affect about 10% of the adult population.[1–3] The disease is characterized by chronic inflammation of the gingival tissues in response to bacterial colonization of the tooth surface. In susceptible individuals, this immune response results in tissue destruction and the loss of supporting bone.[4] Similarly, chronic inflammation can affect dental implants in the form of peri-implantitis, that is, inflammation in peri-implant tissues with loss of supporting bone, which can ultimately lead to implant loss.[5,6] The prevalence of peri-implantitis varies across studies but, according to a recent review, ranged from 13% to 47% among individuals with implants.[6] As with periodontitis, peri-implantitis is considered to be induced by microbial biofilms at the implant surface.[7]

Several factors — environmental, genetic, and epigenetic — contribute to an individual's susceptibility to periodontal disease.[8] Epigenetics is a critical link between genetic and environmental factors. Epigenetic alterations may contribute to individual differences in tissue-specific gene expression and induce or enhance inflammation and susceptibility to disease.[9] However, less is known about how these factors influence peri-implantitis. There is a clinical need for methods to regenerate alveolar bone and suppress inflammation in order to improve the long-term prognosis of teeth and implants affected by periodontitis and peri-implantitis, respectively.[10] The fact that epigenetic mechanisms are reversible makes them attractive targets for new treatment models within tissue regeneration and inflammatory disease.

2 | INFLAMMATORY LESIONS OF PERIODONTITIS AND PERI-IMPLANTITIS

Numerous studies have analyzed how the inflammatory lesion of peri-implantitis differs from that of periodontitis.[6,11] Studies using both human biopsy material and experimental models have concluded that the peri-implantitis lesion is larger than the periodontal lesion, and that their cellular and cytokine compositions differ in important ways (Figure 1).

Although plasma cells and lymphocytes are the dominant cells in both lesions, neutrophils and macrophages occur in greater numbers in peri-implantitis than in periodontitis. Experimental studies have also shown a greater number of osteoclasts in the peri-implantitis

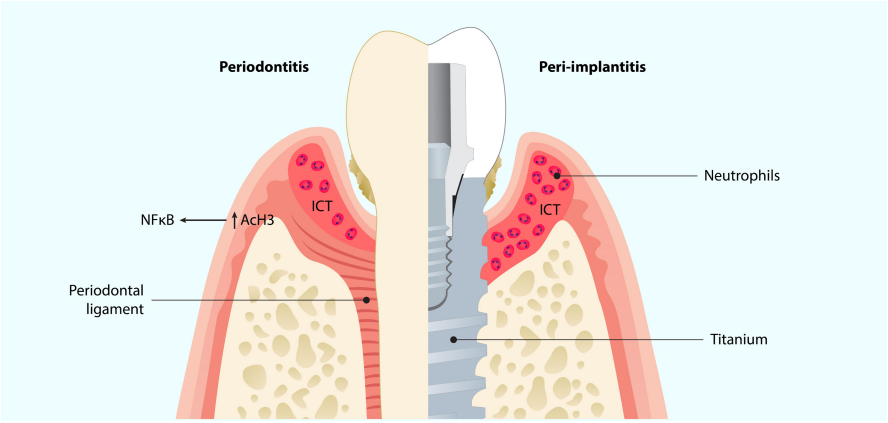

FIGURE 1 Comparison of the inflammatory lesions (ICT) of peri-implantitis and periodontitis, showing major histopathological features. AcH3, acetylated histone H3; NF-κB, nuclear factor kappa B

lesion.[12] In line with these data, a study using biopsies from 40 patients with periodontitis and 40 with peri-implantitis showed that not only were the inflammatory lesions around implants twice as large as those in periodontitis, but the peri-implant lesion also had greater numbers of plasma cells, macrophages, and neutrophils.[5] By contrast, the density of B cells and the density of vessels were greater in periodontitis. An experimental study in dogs from the same group reported a larger lesion in peri-implantitis.[12] Moreover, the levels of myeloperoxidase, a marker for neutrophils, and tartrate-resistant acid phosphatase, a marker for osteoclasts, were higher in peri-implantitis.[12] Similarly, a rat lipopolysaccharide experimental model indicated the presence of osteoclasts, bone resorption, and extensive inflammation in peri-implantitis and suggested that the destruction of peri-implant tissue occurs faster than that of periodontitis tissue.[13] Shedding light on these findings, a review of experimental ligature models in animals illustrated that the inflammatory infiltrate around teeth was separated from the alveolar bone by a connective tissue zone, whereas the inflammatory infiltrate around implants extended all the way to the alveolar crest.[11]

The different pathologies of these lesions are reflected in their signaling: gingival crevicular fluid samples from healthy, periodontitis, or peri-implantitis sites each showed distinct cytokine profiles.[14] In an experimental murine model with *Porphyromonas gingivalis* infection, implants experienced greater bone loss than teeth.[15] Compared with implants without infection, forkhead box P3, a negative regulator of the immune response, decreased in the setting of infection, while tumor necrosis factor alpha, a cytokine for inflammation, increased. Meanwhile, teeth experienced no change in forkhead box P3 or tumor necrosis factor alpha in the setting of infection.[15] Interestingly, the presence of an implant even without infection altered the expression of cytokines compared with healthy teeth; the implant increased the expression of interleukin-10 and forkhead box P3; increased the RANK/osteoprotegerin ratio, an indicator of apoptosis; and decreased the expression of tumor necrosis factor alpha.[15]

Despite similar bacterial etiologies, there are also histopathological differences between peri-implantitis and periodontitis lesions. The spread of the lesion to the crestal bone in peri-implantitis and the lack of an epithelial lining between the biofilm and the apical portion of the infiltrate can be explained by the absence of supra-crestal fibers and a periodontal ligament in peri-implant tissues. In addition, a review summarized the distinct microbiome compositions of the two diseases.[7] It was shown that surface material, roughness, and energy can influence the colonization of bacteria. Because dental implants differ in those aspects from teeth, a specific microbiome may be associated with peri-implantitis.[7,16]

Recently, two reviews reported on the differences between periodontitis and peri-implantitis with respect to epigenetic markers.[17,18] We will explore them in subsequent sections.

