The Role of Epigenetics in Periodontal and Systemic Diseases and Smoking: A Systematic Review

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Citation: Khouly, I.; Braun, R.S.; Ordway, M.; Ghassib, I.; Larsson, L.; Asa’ad, F. The Role of Epigenetics in Periodontal and Systemic Diseases and Smoking: A Systematic Review. Appl. Sci. 2021, 11, 5269. https://doi.org/10.3390/app11115269

Abstract: The aims of this systematic review were to identify and synthesize the evidence for an association in DNA methylation/histone modifications between periodontal diseases and systemic diseases/smoking. Electronic database searches using relevant search terms in PubMed, Embase, MEDLINE, CINAHL, Web of Science, Scopus, and SciELO, and manual searches, were independently conducted to identify articles meeting the inclusion criteria. Nine studies of 1482 participants were included. Periodontitis was compared to metabolic disorders, rheumatoid arthritis (RA), cancer, and smokers, as well as healthy controls. Substantial variation regarding the reporting of sample sizes and patient characteristics, statistical analyses, and methodology was found. IL6 and TNF were modified similarly in RA and periodontitis. While TIMP-3 and GSTP-1 were significantly lower in periodontitis patients and controls than in cancer, SOCS-1, RMI2, CDH1, and COX2 were modified similarly in both cancer and periodontitis. While TLR4 in and CXCL8 were affected in periodontitis independent of smoking habit, smoking might change the transcription and methylation states of ECM organization-related genes, which exacerbated the periodontal condition. There was some evidence, albeit inconsistent, for an association between DNA methylation and periodontal diseases and systemic diseases or smokers compared to healthy patients or non-smokers.

Keywords: DNA methylation; epigenetic; gingival diseases; periodontal disease; systemic disease; smoking; systematic review

1. Introduction

Periodontitis is a biofilm-induced condition that affects tooth-supporting tissues [1], which consists mainly of Gram-negative, anaerobic, and micro-aerophilic bacteria that can colonize the sub-gingival tissues [2,3]. The bacterial biofilm induces an inflammatory host response, which is influenced by environmental, genetic, and epigenetic factors [4–6]. Epigenetic factors refer to alterations in the gene expression that are not encoded in the DNA sequence [7,8], which include chemical alterations of the DNA and histones, resulting in the remodeling of the chromatin and activation or inactivation of a gene [9–11].

To date, the most recognized epigenetic mechanism is DNA methylation, which is regulated by DNA methyltransferases (DNMTs), resulting in the covalent addition of methyl
groups to the 5′ position at the base cytosine (5 mC) and [12]. DNA methylation results in gene silencing [8,13]. Another form of epigenetic change is post-translational modifications of histones, which form the building blocks of the chromatin (i.e., nucleosomes). The most studied histone modification is histone acetylation. Histone acetylation is linked with transcriptional activation [14] and is regulated by histone acetyltransferases (HATs) that add acetyl groups to histones and histone deacetylases (HDACs) that remove acetyl groups [15]. While DNA methylation and histone modifications are linked [12,15], DNA methylation offers a more stable form of gene regulation [16].

The epigenome is dynamic throughout life and can be influenced by environmental and lifestyle factors. Unlike the human genome, the epigenome can be reversed; hence, epigenetic mechanisms have therapeutic potential for improving personalized drug therapy [17]. Moreover, understanding the role of epigenetic in periodontal diseases and systemic diseases could potentially improve wound healing and periodontal tissue regeneration [10].

Due to this dynamic nature of the epigenetic mechanisms, they have been thoroughly investigated in pathological conditions, such as rheumatoid arthritis (RA) [18], metabolic syndrome [19], and cancer [20]. As known, periodontitis shares pathogenesis pathways with RA [21,22] and is associated with metabolic syndrome [23] and an increased risk of several cancers [24]; therefore, studies that investigate the epigenetic modifications in periodontitis patients with these systemic diseases are emerging. Similarly, smoking contributes to the complex network of interactions between an individual’s immune response, bacterial biofilm [25], and epigenetics [26]. As such, smoking affects the host defense system [27–29] and the chromatin structure [26]. Since smoking is a well-established risk factor of periodontitis [30] and affects the chromatin structure [26], recent studies have assessed the epigenetic pattern in smoking periodontitis patients, as well.

In this context, we have developed a systematic review to thoroughly present the potential interactions between epigenetics and periodontal diseases associated with systemic diseases/smoking, which would give a better understanding of this topic to both researchers and clinicians.

2. Methods and Materials
2.1. Reporting Format and Study Registration Registration

The research question and detailed study protocol were designed according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines [31,32]. The protocol was registered in the International Prospective Registrar of Systematic Reviews (PROSPERO) (CRD42018116766).

2.2. PECO Question: Population, Exposure, Comparison, and Outcomes

The focus question for the present systematic review was developed using the population, exposure, comparisons, and outcomes (PECO) criteria.

In human adults of any race (population), what are the effects of DNA methylation and histone modification profiles (outcomes) on periodontal diseases and systemic diseases or smoking (exposures) compared to no periodontal and no systemic diseases and non-smokers (comparison)?

2.3. Eligibility Criteria

2.3.1. Inclusion Criteria

1. Human clinical studies, including both interventional and observational studies: randomized controlled trials, cohort studies, case-control studies, and cross-sectional studies.
2. Studies describing either an association in epigenetic marks (global, site-specific or genome-wide methylation of DNA) or histone modifications (methylations, phosphorylation, acetylation, ubiquitylation, and sumoylation).
3. Studies assessing epigenetic changes from any type of human tissue (e.g., gingival tissues, blood, etc.).
4. Studies comparing periodontal and systemic diseases with non-diseased OR studies comparing periodontal diseases in smokers and non-smokers with non-diseased.

2.3.2. Exclusion Criteria
1. Systematic reviews, case reports, animal trials, and letters to editors.
2. Studies describing epigenetic markers other than DNA methylation and histone modification, such as noncoding RNAs.

2.4. Types of Outcomes Measured
DNA methylation and histone modification, as well as genome-wide methylation, were assessed in human patients with periodontal diseases and systemic diseases/smokers compared to non-diseased or non-smokers, respectively.

2.5. Search Strategy
2.5.1. Electronic Database Search
Electronic database searches were performed in PubMed, Embase, MEDLINE, CINAHL, Web of Science, Scopus, and SciELO and included articles published through 15 February 2021. The search was not limited by any restrictions on language or publication date. Primary and secondary concepts and subject headings were developed by a medical and dental librarian, and the terms were searched for each of the databases, as listed in Supplementary Material SI.

