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Review

Nanoparticle Architecture Governing Antibacterial and Osteoinductive Responses in Bone-Integrating Implants

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Abstract

Metallic nanoparticles (MNPs) have emerged as leading candidates in biomedical applications owing to their unique physicochemical properties and dual functionality, combining potent bactericidal and osteoinductive effects. These bioactivities are intricately governed by structural parameters such as size, shape, crystallinity, and chemical composition, which collectively dictate their interactions with biological systems. These interactions affect key mechanisms including oxidative stress induction, membrane disruption, and modulation of cellular signaling pathways. Despite considerable progress, a comprehensive understanding of the structure property–activity-specific structural relationship in MNPs remains incomplete, hindering the rational design of optimized nanomaterials. This review critically examines recent advances in elucidating the bactericidal and osteoinductive mechanisms of MNPs, with a particular focus on the role of structural determinants. Furthermore, current challenges and future directions for tailoring nanoparticle architecture to enhance clinical performance are discussed. To address this, we conducted a systematic review of the literature published between 2005 and 2024 using Web and Web of Science direct and Scopus databases. Our analysis is structured around a structure →mechanism→outcome perspective, linking nanoparticle features to biological responses. Key insights include the following: (i) nanoparticles below ~20 nm generally enhance bacterial efficiency through enhanced membrane disruption; (ii) surface hydroxyl density above critical thresholds promotes osteogenic signaling; and (iii) safe concentration windows remain narrow, highlighting the importance of dose optimization. We conclude by discussing the translational challenges and future directions for tailoring nanoparticle architectures to advance clinical applications.

Keywords: metallic nanoparticles; bone-integrating implants; surface modification; nanotopography; osteo-imunomodulación; antibacterial mechanisms

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1. Introduction

In recent years, the definition of bioactive material has evolved significantly due to advances in nanotechnology, enabling the development of nanomaterials with customized properties. According to the scientific literature, it is a nanomaterial used to replace part

of a living system or to function intimately with living tissue. It is characterized by being biocompatible, which means it could stimulate favorable biological reactions in relation to its application, exhibiting appropriate mechanical properties such as its weight and density [1]. Among these biomaterials, metallic nanoparticles (MNPs) have generated great interest in biomedical research due to the effectiveness of the biomaterial for its application in the field of bone replacement, generating favorable responses from living tissues upon implantation. This potential is rooted in their tunable physicochemical properties, which, when optimized, can minimize toxicity and promote interfacial bonding [2]. However, it is crucial to note that these properties are not inherent but highly context-dependent. For instance, while dioxide nanoparticles (e.g., in nanotube form) are renowned for their excellent biocompatibility and ability to osseointegrate [3], silver nanoparticles (Ag NPs) exhibit a well-documented dose-dependent cytotoxicity alongside their antibacterial efficacy [4]. This contrast highlights that the capability of MNPs to solve interfacial bonding problems is not universal but is contingent upon a careful design that balances biological activity with safety [5].

Beyond composition, surface functionalization at the nanoscale is a key strategy to enhance this balance. For example, biofunctionalized titanium coatings with collagen-mimetic protein can deliver osteoinductive signals and reduce bacterial adhesion by up to 1000-fold compared to human collagen and 10–100-fold compared to uncoated titanium [6]. Similarly, organic nanomaterials such as chitosan, often combined with peptides (e.g., glycine–aspartic acid) or antibiotics (e.g., vancomycin), can promote bone growth while offering bactericidal protection [6,7]. To begin with, osseointegration is the direct structural and functional connection between bone and an implant surface, while osteoinduction refers to the simulation of progenitor cells to differentiate into bone-forming osteoblasts [8].

However, although the use of bioactive materials in bone tissue regeneration and their implementation as a possible strategy to address health challenges such as antimicrobial resistance has been widely studied, the exact mechanisms through which these nanoparticles exert their effects are still unknown [1]. Therefore, it is essential to develop studies that delve into the influence of the structural factors of nanoparticles, such as size, shape, crystallinity, morphology, and chemical composition, on the bactericidal and osteoinductive effects. Due to the influence that these structural determinants have on the regulation of processes such as the formation of ROS and cell membrane disruption, this will allow for the optimization of nanoparticle design and improve their performance in clinical applications [2]. On the other hand, ensuring the safe biomedical use of nanoparticles is essential to reduce errors in physicochemical stability and cytotoxicity, as it is important to ensure that their implementation contributes both to offering potent antibacterial activity and to ensuring the absence of risks from cellular toxicity and undesirable immune responses, such as chronic inflammation [9]. In this way, the osteoinductive potential of nanoparticles must be analyzed considering their intrinsic structural attributes, along with the mechanisms of interaction with bone system cells, which include components of the extracellular matrix and biochemical signaling pathways. This will contribute to making their implementation efficient in regenerative medicine [10], requiring a deep understanding of these factors to maximize the benefits of these materials in clinical applications [11].

Among the surface modification strategies to enhance the bioactivity of metallic materials, hydroxylation has emerged as an innovative and highly specialized approach, primarily applied in advanced research and biomedical applications. This process involves the formation of hydroxyl groups (-OH) on the metallic surface under specific reaction conditions, distinguishing it from conventional techniques such as anodization, thermal oxidation, or coating deposition. However, although hydroxylation has shown promising results, its implementation in biomedical applications still requires further development to

achieve optimization and widespread adoption [12]. Currently, hydroxylation techniques remain a central topic in cutting-edge research, as their large-scale application has not yet been widely adopted in industrial production or commercial applications. Ongoing development aims to optimize these processes to match or surpass the efficiency of conventional surface treatments. The effects of hydroxylation largely depend on the type of metallic alloy and the intended biomedical application, requiring detailed and specific knowledge in each case [13].

Recent studies provide concrete hydroxylation protocols with verified outcomes. For example, titanium implants prepared with acid-etched in 0.35 M hydrofluoric acid for 15 s at room temperature and a two-step process combining alkaline treatment in sodium hydroxide followed by thermochemical heating demonstrated distinct surface chemistries. Importantly, the hydrofluoric etching and the sodium hydroxide thermos-chemical process effectively induced hydroxylation, while the as-machined and grit-blasted surfaces mainly modified the surface topography without generating hydroxyl groups [14].

The biological significance of hydroxylation extends beyond mere chemical modification; it fundamentally dictates the implant's interfacial bioactivity through electrochemically mediated protein interactions. Upon hydration, the native oxide layer spontaneously generates hydroxyl groups (-OH) that dissociate to create a variable charge surface, rich in both protonated (OH_2^+) and deprotonated (O^-) sites [15]. This electrochemical mosaic actively orchestrates protein adsorption via specific electrostatic forces. Computational studies demonstrate that an increased density of surface hydroxyl groups (-OH) on crystalline rutile (110) facets enhances affinity for specific protein subdomains, such as subdomain IIb of human serum albumin (HAS), through optimized complementarity with charged protein groups (COO^-, NH_3^+) [16,17]. Crucially, this charge-mediated interaction determines biological outcomes: hydrophilic, hydroxyl-rich surfaces limit denaturation of critical proteins like fibrinogen (FIB) by mitigating unfavorable electron transfer, thereby improving hemocompatibility and reducing platelet activation [18]. Furthermore, the conformation of adsorbed proteins is directly influenced; key adhesive motifs such as the RGD sequences within fibronectin (FN) can adopt orientations on hydroxylated surfaces that favor cellular recognition and osteogenic signaling [19]. Consequently, hydroxylation transcends a superficial treatment; it represents a foundational strategy to engineer the crucial biochemical dialogue between implant and host, where protocol-specific OH density modulates the cascade from protein adsorption to osseointegration.

Given the fundamental role of surface hydroxylation in orchestrating the implant host interface through precise protein interaction, this review aims to provide a comprehensive analysis of the impact of structural factors on the bioactivity of metallic nanoparticles, with a special focus on hydroxylated surfaces within bone replacement systems. Through the integration of knowledge from nanotechnology, biomaterials science, and regenerative medicine, this work seeks to identify existing knowledge gaps and explore future directions to optimize biomedical applications based on nanoparticles.

2. Methodology

A literature search was conducted in PubMed, Scopus, and Web of Science Direct between 2000 and 2025 due to the exponential growth in the field of nanomaterials research during this time. The search strategy was based on key terms previously reported in the literature reported in this review. Initially, the search string included terms such as 'metallic nanoparticles', 'metal-oxide nanoparticles', 'antibacterial activity', 'osteogenic response', and 'bond integrating implants', which yielded 293 results. To narrow the focus, more specific terms were subsequently combined using Boolean operators (AND, OR) to focus the search on biomedical applications, biocompatibility, and bone tissue engineering. This

progressive restriction reduced the number of retrieved studies, highlighting the scarcity of publications that simultaneously integrate all structural and functional aspects.

Studies reporting bioactivity, antibacterial effects, or osteogenic responses relevant to bone tissue integration were considered. Publications focusing exclusively on organic nanoparticles (e.g., polymers, lipids, carbon-based systems) or unrelated applications (e.g., sensors, oncology) were excluded. Patents, conference abstracts, and the gray literature were not considered. This search yielded 227 results, distributed across journals and subject areas.

The bibliometric screening revealed a clear gradient: while general descriptors retrieved a broad range of records, more specific combinations drastically reduced the number of eligible publications. For example, the term 'metallic nanoparticles' alone yielded over ten thousand results, whereas the combination 'metallic nanoparticles AND hydroxylation AND bone implants' retrieved a few relevant studies. The reduction underscores the wealth of background knowledge and the dearth of integrative approaches to hydroxylated metallic surfaces for bone regeneration, thereby emphasizing the importance of this review. This search yielded 25 results, associated with full-text articles assessed for eligibility. A flowchart of study selection process was prepared (Figure 1).

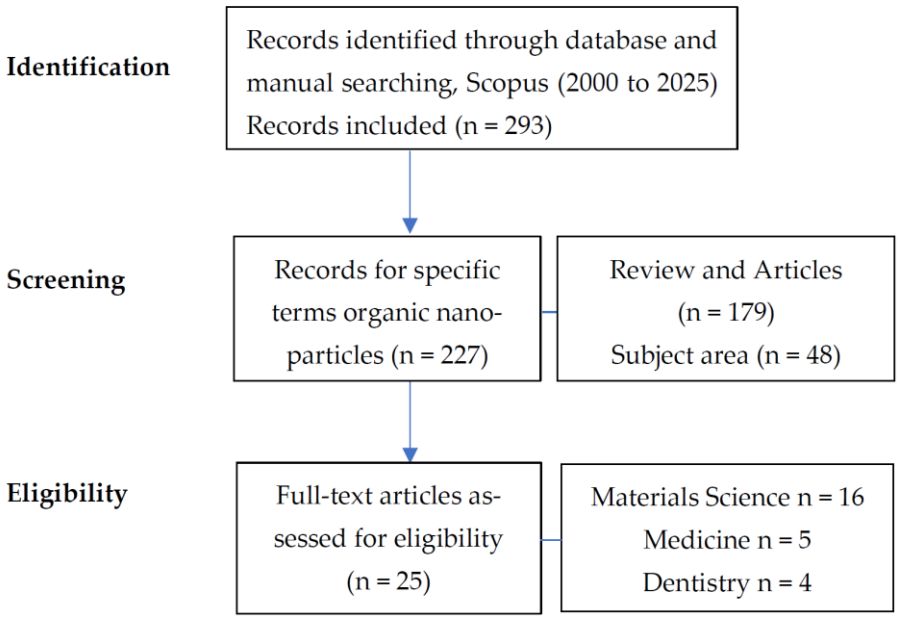

Figure 1. Schematic representation of a flow diagram of the literature selection process.

3. Fundamental Aspects of Bioactivity in Nanoparticles: A Comprehensive Approach

The principles and fundamentals of nanoparticle bioactivity are based on their interaction with biological systems, a process that depends on various structural and physicochemical factors. Among these factors, the size and shape of the nanoparticles, their surface area, surface chemistry, surface charge, biodegradability, functionalization, and antibacterial properties play a crucial role in determining their reactivity and application in biomedical environments. In this regard, according to Abdelkawi et al. [20], adding specific functional groups (such as amines, thiols, and hydroxyls) improves cellular affinity and minimizes undesirable immune responses. Furthermore, using nanoparticles with size ranges between 1 and 100 nanometers increases their reactivity, which enhances their properties, favoring interaction with other biomolecules and cellular structures. In this way, materials with potential characteristics can be obtained for use in medical and therapeutic applications.

In the case of positively charged nanoparticles, they exhibit greater affinity for cellular membranes, enhancing their therapeutic efficacy, as reported by Carrow and Gaharwar [20].