3 | EPIGENETICS: GENERAL PRINCIPLES

The DNA double helix is packaged in the cell nucleus in the form of chromatin. The building block of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around a histone protein complex (Figure 2). The structural arrangement of chromatin affects gene expression: chromatin can be loosely packed, allowing the transcriptional machinery to access and express it, or densely packed, silencing it.[19] The term "epigenetics" refers to chemical alterations to gene expression independent of changes to the DNA sequence, that is, DNA methylation and histone modifications.[20]

Histones can be acetylated or methylated at N-terminal tails that protrude from the nucleosome.[19] These functional groups obstruct the contact between the DNA and histones, loosening their packaging and activating transcription.[19] Acetylation is regulated by histone acetyltransferases that add acetyl groups, and by histone deacetylases that remove them. The balance between histone acetylation and deacetylation at the promoter region of the chromatin is key to the regulation of gene expression and the maintenance of a transcriptionally competent chromatin state.[21,22] The histone acetyltransferases are divided into five distinct families by their sequence divergence at the histone acetyltransferase domain (histone acetyltransferase-1, general control non-depressible 5/PCAF (P300/CBP-associated factor), MYST (Moz, Ybf2/Sas3, Sas2, Tip60), cyclic adenosine monophosphate response element-binding protein/p300, and Rtt109 (regulation of Ty1 transposition gene product 109)). Among all the histone acetyltransferases, p300 is an important histone acetyltransferase that mediates transcriptional

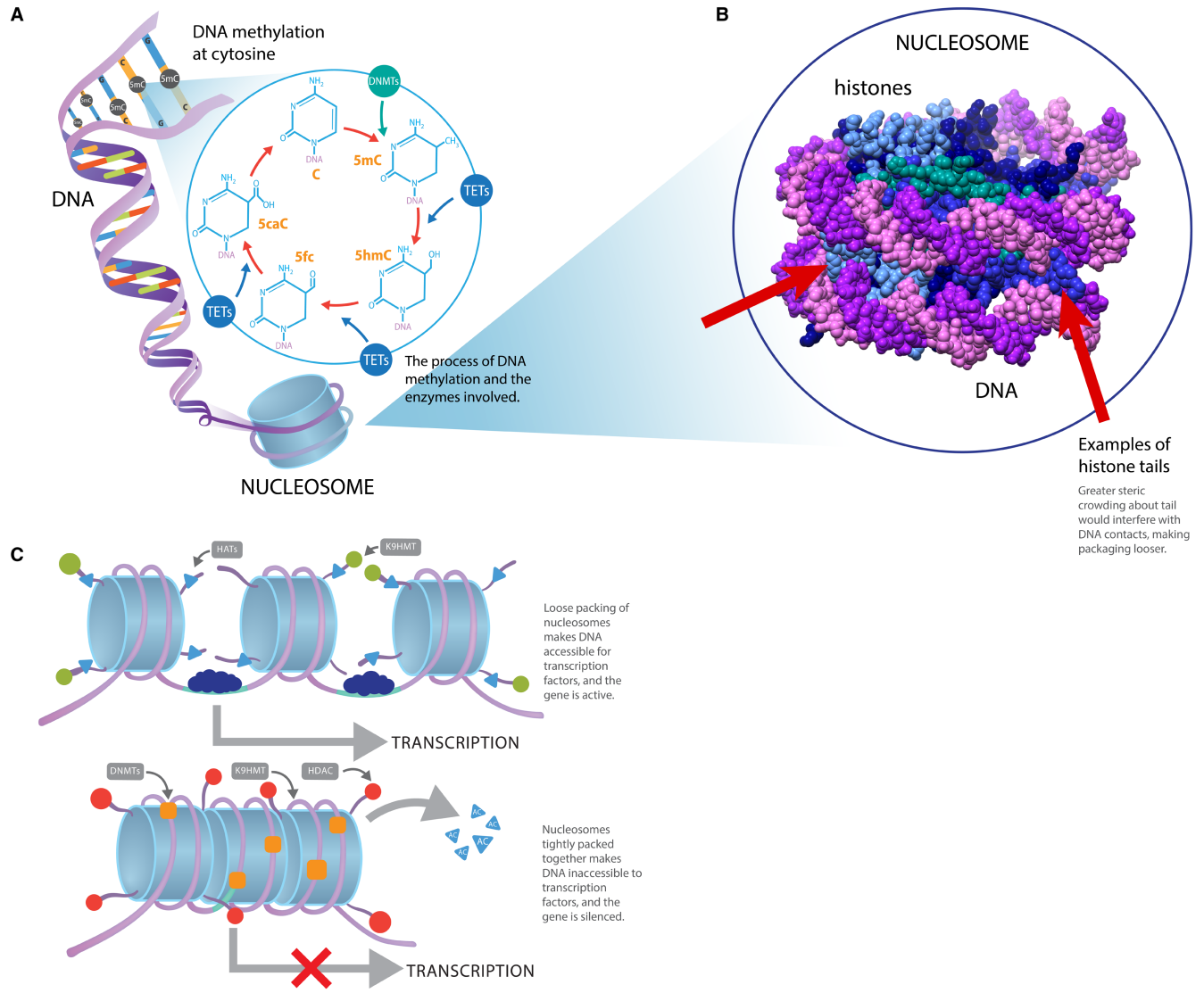

FIGURE 2 The structure and modification of the nucleosome. The histone complex includes two copies each of histones H2A, H2B, H3, and H4, as well as a linker histone H1 that connects the nucleosomes. Along with DNA, these proteins form the primary chromatin structure. A, Chromatin configuration and epigenetic regulation. B, Crystal structure of Protein Data Bank ID 5B2I, showing the nucleasome, rendered in UCSF Chimera.[126] C, Transcriptionally active genes are associated with low levels of DNA methylation and high levels of histone acetylation. Figure adapted with permission from Larsson et al.[9] C, Cytosine; DNMTs, DNA methyltransferases; 5caC, 5-carboxylcytosine; 5fc, 5-Formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; HATs, histone acetyltransferases; HDAC, histone deacetylase; K9HMT, Lysine 9 histone methyltransferase; TET, ten-eleven translocation