2.5.2. Hand Searching
A manual search was conducted for reference lists of relevant papers, published through 15 February 2021, and key periodontal journals published from January 2000 through 15 February 2021, including Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research, Journal of Periodontology, and Oral Health and Preventive Dentistry.

2.5.3. Ongoing and Unpublished Clinical Trials
The US National Institutes of Health Clinical Trials Database: (http://clinicaltrials.gov) and other online databases (www.centerwatch.com/clinicaltrials; www.clinicalconnection.com) were searched for ongoing clinical trials through 15 February 2021. Finally, unpublished studies in the OpenGrey open-access database were searched.

2.6. Data Collection and Analysis
2.6.1. Study Selection
Articles were systematically assessed by three independent reviewers (RB, IK, and MM) using pre-determined eligibility criteria for possible inclusion in the systematic review. After initial selection based on title and abstract, full-text papers were read in detail by the independent reviewers (RB, IK, MM) to ensure that all inclusion criteria were fulfilled. Disagreements were solved by discussion. The reasons for study exclusion were recorded.

2.6.2. Data Extraction and Analysis
Data were extracted by two independent reviewers (RB, MM) using data-extraction tables that were specifically designed for the present review and modified upon a second review process, as required for the presentation of general characteristics and secondary outcomes (Supplementary Material SII). The data tables were organized by systemic diseases and smoking status. The data extraction tables included information on the general characteristics, methodology, and results of the included studies. The extracted data tables were compared and consolidated by the two independent reviewers (RB and MM). The final data extraction tables were reviewed by two reviewers (IG and IK) to ensure accurate data extraction and interpretation of the included studies.
2.6.3. Study Quality

Two reviewers (RB, IG) assessed the study quality independently by following the National Heart, Lung, and Blood Institute’s Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [33]. The tool contains 14 criteria to assess the study design quality. First, each of the criteria was rated as either yes, no, or not reported. Then, each of the included studies received overall scores of good, fair, or poor as outlined in the assessment tool. Any disagreements were discussed, and a third reviewer (IK) moderated any disagreement if needed. Corresponding authors of the included studies were contacted via email for detailed information on study methodology when key criteria were determined to be not reported by the two reviewers.

3. Results

3.1. Literature Selection Process

The electronic database search yielded a total of 1426 results. Removal of duplicates resulted in 604 results (Figure 1). Hand-searching relevant bibliographies and journals failed to identify any additional articles. Of the 604 titles/abstracts screened, a total of 12 [34–45] full-text articles were selected for inclusion in the review based on pre-determined eligibility criteria. Following the full-text review, three [43–45] of the twelve articles were excluded as they did not satisfy the eligibility criteria; Supplementary Material Table S1 summarizes the excluded studies with reasons for exclusion. The remaining nine studies [34–42] were included in the systematic review.

Figure 1. PRISMA Flow Diagram.

3.2. Description of Included Studies

General Characteristics of Included Studies

Nine studies examining a total of 1482 participants were included in the systematic review (Table 1). One of the studies examined CP compared to metabolic disorders [34], two of the studies examined CP compared to RA [35,36], three of the studies examined CP compared to cancer [37–39], and three of the studies examined smokers [40–42]; all nine included studies also included a healthy patient comparison as a third study group. All
nine studies examined DNA modifications (i.e., CpG methylation) as an outcome, and no studies on histone modifications were identified in the search for inclusion in the review.

3.3. Study Design

Four of the included studies were prospective cohort studies [39–42], and five of the studies were retrospective observational studies [34–38].

3.4. Setting and Study Population

Eight of the included studies recruited patients from university-based dental clinics; one of the studies did not report the study setting [40].

Three of the studies were conducted in Brazil [37,41,42], two of the studies in China [38,39], two of the studies in Japan [35,36], one of the studies in Serbia [34], and one of the studies did not report the study location [40].

One of the studies included only Caucasian patients of Serbian nationality [34], two of the studies included only Japanese patients [35,36], two of the studies included only patients from the Southeastern region of Brazil [41,42], and four of the studies did not report ethnic background and/or race of included patients [37–40].

3.5. Assessed Methylated Gene Sites

The methylation sites assessed are listed by study group in Table 2. Key findings were reported for 14-3-3σ [38], COX2 [39], CXCL12 [34], E-cadherin [39], EMC organization related genes [40], GSTP1 [38], IL6 [36], CXCL8 [42], RMI2 [37], SOCS1 [37], TIMP3 [38], TLR2 [41], TLR4 [41], and TNF [35].

3.6. Methods for Detecting DNA Methylation Changes

Bisulfate genomic sequencing [36], bisulfite modification followed by methylation-specific polymerase chain reaction (MSP) [34,38,39,42], direct sequencing of genomic DNA [35], the Illumina NextSeq500 sequencing system [40], Infinium-based DNA Methylation Analysis, PCR, MS-HRM [37], and PCR and Methylation analysis with specific restriction enzymes [41] were applied as methods for detecting epigenetic changes.

3.7. Characteristics of the Outcomes Measured

Table 2 summarizes the study outcomes reported by each of the studies, including the main site-specific methylation level findings, as well as additional observations/outcomes assessed, for example, mRNA expression, clinical assessment correlations, etc. Key findings of individual studies are reported below.
Table 1. Characteristics of the included studies.