On the other hand, quantum effects can also amplify the reactivity of nanoparticles by altering their electronic properties, which is beneficial for catalytic and imaging applications [21]. Moreover, the shape and morphology of NPs also affect their biological behavior. In this regard, it has been demonstrated that nanoparticles with non-spherical geometries, such as rod-shaped or star-shaped structures, exhibit significant differences in cellular uptake and effectiveness in medical applications [22]. In the biomedical field, biocompatibility and biodegradability are essential aspects to minimize adverse effects [23]. Surface modifications of nanoparticles through specific coatings have been implemented to reduce cytotoxicity and immunogenicity. Biodegradability is equally critical to prevent nanoparticle accumulation in tissues, which could lead to undesirable side effects [24].

Another relevant aspect is the antibacterial activity of certain nanoparticles, such as Ag NPs, whose mechanism of action includes the generation of ROS and disruption of bacterial membranes [25]. These properties make NPs promising for the prevention and treatment of infections. Finally, their interaction with the immune system is a determining factor in their therapeutic application, as they can modulate immune responses and act as adjuvants in vaccines or immunomodulation strategies [26]. Overall, the bioactivity of nanoparticles is closely linked to their structural and physicochemical characteristics. Understanding these factors will enable the optimization of their design to enhance their performance in advanced biomedical applications, including regenerative and antimicrobial therapies. This article provides a detailed analysis of these aspects and their implications in the engineering of nanoparticles for use in regenerative medicine and antimicrobial coatings in bone replacement systems. The manuscript comments on the environmental applications conditioned by the small size on the nanometric scale [27,28].

4. Interaction with Biological Systems: Factors and Mechanisms Influencing Bioactivity

The bioactivity is influenced by several factors that directly affect the interactions between nanomaterials and biological systems. In addition to the interactions in nanomaterials stimulated by the presence of ions and bioactive molecules, these systems can modulate cellular responses. In general, the bioactivity of nanomaterials can be modulated by their structural and physical characteristics. It is influenced by the surface properties and nanotopography. Additionally, the nature of the bioactive nanomaterial plays a key role. These factors collectively impact the interaction between nanomaterials and biological systems.

The structural factors of nanomaterials, such as particle size, nanomaterial structure, surface interactions, and nanotopography, are critical in achieving optimal bioactivity outcomes. In this regard, studies have revealed interesting relationships between particle size and the arrangement of atoms on their surface [9]. In the specific case of particle size, it is crucial to ensure that atoms or molecules are exposed on the surface of the nanoparticles, thus improving their interaction with the biological system [29,30]. This occurs when their size is reduced, as it directly favors the interaction of the nanomaterial with the biological system in terms of interaction time, penetration, and circulation time [31]. It is also considered an aspect of great interest for the pharmaceutical industry in applications such as drug delivery, where increasing the surface area by reducing the size facilitates efficient interaction between the cells and the bone, improving the efficiency of drug delivery [29,30].

Smaller nanoparticles can cross biological barriers more effectively, such as the blood–brain barrier, leading to improved drug distribution and higher bioavailability of the therapeutic agents they carry [32]. This enhanced delivery reduces premature drug clearance and degradation, allowing for more controlled release and targeted accumulation

at the disease site, ultimately improving therapeutic efficacy while minimizing systemic toxicity [33]. However, the retention of nanoparticles in the body due to reduced clearance can pose safety concerns that must be addressed during design [34].

A small size favors areas with higher reactivity due to a greater proportion of atoms exposed on the surface, thereby increasing their capacity to interact with biological systems. High reactivity of nanoparticles can trigger harmful effects on cells, leading to cytotoxic and inflammatory processes. In the case of charged nanoparticles, they induce both oxidative stress and mitochondrial dysfunction, altering the expression of genes related to DNA damage, which promotes cytotoxicity [35]. Studies have shown that shape and size directly influence the ability of nanomaterials to be internalized by cells. For example, nanospheres and nanorods smaller than 50 nm exhibit higher toxicity in cell lines due to their efficient internalization and larger intracellular interaction area [36]. Such effects can therefore promote pathological processes, including cancer development. Thus, although nanoparticles offer significant advantages in clinical applications, their potential cytotoxic and inflammatory activity must be thoroughly evaluated to ensure safety in therapeutic use [22].

The responses of biological systems to nanomaterials and the surface properties of nanomaterials are closely related to the surface properties of the nanomaterials. The ligand–receptor binding pathways and nonspecific adhesions are mechanisms commonly used by most nanomaterials to interact with biological systems. In this way, as reported in the literature, two processes can be identified. One mechanism is associated with the surface charge, and another mechanism is related to the surface conditions, depending on the arrangement of water on the surface (hydrophilicity/hydrophobicity) [37].

4.1. Antibacterial Mechanisms: The Interplay of Nanoparticle Properties and Bacterial Target

Research focused on studying surface interactions and the biological response to nanomaterials derived from molecules is a recent field, where their bioactivity is largely determined by their nanostructures, which are designed to meet specific requirements. For example, these materials can recognize specific types of biomolecules, generating high-affinity and selective interactions, such as with peptides, proteins, and others [38].

There are also nanotopographic factors that need to be considered. In this context, it is important to report studies related to the fabrication of scaffolds with osteoinductive capabilities. The nanofibers exposed on their surface have the ability to enhance skin cell migration and induce growth during wound healing. Factors are based on the type of material. Nanoparticles can be categorized into organic lipid-based, inorganic, and carbon-based materials, depending on the type of material.

Based on their compositional characteristics and classification by nanomaterial type, their bioactivity will differ. Below is a synthesis of current findings on bioactive nanomaterials, focusing on their bioactivity and the role of the material in nature.

Gaining insight into how nanoparticles interact with biological systems is crucial for advancing the fields of medicine, environmental sciences, and biotechnology. These interactions depend on multiple factors, including the composition, size, shape, and surface properties of the nanoparticles, all of which collectively affect their bioactivity. A detailed analysis of these factors provides critical insights into how nanoparticles behave within complex biological environments. Building on this knowledge, it is essential to explore the mechanisms that influence their bioactivity, through which nanoparticles exert their biological effects. These mechanisms ultimately determine their antibacterial actions, induction of oxidative stress, interaction with cellular material, intracellular penetration, associated damage, and other key aspects, which will be developed further below.

The bioactivity mechanisms of nanoparticles include the processes and ways in which they interact with biological systems, generating different reactions and specific effects. These mechanisms may involve antibacterial actions, induction of cell growth, drug delivery, and interactions with tissues and cells to enhance biological or therapeutic processes. The mechanisms by which nanoparticles exert their biological activities are discussed below, including those associated with antibacterial action related to silver ions and Ag NPs, mechanisms applied in cell growth induction, and interactions with tissues.

The antibacterial action of specific nanoparticles, such as Ag NPs, can destabilize the bacterial cell membrane and inhibit its growth. The primary factors responsible for this antibacterial activity is the action mechanisms of metals ions and nanoparticles when in contact with bacterial cell structure. For decades, metals and their ions have been used as mater capable of minimizing the risk of bacterial infections, much like metal-based nanomaterials, which also exhibit antimicrobial properties [39,40].

Our analysis synthesizes that the antibacterial efficacy of nanoparticles against Gram-positive and Gram-negative bacteria is fundamentally determined by the interplay between nanoparticle structural characteristic and bacterial cell wall architecture [41,42]. To understand these mechanisms, it is essential to examine the composition of the bacterial cell structure. In this case, the bacteria have a unique and complex cell wall, composed of proteins, lipids, and carbohydrates (Figure 2). Depending on the bacterial structure, bacteria can be classified based on the coloration of their cell wall and are referred to as Gram-positive or Gram-negative bacteria [43]. The variations between Gram-positive and Gram-negative bacteria are related to the structural and molecular composition of their cell walls [44].

These differences primarily occur in the organization of the membrane and cell wall, which are arranged differently between the two types of bacteria. For instance, Gram-negative bacteria possess two distinct lipid membranes, consisting of an inner plasma membrane and an outer membrane, separated by a thin layer of peptidoglycan [45], as shown in detail (Figure 2).

According to Figure 2, it can be observed that the bacterial cell wall is a structure composed of proteins, lipids, and carbohydrates. It acts as a physical barrier to protect the cell from its external environment. Additionally, it is selective when regulating the entry and exit of nutrients from the interior to the exterior. It also allows the removal of toxic compounds for the cell, and, in this context, the composition of the cell wall influences the processes underlying antimicrobial mechanisms [41].

In the specific case of Gram-negative bacteria (Figure 2a), these present a thin peptidoglycan membrane that surrounds the cytoplasmic membrane, which is in turn surrounded by an outer membrane composed of lipopolysaccharides, providing this specific type of Gram-negative bacteria with greater protection against drugs and, in this way, allowing them to resist the presence of antibiotics [46].

The high density of LPS in the outer membrane of Gram-negative bacteria necessitates nanoparticles with high-charge density for effective electrostatic interaction and initial adhesion [47]. Furthermore, the presence of the periplasmic space adds another layer of complexity [48,49]. Consequently, the size-dependent penetration capability of nanoparticles becomes a critical factor for overcoming this barrier, where a smaller diameter facilitates diffusion through porin channels [50].

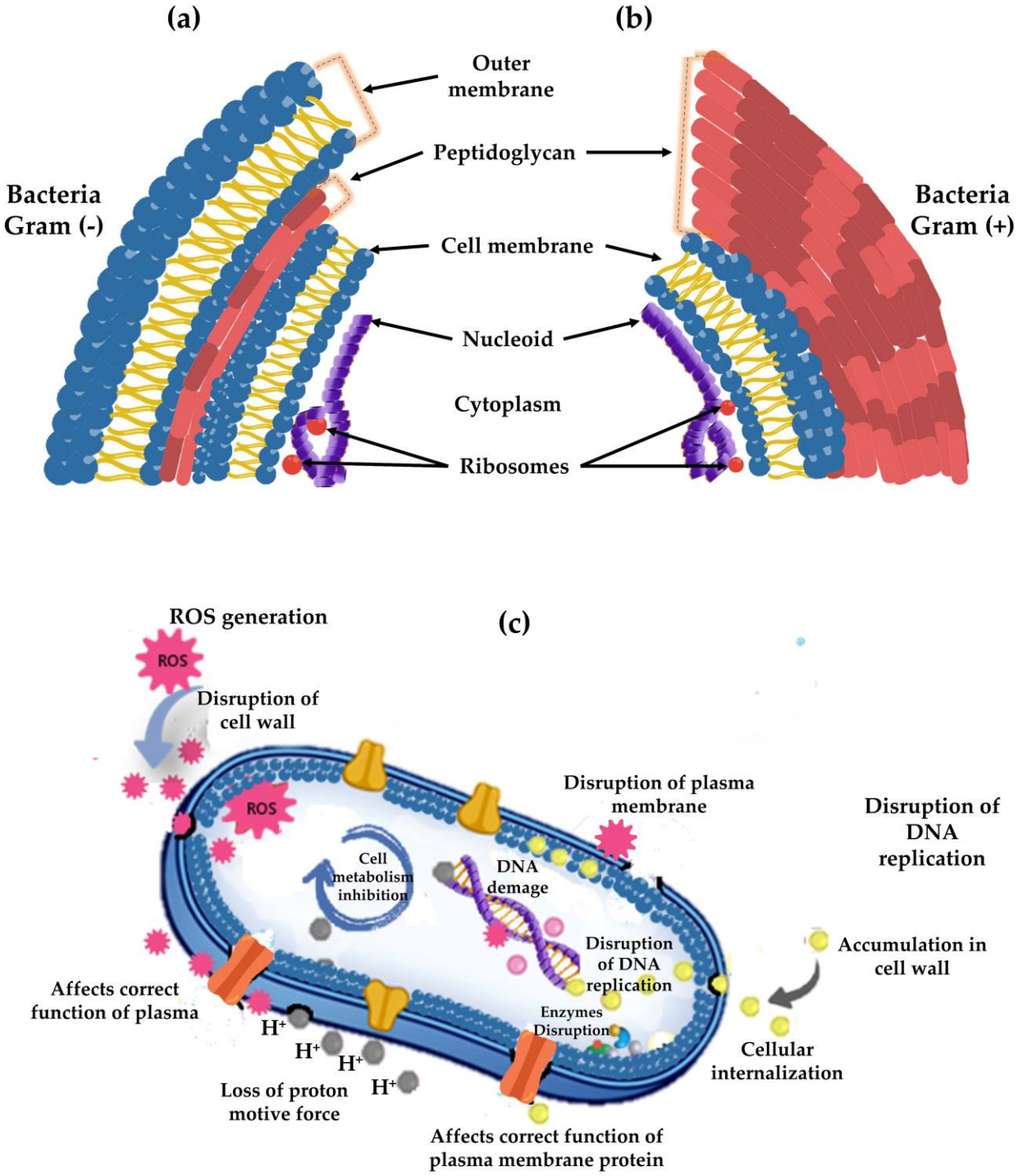

Figure 2. Schematic representation of the cell wall architecture in Gram-negative (**a**) and Gram-positive (**b**) bacteria and the proposed antimicrobial pathways of metal ions (**c**). *Created with Canva.com.*

Another characteristic of Gram-negative bacteria is the presence of the periplasmic space, as observed in Figure 2a. This is a region formed between the extracellular membrane and the cytoplasmic membrane [48]. This region is characterized by having a concentrated gelatinous matrix made up of binding proteins, which are involved in facilitating both the capture of nutrients and their transport. In addition, it defines the characteristics of Gram-negative bacteria due to its organization [49]. Consequently, the size-dependent penetration capability of nanoparticles becomes a critical factor in overcoming this barrier, where smaller diameters facilitate diffusion through porin channels.