activation by participating in the cyclic adenosine monophosphate response element-binding protein/p300 transcriptional coactivation complex.[23] The p300-cyclic adenosine monophosphate response element-binding protein coactivator family, in combination with other proteins, participates in proliferation, differentiation, apoptosis, and transcription through chromatin acetylation.[24] Similar to histone acetyltransferases, histone deacetylases are also divided into four classes and take part in multi-protein complexes that are expressed in many bodily tissues.[25]

DNA itself can be modified by DNA methyltransferases, which add methyl groups to 5-methylcytosine at specific sites in the DNA sequence (ie, CpG sites, or sites with adjacent cytosine and guanine bases).[20,26] When these methyl groups reside at promoters, they can occlude the binding of transcriptional machinery and deactivate transcription; 5-methylcytosine can be further oxidized into 5-hydroxymethylcytosine by the ten-eleven translocation methylcytosine dioxygenases family of enzymes.[27] This oxidation has been suggested as the mechanism for demethylation of DNA so that the cell can reactivate genes (Figure 2).[28]

Importantly, epigenetic mechanisms are reversible and change throughout our lifetimes in response to environmental factors, including the microbiota, smoking, and dietary compounds. It was recently found that biomaterials, material energy, and material topography also influence epigenetic patterns.[9,29,30] Moreover, infection and the host's immune response can induce changes in the epigenome that, in turn, enhance susceptibility to disease. These

epigenetic changes are cell- and tissue-specific, which is relevant for chronic inflammatory diseases like periodontitis and peri-implantitis. These diseases have target tissues in which the inflammation is persistent and tissue destruction occurs: not all teeth or implants are affected. As such, treatments can be targeted as well.

4 | EPIGENETICS AND PERIODONTITIS

Even although epigenetics is a new area of research in periodontology, several studies over the last decade have characterized changes in the epigenetic pattern for periodontal diseases.[9,31–35]

Oral pathogens and bacterial products, such as lipopolysaccharide, have been shown to influence periodontitis by inducing epigenetic changes in gene expression in cells and tissue. For example, *P. gingivalis* and *Fusobacterium nucleatum* can induce acetylation of histone 3 while decreasing the expression of DNA methyltransferase-1.[36] Bacterial activation of pathogen recognition receptors and toll-like receptors, both typically activated in the immune response, can induce histone modifications in oral epithelial cells.[36] These findings are in line with previous research showing that gingival epithelial cells cultured with *P. gingivalis* saw an increase in DNA methylation of the *toll-like receptor-2* promoter.[37] A study conducted by Diomede et al[23] showed that, similar to reports by Martins et al,[36] *P. gingivalis* lipopolysaccharide reduced the expression of DNA methyltransferase-1 in human periodontal ligament cells while upregulating histone acetyltransferase p300 and nuclear factor kappa B, a complex typically activated in response to cellular stress and foreign antigens.[23]

Dysregulation of toll-like receptor expression and consequent changes in the host response against periodontal pathogens can occur, increasing not only inflammation but also a patient's susceptibility to periodontitis.[38] The DNA methylation patterns of the *toll-like receptor-2* and *toll-like receptor-4* promoters have previously been investigated in gingival biopsies, cells, and animal models.[38–41] The *toll-like receptor-4* promoter was reported to be unmethylated in healthy and periodontitis patients, while that of *toll-like receptor-2* included both methylated and unmethylated regions for both groups.[40] However, a higher degree of methylation of *toll-like receptor-2* was found in samples from patients with periodontitis relative to controls, as was a correlation between the level of *toll-like receptor-2* methylation and the number of inflammatory cells within the adjacent connective tissue.[41] Using an in vitro periodontitis and oral gavage model in mice, the presence of *P. gingivalis* was shown to induce methylation of the *toll-like receptor-2* promoter in human gingival epithelial cells.[38] The DNA methylation pattern of other genes in the toll-like receptor signaling pathway (*FADD*, FAS-associated death domain protein; *MAP3K7*, Mitogen-activated protein kinase kinase kinase 7; *MYD88*, Myeloid differentiation primary response 88; *interleukin6R*, interleukin 6 receptor; *PPARA*, Peroxisome Proliferator Activated Receptor Alpha; *IRAK*, Interleukin-1 receptor-associated kinase and *RIPK2*, Receptor-interacting serine/threonine kinase 2) also differed between patients with localized, aggressive periodontitis and healthy controls.[42] The degree of methylation even varied by the severity of disease: patients with moderate disease showed hypermethylation of these genes relative to controls, while patients with severe disease displayed hypomethylation.[42]

The oral pathogen *Treponema denticola* has also been shown to alter epigenetic patterns by inducing hypomethylation of the *matrix metalloprotease-2* promoter, causing chronic activation of pro-matrix metalloprotease-2 expression in periodontal ligament cells.[43] Matrix metalloproteases are key factors in matrix degradation, bone resorption, wound healing, cell proliferation, inflammation, and immunity.[43,44] As a result, hypomethylation by *T. denticola* may influence the activation of matrix metalloproteases and augment the destruction of supporting tissues that occurs in periodontitis.