| Study/Year                  | Design/Study Period | Setting, Location, Funding, and COI | N Patients-SEX | Mean Age in Years (SD/Range) | Racial/Ethnic Background | Eligibility Criteria                                                                                                                                  | Smokers                                                                 |
|-----------------------------|---------------------|-------------------------------------|----------------|-------------------------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| (Grdovic et al., 2016) [34] | Cross-sectional 2013–2015 | University, Serbia Funding: governmental COI: none | 1. CP: 29 13F/16M 1. CP: 48.17 ± 13.48 | 1. CP: 48.17 ± 13.48 2. CP/T2D: 22 6F/16M 2. CP/T2D: 56.36 ± 8.7 3. Controls: 21 13F/8M 3. Controls: 33.43 ± 5.28 | Caucasians of Serbian nationality | Excluded: A systemic disease except for T2D, systemic antibiotic/immunomodulatory therapy within 3 months, therapy of periodontitis during 1.5 years prior to the study, and daily usage of oral antiseptics | Included: Smokers Differences in percentage of DNA methylation between smokers and non-smokers was analyzed |
| (Ishida et al., 2012) [36]  | Observational 2007–2008 | University, Japan Funding: governmental COI: none | 1. CP: 30 20F/10M 1. CP: 30 | 1. CP: 62.3 ± 1.5 (45–76) 2. RA: 30 27F/3M 2. RA: 60.0 ± 2.2 (31–83) 3. Controls: 30 20F/10M 3. Controls: 53.4 ± 2.7 (29–75) | Japanese | Excluded: The history of periodontal therapy within the previous 6 months, the presence of 15 teeth, pregnancy, and DM                                      | Included: Current smokers, former smokers, or never smokers |
| (Kojima et al., 2016) [35]  | Observational 2013–2014 | University, Japan Funding: governmental COI: none | 1. CP: 30 22F/8M 1. CP: 64.4 ± 1.5 | 1. CP: 64.4 ± 1.5 2. RA: 30 25F/5M 2. RA: 61.0 ± 2.0 3. Controls: 30 24F/6M 3. Controls: 63.3 ± 1.8 | Japanese | Excluded: DM, periodontal therapy within the previous 3 months, and the presence of fewer than 15 teeth                                           | Excluded: All smokers                                                                                                 |
| (Loo, Jin, Cheung, Wang, and Chow, 2010) [39] | Prospective Cohort Study 2004–2009 | University, China Funding: governmental COI: none | 1. CP: 110 22 F/88 M 1. CP: 42.9 ± 9.71 18–65 | 1. CP: 42.9 ± 9.71 18–65 2. BRCA: 106 F/M: NR 2. BRCA: 56.2 (26–85) 3. Controls: 108 39F/69M 3. Controls: 42.8 ± 9.69 (18–60) | NR | Included: Free from systemic or chronic disease, current and past non-smokers, no swelling of the lymph nodes, no TMJ disease, no soft tissue abnormalities or severe dental caries, and no furcation involvement or generalized gingival recession | Included: Current and past non-smokers                                                                                          |
| (Wang et al., 2014) [38]   | Observational 2011–2013 | University, China Funding: governmental COI: none | 1. CP: 110 37F/73M 1. CP: 44.2 ± 1.5 (18–65) | 1. CP: 44.2 ± 1.5 (18–65) 2. BRCA: 108 108F/0M 2. BRCA: 56.2 ± 6.8 3. Controls: 180 80F/100M 3. Controls: 45.6 ± 2.4 (18–60) | NR | Included: Systemic or chronic disease, lymph node swelling, TMJ disease, soft tissue abnormalities, severe dental caries, and furcation involvement or generalized gingival recession | Included: Current and past non-smokers                                                                                          |
| (Planello et al., 2016) [37] | Cohort study NR | University, Brazil Funding: public foundation COI: none | 1. CP: 19 54.3% F/45.6% M 1. CP: 47.17 ± 11.31 | 1. CP: 47.17 ± 11.31 2. OSCC: 301 samples (+ adjacent normal samples: 34) F/M: NR 2. SCC: NR 3. Controls: 23 63.3% F/36.3% M 3. Controls: 42.54 ± 11.94 | NR | Included: no systemic disorder that could affect the periodontal condition, not on antibiotics/anti-inflammatory medication (the past 6 months), non-pregnant/lactating and non-alcoholics | Included: Non-smokers                                                                                                           |
| Study/Year                  | Design/Study Period | Setting, Location, Funding, and COI                                                                 | N Patients-SEX | Mean Age in Years (SD/Range) | Racial/Ethnic Background | Eligibility Criteria                                                                 | Smokers                                                                 |
|---------------------------|---------------------|--------------------------------------------------------------------------------------------------|----------------|-----------------------------|--------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| (Oliveira et al., 2009)   | Cohort Study        | University, Brazil Funding: public foundation COI: none                                           | 1. CP/S: 30    | 13F/17M                     |                          | Included: Good general health                                                            | Included: Smokers 30/111 subjects                                      |
|                           | (prospective        |                                                                                                  | 2. CP/NS: 40   | 24F/16M                     |                          | Excluded: Any systemic disorder that would affect the periodontal condition (except smoking), pregnancy or lactation, and systemic antibiotics or anti-inflammatory medication within 6 months |                                                                         |
|                           | observational)      |                                                                                                  | 3. NP/NS: 41   | 22F/14M                     |                          |                                                                                       |                                                                         |
|                           | NR                  |                                                                                                  | 1. CP/S: 47.03 ± 6.49 |                          |                          | NR (Southeastern portion of Brazil)                                                       |                                                                         |
|                           |                      |                                                                                                  | 2. CP/NS: 44.94 ± 9.17 |                          |                          |                                                                                       |                                                                         |
|                           |                      |                                                                                                  | 3. NP/NS: 46.2 ± 14.1 |                          |                          |                                                                                       |                                                                         |
| (De Oliveira et al.,      | Cohort Study        | University, Brazil Funding: public foundation COI: none                                           | 1. CP/S: 11    | 5F/6M                       |                          | Included: Good general health                                                            | Included: Smokers 11/34 subjects                                     |
| 2011) [41]                | (prospective        |                                                                                                  | 2. CP/NS: 12   | 8F/4M                       |                          | Excluded: A systemic condition that could affect periodontal condition (excluding smoking), pregnancy or lactation, systemic antibiotics or anti-inflammatory medications within 6 months |                                                                         |
|                           | observational)      |                                                                                                  | 3. NP/NS: 11   | 7F/4M                       |                          |                                                                                       |                                                                         |
|                           | NR                  |                                                                                                  | 1. CP/S: 45.7 ± 7.4 |                          |                          | NR (Southeastern portion of Brazil)                                                       |                                                                         |
|                           |                      |                                                                                                  | 2. CP/NS: 45.5 ± 10.1 |                         |                          |                                                                                       |                                                                         |
|                           |                      |                                                                                                  | 3. NP/NS: 39.8 ± 15.5 |                         |                          |                                                                                       |                                                                         |
| (Cho et al., 2017)        | Cohort Study        | NR, NR Funding: governmental COI: none                                                             | 1. CP/S: 56    | (52–56)                     |                          | Included: Generally healthy patients                                                       | Included: Smokers 10 of 20 subjects                                   |
| [40]                      | (prospective        |                                                                                                  | 2. CP/NS: 53   | (52–55)                     |                          | Excluded: Acute or AgP, any severe systemic disease that could affect periodontal condition, pregnancy or lactation, systemic antibiotics or anti-inflammatory medications within 6 months |                                                                         |
|                           | observational)      |                                                                                                  | 3. NP/NS: 52   | (47–54)                     |                          |                                                                                       |                                                                         |
|                           | NR                  |                                                                                                  | 4. NP/NS: 44   | (41–55)                     |                          |                                                                                       |                                                                         |

AgP: aggressive periodontitis; BRCA: Breast cancer; COI: Conflict of interest; CP: Chronic Periodontitis; CP/NS: Chronic Periodontitis/Non-smokers; CP/S: Chronic Periodontitis/Smokers; DM: Diabetes Mellitus; F: female; M: male; NP/NS: Non-periodontitis/Non-smokers; NR: no reported; RA: Rheumatoid Arthritis; SCC: Squamous Cell Carcinoma; T2D: type 2 diabetes; TMJ: temporomandibular joint.
Table 2. Description of epigenetic testing and findings.

| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Adjustment (e.g., Age, Sex, Smoking, Number of Teeth etc.) | Type of Tissue (and Site Collected, if Applicable) | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Additional Observations/Outcomes Assessed | Global/Genome-Wide Reporting |
|-----------------|------------------------|--------------------------------|-----------------------------------------------------------|-------------------------------------------------|-----------------------------------|----------------------------------------------|----------------|----------------------------------------|-----------------------------|
| (Grdovic et al., 2016) [34] | 1. CP: 29 2. CP/T2D: 22 3. Controls: 21 | 1. CP: AAP classification, 1999; and no SD. 2. CP/T2D: ADA, 2013 3. Controls: No signs of periodontitis, and no SD; further clinical parameters NR. Nondiabetics: normal parameters on OGTT and HbA1c < 6.5% (ADA, 2013). | Pearson’s correlation coefficient was determined with adjustment for age including all subjects | Buccal Epithelial Cells | CXCL2 | MSP | CXCL2: Highest percent of DNA methylation in CP/T2D. CXCL2 promoter predominantly unmethylated in all groups. Increased frequency of the methylated form and increased percent of methylation of CXCL2 promoter in CP and CP/T2D group vs. controls, although without statistical significance. | A statistically significant relationship between the extent of DNA methylation of the CXCL12 promoter and periodontal parameters, as well as between DNA methylation of CXCL12 and glycosylated hemoglobin. | NR | (mean; SD) |
| | | | | | | | | | 1. CP: PI (Silness-Loe): | 1.66 ± 0.79 * |
| | | | | | | | | | BOP (%): 63.64 ± 27.66 * |
| | | | | | | | | | PPD: 2.77 ± 0.59 mm * |
| | | | | | | | | | CAL: 3.12 ± 1.47 mm * |
| | | | | | | | | | 2. CP/T2D: PI (Silness-Loe): | 2.17 ± 0.78 * |
| | | | | | | | | | BOP (%): 63.21 ± 30.33 * |
| | | | | | | | | | PPD: 2.73 ± 0.91 mm * |
| | | | | | | | | | CAL: 4.06 ± 2.22 mm * |
| | | | | | | | | | 3. Controls: PI (Silness-Loe): | 0.97 ± 0.58 |
| | | | | | | | | | BOP (%): 36.84 ± 20.59 |
| | | | | | | | | | PPD: 1.93 ± 0.45 mm |
| | | | | | | | | | CAL: 0.08 ± 0.28 mm |
| | | | | | | | | | * Statistically significantly different from Control |
| | | | | | | | | | # Statistically significantly different from CP |
| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Type of Tissue (and Site Collected, if Applicable) | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Additional Observations/Outcomes Assessed | Global/Genome-Wide Reporting |
|-----------------|-------------------------|---------------------------------|-----------------------------------------------|----------------------------------|---------------------------------------------|--------------|---------------------------------------|-----------------------------|
| (Ishida et al., 2012) [36] | 1. CP: 30  2. RA: 30  3. Controls: 30 | Adjustment for age, sex, and smoking status | Peripheral Blood | IL6 | Bisulfite genomic sequencing | IL6 gene CpG motifs: −74 bp: Methylation levels significantly lower in RA and CP vs. controls (p = 0.0001). + 19 bp: Differential levels of methylation among groups, not associated with serum levels of IL-6. IL6 CpG motifs: Comparable levels of the methylation between the groups. The hypomethylated status of CpG in the IL6 promoter region may lead to increased levels of serum IL-6, implicating a role in the pathogenesis of RA and CP. RA (vs. CP and controls): fewer remaining teeth (p < 0.05). RA and CP (vs. control): more severe levels of PD and CAL. Both levels of serum IL6 and IL6 production by mononuclear cells were significantly different between individuals with and without the methylation at 74 bp (p = 0.03). | | NR | (mean; SE) | 1. CP: PD: 2.8 ± 0.1 mm * CAL: 3.2 ± 0.2 mm * 2. RA: PD: 3.0 ± 0.1 mm * CAL: 3.2 ± 0.2 mm * 3. Controls: PD: 2.2 ± 0.1 mm CAL: 2.2 ± 0.1 mm | * Statistically significantly different from Control |
| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Adjustment (e.g., Age, Sex, Smoking, Number of Teeth etc.) | Type of Tissue (and Site Collected, if Applicable) | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Additional Observations/Outcomes Assessed | Global/Genome-Wide Reporting |
|----------------|------------------------|--------------------------------|--------------------------------------------------------|-----------------------------------------------|----------------------------------|---------------------------------------------|---------------|----------------------------------------|-----------------------------|
| (Kojima et al., 2016) [35] | 1. CP: 30 2. RA: 30 3. Controls: 30 | 1. CP: AAP classification, 1999. 2. RA: ARA classification, 1987, and the ACR and the ELAR, 2010. 3. Controls: no signs of periodontitis, no CAL > 3 mm. | NR | Peripheral blood | TNF | Direct sequencing of genomic DNA | TNF: Hypermethylated in CP and RA. Significantly higher methylation rates and frequencies at seven and six CpG sites, respectively, and overall methylation rates in RA vs. controls. | Methylation rates and methylation frequencies in patients with RA were not influenced by medication with a steroid that suppressed TNF expression. | NR |
| (Loo, Jin, Cheung, Wang, and Chow, 2010) [39] | 1. CP: 110 2. BRCA: 106 3. Controls: 108 | 1. CP: periodontal disease for over 5 years and had SRP every 6 months. Further classification NR. 2. BRCA: Pathologically confirmed invasive ductal carcinoma BRCA patients. 3. Controls: Periodontitis-free; further clinical parameters NR. | NR | Peripheral blood (control), GT (CP), and BRCA neoplastic tissue | CDH1 and COX2 | MSP | CDH1 and COX2: Hypermethylation highest in BRCA followed by CP, and more frequently than in controls; hypermethylation correlated among the three groups with statistical significance (p < 0.0001). | The relative risk of CP associated with CDH1 and COX2 was 0.1091 and 0.0485. | NR |

