MicroRNA, 2021, *10*, 14-28

REVIEW ARTICLE

MicroRNA in Implant Dentistry: From Basic Science to Clinical Application

Maria Menini[1,*], Elena Dellepiane[1], Francesco Pera[2], Alberto Izzotti[3], Domenico Baldi[1], Francesca Delucchi[1], Francesco Bagnasco[1] and Paolo Pesce[1]

[1]*Division of Fixed and Implant Prosthodontics, Department of Surgical Sciences (DISC), University of Genoa, Genova, Italy;* [2]*Department of Surgical Sciences, University of Turin, Turin, Italy;* [3]*Department of Health Sciences, University of Genoa, Genova, Italy*

ARTICLE HISTORY

Received: September 30, 2020
Revised: March 13, 2021
Accepted: March 18, 2021

DOI:
10.2174/2211536610666210506123240

Abstract: Specific microRNA (miRNA) expression profiles have been reported to be predictive of specific clinical outcomes of dental implants and might be used as biomarkers in implant dentistry with diagnostic and prognostic purposes. The aim of the present narrative review was to summarize current knowledge regarding the use of miRNAs in implant dentistry. The authors attempted to identify all available evidence on the topic and critically appraise it in order to lay the foundation for the development of further research oriented towards the clinical application of miRNAs in implant dentistry.

Keywords: miRNA, implant dentistry, osseointegration, peri-implantitis, dental implants, osteogenesis.

1. INTRODUCTION

The success of implant therapies to rehabilitate partial and total edentulism has been clinically and histologically documented [1] and is based on osseointegration, a direct structural and functional connection between native living bone and the implant surface [2]. Although implant treatment is very successful, biological complications around dental implants such as peri-implant mucositis or peri-implantitis may occur. In the Consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, mucositis has been defined as the condition characterized by bleeding on gentle probing that can be associated with erythema, swelling or suppuration. Differently, peri-implantitis has been defined as a pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and progressive loss of supporting bone [3]. The etiology of peri-implant disease is controversial. In fact, peri-implantitis has often been considered a plaque-associated disease [3]. However, a cause-effect relation between plaque accumulation and bone resorption is far from being demonstrated [4-6].

Nowadays, the aetiology of peri-implantitis is recognized to be multifactorial. In particular, some of the factors that can influence implant success and might be involved in the onset and development of peri-implantitis are the implant design, the surgical and prosthetic protocol, patient's local and systemic conditions, pathogenic microflora, overloading, *etc*. Despite the number of existing hypotheses, the genetic and epigenetic mechanisms controlling peri-implant biological processes remain largely unexplored. The interest in epigenetics in medicine and dentistry has grown impressively during the last decade, and epigenomics is considered one of the most important cutting-edge subjects of scientific discovery [7, 8]. The mainly studied epigenetic molecular mechanisms are microRNA (or miRNAs, or miR) production, DNA methylation, and histone modification.

A recent review by Sartori *et al*. [9] found only one clinical trial evaluating the role of miRNAs in the osseointegration of dental implants. A narrative review by As'ad *et al*. [10] investigated the relationship between peri-implantitis and miRNAs, pointing out that most evidence is only available from a few preclinical studies and highlighting that factors associated with peri-implantitis, such as a history of periodontitis, might increase the susceptibility of certain individuals to develop peri-implantitis *via* sustained DNA-methylation levels or differential miRNA expression. Since the publication of these reviews, other studies, including some clinical trials, have been conducted analyzing miRNAs in implant dentistry, suggesting the need to update the review of the literature on the topic.

1.1. What are miRNAs

MicroRNAs are small endogenous non-coding RNA molecules (ncRNAs) of 18-22 nucleotides responsible for specific regulation of gene expression in a post-transcriptional manner [7, 11]. MicroRNAs are not translated into proteins; instead, they regulate the expression of other genes by either cleaving or repressing the translation of their messenger RNA (mRNA) targets. They control several biological processes, such as development, cell proliferation and differ-

* Address correspondence to this author at the Division of Fixed and Implant Prosthodontics, Department of Surgical Sciences (DISC), University of Genoa, Genova, Italy; E-mail: maria.menini@unige.it

2211-5374/21 $65.00+.00

entiation, apoptosis, carcinogenesis, and response to different extracellular signals and stress, playing a role in a variety of physiological and pathological states [12, 13]. miRNAs are the main regulator of gene transcription and bear relevance in predicting clinical outcomes. Indeed, only about 5% of expressed genes producing mRNA are really translated into proteins, while miRNAs are fully functionally active in cell cytoplasm [14]. In fact, miRNA pathways regulate gene expression by inducing degradation and translational repression of target mRNAs.

A single miRNA can influence the expression of many genes often involved in a functional interacting pathway. In fact, each miRNA may target hundreds of mRNAs, and some targets are affected by multiple miRNAs. miRNAs are fundamental in the maintenance of pluripotency and undifferentiation of adult stem cells; indeed, several miRNAs appear to significantly modulate the differentiation of mesenchymal precursors in osteoblast cells, regulating the activity of transcription factors [15]. miRNAs can be isolated from cells, tissues, and body fluids (including cell-free biological fluids) such as serum, plasma, tears, or urine [16].

miRNAs are currently identified as predictive biomarkers for degenerative diseases, because they do not undergo a post-transcriptional selection, being the controllers of gene transcription themselves. Consequently, compared to genomic or transcriptomic biomarkers, miRNA expression has by far a higher probability of being related with clinical variables representing a new tool for predictive medicine.

In medical literature, numerous studies over the past decade have been devoted to quantitative and qualitative assessment of miRNA expression and have shown considerable changes in their expression profiles in various diseases, but the most remarkable changes were observed in cancer [17]. The results of these studies point to the profiling of miRNA expression as an important tool for diagnostics and treatment of diseases. However, knowledge of the underlying reasons and mechanisms behind changes in miRNA expression is still limited [18].

Current methodologies used for detecting miRNAs (Table **1**) include quantitative PCR (qPCR), *in situ* hybridization, microarrays, and RNA sequencing [19, 20]. Microarray technology is a powerful high-throughput tool capable of monitoring the expression of thousands of small non-coding RNAs at once within tens of samples processed in parallel in a single experiment.

Gene expression using microarrays is evaluated by process of (a) miRNA extraction, (b) labeling, and (c) hybridization on slides containing miRNA probes. Then the slides are scanned with a laser system, and false-color images are generated. The overall result is the generation of a so-called genetic portrait. It corresponds to up- or down-regulated miRNAs in the investigated cell system. The microarray-based methods include multiplex qPCR-based arrays and hybridization-based arrays. The qPCR microarrays use pre-plated PCR primer/probes distributed across 96 or 384 well plates. For a low amount of input material, a microfluidic card is available that requires as low as 1 ng of total RNA, and microfluidic systems are available that enable single-cell miRNA profiling. The hybridization-based arrays have the advantage of allowing a large number of parallel measurements per sample at a relatively low cost. Due to limited specificity, findings from hybridization-based arrays are typically validated with a second method such as qPCR or *in situ* hybridization [21].

Table 1. Advantages and disadvantages of the different miRNA profiling techniques.

miRNA Profiling Techniques	Outcome	Advantages	Disadvantages
RT quantitative PCR (RT-qPCR)	The quantity of one miRNA	• High sensitivity • Specificity • Quantitative	• Requires miRNA annotation • Traditional qPCR will not work on mature miRNAs • Small scale
Microarray hybridization	Relative change in miRNA expression between two conditions	• Large scale screening: the expression of thousands of small ncRNAs can be monitored at once within tens of samples processed in parallel in a single experiment	• Distinguishing the presence of similar sequences may be difficult. • Requires miRNA annotation • Imprecise quantification
RNA sequencing (RNA-seq)	Sequence information for all short RNAs present	• It is able to detect even small differences in miRNA sequence • Does not require miRNA annotation • Gives sequence information	• Requires a large amount of RNA • Complicated • Expensive
In situ hybridization	Localization of one miRNA	• Can localize miRNAs in cells/tissues	• Not quantitative Small scale
Northern Blot	Presence or absence of one miRNA	• Simple and inexpensive • Distinguishes precursors from mature miRNAs	• Requires large quantities of RNA • Small scale

1.2. miRNAs in Dentistry

miRNAs are important regulatory molecules in many biological processes. Many investigations regarding miRNAs have demonstrated their essential roles in physiological and pathological processes in the immune response. Additionally, miRNAs likely contribute to the development and progression of systemic diseases, such as cancer and vascular disease. Consequently, there is considerable interest in the medical literature for the use of miRNAs not only as diagnostic markers but also as potential therapeutic targets for various diseases. However, studies investigating the role of miRNAs in dentistry are still limited. A recent narrative review by Kim *et al*. [22] presents a brief overview of the application of miRNAs as biomarkers for oral cancer and periodontitis. Studies investigating the role of miRNAs in other fields of dentistry are scarcer. In particular, the roles of miRNAs in oral cancer and precancer have been more widely investigated [22, 23]. miRNA expression appears to differ between healthy tissue and squamous cell carcinoma tissues of the oral cavity [24]. According to several recent studies, approximately 30-50 miRNAs have been suggested to be linked with oral cancer using either independent *in vitro* or *in vivo* experiments and human studies [25]. Furthermore, some investigations have linked precancerous lesions and their risk of becoming malignant to changes in miRNA expression [26]. However, there are only a few miRNAs that have been proven to be involved in oral cancer [27]. For example, miR-125b, miR-155, miR-124a, and miR-124b showed altered expressions in oral cancer in different studies [28].

Also, salivary miRNAs have been proposed as useful biomarkers for the detection of oral cancer, periodontal disease, and other systemic diseases [22, 24, 29-33].

miRNAs are also associated with bacterial infections [34] and, thus, are most likely associated with infectious diseases of the oral cavity, for example, dental caries, endodontic infections, and periodontitis.