The epigenetic patterns of several inflammatory cytokines and markers have also been investigated in relation to periodontitis,[9] with variations in DNA methylation between healthy and periodontitis patients being especially large for genes related to immune response.[45] In one study, the methylation levels of CpG sites in 22 inflammatory genes were analyzed in gingival tissue samples from patients with aggressive periodontitis vs controls. A decrease in methylation was found in the promoter regions for *interleukin-17C* and *chemokine ligand-25* in periodontitis patients, resulting in increased expression.[46] These cytokines play important roles in the immune response to bacteria. The different levels of DNA methylation reported by Schulz et al[46] were similar to those reported by Barros and Offenbacher.[31] Given the suggested link between interleukin-17 expression and bone resorption, changes in the methylation pattern and expression of these genes might contribute to the inflammatory response and the loss of attachment seen in periodontitis.[46]

Meanwhile, with respect to the pro-inflammatory cytokine interleukin-6, no difference in methylation of its promoter was found between periodontitis patients and healthy controls.[47] It was previously reported that the *interleukin-6* promoter was partially methylated in gingival tissue samples from both periodontitis and healthy individuals, but that the expression of interleukin-6 was higher in periodontitis patients.[48] Ishida et al[49] also reported an increase in interleukin-6 expression, yet this increase was associated with hypomethylation of only one CpG site in the *interleukin-6* promoter. Similarly, for the inflammatory cytokine tumor necrosis factor alpha, an analysis of 12 CpG sites in the promoter of *tumor necrosis factor alpha* in patients with chronic periodontitis and healthy controls revealed differences in DNA methylation at only one CpG site,[50] although a previous study reported two hypermethylated sites in the *tumor necrosis factor alpha* promoter in chronic periodontitis.[51]

Comparison of the DNA methylation patterns of two inflammatory regulators, suppressor of cytokine signaling-1 and the long interspersed element-1, showed a higher degree of methylation in oral epithelial cells of patients with aggressive periodontitis, relative to healthy subjects.[52] Intragenic CpG islands in *suppressor of cytokine signaling-1* were hypermethylated in periodontal specimens compared with healthy tissue, yet there was no difference in gene expression.[53] Interestingly, the results reported by Planello et al[53] suggested that the increase in DNA methylation of *suppressor of cytokine signaling-1* in periodontitis was not attributable to the

presence of inflammatory cells. Using tissue samples from healthy subjects and periodontitis patients that, at the time of the study, did not show signs of inflammation in the gingival tissue, the levels of methylation for *suppressor of cytokine signaling-1*, *suppressor of cytokine signaling-3*, and *long interspersed element-1* were similar regardless of any previous periodontal inflammation.[54] Similarly, a higher level of DNA methylation in the *cyclooxygenase-2* promoter has been reported for diseased sites compared with healthy sites in patients with periodontitis.[55] Interestingly, periodontal therapy restored the DNA methylation pattern of *cyclooxygenase-2* to a level close to that of healthy patients. By contrast, no changes occurred in the DNA methylation level of *tumor necrosis factor alpha*, *interferon-gamma*, or *long interspersed element-1*.[55] These observations suggest that the treatment of periodontitis and resolution of inflammation may restore some but not all epigenetic modifications to the levels of healthy tissue. Finally, Cho et al[56] also reported on the methylation pattern of inflammatory genes in periodontitis and healthy patients, but the differences were not significant.

Despite many reports on the epigenetic alterations of genes associated with immune response and bone formation, few studies have focused on the expression of epigenetic markers, themselves, in periodontitis. Martins et al[36] reported a downregulation of DNA methyltransferase-1 and upregulation of acetylated histone-3 in epithelial cells close to the inflammatory lesion in a periodontitis model in mice. In contrast, a significant upregulation of DNA methyltransferase-1 and 10-11 translocation-1 mRNA was found in tissue samples from periodontitis patients compared with those from healthy controls.[45] However, it is important to remember that results using tissues reflect the DNA methylation level of genes in several different cell types. The proportion of 10-11 translocation-2-positive cells was even greater in periodontitis lesions than in gingivitis lesions.[57] The increase in ten-eleven translocation (TET) methylcytosine dioxygenases enzymes is of particular interest because they convert 5-methylcytosine to 5-hydroxymethylcytosine and promote demethylation, which, in turn, reactivates genes and increases expression.[27,45] The fact that 10-11 translocation-2 increases in periodontitis relative to gingivitis suggests an association between disease severity and the epigenetic regulation of inflammatory genes.[57] Interestingly, not only did the methylation patterns differ between patients with chronic periodontitis and healthy controls, but this hypermethylation pattern was also found to be located in transcriptional enhancer regions, preventing enhancer activity and gene expression.[57] The DNA methylation pattern found in gingival tissue from periodontitis patients resembled that found in oral squamous cell carcinoma tissue, suggesting that chronically inflamed tissues have a preneoplastic epigenome that may play a role in tumor development.[53] Recently, a role for 10-11 translocation enzymes in the regulation of macrophages in periodontal disease has also been suggested.[58,59]

Jumonji domain-containing protein-3 is a demethylase that binds genes and demethylates them at H3K27, thereby increasing their transcription. Stimulation of macrophages by lipopolysaccharide induces Jumonji domain-containing protein-3, which then influences the polarization of macrophages into either M1 or M2. The polarization of macrophages plays an important role in determining the outcome of an inflammatory response.[60,61] *Porphyromonas gingivalis* lipopolysaccharide treatment caused a decrease in expression of Jumonji domain-containing protein-3, DNA methyltransferase-1, and DNA methyltransferase-3b in keratinocytes, but no difference in gingival fibroblasts.[60] This difference may be a result of the expression of toll-like receptors on epithelial cells, but not on keratinocytes. In the same study, *P. gingivalis* lipopolysaccharide also triggered the toll-like receptor-2 and toll-like receptor-4 signaling pathways, inducing nuclear factor kappa B and downregulating Jumonji domain-containing protein-3.[60] An analysis of the gene expression of Jumonji domain-containing protein-3, DNA methyltransferase-1, and DNA methyltransferase-3b in tissue samples showed no differences between periodontitis patients and healthy controls.[60] In a periodontitis mouse model, adiponectin, a factor secreted by adipose tissue, was found to influence the Jumonji domain-containing protein-3–interferon regulatory factor-4 signaling pathway, which is needed for the polarization of macrophages towards M2; the result was a modified inflammatory response, enhanced bone repair via Jumonji domain-containing protein-3, and reduced periodontal bone loss.[61]