In relation to its participation in the bioactive interaction with NPs, the outer cell membrane is essential for regulating the adsorption, penetration of nanoparticles, and determining the causes of toxicological effects on bacteria [50]. Therefore, understanding the constitution and functioning of this type of cell membrane will allow for the development of efficient nanoparticles in biomedical applications to be used in strategies to combat bacterial infections and in the development of mechanisms for controlled drug delivery [51].

Additionally, according to the previously described information, the cell wall of Gram-negative bacteria not only acts as a physical barrier that blocks the entry of external substances but also blocks the entry of nanoparticles and other antimicrobial compounds, thereby providing them with greater resistance [52].

On the other hand, Gram-positive bacteria (Figure 2b) are made up of a thick cell wall primarily composed of peptidoglycans, teichoic acids, and lipoteichoic acids, which provide structural rigidity and a negative charge. This wall is thick, giving this specific type of Gram-positive bacteria a protective barrier to resist external damage [53]. However, Gram-positive bacteria only rely on their robust cell wall to defend themselves against dehydration and other environmental factors. Additionally, as reported in the literature, positively charged nanoparticles are attracted to the negative charge of the outer membrane of Gram-negative bacteria, allowing the nanoparticles to adhere to and penetrate the membrane depending on their size, shape, and charge [47].

For Gram-positive bacteria, the thick, cross-linked peptidoglycan layer requires nanoparticles with specific size characteristics; smaller nanoparticles demonstrate superior penetration through the mesh-like structure of the peptidoglycan matrix [52].

Additionally, Gram-negative bacteria possess face proteins that play essential roles in adhesion, structural resistance, and defense against environmental factors. Among the main proteins of this type are adhesins, porins, outer membrane proteins, lipoproteins, autotransporters, and flagellins [54]. These proteins work together to ensure the survival of Gram-negative bacteria in hostile environments, promoting colonization, antimicrobial resistance, and biofilm formation [41].

The synthesis demonstrates that nanoparticle density must be optimized against the LPS density of Gram-negative bacteria.

The composition of the Gram-positive bacterial cell wall influences the interaction with the bioactivity of nanoparticles (NPs) in several ways. The negative charge present in the wall facilitates electrostatic binding with positively charged NPs, such as those made from silver and zinc oxide. Additionally, the thick peptidoglycan layer acts as a physical barrier, making it difficult for NPs to penetrate [42], although very small or functionalized particles may be able to cross it.

This synthesis demonstrates that nanoparticle charge density must be optimized against the LPS density of Gram-negative bacteria, while nanoparticle size is the dominant factor for penetration through the peptidoglycan layer of both Gram-positive and Gram-negative types [41].

Metallic nanoparticles can induce the production of ROS, leading to oxidative damage in essential cellular structures, including lipids and proteins [55]. Finally, NPs may interact cooperatively with antibiotics, increasing their effectiveness or disrupting resistance mechanisms such as biofilm formation [23].

The currently accepted antibacterial mechanisms include the cellular induction of oxidative stress, the release of ions, and the disruption of biomolecules [43]. While the mechanisms of the metal compounds discussed so far are well understood, interactions between metal ion compounds and nanoparticles can lead to synergistic effects. These

effects may include enhanced bacterial elimination and a reduction in side effects on the host system [42].

Nanoparticles are well-known and extensively studied nanomaterials, as there are compelling theories about the antibacterial mechanisms involved, such as the induction of oxidative stress, interaction with cellular material, intracellular penetration, associated damage, and ion release [56]. These mechanisms can be described as follows:

Induction of oxidative stress: Ag NPs generate ROS, including hydrogen peroxide and superoxide. These ROS damage cell membranes, proteins, and nucleic acids, leading to bacterial cell death [55].

Interaction with Cell Membranes: In this mechanism, Ag NPs adhere to the bacterial surface, disrupting membrane permeability. This alteration leads to the efflux of ions and essential molecules critical for cell survival [57].

Intracellular penetration and direct damage: After penetrating the cell, nanoparticles interfere with critical processes, such as DNA replication and enzymatic activity, compromising cellular functionality [20].

Controlled release of silver ions: Ag NPs act as reservoirs that gradually release silver ions. These ions enhance the antimicrobial effect over time [58].

Antibacterial nanoparticles also exert their action in proportion to ion release, without disregarding the mechanisms previously mentioned [42,59]. In the case of mechanisms related to silver ions, these are bioprocesses that involve direct interaction with cell membranes, proteins, enzymes, and DNA, in addition to inducing oxidative stress. These mechanisms can be classified as follows:

Interaction with cell membranes: silver ions bind to the bacterial cell membrane, causing its destabilization and eventual rupture [42,60].

Interaction with essential proteins and enzymes: In this case, silver ions bind to thiol groups present in proteins, inhibiting their function and disrupting critical metabolic processes [61].

Interference with DNA: silver ions directly interact with bacterial DNA, inhibiting its replication and repair, thereby compromising cell survival [20].

Induction of oxidative stress: silver ions promote the generation of ROS, which cause damage to essential bacterial structures, such as membranes and nucleic acids [62].

Each of these mechanisms may vary depending on the bacterial cell wall composition (Gram-positive or Gram-negative), the concentration of the agents, and the surrounding environmental conditions. Figure 3 illustrates the antibacterial mechanisms of metal ions and nanoparticles, including the release of metal ions from metallic nanoparticles, the generation of extracellular and intracellular ROS, and metal uptake.

It is important to remember that metals are antibacterial materials, with their significance summarized in their long-term efficacy and bactericidal effect. In the case of traditional antibiotics, they have three primary bacterial functions to fulfill; that is, antibiotics aim to develop interactions during cell wall synthesis, ensure effective DNA replication, and ultimately promote processes related to the proper functioning of the protein translational machinery [44].

However, bacteria tend to develop resistance characteristics against these three targets of antibiotics. In this context, resistance mechanisms include enzymatic expressions capable of degrading, modifying, or inactivating antibiotics, inducing post-translational processes, changes in cellular components, and alterations in the cell efflux pumps [57].

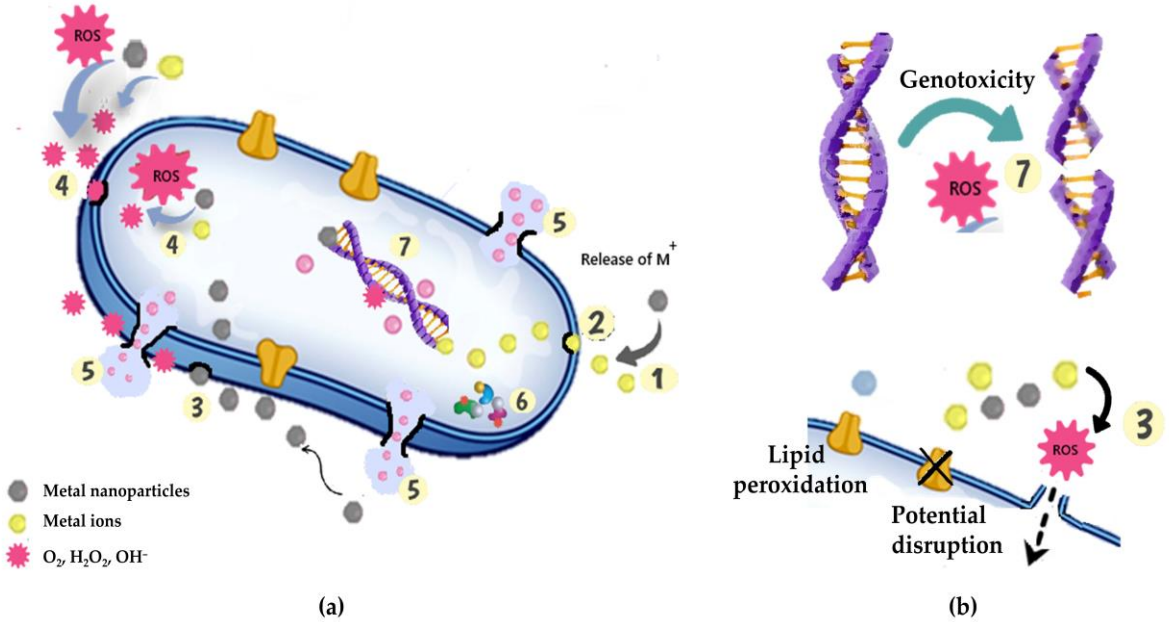

Figure 3. The main antibacterial mechanisms of nanoparticles and metal ions are as follows. (**a**) Illustrates mechanisms: (1) the release of metal ions from nanoparticle surface; (2) direct interaction with the bacterial cell; (3) interaction between metallic nanoparticles and the bacterial cell wall, typically mediated by electrostatic forces, which can result in damage to membrane function and nutrient assimilation; (4) ROS generated both outside and inside the cell contribute to oxidative stress, leading to damage of biomolecules such as lipids; (5) excessive metal accumulation on the bacterial cell envelope, combined with elevated levels of ROS, compromises membrane integrity and results in the leakage of intracellular components; (6,7) after metal absorption, metallic NPs and metal ions can directly interfere with protein and DNA, impairing their functions and disrupting cellular metabolism processes, thereby amplifying oxidative damage through enhanced production of ROS). (**b**) shown in both panels; genotoxicity and peroxidation. *Created with Canva.com.*

4.2. Other Bioactivity Mechanisms: Induction of Cell Growth and Interaction with Tissues

Beyond antibacterial effects, NPs can promote cell proliferation and tissue regeneration, as demonstrated by gold NPs used in wound healing [63]. The physical characteristics of NPs, such as their large surface area, high reactivity, and small size, enable them to interact with tissues through various bioactive mechanisms: interaction with cell receptors [64], controlled release of bioactive substances [64], modification of the extracellular environment [65], response to physical stimuli [66], and immunological responses [67].

Interaction with cell receptors: NPs can activate signaling pathways that promote cell division and growth. This process is enhanced when NPs carry molecules that replicate or amplify natural regulatory signals, triggering a more efficient cellular response essential for tissue development and regeneration [68].

Controlled release of bioactive substances: Engineered NPs can gradually release compounds such as medications and growth factors in a controlled manner. This sustained release triggers cellular proliferation, aiding in tissue regeneration and healing, which is particularly beneficial in regenerative therapies like wound repair [68].

Activation of growth factor signaling pathways: Some NPs can modulate cell influence signaling pathways linked to growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) [69,70]. These factors are essential for cell proliferation and differentiation, playing a crucial role in cellular regeneration and differentiation [64].

Modification of the extracellular environment: Nanoparticles are capable of inducing changes related to the modification of the extracellular matrix, which can stimulate both cell adhesion and proliferation. This alteration is crucial, especially in tissues where interaction

with the matrix plays a key role in regulating cellular activity. Therefore, by promoting these changes, nanoparticles support cell multiplication, contributing to tissue regeneration and repair processes [65].

Physical stimuli, such as magnetic or electric fields: Some nanoparticles can be triggered by physical stimuli like magnetic or electric fields, which may speed up cell proliferation [66]. These fields influence the behavior of cells by modifying their immediate environment, promoting alterations that enhance growth and cell division. Therefore, manipulating nanoparticles physically can be a valuable approach for improving biological processes, including tissue regeneration [71].

Immunological responses in interaction with tissues and cells: The size-dependent properties of nanoparticles are a fundamental determinant of their bioactivity, influencing both their interaction with biological systems and their antibacterial efficacy. Our analysis suggests that a primary structural feature governing NP efficacy is particle size, with diameters under 50 nm, and particularly around 20 nm, demonstrating superior biological activity due to their maximized specific surface area and enhanced cellular penetration capability [67].

This principle is critically demonstrated in antibacterial applications. For instance, a study evaluating Ag NPs of 20, 80, and 113 nm revealed that the 20 nm Ag NPs, with the highest specific surface area, exhibited the greatest toxic effect against microorganisms [67]. This heightened activity is attributed to the smaller particles' increased reactivity and their ability to more efficiently penetrate microbial cells, disrupting vital processes.

