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Review

Bioactive coatings with anti-osteoclast therapeutic agents for bone implants: Enhanced compliance and prolonged implant life

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ABSTRACT

The use of therapeutic agents that inhibit bone resorption is crucial to prolong implant life, delay revision surgery, and reduce the burden on the healthcare system. These therapeutic agents include bisphosphonates, various nucleic acids, statins, proteins, and protein complexes. Their use in systemic treatment has several drawbacks, such as side effects and insufficient efficacy in terms of concentration, which can be eliminated by local treatment. This review focuses on the incorporation of osteoclast inhibitors (antiresorptive agents) into bioactive coatings for bone implants. The ability of bioactive coatings as systems for local delivery of antiresorptive agents to achieve optimal loading of the bioactive coating and its release is described in detail. Various parameters such as the suitable concentrations, release times, and the effects of the antiresorptive agents on nearby cells or bone tissue are discussed. However, further research is needed to support the optimization of the implant, as this will enable subsequent personalized design of the coating in terms of the design and selection of the coating material, the choice of an antiresorptive agent and its amount in the coating. In addition, therapeutic agents that have not yet been incorporated into bioactive coatings but appear promising are also mentioned. From this work, it can be concluded that therapeutic agents contribute to the biocompatibility of the bioactive coating by enhancing its beneficial properties.

1. Introduction

The human skeleton not only serves as a support, protects vital organs in the head and the chest, and enables movement, but it is also responsible for less obvious functions in the body, such as the storage of minerals and stem cells, the production of the necessary specific cells that enable the normal functioning of the whole organism, and endocrine regulation [1–3]. Bone tissue is a dynamic tissue that undergoes remodelling throughout the life span of an organism. The constant process of building and breaking down tissue enables bone vitality and normal movement [4]. However, when the body develops problems with the joints, it is often necessary to replace them with an artificial joint. In order to keep the artificial joint in place for as long as possible, it is important to prevent bone resorption. Bone resorption is a process in which bone minerals dissolve, and the organic bone matrix degrades due to the activation of osteoclasts. Bone resorption is necessary for the bone remodelling process, but in the area of the implant it can lead to the loosening of the implant, requiring revision surgery [5,6]. The stress on the implant, i.e. constant movement and weight-bearing conditions, further contributes to the loosening of the implant, i.e. its long-term anchorage in the human body deteriorates. As a result, 40% of implants fail over time [7,8].

A balance between the formation and breakdown of bone tissue is necessary to maintain healthy bone tissue. When this balance is disturbed, excessive bone resorption, called osteolysis, occurs [9]. The balance between bone formation and resorption tends toward resorption due to the presence of the wear debris particles [10,11]. The presence of wear debris from the implant, such as synthetic polymer (polyethylene,

Abbreviations: 7ND, seven-amino acid truncated protein; BMP-2, bone morphogenic protein-2; c-di-AMP, cyclic diadenylate monophosphate; c-di-GMP, cyclic diguanylate monophosphate; c-Fos, Fos proto-oncogene, AP-1 transcription factor subunit; FPP, farnesyl diphosphate synthase; GGPP, geranylgeranyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; MAPK, mitogen-activated protein kinase; NFAT-c1, nuclear factor for T cell activation - cytoplasmic 1; NF-κB, nuclear factor κB; OPG, osteoprotegerin; PCL, poly(ε-caprolactone); PEI, polyethylenimine; PLGA, poly(lactic-co-glycolic acid); PP2A, protein phosphatase 2A; RANK, receptor activator of nuclear factor kappa B; RANKL, receptor activator of nuclear factor kappa B ligand; RUNX-2, runt-related transcription factor-2; STING, stimulator of interferon genes; TNF-α, tumour-necrosis factor α; TRAP, tartrate-resistant acid phosphatase; Wnt, wingless/integrated proteins.

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polymethyl methacrylate) debris [11,12], metal debris [13,14], and cement debris [11,15], is one of the main factors limiting the longevity of the implant [16,17]. As a multifactorial process, osteolysis is also dependent on the host, i.e. his or her activity [12,18], sex [18,19], age [20], weight [19], reactive oxygen species [21,22], and systemic comorbidities such as diabetes [23–25], immunosuppression [25], periodontitis [26], human immunodeficiency viruses [27,28], rheumatoid arthritis [29], Crohn's disease [30], osteoporosis [31], and estrogen deficiency [32]. The surgical aspect is also among the factors influencing osteolysis, i.e. the placement of the implant in the bone, the presence of bone particles, and the implant debris occurring during surgery [9]. The presence of wear debris particles stimulates the synthesis of cytokines and prostaglandins, which initiate the cascade of events leading to the formation of osteoclasts. Moreover, the balance between the receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) is disturbed, i.e. between type II membrane protein stimulating osteoclast bone resorption, and cytokine receptor inhibiting osteoclastogenesis, respectively [33–35].

There are several effective therapeutic agents that inhibit bone resorption, such as bisphosphonates [36,37], proteins [38,39], statins [40,41], and various nucleic acids [8,42] that regulate the expression of the genes involved in osteoclastogenesis and osteoclast activity. These therapeutic agents have a weakness as regards systemic treatment, namely side effects. Although some bisphosphonates are very effective in clinical use in patients who have medical problems stemming from tumours associated with osteolysis, they can cause side effects such as hypocalcaemia, nephrotoxicity, and osteonecrosis of the jaw in systemic treatment [43–45]. Furthermore, in addition to side effects such as rapid renal clearance and improper biodistribution, systemic administration of nucleic acids is not effective enough due to preferential accumulation in the liver and the weak biostability of molecules encountering nucleases during the long trip to the target cells [8,46].

Therefore, local dosing of therapeutic agents is much more appropriate for inhibiting osteoclast activity and bone resorption in the implant area. It is necessary to optimize the implant material, coating, and selected therapeutic agent to achieve maximum efficacy, which is critical to prolonging implant longevity and postponing revision surgery as long as possible. This review first focuses on bone composition, which is very important for understanding the inhibition of resorption. Second, the main topic is addressed, namely a review of the therapeutic agents with osteoclast inhibitory properties in various bioactive coating materials for bone implants and an evaluation of their potential to inhibit osteoclasts and to be incorporated into bioactive coatings, as well as the potential of therapeutic agents not yet included in bioactive coatings, in the search for the most suitable one.

2. Bone structure

Understanding bone structure, cellular function mechanisms, and signalling pathways is critical for mimicking bone tissue in order to achieve the optimal integration of artificial joint implants with bone. Bones serve as a scaffold to which muscles are attached, and vital organs are protected, while also acting as a reservoir of calcium and phosphate ions for the entire organism [47]. The peculiarity of bone tissue is its ability to constantly transform and rapidly differentiate cells even after the completion of the growth and development of the organism, which allows adaptation to changing functional situations. Its complexity is reflected in both cell differentiation and the biochemical composition of the bone matrix [48].

2.1. Bone matrix

Bones are the primary load-bearing component of the skeletal system [47] due to providing support to the body's anatomical structure, protecting against external strains, and contributing to skeletal movement. These abilities, as well as the ability to adapt to stressful stimuli, reflect the hierarchical structure of bone. The distribution of both mineral and organic phases at the nanoscale, as well as the structure of the entire bone scale, are important for energy dissipation along a bone's entire length. Bone tissue consists of an inorganic phase (50–70%), composed of minerals, water (5–10%), an organic matrix (20–40%), and lipids (less than 3%) [47,49,50]. The mineral phase is responsible for strengthening the collagen composite, resulting in the better mechanical resistance of the tissue and providing a source of $Ca_3(PO_4)_2$ and magnesium ions for mineral homeostasis [51,52]. Approximately 90% of the organic bone matrix is composed of collagen type 1, with the remainder being non-collagenous proteins [49]. Together with non-collagenous proteins, collagen type 1 is responsible for a bone's high elasticity, low compressive strength, and considerable tensile strength, which depends on the close association of the mineral part of the matrix with collagen. These properties make the bone tissue elastic, thus preventing the propagation of stress through the brittle material [47,49,51].

2.2. Bone cell structure

Different cell types are involved in the formation and transformation of bone tissue, which are differentiated directly or indirectly from the mesenchymal stem cells (MSCs) of the bone marrow [53]. The first stage of the differentiation of pluripotent MSCs is represented by osteogenic stem cells or osteoprogenitor cells, which indicate the formation of bone tissue cells and are further modified and specialized into specific bone cells (Fig. 1). These cells are osteoblasts, bone lining cells (also known as endosteal cells), osteocytes, and osteoclasts [53,54].