In particular, some studies focused on the differences in miRNA profiles between peri-implantitis and periodontitis [35]. In this context, miRNAs have been shown to play an important role in the differentiation of periodontal ligament stem cells into an osteogenic lineage [36]. Nahid *et al*. [37] examined the expression of cytokines associated with inflammation and differences in expression of cytokines and miRNAs during infection with live and heat-killed bacteria in THP-1 monocytes. They demonstrated that expression of miR-146a is associated with infections caused by periodontal pathogenic bacteria *in vitro*. Another study indicated that miR-146a is significantly overexpressed in THP-1 cells after stimulation with LPS from Porphyromonas gingivalis [38]. miRNAs have been shown to play an important role in the differentiation of periodontal ligament stem cells into an osteogenic lineage. An *in vitro* study demonstrated that a specific type of miRNA promotes osteogenic differentiation of periodontal ligament stem cells; miRNA-218 acted as a positive regulator of osteogenesis in periodontal ligament stem cells and its ectopic expression promoted the differentiation of periodontal ligament stem cells [39]. From these findings, it seems that miRNA-218 is an important mediator of osteoblast differentiation, thus offering a new target for the development of therapeutic agents against osteogenic disorders.

1.3. Aim

The aim of the present narrative review was to summarize current knowledge regarding the use of miRNAs in implant dentistry. An attempt was made to retrieve all available literature on the topic in order to shed light on the epigenetic mechanisms involved in implant dentistry and in order to identify the possible diagnostic and therapeutic role of miRNAs in this field.

2. METHODOLOGY

Two of the authors (MM and ED) searched the following databases: MEDLINE/PubMed, Google Scholar, Scopus, and Science Citation Index Expanded from Web of Science, without any restriction regarding language and date of publication until the 25th September 2020. The keywords "dental implants," fixture, implants surface, dental implant surface, osseointegration, microRNA, miRNA, miR-, were used and combined using Boolean operators. Only papers in English or Italian language were analysed. Any original study investigating miRNAs in implant dentistry was included. All reference lists of the selected studies were checked for cross-references. Secondary studies (reviews) were carefully analysed, and their reference list was screened for possible original articles to be included. Given the paucity of studies on this topic, the aim was to retrieve all available studies without restrictions regarding study design. Both *in vitro* and *in vivo* studies were included.

3. RESULTS

3.1. miRNAs in Implant Dentistry

The papers identified in the present review work are reported in (Table **2**). In total, 25 papers were retrieved: 14 *in vitro* studies, 3 animal studies, 4 clinical studies, and 4 papers that included both an *in vitro* phase and an animal one. The animal studies used Labrador dogs, rats, and mice as models.

The studies presented great heterogeneity regarding outcomes, study design, and methodology applied.

A paper by Liu *et al*. [40] was excluded from (Table **2**) as the full-text was available in Chinese only. The paper reported that expression levels of miR-29a-3p were suppressed in peri-implant bone in a rat model of hyperlipidemia.

3.2. Dental Implant Surfaces and their Effect on miRNA Expression

Among the 25 studies retrieved, 15 studies (11 *in vitro*, 1 clinical study, and 3 including an *in vitro* phase and an animal phase) investigated the effect of different implant sur-

faces, testifying to the great interest of the dental scientific community (and dental industry) on the topic.

Nine of those papers investigated miRNAs expression in cells next to different implant surfaces and are summarized in the present paragraph. Six papers reported the use of miRNAs to biofunctionalize implant surfaces and will be described in a subsequent paragraph.

Several factors, including the implant surface [41], might affect dental implant success through miRNA expression. Our research group demonstrated using a cDNA microarray analysis that osteoblasts change their gene expression profile *in vitro* according to the type of implant surface in contact with them [42]. In the literature, some *in vitro* studies are present [43, 44] investigating the relation between titanium implant surfaces and Dental Pulp Stem Cells (DPSCs) growth and differentiation evaluating their miRNA expression. The obtained results demonstrated that miRNAs played a pivotal role in the differentiation of mesenchymal stem cells and could be used as a marker of osteogenic differentiation. According to Iaculli, titanium implant surfaces influenced DPSCs differentiation in an osteogenic pattern through modulation of miRNAs expression. Gardin *et al*. [44] demonstrated enhanced osteogenic properties in DPSCs grown onto the nanorough Ti surfaces treated with Non-Washed Resorbable Blasting Media (NWRBM) probably related to an increase in miRNA-196a and VCAM1 mRNA expression compared to Alumina-Blasted/Acid-Etched (AB/AE) implants.

Other *in vitro* studies compared miRNA expression in different cells next to modified implant surfaces [44-47]. In particular, Chakravorty *et al*. [45] showed that the expression profile of miRNAs in osteoprogenitor cells is influenced by the exposure to topographically (Sand-blasted, Large-grit Acid-etched - SLA) and chemically (hydrophilic SLA) modified titanium surfaces.

Ferreira *et al*., with their microarray analysis [46], showed differential expression of 716 mRNAs and 32 miRNAs with functions associated with osteogenesis, demonstrating that oxidative nanopatterning of titanium surfaces induces changes in the metabolism of osteoblastic cells and contributes to the explanation of the mechanisms that control cellular responses to micro- and nano-engineered surfaces.

Sartori *et al*. [47] found that surface nanotopography can affect the expression pattern of miRNAs. In fact, comparing the expression pattern of miRNAs on a surface with nanotopography compared with a smooth surface (control), a total of 117 miRNAs were differentially expressed in human Mesenchymal Stem Cells (hMSCs). Forty-five miRNAs were upregulated, and 72 were downregulated. Several of the miRNAs that were differentially expressed regulate osteogenic genes.

Also, two other *in vitro* studies [48-50] investigated the effect of nanotopography of titanium surfaces on epigenetic mechanisms involving miRNAs. Kato *et al*. [48, 49] found that nanotopography generated by titanium surface oxidation using a mixture of H_2SO_4/ H_2O_2 directs mesenchymal stem cells to osteoblast lineage through regulation of miRNA-SMAD-BMP-2 circuit. In particular, they identified 3 miRNAs (miR-4448, -4708, and -4773) whose downregulation attenuates SMAD1 and SMAD4 degradation, intensifying BMP-2 signal transduction, stimulating osteoblast differentiation. Yang *et al*. [49] used an osteoinductive near square-50 (NSQ50) topography to investigate the role of BMP2 in initiating osteogenic signaling in concert with cues from the integrins. They illustrated the role of BMP2 in the induction of RUNX2 through the repression of miR-23b.

Different from the previously cited papers that were focused on titanium implants, 3 manuscripts evaluated the effect of zirconia implants on miRNA expression [50-53]. In particular, two *in vitro* studies by Palmieri *et al*. [51, 52] investigated the effect of zirconia on miRNA expression of osteoblastic cells. They found a number of miRNAs differently expressed by osteoblast-like cells exposed to titanium compared to zirconium disks, suggesting a better osseointegration potential of titanium surfaces compared zirconia.

Cossellu *et al*. [53] conducted a human cross-sectional study on 90 obese patients correlating the expression of 377 miRNAs in the peripheral blood with titanium and zirconium levels in hair samples. Seven miRNAs (miR-99b, miR-142-5p, miR-152, miR-193a-5p, miR-323-3p, miR-335, miR-494) resulted specifically associated with Zr levels. Although the authors suggest that titanium and zirconium might be present in human hair due to their release from dental implants, it must be underlined that the paper does not report if the patients recruited have been rehabilitated with dental implants.

Many miRNAs influence key osteoinductive pathways controlling Osterix, runt-related transcription factor 2 (RUNX2), and Bone Morphogenetic Protein (BMP)/SMAD function. Others influence the monocyte/macrophage lineage. While significant progress has been made in elucidating the mechanisms associated with the regulation of surface modulation of osteoblast differentiation by miRNAs, thanks to many *in vitro* studies on the topic [44, 46, 47], knowledge gaps are evident in the identification and characterization of miRNAs linked to osseointegration. Given existing knowledge regarding the varied expression of miRNAs and their role in inflammation, it is important to understand how miRNA expression may influence the process of bone accrual at implant surfaces during osseointegration [9]. As miRNAs are vital factors in osteoclastogenesis, osteogenesis, and osteoclast/osteoblast differentiation, they have been investigated in bone-related diseases and bone-remodelling processes. Specifically, miRNAs affect osteoclastogenesis and osteoclast differentiation either by directly regulating osteoclast activity, signaling intermediates or through negative-feedback loops, while they control osteogenic lineage commitment of various stem cells through positive-feedback loops [10].

An *in vitro* study by Du *et al*. [54] evaluated exosomes derived from 3T3L1 preadipocytes (Adipose tissue-Derived Stem Cells - ADSCs). Exosomes are nanovesicles derived

from numerous cell types, and they contain mRNA, miRNA, proteins, and lipids. They act as cell-to-cell messengers and are considered a novel alternative to stimulate bone regeneration. Du *et al*. suggested that exosomes derived from 3T3L1 preadipocytes promoted 3T3L1 cells to undergo osteogenic differentiation *via* reduced miR-223 expression.

4. POSSIBLE CLINICAL APPLICATIONS OF miRNAs IN IMPLANT DENTISTRY

4.1. Diagnostic Role of miRNAs in Implant Dentistry

The evaluation of peri-implant tissue health is mainly based on clinical examinations and diagnostic imaging techniques. Several diagnostic criteria have been proposed for peri-implantitis. As reported in the systematic review by Pesce *et al*. [4], for some authors to define an implant as affected by peri-implantitis, the Probing Depth (PD) has to be ≥ 5 mm or ≥ 6 mm; for others Bleeding On Probing (BOP) or presence of suppuration are necessary to diagnose peri-implantitis or different levels of bone resorption. It must be noted that the value of applying periodontal parameters in the monitoring of peri-implant disease is unclear. Moreover, despite the fact that clinical methods have the advantage of being relatively easy to apply, they are not repeatable if they are not performed by trained and calibrated operators, and they are not able to determine the risk rate and progression of the peri-implant disease [55]. Clinical measurements around implants might be challenged by the force and direction of probing, implant morphology, and prosthetic design. The cyclical episodic progression of peri-implantitis further complicates the assessment of disease progression.