The critical influence of size extends beyond antibacterial action. Research on hydroxyapatite nanoparticles (HAp NPs) showed that 20 nm of HAp NPs significantly enhanced cell growth and inhibited programmed cell death in osteoblast-like cells (MG-63), underscoring the role of minimal size in promoting tissue regeneration and integration [72].

Furthermore, the small size of NPs enables their use in advanced drug delivery systems. Nanoparticles can be engineered to transport therapeutic agents directly to the site of action, minimizing side effects and increasing treatment efficacy [73]. This targeted approach is particularly valuable in combating multidrug-resistant microorganisms, as it enhances the bioavailability and localized effect of antibiotics while reducing the potential for widespread resistance development [62,74]. In summary, this synthesis underscores that nanoparticle size is not merely a physical attribute but a central design parameter that affects efficacy across diverse applications, from inducing bacterial death to promoting cellular growth and enabling targeted therapy [62,74].

Building on these fundamental aspects, surface chemistry emerges as a pivotal determinant of biological responses in metallic implants, particularly titanium and its alloys used in bone replacement systems. Among the various surface modifications, the hydroxylation of titanium surfaces has attracted particular interest, as the density of OH groups can influence zeta potential, protein corona formation, osteoblast adhesion, and downstream signaling pathways. These effects are critical for both osteoinductive and antibiofilm performance. The following Section 4.3 explores the biological implications of the protein corona and titanium surfaces, providing a focused framework to understand how surface functionalization can optimize implant integration and therapeutic outcomes.

4.3. The Protein Corona: Protein Adsorption on Titanium Surfaces

The process of protein adsorption on the surface of titanium and its alloys is complex and fundamental to the success of orthopedic and dental implants. When the biomaterial is incorporated into a biological environment, such as plasma or saliva, a rapidly formed layer of adsorbed proteins influences the cellular response. The protein corona has an

impact. The specific composition of the protein corona influences opsonization, marking the surface for immune cell recognition and modulation of cellular signaling.

In cases where there is a reduction in the adsorption of proteins that promote inflammation, such as immunoglobulins and fibrinogen, on hydroxylated surfaces, osteointegration may be favored by minimizing immune activation. Additionally, it is known that electrostatic interactions between charged hydroxyl groups and proteins affect both the orientation and bioactivity of these proteins, ultimately directing responses such as adhesion, proliferation, and differentiation [75,76].

Among the determining factors in this interaction is the complexity of the environment, characterized by biological fluids with high protein content, including thousands of proteins with varying concentrations and affinities. This generates a dynamic and specific protein layer that varies according to the type of fluid and the material's surface. This approach is supported by studies showing the diversity and specificity of adsorbed proteins in different biological fluids and titanium surfaces [75–77].

On the other hand, the surface also influences the cellular response. In this regard, the chemical properties, topography, surface charge, free energy, and crystalline state (anatase, rutile, amorphous) of titanium significantly affect the quantity, type, and conformation of adsorbed proteins. The chemical composition and exposed functional groups affect protein affinity, while the nano- and microscale topography determines the number of active sites available for adsorption. The surface charge and energy modulate electrostatic interactions and wettability, influencing the strength and stability of protein binding. Furthermore, the crystalline state (anatase, rutile, or amorphous) alters the chemical and physical properties of the surface, affecting both protein adsorption and conformation, as different crystal faces present distinct surface charges and hydroxyl groups that interact with proteins with specificity [8].

Surface modifications such as plastic deformation, UV activation, plasma treatment, or mechanical treatments alter the titanium surface by increasing its surface energy, modifying both hydrophobicity and hydrophilicity, and changing the crystalline structure. These changes directly influence the quantity, type, and conformation of adsorbed proteins, promoting cellular adhesion, osteogenic differentiation, and reducing bacterial colonization. For example, increasing surface energy and modifying topography can enhance the adsorption of specific proteins that promote favorable biological responses [78,79].

Additionally, external factors such as pH, temperature, protein concentration, and the presence of ions (Ca^{2+}, Mg^{2+}) modulate protein adsorption. Aging and storage of the surface may also contaminate it and reduce its bioactivity. In complex biological fluids, protein competition occurs, where proteins with higher affinity displace others (for example, replacing fibronectin or collagen with albumin), determining the final composition of the protein layer and, consequently, the biological response [8].

The interaction between proteins and titanium surfaces is a complex process, fundamental to the success of bone substitution implants. Initial protein adsorption influences the cellular response and bone integration, and it is conditioned by characteristics such as chemistry, topography, and hydroxylation. Understanding these mechanisms and how to modify them is key to designing more functional and biocompatible biomaterials. Therefore, the study of surface hydroxylation and its impact on protein adsorption and biological response is important for optimizing titanium implants.

Hydroxylation of metal surfaces, especially in titanium and its alloys, involves the incorporation of hydroxyl groups (-OH) that significantly modify the physicochemical and biological properties of the material. This modification increases surface reactivity, influences protein adsorption, and modulates cellular interactions, which are fundamental for applications in bone regeneration and implant integration. By controlling hydroxylation,

it is possible to improve osteoblast adhesion and proliferation, favor osteointegration, and reduce bacterial colonization, positioning it as an attractive strategy in the design of bioactive metallic implants.

The hydroxylation mechanism impacts the density of hydroxyl groups (-OH) and the surface zeta potential, increasing the negative charge and enhancing colloidal stability, which in turn regulates protein adsorption and protein corona formation. This protein corona modulates cellular recognition and immune response, directly influencing the biocompatibility of the implant. Furthermore, hydroxylated surfaces can facilitate implant integration and regeneration. Various methods allow the controlled introduction of hydroxyl groups into titanium, each with advantages and limitations.

The most well-known hydroxylation methods are as follows: alkali heat, plasma, and UV/ozone. In general, hydroxylation improves osteoinductive and antibacterial performance. However, to optimize its efficacy, it is essential to evaluate how structural factors such as size, shape, crystallinity, chemical composition, and oxide/hydroxide coverage influence bioactivity. These determinants, along with hydroxylation, modulate interactions with proteins, cells, and bacteria, affecting more complex processes such as antibacterial mechanisms (ROS, membrane disruption, ionic release) and osteogenic mechanisms (BMP/Smad pathways, Wnt/β-catenin, integrins, Piezo). Overall, this paragraph broadly addresses titanium surface hydroxylation as a strategy to improve the bioactivity of implants. It also highlights its affinity for nanostructures such as TiO_2, employed in the functionalization of titanium surfaces, which can enhance protein adsorption and favor interactions beneficial to osteointegration. These modifications are related not only to optimizing the biological response but also to improving antibacterial properties, thus impacting both the functionality and durability of titanium implants in biomedical applications [8].

Given the complex interplay between surface protein properties and biological activity, a system framework is necessary to guide the design and advance implants. Table 1 provides a comprehensive-as-necessary mapping of the key structural determinants of titanium surfaces and nanoparticles to their ensuing physicochemical changes, active biological mechanisms, and final bacterial and osteoinductive outcomes. This summary serves as a foundational reference and offers a strategic overview of how to selectively enhance desired biological responses. The influence of these critical factors will be explored in greater detail in the following sections.

Table 1. Mapping structural determinants of nanoparticles and titanium surfaces to physicochemical changes, biological mechanisms, and bactericidal/osteoinductive outcomes.

Structural Factor	Physicochemical Change	Biological Mechanism	Outcome (Bactericidal/Osteoinductive)	Effect Direction/Strength	Ref.
Size	Increased surface area to volume ratio/higher reactivity.	ROS generation, membrane disruption.	Enhanced bactericidal activity; modulation of osteogenic differentiation.	Positive/strong	[29,30]
Shape (e.g., spheres, rods, wires)	Altered contact points and surface energy; differential protein adsorption	Membrane disruption; altered cell adhesion mechanics.	Variable bactericidal activity (e.g., sharp, round); dictates osteoblast adhesion and proliferation.	Depends on geometry/moderate to strong	[22]
Crystallinity	altered surface energy and defect density; electron–hole pair separation efficiency.	Enhanced catalytic ROS generation; controlled ion release kinetics.	Affects bacterial killing efficiency; influences osteogenic gene expression and bone matrix formation.	Positive/moderate	[8]
Composition (type of metal ions, alloys)	Type of metal/alloys.	Interaction with microbial enzymes/DNA; protein corona formation.	Bactericidal effect (e.g., Ag, Zn); osteoinduction via signaling pathways.	Positive/strong	[6,7]
Surface Hydroxylation (OH-group density)	Surface-OH density.	Enhanced protein adsorption (fibronectin), improved cell adhesion, and may reduce bacterial adhesion.	Supports osteoblast adhesion and maturation; potential for selective bioactivity (osteogenic vs. bacterial).	Positive/moderate	[12]
Nanotopography	Surface roughness, patterning	Influences focal adhesion formation; affects biofilm mechanism.	Modulates bacterial attachment; promotes osteogenic differentiation via contact.	Topography-dependent/moderately strong	[9]

5. Bioactive Characteristics According to the Type of Nanomaterial and Application Type

In general, bioactive nanoparticles are distinguished from sensitive ones by performing a specific biological function, while sensitive nanoparticles respond to external triggers [67]. However, their applications can overlap, as some bioactive nanoparticles can also react to certain conditions, such as the pH level in the body, for example. Biomaterials have a direct influence on the component of cellular activity, which is related to the structural conformation, that is, with their nanopatterns, nanopores, and nanochannels in the nanoparticles. Nanoparticles (NPs) are typically classified based on the material used in their manufacturing process. They can be categorized into organic, lipid-based, inorganic, and carbon-based materials. Within these categories, it is possible to analyze nanomaterials of protein, polymeric, and metallic nature, as well as those containing magnetic minerals [80] (Figure 4).

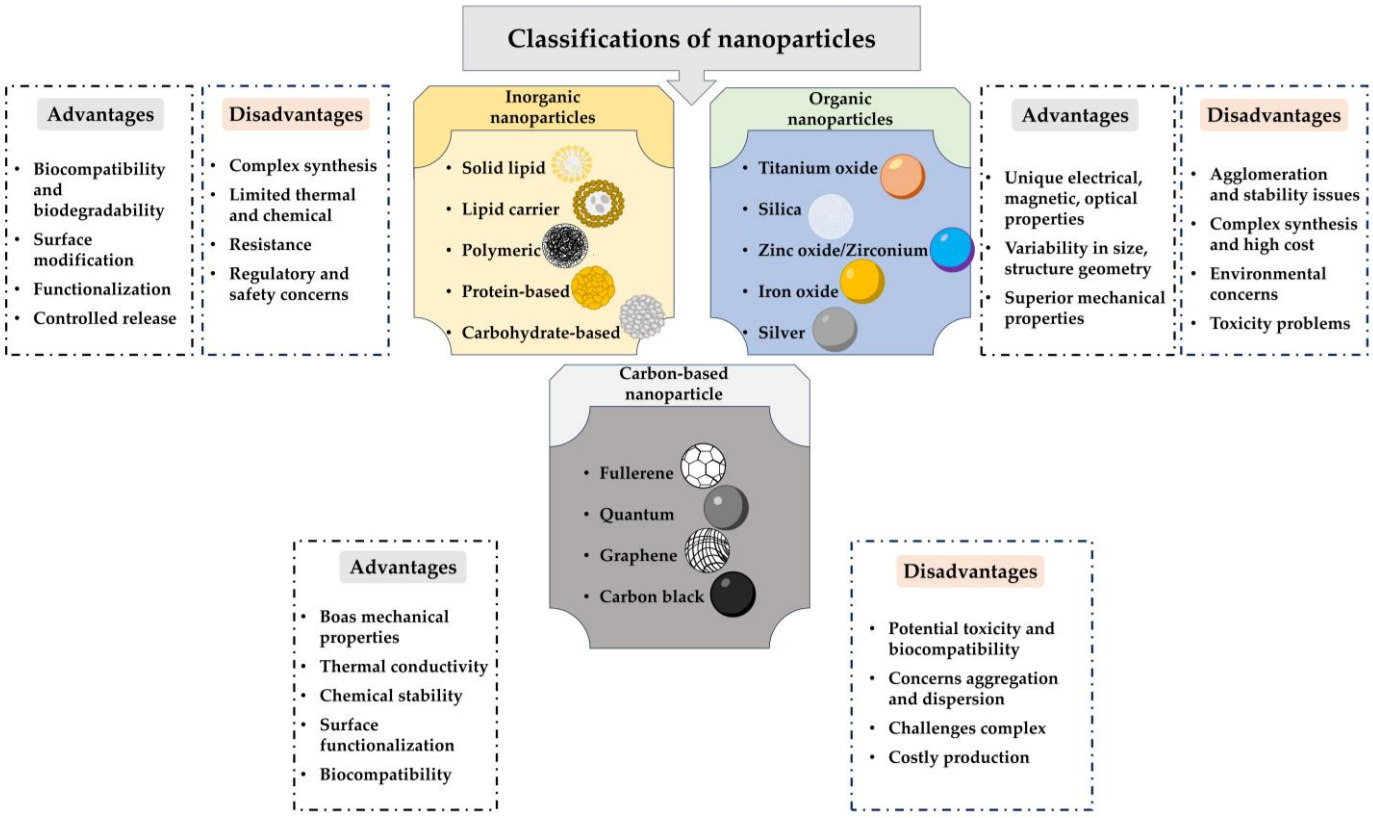

Figure 4. Nanoparticle (NP) classification according to the chemical composition and their functional advantages (disadvantages). *Created with Canva.com.*

The main characteristics of the different types of nanoparticles are generally highlighted below, emphasizing how their unique properties can be applied in biomedical therapies and other areas of nanotechnology.