2.2.1. Osteoblasts

Osteoblasts are the main means of bone formation, and are fully differentiated mononuclear cells formed from preosteoblasts, i.e. osteoprogenitor cells. They are involved in the process of matrix calcification and bone resorption and also regulate the flow of Ca and phosphate into and out of bone tissue [56]. The expression of transcription factors such as runt-related transcription factor-2 (RUNX-2), distal-less homeobox-5, and msh homeobox homolog-2 is required for MSCs to direct their differentiation towards osteoblasts rather than towards the formation of adipocytes, myocytes, and chondrocytes [53]. The transition from a preosteoblastic cell to an osteoblast is caused by stimulating a preosteoblast to differentiate due to soluble factors such as bone morphogenic proteins and wingless/integrated proteins (Wnt). When osteoprogenitor cells are stimulated to differentiate, they cease proliferation and begin to secrete proteins indicative of an osteoblast phenotype, which requires the expression of RUNX-2, the osterix gene, and quite a few components of the Wnt signalling pathway [54,57].

The most important feature that phenotypically distinguishes

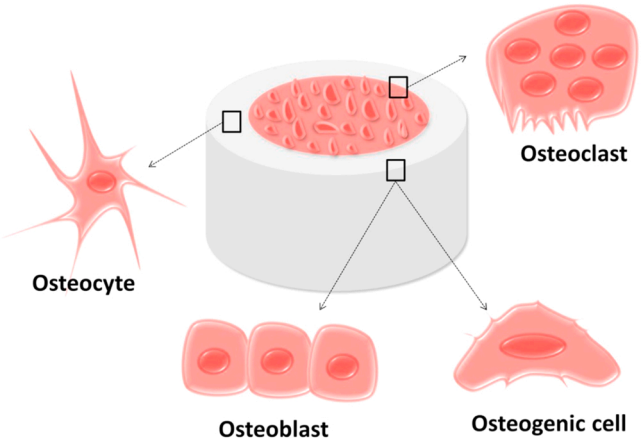

Fig. 1. Types of bone cells.
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osteoblasts from other bone cells is the presence of a greater amount of the membrane protein, i.e. alkaline phosphatase, and the active formation of an osteoid matrix towards the mineralization side of the bone tissue. The osteoid matrix around the osteoblast begins to calcify and approximately 20% of the trapped osteoblasts transform into osteocytes. Osteoblasts that do not transform into bone lining cells or osteocytes undergo apoptosis [47,54,57]. The maintenance of tissue-specific functions, in addition to the expression of tissue-specific genes, is simultaneously influenced by epigenetic events that are closely related to the expression of these genes. These events are coordinated by histone or deoxyribonucleic acid (DNA)-modifying proteins and non-coding ribonucleic acid (RNA) molecules. To understand bone degeneration and regeneration, it is important to identify these processes. In this way, interventions can be developed to prevent or mitigate bone-related disorders [56]. During the process of osteodifferentiation, preosteoblasts transform into mature osteoblasts. These later, depending on biosynthetic activity, functionally differentiate into active osteoblasts, osteoclasts, or endosteal cells [47].

2.2.2. Bone lining cells

The endosteal cells lining the bones originate from mature osteoblasts, which are characterized by a flat morphology and a well-defined location on the bone surface. They represent a group of quiescent cells from the osteo-family that play an important role in tissue transformation and regeneration [58]. In the process of bone remodelling, the initial and most important step is the decomposition of the matrix, even before osteoclasts are recruited to resorbing sites [59]. The involvement of osteoclasts in matrix catabolism has been demonstrated histologically, immunohistochemically, and by gene expression profiling. These assays have shown that matrix degrading enzymes such as matrix metalloproteinase-13, matrix metalloproteinase-14, tissue inhibitor metalloproteinase-1, and tissue inhibitor metalloproteinase-2 are present in these cells. The RUNX-2 gene was also found to be expressed in these cells, confirming that the endosteal cells belong to the osteoblast cell lineage [60,61]. A layer of flattened, elongated cells covers the bone surface, thus protecting the bone from any resorption activity of osteoclasts. Endosteal cells are able to reactivate and form osteoblasts [48]. The cells of the endosteum regulate the passage of Ca into and out of the bone and respond to hormones by producing special proteins that activate osteoclasts [60].

2.2.3. Osteocytes

Osteocytes are terminally differentiated cells derived from mature osteoblasts, which are trapped in a calcified matrix and responsible for the maintenance of bone mass. They are the most abundant cells in bone tissue and account for 90–95% of all bone cells, while osteoblasts account for 4–6% and osteoclasts 1–2% [48,62]. The role of osteocytes in the skeletal system is still not fully understood, but it is assumed that osteocytes are involved as primary mechanosensors in bone tissue. They are thought to act as a network of sensory cells that mediate the effects of mechanical loads through an extensive lacuna-canalicular network. Mechanotransduction, which occurs in osteocytes as mechanical stress, is thought to be triggered by the flow of fluid within the canals created by a pressure gradient between the lacunae when the bone is loaded [54,63]. Environmental stimuli, such as stress, affect fluid movement in the cells, thus triggering the depolarization of the osteocyte process and spreading to other osteocytes through the slit junctions. In response to mechanical stress, cells begin to secrete paracrine factors such as insulin-like growth factor-1 (IGF-1) and express the Fos proto-oncogene, AP-1 transcription factor subunit (c-Fos) gene in response to mechanical stress [48]. Osteocytes have the ability to regulate mineral metabolism and alter the surrounding matrix, as mechanotransduction in osteocytes contributes to the recruitment of osteoblasts or osteoclasts depending on the loading condition [48,54,62,63]. Given the growing number of functions attributed to osteocytes, such as the regulation of phosphate homeostasis, the osteocyte network acts as an endocrine gland. The consequences of defective osteocyte functioning are reflected in the quality of bone, i.e. its brittleness, and can manifest in numerous bone diseases, e.g. osteoporosis, and non-bone diseases such as chronic kidney disease, as well as skeletal and cardiac muscle dysfunction [47,62].

2.2.4. Osteoclasts

Osteoclasts are polynuclear phagocytic cells that form and occupy depressions on the bone surface called Howship's lacunae. Osteoclasts are responsible for the resorption of bone in bone metabolism. They have a characteristic polarized plasma membrane consisting of two separate regions on the basal surface of the osteoclast [54]. These are the folded part of the plasma membrane, where bone resorption occurs, and the sealing area, which binds the folded edge to the extracellular matrix of bone. The combination of the accumulated and sealing regions of the plasma membrane forms resorption lacunae, i.e. emptiness. Osteoclasts can fuse with pre-existing polynuclear osteoclasts and fuse with each other to form *de novo* polynuclear osteoclasts. However, they can also remain as mononuclear cells and serve as a precursor group that is recruited for specific tasks when stimulated by the environment or when mechanical stress changes [53,63]. During bone resorption, fully differentiated osteoclasts express the enzyme tartrate-resistant acid phosphatase (TRAP), which is crucial in the initial phase of immature osteoclast formation. Osteoclasts also express calcitonin receptors, vacuolar proton adenosine triphosphatase, and vitronectin receptors. The successful differentiation of an immature osteoclast requires the expression of several genes, such as a member of the heterodimer of activating protein-1 c-Fos, microphthalmia-related transcription factor, and the nuclear factor for T-cell activation – cytoplasmic 1 (NFAT-c1), which is dependent on calcineurin 1, but this is provided only in the constant presence of the receptor activator of the nuclear factor kappa B (RANK) ligand, also known as RANKL [54,63,64].

Osteoclastogenesis has been shown to require physical contact with stromal cells, as the secretion of some soluble compounds into the medium by stromal cells alone is not sufficient. Simultaneously with the identification of RANKL, a soluble factor, OPG, was also identified as inhibiting RANKL activity. OPG is a soluble bait for RANKL, which binds to it, thereby inhibiting osteoclast differentiation by competitively occupying stromal RANKL sites at earlier and later osteoclast stages [65–68]. Consequently, cells of mesenchymal origin can positively control osteoclast differentiation by increasing the expression of the RANK ligand and decreasing OPG expression, thereby increasing bone resorption. In contrast, a decrease in resorption activity occurs in the opposite case, i.e. when RANKL expression decreases and OPG expression increases. RANKL is an important factor in regulating bone resorption activity and the survival of mature osteoclasts after differentiation itself [47,48,54]. Mature osteoclasts attach peripherally to the bone matrix with integrins, creating a gap between the osteoclast's folded basal border and the bone matrix's surface isolated from the extracellular space, thus creating a microenvironment between the osteoclast and the bone. This microenvironment is acidified by the action of an electrogenic proton pump, which transports H^+ ions to degrade the mineralized components of the bone [54,64]. Until the final decomposition of the bone matrix and the formation of Howship's lacunae, the decomposition of the organic matrix is required, which is broken down into fragments by proteases. These proteases are lysosomal proteolytic enzymes, particularly matrix metalloproteinases, including collagenase, gelatinase B, and cysteine proteinases such as cathepsin B, L, and K [4,69,70]. Substances that act as inhibitors of osteoclast functioning and its differentiation, which are critical for the long-term fixation of the implant with bone, are the focus of the remainder of this review.