Our previous studies [56, 57] suggested the possibility of using miRNAs extracted from peri-implant soft tissue as biomarkers and predictors of implant health. miRNAs were extracted from peri-implant soft tissue samples at 3 months of healing and correlated with clinical parameters (plaque index-PI, bleeding on probing-BOP, probing depth-PD, and bone resorption) recorded during the first year post-implant insertion and at the 5 year follow-up.

Specific miRNA expression profiles were found to be predictive of specific clinical outcomes. In particular, the miRNA mostly involved in the prediction of clinical outcomes was miR-548. Indeed, this miRNA predicted PD and BOP. In fact, it was upregulated in peri-implant soft tissue harvested 3 months post-implant insertion at implant sites that presented augmented PD and BOP at the 5-year follow-up appointment. Moreover, some specific miRNA signatures appeared to be protective from bone resorption despite the presence of plaque accumulation. miRNA expression analysis was found to be far more accurate than periodontal phenotype (thick vs. thin) in predicting peri-implantitis occurrence in the 5-year follow-up [57].

Regarding results of miRNA expression in peri-implantitis, they are mainly confined to findings from animal studies [58], and although the research is developing, scarce studies are available in regard to miRNA expression and peri-implant disease [58].

In the study by Wu *et al*. [58], peri-implantitis was induced by placing cotton floss ligatures around the implant neck in 6 Labrador dogs. miRNAs extracted from soft peri-implant tissue of healthy implants and implants affected by peri-implantitis were evaluated with a split-mouth design. It must be underlined that this experimental model is far from simulating peri-implant disease in humans, and any conclusion must be considered with caution.

Interestingly, most miRNAs involved in peri-implant disease (let-7g, miRNA-27a, miRNA-29a, and miRNA-142) were significantly downregulated with the exception of miRNA-145, which was significantly upregulated. Wu and colleagues [58, 59] demonstrated that let-7g, miRNA-27a, and miRNA-142 influenced the onset, progression, and treatment of peri-implantitis in a canine ligature-induced peri-implantitis model, highlighting the potential biological effects of the differentially expressed miRNAs and the specific enrichment of target genes involved in the MAPK signaling pathway.

One of the clinical studies retrieved [60], instead, investigated a possible association between MiR146a/MiR499 gene polymorphisms and periodontitis/peri-implantitis in a population of 197 Iranian subjects (75 in the chronic periodontitis group, 38 in the peri-implantitis group, and 84 healthy patients), and demonstrated that genotype frequencies in MiR146a and MiR499 were significantly different among the three groups and MiR146a and MiR499 (rs3746444) gene polymorphisms may be genetic determinants for increased risk of chronic periodontitis and peri-implantitis.

Currently, medical research is focused on "liquid biopsies" with the aim of developing non-invasive and site-specific diagnostic tools [61-64]. In the field of implant dentistry, Peri-Implant Crevicular Fluid (PICF) has been proposed for this type of biopsy because it can be easily collected. Usually, in dentistry, proinflammatory cytokines such as Tumor Necrosis Factor (TNF)-α, anti-inflammatory cytokines (*e.g.,* IL-4, IL-10), and chemokines (*e.g.,* IL-8) have been described as important mediators of inflammation and immunity in the pathogenesis of peri-implantitis. Therefore, the evaluation of such cytokines in the PICF has been proposed as a non-invasive means of monitoring the status of peri-implant tissues and the response of peri-implant treatments [65]. However, Duarte *et al.*, in their systematic review [65], concluded that "evidence regarding the PICF levels of anti-inflammatory cytokines, osteoclastogenesis-related cytokines and chemokines as possible predictors of peri-implantitis is too limited." Our research group extracted for the first time miRNAs from PICF and quantified and analyzed miRNAs using microarray technology [66]. We found that miRNAs extracted from PICF were derived from soft peri-implant tissue. In fact, 14 of the miRNAs altered in PICF in relation to bone resorption were also altered in soft peri-implant tissue at the same implant sites at the same time point [66].

Other authors have characterized miRNA patterns in saliva with the aim of using them as biomarkers of oral cancer,

periodontal disease, and other systemic diseases [22, 24]. The possibility to extract miRNAs from PICF provide the added advantage of site-specificity compared to saliva and might be useful for diagnostic and prognostic purposes in implant dentistry. PICF miRNAs may serve as early diagnostic biomarkers of peri-implant disease, providing a promising alternative to traditional tissue sampling. The ease and cost-effectiveness of PICF sampling make it suitable for large-scale screening trials and when repeated sampling is needed at the same implant site for monitoring peri-implant health and disease progression.

4.2. Therapeutic Role of miRNAs in Implant Dentistry

Based on the results of the research about the relationship between miRNA expression and implant surfaces, some studies tried to apply the current knowledge to improve osseointegration and implant therapy success [58, 66-71]. The biofunctionalization of titanium implant surface using miRNAs seems to be a promising approach on the basis of *in vitro* and animal studies.

Wu *et al*. [67], for example, fabricated a microporous titanium oxide surface that provided a larger surface area for miRNA loading and enabled spatial retention of the miRNAs within the pores until cellular delivery. The study demonstrated high miRNA transfection efficiency in Mesenchymal Stem Cells (MSCs) seeded onto the miRNA functionalized surface with no apparent cytotoxicity, promising to lead to more rapid and robust osseointegration of a clinical bone-implant interface. More recently, the same research group [68] fabricated Polyelectrolyte Multilayers (PEMs) using the layer-by-layer approach with a chitosan-miRNA (CS-miRNA) complex and sodium hyaluronate (HA) as the positively and negatively charged polyelectrolytes on Microarc-Oxidized (MAO) titanium surfaces *via* silane-glutaraldehyde coupling. The new biofunctionalized implant exhibited sustained release of CS-antimiR-138 and notably enhanced the *in vitro* osteogenic differentiation of MSCs and *in vivo* osseointegration in the rat model. MiR-138 is a regulator in the development of the osteoblast phenotype [67, 68, 72]. By inhibiting the focal adhesion kinase signaling pathway, antimiR-138 can promote the expression of osteoblast-specific genes, ALP activity, and ECM mineralization of MSCs, and enhance *in vivo* ectopic bone formation, suggesting that antimiR-138 is a good therapeutic molecule for enhancing osteogenesis [72].

Also, another research group [69] used the antimiR-138 delivered MSC sheet to functionalize titanium implants, showing a significant promotion of the expression of endogenous osteogenesis and angiogenesis-related genes and proteins, alkaline phosphatase activity, extracellular matrix mineralization, and collagen secretion. In conclusion, antimiR-138 seems effective in promoting osteogenic differentiation [67-69].

On the other hand, Shao *et al*. [71] evaluated *in vitro* miR-122-modified cell sheets prepared by non-viral transfection and complexed with micro-arc titanium oxide implants to construct a gene-modified tissue-engineered implant. Also, this biofunctionalized implant was found to be effective in promoting osteogenic differentiation of Bone Marrow Mesenchymal Stem Cells (BMMSCs).

Liu *et al*. [70] instead identified that the misexpression of miR204 is one of the mechanisms responsible for the impaired osteogenesis in type 2 diabetes mellitus, and they designed a PLGA-based miR204 delivery system for the titanium implant, which promoted osseointegration in a diabetic rat model *via* promoting BMSCs osteogenesis *in vivo*.

Wang *et al*. [73] realized microarc-oxidized titanium surfaces functionalized with miR-21-loaded chitosan/hyaluronic acid nanoparticles, and in their *in vitro* study, they found that they promoted the osteogenic differentiation of human Bone Marrow Mesenchymal Stem Cells (hBMMSCs).

Si *et al*. [74], in their *in vitro* study, further demonstrated that miR-135b-5p facilitates *in vitro* osteogenesis of human Mesenchymal Stem Cells (hMSCs) by facilitating the Hippo signaling pathway.

Although numerous studies have evaluated the biofunctionalization of dental implants using miRNAs, the materials and methodologies applied are very heterogeneous, and a comparison between the results of different studies is not possible.

miRNAs have also been proposed to realize tools for bone regeneration in peri-implant bone defects. In an animal study, Wu *et al*. [59] constructed a miR-27a-enhanced delivery system to repair the bone defect around implants in a canine peri-implantitis model (5 Labrador dogs with ligature-induced peri-implantitis). The results demonstrated that the miR-27a-treated group could optimize new bone formation and re-osseointegration *in vivo*.

Ren *et al*. [75], using a rat model, found that miR-193a-3p plays an important role in osseointegration during hyperlipidemia and suggested that it might be a therapeutic target for improving dental implantation success rates in patients with hyperlipidemia.

The *in vitro* study by Ma *et al*. [76] found that Human Amnion-Derived Mesenchymal Stem Cells (HAMSCs) promoted osteogenic differentiation of hBMSCs *via* H19/miR-675/APC pathway and suggested that they might be attached into scaffold biomaterials, such as gel or nanofiber, and implanted in the bone defects around dental implants to enhance osteogenesis of existing hBMSCs.

Understanding the epigenetic role of miRNAs in peri-implant soft tissue and bone and how miRNAs are related to peri-implantitis can be important for the use of epigenetics for a more patient-centered and personalized therapeutic approach, and to improve the treatment outcomes of peri-implantitis, especially given that the different treatment of peri-implantitis traditionally proposed have shown unsuccessful outcomes with substantial recurrence in the medium-term follow-up, even in subjects enrolled in regular peri-implant maintenance programs [10, 77, 78].

Table 2. Original studies focusing on miRNAs in implant dentistry.