Organic nanoparticles are defined as solid nanoparticles composed of organic molecules [81]. These NPs are generally considered to have favorable biodegradability and may exhibit reduced toxicity compared to counterparts; however, their biological safety depends on factors such as dose, size, and surface modification. They are composed of lipids, proteins, polymers, and carbohydrates. These properties make them suitable for biomedical applications, including targeted drug delivery, bioimaging, cancer therapy, and biosensors [82]. Moreover, parameters like size, shape, and surface morphology are crucial factors that influence the therapeutic effectiveness of organic nanoparticles [83]. Therefore, the development of effective and non-invasive treatments is directly linked to the use of

superficially modified nanoparticles with the ability to interact with cells and tissues. This modification involves altering the functional groups on their surface, such as amines or thiols. In this way, their cellular penetration and drug delivery can be optimized [84].

Carbon-based nanoparticles: Carbon-based nanoparticles are innovative materials with applications in various fields, such as energy storage, production, and water treatment [85]. Carbon can adopt several allotropic forms such as diamond and graphite, with the latter as the most thermodynamically stable [86]. In this regard, nanoparticles primarily composed of graphene, which is characterized by atomic layers of carbon arranged in a hexagonal pattern, impart high-interest properties to the nanoparticles, such as high mechanical strength [87]. Additionally, the unique structure of these nanoparticles allows them to be used in various applications, including biomedical fields, enhancing cells and tissues.

Therefore, some examples of these nanomaterials include carbon nanotubes (CNTs) and graphene, which stand out for their unique properties, such as high electrical conductivity and remarkable mechanical strength [88] Graphene, for instance, has a two-dimensional structure made up of carbon atoms, arranged in a hexagonal lattice, which provides it with excellent electrical and thermal conductivity, as well as a large surface area [89]. The bioactivity of graphene results from its interaction with biomolecules, facilitating the adsorption and release of therapeutic substances and promoting biological responses, such as cellular stimulation [90].

Fullerenes, or buckminsterfullerenes (C60): These are one of the most recognized forms of fullerenes, characterized by their unique spherical structure that allows the encapsulation of therapeutic molecules [91]. Their bioactivity is linked to their ability to form complexes with drugs, enhancing stability and enabling controlled release [92]. Additionally, fullerenes have antioxidant and anti-inflammatory properties, making them potential candidates for treating inflammatory diseases and for the development of new pharmaceuticals [93]. Their interaction with cells and biomolecules can be manipulated to optimize therapeutic responses [94].

Graphene and graphene oxide (GO) nanoparticles are materials from graphene. Graphene oxide, for instance, exhibits excellent electrical, mechanical, and thermal properties, primarily used in sensors and as a material for controlled drug delivery [95]. GO, with oxygen functional groups on its surface, allows for efficient modifications that facilitate interaction with cells and biomolecules, improving solubility and dispersion in the biological environment [96]. Additionally, these particles have shown great potential in cellular regeneration and cancer treatment, due to their ability to induce specific and targeted biological responses [97].

Carbon nanotube (CNT) nanoparticles are carbon nanotubes formed by graphene sheets rolled into tubes, exhibiting extraordinary mechanical strength and excellent substance absorption capacity [90]. The bioactivity of CNTs is related to their ability to interact with biomolecules and cells, promoting the effective delivery of drugs and therapeutic agents [98]. CNTs have also been studied in nanomaterial therapies, such as cancer treatment and gene delivery, due to their high surface area and ability to modify their functionality and optimize interaction with tissues and cells [99].

Carbon nanofibers (CNFs) have a more flexible structure compared to carbon nanotubes, as they are composed of multiple layers of graphene [100]. They are widely used in the production of composite materials and electronic devices, but they have also been investigated in the biomedical field due to their ability to carry therapeutic molecules [101]. The focus of their bioactivity is on controlled drug delivery systems, where CNFs can be modified to enhance their interaction with cells, thereby increasing the effectiveness of treatments [102]. Additionally, these particles have the potential to create smart medical devices that respond to biological signals [103].

Activated Carbon or Vegetable Carbon particles are known for their adsorption capacity, mainly due to their large surface area and the presence of nanopores in their structure [104]. These characteristics allow them to capture and eliminate a higher amount of chemicals and toxins [105]. In medicine, they are primarily used in water purification and detoxification treatments, demonstrating effectiveness in removing harmful compounds and supporting biological therapeutic processes [106].

In the specific context of bone-integrating replacements, organic nanosystems acquire particular relevance when applied as functional coatings or local delivery platforms in orthopedic and dental implants. Rather than serving as bulk structural materials, they enhance the performance of clinically established metals and alloys such as titanium and Ti-6Al-4V, tantalum, magnesium/zinc alloys, and antibacterial Ag/Cu dopants. By modulating drug release, providing antibacterial activity, and delivering osteoinductive cues directly at the implant tissue interface, these organic nanocarriers bridge generic nanocarrier taxonomies with clinically relevant biological outcomes, including osseointegration (bone–implant bonding) and osteoimmunomodulation (immune responses that favor bone regeneration) [107–109].

In this regard, controlled release of osteoinductive and antibacterial agents from the implant's surface has emerged as one of the most promising applications of organic nanosystems. As reviewed by Banche et al. 2025, nanoencapsulation in vehicles such as liposomes, PLGA polymeric nanoparticles, and micelles overcomes the limitations of systemic administration—including short half-life and off-target side effects by ensuring localized and sustained dosing directly within the osteogenic niche [109,110]. A paradigmatic example is the work of Wu et al. (2020), who developed a polydopamine bioadhesive coating on titanium functionalized with dual nanocarriers. This system enabled sequential and coordinated release of BMP-2 and VEGF, achieving synergistic enhancement of angiogenesis and bone formation in predictive models. Such approaches not only improve the therapeutic efficacy of bioactive molecules but also minimize the required dosages, thereby reducing costs and potential risks [111].

The prevention of peri-implant infection, a leading cause of implant failure, is being revolutionized by engineering of organic nano-coating endowed with intrinsic or release-based antibacterial properties. As comprehensively reviewed by Butler et al. (2023), integrating organic nanocarriers loaded with antimicrobial agents (e.g., Ag^{+}, Cu^{2+}, or Zn^{2+} ions, or conventional antibiotics) enables the achievement of a high local biocidal concentration at the implant–tissue interface while minimizing systemic toxicity [112]. A critical advantage of this nano-encapsulation approach is the mitigation of burst release, thereby prolonging therapeutic activity and reducing the potential for driving antimicrobial resistance. Exemplifying this strategy, Pei Y. et al. (2021) engineered chitosan nanoparticles loaded with copper ions (Cu^{2+}) tethered to the titanium implant surface. Thus nano-composite system demonstrated potent and sustained bactericidal efficacy against prevalent pathogens, such as *S. epidermis* and *E.coli*, while concurrently maintaining favorable cytocompatibility. This "local reservoir" paradigm ensures durable antimicrobial protection throughout the critical post-operative period, safeguarding the initial stage of bone healing [113].

The most advanced frontier in bioactive implant design focuses on osteo-imunomodulación, wherein organic nanosystems are deployed to instruct the immune response and forge a pro-regenerative microenvironment. As established, successful osseointegration is critically dependent on the polarization of incoming macrophages toward an anti-inflammatory and pro-repair (M2) phenotype [114]. Organic nanovehicles are uniquely suited for this task due to their capacity for the spatiotemporally controlled delivery of immunomodulatory cues, such as interleukin-4 (IL-4) or dexamethasone [114,115]. A seminal study function-

alized a titanium implant surface with a nanoscale metal–organic framework (ZEIT-8) to release dexamethasone. This ingenious coating effectively suppressed the initial adverse inflammatory response while simultaneously upregulating key osteogenic markers. This work provides conclusive evidence that nano-confined delivery of immunomodulators can directly bridge advanced material engineering with desired biological outcomes, paving the way for a new generation of "immune-smart" implants [116].

While organic and carbon-based nanoparticles are not the primary focus of this review, it is important to acknowledge their potential as complementary components when integrated with metallic implant systems. Rather than serving as bulk structural substitutes, these nanosystems are increasingly applied as functional coatings, local drug delivery reservoirs, or immune-modulatory interfaces on clinically established metals such as titanium and its alloys. In this way, they expand the therapeutic functionality of metallic implants without altering their mechanical reliability. Therefore, although organic and carbon nanomaterials are discussed here only briefly and remain outside the central scope of this review, their synergistic use alongside metallic platforms represents a promising direction for the next generation of bioactive and multifunctional bone implants.

Inorganic nanoparticles (iNPs) are made up of atoms that are bound through metallic or covalent connections [117]. Unlike carbon-based materials, iNPs do not contain carbon atoms and are typically composed of metals or metal oxides [118]. These particles can be derived from various substances, including semiconductors, ceramics, or magnetic metals (Figure 5). The central structure of iNPs is formed by the crystallization of inorganic salts, arranged in a three-dimensional framework, which grants these nanoparticles increased stability and resistance to external disruptions [119].

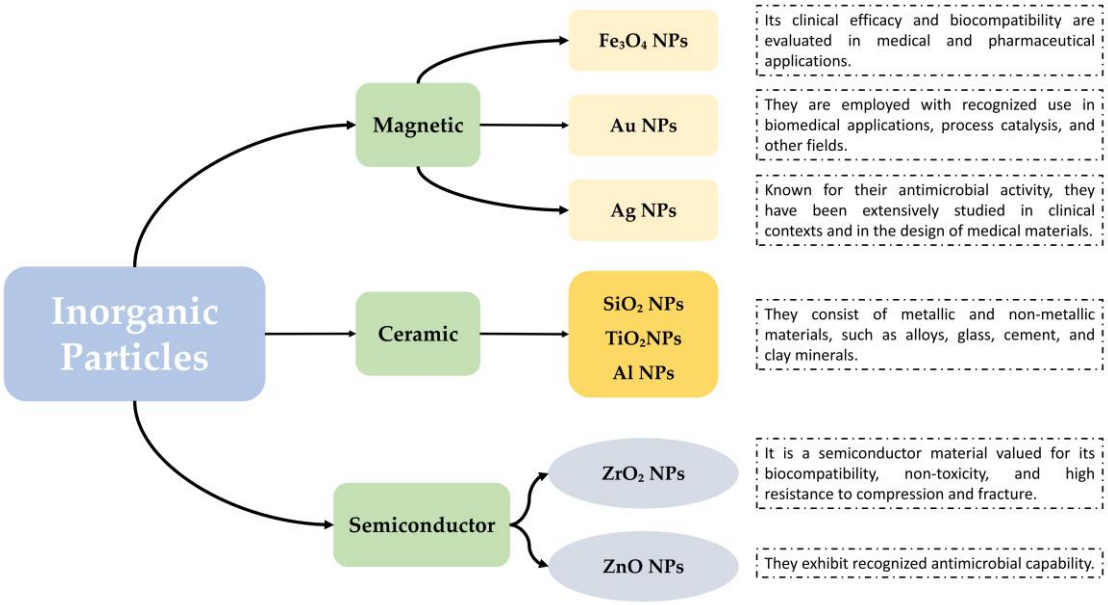

Figure 5. General classification of inorganic nanoparticles (iNPs). *Created with Canva.com*.

The size of the surface plays a crucial role in determining both the characteristics and toxic effects of inorganic nanoparticles (iNPs [120]. This factor is key to understanding the broad spectrum of applications for these particles, including those made from materials like zinc oxide, iron, and silver [24,59,117]. Each type of inorganic nanoparticle has unique attributes that make it ideal for particular applications.

Silver nanoparticles are well-known for their effectiveness in combating microorganisms and have been extensively researched for their potential in medical treatments, particularly for infections and in the production of healthcare-related materials [59,67].

