Epigenetics and its role on implant therapy: a review

L'epigenetica e il suo ruolo nella terapia implantare: review

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Short running title: Epigenetics and implant treatment

One sentence summary of key findings: Implant surfaces influence gene expression in osteoblasts

Key words: epigenomics, microRNAs, implant dentistry, osseointegration, review

ABSTRACT

Aim: To analyse all scientific articles investigating the relations between DNA methylations, histone modifications or micro-RNA production and implant therapy.
Methods: A systematic bibliographical electronic research was carried out on PubMed/Medline, selecting all potentially relevant publications dealing with the influences of implant surface features on gene activation and the influence of epigenetic changes on implant therapy outcomes. Review was conducted according to Cook's principles, with a pre-planned method and using explicit and reproducible criteria. Potentially relevant articles were investigated in a comprehensive search. All presented data were appraised, synthesized, interpreted and discussed.
Results: Of sixty-seven articles found, seventeen met the inclusion criteria. Both in vitro and in vivo studies were included. New implant treatments like KOH alkali-etching, electrolytic etching, ionization, functionalization with miRNAs or anti-microRNAs or osteogenic peptides seem to enhance osteoblast differentiation and genes activation by regulating miRNA production. However, due to the heterogeneity of methodologies and types of cells used, a meta-analysis was not possible to achieve. Besides, epigenetic changes on peri-implant cells induced by smoking still remain unclear.
Conclusions: New titanium implants, functionalized with gene activators, could contribute to develop a new generation of devices, cutting-edge of faster osseointegration. More investigations with other osteoblast-like cell lines, primary cultures, different time points and surfaces functionalized with genetic molecules are needed to get a global comprehension of the epigenetic influence on peri-implant biological mechanisms.

INTRODUCTION

Even though the rehabilitation of partial and total edentulism using dental implants has shown highly satisfactory clinical outcomes[1], 80% of subjects and 50% of implants exhibit mucositis or other biological problems[2,3]. Biological complications around dental implants have been attributed to several factors, from the establishment of a pathogenic microflora[4] to the presence of inflammatory cells close to the implant-abutment interface[5,6]. Despite the amount of existing hypothesis, the genetic mechanisms controlling the peri-implant biological processes remains largely unexplored.

In 2008, at a Cold Spring Harbor meeting, epigenetics was defined as a "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence"[7]. Epigenetic modifications alter the structure of chromatin and influence gene expression without alterations in the sequence of bases. Moreover, epigenetic inheritance influences phenotypes over multiple generations in the absence of any apparent genetic mutation. Epigenetic changes are potentially reversible and can lead to the development and maintenance of cancer, immune or inflammatory diseases. On the other hand, epigenetic influences may play a protective role silencing parasitic DNAs, inactive X chromosome and imprinted genes[8].

Environmental stressors including toxins and microbial exposures[9], adrenaline and psychological stress[10], diet[11], hormones and toxicants[12] can change epigenetic patterns and thereby effect changes in gene activation and cell phenotype.

Epigenetic mechanisms mainly involve DNA methylation, histone modification and mRNA regulation by non-coding RNAs called microRNAs[13] (Fig.1.).

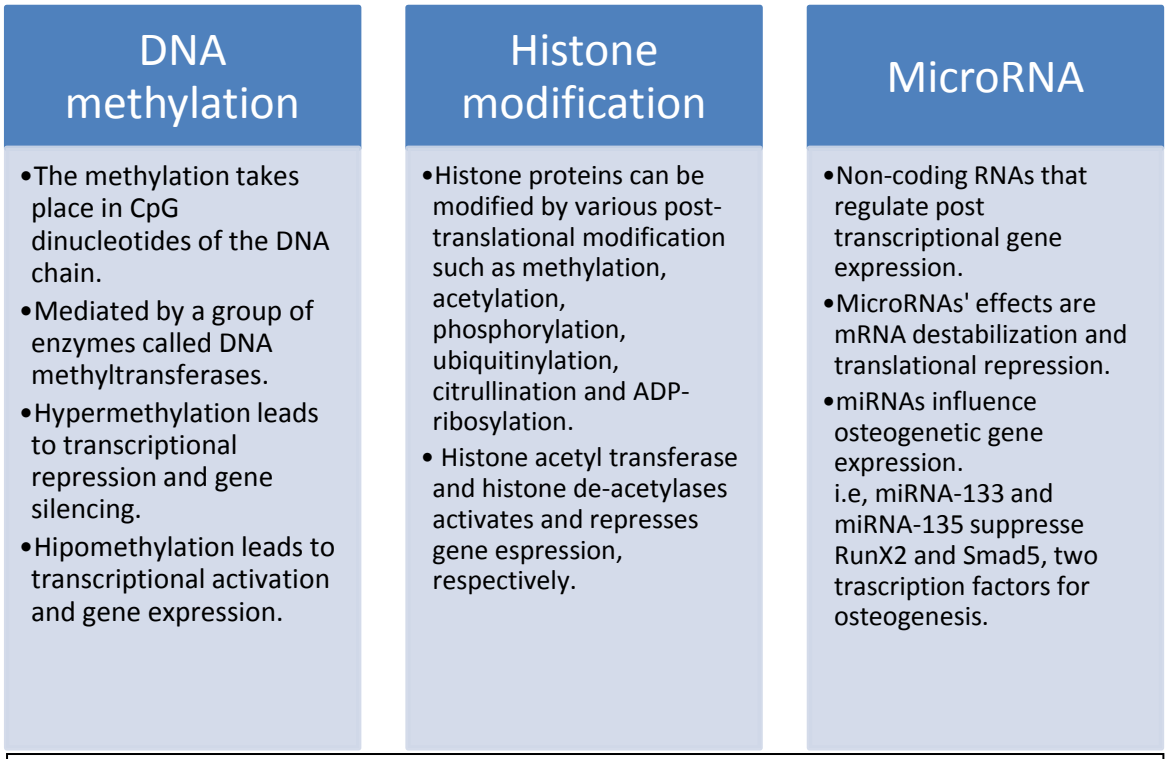

Fig.1. DNA methylation, histone modification and miRNA production influence the DNA expression through chromatin modification and post transcriptional gene repression.

DNA methylation takes place in Cytosine-phosphate-Guanine (CpG) dinucleotides of the DNA chain [14] and consists in a covalent enzymatic transfer of a methyl group from S-Adenosyl Methionine to the C-5 position of a cytosine residues in the CpG island of the promoter region. The methylated DNA sequence in CpG sites causes a more condensed DNA structure leading to transcriptional repression and gene silencing[15].The most known enzyme involved in the DNA methylation is the DNA methyltransferases. When DNA becomes methylated, those methyl groups protrude from the cytosine nucleotides into the major groove of the DNA to displace transcription factors that normally bind to the DNA[16].

Histones can be modified by various post-translational modifications. Acetylation of a core histone results in a more open chromatin's structure that facilitates gene expression. On the other hand, histone deacetylation brings to condensation of chromatin and inhibits gene transcription. Histone methylation can either results in an activated or repressed chromatin state[17]. Has been established that the processes of DNA methylation and histone modification are deeply linked. When the CpG island of a promoter becomes methylated, methyl-CpG-binding-proteins recruit histone deacetylases[18]. A strong electrostatic interaction occurs between the positively charged acetylated histone residues and the negatively charged DNA causing the deacetylation of histone proteins. Due to the condensed nucleosome particle, the gene expression is repressed**.**

One of the most documented epigenetic modifications is the post-transcriptional repression due the production of microRNAs (miRNAs or miR), brief sequences of non-coding RNAs composed by 18-22 nucleotides[19]. They are crucial in regulation of development, proliferation, differentiation, apoptosis and response to different extracellular signals and stress. Besides, they seem to be related with the expression of osteogenic genes like Runx2 and Smad5[20]. MicroRNA pathways regulate gene expression by inducing degradation and/or translational repression of target mRNAs. MicroRNAs influence gene expression by translational repression and gene silencing. If the miRNA production increases, levels of target mRNAs decrease and, therefore, the gene expression is repressed. In the same way, if the miRNA levels decrease, the gene expression is up-regulated. In addition, messengerRNA can bind the promoter of specific microRNAs activating an auto-regulatory feedback loop; thus, when a specific mRNA is up-regulated, the related miRNA is also over-expressed[21]. Each miRNA may target hundreds of mRNAs, and some targets are affected by multiple miRNAs. Probably, 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[19, 22].