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
Palmieri *et al.*	2008	*In vitro* study	Sterile metal disks of machined grade 3 Ti (diameter 3 cm) (control) and sterile disks of Zirconium Dioxide Ceramics (ZDC) (diameter 3 cm) (test) were used	Osteoblast-like cells (MG63) were cultured, collected, and seeded into a set of wells containing sterile disks of control and test surfaces. A miRNA microarray analysis was performed	miRNA expression	BMP4 and 7 are both up-regulated in osteoblasts cultured on Ti disks	Six up-regulated miRNAs in ZDC compared to Ti: mir-214, mir-337, mir-423, mir-339, mir377, mir-193b; and four down-regulated miRNAs: mir-143, mir-17-5p, mir-24, and mir-22
Palmieri *et al.*	2008(b)	*In vitro* study	Sterile metal disks of zirconium dioxide (ZO) (diameter 3 cm) were used	Osteoblast-like cells (MG63) were cultured, collected, and seeded into a set of wells containing sterile disks of ZO. A miRNA microarray analysis was performed	miRNA expression	The most notable regulated genes acting on osteoblasts are NOG, SHOX, IGF1, BMP1, and FGFR1.	18 up-regulated miRNAs: mir-337, mir-423, mir-497, mir-214, mir-377, mir-296, mir-99b, mir-193b, mir-25, mir-324, mir-518a, mir-320, mir-23b, mir-93, mir-23a, mir-422b, mir-330, mir-197; and 3 down-regulated miRNAs: mir-302c, mir-369 5p, mir-10b
Chakravorty *et al.*	2012	*In vitro* study	Three different disks of grade 2 cpTi: smooth polished (SMO), large-grit acid-etched (SLA), and hydrophilic SLA (modSLA)	Primary human osteoprogenitor cells were allowed to interact with the surfaces for 24 h. Then RNA was isolated and analyzed by qRT-PCR	miRNA expression	Modified titanium implant surfaces induce differential regulation of miRNAs, which potentially regulate the TGF-β/BMP and WNT/Ca(2+) pathways during osteogenic differentiation on modified titanium implant surfaces	miR-503, miR-215, miR-10a, miR-125b, miR-1, miR-218, miR-10b, miR-21, miR-16, miR-195, miR-146b-5p, miR-194, miR-7, miR-192, miR-99a, miR-100, miR-125a-5p, miR-137, miR-146a, miR-424, miR-23b, miR-20b, miR-155, miR-378, miR-20a, miR-132, miR-17, miR-26a, miR-134, miR-452, miR-214, miR-24, miR-93, miR-92a, miR-33a, miR-222, miR-127-5p, miR-15a, miR-210, miR-130a, let-7b, miR-22, let-7i, miR-181a
Kadkhodazadeh *et al.*	2013	Clinical cross- sectional study	197 Iranian patients: • 75 Patients with Chronic Periodontitis (CP) • 38 Patients with Peri-Implantitis (PI) • 84 Healthy subjects	DNA was extracted from fresh blood samples from the arm veins of participants, and the genetic polymorphism of the MiR genes was assessed using a Competitive Allele-Specific PCR (KASP) technique	PD, bone loss, genotype frequencies of MiR146a and MiR499	The genotype frequencies in MiR146a and MiR499 were significantly different among the three groups. MiR146a (rs2910146) and MiR499 (rs3746444) gene polymorphisms may be genetic determinants for increased risk of periodontitis and peri-implantitis	MiR146a, MiR499
Wu *et al.*	2013	*In vitro* study	Mesenchymal Stem Cells (MSCs) seeded onto cpTi disks with a miRNA functionalized microporous Ti the surface obtained by lyophilizing miRNA lipoplexes onto a microporous titanium oxide surface formed by Microarc Oxidation (MAO)	The expression of osteogenesis-related genes was evaluated	Transfection Efficiency Cell Viability Lactate Dehydrogenase Activity Cell Morphology (evaluated by FE-SEM) Osteogenesis-related gene expression (real-time PCR) ALP activity Collagen Secretion ECM mineralized nodule	A high miRNA transfection efficiency was observed in MSCs seeded onto the miRNA functionalized surface with no apparent cytotoxicity. Clear stimulation of MSC osteogenic differentiation was observed in terms of up-regulating osteogenic expression and enhancing alkaline phosphatase production, collagen secretion, and ECM mineralization	antimiR-138, miR-29b
Kato *et al.*	2014	*In vitro* study	Disks of grade 2 cpTi with a chemically produced nanotopography and untreated (control) Ti	hMSCs were obtained from the bone marrow of four donors and were cultured in 24-well culture plates on Ti disks with nanotopography and control Ti disks	Cell culture Gene expression of the key bone markers ALP protein detection Extracellular matrix mineralization miR next-generation sequencing on Illumina platforms miR transfection	Ti with nanotopography induces osteoblast differentiation of hMSCs as evidenced by upregulation of osteoblast-specific markers compared with control Ti at day 4. A complex regulatory network involving a miR-SMAD-BMP-2 circuit governs the osteoblast differentiation induced by Ti with nanotopography	miR-4448, -4708, and -4773

(Table 2) contd....

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
Yang *et al*.	2014	*In vitro* study	NSQ5050 nanotopography	hMSCs were isolated from human bone marrow obtained from patients undergoing routine arthroplasty and cultured on NSQ5050 and a planar control	Osteogenic phenotypical expression (qRT-PCR, immunofluorescence staining, western blot)	BMP and BMPR1a are up-regulated ahead of RUNX2 and RUNX2, and its regulatory miRNAs are BMP sensitive. The nanofeatures promote colocalization of integrins and BMP2 receptors in order to enhance osteogenic activity, and vitronectin is important in this interface	miR23-a, miR23-b, miRs-96, miRs-143
Wang *et al*.	2015	*In vitro* study	cpTi specimens with microarc-oxidized surface functionalized with miR-21-loaded chitosan/hyaluronic acid nanoparticles (CS/HA/miR-21 nanoparticle-coated MAO) Ti specimens (test) vs. naked MAO Ti surfaces (control)	hBMMSCs were seeded onto the test and control experimental surfaces	Particle size, zeta potential, surface morphology, gel retardation ability Transfection efficiency Cell viability (cell count) Lactate Dehydrogenase (LDH) activity Cell morphology (FE-SEM) Osteogenic gene expression (real-time PCR)	The miR-21-functionalized MAO Ti surfaces demonstrated cell viability, cytotoxicity, and cell spreading comparable to those exhibited by naked MAO Ti surfaces and led to significantly higher expression of osteogenic genes	miR-21
Cossellu *et al*.	2016	Clinical cross- sectional study	90 Obese/overweight patients	The expression levels of 377 human miRNAs in peripheral blood of 90 subjects was assessed using microarray analysis. Hair samples were analyzed for Ti and Zr content using Inductively Coupled Plasma-Mass Spectrometry	Correlation between Zr and Ti levels traced in hair samples and miRNA expression	Seven miRNAs were specifically associated with Zr levels. miRNAs are relevant molecular mechanisms sensitive to Zr exposure	miR-99b, miR-142-5p, miR152, miR-193a-5p, miR-323-3p, miR-335, miR-494
Ferreira *et al*.	2016	*In vitro* study	Titanium discs exhibiting the following topographies: nanotexture (N), nano+submicrotexture (NS), and rough microtexture (MR)	Osteoblastic cells from human alveolar bone were cultured on commercially pure grade 2 titanium discs	Cell viability Alkaline phosphatase Mineralized matrix formation mRNA and miRNA expression profiling (Microarray and qRT-PCR)	Increase of alkaline phosphatase activity as a function of the surface texture, with higher activity shown by cells adhering onto nanotextured surfaces; differential expression of 716 mRNAs and 32 microRNAs with functions associated with osteogenesis	hsa-miR-101-3p, hsa-miR-106b-5p, hsa-miR-1246, hsa-miR-1290, hsa-miR-134, hsa-miR-136-3p, hsa-miR-136-5p, hsa-miR-15a-5p, hsa-miR-1826 v15.0, hsa-miR-1914-3p, hsa-miR-193a-3p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-21-3p, hsa-miR-21-5p, hsa-miR-218-5p, hsa-miR-26b-5p, hsa-miR-27a-3p, hsa-miR-29b-3p, hsa-miR-301a-3p, hsa-miR-31-3p, hsa-miR-374a-5p, hsa-miR-376a-3p, hsa-miR-376b-3p, hsa-miR-376c-3p, hsa-miR-377-3p, hsa-miR-424-5p, hsa-miR-450a-5p, hsa-miR-494, hur 5, miRNABrightCorner30, mr 1
Gardin *et al*.	2016	*In vitro* study	OsseoFix Non-Washed Resorbable Blasting Media (NWRBM) as experimental nanorough titanium implants, and Alumina-Blasted/Acid-Etched (AB/ AE) as control implants	The analysis of the influence of implant surfaces on the osteoblastic phenotype of Dental Pulp Stem Cells (DPSCs) was performed. The effect on osteogenic differentiation, extracellular matrix (ECM), and cell adhesion molecules production has also been evaluated	Mutagenic potential (Ames test) DPSCs viability (MTT test) Cell morphology (SEM and immunofluorescence) Karyotype analysis Molecular biology analyses (real-time PCR of total RNA and miRNA)	A substantial expression of osteoblast-specific markers and a strong increase of cell adhesion molecules were detected on nanorough Ti surfaces treated with NWRBM. In particular, when DPSCs are seeded on the Ti implants, expression of miR-196a, which is involved in osteoblastic commitment of stem cells, and of Vascular Cell Adhesion Molecule 1 (VCAM1) is strongly enhanced	Mir-26a, mir-100, mir-31, mir-106a, mir-486-5p, mir-196a, mir-218, mir-22, mir-148

(Table 2) contd....

