Influence of epigenetics on periodontitis and peri-implantitis pathogenesis

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Abstract

Periodontitis is a disease characterized by tooth-associated microbial biofilms that drive chronic inflammation and destruction of periodontal-supporting tissues. In some individuals, disease progression can lead to tooth loss. A similar condition can occur around dental implants in the form of peri-implantitis. The immune response to bacterial challenges is not only influenced by genetic factors but also by environmental factors. Epigenetics involves the study of gene function independent of changes to the DNA sequence and its associated proteins, and represents a critical link between genetic and environmental factors. Epigenetic modifications have been shown to contribute to the progression of several diseases, including chronic inflammatory diseases like periodontitis and peri-implantitis. This review aims to present the latest findings on epigenetic influences on periodontitis and to discuss potential mechanisms that may influence peri-implantitis, given the paucity of information currently available.

Introduction

Periodontitis is a widespread disease recently 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, i.e. 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.

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 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 (MPO), a marker for neutrophils, and tartrate-resistant acid phosphatase (TRAP), a marker for osteoclasts, were higher in peri-implantitis.[12] Similarly, a rat lipopolysaccharide (LPS) 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 to implants without infection, FOXP3, a

negative regulator of the immune response, decreased in the setting of infection, while tumor necrosis factor alpha (TNFα), a cytokine for inflammation, increased. Meanwhile, teeth experienced no change in FOXP3 or TNFα in the setting of infection.[15] Interestingly, the presence of an implant even without infection altered the expression of cytokines compared to healthy teeth; the implant increased the expression of interleukin (IL)-10 and FOXP3; increased the RANK/osteoprotegerin (OPG) ratio, an indicator of apoptosis; and decreased the expression of TNFα.[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 recent 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. Since 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.

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 (HATs) that add acetyl groups and by histone deacetylases (HDACs) 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 HATs are divided into five distinct families by their sequence divergence at the HAT domain (HAT1, Gcn5/PCAF, MYST, CBP/p300, and Rtt109). Among all HATs, p300 is an important histone acetyltransferase that mediates transcriptional activation by participating in the CREB-binding protein/p300 transcriptional co-activation complex.[23] The p300-CBP coactivator family, in combination with other proteins, participates in proliferation, differentiation, apoptosis, and transcription through chromatin acetylation.[24] Similar to HATS, HDACs are also divided into 4 classes and take part in multi-protein complexes that are expressed in many bodily tissues.[25]

DNA itself can be modified by DNA methyltransferases (DNMTs), which add methyl groups to cytosine bases (5mC) at specific sites in the DNA sequence (i.e. 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. 5mC can be further oxidized into 5-hydroxymethylcytosine (5hmC) by the ten-eleven translocation (TET)

family of enzymes.[27] This oxidation has been suggested as the mechanism for de-methylation of DNA so that the cell can re-activate 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.

Epigenetics and periodontitis

Even though 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 LPS, 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 DNMT1.[36] And bacterial activation of pathogen recognition receptors (PRRs) and TLRs, 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 *TLR2* promoter.[37] A study by Diomede and co-workers showed that, similar to reports by Martins et

al,[36] *P. gingivalis* LPS reduced the expression of DNMT1 in human periodontal ligament (PDL) cells while upregulating histone acetyltransferase p300 and NF-κB, a complex typically activated in response to cellular stress and foreign antigens.[23]

Dysregulation of TLR 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 *TLR2* and *TLR4* promoters have previously been investigated in gingival biopsies, cells, and animal models.[38–41] The *TLR4* promoter was reported to be unmethylated in healthy and periodontitis patients, while that of *TLR2* included both methylated and unmethylated regions for both groups.[40] However, a higher degree of methylation of *TLR2* was found in samples from patients with periodontitis relative to controls, as was a correlation between the level of *TLR2* 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 *TLR2* promoter in human gingival epithelial cells.[38] The DNA methylation pattern of other genes in the TLR signaling pathway (*FADD*, *MAP3K7*, *MYD88*, *IL6R*, *PPARA*, *IRAK*, and *RIPK2*) 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 *MMP2* promoter, causing chronic activation of pro-MMP2 expression in PDL cells.[43] Matrix metalloproteases (MMPs) 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 MMPs 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 versus controls. A decrease in methylation was found in the promoter regions for interleukin-17C (*IL-17C*) and chemokine ligand-25 (*CCL25*) 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 were similar to those by Barros and Offenbacher.[31,46] Given the suggested link between IL-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 IL-6, no difference in methylation of its promoter was found between periodontitis patients and healthy controls.[47] It had been previously reported that the *IL-6* promoter was partially methylated in gingival tissue samples from both periodontitis and healthy individuals but that the expression of IL-6 was higher in periodontitis patients.[48] Ishida and coworkers also reported an increase in IL-6 expression, yet this increase was associated with hypomethylation of only one CpG site in the *IL-6* promoter.[49] Similarly, for the inflammatory cytokine TNFα, an analysis of 12 CpG sites in the promoter of *TNFα* in patients with chronic periodontitis and healthy controls revealed differences in DNA

methylation only at one CpG site,[50] although a previous study reported two hypermethylated sites in the *TNFα* promoter in chronic periodontitis.[51]