3. Bioactive coatings and osteoclast inhibitors

After a period of time, the artificial joint needs to be replaced. The erosion of the implant material and the formation of small metal or

polyethylene particles that accumulate between the implant and the bone lead to a chronic inflammatory response triggered by macrophages, which enhances osteoclast activation and inhibits osteoblast functioning. This leads to aseptic loosening, which occurs due to periprosthetic osteolysis. An inflammatory response and bone resorption can be prevented with osteoclast inhibitors. Osteoclast inhibitors include agents such as bisphosphonates, proteins, statins, and some other molecules that are intended to inhibit osteoclasts and prevent their differentiation and proliferation. Moreover, other compounds such as RNA molecules and recombinant proteins are known to have the same effect as the above-mentioned compounds [71–73].

Osteoclast inhibitors affect both osteoblast-associated cells, which are regulators of the cells from which all immune and blood cells originate, and osteoclasts derived from the same origin as myeloid precursor cells from which macrophages and myeloid dendritic cells are derived [74,75]. Therefore, such therapeutic agents also interact with the immune system [76]. Research has shown that RANKL, essential in osteoclastogenesis, stabilizes the innate immune system challenged by endotoxin, induces tolerance, and suppresses proinflammatory cytokine production in macrophages. This means that RANKL inhibits the production of proinflammatory cytokines by macrophages and can be used as a prophylactic molecule to prevent endotoxic shock [74,77]. The most used bisphosphonates, among other therapeutic agents, can elicit an immune response. Although understanding the breadth of bone-immune cell interactions is just beginning and much more research is needed in this direction [74,76], it is known that oral dosing of bisphosphonates can cause gastrointestinal reactions in patients with inflammatory bowel disease [78].

3.1. Bisphosphonates

Bisphosphonates are drugs that bind to $Ca_3(PO_4)_2$ minerals with high and long-lasting affinity. Upon bisphosphonates entering into cells, they block osteoclastic bone resorption, resulting in the stabilization of bone mass and the preservation of the structure. The high affinity between bisphosphonates and the bone matrix of hydroxyapatite also causes bisphosphonates to bind to healthy bone tissue, thus side effects may occur during systemic treatment [36,79]. Bisphosphonates are generally divided into two categories, depending on the presence of nitrogen in the two covalently-bonded groups attached to the geminal carbon. Simple bisphosphonates consist of two phosphate groups bonded to a carbon atom to which two side chains (R^1 and R^2) are bonded [80–82]. In etidronate, R^1 is a hydroxyl, while R^2 is a methyl. Even more effective in inhibiting bone resorption than etidronate are clodronate and tiludronate, where the R^1 and R^2 consist of chlorine atoms and R^1 is a hydrogen atom and R^2 is a chlorophenyl group. Non-nitrogenous bisphosphonates inhibit the activity of osteoclasts by inducing their apoptosis *in vitro* and *in vivo*, meaning osteoclast retraction, condensation, and cellular fragmentation [80,81,83–88].

On the other hand, nitrogen-containing bisphosphonates contribute to increased potency in inhibiting osteoclast activity as well as the differentiation thereof. This group includes alendronate, pamidronate, ibadronate, and olpadronate, which contain nitrogen in the alkyl chain, and risedronate, zoledronate, and minodronate, in which nitrogen is present in the heterocyclic ring structure [89–91]. Nitrogen-containing bisphosphonates inhibit osteoclastogenesis and affect osteoclast survival by blocking the enzyme farnesyl diphosphate synthase (FPP), which is critical for the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (mevalonate) pathway (Fig. 2). The FPP synthase directly induces the synthesis of FPP and indirectly induces the synthesis of geranylgeranyl pyrophosphate (GGPP), which further induces the prenylation (the attachment of isoprenoid groups) of mostly geranylgeranylated hydrolase enzymes, which bind to the nucleotide guanosine triphosphate (GTPases). Consequently, the inhibition of FPP synthase prevents the synthesis of FPP and GGPP, which prevents the proper localization of GTPases and results in their accumulation in the cell. An abnormal distribution of unprenylated GTPases disrupts balance, signal transduction, and osteoclast functioning [89,92–94]. The following sections summarize the effects of various bisphosphonates in bioactive coatings.

3.1.1. Alendronate

Bisphosphonates are assumed to affect the progression of bone morphogenic protein-2 (BMP-2)-induced bone healing. This hypothesis was tested in an *in vitro* study using ovariectomized rats, in which osteoporosis was accelerated by ovariectomy. The effect of alendronate,

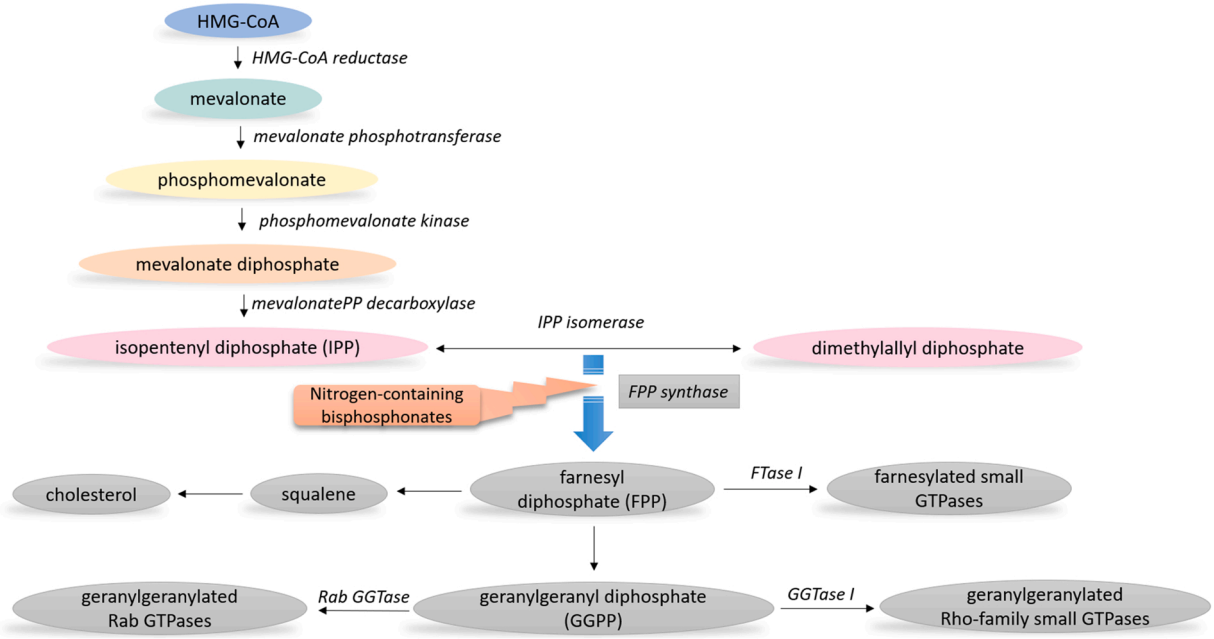

Fig. 2. Schematic diagram of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase pathway (mevalonate pathway). Following the arrows, statins inhibit HMG-CoA reductase by preventing the prenylation of proteins. Nitrogen-containing bisphosphonates subsequently affect the mevalonate pathway by inhibiting FPP synthase, thereby inhibiting FPP and GGPP synthesis [80].

a drug of the bisphosphonate family, was tested together with BMP-2 and L51P, which is an *in vitro*-developed version of BMP-2 with the substitution of leucine with proline at the 51st amino acid site. The results showed that in rats receiving alendronate and 10 μg BMP-2 or 10 μg L51P/1 μg BMP-2, osteoblast formation was increased, and the newly formed bone volume exceeded the removed volume replaced by the implant [79]. Given that bisphosphonates have a high affinity for hydroxyapatite, alendronate is a suitable therapeutic agent for binding to hydroxyapatite. Alendronate incorporated into the hydroxyapatite coating significantly reduces the number of osteoclasts. Moreover, it was reported that such a coating also mitigates the corrosion of implants made of (commercially pure) titanium and its alloy (Ti6Al4V) [95,96]. Furthermore, the presence of alendronate increased the hydrophilicity of the coating, which in turn promotes osteoblast adhesion and the osseointegration process. The hydroxyl groups, amine groups, and phosphate groups of this active substance significantly affect the increased hydrophilicity of the surface (the water contact angle dropped by approximately half compared to the unmodified titanium implant) [97].