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
Iaculli *et al*.	2017	*In vitro* study	5 titanium disks with a sandblasted and acid-etched surface (Control Group); 5 titanium disks with a sandblasted and acid-etched surface, then treated with inorganic ions (Test Group)	DPSCs were cultured on sandblasted and acid-etched titanium disks, with (Test) or without the presence of ions (Control). miRNA expression (real-time PCR) of DPSCs was analyzed	DPSCs differentiation in an osteogenic pattern was evaluated through the expression of miR-133, miR-135, Runx2, Smad5, and Osteocalcin	The evaluated ionized sandblasted and acid-etched surface (Test) seemed to markedly enhance the cell differentiation	miRNAs 133a, 133b, 135°
Liu *et al*.	2017	*In vitro* and Animal study	Animal phase: Sprague Dawley (SD) rats (male, 12 months old) received titanium and were divided into three groups: 1) control group, rats received original implant; 2) PLGA-control, rats received an implant with PLGA sheet containing scramble sequence-conjugated AuNP; 3) PLGA-inhibitor, rats received an implant with PLGA sheet containing miR204-inhibitor-conjugated AuNP	The miR204 inhibitor was conjugated with gold nanoparticles (AuNP-antagomiR204) and dispersed in the poly lactic-co-glycolic acid (PLGA) solution. The AuNP-antagomiR204 containing PLGA solution was applied for coating the surface of the titanium implant. *in vitro* phase: BMSCs were seeded on the functionalized titanium discs. Animal phase: The rats in which type 2 diabetes mellitus (T2DM) was induced were kept for 8 weeks recovery before osseointegration strength test, microcomputerized tomography (microCT), and histological analysis. RNAs from cells or tissues were isolated and were detected	*in vitro phase:* release and uptake of the auNP-miRNA from PLGA sheets Cell adhesion Cytotoxicit>*Animal phase:* Removal torque Bone volume fraction, BIC rate, number of trabeculae, and trabecular thickness (microCT) Expression of miR204 and osteogenesis-related genes including Bmp, Opg, Alp, Runx2, and Col 1 by Real-time quantitative polymerase chain reaction (PCR) analysis	miR204 misexpression accounted for the deficient osseointegration in diabetes mellitus, while PLGA sheets aided the release of AuNP-antagomiR204, which promoted osseointegration	miR204
Menini *et al*.	2017	Clinical prospective study	7 patients (14 implants)	Patients were rehabilitated with fixed delayed-loading implant-supported prostheses. Samples of soft peri-implant tissue have been taken 3 months after implant insertion and correlated with periodontal parametres (PI, BOP, bone resorption) recorded during the first year since implant placement	Correlation between miRNA profile and peri-implant health parameters and different implant surfaces	Soft tissue inflammation (BOP) was more related to miRNA expression profile than to PI or to the implant surface. The type of implant surface affected miRNA expression profile. Some specific miRNA signatures appeared to be protective from bleeding and bone resorption despite the presence of plaque accumulation	miR-9, miR-100, miR-128, miR-144, miR-145, miR-182, miR-194, miR-367, miR-429, miR-495, miR-579, miR-607, miR-655, miR-889, miR-924, miR-1271, miR-1298, miR-1324
Si *et al*.	2017	*In vitro* study	hMSCs and Human Calvarial Osteoblasts (HCO) cells	hMSCs and HCO cells were cultured *in vitro*, followed by the transfection of the miR-135b-5p mimic or inhibitor using Lipofectamine 2000	Cell viability (MTT) Calcium deposits (Alxarin red staining) miRNA and mRNA expression (real-time PCR Protein levels (western blot)	miR-135b-5p was highly expressed in osteoblasts compared with hMSCs. miR-135b-5p promotes hMSC differentiation into mature osteoblasts by controlling LATS1 and MOB1B expression and subsequently activating the HIPPO signaling pathway	miR-135b-5p
Wu *et al*.	2017	Animal study	5 Healthy adult male Labrador dogs	24 ITI implants were inserted, and peri-implantitis was induced by ligatures	Radiographic analysis; Gingival tissue collected from both healthy and peri-implantitis sites, and miRNA analysis was performed	Let-7g, miR-27a, and miR-145 may play important roles in peri-implantitis and are worth further investigation	cfa-miR-452, cfa-miR-375, cfa-miR-98, cfa-miR-145, cfa-let-7e, cfa-miR-142, cfa-miR-500, cfa-miR-7, cfa-let-7g, cfa-miR-486, cfa-miR-152, cfa-miR-127, cfa-miR-1271, cfa-miR-101, cfa-miR-16, cfa-miR-200a, cfa-miR-140, cfa-miR-27b, cfa-miR-146a, cfa-miR-204, cfa-miR-29a, cfa-miR-26a, cfa-miR-451, cfa-miR-125a, cfa-miR-93, cfa-miR-361, cfa-miR-27a, cfa-miR-532, cfa-let-7c, cfa-miR-23a, cfa-miR-211, cfa-miR-92b,

(Table 2) contd....

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
							cfa-miR-342, cfa-miR-340, cfa-miR-374a, cfa-miR-9, cfa-miR-429, cfa-miR-20a
Du *et al*.	2019	*In vitro* study	3T3L1 murine preadipocyte cell line	Exosomes derived from 3T3L1 preadipocytes (3T3L1-exo) were purified and characterized. The effects and potential mechanisms of 3T3L1-exo on 3T3L1 cell ossification were examined	Osteogenic differentiation evaluated through Alizarin Red Staining (ARS), reverse transcription-quantitative polymerase chain reaction (RT-qPCR), western blotting, electron microscopy Osteogenic miRNA expression (RT-qPCR)	3T3L1-exo promoted 3T3L1 bone formation by decreasing miR-223 through a competitive mechanism, another miRNA, or another factor	miR-223
Sartori *et al*.	2018	*In vitro* study	Silica-based glass wafers with different nanoroughness were tested. Smooth glass disk surface and nano glass disk surface were used. The first ones after 7 days were considered the control group with the exception of the ALP activity test, where the smooth surface day 3 was the control	Human Mesenchymal Stem Cells (hMSCs) were plated on different surfaces and compared at 3, 7, and 14 days	Alkaline Phosphatase (ALP) activity, expression of genes (osterix [OSX], runt-related transcription factor 2 [RUNX2], bone morphogenetic protein 2 [BMP2], and ALP), and expression of miRNAs. Western blot was also used to detect osteogenic proteins (BMP2, OSX, and osteocalcin [OCN]). Scanning electron microscopy of cells plated onto the surfaces	Nanotopography surfaces affected MSC differentiation to osteoblasts. Several miRNAs were differentially regulated by surface topography.	Some of the most important miRNAs shown in this study were hsa-miR-135-5p, hsa-miR196a-5p, hsa-miR-26a-5p, hsa-miR-148b-3p, hsa-miR-122-5p. The complete list of miRNAs analyzed can be found in the original article by Sartori *et al*.
Shao *et al*.	2018	*In vitro* study	Bone Marrow Mesenchymal Stem Cells (BMMSCs) derived from femur and tibia of 18 one-week-old Sprague-Dawley rats divided into a control group, an miR-122 control group, and a miR-122 group (n=6)	miR-122-modified cell sheets were prepared by non-viral transfection and complexed with micro-arc titanium oxide implants to construct a gene-modified tissue-engineered implant, with its surface morphology observed by Scanning Electron Microscopy (SEM)	Morphology of the implant (SEM) Osteogenic potential evaluated by Alkaline Phosphatase (ALP), Sirius Red, alizarin red staining, polymerase chain reaction, and western blot analysis	miR-122 effectively promoted osteogenic differentiation of the BMMSC sheet	MiR-122
Yan *et al*.	2018	*In vitro* and animal study	*In vitro phase*: Commercially pure Ti plates (Φ10 × 1 mm) and rods (Φ1 × 10 mm) *in vivo phase*: immunocompromised mice	*In vitro phase*: the antimiR-138 delivered MSC (Mesenchymal Stem Cells) sheet was wrapped around the Ti implant (MSIC); *In vivo phase:* The constructed MSICs were subcutaneously implanted into the dorsal surface of immunocompromised mice. At 4 and 8weeks after implantation, the MSICs were harvested	*In vitro phase:* miR-138 silencing efficiency in the MSICs Osteogenesis related gene expression Expression of osteogenic and angiogenic biomarkers (ALP activity, collagen secretion, and ECM mineralization) Osteogenesis related gene expression (RT-PCR) *in vivo phase:* micro-computerized tomography (Micro-CT), histological analysis (hematoxylin-eosin staining, Masson's Trichrome staining, and immunofluorescent staining) *in vivo* osteogenesis and angiogenesis of the MSICs	*In vitro,* the antimiR-138 delivered MSIC significantly promotes the expression of endogenous osteogenesis and angiogenesis-related genes and proteins, alkaline phosphatase activity, extracellular matrix mineralization, and collagen secretion compared to the antimiR-control and the nothing delivered control. The *in vivo* ectopic implantation assay displayed a massive peri-implant bone formation with good vascularization	MiR-138
Menini *et al*.	2019	Clinical prospective study	7 Patients (14 implants)	Patients were rehabilitated with fixed delayed-loading implant-supported prostheses. Samples of soft peri-implant tissue were taken 3 months after implant insertion and correlated with periodontal parameters (PI, BOP, PD, bone resorption) recorded at the 5-year follow-up appointment	Correlation between miRNA profile and peri-implant health parameters	Some specific miRNA signatures appeared to be "protective" from bone resorption despite the presence of plaque accumulation	hsa-miR-4677-5p, hsa-miR-3914, hsa-miR-4679, hsa-miR-378b, hsa-miR-4434, hsa-miR-32-3p/mmu-miR-32-3p/rno-miR-32-3p, hsa-miR-1/mmu-miR-1a-3p, hsa-miR-4778-5p, hsa-miR-99b-3p/mmu-miR-99b-3p/rno-miR-99b-3p, hsa-miR-7-5p/mmu-miR-7a-5p/

(Table 2) contd....