A previous study showed that the immune response to bacteria may be influenced by stressful events in early life.[62] As demonstrated in an experimental periodontitis lipopolysaccharide and ligature model in rats, such events increased the susceptibility to chronic inflammation later in life. Animals that had been separated daily from their mothers as pups eventually had greater alveolar bone loss and lower levels of transforming growth factor beta. These animals also had a higher expression of glucocorticoid receptor, a marker for stress reactivity, in the hippocampus. By contrast, rats that had been handled daily or undisturbed as pups had a higher degree of DNA methylation at specific CpG sites in the glucocorticoid receptor promoter, resulting in a lower level of glucocorticoid receptor expression.[62] These results may shed light on the socioeconomic disparities of periodontal disease, as minority and low-income individuals experience greater social stressors and higher disease rates.[2]

Taken together, most studies on the influence of epigenetics on periodontitis have compared diseased sites with healthy sites. Thus, it is not clear if the epigenetic changes are specific to periodontitis or if they are features of gingival inflammation more generally. Studies evaluating differences between periodontitis and longstanding gingivitis lesions are needed.

5 | EPIGENETICS AND PERI-IMPLANTITIS

To the best of our knowledge, no characterization of the epigenetic pattern of the peri-implantitis lesion has yet been made. A previous review on epigenetics in implant therapy found only eight articles on the role of miRNAs in implant dentistry and no reports on DNA methylation or histone modifications in response to implants.[63] Interestingly, it was recently reported that the global DNA methylation level was higher in gingival tissues than in bone, regardless of

whether the bone was from periodontally healthy patients or from around failed implants resulting from peri-implantitis.[64] The authors suggested that these findings could reflect a different epigenetic response between various tissues in the same microenvironment.

6 | EPIGENETICS AND TITANIUM PARTICLES

In contrast to epigenetic influences on peri-implantitis, there has been a great focus on titanium particles found in the tissue surrounding implants with peri-implantitis and their influence on the disease.[65,66] In gingival tissue where *P. gingivalis* was present, titanium ions from implants were shown to increase the expression of C-C motif chemokine ligand 2, an inflammatory cytokine, and to elevate the ratio of RANKL to osteoprotegerin.[67] In addition, titanium ions elevated toll-like receptor-4 expression, which may increase the host response to microorganisms. Titanium concentrations have also been associated with global methylation levels independent of peri-implantitis, suggesting that titanium particles may affect the level of DNA methylation.[68] As such, the presence of titanium in tissue samples taken near titanium implants, as well as that of titanium ions that can form particles, can induce a pro-inflammatory response.[69]

There are also several studies on the influence of titanium dioxide particles on epigenetic mechanisms.[30] The most prominent connection between titanium and epigenetic modification has been the DNA damage pathway. When the double-stranded helix breaks, H2A histone family member X is phosphorylated (becoming γH2A histone family member X) and is recruited to the damaged site; as such, γH2A histone family member X is an early marker for DNA damage.[70] The efficacy of γH2A histone family member X's response to DNA damage is epigenetically controlled by the acetylation of histones other than itself: the acetylation of histone H3 at lysine 56 enhances the DNA damage response in stem cells.[70] As a result, the γH2A histone family member X/histone H3 acetylated at lysine 56 interaction has been proposed as an important factor for the control of cells' hypersensitivity to DNA damage repair.

As it relates to peri-implantitis, exposure of cells to titanium dioxide particles may directly influence histone acetylation, inhibiting the repair of double-stranded breaks. In vitro, a relatively low concentration of 10 μg/mL of titanium dioxide induced γH2A histone family member X in fibroblasts compared with other compounds, like terbium-doped-gadolinium oxide, which required 1000 μg/mL to induce γH2A histone family member X, or poly(lactic-co-glycolic acid) nanoparticles, which did not induce any DNA damage.[71] Interestingly, nano-sized particles of titanium dioxide induced γH2A histone family member X in fibroblasts more efficiently than larger ones, and the induction of γH2A histone family member X occurred independently of reactive oxygen species produced by inflammatory cells.[72] Similar to fibroblasts, titanium particles isolated from commercially available dental implants have been shown to induce the activation of checkpoint kinase 2 and accumulation of breast cancer gene 1 in a culture of oral epithelial cells.[73] Following DNA damage, the recruitment and accumulation of breast cancer gene 1 to the nuclear foci was mediated by the phosphorylation of γH2A histone family member X.[74–76] In addition to being cytotoxic to fibroblast and oral epithelial cells, low doses of titanium dioxide particles induced expression of pro-inflammatory markers.[77] Also, stimulating these cells with lipopolysaccharide following titanium dioxide stimulation enhanced the expression of the inflammatory cytokine tumor necrosis factor alpha. In line with this focus, there are numerous studies on how surface topography (eg, of implants) impacts the epigenetic pattern.[30] In any case, a more thorough characterization of the epigenetic pattern of the peri-implant lesion in response to titanium particles is necessary to make any clinical correlations valid.