Comparison of the DNA methylation patterns of two inflammatory regulators, suppressor of cytokine signaling 1 (SOCS1) and the long interspersed element-1 (LINE-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 *Socs1* were hypermethylated in periodontal specimens compared to healthy tissue, yet there was no difference in gene expression.[53] Interestingly, the results by Planello et al suggested that the increase in DNA methylation of *Socs1* in periodontitis was not due to the presence of inflammatory cells.[53] 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 *Socs1*, *Socs3*, and *LINE-1* were similar regardless of any previous periodontal inflammation.[54] Similarly, a higher level of DNA methylation in the *COX-2* promoter has been reported for diseased sites compared to healthy sites in patients with periodontitis.[55] Interestingly, periodontal therapy restored the DNA methylation pattern of *COX-2* to a level close to that of healthy patients. In contrast, no changes occurred in the DNA methylation level of *TNFα*, *IFNγ*, or *LINE-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 and co-workers also reported on the methylation pattern of inflammatory genes in periodontitis and healthy patients, but the differences were not significant.[56]

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 and coworkers reported a down-regulation of DNMT1 and

up-regulation of acetylated histone 3 in epithelial cells close to the inflammatory lesion in a periodontitis model in mice.[36] In contrast, a significant up-regulation of DNMT1 and TET1 mRNA was found in tissue samples from periodontitis patients compared to 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 TET2-positive cells was even greater in periodontitis lesions than in gingivitis lesions.[57] The increase in TET enzymes is of particular interest since they convert 5mC to 5hmC and promote demethylation, which, in turn, re-activates genes and increases expression.[27,45] The fact that TET2 rises 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 also was 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 pre-neoplastic epigenome that may play a role in tumor development.[53] Recently, a role for TET enzymes in the regulation of macrophages in periodontal disease has also been suggested.[58,59]

JMJD3 is a demethylase that binds genes and demethylates them at H3K27, thereby increasing their transcription. Stimulation of macrophages by LPS induces JMJD3, 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] *P. gingivalis* LPS treatment caused a decrease in expression of JMJD3, DNMT1, and DNMT3b in keratinocytes but no difference in gingival fibroblasts.[60] This difference may be due to the expression of TLRs on epithelial cells but not on keratinocytes. In the same study, *P. gingivalis*

LPS also triggered the TLR2 and 4 signaling pathways, inducing NFkB and downregulating JMJD3.[60] An analysis of the gene expression of JMJD3, DNMT1, and DNMT3b in tissue samples showed no differences between periodontitis patients and healthy controls.[60] In a periodontitis mouse model, adiponectin (APN), a factor secreted by adipose tissue, was found to influence the JMJD3-IRF4 signaling pathway, which is needed for the polarization of macrophages towards M2; the result was a modified inflammatory response, enhanced bone repair via JMJD3, and reduced periodontal bone loss.[61]

A recent study showed that the immune response to bacteria may be influenced by stressful events in early life.[62] As demonstrated in an experimental periodontitis LPS 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 TGF-β. These animals also had a higher expression of glucocorticoid receptor (Gr), a marker for stress reactivity, in the hippocampus. In contrast, rats that had been handled daily or undisturbed as pups had a higher degree of DNA methylation at specific CpG sites in the Gr promoter, resulting in a lower level of Gr expression.[62] These results may shed light on the socioeconomic disparities of periodontal disease, as minority and low-income individuals suffer 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.

Epigenetics and peri-implantitis

To the knowledge of the authors of this review, no characterization of the epigenetic pattern of the peri-implantitis lesion has yet been made. A recent review on epigenetics in implant therapy found only 8 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 due to peri-implantitis.[64] The authors suggested that these findings could reflect a different epigenetic response between various tissues in the same microenvironment.

Epigenetics and titanium particles

In contrast with 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 CCL2, an inflammatory cytokine, and to elevate the ratio of RANKL to OPG.[67] In addition, titanium ions elevated TLR4 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 (TiO_2) 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, histone H2A.X is phosphorylated (becoming γH2AX) and is recruited to the damaged site; as such, γH2AX is an early marker for DNA damage.[70] The efficacy of γH2AX's response to DNA damage is epigenetically controlled by the acetylation of histones other than itself: the acetylation of histone H3 at lysine 56 (H3K56ac) enhances the DNA damage response in stem cells.[70] As a result, the γH2AX/H3K56ac 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 TiO_2 particles may directly influence histone acetylation, inhibiting repair of double-stranded breaks. *In vitro*, a relatively low concentration of 10 μg/mL of TiO_2 induced γH2AX in fibroblasts compared with other compounds, like terbium-doped-gadolinium oxide (Tb-Gd2O3), which required 1,000 μg/mL to induce γH2AX, or poly(lactic-co-glycolic acid) (PLGA) nanoparticles, which did not induce any DNA damage.[71] Interestingly, nano-sized particles of TiO_2 induced γH2AX in fibroblasts more efficiently than larger ones, and the induction of γH2AX occurred independently of reactive oxygen species (ROS) produced by inflammatory cells.[72] Similar to fibroblasts, titanium particles isolated from commercially available dental implants have been shown to induce the activation of CHK2 and accumulation of BRCA1 in a culture of oral epithelial cells.[73] Following DNA damage, the recruitment of BRCA1 to the nuclear foci was mediated by the phosphorylation of γH2AX.[74–76] In addition to being cytotoxic to fibroblast and oral epithelial cells, low doses of TiO_2 particles induced expression of pro-inflammatory markers.[77] Also, stimulating these cells with LPS following TiO_2 stimulation enhanced the expression of the inflammatory cytokine TNFα. In line with this focus, there are numerous studies on how surface topography, e.g. 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.