In the initial phase of the osteointegration of the implant, the appropriate ratio between osteoclasts and osteoblasts is crucial. However, the long-term inhibition of osteoclasts is important, which thus requires the long-term release of bisphosphonates. A thin layer of hydrophobic poly(lactic-co-glycolic acid) (PLGA) coating has been shown to contribute to the long-term release of alendronate from the bioactive glass coating, extending the release time from 8 to 60 days [98]. Controlled release of alendronate can be achieved by encapsulating alendronate-loaded hydroxyapatite crystals in a porous poly(ε-caprolactone) (PCL) scaffold. Such a formulation enables the controlled release of alendronate from the implant for 28 days. Furthermore, the addition of Fe_3O_4 nanoparticles improves both the biostability of the complex crystals of alendronate, hydroxyapatite, and Fe_3O_4, as well as the release profile of alendronate [99]. Prolonged release of alendronate can be achieved by incorporating it into a coating of carbonated calcium-deficient hydroxyapatite and polylactic acid. The presence of carbonated calcium-deficient hydroxyapatite in this biocompatible coating prolongs the release of alendronate to about 9 months, while it is released from polylactic acid within a few hours [100].

The mesoporosity of the TiO_2 coating of a titanium (99.5%, grade IV) implant also allows sustained release. Compared to approximately 550 ng/cm^2 of alendronate immobilized in hydrophilic pores, 900 ng/cm^2 of raloxifene (which is not a bisphosphonate) can be immobilized in hydrophobic pores. Both agents induce bone formation, with raloxifene inducing apatite formation inside the coating and alendronate inducing increased bone density outside the coating [101]. Since combination therapy has been shown to be more effective than separate treatment with alendronate and raloxifene [102], it is highly likely that the combination of both agents in the coating would significantly improve the osseointegration and osteoconduction of the implant. In addition to controlled release, proper dosing is also important. In an *in vivo* study in dogs, an amount of 0.06 mg/cm^2 alendronate on the hydroxyapatite coating of three-dimensional printed porous cylindrical fabricated implant from titanium alloy (Ti6Al4V) was sufficient to increase bone formation around the implant by 92%. In contrast, 0.02 mg/cm^2 was not found to have a significant effect on bone formation and is thus insufficient [103].

Compared to the systemic administration of alendronate, the results of an *in vivo* study using rats showed that local administration of alendronate was more effective as it significantly enhanced the pull-out force of the implant. The pull-out force increased by 39% compared to the pull-out force in cases with systemic delivery [104]. A modification of alendronate in the form of calcium alendronate has been shown to be an effective inhibitor of the differentiation of RAW 264.7 cells into mature osteoclasts. In an *in vitro* study on precursor osteoclast cells, calcium alendronate successfully inhibited the formation of multinucleated cells, although it induced an osteoclast phenotype with a RANKL-containing medium. On the other hand, high concentrations of calcium alendronate may be cytotoxic to adipose-derived MSCs [105].

3.1.2. Zoledronate

In an *in vivo* study employing ovariectomized rats, zoledronate was included in the bioactive coating of 3,4-dihydroxy-L-phenylalanine. It was found that a coating containing 3,4-dihydroxy-L-phenylalanine and zoledronate did not show the expected synergistic effect. However, microarray analysis revealed that the 3,4-dihydroxy-L-phenylalanine coating itself successfully inhibited the expression of genes associated with osteoclast differentiation and acted according to a similar principle as zoledronate [106]. The efficacy of zoledronate was substantiated in an *in vitro* study using a rat model implanted with a combined material of α-tricalcium phosphate and collagen sponge. The rats were divided into three groups four weeks after implantation, with the first group being injected with zoledronate, the second with interferon-γ, while the third group was the control group, as it did not receive any active substances. Compared with the control group, the groups injected with interferon-γ and zoledronate showed a remarkable attenuation of severe osteoclastogenesis, resulting in a significant increase in bone mass [107]. Zoledronate can bind to the fibrinogen matrix and thus be incorporated into bioactive coatings, allowing local treatment and a reduction in side effects [108].

In an *in vitro* study, it was found that a bilayer coating of zoledronate in combination with $Ca_3(PO_4)_2$ on a magnesium-strontium alloy scaffold can accelerate the proliferation and osteogenic differentiation and mineralization of preosteoblasts, while inducing the apoptosis of osteoclasts and inhibiting their differentiation [109]. Bisphosphonates can cause the apoptosis of both osteoclasts and osteoblasts at higher concentrations or with burst release. Therefore, the pulse electrodeposition technique of coating and incorporating zoledronate proves to be more suitable than the method involving soaking, as it allows a more controlled and slower release. In addition, an appropriate coating is necessary in the case of magnesium-based implants as such slows down the release of magnesium ions [110]. The presence of zoledronate in the hydrogel has been shown to significantly improve the bone fixation of the implant. In an *in vivo* study in rats, an implant coated with a zoledronate-incorporated hydrogel in the bone withstood 42% greater force 31 days after implantation compared to an implant without zoledronate [111]. The improved fixation of an implant with a bioactive fibrinogen coating containing zoledronate was also demonstrated in an *in vivo* human study, where pins with the aforementioned coating and with a coating of hydroxyapatite were compared [112].

The fixation of the implant depends, among other factors, on the concentration of the bisphosphonate. The presence of a higher concentration of zoledronate affects bone density, which in turn decreases significantly in the immediate vicinity and increases with a decrease in concentration and with an increase in the distance from the source of zoledronate [113]. This could be due to the effect of higher concentrations of bisphosphonates on the activity of osteoblasts, which are imperative cells in the bone integration of the implant [114]. One of the more promising drug delivery system is TiO_2 nanotubes. The nanotube structure allows a larger surface area for zoledronate application as well as more controlled release. The nanotube structure, together with zoledronate, contributes to both implant stability and the better integration of the implant with the bone tissue [115]. The combination of BMP-2 and zoledronate in a polycondensed deoxyribose isobutyrate ester polymer coating has been shown to induce bone formation and healing in allograft, suggesting potentially synergistically improved orthopaedic outcomes with implants [116]. As zoledronate has an antitumor effect on malignant bone tumours, without causing systemic toxicity, this bisphosphonate is suitable for application to implants in patients with bone cancer [117]. Zoledronate-loaded $Ca_3(PO_4)_2$ coating on magnesium-strontium alloys has been found to synergistically inhibit giant cell tumours of bone by inducing their apoptosis, increasing oxidative stress, and inhibiting stromal cells-mediated pre-osteoclasts

migration and osteolysis [118]. Regarding the formation of new bone tissue at the implant interface, strontium coatings have been shown to be significantly more effective than zoledronate coatings [119]. On the other hand, the inhibitory action of zoledronate in the coating on osteoclast activity is more effective than that of strontium [120].

3.1.3. Other bisphosphonates

A zoledronate-coated implant, with zoledronate immobilized on cross-linked fibrinogen, showed greater implant fixation compared with a pamidronate-coated implant also immobilized on cross-linked fibrinogen. In an *in vivo* study, these two bisphosphonates were immobilized by ethyl dimethyl-aminopropylcarbodiimide/imidazole immobilization [121]. The presence of pamidronate in hydrophobic coatings such as PCL decreases the hydrophobicity of the coating, which contributes to the faster degradation of this coating (usually 2–3 years is the time needed to degrade a PCL coating due to its high hydrophobicity) [122]. The method of application is also important in achieving a sufficiently high concentration of the bisphosphonate. The results of comparing the adsorption of pamidronate in a simulated body fluid-grown hydroxyapatite coating on titanium (99.7% pure) showed higher adsorption from the co-precipitated solution of pamidronate dissolved in the simulated body fluid compared to its aqueous solution [123]. Polyvinyl chloride also has the property of mitigating degradation, which enables release with a higher degree of control of the bisphosphonate risedronate compared to the release profile of risedronate from chitosan. On the other hand, the combination of risedronate and chitosan provides a greater material elasticity. However, the problem with chitosan-based coatings is their faster biodegradability and consequently burst release, compared to polyvinyl chloride-based coatings [124].