7 | CLINICAL APPLICATION OF EPIGENETICS IN PERIODONTITIS AND PERI-IMPLANTITIS

7.1 | Epi-drugs

The fact that epigenetic mechanisms are reversible makes them attractive targets for new treatment models. Many epigenetic molecules, or epi-drugs, have already been approved by the U.S. Food and Drug Administration, like histone deacetylase inhibitors for cancer treatment. Histone deacetylase inhibitors are small compounds that inhibit the function of histone deacetylases by blocking their binding to target sites, thereby increasing histone acetylation and enhancing gene transcription.[78] Trichostatin A, entinostat sodium butyrate, suberoylanilide hydroxamine (or vorinostat), and valproic acid are all histone deacetylase inhibitors that are currently in clinical studies.[24,79,80]

Reports on the use of epi-drugs for the treatment of inflammatory diseases have recently emerged. An inhibitory effect of histone deacetylase inhibitors on bone destruction and inflammation was reported for rheumatoid arthritis, suggesting a treatment option that simultaneously targets both pathways.[81] In line with these findings, histone deacetylase inhibitors have been reported to decrease bone loss, not only for rheumatoid arthritis, but also for periodontitis.[82,83] Trichostatin A, valproic acid, and entinostat have been investigated for potential use in regulating bone formation and were suggested as suitable agents for both local and systemic treatment of bone loss.[79]

In a previous study, periodontitis gingival tissue was shown to have increased mRNA expression of histone deacetylase-1, histone deacetylase-5, histone deacetylase-8, and histone deacetylase-9; of these, the histone deacetylase-1 protein was found in significantly higher quantities in diseased tissue than in healthy tissue.[84] Histone deacetylase-1 was also found in inflammatory cells, suggesting a role in regulating inflammation.[84] Treatment of human periodontal ligament cells with trichostatin A decreased expression of histone deacetylase-3, increased acetylation of histone H3, and induced osteogenic differentiation.[85] Treatment of periodontal ligament fibroblasts with sodium butyrate induced the expression

of osteoblast-related proteins and inhibited the production of pro-inflammatory cytokines.[80]

Other epi-drugs target the DNA methylation pathway. 5-aza-2′-deoxycytidine inhibits DNA methylation and was reported to increase the responsiveness of gingival fibroblasts to transforming growth factor beta-1 and increase DNA methyltransferases.[86] Gingival epithelial cells exposed to *P. gingivalis* and *F. nucleatum* showed decreased expression of DNA methyltransferases and histone deacetylase.[37] When the cells were treated with 5-aza-2′-deoxycytidine prior to exposure to *F. nucleatum*, their expression of *human beta-defensin-2* and *chemokine (C-C motif) ligand 20* was enhanced relative to no treatment; both genes are typically upregulated in response to bacteria.[37] Treatment with an histone deacetylase inhibitor, however, increased the expression of both genes, as well as histone acetylation in response to *F. nucleatum* and *P. gingivalis*. This effect could represent a new tool for improving wound healing and periodontal tissue regeneration. Similarly, treatment of human bone marrow stromal cells with either trichostatin A or 5-aza-2′-deoxycytidine induced the cells to differentiate into osteogenic and chondrogenic populations, respectively.[87] Treatment with 5-aza-2′-deoxycytidine of osteoblasts grown on titanium discs of two different surface characteristics decreased DNA methylation on both surfaces and induced gene expression of alkaline phosphatase.[88] Decitabine (5-aza-2′-deoxycytidine) was found to reduce bone loss in a mouse periodontitis model by inhibiting osteoclastogenesis.[89]

Another challenge in periodontal tissue regeneration is reducing inflammation. Histone deacetylase inhibitor 1179.4b was able to suppress alveolar bone loss but not gingival inflammation.[83] By contrast, the bromodomain and extra-terminal motif inhibitor JQ1 inhibited both the inflammatory response and alveolar bone loss.[90] Bromodomain and extra-terminal motif proteins contain bromodomains that sense acetylated histones and can recruit epigenetic regulators of gene expression.[90] A previous review reported that histone deacetylase inhibitors influence not only osteoclast differentiation, but also maturation and activity.[91] Transforming growth factor beta-1 is a key factor in regulating wound healing, an event important to tissue regeneration (eg, after periodontitis surgery and implant placement). Treating oral fibroblasts with 5-aza-2′-deoxycytidine demethylation agent prior to treatment with transforming growth factor beta-1 increased DNA methyltransferase-1 and DNA methyltransferase-3b expression, increased the fibroblasts' response to transforming growth factor beta-1, and induced the expression of transforming growth factor beta-1's targets.[86]

7.2 | Epigenetics in bone regeneration

An important aspect of treating periodontitis and peri-implant defects is the improvement of bone regeneration. A primary focus in this field, therefore, has been improving the osteogenic potential of scaffolds and bone-grafting materials.[78] Cell-based techniques using stem cells and induced pluripotent stem cells have become particularly popular in tissue regeneration.[92] Stem cell differentiation was extremely sensitive to changes in epigenetic mechanisms.[93] Dental pulp stem cells can differentiate into osteogenic cells, and the fact that they are easy to access has made them an alluring source for cell therapy. It was recently shown that treating dental pulp stem cells with histone deacetylase inhibitors enhanced matrix mineralization and the expression of osteogenic differentiation markers, such as osteopontin and bone sialoprotein, yet decreased expression of osteocalcin.[94] In addition, histone deacetylase-1 and histone deacetylase inhibitor-2 were identified as important regulators in osteoblast differentiation.[94] Targeting epigenetic mechanisms, may, therefore, present new models for improving bone and soft tissue regeneration.

10-11 translocation-2 and the enzyme thymine-DNA glycosylase were able to induce changes in both the 5-methylcytosine and 5-hydroxymethylcytosine patterns in myeloid stem cells.[95] In later stages of cell differentiation, 10-11 translocation-2 and thymine-DNA glycosylase further regulated histone modifications of genes and determined if the cells differentiated into macrophages or osteoclasts.[95] Targeting this signaling pathway may present a mechanism for regulating bone resorption by influencing cell differentiation towards the macrophage lineage. 10-11 translocation-1 and 10-11 translocation-2 also regulated the differentiation of mesenchymal stem cells into osteoblasts by demethylating and activating *Sp7*, which encodes an important transcription factor for bone formation and osteoblast differentiation.[96] Furthermore, it was shown that this process also involved altering the histone methylation and acetylation patterns of the *Sp7* promoter. These findings showed that although these different epigenetic mechanisms by themselves can induce changes in gene expression, they also interact to regulate gene expression and, hence, cell differentiation and function.