Clinical application of epigenetics in periodontitis and peri-implantitis

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 HDAC inhibitors (HDACi) for cancer treatment. HDACi are small compounds that inhibit the function of HDACs by blocking their binding to target sites, thereby increasing histone acetylation and enhancing gene transcription.[78] Trichostatin A (TSA), Entinostat (MS-275) sodium butyrate, suberoylanilide hydroxamine (SAHA, or Vorinostat), and valproic acid (VPA) are all HDACi 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 HDACi 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, HDACi have been reported to decrease bone loss not only for rheumatoid arthritis but also for periodontitis.[82,83] TSA, VPA, and MS-275 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 recent study, periodontitis gingival tissue was shown to have increased mRNA expression of HDAC1, 5, 8 and 9; of these, the HDAC1 protein was found in significantly higher quantities

in diseased tissue than in healthy tissue.[84] HDAC1 was also found in inflammatory cells, suggesting a role in regulating inflammation.[84] Treatment of human PDL cells with TSA decreased expression of HDAC3, increased acetylation of histone H3, and induced osteogenic differentiation.[85] Treatment of PDL 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 (5-aza) inhibits DNA methylation and was reported to increase the responsiveness of gingival fibroblasts to TGF-β1 and increase DNMTs.[86] Gingival epithelial cells exposed to *P. gingivalis* and *F. nucleatum* showed decreased expression of DNMT and HDAC [37]. When the cells were treated with 5-aza prior to exposure to *F. nucleatum*, their expression of human beta-defensin-2 (*hBD-2*) and *CCL20* was enhanced relative to no treatment; both genes are typically up-regulated in response to bacteria.[37] Treatment with an HDAC 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 TSA or 5-aza induced the cells to differentiate into osteogenic and chondrogenic populations, respectively.[87] Treatment with 5-aza-dC of osteoblasts grown on titanium discs of two different surface characteristics decreased DNA methylation on both surfaces and induced gene expression of alkaline phosphatase (ALP).[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. HDACi 1179.4b was able to suppress alveolar bone loss but not gingival inflammation.[83] In contrast, the BET inhibitor JQ1 inhibited both the inflammatory response and alveolar bone loss.[90] BET proteins

contain bromodomains that sense acetylated histones and can recruit epigenetic regulators of gene expression.[90] A recent review reported that HDACi influence not only osteoclast differentiation but also maturation and activity.[91] TGF-β1 is a key factor in regulating wound healing, an event important to tissue regeneration, e.g. after periodontitis surgery and implant placement. Treating oral fibroblasts with 5-aza demethylation agent prior to treatment with TGF-β1 increased DNMT1 and DNMT3b expression, increased the fibroblasts' response to TGF-β1, and induced the expression of TGF-β1's targets.[86]

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 HDACi enhanced matrix mineralization and the expression of osteogenic differentiation markers, such as osteopontin and bone sialoprotein, yet decreased expression of osteocalcin.[94] In addition, HDAC1 and HDAC2 were identified as important regulators in osteoblast differentiation.[94] Targeting epigenetic mechanisms may, therefore, present new models for improving bone and soft tissue regeneration.

TET2 and the enzyme thymine-DNA glycosylase were able to induce changes in both the 5mC and 5hmC patterns in myeloid stem cells.[95] In later stages of cell differentiation, TET2 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. TET1 and TET2 also regulated the differentiation of mesenchymal stem cells into osteoblast 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 PDL also have the potential to differentiate into osteoblasts, and RUNX2 is a key factor in this process.[97] HDAC1, 2, 3, 4, and 6 were all shown to be present in human PDL cells.[85] HDACs 3, 6 and 7 were involved in regulating the expression of RUNX2, and HDACi induced acetylation of the RUNX2 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 PDL cells and dental pulp stem cells.[78] *P. gingivalis* LPS induced an increase of DNMT1 and down-regulation of RUNX2 expression in human PDL cells, suggesting that the inhibitory effect of LPS on osteoblastic differentiation may be a consequence of DNA hypomethylation of RUNX2.[97] Treatment of human gingival fibroblasts with a DNA methylation inhibitor induced hypomethylation of RUNX2 and ALP, and subsequent treatment of these cells with BMP-2 induced the expression of RUNX2 and ALP as well as differentiation into osteoblasts.[98]

PDL stem cells extracted from periodontitis patients and healthy subjects were investigated with respect to the expression of histone acetyltransferase GCN5.[99] Cells from periodontitis patients showed a down-regulation of GCN5 and a decrease in osteogenic differentiation potential compared to cells from controls. Knockdown of GCN5 decreased expression of RUNX2 and ALP, while overexpression restored the osteogenic potential of the cells.[99] Mechanistically, GCN5 induced acetylation of histone H3 at lysines 9 (H3K9) and 14 (H3K14) near the *DKK1* gene, thereby increasing its expression. DKK1 is an inhibitor of the Wnt/β-catenin signaling pathway, which is important in the regulation of osteogenic differentiation of PDL stem cells. Interestingly, treatment with aspirin inhibited both GCN5 expression and inflammation in LPS-induced periodontitis rats while up-regulating DKK1 and reducing bone loss.[99] Inhibition of HDACs using TSA enhanced the osteogenic differentiation of human PDL cells. There was not only an up-regulation of osteoblast-related genes but also an increase in ALP activity, mineral formation, and RUNX2 production.[78,85] Furthermore, when TSA-treated human PDL cells were implanted in a scaffold, bone formation was enhanced for up to 8 weeks.[78] TSA has also been shown to enhance osteogenic differentiation in mesenchymal stem cells and periodontal repair by interfering with the NFkB-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]

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 HDACi were found to induce woven bone formation at the interface with the scaffold.[103] Two studies on the use of microarc oxidation

(MAO) titanium implant surfaces as a delivery model for miRNAs have been published.[104,105] Wang and co-workers fabricated chitosan-hyaluronic acid nanoparticles to deliver miRNA-21 into human bone marrow mesenchymal stem cells and, thereby, increased the expression of osteogenic genes.[104] Wu and co-workers attached miRNA-29b and anti-miRNA-138 lipoplexes onto an MAO titanium implant and induced osteogenic differentiation in rat bone marrow mesenchymal stem cells.[105] These studies suggest a novel tool for improving the osseointegration of implants and a method for delivering epi-drugs.