**Periodontitis compared to those with cancer vs. healthy controls**
Table 2. Cont.

| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Methodology | Results/Conclusions | Clinical Parameters |
|-----------------|-------------------------|---------------------------------|-------------|---------------------|---------------------|
| (Wang et al., 2014) [38] | 1. CP: 110 2. BRCA: 108 3. Controls: 180 | 1. Periodontitis: periodontal disease for over 5 years and had SRP every 6 months. Further classification NR. 2. BRCA: Pathologically confirmed invasive ductal carcinoma BRCA patients. 3. Controls: No signs of periodontitis, and no SD; further clinical parameters NR. | Peripheral blood (from all groups), GT (from CP), and BRCA neoplastic tissue | TIMP3 and GSTP1: Hypermethylation of periodontitis and controls were similar, but both were significantly lower than those for BRCA (p < 0.0001). 14-3-3 sigma: Methylation in chronic inflammatory gum disease was higher than in the cancer and controls (p < 0.0001). | Epigenetic silencing of 14-3-3 sigma occurred more frequently in the chronic inflammation group than in cancer patients and healthy controls. |
|                 |                         |                                 | MSP         |                     | (mean; SD)         |
|                 |                         |                                 |             |                     | 1. CP: PD: 5.5 ± 2.2 mm * Calculus (%): 63.0 ± 11.8 BOP (%): 61.3 ± 11.4 * CAL: 5.5 ± 0.9 mm 2. BRCA: NR 3. Control: PD: 2.1 ± 0.9 mm Calculus (%): 34.1 ± 10.6) BOP (%): 29.53 ± 7.2 CAL: 0.0 mm | * Statistically significantly different from Control |
| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Adjustments (e.g., Age, Sex, Smoking, Number of Teeth etc.) | Type of Tissue (and Site Collected, if Applicable) | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Clinical Parameters |
|----------------|-------------------------|---------------------------------|-------------------------------------------------------------|--------------------------------------------------|-----------------------------------|-----------------------------------------------|--------------|---------------------|
| (Planello et al., 2016) [37] | | | | | | | | |
| | 1. CP: 19 2. Controls: 23 3. OSCC: 301 samples (+adjacent normal samples: 34) | | | | | | | |
| | NR | | | | | | | |
| | NR | | | | | | | |

**Table 2. Cont.**

1. CP: AAP classification, 1999 and 2004. 2. Controls: No clinical sign/symptoms of periodontal disease. 3. OSCC: from the oral cavity, SCC and adjacent normal was obtained from TCGA.

**Methodology**

**Results/Conclusions**

**Clinical Parameters**

**Additional Observations/Outcomes Assessed**

**Global/Genome-Wide Reporting**

Global methylation pattern in CP was significantly altered when compared to normal tissue. In total, 127 hypermethylated putative enhancers in CP were obtained. DNA-seq profiles of over 100 human cell lines from 79 different cell types were generated through the ENCODE project. DNA hypermethylation directly disrupts the transcriptional enhancer activity, leading to the shortening of telomere in the described chronic conditions.
Table 2. Cont.

| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Methodology | Results/Conclusions | Clinical Parameters |
|-----------------|-------------------------|--------------------------------|--------------|---------------------|---------------------|
| (Oliveira et al., 2009) [42] | 1. CP/S: 30 2. CP/NS: 40 3. NP/NS: 41 | NR | GT | CXCL8 | Smoker/Non-Smoker Study Groups |
|                  | 1. CP/S: At least three teeth exhibiting sites ≥5 mm CAL, at least 2 different quadrants; smokers: 5 cigarettes/day for at least 5 years. 2. CP/NS: At least three teeth exhibiting sites ≤5 mm CAL, in at least 2 different quadrants; never smoked. 3. NP/NS: No signs of periodontal disease (absence of CAL, no sites with PD > 3 mm); and never smoked. | NR | GT | CXCL8 | Higher levels of CXCL8 mRNA vs. controls in gingival cells (p = 50.007). No significant differences among groups were observed in gingival cells and blood cells. Smoking was not found to interfere with the loss of methylation in the cells collected in the mouthwash. | NR |
|                  | | | | | (Mean; SD) 1. CP/S: PD: 7.8 ± 2.02 mm 2. CP/NS: PD: 7.9 ± 2.18 mm 3. NP/NS: NR |
| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Adjustment (e.g., Age, Sex, Smoking, Number of Teeth etc.) | Type of Tissue (and Site Collected, if Applicable) | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Additional Observations/Outcomes Assessed | Global/Genome-Wide Reporting |
|-----------------|-------------------------|--------------------------------|----------------------------------------------------------|-------------------------------------------------|-----------------------------------|-----------------------------------------------|--------------------------|---------------------------------------------|-----------------------------|
| (De Oliveira et al., 2011) [41] | 1. CP/S: 11 2. CP/NS: 12 3. NP/NS: 11 | 1. CP/S: at least three teeth exhibiting sites ≥5 mm CAL in at least 2 different quadrants; smokers: 5 cigarettes/day for at least 5 years. 2. CP/NS: At least three teeth exhibiting sites ≤5 mm CAL in at least 2 different quadrants, and never smoked. 3. NP/NS: No signs of periodontal disease, (absence of CAL, and PPD ≤3 mm), and never smoked. | NR | GT | TLR2 and TLR4 | PCR and Methylation analysis with specific restriction enzymes | TLR2: no difference among groups (p = 40.05). TLR4: no statistical differences were found among these groups (p > 0.05). | N/A | NR |
| (Cho et al., 2017) [40] | 1. CP/S: 5 2. CP/NS: 5 3. NP/S: 5 4. NP/NS: 5 | 1. CP/S: PD ≥ 6 mm, BOP, and ABL. Smoked for at least 5 years; quantity/frequency NR. | NR | GT | ECM related organization genes | Illumina NextSeq500 sequencing system | ECM organization-related genes: Smoking is closely associated with DNA methylation with PPI analysis: performed for 17 genes exhibiting inverse correlation of DNA methylation with | Between NN and SN: In total, 84 genes were differently methylated; 36 were hypermethylated, | NR |
Table 2. Cont.

| Author and Year | N Cases per Study Group | Criteria for Subject Allocation | Adjustment (e.g., Age, Sex, Smoking, Number of Teeth etc.) | Type of Tissue and Site Collected, if Applicable | Methylation Sites (Genes) Assessed | Methods for Detecting DNA Methylation Changes | Main Findings | Additional Observations/Outcomes Assessed | Global/Genome-Wide Reporting |
|------------------|-------------------------|--------------------------------|----------------------------------------------------------|-----------------------------------------------------|-------------------------------------|---------------------------------------------|----------------|-------------------------------------------|-----------------------------|
|                  |                         |                                |                                                          |                                                     |                                     |                                             |                |                                            |                             |
|                  |                         | 2. CP/NS: PD ≥ 6 mm, BOP, and ABL. Never smoked. |                                                          |                                                     |                                     |                                             |                |                                            |                             |
|                  |                         | 3. NP/S: No BOP, no ABL, and PPD ≤ 4 mm. Smoked for at least 5 years; quantity/frequency NR. |                                                          |                                                     |                                     |                                             |                |                                            |                             |
|                  |                         | 4. NP/NS: No BOP, no ABL, and PPD ≤ 4 mm. Never smoked. |                                                          |                                                     |                                     |                                             |                |                                            |                             |

ECM organization-related genes. Differential gene expression pattern; i.e., increased in CP/S vs. CP/NS and NP/S.