Cells derived from periodontal ligament also have the potential to differentiate into osteoblasts, and runt-related transcription factor-2 is a key factor in this process.[97] Histone deacetylase-1, histone deacetylase-2, histone deacetylase-3, histone deacetylase-4, and histone deacetylase-6 were all shown to be present in human periodontal ligament cells.[85] Histone deacetylase-3, histone deacetylase-6, and histone deacetylase-7 were involved in regulating the expression of runt-related transcription factor-2, and histone deacetylase inhibitors induced acetylation of the runt-related transcription factor-2 gene, increased its expression, and, in turn, induced the expression of genes related to osteogenesis and bone formation.[78] These inhibitors also enhanced mineralization, bone regeneration, and osteogenic differentiation of periodontal ligament cells and dental pulp stem cells.[78] *Porphyromonas gingivalis* lipopolysaccharide induced an increase of DNA methyltransferase-1 and downregulation of runt-related transcription factor-2 expression in human periodontal ligament cells, suggesting that the inhibitory effect of lipopolysaccharide on osteoblastic differentiation may be a consequence of DNA hypomethylation of runt-related transcription factor-2.[97] Treatment of human gingival fibroblasts with a DNA methylation inhibitor

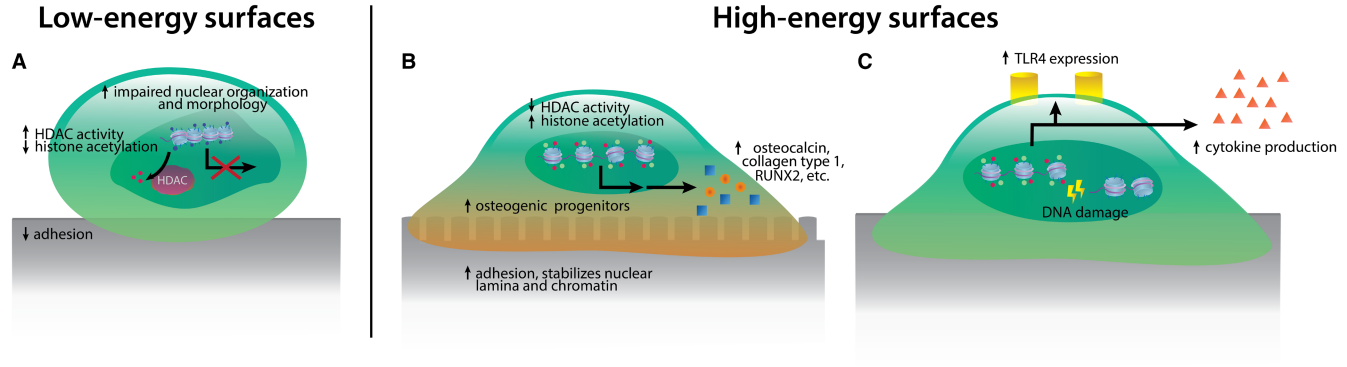

FIGURE 3 Surface characteristics and epigenetic patterns. A, Cells grown on a soft material, or low-energy surface, have transcriptionally inactive chromatin, while B, Cells grown on a stiff, or high-energy, surface, have transcriptionally active chromatin. C, Contact with titanium activates the DNA damage pathway. Figure adapted with permission from Larsson et al.[30] HDAC, histone deacetylase; RUNX2, runt-related transcription factor-2; TLR4, toll-like receptor-4

induced hypomethylation of runt-related transcription factor-2 and alkaline phosphatase, and subsequent treatment of these cells with bone morphogenetic protein-2 induced the expression of runt-related transcription factor-2 and alkaline phosphatase, as well as differentiation into osteoblasts.[98]

Periodontal ligament stem cells extracted from periodontitis patients and healthy subjects were investigated with respect to the expression of histone acetyltransferase general control non-depressible 5.[99] Cells from periodontitis patients showed a downregulation of general control non-depressible 5 and a decrease in osteogenic differentiation potential compared with cells from controls. Knockdown of general control non-depressible 5 decreased expression of runt-related transcription factor-2 and alkaline phosphatase, while overexpression restored the osteogenic potential of the cells.[99] Mechanistically, general control non-depressible 5 induced acetylation of histone H3 at lysines 9 and 14 near the *Dickkopf-1* gene (Dickkopf WNT Signaling Pathway Inhibitor 1), thereby increasing its expression. Dickkopf-1 is an inhibitor of the Wnt/β-catenin signaling pathway, which is important in the regulation of osteogenic differentiation of periodontal ligament stem cells. Interestingly, treatment with aspirin inhibited both general control non-depressible 5 expression and inflammation in lipopolysaccharide-induced periodontitis rats while upregulating Dickkopf-1 and reducing bone loss.[99] Inhibition of histone deacetylases using trichostatin A enhanced the osteogenic differentiation of human periodontal ligament cells. There was not only an upregulation of osteoblast-related genes, but also an increase in alkaline phosphatase activity, mineral formation, and runt-related transcription factor-2 production.[78,85] Furthermore, when trichostatin A-treated human periodontal ligament cells were implanted in a scaffold, bone formation was enhanced for up to 8 weeks.[78] Trichostatin A has also been shown to enhance osteogenic differentiation in mesenchymal stem cells and periodontal repair by interfering with the nuclear factor kappa B-pathway.[100]

Smoking and diabetes also had epigenetic effects on osseointegration and bone regeneration by targeting DNA methylation in the former and histone acetylation in the latter.[101,102]

7.3 | Delivery models for epi-drugs and miRNA

Identifying a method for local and sustainable delivery of epi-drugs to the site of periodontitis and peri-implantitis is crucial for new treatment models. Collagen sponges and macroporous biphasic calcium phosphate scaffolds mixed with histone deacetylase inhibitors were found to induce woven bone formation at the interface with the scaffold.[103] Two studies on the use of microarc oxidation titanium implant surfaces as a delivery model for miRNAs have been published.[104,105] Wang et al[104] fabricated chitosan-hyaluronic acid nanoparticles to deliver miRNA-21 into human bone marrow mesenchymal stem cells and, thereby, increased the expression of osteogenic genes. Wu et al[105] attached miRNA-29b and anti-miRNA-138 lipoplexes onto a microarc oxidation titanium implant and induced osteogenic differentiation in rat bone marrow mesenchymal stem cells. These studies suggest a novel tool for improving the osseointegration of implants and a method for delivering epi-drugs.