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
							rno-miR-7a-5p, hsa-miR-3146, hsa-miR-4439, hsa-miR-539-3p, hsa-miR-222-3p/mmu-miR-222-3p/rno-miR-222-3p, hsa-miR-124-5p/mmu-miR-124-5p/rno-miR-124-5p, hsa-miR-4689
Wu *et al*.	2019	Animal study	6 Male Labrador dogs	For inflammation-related and osteogenesis-related miRNA identification in peri-implantitis disease, an experimental peri-implantitis model was established in Labrador dogs, and miRNA sequencing analysis was performed with no subsequent treatment. The bilateral mandibular fourth premolar and first molar were extracted from the dogs. Three months later, 20 (2 per side for each dog) standard ITI implants were inserted. To validate whether miR-27a regulates bone regeneration, a surgery was conducted to repair peri-implant bone defects using miRNA-based tissue-engineered constructs in 5 Labrador dogs (one Labrador dog died before surgery)	Radiographic evaluation, histological analysis, micro-CT, sequential fluorescent labeling, inflammation-related and osteogenesis-related miRNA identification	The miR-27a-treated group could optimize new bone formation and reosseointegration *in vivo*	MiR-27a
Ma *et al*.	2020	*In vitro* and animal study	*in vitro phase:* Human Amnion-Derived Mesenchymal Stem Cells (HAMSCs) and Human Bone Marrow Mesenchymal Stem Cells (HBMSCs) collected from human mandible samples>*Animal phase:* 16 female nude rats	*In vitro phase: a* coculture of HAMSCs and HBMSCs and transfection of HAMSCs with the miRNA duplexes was carried out. Osteogenesis was induced>*Animal phase:* A critical-size mandible defect (5x5 mm) was made in the rats. Cells ($5x10^4$ HAMSCs or HAMSCs shH19 and $5x10^4$ were attached to each HA/TCP biomaterial. The complexes were subcutaneously implanted into the mandibular defect area	*In vitro phase:* ALP activity Mineralized matrix formation determined by alizarin red staining quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) Western blot analysis Immunofluorescence staining Transfection efficacy of miR-675 (detected by RT-PCR). *in vivo phase:* 3D micro-computerized tomography (micro-CT) scanning Histological observation	HAMSCs promote osteogenic differentiation of HBMSCs *via* H19/miR675/APC pathway. MiR-675 serves as a downstream of LncRNA-H19 in HAMSCs-droved osteogenesis	miR-675
Ren *et al*.	2020	Animal study	Wistar rats with hyperlipidemia	Specific Pathogen-Free (SPF) Wistar rats were used to develop hyperlipidemia. Threaded titanium implants were inserted into the site 5 mm away from the bilateral distal femoral metaphysis. Bone implant tissues were dissected on day 14 and day 28. After lentivirus injection, samples were obtained on day 14	Role of colon cancer antigen 3 (Sdccag3) in the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and in implant osseointegration: Hematoxylin-Eosin staining Micro-Computed Tomography Analysis Alkaline Phosphatase (ALP) Alizarin Red S (ARS) Oil Red O (ORO) Gene expression: microarray, Quantitative Reverse Transcriptase Polymerase Chain Reaction Analysis Western Blot Analysis Immunofluorescence Assay for Sdccag3 Protein in BMSCs	Sdccag3 promotes implant osseointegration, and its related lncRNA-MSTRG.97162.4 and miR193a-3p play an important role in osseointegration during hyperlipidemia	miR-193a-3p

(Table 2) contd....

Study	Year	Study Design	Sample	Methods	Outcomes	Findings	miRNAs Identified
					RNA Pulldown Assays of lncRNAMSTRG.97162.4 and Sdccag3 in BMSCs Lentivirus construction and functional analysis *in vitro* and *in vivo*		
Wu *et al.*	2020	*In vitro* and animal study	Commercially pure Ti discs and screw Ti rods with a CS-antimiR-138/HA PEM coating *In vitro phase:* Mesenchymal Stem Cells (MSCs) *In vivo phase:* Twenty female Sprague-Dawley rats divided into 5 groups: antimiR-138 group, antimiR-control group, CS group, MAO group, and Polished Ti (PT) group	Polyelectrolyte Multilayers (PEMs) were fabricated using the layer-by-layer approach with a chitosan-miRNA (CS-miRNA) complex and sodium hyaluronate (HA) as the positively and negatively charged polyelectrolytes on microarc-oxidized (MAO) Ti surfaces *via* silane-glutaraldehyde coupling. *In vitro phase:* MSCs were seeded on the CS-antimiR-138/HA PEM-functionalized microporous Ti surface *In vivo phase:* Ti implants were inserted in the femurs of rats. Femurs containing implants were then extracted	*In vitro phase:* Characterization of the CS-antimiR-138/HA PEM-functionalized microporous Ti (SEM) Quantification of the loading and release of miRNA from CS-antimiR-138/HA PEM-functionalized microporous Ti Transfection efficiency Cytotoxicity Cell morphology Expression of osteogenisis-related genes (Real-time PCR) ALP activity ECM mineralized nodule displaying *In vivo phase:* Micro-CT evaluation Qualitative analysis of bone formation and BIC quantification (Van Gieson staining) Analysis of the bone-to-implant interface (FE-SEM and EDX)	A sustained miRNA release was obtained over a timeframe of approximately 2 weeks. *in vitro* transfection revealed that the CS-antimiR-138 nanoparticles were taken up efficiently by the cells and caused significant knockdown of miR-138 without showing significant cytotoxicity. The CS-antimiR-138/HA PEM surface enhanced the osteogenic differentiation of MSCs in terms of enhanced alkaline phosphatase, collagen production, and extracellular matrix mineralization. Substantially enhanced *in vivo* osseointegration was observed in the rat model	antimiR-138

5. DISCUSSION

This narrative review investigated the application of miRNAs in dentistry, focusing on their application in implant therapy. Many researchers focused their attention on miRNAs rather than other epigenetic mechanisms because of the more precise results of this kind of analysis due to the functional link between specific miRNAs and target mRNAs.

Two other recent narrative reviews on the use of miRNAs in implant dentistry are available [9, 10]. Among them, the work of Sartori describes the relationship between miRNAs and osseointegration, listing miRNAs with a reported role in osteogenesis and osseointegration and highlighting which miRNAs are reported to be upregulated and downregulated in relation to osteogenesis. The review by Asa'ad instead investigates the epigenetic mechanisms that play a role in periodontal and peri-implant tissue breakdown and may have utility as potential therapeutic applications in alveolar bone regeneration.

Compared to the previous review works, our manuscript has been updated, including 12 additional original papers that were not included in the previous reviews.

The majority of the studies found in the literature are pilot pre-clinical *in vitro* or animal studies. It must be underlined that *in vitro* and animal experimental models are far from properly simulating the clinical situation, and any conclusion must be taken with caution and validated by clinical trials.

The pre-clinical available data lay the foundation for the possible future use of miRNAs in implant dentistry. In particular, they could be used as implant coatings to enhance osseointegration. Currently, the miRNAs used as activators for implant surfaces are miR122, miR-21, miR-29b, antimiR204, and antimiR138 [15], but further studies are required to define the role of time and to investigate the effects of miRNA-functionalized surfaces with clinical trials.

On the other hand, the results obtained reveal that several miRNAs can be modulated as a consequence of surface modification, and more studies should be addressed to elucidate their role in osteoblast metabolism in order to realize implant surfaces that are able to promote the best osseointegration possible.

Despite the current difficulty in defining which implant surface is the best to stimulate osteogenesis genetically, there is evidence that implant surface features can upregulate or downregulate genes related to osseointegration.

No papers were found on the possible role of smoke in affecting peri-implant tissue health through miRNA expression. Exposure to cigarette smoke extensively affects microRNA expression in several organs [79]. The role of miRNAs in the augmented risk of peri-implant bone loss in smokers should be investigated.

Only a few clinical trials are available at the moment to shed light on possible biomarkers that may be predictable of dental implants' clinical outcomes [53, 56, 57, 60]. Among these, the research conducted by our group [56, 57] represents the first important step to understanding the use of miRNAs as biomarkers in our clinical field. In fact, specific miRNA profiles were found to predict the onset of peri-implantitis. In particular, the possibility to extract miRNAs

from PICF is especially promising as it provides a non-invasive and site-specific liquid biopsy that might be repeated over time without any noxious effect at the implant site. However, the small sample size of this study (7 patients only) induce to interpret the results with caution and suggests the need to deepen the topic on a larger and more representative sample size.

Finally, epigenetic modifications can be used to treat stem cells and help them differentiate into the desired lineage *in vitro*, which can then be seeded into the scaffold used in bone tissue regeneration and eventually implanted into the area where bone regeneration is desired.

The importance of epigenetic mechanisms in determining the clinical outcome of dental implants will probably largely influence the industry and the clinical application of implant treatment in the future.

CONCLUSION

miRNAs have a crucial role as biomarkers in several diseases, and they are considered regulators of several biologic processes, including bone remodelling and osseointegration. A better understanding of the molecular phenomena regulating osseointegration will help improving implant treatment, accelerating bone healing, and understanding bone resorption and implant loss.

miRNAs might be used for diagnostic and prognostic purposes in implant dentistry and as therapeutic agents for a more personalized and patient-centered therapeutic approach.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Ali Z, Baker SR, Shahrbaf S, Martin N, Vettore MV. Oral health-related quality of life after prosthodontic treatment for patients with partial edentulism: A systematic review and meta-analysis. J Prosthet Dent 2019; 121(1): 59-68.e3. http://dx.doi.org/10.1016/j.prosdent.2018.03.003 PMID: 30006220

[2] Guglielmotti MB, Olmedo DG, Cabrini RL. Research on implants and osseointegration. Periodontol 2000 2019; 79(1): 178-89. http://dx.doi.org/10.1111/prd.12254 PMID: 30892769

[3] Berglundh T, Armitage G, Araujo MG, *et al.* Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45(20) (Suppl. 20): S286-91. http://dx.doi.org/10.1111/jcpe.12957 PMID: 29926491

[4] Pesce P, Menini M, Tealdo T, Bevilacqua M, Pera F, Pera P. Peri-implantitis: A systematic review of recently published papers. Int J Prosthodont 2014; 27(1): 15-25. http://dx.doi.org/10.11607/ijp.3785 PMID: 24392473

[5] Pesce P, Canullo L, Grusovin MG, de Bruyn H, Cosyn J, Pera P. Systematic review of some prosthetic risk factors for periimplantitis. J Prosthet Dent 2015; 114(3): 346-50. http://dx.doi.org/10.1016/j.prosdent.2015.04.002 PMID: 26050027

[6] Menini M, Setti P, Pera P, Pera F, Pesce P. Peri-implant tissue health and bone resorption in patients with immediately loaded, implant-supported, full-arch prostheses. Int J Prosthodont 2018; 31(4): 327-33. http://dx.doi.org/10.11607/ijp.5567 PMID: 29953561

[7] Williams SD, Hughes TE, Adler CJ, Brook AH, Townsend GC. Epigenetics: A new frontier in dentistry. Aust Dent J 2014; 59 (Suppl. 1): 23-33. http://dx.doi.org/10.1111/adj.12155 PMID: 24611746