Main Findings:
- Gene expression between (NN and NP) and (SN and SP).
- In total, 157 genes were identified as potentially affected by PPI. Among them, 50 genes were differentially expressed and 107 genes showed no change between the non-smoker and smoker groups.

Additional Observations/Outcomes Assessed:
- In total, 157 genes were identified as potentially affected by PPI. Among them, 50 genes were differentially expressed and 107 genes showed no change between the non-smoker and smoker groups.

Global/Genome-Wide Reporting:
- 115 genes were differentially methylated between NN and NP.
- 48 were differentially methylated between SN and SP.
- In total, 157 genes were identified as potentially affected by PPI. Among them, 50 genes were differentially expressed and 107 genes showed no change between the non-smoker and smoker groups.

2-CP/NS: PD ≥ 6 mm, BOP, and ABL. Never smoked.
3-NP/S: No BOP, no ABL, and PPD ≤ 4 mm. Smoked for at least 5 years; quantity/frequency NR.
4-NP/NS: No BOP, no ABL, and PPD ≤ 4 mm. Never smoked.

AAP: American Academy of Periodontology; ABL: Alveolar Bone Loss; NR: ACR: American College of Rheumatology; ADA: American Diabetic Association; AgP: Aggressive Periodontitis; ARA: American Rheumatism Association; BOP: Bleeding on Probing; BRCA: Breast Cancer; CAL: Clinical Attachment Level; CDH1: E-Cadherin; COBRA: bisulfite restriction analysis; COX2: cyclooxygenase-2; Cpg: cytosine-guanine; CXCL8: Interleukin 8; CXCL12: CXC chemokine receptor 12; DE: Differential expression genes; DM: Differently methylated genes; ELAR: European League Against Rheumatism; EMC: extracellular matrix organization; ECM: Extracellular Matrix; FMBS0: full-mouth bleeding score; GCF: Gingival Crevicular Fluid; GSTP1: glutathione S-transferase pi gene 1; GT: Gingiva tissue; IL6: interleukin 6; MS-HRM: Methylation Sensitive High-resolution melting; MSP: Bisulfite modification followed by methylation specific polymerase chain reaction; NN: Non-smokers/Non-periodontitis; NP: Non-smokers/Periodontitis; NR: Not reported; PB: Peripheral blood; PCR: Polymerase chain reaction; PD: Probing Depth; RA: Rheumatoid Arthritis; RMI2: RecQ Mediated Genome Instability 2; S: Smokers; SD: systemic diseases; SOCS1: suppressors of cytokine signaling 1; SP: Smokers/Periodontitis; SRP: Scaling and Root Planing; T2D: type 2 diabetes; TCGA: The Cancer Genoma Atlas; TIMP3: Metalloproteinase Inhibitor 3; TLR2: Toll-like receptors 2; TLR4: Toll-like receptors 4; TNF: tumor necrosis factor; TNFA: tumor necrosis factor alfa.
3.7.1. Individual Study Outcomes

DNA Methylation of Candidate Genes

(a) Methylation of candidate genes in periodontitis and metabolic disorder.

Grdovic et al. (2016) evaluated the DNA methylation status of \textit{CXCL12} in buccal mucosa epithelial cells from patients with CP, compared to diabetes with CP patients (D/P) and healthy controls [34]. The authors reported that the \textit{CXCL12} promoter was predominantly unmethylated in all groups. However, both the CP and D/P groups had increased frequency and percent methylation of the \textit{CXCL12} promoter compared to healthy controls, albeit no statistically significant difference [34].

(b) Methylation of candidate genes in periodontitis and rheumatoid arthritis.

Ishida et al. (2012) evaluated the DNA methylation status of 19 IL6 CpG motifs in peripheral blood from patients with CP compared to RA and controls [36]. The authors found that the methylation levels at the CpG motif at $-74\text{ bp}$ and $+19\text{ bp}$ were significantly lower in patients with CP and RA compared to controls; the methylation levels at $-74\text{ bp}$, but not $+19\text{ bp}$, were also associated with serum \textit{IL6} and \textit{IL6} production by mono-nuclear cells, and the other 17 CpG motifs exhibited comparable methylation levels between groups [36].

Kojima et al. (2016) evaluated the DNA methylation status of 12 CpG motifs in the \textit{TNF} gene promoter region in peripheral blood from participants with CP compared to RA and controls [35]. The authors reported that both the CP and RA groups showed significantly higher methylation rates and frequencies than the control groups at $-72\text{ bp}$, and the RA group additionally exhibited significantly higher methylation rates and/or frequencies at 6 additional CpG motifs compared to controls. The levels of \textit{TNF} produced were significantly different between individuals with and without methylation at $-163\text{ bp}$ (significantly higher hypermethylation rate and frequency in the RA group) [35].

(c) Methylation of candidate genes in periodontitis and cancer.

Loo et al. (2010) observed the hypermethylation status of \textit{E-cadherin} and \textit{COX2} genes in blood samples and gingival tissues in CP patients compared to neoplastic tissues from breast cancer patients and blood samples from healthy controls [39]. The authors reported hypermethylation of candidate genes in both CP and cancer groups, with higher methylation frequencies in the cancer patients compared to CP patients and \textit{E-cadherin} methylation compared to \textit{COX2} gene methylation [39].

Wang et al. (2014) analyzed the methylation status of \textit{TIMP1}, \textit{GSTP1}, \textit{14-3-3\textsigma}} genes in peripheral blood and gingival tissues from periodontitis patients compared to neoplastic tissues from breast cancer patients and blood samples from healthy controls [38]. The authors reported that the hypermethylation frequencies of \textit{TIM-3} and \textit{GSTP1} were similar in the periodontitis and control groups, but both were significantly lower than those for malignant disease patients. The methylation frequency of \textit{14-3-3\textsigma}} periodontitis was significantly higher than in the cancer and control groups [38].

Planello et al. (2016) profiled the DNA methylome of gingival tissue from CP patients, compared to oral squamous cell carcinoma samples (OSCC) and healthy controls [37]. The authors observed significant overlap between the altered DNA methylation patterns in CP and OSCC; specifically, the authors reported that for \textit{SOCS1} and \textit{RMI2}, hypermethylated CpG sites in CP were also hypermethylated in OSCC, and hypomethylated CpG sites in CP were also hypomethylated in OSCC [37].