Risedronate loaded into a polyelectrolyte complex coating of cationic homopolypeptide poly(L-lysine) and two cellulose sulphates with low and high degrees of substitution was released within a few hours. Moreover, risedronate incorporated into the aforementioned polyelectrolyte complex coating was found to be toxic to human MSCs, which may be a consequence of the cell toxicity of risedronate itself [125]. Compared to alendronate, risedronate is a bisphosphonate with low mineral binding affinity. Alendronate and risedronate also differ in their suitability, as the presence of risedronate in the hydroxyapatite coating resulted in a significant systemic effect and is more suitable for non-peri-implant bones compared to alendronate. In contrast, alendronate is more suitable for peri-implant bones and also has a stronger osteoinductive effect [126]. The synergistic effect of the two bisphosphonate combinations was demonstrated in an *in vivo* study in rats, where the incorporation of pamidronate and ibandronate into the fibrinogen coating significantly improved implant fixation compared with the hydroxyapatite-coated control implant [127]. The same results were obtained when comparing the pull-out forces between fibrinogen-coated screws and fibrinogen-coated screws with incorporated bisphosphonates [128].

Pamidronate and ibandronate together in a fibrinogen matrix also improve the fixation of dental implants, as shown in a clinical trial involving sixteen individuals. They received one dental implant with bisphosphonates in the fibrinogen matrix and one with a coating without bisphosphonates. After six months, significantly higher implant stability was shown for the dental implants with bisphosphonates, indicating potential for their use in orthopaedic implants [129]. One way to incorporate bisphosphonates into the coating is by the electrolytic deposition technique, as used in a study involving etidronate. The electrolytic deposition technique, which can be performed at room temperature, enables the stability of the etidronate molecules to be maintained and the release to be prolonged [130].

3.2. Nucleic acids as osteoclast inhibitors

Nucleic acids represent one of the potentially promising therapeutic agents in the treatment of bone diseases, acting as enhancers and silencers of genes [131]. Based on their structure, they are classified into nucleotide and nucleoside sequences. The nucleotide sequences consist of a nitrogen-containing base, a sugar molecule, and a phosphate group, while the nucleoside sequences do not contain a phosphate group. The phosphate group is crucial for linking the nucleotides in sequence to form a DNA or RNA molecule. Due to their composition, cyclic dinucleotides also belong to the group of nucleic acids that act as osteoclast inhibitors. The cyclic structure consists of two riboguanosine residues or two riboadenosine residues linked by 3'−5' phosphodiester bonds or one riboguanosine residue and one riboadenosine residue linked by 2'−5' or 3'−5' phosphodiester bonds (Fig. 3) [132,133]. The function and role of cyclic dinucleotides in osteoclast inhibition are described in more detail below.

3.2.1. Deoxyribonucleic acid (DNA)

DNA can also serve as a sequence that acts as a bridge to bind growth factors, nucleic acids, and other larger molecules to a bioactive coating or scaffold [42,134,135]. Although DNA is more stable than RNA (usually single-stranded) [136,137], its double-stranded structure requires it to be properly packaged for gene therapy. Greater stability is achieved by inserting the DNA of the desired gene into a vector, called a plasmid (small circular DNA), which is a nonviral gene delivery system. Although non-viral vectors have minimal toxic or immunological complications, the weak point of the plasmid is the presence of antibiotic resistance genes in the plasmid backbone, as they pose a safety risk and can also reduce the efficiency of gene delivery [42,138]. This viral gene delivery system is represented by viral vectors such as adenovirus containing double-stranded DNA and an adeno-associated virus containing single-stranded DNA. These are more effective than plasmids in transferring genetic material into a cell, but the risk of toxic effect and even death is higher [42,139,140].

Osteoblasts and osteoclasts are distinguished (in addition to morphological features) by differences in gene expression. Osteoblasts, which are desirable cells and contribute to osseointegration, express members of the ephrin family, particularly ephrin receptor B4. Osteoclasts, which are undesirable cells because they initiate bone resorption, express the target gene ephrin B2 of the nuclear factor of activated T-cells. An *in vivo* study in mice showed that ephrin B2, expressed by osteoclasts, acts through its ephrin receptor B4 in osteoblasts to promote osteoblast differentiation. In this regard, reverse signalling with osteoblast-derived ephrin receptor B4 inhibits osteoclast differentiation by inhibiting the osteoclastogenic c-Fos – NFAT-c1 cascade. However, the overexpression of ephrin receptor B4 has also been shown to increase bone mass in transgenic mice [141,142]. In summary, the overexpression of ephrin B2 increases osteoblast differentiation by forward signalling and inhibits osteoclastogenesis by reverse signalling. Therefore, ephrin B2 is a promising agent in gene therapy for bone repair [143]. Porous collagen scaffolds have been identified as effective gene-activated matrices for polyplex uptake, consisting of a non-viral vector, polyethylenimine (PEI) and an ephrin B2 DNA plasmid. Such a scaffold containing the ephrin B2 plasmid was also found to induce the overexpression of ephrin B2, thereby forcing MSCs to differentiate into an osteoblast cell line [144]. Another DNA plasmid that has been shown to be a successful osteoclast inhibitor is the recombinant Rho-inhibiting C3 toxin, i.e. C2IN-C3lim-G205C. In an *in vitro* study, the presence of this plasmid DNA was found to successfully inhibit the activity of the pre-osteoclastic cell line RAW 264.7, while osteoblast activity and proliferation remained unchanged [145].

3.2.2. Ribonucleic acids (RNA)

In addition to the RNA molecules involved in transcription, translation, and protein coding, there are also small noncoding RNAs that regulate gene expression posttranscriptionally. Small endogenous RNAs, microRNAs (miRNA), found in some viruses, plants, and higher eukaryotes, regulate gene expression, which in turn affects the phenotype of the organism [146]. They function by base-pairing with

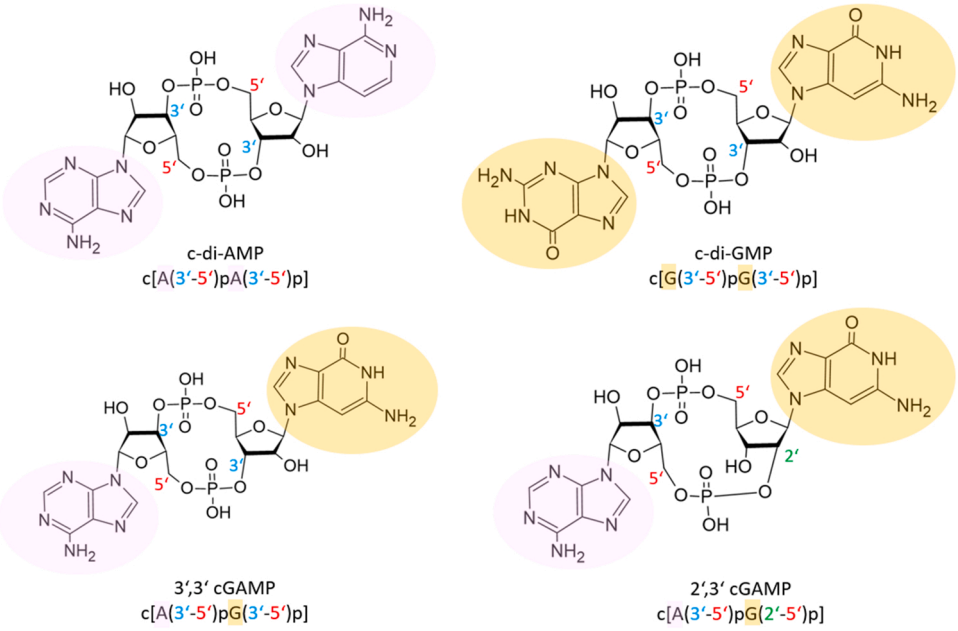

Fig. 3. Structural overview of cyclic dinucleotides: two riboadenosine residues linked by 3'−5' phosphodiester bonds (c-di-AMP); two riboguanosine residues linked by 3'−5' phosphodiester bonds (c-di-GMP); riboadenosine residue and riboguanosine residue linked by two 3'−5' phosphodiester bonds (3',3' cGAMP); riboadenosine residue and riboguanosine residue linked by 2'−5' and 3'−5' phosphodiester bonds (2',3' cGAMP).

complementary sequences in messenger RNA (mRNA) molecules. Another type of RNA regulatory molecule are small interfering RNA (siRNA) and short hairpin RNA (shRNA), which are exogenous double-stranded molecules. These molecules have the form of a long bimolecular duplex and an elongated hairpin, respectively [146]. Both siRNA and shRNA are effective in posttranscriptional regulation, but the latter is more cost-effective, easier to deliver, and has a longer-lasting effect in silencing target genes. Additionally, shRNA is double-stranded, but unlike the siRNA molecule, it contains a hairpin loop. The advantages and disadvantages of incorporating the above RNA molecules into bioactive coatings as a therapeutic strategy for osteoclast inhibition are listed in Table 1.