[8] Barros SP, Offenbacher S. Epigenetics: Connecting environment and genotype to phenotype and disease. J Dent Res 2009; 88(5): 400-8. http://dx.doi.org/10.1177/0022034509335868 PMID: 19493882

[9] Sartori EM, das Neves AM, Magro-Filho O, *et al.* The role of microRNAs in the osseointegration process. Int J Oral Maxillofac Implants 2019; 34(2): 397-410. http://dx.doi.org/10.11607/jomi.6581 PMID: 30883619

[10] Asa'ad F, Monje A, Larsson L. Role of epigenetics in alveolar bone resorption and regeneration around periodontal and peri-implant tissues. Eur J Oral Sci 2019; 127(6): 477-93. http://dx.doi.org/10.1111/eos.12657 PMID: 31701573

[11] Shookhoff JM, Gallicano GI. The emerging role of microRNAs in adult stem cells. Stem Cell Biology and Regenerative Medicine. Humana Press: New York, NY, USA; Springer: Berlin, Germany 2011; 1: pp. 57-94. http://dx.doi.org/10.1007/978-1-61779-002-7_3

[12] Pasquinelli AE, Reinhart BJ, Slack F, *et al.* Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000; 408(6808): 86-9. http://dx.doi.org/10.1038/35040556 PMID: 11081512

[13] Osada H, Takahashi T. MicroRNAs in biological processes and carcinogenesis. Carcinogenesis 2007; 28(1): 2-12. http://dx.doi.org/10.1093/carcin/bgl185 PMID: 17028302

[14] Izzotti A. Molecular medicine and the development of cancer chemopreventive agents. Ann N Y Acad Sci 2012; 1259: 26-32. http://dx.doi.org/10.1111/j.1749-6632.2012.06646.x PMID: 22758633

[15] Di Gianfilippo R. The use of MiRNAs as activators of dental implant surfaces, a review. Int J of Bone Mineral Metabolism 2018; 1(1): 2-9.

[16] Weber JA, Baxter DH, Zhang S, *et al.* The microRNA spectrum in 12 body fluids. Clin Chem 2010; 56(11): 1733-41. http://dx.doi.org/10.1373/clinchem.2010.147405 PMID: 20847327

[17] Katchy A, Williams C. Expression profiles of estrogen-regulated microRNAs in breast cancer cells. Methods Mol Biol 2016; 1366: 373-93. http://dx.doi.org/10.1007/978-1-4939-3127-9_30 PMID: 26585151

[18] Gulyaeva LF, Kushlinskiy NE. Regulatory mechanisms of microRNA expression. J Transl Med 2016; 14(1): 143. http://dx.doi.org/10.1186/s12967-016-0893-x PMID: 27197967

[19] Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018; 141(4): 1202-7. http://dx.doi.org/10.1016/j.jaci.2017.08.034 PMID: 29074454

[20] Kebschull M, Papapanou PN. Mini but mighty: MicroRNAs in the pathobiology of periodontal disease. Periodontol 2000 2015; 69(1): 201-20. http://dx.doi.org/10.1111/prd.12095 PMID: 26252410

[21] Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: Approaches and considerations. Nat Rev Genet 2012; 13(5): 358-69. http://dx.doi.org/10.1038/nrg3198 PMID: 22510765

[22] Kim SH, Lee SY, Lee YM, Lee YK. MicroRNAs as biomarkers

for dental diseases. Singapore Dent J 2015; 36: 18-22. http://dx.doi.org/10.1016/j.sdj.2015.09.001 PMID: 26684491

[23] Liu C, Tong Z, Tan J, Xin Z, Wang Z, Tian L. MicroRNA-21-5p targeting PDCD4 suppresses apoptosis *via* regulating the PI3K/AKT/FOXO1 signaling pathway in tongue squamous cell carcinoma. Exp Ther Med 2019; 18(5): 3543-51. http://dx.doi.org/10.3892/etm.2019.7970 PMID: 31602231

[24] Menini M, De Giovanni E, Bagnasco F, *et al.* Salivary micro-RNA and oral squamous cell carcinoma: A systematic review. J Pers Med 2021; 11(2): 101. http://dx.doi.org/10.3390/jpm11020101 PMID: 33557138

[25] Gombos K, Horváth R, Szele E, *et al.* miRNA expression profiles of oral squamous cell carcinomas. Anticancer Res 2013; 33(4): 1511-7. PMID: 23564792

[26] Roy R, De Sarkar N, Ghose S, *et al.* Association between risk of oral precancer and genetic variations in microRNA and related processing genes. J Biomed Sci 2014; 21: 48. http://dx.doi.org/10.1186/1423-0127-21-48 PMID: 24885463

[27] Babu JM, Prathibha R, Jijith VS, Hariharan R, Pillai MR. A miR-centric view of head and neck cancers. Biochim Biophys Acta (BBA)- Rev Can 2011; 1816(1): 67-72.

[28] Heah KG, Shobri NRBT, Suhaimi SNB, *et al.* A review of microRNA associated with oral cancer. J Int Dental and Medical Res 2019; 12: 738-43.

[29] Li Y, Zhou X, St John MA, Wong DT. RNA profiling of cell-free saliva using microarray technology. J Dent Res 2004; 83(3): 199-203. http://dx.doi.org/10.1177/154405910408300303 PMID: 14981119

[30] Park NJ, Zhou H, Elashoff D, *et al.* Salivary microRNA: Discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res 2009; 15(17): 5473-7. http://dx.doi.org/10.1158/1078-0432.CCR-09-0736 PMID: 19706812

[31] Patel RS, Jakymiw A, Yao B, *et al.* High resolution of microRNA signatures in human whole saliva. Arch Oral Biol 2011; 56(12): 1506-13. http://dx.doi.org/10.1016/j.archoralbio.2011.05.015 PMID: 21704302

[32] Liu CJ, Lin SC, Yang CC, Cheng HW, Chang KW. Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma. Head Neck 2012; 34(2): 219-24. http://dx.doi.org/10.1002/hed.21713 PMID: 22083872

[33] Yoshizawa JM, Wong DT. Salivary microRNAs and oral cancer detection. Methods Mol Biol 2013; 936: 313-24. http://dx.doi.org/10.1007/978-1-62703-083-0_24 PMID: 23007518

[34] Drury RE, O'Connor D, Pollard AJ. The clinical application of microRNAs in infectious disease. Front Immunol 2017; 8: 1182. http://dx.doi.org/10.3389/fimmu.2017.01182 PMID: 28993774

[35] Ogata Y, Matsui S, Kato A, Zhou L, Nakayama Y, Takai H. MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. J Oral Sci 2014; 56(4): 253-60. http://dx.doi.org/10.2334/josnusd.56.253 PMID: 25500922

[36] Asa'ad F, Garaicoa-Pazmiño C, Dahlin C, *et al.* Expression of MicroRNAs in periodontal and peri-implant diseases: A systematic review and meta-analysis. Int J Mol Sci 2020; 21(11): 4147. http://dx.doi.org/10.3390/ijms21114147 PMID: 32532036

[37] Nahid MA, Rivera M, Lucas A, Chan EKL, Kesavalu L. Polymicrobial infection with periodontal pathogens specifically enhances microRNA miR-146a in ApoE-/- mice during experimental periodontal disease. Infect Immun 2011; 79(4): 1597-605. http://dx.doi.org/10.1128/IAI.01062-10 PMID: 21263019

[38] Honda T, Takahashi N, Miyauchi S, Yamazaki K. *Porphyromonas gingivalis* lipopolysaccharide induces miR-146a without altering the production of inflammatory cytokines. Biochem Biophys Res Commun 2012; 420(4): 918-25. http://dx.doi.org/10.1016/j.bbrc.2012.03.102 PMID: 22480686

[39] Sun M, Ma Y, Cai Z, Yang Z. MiR-218 promotes osteogenic differentiation of periodontal ligament stem cell through activation of Wnt signaling by targeting SFRP2. Int J Clin Exp Pathol 2016; 9: 10188-96.

[40] Liu F, Wang ZF, Liu FF, Xu JZ, Liu Q, Lan J. MicroRNA-29a-3p regulates osteoblast differentiation and peri-implant osseointegration in a rat model of hyperlipidemia by modulating Frizzled 4 expression. Hua Xi Kou Qiang Yi Xue Za Zhi 2019; 37(2): 200-7. PMID: 31168988

[41] Menini M, Dellepiane E, Chvartszaid D, Baldi D, Schiavetti I, Pera P. Influence of different surface characteristics on peri-implant tissue behavior: A six-year prospective report. Int J Prosthodont 2015; 28(4): 389-95. http://dx.doi.org/10.11607/ijp.4066 PMID: 26218023

[42] Baldi D, Longobardi M, Cartiglia C, *et al.* Dental implants osteogenic properties evaluated by cDNA microarrays. Implant Dent 2011; 20(4): 299-305. http://dx.doi.org/10.1097/ID.0b013e318225f22b PMID: 21778890

[43] Iaculli F, Di Filippo ES, Piattelli A, Mancinelli R, Fulle S. Dental pulp stem cells grown on dental implant titanium surfaces: An *in vitro* evaluation of differentiation and microRNAs expression. J Biomed Mater Res B Appl Biomater 2017; 105(5): 953-65. http://dx.doi.org/10.1002/jbm.b.33628 PMID: 26856387

[44] Gardin C, Ferroni L, Piattelli A, *et al.* Non-Washed Resorbable Blasting Media (NWRBM) on titanium surfaces could enhance osteogenic properties of MSCs through increase of miRNA-196a and VCAM1. Stem Cell Rev Rep 2016; 12(5): 543-52. http://dx.doi.org/10.1007/s12015-016-9669-1 PMID: 27318850

[45] Chakravorty N, Ivanovski S, Prasadam I, Crawford R, Oloyede A, Xiao Y. The microRNA expression signature on modified titanium implant surfaces influences genetic mechanisms leading to osteogenic differentiation. Acta Biomater 2012; 8(9): 3516-23. http://dx.doi.org/10.1016/j.actbio.2012.05.008 PMID: 22588073