Several authors investigated the role of epigenetics in chronic or aggressive periodontitis[23, 24, 25, 26]. Authors demonstrated that the expression levels of cytokines and chemokines [27, 28], toll-like receptors[29], protease-activated receptor[30], interleukin-8[31] and cyclooxygenase-2[24] could be affected by oral bacteria. A recent study documented that the presence of different oral bacteria resulted in differential methylation profile in gingival epithelia[32] while another article demonstrated a hypomethylated oral epithelia in patients with generalized aggressive periodontitis[23].

Despite some available data regarding epigenetics and periodontology, the knowledge on epigenetics related to implant dentistry lack of evidence. Only a few experimental in vivo and in vitro studies are available in literature. Therefore, aim of this review was to evaluate the available evidence investigating the potential effects of DNA methylations, histone modifications or micro-RNA production on implant survival, osseointegration, peri-implant mucositis, perimplantitis or implant-abutment leakage.

MATERIALS AND METHODS

This review was conducted according to Cook's principles, with a pre-planned method and using explicit and reproducible criteria[33]. Potentially relevant articles were investigated in a comprehensive search. All presented data were appraised, synthesized, interpreted and discussed[33].

A systematic bibliographical electronic research was carried out on PubMed/Medline, selecting all potentially relevant publications dealing with the influences of implant surface features on gene activation and the influence of epigenetic changes on implant therapy outcomes.

Search strategy

The systematic search was performed using the following terms and boolean connectors: *((epigenetics) OR (dna methylation) OR (dna methyl transferase) OR (histone deacetylation) OR (histone deacetylase) OR (histone methyl transferase) OR (histone demethylase) OR (micro-rna)) AND ((dental implant) OR (dental implants) OR (implantology) OR (implant dentistry) OR (implant failure) OR (platform shifting) OR (platform switching) OR (implant-abutment connection) OR (osseointegration) OR (mucositis) OR (perimplantitis)).*

The electronic outcome was: *(("epigenomics"[MeSH Terms] OR "epigenomics"[All Fields] OR "epigenetics"[All Fields]) OR ("dna methylation"[MeSH Terms] OR ("dna"[All Fields] AND "methylation"[All Fields]) OR "dna methylation"[All Fields]) OR ((("dna"[MeSH Terms] OR "dna"[All Fields]) AND methyl[All Fields] AND ("transferases"[MeSH Terms] OR "transferases"[All Fields] OR "transferase"[All Fields])) OR (("histones"[MeSH Terms] OR "histones"[All Fields] OR "histone"[All Fields]) AND deacetylation[All Fields]) OR ("histone deacetylases"[MeSH Terms] OR ("histone"[All Fields] AND "deacetylases"[All Fields]) OR "histone deacetylases"[All Fields] OR ("histone"[All Fields] AND "deacetylase"[All Fields]) OR "histone deacetylase"[All Fields]) OR ((("histones"[MeSH Terms] OR "histones"[All Fields] OR "histone"[All Fields]) AND methyl[All Fields] AND ("transferases"[MeSH Terms] OR "transferases"[All Fields] OR "transferase"[All Fields])) OR (("histones"[MeSH Terms] OR "histones"[All Fields] OR "histone"[All Fields]) AND demethylase[All Fields]) OR ("micrornas"[MeSH Terms] OR "micrornas"[All Fields] OR ("micro"[All Fields] AND "rna"[All Fields]) OR "micro rna"[All Fields])) AND (("dental implants"[MeSH Terms] OR ("dental"[All Fields] AND "implants"[All Fields]) OR "dental implants"[All Fields] OR ("dental"[All Fields] AND "implant"[All Fields]) OR "dental implant"[All Fields]) OR ("dental implants"[MeSH Terms] OR ("dental"[All Fields] AND "implants"[All Fields]) OR "dental implants"[All Fields]) OR implantology[All Fields] OR ("Implant Dent"[Journal] OR ("implant"[All Fields] AND "dentistry"[All Fields]) OR "implant dentistry"[All Fields]) OR (implant[All Fields] AND failure[All Fields]) OR (platform[All Fields] AND shifting[All Fields]) OR (platform[All Fields] AND switching[All Fields]) OR (implant-abutment[All Fields] AND connection[All Fields]) OR ("osseointegration"[MeSH Terms] OR "osseointegration"[All Fields]) OR ("mucositis"[MeSH Terms] OR "mucositis"[All Fields]) OR perimplantitis[All Fields])*

Selection of studies

Inclusion criteria: All scientific in vivo and in vitro publications investigating the impact of genetic expression levels on implant rehabilitations were included. According to Mulrow[34], studies with both direct and indirect evidence were also included. No filters like language or time limitation were applied.

Exclusion criteria: Published studies not meeting the inclusion criteria and all those not providing any information concerning dental implant therapy, up-regulation or down-regulation of genes or their products were excluded. All scientific articles resulted from the use of confounding words and not meeting the inclusion criteria were excluded; in example, articles aimed to investigate oncologic issues, mucositis different from peri-implant mucositis, or orthopaedic prosthetic joint were excluded.

Development of the review

This study was conducted over three phases. The first phase was the screening of titles and abstracts. The second phase consisted in the screening of full-text articles. Finally, the third phase consisted in the review of included articles. Two authors (RDG, CDG) worked independently and compared their results at the end of each phase. One author (GPP) supervised each phase in the role of quality control.

Two authors (RDG, GPP) worked on the critical analysis of included studies. For each included study, several data regarding activated genes, influences of implant materials on genes expression, types of implant materials, types of surface modifications, number of patients or types of cells studied were re-wrote.

RESULTS

The electronic search found sixty-seven articles (Fig.2.). During the first phase, which consists in the screening of titles and abstracts, eighteen studies were excluded because aimed to study oral mucositis during radiotherapy or chemotherapy, mucositis in patients with neoplastic diseases like cancer or leukaemia, tolerability of temsirolimus, graft-versus-host disease, fluorouracil toxicity, hypomethylating agent therapy for neoplastic diseases and radiation-induced tissue damages.

Forty-nine articles were downloaded and studied (Fig.2.). During the second phase, which consists in the full-text examination, thirty-two articles were excluded because not investigating the effect of gene expression changing on implant therapy, but evaluating data regarding: oral or neck cancer (seven articles), papilla stem cells (one article), nano-hydroxyapatite (one article), endometrial epithelial cells (one article), systemic influence of titanium and zirconia (one article), chemical drugs for systemic diseases (one article), osteogenic peptides but not associated with implant surfaces (two articles), muscle cells (one article), immunoglobulins (one article), myelodysplastic syndromes (one article), orthopaedic defects (five articles), critical limb ischemia (one article), cementoblasts (one article), bone-ligament cells (one article), calvarial bone defect (one article), genetic effects of adrenaline (one article), evaluation of systemic miRNAs (one article), or publications without information regarding gene expression (one article), or aimed to discuss different methods to detect RNAs from cells (three articles).

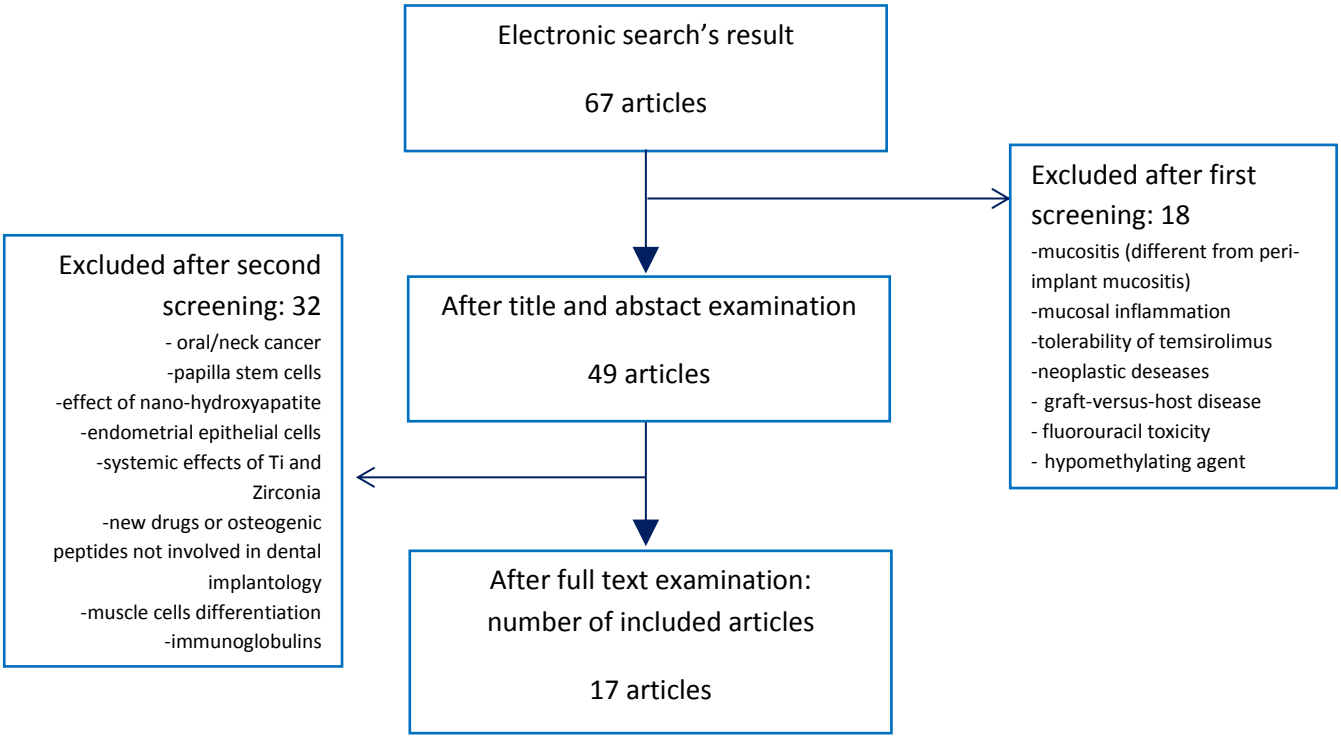

Fig.2. Flow-chart. Search strategy, screening for eligibility and final number of included publications: The electronic search found 67 studies regarding changing in gene expression and implantology. After title and abstract screening, 18 of them were excluded because focussed mainly on mucositis after radio/chemotherapy, mucosal inflammation, neoplastic deseases, hypomethylating agent therapy for neoplastic diseases and radiation-induced tissue damages. 49 articles were downloaded and studied. 32 of them were excluded because not investigating the effect of DNA methylations, histone modifications or micro-RNA production on implant survival, osseointegration, peri-implant mucositis, perimplantitis or implant-abutment leakage. Thus, this systematic review was finalized with

Thus, this review was finalized on seventeen scientific articles (Fig.3.) which were evaluated during the phase three. Fifteen of seventeen articles were in vitro or in vivo studies [35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49]. One was a randomized clinical trial with immune-histochemical analysis [50]. One article was a narrative review [51].

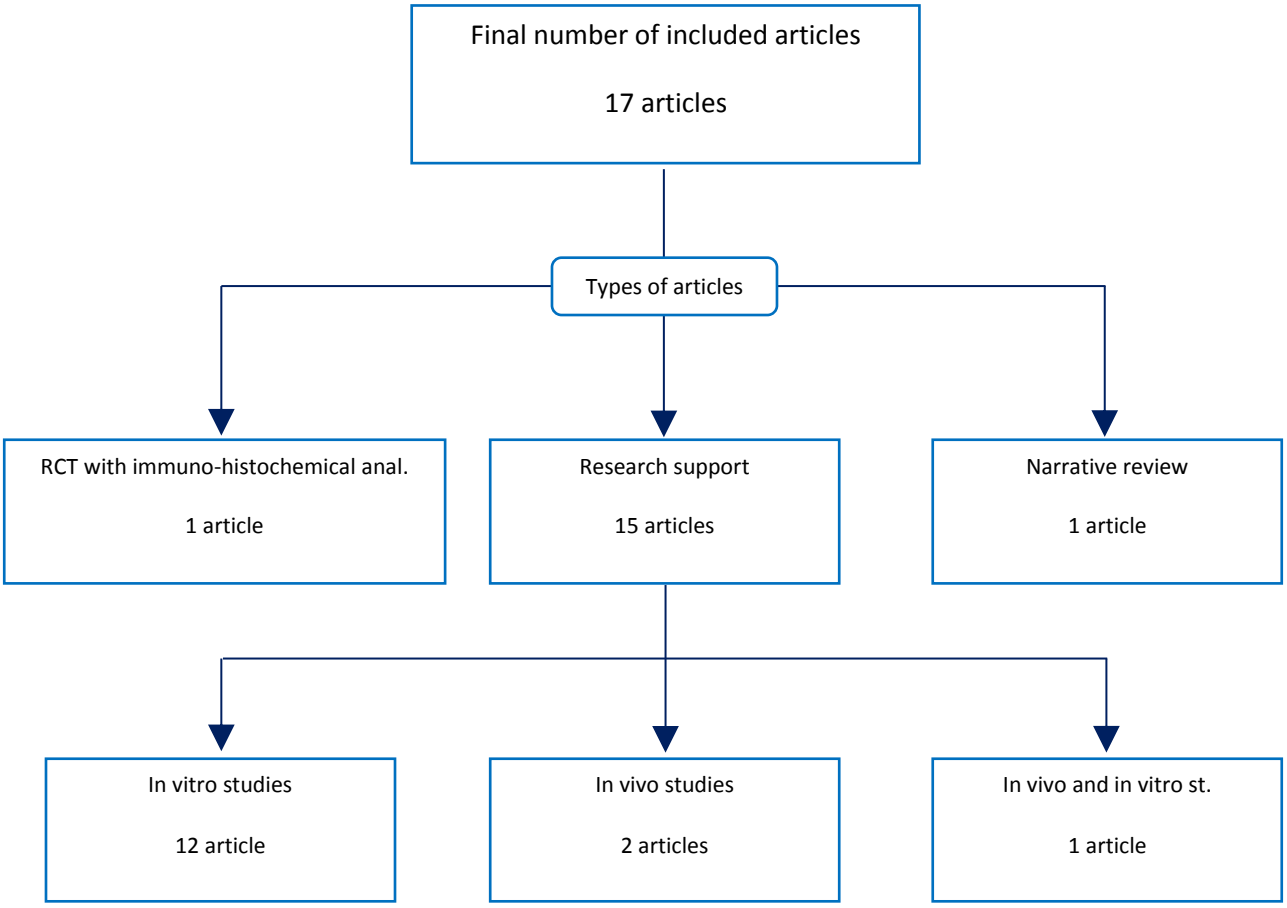

Fig.3. Types of included articles. Out of 17 included articles: 12 were in vitro studies, 2 were in vivo studies, 1 was both an in vivo and in vitro study, 1 was a RCT with immune-histocemical and RNA analyses, 1 was a narrative review. All 17 articles were reviewed.

In vivo and in vitro studies

Fifteen of the seventeen included publications aimed to compare changing in gene expression profiles of cells cultured on different implant surfaces[35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49]. (Tab. 1.)

Eight articles documented the miRNA production[36, 40, 41, 42, 44, 45, 46, 47]. Seven articles didn't provided information on miRNA production[35, 37, 38, 39, 43, 48, 49]. No one of the included articles reported information on chromatin alteration due to DNA methylation or histone modification.

Cell population/Implant surfaces

A large variability of cells selection was tested. Out of fifteen studies, twelve were in vitro studies: four articles used MG-63 osteoblast like cells for cell culture[37, 42, 45, 46] while other four studies used human alveolar stem cells from human donors[40, 41, 44, 48]. Human mesenchymal stem cells[49], dental pulp stem cells[36], rat bone marrow cells[47], marrow stromal cells obtained from iliac crest[35] were used in one study each.

Two of fifteen articles were in vivo studies aimed to investigate genetic expression levels of implant-adherent cells taken from rats' tibia[43] and from humans[38].

One of fifteen articles was both an in vitro and in vivo study using MG-63 cells and implant adherent cells from beagle dogs' tibias[39].

Iliac crest cells grown on KOH alkali-etched, NaOH alkali-etched or not-etched surfaces

One article challenged experimental titanium alkali-etched surfaces with human bone marrow stromal cells[35]. Cells were cultured on KOH alkali-etched, on NaOH alkali-etched or on not-etched surfaces. Bone sialo protein and matrix metalloprotease 2 levels were found enhanced on alkali-etched metals compared to not-etched surfaces. Higher expression of osteogenic genes was found in cells grown on KOH alkali-etched surfaces.

Dental pulp stem cells grown on ionized or not ionized SLA surfaces

One study evaluated the differentiation and gene activation of dental pulp stem cells grown on ionized SLA (sandblasted acid etched) surfaces, or on not ionized surfaces[36]. Authors found that ionized surfaces induced a more marked cell differentiation. Besides, higher levels of Runx-2, Smad5 and osteocalcin were found in cells grown on ionized surfaces.

MG-63 cells grown on electrolytic etched, sandblasted acid etched or machined surfaces

One article cultured MG-63 cells on electrolytic etched, sandblasted acid etched or machined surfaces[37]. Levels of alkaline phosphatase, osteocalcin, Runx-2, ospeopontin and collagen type Iα1 were higher in electrolytic group.

Human alveolar bone cells grown on SLA, modified SLA or smooth surfaces

Human alveolar bone cells were grown on SLA (saldblasted acid etched), hydrophilic SLA or smooth surfaces[40]. Authors found that the majority of miRNAs were down-regulated in response to the SLA and modSLA surfaces compared to the SMO one, with only relatively changes found between SLA and modSLA.

Alveolar stem cells grown on microarc oxidated surface or chitosan/hyaluronic acid surface with miRNA-21

Human bone marrow mesenchymal cells were cultured on microarc oxidated surface or on CS/HA/miR-21 surfaces[41]. After PCR analysis, levels of collagen type IIIα1, osteocalcin, Runx-2, ospeopontin and collagen type Iα1 were higher in cells grown in contact with CS/HA/miR-21 surfaces.

MG-63 cells grown on zirconia or machined titanium surfaces

One article evaluated miRNA production and gene expression of MG-63 cells grown on zirconia or machined titanium surfaces[42]. Osteogenic gene activation were higher in machined titanium group and bone morphogenic protein-4 and -7 were more expressed in cells grown on titanium surfaces.

Human alveolar bone cells grown on nanotextured, nano-submicrotextured, rough microtextured or smooth surfaces

In one article, human alveolar cells were cultured on titanium surfaces with different rough scale[44]. It was not possible to define a certainly better surface. However, the nanotextured surface group showed the highest alkaline phosphatase production while the microtextured surface group had the greatest amount of calcium and mineralized nodules.

MG-63 cells grown on zirconia surfaces

In one article, MG-63 cells were cultured on zirconia wells and, than, their miRNA production was evaluated[45]. Eighteen miRNAs involved in the repression of osteogenic genes were found up-regulated while only three miRNAs were down-regulated. Authors did not provide information about control groups.

MG-63 cells grown on anatase surfaces

One study evaluated the osteogenic gene activation and differentiation of MG-63 cells grown on anatase surfaces[46]. Authors found that nine miRNAs were up-regulated and ten down-regulated. Due to down-regulated miRNAs, three genes were more expressed and they are fibrillin 1, insulinlike growth factor-binding protein4 and calcitonin. Due to up-regulated miRNAs, three genes were repressed and they are collagen 9α2, ADAMTS4 member of metalloproteinases and alkaline phosphatase. Authors did not provide information about control group.

Rat bone marrow cells grown on surfaces functionalized with miRNA-29b, antimiRNA138 or without functionalization

One article cultured rat bone cells on microporus titanium oxide surfaces with or without functionalization[47]. For bone morphogenic protein, osteocalcin, osterix, and Runx2, the antimiR-138 functionalized surface induced higher expression. For collagen type Iα1, the miR-29b functionalized surface induced higher expression than using antimiR-138, whereas this trend is reversed after 14 days of culture. The miR-29b functionalized surface induces higher expression of alkaline phosphatase.

Human alveolar bone cells grown on microsandblasted, macrosandblasted or machined surfaces

In one article, primary cultures of osteoblasts derived from human mandibular bone were cultured on titanium microsandblasted, macrosandblasted or machined surfaces[48]. Gene activation was evaluated with RT-PCR analysis. All blasted surfaces showed higher DNA activation than the machined surfaces. However, TGFβ2, osteopontin, Runx-2 and bone sialoprotein levels were higher in macrosandblasted groups.

Human mesenchymal stem cells and human osteoblasts grown on titanium-alluminium-vanadium surfaces with different rough scale or on polystyrene surface

One study evaluated gene activation of human mesenchymal stem cells and human osteoblasts grown on titanium-alluminium-vanadium surfaces[49]. Ti6A14V #9 surface showed greater ALP, osteocalcin, VEGF-A, FGF-2, bone morphogenic protein and osteoprotegerin production. Integrin expression also varied with the surface. MessengerRNAs for all integrin subunits except ITGA-5 were higher when cells were cultured on Ti6A14V substrates than on polystyrene ones.

Human cells adherent to surgically placed tioblast or osseospeed implants

One article was performed placing tioblast and osseospeed implants in human smoker and nonsmoker patients. Implants were harvested after two and four days of submerged healing, and implant adherent cells were studied[38]. Interestingly, authors found that the variable of time influences gene expression more that the effect of different surfaces or nicotine. Similar trends in gene expression were noted in implant-adherent cells regardless of implant surface and smoking status.

Rat cells adherent to surgically placed nano- or micro-rughened implants

In one in vivo study, nano- or micro roughened implants were placed in rats' tibias and were harvested after two and four days of submerged healing[43]. Implant adherent cells were studied in order to investigate the influence of different surfaces on gene expression. Significant differences at the gene level were not noted when comparing the two implant surfaces at each timepoint. However, genes were differentially regulated at different days for both implant surfaces.

MG-63 cells and beagle cells grown on implants with or without nanotubes

Only one study evaluated the genetic effect of implant surfaces both in vivo and in vitro[39]. The in vitro experiment was conducted with MG-63 cells while the in vivo phase was performed placing and harvesting implants in beagle tibias. The surfaces compared were SLA without nanotubes, SLA with nanotubes of 30nm, SLA with nanotubes of 50nm and SLA with nanotubes of 80nm. SLA+80nm showed to induce the highest gene expression compared with the other sizes of nanotubes. SLA+80nm also showed to induce the formation of the higher number of filopodia, lamellipodia and cellular extensions.

Comparative analysis

Nine studies found that specific surfaces induced osteoblast differentiation, expression of osteogenic genes or repression of those miRNAs which down-regulate osteogenic genes[35, 36, 37, 39, 41, 42, 47, 48, 49] while six articles did not indicate a better surface[38, 40, 43, 44, 45, 46]. Two articles documented similar trends in gene expression regardless of implant surface; they found that the effect of time influences gene expression more than the surfaces[38, 43]. Only two publications studied the genetic effect of zirconia surfaces. In the first study, zirconia surfaces were associated with worse genetic activation than machined titanium ones[42]. In the second one the number of miRNAs up-regulated was much higher than the number of miRNAs down-regulated[45].