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 γH2AX-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 DNMT1 and lower histone acetylation than smooth titanium.[29] In addition, the methylation level of the ALP 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 recent study, pre-osteoblastic 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, likely due 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 OPG 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 BMPs.[116]

Even though 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]

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 toward 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 TET enzymes and the 5hmC pathway were influenced by nutritional compounds such as vitamin C, and that microbiome-produced metabolites like folate also influenced enzymes regulating 5hmC.[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 anti-cancerous properties and has been linked to both DNA methylation and histone acetylation.[124] Recently, the influence of modified curcumin CMC2.24 was

investigated for its effect on periodontitis. Administration of CMC2.24 decreased inflammatory cytokines, MMPs, and alveolar bone loss in an experimental murine periodontitis model.[125] It was suggested as an anti-inflammatory treatment model for periodontitis.

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.

Figure legends.

Figure 1. Comparison of the inflammatory lesions (ICT) of peri-implantitis and periodontitis, showing major histopathological features.

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]

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]

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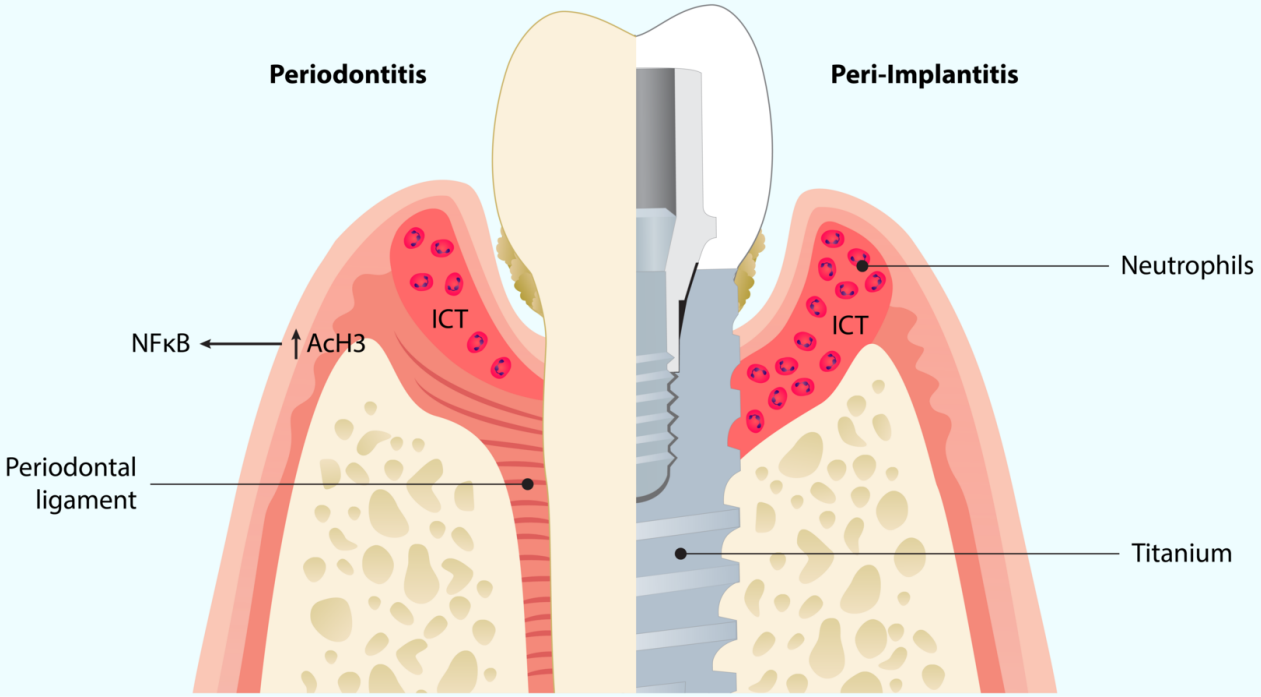