With therapeutic agents, establishing a system that allows controlled release is often a problem. In therapeutic treatments with RNA, or more specifically siRNA, this alone is not important. The RNA is fully released from the calcium/siRNA-coated titania nanotube surface within 14 days, with an initial burst release of about 80% in the first 2 h. This time frame is adequate to achieve transfection and gene manipulation [161]. The inflammatory response of cells to the fine implant particles can be inhibited by the autophagy inhibitor chloroquine or the short interference RNA siRNA-CD147, which silences the basigin (CD147) gene important in osteoclastogenesis. It was discovered that silencing basigin gene with siRNA and chloroquine can reduce particle-induced autophagy and soluble RANKL expression [162]. Significant suppression of the expression of RANK was observed in an *in vitro* experiment on osteoclast-precursors and osteoclasts in which cells were exposed to PLGA microparticles consisting of PLGA and RANK siRNA/branched PEI complex [163]. The presence of branched PEI increases the stability and loading efficiency of siRNA in PLGA microparticles, with the encapsulation efficiency of siRNA being approximately 80% [163,164]. Considering the different ratios of PEI and siRNA, it was found that a mixture of 0.4 μL of 1 mg/mL PEI and 2.5 μL of 20 μM siRNA was required for the complete capture of siRNA into PEI [163]. Protein phosphatase 2 A (PP2A), the major serine-threonine phosphatase, is highly expressed in human periprosthetic membranes with aseptic loosening and in a mouse model with titanium particle-induced osteolysis. A selective PP2A inhibitor (siRNA PP2A) inhibits osteoclastogenesis and attenuates osteoclastic resorption by inhibiting the RANKL-induced nuclear factor κB (NF-κB) signalling pathway and the c-Jun N-terminal kinase signalling pathway [165]. Implant wear particles also cause osteoblast apoptosis, which is the cause of the aseptic loosening of the implant. This osteoblast apoptosis can be drastically reduced by the autophagy inhibitor 3-methyladenine and siRNA of the autophagy-related 5 (ATG5) gene. ATG5 siRNA suppresses the expression of the ATG5 gene, which is required for autophagosomes formation, resulting in reduced apoptosis [166].

Another type of RNA molecule able to affect osteoclastogenic activity is shRNA. Included in the plasmid and incorporated into bioactive glass coating, it can reduce the expression of tumour-necrosis factor α (TNF-α) by 12-fold and increase the expression of alkaline phosphatase by 4-fold. Gene regulation, i.e. the reduction of TNF-α expression, using retrovirus-mediated shRNA significantly inhibits osteoclast differentiation, leading to reduced bone resorption in the periprosthetic environment [72].

Table 1
Advantages and limitations of siRNA, shRNA, and miRNA.

Class	length (nt)	PROS	CONS
siRNA	20–27 [147]	perfectly complementary to the target mRNA [8,148]	not stable *in vivo*; degradation within a few days [149]; off-target effects; low efficacy in silencing the intended targets; may activate innate immunity [8,148, 150,151]
shRNA	50–100 [152]	perfectly complementary to the target mRNA [8,148]	off-target effects; low efficacy in silencing the intended targets; may exert target-independent toxicity [8,148,150,151]
miRNA	~22 [153–155]	stable in blood plasma [156, 157]; easy to test *in vivo* [158]; more cost-effective compared to recombinant protein approaches; imperfect binding (resulting in targeting multiple targets within a given cell pathway) [8,159,160]	imperfect binding (resulting in multiple targets being targeted within a given cell pathway) [8,159,160]

The inhibition of osteolysis can be achieved by using an inhibitor of the recently discovered microRNA miR-106b, which inhibits the expression of miR-106b, thereby reducing the rate of joint inflammation and joint bone destruction. The results of an *in vivo* study in mice with rheumatoid arthritis showed that the inhibitory miR-106b was able to inhibit synovial inflammation, regulate RANKL/OPG signalling, and reduce the number of mature osteoclasts [167]. Pure-grade Ti (99.99% purity) coated with strontium-substituted hydroxyapatite nanoparticles possesses hydrophilic properties and has an inhibitory effect on osteoclast resorption and activity. Moreover, the presence of miR-21 further enhances the beneficial properties of the coating by inducing the expression of osteogenesis-related genes such as collagen type 1, RUNX-2, osteocalcin, and osteopontin. Consequently, due to the synergistic effect of strontium-substituted hydroxyapatite and miR-21, this coating promotes angiogenesis and osteogenesis, while inhibiting osteoclast functioning. To achieve such results in an *in vitro* and *in vivo* study, miR-21 needs to be encapsulated in nanocapsules mixed with O-carboxymethyl chitosan to form a gel that serves as a coating [168]. The functional coating does not necessarily have to consist of additional components, but only miRNA lipoplexes may be sufficient. The latter proved to be an effective coating on a microporous titanium (commercially pure) implant. Lyophilization of miRNA lipoplexes on a microporous titanium oxide surface formed by microarc oxidation filled 2–5 μm pores on the implant surface with 140 nm lipoplexes. Such an implant, in which the lipoplexes contained miR-29b and antimir-138, was used to manipulate the MSC phenotype and inhibit microRNAs produced by osteoclast functioning, thereby promoting bone resorption [169].

3.2.3. Cyclic dinucleotides

Cyclic dinucleotides such as cyclic diadenylate monophosphate (c-di-AMP) and cyclic diguanylate monophosphate (c-di-GMP) have recently proven to be very promising. Cyclic dinucleotides originate from the intestinal microbiota and modulate bacterial survival, colonisation, and biofilm formation [170–173]. Since cyclic dinucleotides are likely to be absorbed from the gastrointestinal tract and transferred to the bone marrow, they could very likely affect the differentiation and metabolism of osteoclasts derived from monocyte/macrophage lineages [174]. The presence of cyclic dinucleotides also reduces inflammation in the digestive tract by indirectly regulating the stimulator of interferon gene (STING) signalling [175]. STING is an endoplasmic reticulum-associated membrane protein that recognizes "self"-DNA in the cytosol, i.e. DNA from pathogens after infection or DNA from necrotic or apoptotic cells. In the case of chronic STING activation, an inflammatory response may occur, and inflammatory diseases may even develop [132,176].

In an *in vitro* study in which bone marrow-derived macrophages were differentiated into osteoclasts using macrophage colony stimulating factor and an NF-κB receptor activator, cyclic dinucleotides were found to inhibit dose-dependent osteoclast differentiation. Cyclic dinucleotides display immunomodulatory activity by inducing the expression of interferon-1 via the STING signalling pathway in macrophages, making the STING signalling pathway critical for inhibiting osteoclast differentiation. The results also showed that cyclic dinucleotides can only affect the differentiation of early osteoclasts and cannot affect the differentiation of mature osteoclasts and osteoblast precursors. Moreover, cyclic dinucleotides inhibited the expression of c-Fos, TRAP, cathepsin K, and NFAT-c1, which are involved in the RANKL signalling pathway, at an early stage of osteoclast differentiation. Given that no cytotoxicity was present, cyclic dinucleotides are one of the more promising agents that would be worth testing as a component in bioactive coatings [174].

3.3. Proteins as osteoclast inhibitors

Molecules consisting of an amino acid sequence, i.e. proteins, are also actively involved in maintaining cellular functions and pathways of cellular processes. Therefore, they represent one of the possible types of therapeutic agents that can influence the activity of osteoclasts. Calreticulin, a 417-amino-acid-long protein with a mass of 46 kDa and intermediate P- and C-terminal domains with multiple interaction sites affecting Ca, also has a potent anti-osteoclastogenic effect. Both *in vitro* and *in vivo* experiments in rats demonstrated the beneficial effect of recombinant human calreticulin, a known intracellular protein, as it inhibits key pro-osteoclastogenic transcription factors such as c-Fos and NFAT-c1 precursor in osteoclasts. It was also found that the protein was released from hydrogel in a sustained manner during 4 days of immersion. Recombinant human calreticulin acts as an inhibitor of inflammatory osteolysis and inhibits osteoclast formation mediated by RANKL [73]. Monocytes, especially macrophages, are a cause of chronic inflammation due to the presence of implant particles, which represent a foreign substance to bone tissue. One of the most important agents in the regulation of macrophages is the chemokine (C-C motif) ligand-2 (CCL2) [177].