[46] Wimmers Ferreira MR, Rodrigo Fernandes R, Freire Assis A, *et al.* Oxidative nanopatterning of titanium surface influences mRNA and microRNA expression in human alveolar bone osteoblastic cells. Int J Biomater 2016; 2016: 9169371. http://dx.doi.org/10.1155/2016/9169371 PMID: 27200092

[47] Sartori EM, Magro-Filho O, Silveira Mendonça DB, *et al.* Modulation of Micro RNA expression and osteoblast differentiation by nanotopography. Int J Oral Maxillofac Implants 2018; 33(2): 269-80. http://dx.doi.org/10.11607/jomi.5372 PMID: 29534118

[48] Kato RB, Roy B, De Oliveira FS, *et al.* Nanotopography directs mesenchymal stem cells to osteoblast lineage through regulation of microRNA-SMAD-BMP-2 circuit. J Cell Physiol 2014; 229(11): 1690-6. http://dx.doi.org/10.1002/jcp.24614 PMID: 24619927

[49] Kato RB, Roy B, De Oliveira FS, *et al.* Erratum: Nanotopography directs mesenchymal stem cells to osteoblast lineage through regulation of microRNA-SMAD-BMP-2 circuit. J Cell Physiol 2016; 231(11): 2548. http://dx.doi.org/10.1002/jcp.25474 PMID: 27420802

[50] Yang J, McNamara LE, Gadegaard N, *et al.* Nanotopographical induction of osteogenesis through adhesion, bone morphogenic protein cosignaling, and regulation of microRNAs. ACS Nano 2014; 8(10): 9941-53. http://dx.doi.org/10.1021/nn504767g PMID: 25227207

[51] Palmieri A, Pezzetti F, Brunelli G, *et al.* Short-period effects of zirconia and titanium on osteoblast microRNAs. Clin Implant Dent Relat Res 2008; 10(3): 200-5. http://dx.doi.org/10.1111/j.1708-8208.2007.00078.x PMID: 18241218

[52] Palmieri A, Pezzetti F, Brunelli G, *et al.* Zirconium oxide regulates RNA interfering of osteoblast-like cells. J Mater Sci Mater Med 2008; 19(6): 2471-6. http://dx.doi.org/10.1007/s10856-008-3386-5 PMID: 18253813

[53] Cossellu G, Motta V, Dioni L, *et al.* Titanium and zirconium levels are associated with changes in microRNAs expression: Results from a human cross-sectional study on obese population. PLoS One 2016; 11(9): e0161916. http://dx.doi.org/10.1371/journal.pone.0161916 PMID: 27611787

[54] Du W, Su L, Zhang N, Wang H. Exosomes derived from preadipocytes improve osteogenic differentiation, potentially *via* reduced miR-223 expression. Mol Med Rep 2019; 19(2): 951-8. PMID: 30569151

[55] Ghassib I, Chen Z, Zhu J, Wang HL. Use of IL-1 β, IL-6, TNF-α, and MMP-8 biomarkers to distinguish peri-implant diseases: A

systematic review and meta-analysis. Clin Implant Dent Relat Res 2019; 21(1): 190-207.
http://dx.doi.org/10.1111/cid.12694 PMID: 30508312

[56] Menini M, Dellepiane E, Baldi D, Longobardi MG, Pera P, Izzotti A. Microarray expression in peri-implant tissue next to different titanium implant surfaces predicts clinical outcomes: A split-mouth study. Clin Oral Implants Res 2017; 28(9): e121-34.
http://dx.doi.org/10.1111/clr.12943 PMID: 27492799

[57] Menini M, Pesce P, Baldi D, *et al.* Prediction of titanium implant success by analysis of microrna expression in peri-implant tissue. A 5-year follow-up study. J Clin Med 2019; 8(6): 888.
http://dx.doi.org/10.3390/jcm8060888 PMID: 31234311

[58] Wu X, Chen X, Mi W, Wu T, Gu Q, Huang H. MicroRNA sequence analysis identifies microRNAs associated with peri-implantitis in dogs. Biosci Rep 2017; 37(5): 1-12.
http://dx.doi.org/10.1042/BSR20170768 PMID: 28864780

[59] Wu X, Gu Q, Chen X, Mi W, Wu T, Huang H. MiR-27a targets DKK2 and SFRP1 to promote reosseointegration in the regenerative treatment of peri-implantitis. J Bone Miner Res 2019; 34(1): 123-34.
http://dx.doi.org/10.1002/jbmr.3575 PMID: 30151888

[60] Kadkhodazadeh M, Jafari AR, Amid R, *et al.* MiR146a and MiR499 gene polymorphisms in Iranian periodontitis and peri-implantitis patients. J Long Term Eff Med Implants 2013; 23(1): 9-16.
http://dx.doi.org/10.1615/JLongTermEffMedImplants.2013007073 PMID: 24266439

[61] Izzotti A, Carozzo S, Pulliero A, Zhabayeva D, Ravetti JL, Bersimbaev R. Extracellular MicroRNA in liquid biopsy: Applicability in cancer diagnosis and prevention. Am J Cancer Res 2016; 6(7): 1461-93.
PMID: 27508091

[62] Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019; 20(2): 71-88.
http://dx.doi.org/10.1038/s41576-018-0071-5 PMID: 30410101

[63] Chen M, Zhao H. Next-generation sequencing in liquid biopsy: Cancer screening and early detection. Hum Genomics 2019; 13(1): 34.
http://dx.doi.org/10.1186/s40246-019-0220-8 PMID: 31370908

[64] Mendioroz M, Martínez-Merino L, Blanco-Luquin I, Urdánoz A, Roldán M, Jericó I. Liquid biopsy: A new source of candidate biomarkers in amyotrophic lateral sclerosis. Ann Clin Transl Neurol 2018; 5(6): 763-8.
http://dx.doi.org/10.1002/acn3.565 PMID: 29928659

[65] Duarte PM, Serrão CR, Miranda TS, *et al.* Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. J Periodontal Res 2016; 51(6): 689-98.
http://dx.doi.org/10.1111/jre.12354 PMID: 26774043

[66] Menini M, Pesce P, Pera F, *et al.* MicroRNAs in peri-implant crevicular fluid can predict peri-implant bone resorption: Clinical trial with a 5-year follow-up. Int J Oral Maxillofac Implants 2020.

[67] Wu K, Song W, Zhao L, *et al.* MicroRNA functionalized microporous titanium oxide surface by lyophilization with enhanced osteogenic activity. ACS Appl Mater Interfaces 2013; 5(7): 2733-44.
http://dx.doi.org/10.1021/am400374c PMID: 23459382

[68] Wu K, Liu M, Li N, *et al.* Chitosan-miRNA functionalized microporous titanium oxide surfaces *via* a layer-by-layer approach with a sustained release profile for enhanced osteogenic activity. J Nanobiotechnology 2020; 18(1): 127.
http://dx.doi.org/10.1186/s12951-020-00674-7 PMID: 32907598

[69] Yan J, Chang B, Hu X, Cao C, Zhao L, Zhang Y. Titanium implant functionalized with antimiR-138 delivered cell sheet for enhanced peri-implant bone formation and vascularization. Mater Sci Eng C 2018; 89: 52-64.
http://dx.doi.org/10.1016/j.msec.2018.03.011 PMID: 29752119

[70] Liu X, Tan N, Zhou Y, *et al.* Delivery of antagomiR204-conjugated gold nanoparticles from PLGA sheets and its implication in promoting osseointegration of titanium implant in type 2 diabetes mellitus. Int J Nanomedicine 2017; 12: 7089-101.
http://dx.doi.org/10.2147/IJN.S124584 PMID: 29026303

[71] Shao D, Wang C, Sun Y, Cui L. Effects of oral implants with miR-122-modified cell sheets on rat bone marrow mesenchymal stem cells. Mol Med Rep 2018; 17(1): 1537-44.
PMID: 29257226

[72] Eskildsen T, Taipaleenmäki H, Stenvang J, *et al.* MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells *in vivo*. Proc Natl Acad Sci USA 2011; 108(15): 6139-44.
http://dx.doi.org/10.1073/pnas.1016758108 PMID: 21444814

[73] Wang Z, Wu G, Feng Z, *et al.* Microarc-oxidized titanium surfaces functionalized with microRNA-21-loaded chitosan/hyaluronic acid nanoparticles promote the osteogenic differentiation of human bone marrow mesenchymal stem cells. Int J Nanomedicine 2015; 10(10): 6675-87.
PMID: 26604744

[74] Si YJ, Ren QH, Bi L. miR-135b-5p regulates human mesenchymal stem cell osteogenic differentiation by facilitating the Hippo signaling pathway. Int J Clin Exp Pathol 2017; 10(7): 7767-75.
PMID: 31966624

[75] Ren H, Huo F, Wang Z, *et al.* Sdccag3 promotes implant osseointegration during experimental hyperlipidemia. J Dent Res 2020; 99(8): 938-48.
http://dx.doi.org/10.1177/0022034520916400 PMID: 32339468

[76] Ma X, Bian Y, Yuan H, *et al.* Human amnion-derived mesenchymal stem cells promote osteogenic differentiation of human bone marrow mesenchymal stem cells *via* H19/miR-675/APC axis. Aging (Albany NY) 2020; 12(11): 10527-43.
http://dx.doi.org/10.18632/aging.103277 PMID: 32434960

[77] Razzouk S, Sarkis R. Smoking and diabetes. Epigenetics involvement in osseointegration. N Y State Dent J 2013; 79(2): 27-30.
PMID: 23691725

[78] Monje A, Asa'ad F, Larsson L, Giannobile WV, Wang HL. Epigenetics: A missing link between periodontitis and peri-implantitis? Int J Periodont Restor Dent 2018; 38(4): 476-7.
http://dx.doi.org/10.11607/prd.2018.4.e PMID: 29999503

[79] Izzotti A, Longobardi M, La Maestra S, *et al.* Release of microRNAs into body fluids from ten organs of mice exposed to cigarette smoke. Theranostics 2018; 8(8): 2147-60.
http://dx.doi.org/10.7150/thno.22726 PMID: 29721069