(d) Methylation of candidate genes in periodontitis and smokers/non-smokers.

Oliveira et al. (2009) analyzed the DNA methylation status of the \textit{CXCL8} gene promotor region in epithelial oral mucosa cells from CP smokers, CP non-smokers, and non-periodontitis non-smokers [42]. The authors reported that independent of smoking status, the CP group had higher percentages of hypomethylation and higher expression of \textit{CXCL8} mRNA than non-periodontitis non-smoker controls. Additionally, there was no
significant difference in results from gingival cells compared to blood leukocytes obtained from a sub-sample [42].

De Oliveira et al. (2011) analyzed the DNA methylation status in the TLR2 and TLR4 gene promoter regions in gingival tissue from CP smokers, CP non-smokers, and non-periodontal non-smokers [41]. The authors reported that the CpG sites analyzed were unmethylated in the majority of DNA samples of the three groups, and there was no statistically significant difference in DNA methylation and mRNA transcript levels between the groups [41].

Cho et al. (2017) analyzed the methylome and transcriptome of CP smokers, CP non-smokers, and non-periodontitis smokers and non-smokers [40]. The authors reported that smoking is associated with DNA methylation status and transcription of extracellular-matrix organization-related genes [40].

Genome Wide Methylation

Two studies reported the global and genome-wide methylation results [37,40]. DNA sequencing data showed that over 100 cell lines and 79 cell types exhibited DNA hypermethylation in CP [37].

In the presence of a chief disease-modifying factor, such as smoking, high throughput gene and methylation analysis in reference to the former’s expression was able to decipher a pattern of expression in ECM degradation, adaptive immunity and skin pattern related genes among smokers and non-smokers who either have periodontitis or do not [40].

3.8. Pooled Data

3.8.1. DNA Methylation of Candidate Genes

An inter-study comparison of candidate gene methylation could not be conducted as each of the candidate genes included were examined in only one of the included studies.

3.8.2. Global and Genome-Wide Methylation

An inter-study comparison of global and genome-wide methylation could not be conducted as the methodology for assessing outcomes was inconsistent across included studies.

3.9. Study Quality

Following the quality assessment guidelines [33], four studies [34-36,42] were assessed as overall good quality (Supplementary Material Table S2), four studies [39-41] were assessed as fair quality, and two studies [37] were assessed as poor quality. While the studies assessed as poor quality [37,38] lacked multiple exposure and outcome assessments, they were included in the review on the basis of pre-determined eligibility criteria; these two studies also presented extensive genomic and epigenomic data, which was determined as an additional strength of the study. A total of three quality assessment criteria (items #3, #7, and #11) were reported by all included studies. Four quality assessment criteria were rarely reported (items #5, #8, #12, and #14), and one of the criteria (item #10) was not reported by any of the included studies.

4. Discussion

4.1. Patients with Systemic Diseases

Rheumatoid arthritis (RA) and periodontitis share a common pathogenesis pathway [21]; therefore, the epigenetic regulation in RA has been assessed and compared to periodontitis. Kojima et al. (2016) evaluated the DNA methylation of 12 CpG motifs in the TNF-α gene promoter region from peripheral blood of the Japanese population with CP compared to RA and controls [35], taking into consideration that TNF-α is an inflammatory cytokine that is elevated in active and progressing periodontitis [46-48]. Results of this study revealed that both CP and RA groups had higher DNA methylation rates and fre-
quences than the control groups at −72 bp [35], suggesting a pattern of hypermethylated CpG motifs in the TNF-α gene promoter in blood cells in Japanese adults with CP and RA.

In an earlier study on periodontitis and RA, Ishida et al. (2012) assessed the DNA methylation status of 19 CpG motifs in the IL-6 gene promoter from the peripheral blood of patients with CP compared to RA and controls [36]. Notably, IL-6 is a multifunctional pro-inflammatory cytokine that is highly expressed in periodontitis [49]. Ishida’s group reported significantly lower DNA methylation levels at −74 bp and +19 bp in CP and RA patients when compared to controls. Moreover, the methylation levels at −74 bp but not +19 bp were also associated with the production of IL-6 by mononuclear cells, suggesting that hypomethylation of a single CpG motif in the IL-6 promoter region might result in increased levels of IL-6 in the serum. This observation could indicate the role of DNA methylation status at this specific CpG motif in the pathogenesis of RA and periodontitis [36].

The relationship between diabetes mellitus and periodontitis is well-established by the fact that periodontitis is the sixth complication of diabetes mellitus [50] and diabetes being a major risk factor for periodontitis [51]; therefore, the epigenetic modifications were evaluated in periodontitis and diabetic patients. In this context, Grdovic et al. (2016) evaluated DNA methylation of CXCL12 promoter in epithelial cells from CP, diabetes/CP patients and healthy controls. CXCL12 is a pro- and anti-inflammatory chemokine [34], and its role in the development and progression of both periodontitis and diabetes remains elusive as its effects range from protective to destructive [34]. Although Grdovic et al. (2016) demonstrated an increased DNA methylation percentage in the CXCL12 promoter in healthy patients with periodontitis and in diabetic patients with periodontitis, compared to healthy controls, these results failed to show a statistically significant difference [34]. Nonetheless, it can be inferred that chronic inflammation contributes to the change of DNA methylation of CXCL12 promoter in buccal epithelial cells, which might play a role in the development and progression of periodontal disease.

Since epidemiological data have demonstrated that CP patients tend to have a significantly higher incidence of squamous cell carcinoma [52,53], Planello et al. (2016) analyzed DNA methylation in CP and healthy controls compared to oral squamous cell carcinoma samples [37]. Their results demonstrated hypermethylated CpG sites of SOCS1 and RMI2 in samples from periodontitis and in samples from squamous cell carcinoma, and hypomethylated sites consistent in both periodontitis and squamous cell carcinoma. These findings might suggest the emergence of the pre-neoplastic epigenome in CP.

In a recent systematic review, periodontitis was associated with an increased longitudinal risk of cancer mortality [54]. Due to the possible association between chronic periodontitis and cancer on the molecular level [55,56], epigenetic changes in periodontitis and breast cancer have been evaluated. Wang et al. (2014) evaluated the DNA methylation of TIMP3, GSTP1, and 14-3-3 in CP, healthy controls, and patients with breast cancer, since these three genes were previously investigated in the diagnosis and treatment of cancer. The results of their study demonstrated that hypermethylation levels of TIMP3 and GSTP1 were significantly lower in periodontitis patients and controls than those with breast cancer [38]. However, the epigenetic silencing of 14-3-3 occurred more frequently in the CP group compared to the healthy and breast cancer groups, suggesting that 14-3-3 plays a role in the development of CP.