In contrast, zinc oxide nanoparticles are highly regarded for their photocatalytic abilities and are frequently incorporated into cosmetic products and sunscreens for their protective properties [121].

Iron nanoparticles have become increasingly important in biomedicine, particularly for their role in magnetic therapies and as agents for enhancing imaging in magnetic resonance [122]. Ongoing studies are focused on evaluating their clinical effectiveness and confirming their safety and compatibility for use in both medical and pharmaceutical applications.

Metal nanoparticles represent a distinct category of nanoparticles that are produced through either degradative or synthetic methods. Most of the metals used in the production of these nanoparticles are easy to synthesize. This means that the metals chosen for the production of metal nanoparticles are readily available and also undergo a straightforward manufacturing process.

Below are the metals commonly used in the production of metal nanoparticles: silver (Ag) [25,123], gold (Au) [124], iron (Fe) [125], cadmium (Cd) [126], zinc (Zn) [121], cobalt (Co) [127], aluminum (Al) [128], and copper (Cu) [129] All of these are employed with recognized use in biomedical applications, process catalysts, and other fields.

The surface configuration, volume, quantum effects, and small size of metallic nanoparticles lead to significant structural changes. These alterations are manifested in the nanoparticles through modifications in quantum effects, high sensitivity to ultraviolet-visible light, as well as the generation of predominant electrical, catalytic, and thermal properties in this type of nanoparticle [21,130].

Due to their small size, metallic NPs exhibit a higher proportion of atoms on their surface [80]. Furthermore, by modifying certain physical factors of these nanoparticles, an interesting correlation between surface area and volume can be observed, which influences properties such as conductivity and detection in the ultraviolet–visible range [131–133]. Other characteristics that are directly affected by changes in surface area are also reported, including the melting point of the nanoparticles, their affinity for organic, polymeric, and biological compounds, as well as electronic affinities and magnetic properties, among others [122].

In this framework, metallic NPs such as Ag NPs and Au NPs exhibit distinct properties based on their surface and size characteristics, which directly influence their biological activity and toxicity. Table 2 shows the limited toxicity concentration of Au NPs and Ag NPs, along with the detected effects in various experimental models. In studies with Ag NPs, effects such as a significant decline in cell viability at high concentrations, gene expression alterations, and the inhibition of neurite outgrowth have been observed, highlighting the toxicity risk related to their high surface reactivity due to increased surface area [29,30].

Furthermore, Ag NPs are capable of eliciting oxidate stress, leading to the generation of ROS and apoptosis, particularly at higher concentrations. Furthermore, it has been reported that Ag NPs induce oxidative stress, leading to the generation of ROS and apoptosis, particularly at higher concentrations. In specific studies, Au NPs were found to have no significant adverse effects at concentrations of up to 800 μg/mL in *C. elegans* and 300 μg/mL in human keratinocyte cells. This suggests that they have a relatively low toxicity profile under these experimental conditions [134].

Table 2. Toxicity concentration and observed toxicity range of silver nanoparticles (Ag NPs) and gold nanoparticles in various experimental models.

Metallic Nanoparticle	Assays	Value (µg/mL)	Exposure Time (h)	Medium	Environment/Setting	Observed Effects	Ref.
Ag NPs (Silver Nanoparticles)	OECD	0, 250, 500, 1000, 5000	3	10% FBS trisodium citrate and sodium lauryl sulfate	In vitro (human keratinocyte HaCat cells)	Significant decline in viable cell number at high concentrations	[135]
	Fluorescein-diacetate (FDA)/ethidium bromide (Et-Br) test	0, 250, 500, 1000, 5000	24	RPMI + 10% FBS	In vitro (murine dendritic cells)	Alteration in gene expression; 1000+ genes affected	[136]
	MTT assay	12,100	24 and 48	DMEM + 10% FBS	In vitro (human lung epithelial cell line A549)	Intracellular production of ROS but did not induce either apoptosis or necrosis	[137]
	MTT and resazurin reduction assay	1, 6, and 12	24	DMEM + 10% FBS	In vitro (human lung epithelial cell line A549)	Decreased cell viability, changes in cell morphology and confluence	[138]
	Lactate dehydrogenase release assay (LDH)	0, 10, 20, 50, and 100	12 and 24	RPMI 1640 + 10% FBS	In vitro (human lung epithelial cell line A549)	Time and dose-dependent toxicity, induction of cell necrosis	[139]
	MTT, lipid peroxidation assay, ROS detection	0, 0.31, 0.62, 1.25, 2.50, 5.00	72	Hams F12 basal media + 10 mM HEPES + 5% FBS	In vitro (breast carcinoma cell line SUM159 cells)	Induction of cell death by lipid peroxidation, proteotoxic stress, and necrotic cell death	[140]
Au NPs (Gold Nanoparticles)	Cytotoxicity (cell impedance), genotoxicity (micronucleus assay)	0.5, 1, 2 and 5 nM	72	DMEM + 10%FBS	In vitro (Caco-2 cells)	Dose-dependent genotoxicity observed for all Au NPs tested	[134]
Au NPs (Gold Nanoparticles)	Flowmetry with An-nexin V and propidium iodide	10, 50, and 100	24	DMEM + 10% FBS	In vitro (MG-63 cells)	High cell viability (>90%), with less than 3% early apoptosis, 6% late apoptosis, and 1% necrosis	[141]

OECD—Guideline for the testing of Chemicals, acute oral toxicity fixed dose procedure (test No 420). DMEM—Modified Eagle's Medium. MTT—(3-(4,5-Dimethylthiazol-2 yl)-2,5-diphenyltetrazolium bromide). FDA—Food and Drug Administration. FBS—Fetal Bovine Serum. RPMI—Roswell Park Memorial Institute cell culture medium. SUM-159—human breast cancer cell line. HEPES—(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid).

Magnetic Metallic Nanoparticles (MNPs) are a subgroup of inorganic nanoparticles whose nature can be manipulated by an applied external magnetic field [66]. MNPs can consist of a single domain, typically measuring between 10 and 20 nm, or multiple domains when the size exceeds 20 nm [71,142].

The composition of MNPs involves pure magnetic materials or specific combinations of metals and alloys. For the production of MNPs, materials with high saturation magnetization are frequently used. These composition may include pure metals such as cobalt (Co), iron (Fe), and nickel (Ni), as well as some alloys like iron–cobalt (FeCo), alnico, a combination of aluminum (Al), nickel (Ni), cobalt (Co), and iron (Fe), and permalloy ($Ni_{60}Fe_{40}$) [143].

In the case of biomedical applications, pure metals present some limitations due to their high toxicity and oxidative properties [144]. In contrast, iron oxides (IOs) are highly popular and widely applicable materials in the biomedical industry, such as iron oxides (Fe_3O_4 and γ-Fe_2O_3), magnetite, and maghemite, recognized for their stable behavior both chemically and colloidally, as well as their good biocompatibility in biological environments [143]. Another interesting property of MNPs is the strong influence of quantum confinement of electrons, the crystalline condition of their structure, and the effect of surface changes; the latter property is reflected in the loss of the crystalline structure in MNPs [142,145]. Furthermore, MNPs possess other interesting anisotropic magnetic properties, such as high heating efficiency, low Curie temperature, and magnetic coercivity [146].

MNPs are widely used in various biological applications. In the case of iron oxide NPs, they exhibit an antibacterial mechanism, mainly based on the production of ROS,

and induce chlorosis [147]. Furthermore, magnetite nanoparticles (NPsFe_3O_4) and their oxidized form to maghemite (γ-Fe_2O_3) are extensively studied in the biological field due to their ease of biodegradability, functionalization, biocompatibility, and low-cost synthesis [148].

Magnetic metal nanoparticles (MNPs) made of magnetite (Fe_3O_4), such as iron oxide (IO), have become important in various applications due to their magnetic properties and their ability to be manipulated by magnetic fields. However, their clinical and therapeutic use requires a thorough evaluation of their toxicity. Table 3 presents the toxicity limit concentration of various types of MNPs and the effects observed in different experimental models. In vitro and in vivo studies have demonstrated that Fe_3O_4 MNPs exhibit a wide range of biological effects, including the production of ROS, alterations in cell membranes, erythrocyte apoptosis, oxidative stress, and cellular dysfunction, depending on the concentration and characteristics of the nanoparticle coating. These effects highlight the importance of understanding the risks associated with MNPs for their safe and effective use in biomedical applications.

Table 3. Concentration and observed toxicity range of iron oxide nanoparticles (Fe_3O_4) in various experimental models.

Type of Magnetic Nanoparticle (MNP)	Assays	Value (µg/mL)	Exposure Time (h)	Medium	Environment/Setting	Observed Effects	Ref.
Fe_3O_4 (magnetite)	Intracellular ROS, Ca^{2+}, 2, 3-DPG, ATP, and RBC deformability	25,000,000	12	N/A	In vitro (erythrocyte cells)	Increased production, ROS, cell membrane changes, and erythrocyte apoptosis.	[149]
	ROS, phosphatidylserine exposure, hematology analysis, blood serum biochemistry and hemorheology analysis	12 mg/kg	144	N/A	In vivo (rats)	Erythrocyte apoptosis, oxidative stress, and cellular function disruption.	[149]
Fe_3O_4 (magnetite, uncoated)	XTT assay	0.1100000	24 and 72	RPMI medium + 10% Horse serum + 5% FPS	In vitro (PC12 cells—tumor origin	No significant cellular interaction. No cytotoxic effects up to 0.1 mg/mL.	[150]
Fe_3O_4 (Na-oleate-coated Fe_3O_4)	XTT assay	250	24 and 72	RPMI medium + 10% Horse serum + 5% FPS	In vivo (PC12 cells—tumor origin	Cell viability reduced to 70% at 0.1 mg/mL after 72 h of exposure.	[150]
Fe_3O_4 (dextran-coated magnetite)	Hematological and biochemical analysis	62, 5–125–250–500	24–72 h and 21–28 days	N/A	In vivo (male brown Norway rats)	Hematological tests showed a significant increase in leukocytes, red blood cells, hemoglobin, and hematocrit compared to the values obtained for the control group for the group exposed to concentrations of (250 and 500 µg/mL).	[151]
	Fluorescein diacetate (FDA)	62, 5–125–250–500	24 h, 72 h, and 7 days	DMEM + 10 FBS	In vitro (HeLa cells)	There were no representative differences in cell viability values compared to the control cell culture and the cell culture at 0 d for cells incubated in suspensions of 62.5 and 125 µg/mL, at all-time intervals tested.	[151]
$CoFe_2O_4$ (cobalt ferrite)	ROS, catalase (CAT), glutathione S-transferase (GST), and acid phosphatase (AP)	10–500 µM	96	N/A	In vivo (zebrafish larvae)	Hatching delay, membrane damage, severe apoptosis in head, heart, and tail, and oxidative stress due to increased ROS.	[152]
Fe_3O_4 (DMSA-coated)	AlamarBlue assay, ROS, Caspase-3	0.5	24	DMEM + 10% HS	In vitro (hepatocytic cells)	No significant effects on cell viability or cell cycle.	[152]

2,3-DPG—2,3-diphosphoglycerate. ROS—reactive oxygen species. RBC—red blood cell XIT—experimental iron treatment: FeNP-Treated (ferric nanoparticle-treated). IONP-Treated—(iron oxide nanoparticle-treated). DMEM—Modified Eagle's Medium. PC12—cell line derived from a rat pheochromocytoma (adrenal gland tumor). RPMI—liquid cell culture medium (Roswell Park Memorial Institute). FBS—fetal bovine serum. DMSA—dimercaptosuccinic acid. HS—cell culture medium Horse Serum.

Ceramic nanoparticles are composed of a combination of metallic and non-metallic materials. These materials can include metal alloys, as well as substances such as glass, cement, and clay minerals. The mechanical properties of ceramic-based nanoparticles are favorable, as they exhibit resistance to chemical reagents and are also good thermal and electrical insulators [153]. In terms of mechanical properties, they are hard and brittle.

The composition of ceramic nanoparticles can encompass a wide array of inorganic substances, as well as nanostructures based on metallic materials, oxides, and metal sulfides, presenting a variety of dimensions, shapes, and porosities. Due to their versatility and biocompatibility, these nanoparticles are widely used in industries such as dentistry, where they are employed in the synthesis of metal–ceramic alloy nanomaterials for crowns, as well as calcium hydroxide and phosphate compounds for endodontic filling materials [100].

Semiconductor materials (SCMs) are solid materials characterized by a crystalline structure, electrical conductivity properties, as well as electronic energy band gaps, exhibiting both conductive and insulating properties [154]. Materials such as zinc oxide (ZnO) and zirconium oxide (ZrO_2) are commonly used in the synthesis of semiconductor nanoparticles (NPs) [155].