Figure 1.png

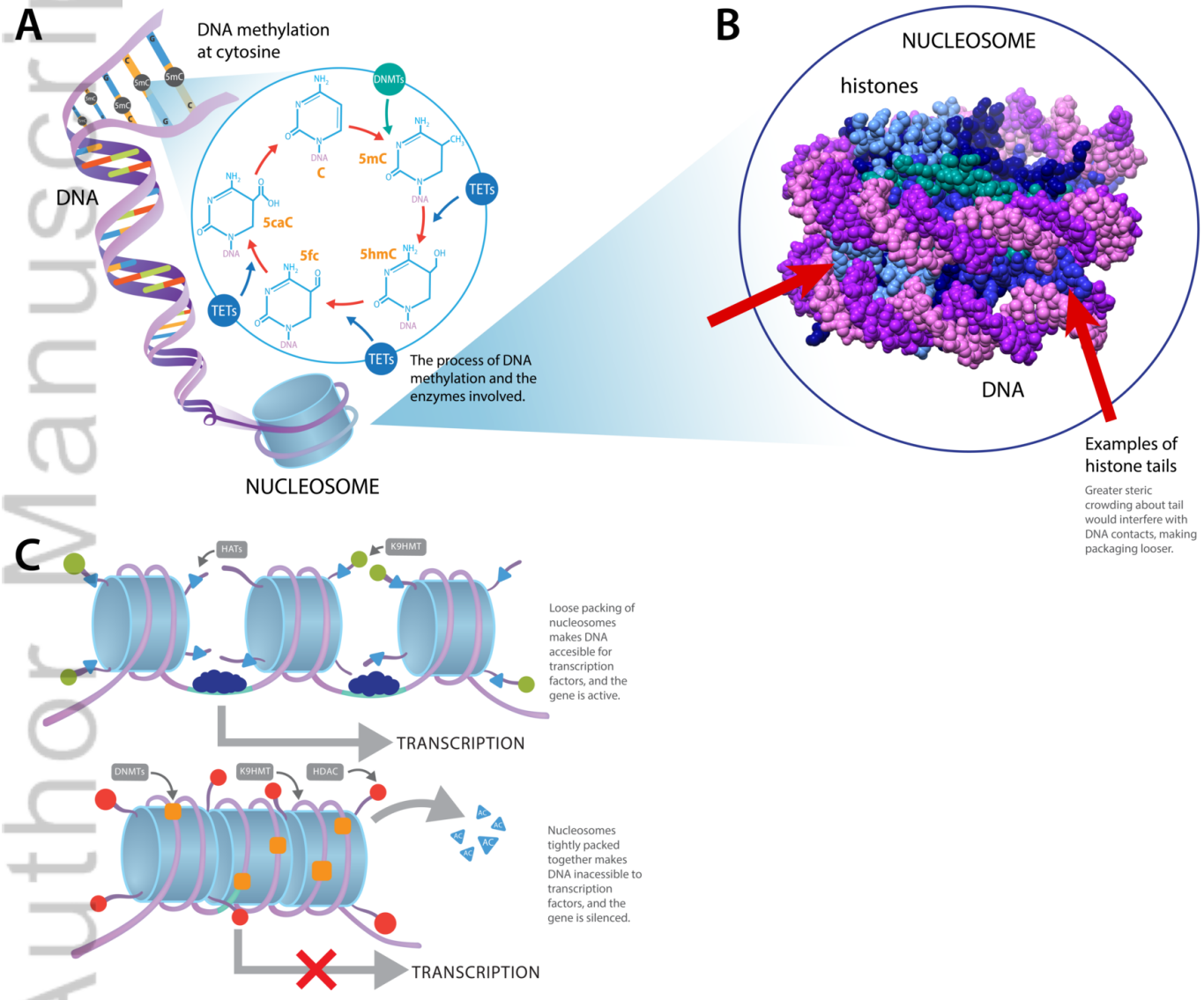

Figure 2.png

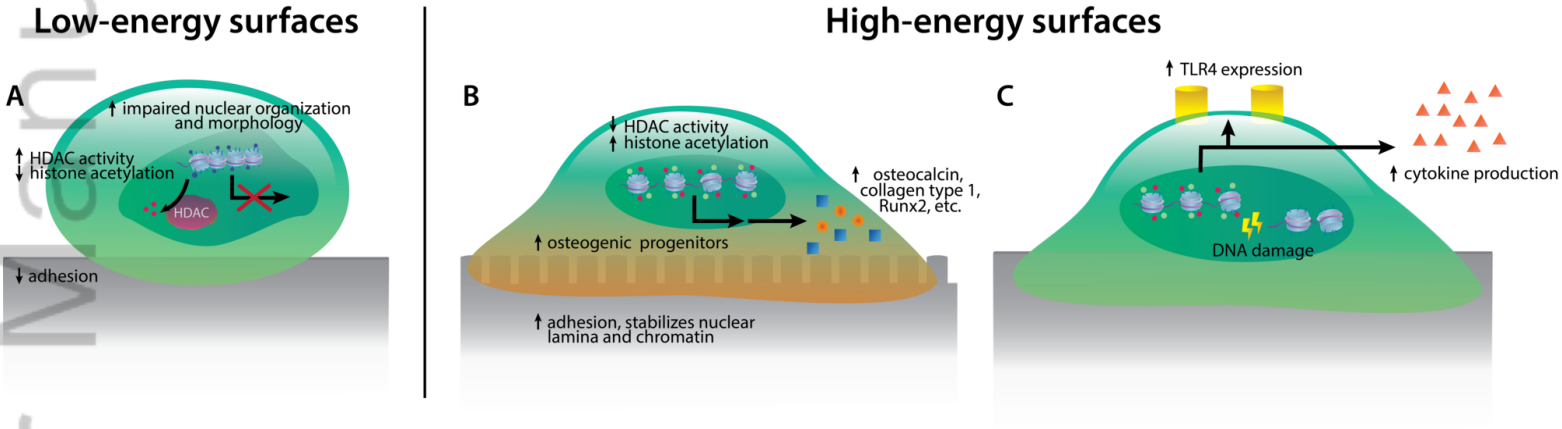

Figure 3.png

Influence of epigenetics on periodontitis and peri-implantitis pathogenesis

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Abstract

Periodontitis is a disease characterized by tooth-associated microbial biofilms that drive chronic inflammation and destruction of periodontal-supporting tissues. In some individuals, disease progression can lead to tooth loss. A similar condition can occur around dental implants in the form of peri-implantitis. The immune response to bacterial challenges is not only influenced by genetic factors but also by environmental factors. Epigenetics involves the study of gene function independent of changes to the DNA sequence and its associated proteins, and represents a critical link between genetic and environmental factors. Epigenetic modifications have been shown to contribute to the progression of several diseases, including chronic inflammatory diseases like periodontitis and peri-implantitis. This review aims to present the latest findings on epigenetic influences on periodontitis and to discuss potential mechanisms that may influence peri-implantitis, given the paucity of information currently available.

Introduction

Periodontitis is a widespread disease recently 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, i.e. 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.

