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Hydroxyapatite modified with therapeutic ions as a smart material for implantology and regenerative applications. A review

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Abstract

Hydroxyapatite is a material widely used in biomaterial engineering due to its biocompatibility, bioactivity and chemical similarity to the mineral fraction of bone tissue. Despite its numerous advantages, its limited biological activity and mechanical properties prompt the search for modifications that will increase its functionality in clinical applications. One effective approach is ion substitution in the HA crystal structure, which can occur in both cationic (e.g. Ca^{2+}) and anionic (e.g. OH^{-}) sites.

This review article discusses the effect of substitution of selected therapeutic ions – copper (Cu^{2+}), strontium (Sr^{2+}), cobalt (Co^{2+}), zinc (Zn^{2+}) and magnesium (Mg^{2+}) – on the physicochemical and biological properties of hydroxyapatite. Their impact on physical and chemical properties and biological activity was analysed. Ion substitution enables the design of multifunctional materials that can support regenerative processes, prevent infections and modulate cellular response. A review of current literature data confirms that modified HA may be a promising platform for advanced implant systems and drug delivery systems.

Keywords: hydroxyapatite, therapeutic ions, bioactivity

1. Introduction

The focus of biomaterials development is directed toward bone tissue regeneration, which is associated with osteoconductive properties. Calcium phosphates (CaP), including hydroxyapatite (HAp) are among the materials that meet the requirements for orthopedic implants. Apatites are characterized by the general formula $Me_{10}(X)_6(Y)_2$, where Me represents divalent cations (Ca^{2+}, Mg^{2+}), X represents divalent or trivalent anions (PO_4^{3-}, CO_3^{2-}), and Y represents monovalent anions (OH^-, F^-, Cl^-). They are among some of the most widespread, naturally occurring phosphate minerals, and moreover, it is a natural component of vertebrate bones and teeth. One of the most important properties that apatites have is their ability to accept ionic substituents (Cazalbou et al., 2005; Stötzel et al., 2009).Currently, a lot of research is focused on investigating new solutions that can improve osteogenic properties, resulting in better bone regeneration. For many years, the main direction in the development of bone graft materials was the use of pharmacological agents with which calcium phosphates were modified. However, due to the danger of undesirable bone formation, the search for new concepts began (Ressler et al., 2021).One such solution is the incorporation of elements such as sodium, magnesium, zinc, strontium into the apatite structure. Incorporating dopants in the form of these ions into bioactive ceramics can be an effective solution for clinical applications (Bose et al., 2013; Minardi et al., 2015)however, they have recently become the target of scrutiny over safety. The importance of trace elements in natural bone health is well documented. Ions, for example, lithium, zinc, magnesium, manganese, silicon, strontium, etc., have been shown to increase osteogenesis and neovascularization. Incorporation of dopants (trace metal ions.

This review focuses on ion-modified hydroxyapatite, which may have a positive effect on bone regeneration. Elements whose incorporation into the structure of hydroxyapatite may have a beneficial effect on bone tissue development will also be discussed.

1.1. Scope and Method

This article is a narrative review of the literature. Publications were searched in the Web of Science, Scopus, and PubMed databases between based on the most recent years, using the keywords: “hydroxyapatite,” “ion substitution,” “copper,” “strontium,” “zinc,” “cobalt,” “magnesium,” and “therapeutic ions.” