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Review Article

The role of miRNAs in titanium implants-molecular and pathophysiological mechanisms

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Abstract

Implants are structures made of alloplastic materials. They are widely implemented in both orthopedics and dentistry in order to rehabilitate different parts of the body with predictable outcomes. Although plenty materials have been introduced, titanium still remains the gold standard in *Implantology*. MiRNAs constitute a group of small non-coding RNA sequences composed of approximately 22 nucleotides. It is a well-known fact that they play a significant role in various biological processes such as osseointegration and peri-implant health maintenance, promoting the differentiation of mesenchymal stem cells into osteoblast cells. They regulate bone remodeling and signaling pathways of bone formation as well. Moreover, miRNAs can be utilized as activators of implant surfaces, biomarkers in the diagnostic process and finally occupy a prominent place in the therapeutic approach in Implantology. Even though the importance of miRNAs in Implantology is obvious, more studies are needed for a complete comprehension of the effects triggered by miRNAs.

Keywords: Titanium implants, miRNAs, osseointegration, peri-implantitis

Introduction

MiRNAs represent a group of small sequence non-coding RNAs with a length of 19-25 nucleotides which regulate the expression of a great number of genes by binding to the "3'-untranslated region of target mRNA". This results to degradation and/or translation repression of target mRNAs. The generation of functional single-stranded miRNAs from genomic DNA is a complex, multi-step process that involves many different enzymes both in the nucleus and cytoplasm. More specifically, miRNAs are transcribed as long transcripts which are processed by RNase III type enzyme Drosha into pre-miRNAs in the nucleus. Next, pre-miRNAs are transported to the cytoplasm where they undergo processing by a second RNase III family enzyme Dicer resulting to 22nt RNA duplexes with 2 nt 3' overhangs[1].

Each miRNA may regulate the expression of different genes, while the expression of a certain gene is controlled by multiple miRNAs. Particularly, miRNAs regulate multiple biological processes such as cell proliferation, differentiation and apoptosis as a response to extracellular signals. They participate in the progress of pathological processes and diseases (such as cancer where they function either as oncogenes or as tumor suppressors, rheumatoid arthritis, developmental abnormalities, cardiovascular disorders and schizophrenia). In addition to this, mRNAs can connect to the promoter of particular miRNAs stimulating an auto-regulatory feedback loop, thereby when a particular mRNA is up-regulated, the corresponding miRNA is over-expressed as well[2].

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Implants are structures made of alloplastic materials which are inserted surgically into the bone with the ultimate aim of prosthetic rehabilitation. They are strongly related to the concept of osseointegration which was first described by the Swedish physician Per-Ingrar Branemark during his studies on rabbits. He and his team found that titanium chambers placed in the lower leg bones of rabbits could not be removed from the bones after a certain healing period. Thus, osseointegration can be defined as the direct structural and functional connection between vital bone and the surface of the load-bearing implant without intervening soft tissue[3,4].

One of the main criteria determining the biocompatibility of an implant is the material it is made of. Titanium is the gold standard material in implantology used since 1960s. The combination of mechanical and biological properties makes titanium the material of choice since it provides biocompatibility, mechanical strength, rare allergic and toxic reactions, corrosion and oxidation resistance. However, ceramics such as Zr-based ceramics have been also successfully introduced; Zirconium oxide (ZrO_2) is a bio-inert metal oxide which can fulfil the aesthetic and functional requirements in the field of implantology[5].

The role of mirnas as activators of implant surfaces

The clinical success of implants is directly related to their early osseointegration. In order to minimize the implant failure and accelerate osseointegration many types of surface treatments have been proposed seeking to achieve higher Bone to Implant Contact (BIC). Since geometry and surface topography are of great significance for the short and long term success, modern implants have mechanically and chemically treated surfaces aiming to reach the desirable roughness. Various methods have been advocated such as roughening by acid-etching, grit blasting, anodization, titanium plasma spraying, hydroxyapatite coating, use of fluoride and bisphosphonates[6]. While the aforementioned surface modifications have been shown to present questionable osteo-inductivity, which is the ability of the material to induce osteogenic differentiation of mesenchymal stem cells toward osteoblasts and subsequent bone formation, studies that are searching for the effectiveness of implant surface treatment with miRNAs appear to show promising results[7].

In a recent study, Zhai et al. (2020), found that exosomes derived from osteogenically pre-differentiated human mesenchymal stem cells(hMSCs) could induce osteogenic differentiation when applied on 3D printed titanium scaffolds through the up-regulated miRNAs that they contained. At least a couple of osteogenic differentiation signaling pathways such as PI3K/Akt and MAPK could be activated and consequently this study provided a promising point of view for tissue regeneration[8].

The key role of exosomes on osseointegration and intracellular communication has been studied extensively in numerous studies, however, Zhang et al. (2021), explored the close relationship between osteoimmunity and osseointegration and noticed that macrophage cell derived exosomes could promote osteogenic differentiation of osteoblast precursor cells. Also, activation of signaling pathways such as mTOR, AMPK, WNT that are of great significance in osseointegration were observed when exosomes containing miRNAs were implemented on titanium surfaces[9].

Furthermore, Liu et al. (2021) found that exosomes over-expressing specific miRNAs such as miR-181b could promote macrophage polarization and as a result suppress inflammatory response and have beneficial effect on osteogenesis and osseointegration as well[10]. Another work demonstrated that macrophages-derived exosomes when stimulated with titanium nanotube arrays, can promote angio-osteogenesis, increase the expression of osteogenesis-related genes and ALP activity through the interplay between miR-3472e and target gene Akt1[11]. The aforementioned studies indicate that surface morphology of titanium implants can strongly influence the composition of the derived exosomes.

It is shown that multiple miRNAs can be used to functionalize titanium implant surfaces stimulating gene expression which is closely related to osteoblast differentiation, osseointegration and tissue mineralization. Huang et al. (2010), found in their work that miR-204 acted as a negative regulator of osteoblast differentiation in BMSCs through binding in the 3-UTR of RUNX2 and finally promoting RUNX2 repression[12]. On the other hand, antimiR-204 inhibits miR-204 action and in this way, Liu et al. (), studied the role of miR-204 inhibitor on osseointegration of titanium implants in patients suffering from type 2 diabetes mellitus, where the success rate is much more compromised than in the general population[10,13]. In this study, reasearchers utilized a combination of miR-204 inhibitor and gold nanoparticles (AuNP-antagomiR-204) and distributed them in the poly(lactic-co-glycolic acid) (PLGA) solution[13]. Consequently, this solution was applied as a coating on titanium implants and the results revealed that PLGA solution facilitated the release of AuNP-antagomiR-204 complex and the absorption from the neighboring BMSCs. In addition to this, in vivo the increase of osseointegration and the defective expression of antimiR-204 which was largely responsible for the insufficient osseointegration in type 2 diabetes mellitus was confirmed[13].

It has been previously shown that miR-21 is one of the most widely studied microRNAs. Geng et al. (2020), focused their studies on titanium surfaces treated with acid and uniformly distributed miR-21 nanocapsules[14]. Both *in vitro* and *in vivo* results showed that this specific miRNA coating can assist vascularization (high levels of CD31), osteogenic differentiation and fast remodeling (expression of proteins related to osteogenesis and osteoclastogenesis)[14]. All the aforementioned factors resulted to an improved bone to

implant contact (BIC) and high bonding strength in the early stages of surgery which was nearly twice the strength of non treated Ti-surfaces. The same authors, found in their previous studies that miR-21 can promote osteogenic differentiation of mesenchymal stem cells through the PI3K/b-catenin pathway[15]. In another study, researchers observed the effect of a composite coating fabricated by a deposition of SrHA (strontium-hydroxyapatatite) and miR-21 nanocapsules on titanium surfaces. *In vivo* and *in vitro* evaluations demonstrated a positive outcome on osteogenic differentiation and more specifically, the composite coating showed improved osteoblast differentiation, CD31 expression, angio-osteogenesis and reduced levels of RANKL (as compared to miR-21 coating), ending up to new bone formation, enhanced osseointegration and BIC[15].

In another study by Yan et al. (2018), it has been reported on the application of antimiR-138 on mesenchymal stem cell sheet (MSC) to activate dental titanium implants and formed the MSC sheet implant complex (MSIC)[16]. The ultimate goal of this action was to improve the osseointegration in compromised bone conditions and bone diseases. *In vitro*, the produced complex presented highly upgraded osteogenesis, angiogenesis and more specifically, higher expression of morphogenetic proteins, and over-expression of vascular endothelial growth factor (VEGF) was witnessed. Alkaline phosphatase activity, mineralization of extracellular matrix and collagen secretion were higher compared to the control group. In vivo, the increased periimplant regeneration was the most remarkable fact and higher expression of osteogenesis and angiogenesis factors including RUNX2, OSX, OPN, VEGF and CD3 were found[16].

Wu et al. (2020), manufactured polyelectrolyte multilayers(PEMs) with chitosan-miRNA (CS-miRNA) complex and sodium hyaluronate(HA) as positively and negatively charged polyelectrolytes on microarc oxidized (MAO) Ti surfaces, through a silane-glutraldheyde coupling process[17]. The novel functionilized Ti implant exhibited prolonged release of the complex CS-antimiR-138 and enhancement in vitro of osteogenic differentiation of MSCs associated with the increase of ALP, collagen production and mineralization of extracellular matrix but also improved *in vivo* osseointegration was noticed[17]. Similar findings were presented by the study of Song et al. (2018), who demonstrated the therapeutic modality of Ca/antimiR-138 complex on implant surfaces pretreated with anodization, which positively regulated the osteogenic differentiation of hMSCs[18].

Liu et al. (2019), found in their study that high-fat environment had a negative impact on osteogenic differentiation of BMSCs and on the expression levels of multiple proteins whereas the presence of miRNA-29-3p played a vital role in the promotion of osteogenic differentiation in hyperlipidemic rat models[19]. It had the property to proliferate ALP and RUNX2 levels in peri-implant tissues and finally improve implant osseointegration[19]. Additionally, miRNA-29b and miR-355-5p lipidoid nanoparticles can be used as promising tools to increase the osteogenic properties of titanium implants[20,21]. Similarly, Shao et al. (2018) analyzed the role of miR-122 modified cell sheets complex with micro-arc titanium oxide implants in the contribution to BMMSCs transformation to osteoblasts[22]. They concluded that miR-122 can improve excellently the osteogenic differentiation of cell sheets mainly through ERK 1/2 signaling pathway, accelerate and enhance osteogenesis providing new perspectives on dealing with poor healing conditions and high implant failure rate[22].

Eventually, miR-modified Ti surfaces can present positive effect on gene expression related to osseointegration, promotion of osteogenic differentiation, tissue mineralization, osteogenesis and reduction of osteoclastic activity as well. Tested surfaces have shown high expression levels of RUNX2, OPN, OCN, BMP, OSX, ALP, COL1 and 3. However, there is a necessity for further studies in order to completely define the interaction between miRNAs and titanium surfaces since this knowledge can drastically improve the outcome of the implants and raise the success rates.

The role of titanium implant surfaces on mirnas expression

As aforementioned, surface topography is one of the most important parameters influencing osseointegration and osteo-inductivity. Although many studies show that rough surfaces have increased BIC compared to smooth surfaces, simultaneously it is mentioned in other studies that they induce greater plaque accumulation with no adverse impact on soft tissue health[23]. Menini et al. (2017), showed that modified implant surfaces displayed less bone resorption in the first year despite higher level of plaque accumulation[24]. MicroRNA expression was evaluated by microarray analyses indicating that implant characteristics influence the peri-implant tissues and provoke the expression of multiple miRNAs which can act protectively in cases with different clinical parameters[24].

In another study, it was shown that oxidative nanopatterning of titanium surfaces can promote the metabolism of osteoblastic cells and affect the expression of genes that regulate the osteogenesis. Three different topographies, nanotexture, nano-submicrotexture and rough micro texture were evaluated by using microarrays. Among the textures utilized, alkaline phosphatase was found higher on cells located onto nanotexture and calcium on rough microtexture surfaces. The expression of multiple genes associated with osteogenesis (*NOTCH1, PHOSPHO1, SMURF2, COL24A1*) was marked in different pattern on the surfaces.

It was also observed that several miRNAs and mRNAs that participate in osteogenesis were differentially expressed as a consequence of surface modification. Particularly, 716 mRNAs and 32 miRNAs were involved, regulating the mineralization, apoptosis and regulation of cell proliferation.

Some of the miRNAs noted were miR-136-3p, miR-134, miR-494, miR-31, miR-424-5p and miR-484[25].

Chakravorty et al. (2012), demonstrated that different levels of miRNA expression were induced between modified titanium implant surfaces and smooth surfaces[26]. The authors demonstrated not only the fact that miRNA expression pattern is strongly related to the surface composition, but also that miRNAs influence the genetic mechanisms of osteogenic lineage differentiation and commitment on the treated surfaces. Three surfaces were included in the study, the sand-blasted, large gritetched (SLA), the hydrophlic SLA (mod-SLA) and smooth surface. It was proved that modified surfaces (SLA,mod-SLA) could induce higher osteogenic differentiation, but the molecular mechanisms responsible for the pathways TGF-b/BMP and WNT which were involved, are not clarified yet. It was found that modified surfaces could induce over-expression of non-canonical Wnt pathway genes (WNT5A, FZD6) but BMP-2, BMP-6, ACVR1 from the TGF-b/BMP pathway and integrin a2b1 showed higher expression levels in osteoprogenitor cells. This pattern showed a higher expression of miRNAs on modSLA, SLA and finally SMO surfaces in descending order. In particular, most miRNAs were down-regulated on modified surfaces with miR-22, miR-93 and miR-17 being among the most significant down-regulated miR-NAs and these findings are in line with previous reports. Eventually, the authors found that several genes of signaling pathways are supposed to be the targets of miRNAs that were down regulated on modified surfaces[26]. Mizuno et al. (2008), reported in their study that miR-125b could regulate cell proliferation and inhibit osteoblastic proliferation in mouse mesenchymal stem cells, which is in agreement with the view of Chakravorty et al. (2012), who found down-regulation of the same miRNA in modified surfaces[27].

In the same way, Iaculli et al. (2017), evaluated the influence of treated titanium surfaces on human dental pulp stem cell's osteogenic differentiation through the regulation of expression of miRNAs (miR-133 and miR-135), of SMAD, RUNX2, osteocalcin and the results revealed a positive role of treated surfaces with higher osseointegration[28]. According to Gardin et al. (2021), nano-modifications of titanium surfaces have the ability to promote exosome secretion and therefore increase specific miRNA expression, thus stimulating the in vitro endothelial and cell proliferation, and optimize in vivo angiogenesis and scaffold osseointegration[29].

In a recent study conducted by Kim et al. (2006) demonstrated that implant surface characteristics can influence gene expression from osteoblast-like-cells (MG-63, SaoS-2) which encode bone formation related proteins that affect cell proliferation, differentiation and adhesion[30]. This findings were also confirmed by Lin et al. (2021), who compared two different surfaces, smooth and surfaces with moderate roughness in order to reveal the pathway through which titanium surfaces modulate miRNA expression and consequently osteogenic differentiation of hBMSCs. Among miRNAs expressed, only miR-181d-5p was correlated with osteogenic gene expression and it was confirmed that downregulation of miR-181d-5p could enhance osteogenic differentiation of mesenchymal stem cells[31]. Moreover, it was noted that this specific miR-inhibitor enhanced the inferior bone formation on smooth surfaces, data which can be implemented on dental implant necks that have smooth collars to enhance peri-implant tissues, whereas miR-mimic reduced superior formation on rough surfaces[31].

On the other hand, Palmieri et al. (2008), compared the miRNA expression after 24 hour of culturing of osteoblast like-cells on Ti and ZrO_2 surfaces using microarray technique. The results showed that miRNAs expressed by osteoblasts which were detected on titanium surfaces regulated genes related to bone formation to a greater extent than ZrO_2 surfaces. The most notable genes were BMP-4 and 7 which are both members of the transforming growth factor-beta family (TGF-b) and they are able to promote bone growth, neoangiogenesis and early development. This data could provide advantages regarding the early phase of osseointegration, hence immediate implant loading which is defined as the placement of the final or provisional restoration immediately after surgery or within 24 hours can be amplified as well[32].

Liu et al. (2020), chose three kinds of titanium implant surfaces (SLA, AOS, MAO) to evaluate the osteogenic activity of human bone marrow mesenchymal stem cells (hb-MSCs) and it was shown that MAO (micro-arc oxidized) surfaces were more promising to regulate osteogenic differentiation of hb-MSCs than the others, promoting the phosphorylation of p38, JNK, ERK 1/2 and also activating p38 MAPK and ERK pathway[33]. Additionally, it was verified that ERK 1/2 was the most crucial target of the pathway, focusing on miR-1827 which has been proposed as an inhibitor of osteogenesis. However, the inhibitory role of this miRNA was reversed by the over-expression of Osterix which was the direct target of miR-1827. Overall, this study presented that MAO coating could activate the ERK 1/2-miR-1827-Osterix pathway[33].

The role of mirnas in periodontal and peri-implant diseases

MiRNAs appear to be one of the main modulators of bone homeostasis since they affect bone remodeling and eventually control osteogenic and osteoclastic activity, through positive and negative feedback loops respectively. In addition to this, as a consequence of their role, miRNAs intervene in alveolar bone resorption which is a major characteristic of periodontitis and also peri-implantitis. Both of these conditions are destructive diseases affecting teeth and peri-implant tissues. They are induced by bacterial biofilms consisting mostly of Gram(-) anaerobic bacteria that are established over subgingival tissues. On their turn, biofilms provoke an inflammatory response of the host which is influenced by environmental, genetic and epigenetic factors[34].

The increasing use of dental implants is resulting to the high frequency of peri-implantitis. Many studies have been performed in order to identify the expression of miRNAs mainly between conditions of health and disease. Asa'ad et al. (2020), found in their meta-analysis different expression of miRNAs between periodontitis-peri-implantitis and healthy patients, and to be more specific results showed an over expression of most miRNAs in periodontitis with miRNA-146a and miR-NA-142-3p being the leading characters on both microarray and rt-PCR methods[35]. It was also indicated that miRNA expression can be affected by multiple systematic conditions considering obesity, diabetes and cardiovascular diseases. Furthemore, it was noted that miRNA-30e, miRNA-130a, miRNA-142-3p and miRNA-210 were up-regulated in periodontal patients with obesity. MiRNA-24-3p and miRNA-155 were also upregulated in periodontal disease, but their role is not clear at the moment[35].

Motedayyen et al. (2015) presented an over expression of miRNA-146a in patients with chronic periodontitis and this was related to the decrease of pro-inflammatory cytokines like TNF-a, IL-6 and IL-1a which was strongly associated with the regulation of cytokines through a negative feedback loop[36]. The role of miRNA-146a was also assessed by Ghotloo et al. (2019), who suggested that there is a close relationship between generalized aggressive periodontitis and miRNA-146a and inferred that it could serve as an indicator of periodontal disease and inflammation[37].

In another study, the expression levels of miRNA-18b, miRNA-19b, miRNA-30a, miRNA-let7a, miRNA-301 were found lower in healthy tissues in comparison to chronic periodontitis. However, it is shown that in severe periodontitis miRNAs are able to regulate even opposite conditions through their ability to target multiple genes[38].

Regarding the expression of miRNAs in peri-implantitis the results are limited to two animal studies in which most of the miRNAs were down-regulated (miRNA-let-7g, miRNA-27a, miRNA-29a, miRNA-142) apart from miRNA-145 which was excessively up-regulated. Wu et al. (2017), revealed the prominent role that miRNAs can have in inflammation, osteoclastogenesis and peri-implantitis[39]. The results of qRT-PCR showed that miRNA-let-7g, miRNA-27a, miRNA-142 affected not only the beginning but also the progression and treatment of peri-implantitis in a canine ligature-induced peri-implantitis. These miRNAs promoted the expression of genes enrolled in MAPK signaling pathways which commonly present an enrichment in peri-implantitis lesions[35,39].

Indeed, the early diagnosis of peri-implantitis is of great importance for the successful treatment and maintenance of peri-implant tissues in healthy status[40]. Urvasizoglu et al. (2021) collected saliva samples from patients with titanium-aluminum-vanadium dental implants who had peri-implantitis and identified possible biomarkers for peri-implantitis[41]. They confirmed through qRT-PCR the deregulation of specific types of miRNAs ending up to the fact that that miR-4484 might be used as an early diagnosis biomarker for this condition[41]. On the other hand, based on the results of a systematic review which compared the concentration levels of certain biomarkers like cytocins (IL-1b, IL-6 and IL-10), antioxidants, urate, ascorbate between healthy and peri-implant tissues, there was no clear answer whether biomarkers can be used predictably in this direction[42].

Chapparo et al. (2021), attempted to explore the diagnostic potential of extracellular vesicles, their subpopulations and their transcriptional cargo microRNAs in peri-implant crevicular fluid of healthy, peri-implantitis and peri-implant mucositis implants[43]. They found a rise of extracellular vesicles with a decrease of miRNA-21-3p and miRNA-150-5p in peri-implantitis[43]. In the same manner, He et al. (2021), performed next-generation sequencing in order to evaluate the miRNA expression profiles in crevicular fluid of peri-miniscrew implants in orthodontic patients and the results revealed that different profiles were detected between healthy, peri-implant and periodontal patients[44]. The results showed that miR-4291, miR-1245-3p and miR-1825 had higher expression levels in peri-implant patients and authors proposed that these miRNAs can be used as biomarkers and therapeutic targets for peri-implantitis[44].

Conclusions

The significant place that miRNAs hold in clinical implantology is obvious especially when we take into consideration the fact that miRNAs regulate gene expression not only at transcriptional but post-transcriptional level as well. The identification of specific miRNAs that act as positive or negative regulators of osteogenesis may contribute positively to implantology since such miRNAs can be utilized as therapeutic agents. Their use can be extended to different aspects of medicine and pharmacology such as improvement of surface characteristics and implant surface activation with the ultimate goal of enhanced healing and maintenance of peri-implant tissues, promotion of bone formation and successful outcome of the therapeutic strategy. MiRNAs can conduce to the field of regenerative medicine and assist the treatment of peri-implantitis by regulating the immune response to the infection.

In addition to this, miRNAs can play a vital role in predicting clinical outcomes, thus they can be utilized as diagnostic and prognostic tools affecting a small part or the whole treatment plan. Although there is a great number of compromising factors for the implant success (smoking, bruxism, frequency of follow-ups, bone quantity and quality, systematic diseases etc.), the incorporation of miRNAs as predictors for the predisposition of peri-implant tissues to achieve smooth osseointegration would be extremely beneficial. Totally, the above findings offer the advantage of a more personalized therapeutic approach ensuring in this way a patient-centered treatment. In conclusion, more studies are needed to clarify the relationship between miRNAs and titanium implants.

Authors' contributions

VIM: Drafted the manuscript, reviewed literature. GIL: Proof-edited the manuscript and gave final permission for publication. All authors read and approved the manuscript.

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