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 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 (MPO), a marker for neutrophils, and tartrate-resistant acid phosphatase (TRAP), a marker for osteoclasts, were higher in peri-implantitis.[12] Similarly, a rat lipopolysaccharide (LPS) 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 to implants without infection, FOXP3, a

negative regulator of the immune response, decreased in the setting of infection, while tumor necrosis factor alpha (TNFα), a cytokine for inflammation, increased. Meanwhile, teeth experienced no change in FOXP3 or TNFα in the setting of infection.[15] Interestingly, the presence of an implant even without infection altered the expression of cytokines compared to healthy teeth; the implant increased the expression of interleukin (IL)-10 and FOXP3; increased the RANK/osteoprotegerin (OPG) ratio, an indicator of apoptosis; and decreased the expression of TNFα.[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 recent 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. Since 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.

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 (HATs) that add acetyl groups and by histone deacetylases (HDACs) 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 HATs are divided into five distinct families by their sequence divergence at the HAT domain (HAT1, Gcn5/PCAF, MYST, CBP/p300, and Rtt109). Among all HATs, p300 is an important histone acetyltransferase that mediates transcriptional activation by participating in the CREB-binding protein/p300 transcriptional co-activation complex.[23] The p300-CBP coactivator family, in combination with other proteins, participates in proliferation, differentiation, apoptosis, and transcription through chromatin acetylation.[24] Similar to HATS, HDACs are also divided into 4 classes and take part in multi-protein complexes that are expressed in many bodily tissues.[25]

DNA itself can be modified by DNA methyltransferases (DNMTs), which add methyl groups to cytosine bases (5mC) at specific sites in the DNA sequence (i.e. 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. 5mC can be further oxidized into 5-hydroxymethylcytosine (5hmC) by the ten-eleven translocation (TET)

family of enzymes.[27] This oxidation has been suggested as the mechanism for de-methylation of DNA so that the cell can re-activate 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.

Epigenetics and periodontitis

Even though 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 LPS, 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 DNMT1.[36] And bacterial activation of pathogen recognition receptors (PRRs) and TLRs, 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 *TLR2* promoter.[37] A study by Diomede and co-workers showed that, similar to reports by Martins et

al,[36] *P. gingivalis* LPS reduced the expression of DNMT1 in human periodontal ligament (PDL) cells while upregulating histone acetyltransferase p300 and NF-κB, a complex typically activated in response to cellular stress and foreign antigens.[23]

Dysregulation of TLR 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 *TLR2* and *TLR4* promoters have previously been investigated in gingival biopsies, cells, and animal models.[38–41] The *TLR4* promoter was reported to be unmethylated in healthy and periodontitis patients, while that of *TLR2* included both methylated and unmethylated regions for both groups.[40] However, a higher degree of methylation of *TLR2* was found in samples from patients with periodontitis relative to controls, as was a correlation between the level of *TLR2* 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 *TLR2* promoter in human gingival epithelial cells.[38] The DNA methylation pattern of other genes in the TLR signaling pathway (*FADD*, *MAP3K7*, *MYD88*, *IL6R*, *PPARA*, *IRAK*, and *RIPK2*) 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 *MMP2* promoter, causing chronic activation of pro-MMP2 expression in PDL cells.[43] Matrix metalloproteases (MMPs) 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 MMPs 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 versus controls. A decrease in methylation was found in the promoter regions for interleukin-17C (*IL-17C*) and chemokine ligand-25 (*CCL25*) 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 were similar to those by Barros and Offenbacher.[31,46] Given the suggested link between IL-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 IL-6, no difference in methylation of its promoter was found between periodontitis patients and healthy controls.[47] It had been previously reported that the *IL-6* promoter was partially methylated in gingival tissue samples from both periodontitis and healthy individuals but that the expression of IL-6 was higher in periodontitis patients.[48] Ishida and coworkers also reported an increase in IL-6 expression, yet this increase was associated with hypomethylation of only one CpG site in the *IL-6* promoter.[49] Similarly, for the inflammatory cytokine TNFα, an analysis of 12 CpG sites in the promoter of *TNFα* in patients with chronic periodontitis and healthy controls revealed differences in DNA

methylation only at one CpG site,[50] although a previous study reported two hypermethylated sites in the *TNFα* promoter in chronic periodontitis.[51]

Comparison of the DNA methylation patterns of two inflammatory regulators, suppressor of cytokine signaling 1 (SOCS1) and the long interspersed element-1 (LINE-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 *Socs1* were hypermethylated in periodontal specimens compared to healthy tissue, yet there was no difference in gene expression.[53] Interestingly, the results by Planello et al suggested that the increase in DNA methylation of *Socs1* in periodontitis was not due to the presence of inflammatory cells.[53] 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 *Socs1*, *Socs3*, and *LINE-1* were similar regardless of any previous periodontal inflammation.[54] Similarly, a higher level of DNA methylation in the *COX-2* promoter has been reported for diseased sites compared to healthy sites in patients with periodontitis.[55] Interestingly, periodontal therapy restored the DNA methylation pattern of *COX-2* to a level close to that of healthy patients. In contrast, no changes occurred in the DNA methylation level of *TNFα*, *IFNγ*, or *LINE-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 and co-workers also reported on the methylation pattern of inflammatory genes in periodontitis and healthy patients, but the differences were not significant.[56]

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 and coworkers reported a down-regulation of DNMT1 and

up-regulation of acetylated histone 3 in epithelial cells close to the inflammatory lesion in a periodontitis model in mice.[36] In contrast, a significant up-regulation of DNMT1 and TET1 mRNA was found in tissue samples from periodontitis patients compared to 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 TET2-positive cells was even greater in periodontitis lesions than in gingivitis lesions.[57] The increase in TET enzymes is of particular interest since they convert 5mC to 5hmC and promote demethylation, which, in turn, re-activates genes and increases expression.[27,45] The fact that TET2 rises 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 also was 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 pre-neoplastic epigenome that may play a role in tumor development.[53] Recently, a role for TET enzymes in the regulation of macrophages in periodontal disease has also been suggested.[58,59]