In order to inhibit an inflammatory response, a recombinant seven-amino acid truncated protein (7ND) that inhibits CCL2 signalling was used in an *in vivo* study. The 7ND protein was applied to hollow titanium rods using a layer-by-layer technique and inserted into a mouse femur. Polyethylene particles were infused into titanium rods to induce an inflammatory response. The results showed that the use of 7ND reduced the systematic recruitment of macrophages, the number of osteoclasts, and consequently bone loss. These results suggest that 7ND, as a prospective coating, attenuates bone loss caused by excessive inflammatory stimuli and may also alter the homeostatic composition of bone [178].

The efficiency of alendronate, a bisphosphonate, and recombinant Fc-labelled OPG (OPG-Fc) was compared in an *in vivo* experiment in rats by intravenous administration. The screws were inserted into the rat's tibia. The rats were then divided into two groups, one with OPG-Fc administered and one with alendronate administered. There was no significant difference between the efficacy of OPG-Fc and alendronate, but the effect of 8 mg/kg OPG-Fc was as effective as a high dose of alendronate (200 μg/kg). This means that the RANKL targeted drug, i.e. OPG-Fc, is comparable to very high doses of the bisphosphonate alendronate, and successfully improves the attachment of the implant to the bone [179]. In an *in vivo* study in rats, it was predicted that the activation of the Wnt/β-catenin pathway by the inhibition of glycogen synthase kinase-3β would reduce bone loss by regulating osteoblast and osteoclast differentiation. This hypothesis was confirmed by the use of the glycogen synthase kinase-3β inhibitor AR28. The mRNA levels of β-catenin, RUNX-2, osterix gene, collagen type 1 α1, OPG, and alkaline phosphatase increased and contributed to osteoblast proliferation and decreased osteoclast numbers [180].

3.4. Statins

Statins are one such therapeutic agent that has an anabolic effect on bone formation through osteoblast differentiation, which is also reflected in increased osteocalcin mRNA expression [181,182]. In this way, they inhibit bone resorption by preventing the prenylation of GTPases. Statins induce osteoclast apoptosis and inhibit bone resorption [183,184], making them promising therapeutic agents for use in bioactive coatings on orthopaedic and dental implants.

Not only titanium but also polymethyl methacrylate particles from the implant promote the release and phagocytic activity of monocytes and macrophages around the periprosthetic tissue and generate a variety of inflammatory cytokines, including TNF-α, matrix metalloproteinase, interleukin-1α, and interleukin-6 . The expression of these proteins can be inhibited by ulinastatin. In *in vitro* and *in vivo* mouse models, a decrease in the secretion of anti-inflammatory cytokines such as matrix metalloproteinase-9, interleukin-6, TNF-α, RANK, and cathepsin K was observed in the presence of ulinastatin, which is associated with a decrease in NF-κB activity and mitogen-activated protein kinase (MAPK), implying a decrease in inflammatory osteolysis [185]. A

similar effect to that obtained with ulinastatin was obtained in an *in vitro* study in which cytokine activation and response to polymethyl methacrylate particles were observed in the presence of pitavastatin. An enzyme-linked immunosorbent assay (ELISA) showed the inhibition of mRNA and the expression of interleukin-1, interleukin-6, and TNF-α as a result of the presence of pitavastatin [186]. The family of statins also includes simvastatin, which, in addition to its beneficial effects on osteoblast differentiation and proliferation [187], has positive effects as an osteoclast inhibitor. Simvastatin has been found to promote MC3T3-E1 mouse cell (preosteoblast cell) activity at concentrations below 0.01 g/L, with a cytotoxicity threshold at 0.05 g/L. Simvastatin, currently prescribed to inhibit cholesterol biosynthesis, also appears to be a potentially effective osteoclast inhibitor as a promoter of osteogenic differentiation [188].

3.5. Strontium

Strontium, which can regulate both osteoblastogenesis and osteoclastogenesis, as well as osteogenesis and adipogenesis, is an effective antiresorptive agent [189,190]. The compact strontium phosphate ($SrPO_4$) coating contains a series of crystalline particles that give it a unique three-dimensional structure, which contributes to the modulation of osteoblast and osteoclast activity and consequently promotes bone formation. The release of strontium from the coating was found to have a positive effect on osteoblast proliferation and differentiation [191]. The incorporation of strontium, specifically $SrCl_2$, into the silica-hybrid sol-gel coating increased the hydrophilicity and roughness of the coating, affecting protein affinity profiles and cell adhesion [192]. The same findings regarding the positive effect of Sr coating on titanium (99.7% pure) scaffolds on bone formation and the balance between osteoblasts and osteoclasts were reached through an experiment in which the signalling pathways were investigated in more detail in the presence of Sr [193]. The results showed that Sr inhibited osteoclast-associated gene expression. In addition, Sr also inhibited protein kinase B and NFAT-c1, suggesting an inhibitory effect on osteoclasts [193,194]. Furthermore, strontium in combination with hydroxyapatite has shown promise as a coating component. A coating of Sr and hydroxyapatite not only had strong effects on bone formation and the improvement of biomechanical strength in an *in vivo* experiment using rats, but also improved the osseointegration [195].

The same beneficial properties were found in an *in vivo* rabbit model [196] and *in vitro* using MG63 cells [194,196]. The Sr-doped bioactive hydroxyapatite coating significantly improved cell spreading compared to 99.99% pure titanium implants, resulting in better cellular communication through pseudopods [196]. Similar to titanium scaffolds with the aforementioned coating, but also with polyetherketoneketone scaffolds with the same coating, good intrinsic bone mechanical quality was achieved at the microlevel. This is due to the local release of Sr, which allows dual effect in enhancing osteogenesis and inhibiting osteoclastogenesis and improved bonding strength between the scaffold and the bone [197]. Although Ca is much higher in bone than Sr, Ca, and $Ca_3(PO_4)_2$ does not have as strong an effect on cell adhesion, osteogenic differentiation, inhibition of osteoclast fusion, and their function as Sr and Sr-substituted $Ca_3(PO_4)_2$ coating [198–202]. Sr-containing surface and coating modifications, such as Sr-loaded TiO_2 nanotubes loaded with silk fibroin and silver coating and Sr-incorporated TiO_2 surface in general, extend the potential beneficial properties [203,204]. The presence of Sr and silk fibroin inhibits osteoclasts, while silver inhibits the proliferation of *E. coli* and *S. aureus* [203].

Another example of enhanced beneficial properties is a complex combined coating of Sr-substituted hydroxyapatite and Zn-substituted β-tricalcium phosphate, in which Sr-substituted hydroxyapatite inhibited osteoclast function and Zn-substituted β-tricalcium phosphate improved osteoblast viability [205]. A synergistic and multidirectional effect on osteoclastogenesis and osseointegration was also found in the combination of bioactive elements such as strontium, silicon, and magnesium in the ceramic coating, with significantly higher bonding strength on Ti6Al4V than pure hydroxyapatite coating [206]. When comparing the concentrations of Sr in collagen type 1 coating on magnesium – zirconia alloys, it was found that the beneficial properties of the implant were more enhanced at higher concentrations (5 wt%) than at lower (2 wt%) [207]. Not only does strontium improve the biocompatibility of the implant, but it is also important that the coating allows for prolonged release, which was achieved with dopamine, sodium alginate and strontium-grafted titanium implant [208].

3.6. Other chemicals inhibiting osteoclasts incorporated in bioactive coatings

An *in vivo* study of ethyl 2,5-dihydroxybenzoate in PLGA coatings found that topical administration of the drug decreased bone resorption and increased bone formation around the implant, improving fixation. In addition to bisphosphonates, ethyl 2,5-dihydroxybenzoate, which stimulates bone formation and inhibits bone resorption, is effective in the same manner as strontium ranelate [209]. RANKL/OPG signalling and osteoclast inhibition are affected by strontium ranelate [210], but serious side effects such as Steven-Johnson syndrome and toxic epidermal necrolysis, which occur at concentrations greater than 0.5 mM, are a concern [211,212]. Polydopamine also proved to be a promising coating compared to uncoated Ti6Al4V substrate in both *in vitro* and *in vivo* studies. Polydopamine coating was found to reduce the number of osteoclasts and the activity of TRAP, which is involved in osteoporosis. However, the results of an experiment with a polyphenolic tannic acid coating showed that it had a stronger effect on reducing osteoclast development and the activity of TRAP, which inhibits osteoclastogenesis [213]. Stronger inhibition of osteoclast activity was found in the case of the addition of strontium to the polyphenol tannic acid coating [214].