In the case of zinc *oxide nanoparticles (ZnO NPs)* along with titanium dioxide nanoparticles (TiO_2), they are known for their microbial inhibition capability [156]. This property is attributed to the susceptibility of these metal oxide nanostructures to ultraviolet light [157]. Zinc oxide also exhibits positive effects on tissue regeneration, antibacterial activity, and enhancement of mechanical properties [158]. The bactericidal mechanism of ZnO NPs is associated with structural disruption of the cell membrane, triggered by their interaction with ROS within the cell. Specifically, when the surface charge of ZnO NPs (with an isoelectric point above 9) interacts with the negative charge of bacteria, it can cause permanent disruption of the bacterial membrane [159].

Given their broad applications, it is crucial to assess the possible toxicological impacts of ZnO NPs in biological systems. Table 4 below presents the toxicity threshold concentrations for various ZnO NPs and the observed effects in cardiovascular endothelial cell models. In vitro studies show that ZnO NPs, with sizes ranging from 9.1 nm to 100 nm, can reduce cell viability, trigger apoptotic and necrosis processes, and stimulate the Fas signaling pathway in a dose and time-dependent manner. Other studies highlight the role of oxidative stress, DNA damage, and the destabilization of the endothelial barrier as a result of ZnO NP exposure. These observations emphasize the relevance of understanding both the beneficial and detrimental effects of ZnO NPs in biomedical contexts, as well as the necessity of precisely regulating their concentration and exposure duration to ensure safe application.

Table 4. Toxicity concentration and observed toxicity range of zinc oxide nanoparticles (ZnO) in various experimental models.

Metallic Nanoparticle Types by Size (nm)	Assays	Value (μg/mL)	Exposure Time (h)	Medium	Environment/Setting	Observed Effects	Ref.
ZnO (Zinc oxide 70 nm)	MTT + LDH	8, 15, 25, and 50	12	DMEM + 10% FBS	Human aortic endothelial cells (HAECs)	ZnO NPs decrease cell viability, induce necrosis and apoptosis, and activate the Fas pathway in a dose- and time-dependent manner.	[160]
ZnO (Zinc oxide 100 nm)	WST-1 assay + LDH assay	2, 4, 8, 16, and 32	24	NM110 + 2% FBS	Cardiovascular endothelial cells	The oxidative stress and inflammatory response triggered by ZnO NPs were not linked to ER stress. Exposure Time: 24 h.	[161]
ZnO (Zinc oxide < 50 nm)	MTS assay	0, 5, 10, 15, 20, 25, 30, and 50	3, 6, 12, and 24	DMEM + 10% FBS	Human umbilical vein endothelial cells (HUVECs)	Ferroptosis in HUVEC in a dose- and time-dependent manner, with clear biomarkers of oxidative stress and ionic overload.	[162,163]

Table 4. *Cont.*

Metallic Nanoparticle Types by Size (nm)	Assays	Value (μg/mL)	Exposure Time (h)	Medium	Environment/Setting	Observed Effects	Ref.
ZnO (Zinc oxide 45–55 nm)	MTT + comet assay +	10, 20, and 50	24	EGCM/FBS	HUVECs	The subcellular toxicity of ZnO NPs results in DNA damage and loss of cell function.	[164]
ZnO (Zinc oxide 20 nm and 90–210 nm)	Trypan blue dye exclusion assay + ELISA	0, 20, 50 and 150	4	N/A-	HCAECs	Exposure to ZnONPs promoted a decrease in cell viability and an increase in 8-OHdG and IL-6 levels.	[165]
ZnO (Zinc oxide 21.46 nm)	MTT assay	0–1000	24	90% RPMI-1640	HUVECs	Exposure to ZnONPs does not present cytotoxicity in HUVEC lines.	[163]

EGCM—elicited granulocyte cell model. DMEM—Modified Eagle's Medium. FBS—fetal bovine serum. MTT—(3-(4,5-dimethylthiazol-2 yl)-2,5-diphenyltetrazolium bromide). HCAECs—human coronary artery endothelial cells. HUVECs—human umbilical vein endothelial cells. NM 110—commercial culture medium specific for endothelial cells. RPMI—liquid cell culture medium (Roswell Park Memorial Institute). WST—water-soluble tetrazolium salts. 8-OHdG 8—8-hydroxy-2′-deoxyguanosine. ER—endoplasmic reticulum. LDH—cytotoxicity marker.

Regarding zirconium oxide (ZrO_2), it is known to be a semiconductor material valued for its biocompatibility, non-toxicity, and high resistance to compression and fracture. These properties make it widely used in dental implants and certain biomaterials. Additionally, ZrO_2 is applied in thermal coatings and energy storage, making it a versatile material for biomedical applications [155]. ZrO_2 NPs can also exhibit antimicrobial activity through electrostatic interactions with bacterial cells [166].

Other important features of Metal Oxide-Based Nanoparticles are their richness in ions, meaning that the nanoparticles maintain a structure containing a combination of negative oxygen ions and positive metal ions, forming strong and stable ionic interactions driven by electrostatic forces [167]. Among the most common metal oxide-based nanomaterials, titanium oxide ($TiO_{2)}$, aluminum oxide (Al_2O_3), and silicon dioxide (SiO_2) stand out [121,168].

The versatility of TiO_2 nanoparticles in the biomedical sector is remarkable, with applications ranging from the manufacture of resins and food materials to their use in pharmaceutical synthesis processes [169].

Another relevant application is the nanofunctionalization of TiO_2 nanoparticles in biomimetic scaffolds for bone tissue regeneration. The success of titanium metal structure functionalization with TiO_2 has been demonstrated, showing a reduction of up to 99.4% in the presence of bacteria (*S. aureus*) and induction of mesenchymal stem cell proliferation, which is 4.3 times higher than conventional titanium scaffolds [170]. Furthermore, TiO_2 nanoparticles are also used in industrial applications, such as the manufacturing of bactericidal coatings for hospital surfaces, due to their ability to function as a semiconductor with a wide bandgap [132].

Given their widespread use, it is crucial to evaluate the potential toxicological effects of TiO_2 NPs, particularly when applied in biological systems. Table 5 below presents the toxicity limit concentration of different sizes of TiO_2 NPs and observed effects in cardiovascular endothelial cell models. In vitro and in vivo studies have demonstrated that TiO_2 NPs, with dimensions from 10 to 50 nm, can induce a diversity of biological effects, including increased expression of adhesion molecules, apoptosis, DNA damage, oxidative stress, and inflammation, especially at higher concentrations and exposure times. These findings highlight the importance of managing the concentration and exposure times of TiO_2 NPs to reduced possible risks and promote their secure application in biomedical settings.

Table 5. Concentration and observed toxicity range of titanium dioxide nanoparticles (TiO_2) in various experimental models.

Metal oxide Nanoparticle Types by Size (nm)	Assays	Value (μg/mL)	Exposure Time (h)	Medium	Environment/Setting	Observed Effects	Ref.
TiO_2 (Titanium Dioxide 50 nm)	Oxidative stress and ROS production	50 and 200	1	H2DCFDA	Human umbilical vein endothelial cells (HUVECs)	TiO_2 NPs can increase the expression of adhesion molecules in HUVECs. Exposure Time: 3 and 24 h.	[171]
TiO_2 (Titanium Dioxide 10–30 nm)	Cell viability assay (WST-1 tetrazolium salts)	0, 1, 5, 25, 50, and 100	24	1% PBS + 10% DMSO	HUVECs	For TiO_2, oxidative stress plays a key role in toxicity, and the total antioxidant capacity tends to increase with longer exposure. Exposure Time: 24 h.	[172]
TiO_2 (Titanium Dioxide 10, 30, 50, and 100 nm)	CCK-8 assay	1, 5, and 25	24	MEM with Earle's salts + 10% FBS+ 1% HEPES	HUVECs	Prolonged exposure to high levels of nano-TiO_2 may pose a significant risk to human cardiovascular health by inducing apoptosis in cardiovascular endothelial cells. Exposure Time: 24 h.	[173]
TiO_2 (Titanium Dioxide 10, 30, 50, and 100 nm)	Comet assay	1, 5, and 25	4	MEM with Earle's salts + 10% FBS+ 1% HEPES	HUVECs	TiO_2 NPs are capable of causing DNA damage and an increase in the micronucleus with a positive dose-dependent and negative size-dependent effect.	[174]

H_2DCFDA—2′,7′-dichlorodihydrofluorescein diacetate. HUVECs—human umbilical vein endothelial cells. ROS—reactive oxygen species. WST—water-soluble tetrazolium salts. DMS—dimethyl sulfoxide. PBS—phosphate-buffered saline. CCK-18—cell counting kit-8. HEPES—4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. FBS—fetal bovine serum. MEM—minimum es-sential medium.

6. Challenges and Future Trends

In the case of clinical translation for implants, it is subject to compliance with international regulations that ensure the safety, efficacy, and quality of medical and dental implants. This framework is based on standards such as ISO 10993 [175], which focuses on the biological evaluation of medical devices (biocompatibility), and others like according to ISO 19227 [176–178], which addresses the cleaning of orthopedic implants. Together, these standards and complementary regulations establish the minimum requirements that must be met from design and manufacturing to implantation in the human body, ensuring biocompatibility, functionality, and long-term safety [179].

The ISO 10993 standard, known as the 'Biological Evaluation of Medical Devices' or 'Biocompatibility Testing,' addresses fundamental topics such as the acceptance and control of biological risks arising from the interaction between the implantable device and the organism. Complementary resolutions, such as RDC No. 725/2022, align with this international standard by establishing the regulatory framework for the biological evaluation of health materials, in accordance with ISO 10993-1:2018, [175] which defines risk evaluation and testing procedures [178].

It is true that standards such as ISO 10993-1 do not explicitly mention specific regulations for coatings with nanomaterials required in titanium implants or titanium alloys. Instead, they focus on biological evaluation within a risk management process, without detailing specific requirements for coatings. The emphasis is on biological evaluation as part of a risk management process. Under this approach, biological evaluation is not an isolated end but rather an integrated activity that essentially verifies the safety of the device design, considering all potential biological risks associated with its use. The main objective is to identify, evaluate, control, and ensure patient safety throughout the product's lifecycle [176–178].

The set of regulations applicable to implants ensures that innovation in materials and techniques can be successfully translated into clinical practice safely. This is further supported by specific regulatory processes, such as obtaining the CE mark (Europe) or FDA

approval (U.S.), which are essential to demonstrate compliance with international standards. However, although these regulations provide a general framework for risk management, they do so without specifications for titanium implants, alloys, and nanofunctionalized coatings. Their focus on hazard analysis suggests the need to address translational challenges [180].

However, beyond the basic biocompatibility testing established by international standards, the clinical validation of metal implant surfaces functionalized with nanomaterials requires addressing critical aspects that are not yet sufficiently standardized [175,180]. These include the implant's compatibility with different sterilization methods, long-term stability properties against corrosive processes and ion release, validation of bactericidal efficacy, such as biofilm formation on implants, and the definition of regulations for the development of devices functionalized with nanomaterials [180]. These elements serve as a bridge between innovation in coatings and safe clinical implementation, addressing a regulatory and translational gap highlighted by the literature and regulatory bodies. In this context, it is essential to consider critical aspects that are still poorly standardized, such as the compatibility of titanium implants functionalized with nanomaterials and sterilization methods.

Compatibility with sterilization methods in titanium implants functionalized with nanomaterials can alter both their surface and functional properties. Thermal methods, such as steam sterilization using autoclave systems, can promote oxidation reactions in metallic nanoparticles, as well as undesirable degradation reactions, compromising, for example, controlled drug release processes.

In the case of analyzing the effect of cleaning and sterilization on the surface properties of titanium implants and their cellular response, there are interesting findings by Park (2012) [181], where the removal of small organic molecules present on titanium surfaces was observed. Additionally, alterations in surface properties due to sterilization processes were confirmed, along with changes in wettability and roughness analyzed by XPS. Both results impacted the osteogenic differentiation of MG63 osteoblast-like figure cells, concluding the limited feasibility of reusing sterilization, as it may promote different tissue responses found in specimens that had never been implanted before [79,181].

Finally, a deficiency is identified in the general technical regulations, specifically regarding the sterilization and disinfection processes of titanium alloys functionalized with nanomaterials. This gap exists because it is still a very specific topic, and existing standards have focused on general practices and principles for high-level sterilization and disinfection of medical items, such as in Technical Standard No. 199 according to ASTMG199-09 [182] and ANSI/AAMI standards for medical equipment [180].