JMJD3 is a demethylase that binds genes and demethylates them at H3K27, thereby increasing their transcription. Stimulation of macrophages by LPS induces JMJD3, 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] *P. gingivalis* LPS treatment caused a decrease in expression of JMJD3, DNMT1, and DNMT3b in keratinocytes but no difference in gingival fibroblasts.[60] This difference may be due to the expression of TLRs on epithelial cells but not on keratinocytes. In the same study, *P. gingivalis*

LPS also triggered the TLR2 and 4 signaling pathways, inducing NFkB and downregulating JMJD3.[60] An analysis of the gene expression of JMJD3, DNMT1, and DNMT3b in tissue samples showed no differences between periodontitis patients and healthy controls.[60] In a periodontitis mouse model, adiponectin (APN), a factor secreted by adipose tissue, was found to influence the JMJD3-IRF4 signaling pathway, which is needed for the polarization of macrophages towards M2; the result was a modified inflammatory response, enhanced bone repair via JMJD3, and reduced periodontal bone loss.[61]

A recent study showed that the immune response to bacteria may be influenced by stressful events in early life.[62] As demonstrated in an experimental periodontitis LPS 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 TGF-β. These animals also had a higher expression of glucocorticoid receptor (Gr), a marker for stress reactivity, in the hippocampus. In contrast, rats that had been handled daily or undisturbed as pups had a higher degree of DNA methylation at specific CpG sites in the Gr promoter, resulting in a lower level of Gr expression.[62] These results may shed light on the socioeconomic disparities of periodontal disease, as minority and low-income individuals suffer 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.

Epigenetics and peri-implantitis

To the knowledge of the authors of this review, no characterization of the epigenetic pattern of the peri-implantitis lesion has yet been made. A recent review on epigenetics in implant therapy found only 8 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 due to peri-implantitis.[64] The authors suggested that these findings could reflect a different epigenetic response between various tissues in the same microenvironment.

Epigenetics and titanium particles

In contrast with 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 CCL2, an inflammatory cytokine, and to elevate the ratio of RANKL to OPG.[67] In addition, titanium ions elevated TLR4 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 (TiO_2) 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, histone H2A.X is phosphorylated (becoming γH2AX) and is recruited to the damaged site; as such, γH2AX is an early marker for DNA damage.[70] The efficacy of γH2AX's response to DNA damage is epigenetically controlled by the acetylation of histones other than itself: the acetylation of histone H3 at lysine 56 (H3K56ac) enhances the DNA damage response in stem cells.[70] As a result, the γH2AX/H3K56ac 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 TiO_2 particles may directly influence histone acetylation, inhibiting repair of double-stranded breaks. *In vitro*, a relatively low concentration of 10 μg/mL of TiO_2 induced γH2AX in fibroblasts compared with other compounds, like terbium-doped-gadolinium oxide (Tb-Gd2O3), which required 1,000 μg/mL to induce γH2AX, or poly(lactic-co-glycolic acid) (PLGA) nanoparticles, which did not induce any DNA damage.[71] Interestingly, nano-sized particles of TiO_2 induced γH2AX in fibroblasts more efficiently than larger ones, and the induction of γH2AX occurred independently of reactive oxygen species (ROS) produced by inflammatory cells.[72] Similar to fibroblasts, titanium particles isolated from commercially available dental implants have been shown to induce the activation of CHK2 and accumulation of BRCA1 in a culture of oral epithelial cells.[73] Following DNA damage, the recruitment of BRCA1 to the nuclear foci was mediated by the phosphorylation of γH2AX.[74–76] In addition to being cytotoxic to fibroblast and oral epithelial cells, low doses of TiO_2 particles induced expression of pro-inflammatory markers.[77] Also, stimulating these cells with LPS following TiO_2 stimulation enhanced the expression of the inflammatory cytokine TNFα. In line with this focus, there are numerous studies on how surface topography, e.g. 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.

Clinical application of epigenetics in periodontitis and peri-implantitis

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 HDAC inhibitors (HDACi) for cancer treatment. HDACi are small compounds that inhibit the function of HDACs by blocking their binding to target sites, thereby increasing histone acetylation and enhancing gene transcription.[78] Trichostatin A (TSA), Entinostat (MS-275) sodium butyrate, suberoylanilide hydroxamine (SAHA, or Vorinostat), and valproic acid (VPA) are all HDACi 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 HDACi 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, HDACi have been reported to decrease bone loss not only for rheumatoid arthritis but also for periodontitis.[82,83] TSA, VPA, and MS-275 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 recent study, periodontitis gingival tissue was shown to have increased mRNA expression of HDAC1, 5, 8 and 9; of these, the HDAC1 protein was found in significantly higher quantities

in diseased tissue than in healthy tissue.[84] HDAC1 was also found in inflammatory cells, suggesting a role in regulating inflammation.[84] Treatment of human PDL cells with TSA decreased expression of HDAC3, increased acetylation of histone H3, and induced osteogenic differentiation.[85] Treatment of PDL 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 (5-aza) inhibits DNA methylation and was reported to increase the responsiveness of gingival fibroblasts to TGF-β1 and increase DNMTs.[86] Gingival epithelial cells exposed to *P. gingivalis* and *F. nucleatum* showed decreased expression of DNMT and HDAC [37]. When the cells were treated with 5-aza prior to exposure to *F. nucleatum*, their expression of human beta-defensin-2 (*hBD-2*) and *CCL20* was enhanced relative to no treatment; both genes are typically up-regulated in response to bacteria.[37] Treatment with an HDAC 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 TSA or 5-aza induced the cells to differentiate into osteogenic and chondrogenic populations, respectively.[87] Treatment with 5-aza-dC of osteoblasts grown on titanium discs of two different surface characteristics decreased DNA methylation on both surfaces and induced gene expression of alkaline phosphatase (ALP).[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. HDACi 1179.4b was able to suppress alveolar bone loss but not gingival inflammation.[83] In contrast, the BET inhibitor JQ1 inhibited both the inflammatory response and alveolar bone loss.[90] BET proteins