Secondary plant metabolites, such as flavonoids, have known antioxidant, anti-inflammatory, and antibacterial properties. These include quercitrin, which is extracted from Tartary buckwheat and oak [215,216]. Quercitrin is a flavonoid that successfully reduces osteoclast-associated gene expression. The results of an *in vitro* and *in vivo* study using titanium surfaces (commercially pure, grade IV) blasted with titanium dioxide (TiO_2) microparticles and covalently coated with quercitrin showed a decrease in the gene expression of functional osteoclast markers. Therefore, quercitrin indirectly provides a more balanced ratio of bone resorption and regeneration, which could ultimately lead to improvements in both dental and orthopaedic implants [217]. The same antioxidant and osteoclast inhibitory effects have also been noted with vitamins. Vitamin E, together with an ultraviolet-irradiated precursor of vitamin D (7-dehydrocholesterol), was found to decrease RANKL mRNA levels, which could imply the indirect inhibition of bone resorption. Titanium implants (commercially pure, grade IV) coated with 7-dehydrocholesterol and supplemented with vitamin E were found to be biocompatible with human gingival fibroblasts and to inhibit osteoclastogenesis [218]. Vitamin D is synthesized in this drug combination, which has a positive effect on osseointegration and an inhibitory effect on osteoclastogenesis [219–222].

3.7. Promising chemicals inhibiting osteoclast activity not (yet) included in bioactive coatings

Osteoclastogenesis and osteolysis can also be inhibited by the inhibition of PP2A. *In vitro* experiments have shown that okadaic acid successfully inhibits PP2A, which in turn activates the Wnt/β-catenin signalling pathway important for osteoblastogenesis. Significantly increased β-catenin expression was observed compared to samples exposed to titanium particles in the absence of okadaic acid. Furthermore, an *in vivo* study in mice showed wear debris-induced resorption of periosteum was inhibited in animals injected with okadaic acid [223]. In

the presence of titanium particles, the degradation of β-catenin, which is required for the Wnt/β-catenin signalling pathway, occurs due to implant wear. An *in vivo* study in mice showed that melatonin (administered intraperitoneally) reduces the degradation of β-catenin, resulting in the alleviated depression of osteoblastic differentiation and mineralization, as well as decreased bone resorption at the osteolytic site [224]. The negative effects of titanium particles on MSCs can be reduced with icariin, as it also inhibits the degradation of β-catenin. *In vitro* and *in vivo* studies in mice showed increased bone mass and decreased bone loss at osteolytic sites in the presence of icariin (in the medium and by gavage, respectively) caused by titanium particles. [225].

As mentioned in Section 3.6, the presence of vitamin E in bioactive coating inhibits osteoclast activity [218]. The same inhibitory effect on inflammatory osteolysis was observed *in vitro* using human osteoblastic SaOS2 cells. The vitamin was cross-linked with ultra-high-molecular-weight polyethylene particles. The expression of sclerostin and dickkopf-1 was reduced in cells in the presence of vitamin E particles. Sclerostin is an osteocyte-soluble factor that negatively regulates Wnt signalling, and dickkopf-1 blocks the interaction of Wnt with β-catenin, resulting in the degradation of β-catenin [226]. NF-κB signalling is also important in inhibiting osteoclast functioning. *In vivo* studies have shown that emodin injection reduces bone resorption and osteoclast numbers due to poorer NF-κB signalling. Emodin, a type of anthraquinone compound, is an agent derived from rhubarb root that has been shown to be an effective osteoclast inhibitor [227] and is a promising agent for incorporation in bioactive coatings. Sudachitin also belongs to the group of plant-derived active substances with an inhibitory effect on osteoclasts. Sudachitin is a polymethoxyflavonoid derived from *Citrus sudachi* that successfully inhibits the differentiation of pre-osteoblasts or early osteoclasts into mature osteoclasts. A disadvantage of sudachitin is that it significantly increases the expression of RANKL in osteoblasts, which in turn triggers the mechanism of cell differentiation into osteoclasts [228].

Accelerated bone and cartilage formation from MSCs is also affected by the permanent loss of gamma-secretase activity and Notch2 signalling, leading to pathological bone harvesting and the depletion of MSC from the bone marrow. However, for bone regeneration, the inhibition of gamma-secretase and Notch2 activity is welcome. Nirogacestat, an inhibitor of the gamma-secretase signalling pathway, has been shown to inhibit osteoclast formation from bone marrow macrophages *in vitro* without causing cytotoxicity. It also inhibits Notch2 signalling as well as RANKL-induced protein kinase B signalling [229]. Potent inhibition of osteoclast differentiation, osteoclast-specific gene expression, and bone resorption can be affected by applying a dual tyrosine inhibitor and phosphoinositide kinase called PP121. It suppresses the induction of NFAT-c1 by proto-oncogene tyrosine-protein kinase/MAPK (extracellular signal-regulated kinase and p38 kinase)/protein kinase B. In an *in vitro* experiment using bone marrow-derived macrophages, PP121 was found to inhibit osteoclast formation without causing cytotoxicity [230].

The RANK and its ligand RANKL are important regulators of osteoclast activity and bone resorption and influence osteolysis around the implant. In an *in vivo* human study in which 110 patients with an artificial joint were injected with 60 mg of denosumab every 6 months, denosumab was shown to be effective in reducing osteolysis. Denosumab is a monoclonal antibody that acts as an inhibitor of the RANK ligand and would most likely prove to be an effective inhibitor of osteolysis and osteoclastogenesis if incorporated into bioactive coatings [231]. Particle-induced osteolysis and osteoclastogenesis can be inhibited by caffeic acid phenyl ester. Caffeic acid phenyl ester acts as an inhibitor of NF-κB and NFAT-c1, which significantly reduces superficial bone resorption and local volumetric bone loss even at low doses, as shown by the results of an *in vivo* study [232]. In addition to caffeic acid phenyl ester, parthenolide has also shown promise as an inhibitor of NF-κB. It acts as an inhibitor of NF-κB and sesquiterpene lactone and has been shown to be a beneficial therapeutic agent in an *in vivo* study in mice. In test organisms treated with parthenolide, bone mass increased, signifying decreased bone resorption, decreased osteoclast proliferation, and decreased aseptic loosening [233].

4. Conclusions

There are numerous different therapeutic agents that are used for the purpose of inhibiting bone resorption, that is, to prevent the activity of osteoclasts. They are used in systemic treatment, which means that in this case the therapeutic agent is administered orally or by injection. In both methods, the drug travels through the body, so the drug is lost throughout the body, and an insufficient amount reaches the desired location. In addition to insufficient effectiveness, there are also potential side effects. When taken orally, one possible side effect is irritation of the gastrointestinal tract, manifested in gastritis, vomiting, and diarrhoea. When injected, the path of the drug through the blood system is an obstacle as an insufficient amount reaches the desired site. When the dose is increased, it also affects other cells in the body, and side effects occur.

An effective solution is to incorporate therapeutic agents into bioactive implant coatings that provide localized treatment, avoiding both side effects and spread through the body. Many of them are still used in systemic treatment but seem to be very promising in local treatment. Such therapeutic agents include bisphosphonates, statins, and others. On the other hand, in the case of nucleic acids, local treatment is most appropriate due to the direct entry of these larger molecules into cells and avoidance of their possible degradation due to the presence of nucleases in the body. Based on the literature reviewed, it can be concluded that it is still a great challenge to select the most appropriate therapeutic agent and determine the concentration required for satisfactory bone resorption inhibition. There is still a lack of research into combinations of therapeutic agents and bioactive coating materials, and most importantly, the release time period. For example, it was mentioned that cyclic dinucleotides cause inflammation and play an important role in bacterial adhesion. The question is at what concentration inflammation occurs and at what induction of bacterial adhesion. Although the inhibition of osteoclasts is effective, concentrations need to be better defined for all therapeutic agents, not just cyclic dinucleotides. Given that many antiresorptive agents not only inhibit resorption but also contribute to faster bone regeneration, which means enhanced osteoinduction, they represent a very promising group of agents for use as coatings of 3D scaffolds in bone tissue engineering. Considering that there are a number of effective therapeutic agents in the fight against bone resorption in the vicinity of the implant, much research is still needed to optimize parameters such as concentrations, controlled release, and material combinations to incorporate these therapeutic agents.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to legal or ethical reasons.

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