7.4 | Modifying surface structure to improve implant–bone interactions

In implant therapy, promoting tissue integration, especially between bone and implant, is a primary goal. In this process, early attachment of epithelial cells and fibroblasts is important for making a seal around the implant to promote osseointegration and prevent bacteria from colonizing the implant surface.[106,107] An important factor in the regulation of cell adhesion, migration, proliferation, and differentiation is the surface topography.[108,109] Interestingly, cells grown on a stiff surface have transcriptionally active chromatin, while cells grown on a soft material have transcriptionally inactive chromatin (Figure 3).[110] Using titanium discs with either smooth or rough surfaces, it was shown that surface characteristics influence not only DNA damage and the DNA repair pathway, but also epigenetic factors.[29] Total γH2A histone family member X-positive cells on rough titanium decreased in proportion over time, while such cells grown

on smooth titanium did not. Rough titanium surfaces also induced more cytoplasmic staining of DNA methyltransferase-1 and lower histone acetylation than smooth titanium.[29] In addition, the methylation level of the alkaline phosphatase gene was lower in osteoblast cells grown on smooth titanium surfaces than in cells grown on modified titanium surfaces.[88] Interestingly, surface decontamination using mechanical methods was found to further influence epigenetic markers.[111]

In a previous study, preosteoblastic cells were grown on titanium discs with various surfaces: machined, dual acid-etched, and acid-etched nanohydroxyapatite-blasted.[112] Nanohydroxyapatite-blasted discs had greater cell adhesion, more cell spreading, and lower apoptosis, probably attributable to its better absorption of protein from serum, an important early factor for cell adaption and attachment to the titanium surface. Nanohydroxyapatite also promoted intracellular signaling networks, important for cell–surface interactions.[112] Changing a titanium surface's nanostructure promoted adipocytes towards osteogenic differentiation,[106,113] and altering the surface and the construction of titanium tubes induced periodontal regeneration and enhanced periodontal ligament structure.[114,115] Adding a coating of osteoprotegerin also increased early osteoblast differentiation and mineralization.[109]

Many studies have also reported a correlation between changes in gene expression and different implant surfaces.[63] The recently developed zirconia implant surface was shown to induce a different level of expression of 10 miRNAs that were involved in the regulation of osteogenic and bone remodeling genes, such as bone morphogenetic proteins.[116]

Even although research on how surface topography and material energy affect the epigenome is still in its infancy, the present literature suggests that materials and nanotechnology can promote tissue regeneration and cellular functions, like attachment and osseointegration, via epigenetics. This role can be regulated by altering the titanium surface itself. These findings illustrate the importance of understanding material "structures" as well as cellular functions in order to obtain the best outcome for periodontal regeneration.[30]

8 | FUTURE CONCEPTS OF EPIGENETICS AND INFLAMMATION

While epi-drugs may be potent against cancer, they have side effects. For peri-implantitis and bone regeneration, they may be avoided by instead using topography and material energy to induce changes in the epigenetic pattern of cells in contact with the implant or scaffold.

Other methods may achieve the same goals. Recently, dietary substances as substitutes for epi-drugs have received interest as potential treatment options. Nutritional components are known to induce changes in the epigenetic pattern, and the term "epigenetic diet," or "epi-diet," has been coined.[117] So far, they have been studied mostly in relation to cancer,[118] but the close association of inflammation, cancer, and epigenetics suggests the use for an epi-diet in the treatment of inflammatory diseases, too. The idea of diet as an epigenetic tool for the prevention of chronic diseases was discussed in a recent review.[119] Since 2004, the term "immunonutrition" has described nutrients shown to influence the immune response towards an anti-inflammatory reaction.[120] Epigallocatechin-3-gallate in green tea, polyphenols, and omega-3-polyunsaturated fatty acids in fatty fish were suggested to be anti-inflammatory as well as preventative of cancer.[120] Interestingly, it has been suggested that the epigenetic pattern is more susceptible to changes in nutrition during times of inflammation and in ways that may be organ- or tissue-specific.[121] Recently, it was shown that 10-11 translocation enzymes and the 5-hydroxymethylcytosine pathway were influenced by nutritional compounds such as vitamin C, and that microbiome-produced metabolites like folate also influenced enzymes regulating 5-hydroxymethylcytosine.[122]

Few studies on diet and inflammation are available, but there are studies on the effects of dietary compounds on the oral mucosa. Vegetarians and omnivores have different DNA methylation patterns in cells of the buccal mucosa.[123] Curcumin is a compound with anti-inflammatory, wound-healing, and anticancerous properties that has been linked to both DNA methylation and histone acetylation.[124] the influence of modified curcumin CMC2.24 was investigated for its effect on periodontitis. Administration of CMC2.24 decreased inflammatory cytokines, matrix metalloproteases, and alveolar bone loss in an experimental murine periodontitis model.[125] It was proposed as an anti-inflammatory treatment model for periodontitis.

9 | CONCLUSIONS AND FUTURE DIRECTIONS

Evidence continues to emerge on the pathogenesis of periodontal and peri-implant diseases. While the host responses in both diseases share some similarities, their differences reflect the unique make-up of the tooth–periodontium and implant–alveolar bone biointerfaces. As such, we cannot translate all the protocols of one directly to the management of the other. More longitudinal clinical studies that monitor the progression of peri-implant diseases are necessary to better understand the triggers of the disease, its progression, and its epigenetic and other mechanisms. This information could allow us to stratify our patients by level of risk and manage them in a more personalized fashion based on their disease activity and lifestyles.

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