Regarding titanium surfaces and their alloys, the European Union emphasizes the need for specific risk evaluation, improvement and validation of detection methods, control, and regulatory harmonization to ensure safety [175]. Some general aspects of risk evaluation, regulation, and the improvement of detection and control processes for nanomaterials in the EU, which are also relevant to titanium surfaces and alloys, include the following: Risk evaluation, which should be conducted on a case-by-case basis using current methods adapted to the specificities of nanomaterials, though there is a recognized to explore specific aspects further to ensure their safety [183]. European regulation, under the REACH framework (Registration, Evaluation, Authorization, and Restriction of Chemicals), includes nanomaterials but requires adjustments and specific requirements, including modifications and guidelines for their registration and evaluation [175,183].

In the registration process, manufacturers and importers to register nanomaterials with information on their properties, uses, and risks. Clarity regarding nanomaterials' forms may affect the registration process [183]. Regarding evaluation, the European Chemicals

Agency (ECHA) evaluates the registrations and identifies potential risks. Working groups on nanomaterials provide guidance and improve the scientific and technical evaluation of these materials [180,183]. Finally, nanomaterials may be subject to specific authorizations or restrictions based on risk assessments related to occupational health, environmental impacts, and public health [179].

There are challenges in the validation and standardization of methods for the detection, characterization, and analysis of nanomaterials in complex matrices, such as metal surfaces or implants, which makes comparability difficult. The standardization of measurement and testing methods is promoted to support risk determination, which is essential for materials used in biomedical applications such as titanium and its alloys. These actions are crucial for advancing both the safety and effective regulation of nanomaterials in biomedical applications and other related sectors.

7. Translational Readiness: Challenges and Pathway

The clinical translation of nano-functionalized bone implants must overcome major technical and regulatory barriers. Beyond standard biocompatibility (ISO 10993), implants must withstand long-term cyclic loading without significant corrosion or metal ion release (e.g., Ti, Ag, Mg ions) into the biological system [179]. Additionally, biofilm formation under mechanical shear remains a critical issue, as bacterial remains a critical issue, as bacteria can adhere to the implant surface under movement, leading to infection. Sterilization methods (e.g., gamma radiation, ethylene oxide, steam) must also be carefully chosen, as they can damage nanocoating, alter drug release profiles, or oxidize surfaces [180].

Regulatory frameworks (e.g., FDA, EU) still lack specific guidelines for nanocoated implants. Key gaps include standardized tests for coating adhesion, scratch resistance, endotoxin limits, and corrosion under biomechanical stress [175,180]. Each new implant material may require case-by-case risk evaluation under systems like the EU [183].

Future success depends on smart immune modulation design. Controlling the host immune response with special attention to macrophages from inflammatory (M1) to healing (M2) phenotypes is important. This can be achieved through engineered super face (e.g., TiO_2, nanostructure [107]) agents, Zn^{2+} releasing coating) [184] Hybrid systems (e.g., polymeric nanoparticles with BMP-2 on ta-doped Ti implants) allow precise special release of osteogenic and immune signals. Surface hydroxylation of titanium is another promising strategy, improving protein adsorption for bone healing while reducing bacterial attachment [7,185].

The use of NPs faces significant challenges, including issues of efficiency and potential compatibility problems, as well as long-term bactericidal effects on materials within the biomedical industry. Further studies are essential to assess risks and develop suitable materials. Research into the bioactivity mechanisms of nanoparticles represents a promising and innovative field.

Understanding how these particles interact with biological environments is essential to enhancing their benefits and reducing potential risks. Below, we highlight some of the ongoing research challenges, difficulties, and research trends in the field of NP bioactivity. A relevant obstacle in current research is osteo-immunomodulation, a topic that deserves further exploration, as it reflects the present landscape of studies with nanomaterials and helps identify future challenges in the biomedical industry and scientific research in general.

Osteo-immunomodulatory nanoparticles for bone regeneration: Biomaterials, including nanomaterial-based materials, exhibit innovative and impactful properties for the biomedical industry due to their high biocompatibility and plasticity. These aspects have led to advancements in the development of nanomaterial-based treatments that help mitigate bone diseases. This point is particularly interesting because it demonstrates that the

application of nanomaterials is not limited solely to antibacterial activity, cellular induction, and drug delivery. On the contrary, there is an expansion into new areas of development, such as their role in guided bone regeneration (GBR). In this context, recent studies have investigated the presence of nanomaterials in processes like bone regeneration, bioactivity, and interaction with the biological environment. Other important aspects include the recognition of the influence of nanomaterials in the creation of highly biocompatible microenvironments, the strengthening of mechanical properties, their role as an essential barrier, and the promotion of osteogenesis and angiogenesis [186].

Currently, it is crucial to deepen the study of the immunological microenvironment in biomaterial-mediated bone regeneration, since implanted cells or scaffolds do not always successfully integrate into host tissues due to an unfavorable immune response. This challenge underscores the need to search for biomaterials capable of creating an ideal environment for osteogenesis, an emerging concept known as "osteo-immunomodulation" [184]. In this context, nanoparticles, as nanomaterials, play a key role in developing drug delivery strategies, enabling the controlled release of functional chemicals and proteins that can modulate local immunological microenvironments. In this area, multifunctional nanoparticles, such as those based on traditional metals like titanium, are considered an innovative and promising platform for tissue regeneration due to their diverse functionalities [187].

It is anticipated that new research will advance the development of nanomaterials that enhance osteoinduction and promote a regenerative microenvironment for bone regeneration. As the field of nanomaterials progresses at a remarkable pace, numerous unexplored interconnections between their various applications are emerging, which are essential to unlock their transformative potential in modern medicine. The delivery of biological molecules via nano-engineered biomaterials promises to revolutionize tissue engineering by offering NPs unprecedented precision in regulating the cellular responses critical for regeneration [188].

Simultaneously, the development of multifunctional surface topographies, such as micro/nano texturization and electrolytic plasma oxidation, emerges as a key strategy to modulate complex biological interactions, enhancing the integration of biomaterials in specific cellular environments [189,190]. Additionally, advancements in functional NPs for 3D-printed biodegradable implants are expanding the possibilities for personalized therapeutic devices, specifically designed to meet each patient's unique requirements [191].

Meanwhile, the use of metallic NPs in cancer therapy presents a promising frontier in precision medicine, and bioinspired composite materials are emerging as sustainable, cutting-edge solutions for the design of biomedical structures [192]. Together, these advances blend the best of nanotechnology, biotechnology, and materials engineering, not only redefining the treatment of complex diseases but also opening a broad range of possibilities for more personalized, effective, and ethical healthcare in the near future [193]. The following section will delve into how these advancements, such as osteoinduction through nanoparticles and the customization of therapeutic devices, are transforming modern medicine.

Surface engineering of metallic nanoparticles represents a key area in addressing the current challenges and trends in biomedical nanotechnology. These NPs, which include metals such as Pt, Cu, Au, Ag, as well as elements like Gd, Er, and Zn, possess exceptional properties in terms of stability, specificity, and sensitivity [194–196]. Nevertheless, one of the main and most useful challenges for future applications lies in the manipulation of their surface, as it determines their functionality and applicability in the medical field. Among other novel and attractive characteristics of these nanoparticles is their functionalization capacity, which enables the incorporation of molecules of various natures [197].

Currently, research efforts focused on surface manipulation of NPs reflect a key trend in the advancement of precision delivery systems. These strategies aim to facilitate the precise transport of therapeutic agents from nanoparticles to particular tissues or cells. For this purpose, the use of structures such as ligands (antibodies or peptides) is essential, as they increase precision in both treatment and diagnosis. At the same time, progress is being made in the fabrication of multifunctional nanoparticles capable of performing diagnosis, drug release, and therapeutic actions, thus optimizing treatments and minimizing side effects [1].

Future research is also directed toward developing anticorrosive properties and harnessing the broad range of optical and electronic features exhibited by nanoparticles, which can be tailored based on specific physical parameters. This control over their surface and physical characteristics enhances their applicability in diagnostics, therapeutic treatment, and other biomedical areas [197]. Therefore, it is expected that the study of the properties of metallic nanoparticles will enable the development of new biomedical applications. However, it is also necessary to address other challenges related to safety, toxicity, and stability in different environments. These aspects are essential to establish reliable synthesis protocols, as well as to perform risk and toxicity assessments, which are critical for advancing toward safe clinical applications.

Another noteworthy trend is the integration of metallic NPs with emerging technologies, including artificial intelligence and nanotechnology. This outlook offers significant potential, as these tools could support the development of personalized biomedical applications, enabling greater specificity in diagnostics and improved effectiveness in therapeutic treatments. An example of their applicability, analyzed through surface modifications of nanoparticles, is reflected in their ability to produce images using modalities such as photoacoustic, magnetic resonance imaging, and tomography. Moreover, these NPs can be employed as contrast agents and as a multifunctional therapeutic platform, defined as theranostics. As safe application is essential, particular attention must be given to minimizing the risk of allergic reaction and toxic effects [1].

Finally, innovation in fabrication techniques and the incorporation of novel materials are expected to expand the range of applications, including gene editing, nanorobotics, and combined therapies, consolidating metallic nanoparticles as key tools in 21st-century medicine [1].

8. Conclusions

This review broadens our understanding and promotes the potential use of nanoparticles to stimulate specific cells to help heal the body to repair its own tissues in a natural way. The analysis of the state of the art carried out reveals that there is a critical regulatory gap. There are no specific standardized regulations for the biocompatibility of nano-functionalized implants, nor for addressing their application in the clinical sector, particularly the effects of the sterilization methods used, corrosion, and long-term ion release, etc. On the other hand, no studies are reported in the scientific literature with an approach that integrates all aspects related to the use and influence of the structural characteristics of metallic nanoparticles and metal oxides. In this context, it is very important to consider the bioactivity of nanoparticles, as well as the synergistic interaction of the multiple factors (size, shape, area, surface chemistry, charge, functionalization, biodegradability) that determine their reactivity and application in biomedical settings. Another aspect to consider is the dynamics of antibacterial mechanisms and antibacterial efficiency, determined by the interaction between the structural characteristics of nanoparticles and the architecture of the bacterial cell wall. It is established that, for Gram-negative bacteria (high LPS density), the charge density of nanoparticles is critical for initial electrostatic adhesion. In the specific

case of Gram-positive bacteria, to penetrate the thick peptidoglycan wall, a nanoparticle size of less than 50 nm (ideally ~20 nm) is recommended. Regarding bioactivity and cell growth induction, this work goes beyond the antibacterial applications of nanoparticles, addressing in depth how they can actively promote cell proliferation and tissue regeneration. Furthermore, the keys related to surface hydroxylation are presented as an advanced and promising surface modification alternative for improving the bioactivity of titanium implants. In this context, emphasis is placed on the importance of the density of OH groups in modulating protein corona formation and, ultimately, cellular response and the reduction in bacterial colonization. The protein corona is a biological determinant of the complex process of protein adsorption on surfaces and is fundamental to the success of titanium implants.

This work identifies osteoimmunomodulation as an emerging and crucial field of research. The success of bone regeneration depends on the modulation of the immune response (macrophage polarization). It is also proposed that nanoparticle systems constitute ideal platforms for the controlled (space-time) release of immunomodulatory signals directly at the implant–tissue interface, creating a pro-regenerative microenvironment. The role of organic and carbon nanoparticles in implants is not structural, but rather their use serves as functional coatings for metallic implants and potential reservoirs for the controlled release of drugs, osteoinductive agents, and agents with bactericidal activity. Finally, regarding future trends and innovative visions, hybrid and intelligent systems are highlighted. Their uses in thermoplatforms that employ metallic nanoparticles for a dual purpose include the following: diagnosis and therapy. Also, the use of artificial intelligence or machine learning could be implemented. This software can be used as a design tool in this field. In this sense, the designer (or even users) would insert in the program the parameters related to the specific characteristics of the nanoparticles, and the program would return information about how the nanoparticle design should be developed: biofunctionality properties, type of materials, obtaining protocols, etc. All of this could allow us to limit and eliminate all irrelevant information, allowing us to develop implants that enhance their predictive accuracy and reduce experimental costs. Despite the promising advances previously described, unfortunately, there is still a significant challenge based on the need for proposing personalized "tailored-made" solutions (age and gender of the patient, etc.), which would require multiple efforts, more knowledge, and synergy including multidisciplinary research teams and STEM (engineers, medical, chemists, pharmacists, microbiologists, etc.).

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Abbreviations

NPs	Nanoparticles
MNPs	Metallic Nanoparticles
ROS	Reactive Oxygen Species
LPS	Lipopolysaccharides
DNA	Deoxyribonucleic Acid
PDGF	Platelet-Derived Growth Factor
FGF	Fibroblast Growth Factor
HAp	Hydroxyapatite
CNTs	Carbon Nanotubes
C60	Fullerenes or Buckminsterfullerenes
GO	Graphene Oxide
CNT	Carbon Nanotube
CNFs	Carbon Nanofibers
iNPs	Inorganic Nanoparticles
ZnONPs	Zinc Oxide Nanoparticles

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