Randomized clinical trial

One article out of the seventeen evaluated in the present review was a randomized clinical trial[50]. Purpose of the study was to investigate the expression levels of osteogenic genes after two different treatments (Tab.2.). Twenty-six patients with one buccal implant dehiscence defect each were randomly treated with particulate allograft bone (control) or particulate allograft bone and pericardium membrane (test). After 6 months of healing, analysis of bone volume and gene expression was performed. After bone volume evaluation, greater volume levels were found in the test group (treatment with bone and membrane) than in control group (treatment with bone without membrane). Then, bone biopsies were harvested and processed. Genetic expression of osteogenic genes was evaluated with an immune-histochemical analysis. Positive periostin (POSTN), sclerostin, and runt-related transcription factor-2 (Runx2) immunoreactivities were detected in both the control and test groups without statistically differences. Tartrate-resistant acid phosphatase (TRAP) positive was mostly noted in the control group. Analysis of DNA methylation, histone modifications and miRNA production was not provided.

Narrative review

A narrative review, investigating the epigenetic effect of smoking and diabetes on osseointegration, was also included and evaluated in the present study[51] (Tab.3.). It resulted that global DNA methylation is

influenced by smoking behaviour. Smoking resulted to have an impact on bone metabolism and estrogen production, leading to a phenotype of low-bone mineral density. In fact, gene expression of bone matrix proteins, including osteopontin, Type II collagen, bone morphogenetic protein-2 and osteoprotegerin resulted significantly down-regulated by smoking components.

Diabetes was associated with decreased gene expression of bone matrix proteins (osteocalcin, PTHrP), of transcription factors involved in osteoblast differentiation (Runx2, osterix) and of osteoprotegerin through the receptor activator of nuclear factor-kappaB ligand mRNA ratio[52]. Histone lysine methylation and other post transcriptional changes, has been implicated in aberrant gene regulation associated with the pathology of diabetes and its complications. Moreover, serum osteocalcin levels have been found to be significantly increased in patients with Type II diabetes[53]. As a result, alteration of bone healing in diabetes and epigenetic modifications are deeply connected.

DISCUSSION

Genetic information is encoded not only by the linear sequence of DNA, but also by epigenetic modifications[9].

Some miRNAs are key regulators for the development of osteoblasts by targeting anti-osteogenic factors such as histone deacetylase-4 and modulating bone extracellular matrixproteins (ECM)[54]. MicroRNAs induce degradation and translational repression of target mRNAs. Therefore, production of miRNAs and post-transcriptional gene expression are inversely correlated. Indeed, antimiR-138 has been shown to enhance in vivo bone formation by inhibiting miRNA-138[55].

To better understand the epigenetic influence on implant therapy, this review was conducted to evaluate the available evidence investigating the potential effects of DNA methylations, histone modifications or micro-RNA production on implant survival, osseointegration, peri-implant mucositis, perimplantitis or implant-abutment leakage.

It has been demonstrated that surface roughness, cellular attachment, and osteoblast activity are directly correlated[56]. Sandblasted and acid etched surfaces (SLA), largely used in today's implants, influence positively genetic expression if compared with smooth surfaces, machined surfaces and zirconia surfaces. To achieve even better clinical results and to accelerate the healing period required for the prosthetic loading, several innovative methods for implant surface modification were studied, including alkali-etching[35], ionization[36], electrolytic etching[37], surfaces with nanotubes[39], isotonic solution and N_2 treatments [40], surfaces functionalized with miRNAs[41, 47], hydrofluoric treatments[43], anatase coating[46] and others. Most of the authors documented better results with processed surfaces in terms of osteoblast differentiation, expression of osteogenic genes or repression of those miRNAs which down-regulate osteogenic genes [35, 36, 37, 39, 41, 43, 44, 45]. In light of these findings, surface presented could represent a pivotal advancement to obtain a faster osseontegration, more predictable post-extractive implants or immediate loading. However, the reviewed articles are not immune to bias.

Type of studied cells

First, most of the reported data derived from studies performed on MG-63 cells or animal cells, which are not normal human osteoblasts. Notwithstanding this, the advantages of using a cell line, like MG-63, are

related to the fact that the reproducibility of the data is higher because there is not the variability in the patient studied. Primary cell cultures provide a source of normal cells, but they also contain contaminating cells of different types and cells in variable differentiation states.

Second, most of the reviewed studies are in vitro studies[35, 36, 37, 40, 41, 42, 44, 45, 46, 47, 48, 49]. On one hand, in vitro studies are not influenced by systemic interindividual variability of human patients; indeed, adrenaline released after a psychological stress may inhibit osteogenic differentiation through histone acetylation and down-regulation of miR-21[10]. On the other hand, in vivo human studies, with a large sample and long follow-up are absolutely required for a better comprehension of epigenetic influences.

Effect of time

The influence of time affects gene expression more than different surfaces[38, 43]. Recently, in an in vivo study, similar trends in gene expression were noted in implant-adherent cells regardless of implant surface and smoking status only if they were evaluated at the same early time point. However, when the time-course was evaluated, statistically differences in genetic patterns was identified.

In the light of these considerations, all further studies have to include two or more time points to evaluate how big is the influence of time if compared with the influence of different surfaces.

Smoking and nicotine

Thalji and co-workers, in their in vivo study, noted that the impact of smoking did not occur at early time points[38]. Potentially, detrimental effects were likely to occur at a later stage and upon exposure of the implants to the oral environment. Implants submerged and never exposed to the oral environment prior to retrieval did not suffer the negative effect of nicotine. The absorption of nicotine through the oral mucosal tissues is pH dependent. Since the pH of tobacco smoke in most cigarettes is acidic, nicotine is primarily ionized resulting in minimal absorption of nicotine from cigarette smoke. Thalji's data indicate that gene expression profiles of submerged implant adherent cells were similar among smokers and non-smokers.

Interestingly, the effects of smoking may have been negated or delayed by the implant surface topography. In a long-term retrospective study, Balshe et al. compared the survival rates of smooth and rough surface dental implants among smokers and non-smokers[57]. Smoking was identified as significantly associated with implant failure only in the smooth surface group. Similar results were reported by Sayardoust et al. in patients with periodontitis, where the smokers' likelihood ratio for implant failure was 6.40 for smooth surface implants and 0 for oxidized implants[58].

Yamano et al, in a rat model, showed that while no differences were noted on bone-to-implant contact after 2 weeks of systemic nicotine exposure, significant differences were observed after 4 weeks[59]. They noted significantly decreased expression of Bmp2, Bsp, Opn, Col2, Cbfal in peri-implant tissues in rats exposed to nicotine compared with controls at 4 weeks. This demonstrates that the systemic effects of nicotine on peri-implant healing occur at later stages. Therefore, future studies should include a period longer than 2 weeks to evaluate the smoking's effects on osseointegration.

All original articles investigating the role of genetic changes on implant therapy outcomes present an indirect evidence[34]. Besides, due to the heterogeneity of methods, of types of cells used and of evaluation

time points, a meta-analysis was not possible to achieve. Even if all included articles agreed with the idea that implant features influence osteoblast differentiation, only a weak evidence could be obtained in the present systematic qualitative review.

CONCLUSION

This systematic qualitative review shows that positive genetic stimulation is associated with surface treatments like alkali-etching, ionization, electrolytic etching, surfaces with nanotubes, isotonic solution and N_2 treatments; osteogenic inhibition was found around zirconia surfaces and anatase coating.

Micro and nanoporous surfaces may provide a larger surface area for loading miRNAs, anti-miRNAs, peptides or other osteogenic drugs. Implant surface could be used like a carrier to position functional groups or biomolecules contributing to achieve a faster osseointegration.