contain bromodomains that sense acetylated histones and can recruit epigenetic regulators of gene expression.[90] A recent review reported that HDACi influence not only osteoclast differentiation but also maturation and activity.[91] TGF-β1 is a key factor in regulating wound healing, an event important to tissue regeneration, e.g. after periodontitis surgery and implant placement. Treating oral fibroblasts with 5-aza demethylation agent prior to treatment with TGF-β1 increased DNMT1 and DNMT3b expression, increased the fibroblasts' response to TGF-β1, and induced the expression of TGF-β1's targets.[86]

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 HDACi enhanced matrix mineralization and the expression of osteogenic differentiation markers, such as osteopontin and bone sialoprotein, yet decreased expression of osteocalcin.[94] In addition, HDAC1 and HDAC2 were identified as important regulators in osteoblast differentiation.[94] Targeting epigenetic mechanisms may, therefore, present new models for improving bone and soft tissue regeneration.

TET2 and the enzyme thymine-DNA glycosylase were able to induce changes in both the 5mC and 5hmC patterns in myeloid stem cells.[95] In later stages of cell differentiation, TET2 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. TET1 and TET2 also regulated the differentiation of mesenchymal stem cells into osteoblast 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 PDL also have the potential to differentiate into osteoblasts, and RUNX2 is a key factor in this process.[97] HDAC1, 2, 3, 4, and 6 were all shown to be present in human PDL cells.[85] HDACs 3, 6 and 7 were involved in regulating the expression of RUNX2, and HDACi induced acetylation of the RUNX2 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 PDL cells and dental pulp stem cells.[78] *P. gingivalis* LPS induced an increase of DNMT1 and down-regulation of RUNX2 expression in human PDL cells, suggesting that the inhibitory effect of LPS on osteoblastic differentiation may be a consequence of DNA hypomethylation of RUNX2.[97] Treatment of human gingival fibroblasts with a DNA methylation inhibitor induced hypomethylation of RUNX2 and ALP, and subsequent treatment of these cells with BMP-2 induced the expression of RUNX2 and ALP as well as differentiation into osteoblasts.[98]

PDL stem cells extracted from periodontitis patients and healthy subjects were investigated with respect to the expression of histone acetyltransferase GCN5.[99] Cells from periodontitis patients showed a down-regulation of GCN5 and a decrease in osteogenic differentiation potential compared to cells from controls. Knockdown of GCN5 decreased expression of RUNX2 and ALP, while overexpression restored the osteogenic potential of the cells.[99] Mechanistically, GCN5 induced acetylation of histone H3 at lysines 9 (H3K9) and 14 (H3K14) near the *DKK1* gene, thereby increasing its expression. DKK1 is an inhibitor of the Wnt/β-catenin signaling pathway, which is important in the regulation of osteogenic differentiation of PDL stem cells. Interestingly, treatment with aspirin inhibited both GCN5 expression and inflammation in LPS-induced periodontitis rats while up-regulating DKK1 and reducing bone loss.[99] Inhibition of HDACs using TSA enhanced the osteogenic differentiation of human PDL cells. There was not only an up-regulation of osteoblast-related genes but also an increase in ALP activity, mineral formation, and RUNX2 production.[78,85] Furthermore, when TSA-treated human PDL cells were implanted in a scaffold, bone formation was enhanced for up to 8 weeks.[78] TSA has also been shown to enhance osteogenic differentiation in mesenchymal stem cells and periodontal repair by interfering with the NFkB-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]

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 HDACi were found to induce woven bone formation at the interface with the scaffold.[103] Two studies on the use of microarc oxidation

(MAO) titanium implant surfaces as a delivery model for miRNAs have been published.[104,105] Wang and co-workers fabricated chitosan-hyaluronic acid nanoparticles to deliver miRNA-21 into human bone marrow mesenchymal stem cells and, thereby, increased the expression of osteogenic genes.[104] Wu and co-workers attached miRNA-29b and anti-miRNA-138 lipoplexes onto an MAO titanium implant and induced osteogenic differentiation in rat bone marrow mesenchymal stem cells.[105] These studies suggest a novel tool for improving the osseointegration of implants and a method for delivering epi-drugs.

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 γH2AX-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 DNMT1 and lower histone acetylation than smooth titanium.[29] In addition, the methylation level of the ALP 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 recent study, pre-osteoblastic 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, likely due 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 OPG 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 BMPs.[116]

Even though 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]

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 toward 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 TET enzymes and the 5hmC pathway were influenced by nutritional compounds such as vitamin C, and that microbiome-produced metabolites like folate also influenced enzymes regulating 5hmC.[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 anti-cancerous properties and has been linked to both DNA methylation and histone acetylation.[124] Recently, the influence of modified curcumin CMC2.24 was

investigated for its effect on periodontitis. Administration of CMC2.24 decreased inflammatory cytokines, MMPs, and alveolar bone loss in an experimental murine periodontitis model.[125] It was suggested as an anti-inflammatory treatment model for periodontitis.

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.

Figure legends.

Figure 1. Comparison of the inflammatory lesions (ICT) of peri-implantitis and periodontitis, showing major histopathological features.

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]

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]

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