In an earlier investigation, Loo et al. (2010) evaluated the DNA methylation of E-cadherin and COX2 promoter in CP, breast cancer patients, and healthy patients. Both genes are key indicators of cancer and also have links to periodontitis [39]. In fact, COX-2 is reportedly downregulated in chronic periodontitis, which might serve as a new setpoint to restrict further tissue destruction [57]. The results by Loo et al. (2016) revealed that the promoters of Cadherin and COX2 were hypermethylated the most frequently in breast cancer, followed by periodontitis and the least frequently in controls [39]. These findings suggest that epigenetic changes presented in CP patients might demonstrate irreversible destruction in the tissues or organs similar to the effects of cancer. DNA
hypermethylation in CP might be associated with DNA hypermethylation which is related to cancer risk factors.

4.1.1. Smokers and Non-Smokers

Since smoking is considered a major risk factor for periodontitis [58] and also influences epigenetic changes [59], DNA methylation patterns were evaluated in smokers with periodontitis. Toll-like receptors (TLRs) were evaluated, given their important role in the inflammatory process in periodontitis [60]. Gingival tissue samples from smokers and non-smokers affected by periodontitis as well as healthy patients were found to have major unmethylation of the TLR4 gene promoter, whereas the results for the TLR2 gene promoter were inconclusive given the mosaic of methylated and unmethylated DNA in the majority of samples [41]. There was also no statistically significant correlation between the DNA methylation status and mRNA transcription levels of both genes between the groups [41]. In another study, individuals with periodontitis displayed a higher percentage of hypomethylation of the CXCL8 gene, compared to healthy controls, independent of smoking habit [42]. This is in concordance with previously published papers that reported an increased expression of CXCL8, indicating its role in tissue destruction in periodontal diseases [61,62].

In another investigation assessing the methylation status of the extracellular matrix (ECM) organization-related genes, findings indicated that smoking might change the transcription and methylation status of ECM organization-related genes, which aggravated the periodontal disease [40]. These findings suggested that smoking-related changes in DNA methylation and successive changes in the expression of the ECM component-related genes may result in increased susceptibility to periodontitis in smokers by influencing ECM organization, which in turn may affect disease characteristics.

In a more recent investigation, the DNA methylation pattern of the SOCS1 promoter, a potent inhibitor of cytokine signaling [63], was evaluated in epithelial cells from the saliva of smoking and non-smoking patients with periodontitis [64]. Results showed that cells from the saliva of periodontitis patients who smoked were 7.08 times more likely to show a methylated SOCS1 promoter in comparison to periodontitis patients who are non-smokers, suggesting that SOCS1 may be a consequence of tobacco exposure and not periodontitis. This finding that methylation of SOCS1 promoter is solely due to tobacco is consistent with previous studies on SOCS1 and periodontitis in which SOCS1 hypomethylation was reported in chronic and aggressive periodontitis [63,65].

4.1.2. Limitations and Confounding Variables

The results of the present systematic review need to be interpreted with caution due to certain limitations. First, the number of available studies on the DNA methylation in periodontitis patients who have a systemic disease or smoke is quite limited to generalize conclusions. Most of the studies lacked information on the ethnic background of the study participants, which is an important factor that affects epigenetics. Regarding studies on smoking, there was inconsistency between the studies in identifying what a (smoker) patient was, i.e., how many cigarettes they smoked a day. These studies did not stratify smoking patients into slight, moderate, and heavy, which could affect the conclusions of the included studies.

4.1.3. Future Research

There is a need for further properly designed and conducted clinical human studies to evaluate the DNA methylation and periodontal and systemic diseases as well as smoking. Future investigations should further aim to identify DNA methylation of specific genes as potential diagnostic biomarkers for periodontitis in patients with systemic diseases at the chairside in dental practice. Furthermore, future research should aim to further understand the relationship in DNA methylation of specific genes between patients with periodontitis, systemic diseases, and smokers, in order to utilize this epigenetic modification as a tool for
patient stratification and thereby a more personalized treatment approach based on the disease susceptibility. Moreover, experimental and clinical trials should be performed to further study the epigenetic association of short- and long-term interactions of biomaterials with gingival tissue.

4.1.4. Clinical Relevance

Understanding the link between periodontal diseases and systemic diseases/smoking through epigenetics will aid in increasing the knowledge and understanding of the pathogenesis and progression of periodontitis in patients with systemic diseases and smokers. In addition, the fact that epigenetic mechanisms are reversible makes them attractive targets and offers new treatment strategies. By identifying the pathways that can be targeted to promote epigenetic changes, periodontal tissue destruction can be limited. Moreover, epigenetic drugs, also known as epidrugs, can be used as an adjunct to periodontal therapy in order to improve the outcomes of periodontal therapy in these systemically compromised patients.

5. Conclusions

There was some evidence, albeit inconsistent, for an association between DNA methylation and periodontal diseases and systemic diseases or smokers compared to non-diseased or non-smokers. However, due to the limited number of studies and the heterogeneity in study design, population and outcomes, no definite conclusion may be drawn between DNA methylation and periodontal diseases and systemic diseases or smokers. The DNA modifications in periodontal and systemic diseases in human adults may lead to the identification of potential disease therapies. Therefore, further research in DNA methylation and periodontal diseases and systemic diseases or smokers in humans is needed in developing alternative therapeutic approaches to treat systemic conditions and periodontal diseases by improving wound healing and periodontal tissue regeneration.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11115269/s1, Supplemental SI: Search protocol used in the systematic review; Supplemental SII: Extracted information on study characteristics; Table S1: Studies Excluded After Full-text Review; Table S2: Risk of Quality Assessment.

Author Contributions: Conceptualization, I.K.; data curation, I.K., R.S.B., M.O., I.G., F.A. and L.L.; formal analysis, I.K., R.S.B., M.O., I.G.; writing—original draft, I.K., R.S.B., M.O., F.A. and L.L.; writing—review and editing, I.K., R.S.B., M.O., I.G., F.A. and L.L. All authors have read and agreed to the published version of the manuscript.

Funding: F.A. is supported by the Osteology research scholarship (Osteology Foundation, Lucerne, Switzerland) and has received additional funding from Wilhelm and Martina Lundgren’s Science Fund Foundation, Sweden (2019-2906).

Acknowledgments: The authors are grateful to Richard McGowan (NYU Health Sciences Library Liaison and NYU College of Dentistry) for his assistance with the electronic database search process.

Conflicts of Interest: The authors declare no conflict of interest.

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