Treated surfaces, early checkpoints and submerged healing seem to be less related to the negative epigenetic effects of smoking.

More investigations with other osteoblast-like cell lines, primary cultures, different time points and surfaces functionalized with genetic molecules are needed to get a global comprehension of the epigenetic influence on peri-implant biological mechanisms.

Conflict of interest: The author declared no conflict of interest.

Tab.1. EFFECTS OF DIFFERENT SURFACES ON GENETIC EXPRESSION							
Author	**Year**	**Type**	**Surfaces tested**	**Type of cells**	**Evaluation of miRNAs**	**Gene expression and molecular mechanism**	**Conclusion**
Giannoni P.	2009	In vitro, research support.	KOH alkali-etched; NaOH alkali-etched; not etched surface.	Bone marrow stromal cells from iliac crest.	not provided	High expression of BSP was found on KOH etched surfaces.	KOH modifications seem to allow the best osteogenic differentiation of human mesenchymal stromal cells.
Iaculli F.	2016	In vitro, research support.	SLA surface without ionization (control); Ionized SLA surface (test).	Dental pulp stem cells.	provided	miRNA-133a, miRNA-133b and miRNA-135 influenced the expression of Runx2 and Smad5 genes.	Ionized sandblasted and acid-etched surface seemed to markedly enhance the development and differentiation of osteoblast cells.
Meng W.	2013	In vitro, research support.	EE; SLA; M.	MG-63 osteosarcoma cells.	n.p.	Gene expression is related to features of implant surface and the level of osteoblast differentiation.	Hierarchical micro-/nanostructured titanium surface treated by EE enhanced the ALP, OCN, Runx2, OPN activity, and COL1 mRNA gene expression of osteoblast.
Thalji G.	2015	In vivo (human).	TiO versus OS; smoker versus non-smoker.	Implant adherent cells (alveolar bone cells).	n.p.	The variable of time influences the gene expression more that the effect of nicotine. Modified surfaces could soften the negative effect of nicotine. High number of genes has been investigated.	At early time points, similar trends in gene expression were noted in implant-adherent cells regardless of implant surface and smoking status.
Ding X.	2015	In vitro and in vivo (animal, beagle dog).	SLA + 30nm; SLA + 50nm; SLA + 80nm; SLA without nanotubes.	(in vitro study) MG-63 osteosarcoma cells. (in vivo study) beagles' tibias cells.	n.p.	Nanotube diameters influence cell phenotype (filopodia and lamellipodia) and ALP, Runx2 and OCN genes expression.	SLA + 80 nm surface is the most favourable for promoting the activity of osteoblasts and early bone bonding.
Chakravorty N.	2012	In vitro, research support.	SLA; ModSLA; SMO.	Human alveolar bone cells.	provided	Highest level of bone expression in modSLA and SLA. 35 different types of miRNA were downregulated in modSLA, and 32 in SLA surfaces. High number of genes and miRNAs has been investigated.	The majority of miRNA were down-regulated in response to the SLA and modSLA surfaces compared to the SMO one, with only relatively changes found between SLA and modSLA.
Wang Z.	2015	In vitro, research support.	MAO; CS/HA/miR-21.	Human bone marrow mesenchymal stem cells (alveolar cells).	Provided only miRNA-21	miRNA-21 induces upregulation of osteogenesis-related genes like COL1, COL3, Runx2, OPN, and OCN.	Titanium surfaces functionalized with miRNA-21 presented a significantly higher expression of osteogenic genes.
Palmieri A.	2008a	In vitro, research support.	Machined Ti; Zirconia.	MG-63 cells.	provided	Six miRNAs were found up-regulated in zirconia compared to Titanium (miR-214,miR-337,miR-423,miR-339,miR377, and miR-193b), and four down-regulated	Ti surfaces could provide some advantages to earlier osteogenesis useful for immediate loading.

						(miR-143, miR-17-5p, miR-24, and miR-22). Bone related genes BMP4 and 7 were more expressed in osteoblasts exposed to Ti surface.	
Thalji G.	2013	In vivo (animal, rat tibia model).	AT-1; AT-2.	Rat's tibia cells.	n.p.	Significant differences at the gene level were not noted when comparing the two implant surfaces at each timepoint. However, genes were differentially regulated at day 4 vs.day 2 for both implant surfaces. High number of genes and miRNAs has been investigated.	The number of genes that were associated with the inflammatory or immune response category was greater for AT-1 than AT-2.
Wimmers Ferreira M.R.	2016	In vitro, research support.	Nanotextured; NanoSubmicrotextured; Rough microtextured; Smooth surface.	Human alveolar bone cells.	provided	The nanotextured surface group showed the highest alkaline phosphatase activation. The rough microtextured surface group had the greatest amount of calcium produced. NOTCH1 gene increased its expression in nanosubmicrotexture surfaces.	Oxidative nanopatterning of titanium surfaces induces changes in the metabolism of osteoblastic cells and cell responses.
Palmieri A.	2008b	In vitro, research support.	Zirconium Oxide; Control group not provided.	MG-63 cells.	provided	Zirconia disks up-regulated 18 miRNAs and down-regulated 3 miRNAs related to osseogenetic genes. The most notable osseogenic genes influences by zirconia are NOG, SHOX, IGF1, BMP1 and FGFR1.	Zirconium oxide surfaces influence genic expression, however speculations about clinical outcomes of zirconia implants were not provided.
Palmieri A.	2008c	In vitro, research support.	Anatase coating; Control group not provided.	MG-63 cells	provided	There were 9 up-regulated miRNAs and 10 downregulated miRNAs. PRDX1, COL9A2, ADAMTS4, SHOX and ALPL, AMBN, TUFT1 were up-regulated. PHEX, FBN1, IGFBP4, CALCA, TFIP1 and PTH were down-regulated.	Anatase colloidal solution regulates osteogenic genes and miRNAs, however clinical speculations were not provided.
Wu K.	2013	In vitro, research support.	MAO+miR29b; MAO+antimiR138; Nacked MAO surface.	Rat bone marrow cells.	provided	For the genes BMP, OCN, OSX, and Runx2, the antimiR-138 functionalized surface induces higher expression. For COL1, the miR-29b functionalized surface induces higher expression than using antimiR-138, whereas this trend is reversed after 14 days of culture. The miR-29b functionalized surface induces higher expression of ALP.	MicroRNA-29b enhances osteogenic activity and antimicroRNA-138 inhibits miR-138, inhibitors of endogenous osteogenesis. Clear stimulation of osteogenic process was observed, in terms of up-regulating osteogenic expression and enhancing alkaline phosphatase production, collagen secretion and mineralization.
Marinucci L.	2006	In vitro, research support.	Machined; Microsandblasted; Macrosandblasted.	Human alveolar bone cells.	n.p.	All blasted surfaces showed significantly higher DNA synthesis than the machined surfaces. Other mRNA transcripts were increased in osteoblasts cultured on rough	Macro-sandblasted titanium showed best results in favouring osteoblast differentiation.

						titanium surfaces, particularly the macrosandblasted surface.	
Olivares-Navarette R.	2014	In vitro, research support.	TCPS; Ti6A14V #5; Ti6A14V #9; Ti6A14V #12.	Human mesenchymal stem cells; human osteoblasts.	n.p.	Test #9 showed greater ALP activity, OCN and osteoprotegerin production. BMP2 and BMP4 were highest in cultures grown on #9, as were VEGF-A and FGF-2. Integrin expression also varied with the surface. mRNAs for all integrin subunits except ITGA-5 were higher when cells were cultured on test substrates than on TCPS.	Osteoblast lineage cells are sensitive to specific micro/nanostructures.

List of abbreviated surfaces:

SLA: sandblasted acid-etched Titanium surface
modSLA: SLA surface with an N_2 protection and stored in an isotonic saline solution
MAO: microarch-oxidated titanium
CH/HA/mR-21: chitosan/hyaluronic acid surface with miRNA-21
Ti6A14V: Micron-scale rough Titanium alloy
EE: micro/nanostructured surface electrolytic etched
M: Machined surface
TiO: TiOBlast; surface blasted with TiO_2
OS: Osseospeed; surface blasted with TiO_2 then treated with hydrofluoric acid
SMO: smooth polished surface
AT-1: oxalic acid and hydrofluoric acid treated surface
AT-2: oxalic acid treated surface
Ti6A14V: Micron-scale rough titanium alloy (# indicates different dimension of roughness parameters).
TCPS: polystyrene surface

List of abbreviated genes and proteins:

ALP: alkaline phosphatase
BMP: bone morphogenic protein
BSP: bone sialoprotein
Runx: runt-related transcription factor
OCN: osteocalcin
PTHrp: Parathyroid hormone-related protein
PTH: Parathyroid hormone
BIC: bone-implant contact
POSTN: periostin related factor
VEGF: vascular endothelial growth factor
COL1: collagen type Iα1
COL3: collagen type IIIα1
OPN: osteopontin
FGF: fibroblast growth factor
ITGA: integrin subunit
SHOX: Short stature HOmeoboX-containing gene
IGF: insulin-like grow factor
NOG: noggin gene
PRDX: peroxiredoxin
ADAMTS: gene encoding for disintegrin and metalloproteinase with thrombospondin motifs
AMBN: ameloblastin
PHEX: Phosphate-regulating neutral endopeptidase
FBN: fibrillin
CALCA: Calcitonin Related Polypeptide Alpha
TFIP: Tissue factor pathway inhibitor
OSX: Osteoblast-specific transcription factor, osterix

Tab.1. Effects of different surfaces on genetic expression. Types of cells used, tested surfaces, gene influenced and conclusions of different authors are presented.

Tab.2. COMPARISON OF CLINICAL TREATMENTS								
Author	**Year**	**Type**	**N° of patients**	**Bone defect**	**Clinical treatments tested**	**Evaluat. of miRNAs**	**Results**	**Conclusion**
Fu J.H.	2015	RCT with immuno-histochemical and RNA analyses.	26 patients 13 (test) 13 (contr)	Buccal implant dehiscences in maxilla.	Defects treated with bone particulate allograft (control) or bone and pericardium membrane (test).	Not provided.	No significant differences in POSTN, Runx2 and VEGF expressions between test and control groups were found. Epigenetic mechanism was not provided.	Bone preserved with the membrane was bigger in volume but less mineralized and more fibrous. No significant differences in mRNA expression between the two groups were found.

Tab.2. Comparison of genetic stimulation of two clinical treatments for implant dehiscence. Dehiscences treated with both bone and membrane showed bigger bone volume and immature bone. Dehiscences treated with only bone showed fasted healing and lower bone volume. No significant differences were found in RNA analyses.

Tab.3. NARRATIVE REVIEW			
Author	**Year**	**Type**	**Mechanism**
Razzouk S.	2013	Narrative review.	Smoking down-regulates osteopontin, Type 2 collagen, BMP-2 and osteoprotegerin. Diabetes influences the expression of PTHrP, OCN, Runx2 and OSX.

Tab.3. Narrative review about the epigenetic influence of smoking and diabetes on osseointegration. Smoking and diabetes can lead to a low-quality bone and altered microarchitecture through the histone deacetylation and DNA methylation of different genes.

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REFERENCES

1. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J Clin Periodontol. 2002;29 Suppl 3:197-212.
2. Roos-Jansåker A-M, Lindahl C, Renvert H, Renvert S. Nine to fourteen year follow-up of implant treatment. Part I: implant loss and associations to various factors. J Clin Periodontol 2006a;33:283–9.
3. Roos-Jansåker A-M, Lindahl C, Renvert H, Renvert S. Nine to fourteen year follow-up of implant treatment. Part II: presence of peri-implant lesions. J Clin Periodontol 2006b; 33:290–5.
4. Lindhe J, Meyle J. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontol. 2008 Sep;35(8 Suppl):282-5.
5. Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, Cochran DL. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res. 2006 May;85(5):473-8.
6. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. J Periodontol. 2003 Mar;74(3):346-52.
7. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev. 2009;23(7):781-83.
8. Fazzari MJ, Greally JM (2004). Epigenomics: beyond CpG islands. Nat Rev Genet 5:446-455.
9. Barros SP, Offenbacher S. Epigenetics: Connecting environment and genotype to phenotype and disease. J Dent Res. 2009;88:400-08.
10. Chen D, Wang Z. Adrenaline inhibits osteogenesis via repressing miR-21 expression. Cell Biol Int. 2017 Jan;41(1):8-15.
11. Bishop KS, Ferguson LR. The interaction between epigenetics, nutrition and the development of cancer. Nutrients. 2015 Jan 30;7(2):922-47. doi: 10.3390/nu7020922.
12. Martos SN, Tang WY, Wang Z. Elusive inheritance: Transgenerational effects and epigenetic inheritance in human environmental disease. Prog Biophys Mol Biol. 2015 Jul;118(1-2):44-54. doi: 10.1016/j.pbiomolbio.2015.02.011. Epub 2015 Mar 16.
13. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004; 429:457-63.
14. Ji-Yun Seo, Yoon-Jung Park, Young-Ah Yi, Ji-Yun Hwang, In-Bog Lee, Byeong-Hoon Cho, et al. Epigenetics: General characteristics and implications for oral health. Restor Dent Endod. 2015;40(1):14-22.
15. Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nat Rev Genet. 2009;10:805-11.

16. Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM (2000). CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature 405:486-489.

17. Bayarsaihan D. Epigenetic mechanisms in inflammation. J Dent Res. 2011;90:917.

18. Ari G, Cherukuri S, Namasivayam A. Epigenetics and Periodontitis: A Contemporary Review. J Clin Diagn Res. 2016 Nov;10(11):ZE07-ZE09.

19. Shookhoff JM, Gallicano GI. The emerging role of microRNAs in adult stem cells. Turksen K, editor. Stem cell biology and regenerative medicine, vol 1, Humana Press: Springer; 2011. pp 57–94.

20. Fakhry M, Hamade E, Badran B, Buchet R, Magne D. Molecular mechanisms of mesenchymal stem cell differentiation towards osteoblasts. World J Stem Cells 2013;5:136–148.

21. Sylvestre Y, De Guire V, Querido E, et al. An E2F/miR-20a autoregulatory feedback loop. J Biol Chem 2007; 282:2135– 2143.

22. Gordeladze JO, Reseland JE, Duroux-Richard I, Apparailly F, Jorgensen C. From stem cells to bone: phenotype acquisition, stabilization, and tissue engineering in animal models. LAR J 2009; 51:42–61.

23. Andia DC, de Oliveira NF, Casarin RC, Casati MZ, Line SR, de Souza AP. DNA methylation status of the IL-8 gene promoter in aggressive periodontitis. J Periodontol. 2010;81:1336-41.

24. Zhang S, Barros SP, Niculescu MD, Moretti AJ, Preisser JS, Offenbacher S. Alteration of PTGS2 promoter methylation in chronic periodontitis. J Dent Res. 2010;89:133–7.

25. Zhang S, Crivello A, Offenbacher S, Moretti A, Paquette DW, Barros SP. Interferon-gamma promoter hypomethylation and increased expression in chronic periodontitis. J Clin Periodontol. 2010;37:953-61.

26. Miao D, Godovikova V, Qian X, Seshadrinathan S, Kapila YL, Fenno JC. Treponema denticola upregulates MMP-2 activation in periodontal ligament cells: interplay between epigenetics and periodontal infection. Arch Oral Biol. 2014 Oct;59(10):1056-64.

27. Yin L, Sanson B, An J, Hacker BM, Silverman GA, Dale BA, et al. Differential effects of peripopathogens on host protease inhibitors SLP1, elafin, SCCA1, SCCA 2. J Oral Microbiol. 2010;2:1–12.

28. Chung WO, An JY, Yin L, Hacker BM, Rohani MG, Dommisch H, et al. Interplay of proteaseactivated receptors and NOD pattern recognition receptors in epithelial innate immune responses to bacteria. Immunol Lett. 2010;131:113–9.

29. Darveau RP, Pham TT, Lemley K, Reife RA, Bainbridge BW, Coats SR, Howald WN, Way SS, Hajjar AM. Porphyromonas gingivalis lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. Infect Immun. 2004 Sep;72(9):5041-51.

30. Chung WO, Hansen SR, Rao D, Dale BA. Protease-activated receptor signaling increases epithelial antimicrobial peptide expression. J Immunol. 2004;173:5165–70.

31. Oliveira NF, Damm GR, Andia DC, Salmon C, Nociti FH, Jr, Line SR, et al. DNA methylation status of the IL8 gene promoter in oral cells of smokers and non-smokers with chronic periodontitis. J Clin Periodontol. 2009;36:719–25.

32. Yin L, Chung WO. Epigenetic regulation of human ß-defensin 2 and CC chemokine ligand 20 expression in gingival epithelial cells in response to oral bacteria. Mucosal Immunol. 2011;4:409– 19.

33. Cook DJ, Mulrow CD, Haynes RB. Systematic reviews: synthesis of best evidence for clinical decisions. Ann Intern Med. 1997 Mar 1;126(5):376-80.

34. Mulrow C, Langhorne P, Grimshaw J. Integrating heterogeneous pieces of evidence in systematic reviews. Ann Intern Med. 1997 Dec 1;127(11):989-95.

35. Giannoni P, Muraglia A, Giordano C, Narcisi R, Cancedda R, Quarto R, Chiesa R. Osteogenic differentiation of human mesenchymal stromal cells on surface-modified titanium alloys for orthopedic and dental implants. Int J Artif Organs. 2009 Nov;32(11):811-20.

36. 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. 2016 Feb 9. doi: 10.1002/jbm.b.33628.

37. Meng W, Zhou Y, Zhang Y, Cai Q, Yang L, Wang B. Effects of hierarchical micro/nano-textured titanium surface features on osteoblast-specific gene expression. Implant Dent. 2013 Dec;22(6):656-61.

38. Thalji G, Cooper LF, Nares S. Gene Expression Profiles of Early Implant Adherent Cells in Smokers and Nonsmokers. J Oral Implantol. 2015 Dec;41(6):640-5.

39. Ding X, Zhou L, Wang J, Zhao Q, Lin X, Gao Y, Li S, Wu J, Rong M, Guo Z, Lai C, Lu H, Jia F. The effects of hierarchical micro/nanosurfaces decorated with TiO2 nanotubes on the bioactivity of titanium implants in vitro and in vivo. Int J Nanomedicine. 2015 Nov 9;10:6955-73.

40. 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 Sep;8(9):3516-23. doi: 10.1016/j.actbio.2012.05.008.

41. Wang Z, Wu G, Feng Z, Bai S, Dong Y, Wu G, Zhao Y. 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 Oct 27;10:6675-87.

42. Palmieri A, Pezzetti F, Brunelli G, Lo Muzio L, Scarano A, Scapoli L, Martinelli M, Arlotti M, Guerzoni L, Rubini C, Carinci F. Short-period effects of zirconia and titanium on osteoblast microRNAs. Clin Implant Dent Relat Res. 2008a Sep;10(3):200-5.

43. Thalji G, Gretzer C, Cooper LF. Comparative molecular assessment of early osseointegration in implant-adherent cells. Bone. 2013 Jan;52(1):444-53.

44. Wimmers Ferreira MR, Rodrigo Fernandes R, Freire Assis A, Dernowsek JA, Passos GA, Variola F, Fittipaldi Bombonato-Prado K. Oxidative Nanopatterning of Titanium Surface Influences mRNA and MicroRNA Expression in Human Alveolar Bone Osteoblastic Cells. Int J Biomater. 2016;2016:9169371.

45. Palmieri A, Pezzetti F, Brunelli G, Zollino I, Lo Muzio L, Martinelli M, Scapoli L, Arlotti M, Masiero E, Carinci F. Zirconium oxide regulates RNA interfering of osteoblast-like cells. J Mater Sci Mater Med. 2008b Jun;19(6):2471-6.

46. Palmieri A, Pezzetti F, Brunelli G, Arlotti M, Lo Muzio L, Scarano A, Rubini C, Sollazzo V, Massari L, Carinci F. Anatase nanosurface regulates microRNAs. J Craniofac Surg. 2008c Mar;19(2):328-33.

47. Wu K, Song W, Zhao L, Liu M, Yan J, Andersen MØ, Kjems J, Gao S, Zhang Y. MicroRNA functionalized microporous titanium oxide surface by lyophilization with enhanced osteogenic activity. ACS Appl Mater Interfaces. 2013 Apr 10;5(7):2733-44.

48. Marinucci L, Balloni S, Becchetti E, Belcastro S, Guerra M, Calvitti M, Lilli C, Calvi EM, Locci P. Effect of titanium surface roughness on human osteoblast proliferation and gene expression in vitro. Int J Oral Maxillofac Implants. 2006 Sep-Oct;21(5):719-25. Erratum in: Int J Oral Maxillofac Implants. 2007 Jan-Feb;22(1):19.

49. Olivares-Navarrete R, Hyzy SL, Berg ME, Schneider JM, Hotchkiss K, Schwartz Z, Boyan BD. Osteoblast lineage cells can discriminate microscale topographic features on titanium-aluminum-vanadium surfaces. Ann Biomed Eng. 2014 Dec;42(12):2551-61.

50. Fu JH, Rios H, Al-Hezaimi K, Oh TJ, Benavides E, Wang HL. A randomized clinical trial evaluating the efficacy of the sandwich bone augmentation technique in increasing buccal bone thickness during implant placement. II. Tomographic, histologic, immunohistochemical, and RNA analyses. Clin Oral Implants Res. 2015 Oct;26(10):1150-7. doi: 10.1111/clr.12481.

51. Razzouk S, Sarkis R. Smoking and Diabetes Epigenetics Involvement in Osseointegration. N Y State Dent J. 2013 Mar;79(2):27-30.

52. Lozano D, de Castro LF, Dapia S, Andrade-Zapata, Manzarbeitia F, Alvarez-Arroyo MV, Gómez-Barrena E, Esbrit P. Role of parathyroid hormone-related protein in the decreased osteoblast function in diabetes-related osteopenia. Endocrinology 2OO9;15O:2O27-35.

53. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010, 142:296-308.

54. Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J Biol Chem. 2009 Jun 5;284(23):15676-84.

55. Eskildsen T, Taipaleenmäki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci U S A. 2011 Apr 12;108(15):6139-44.

56. Mustafa K,Wennerberg A,Wroblewski J, Hultenby K,Lopez BS, Arvidson K.Determining optimal surface roughness of TiO blasted titanium implant material for attachment,proliferation and differentiation of cells derived from human mandibular alveolar bone. Clin Oral Implants Res 2001;12:515–525.

57. Balshe AA, Eckert SE, Koka S, Assad DA, Weaver AL. The effects of smoking on the survival of smooth- and rough-surface dental implants. Int J Oral Maxillofac Implants. 2008;23:1117-1122.

58. Sayardoust S, Grondahl K, Johansson E, Thomsen P, Slotte C. Implant survival and marginal bone loss at turned and oxidized implants in periodontitis-susceptible smokers and never-smokers: a retrospective, clinical, radiographic case-control study. J Periodontol. 2013;84:1775-1782.

59. Yamano S, Al-Sowygh ZH, Gallucci GO, Wada K, Weber HP, Sukotjo C. Early peri-implant tissue reactions on different titanium surface topographies. Clin Oral Implants Res. 2011;22:815-819.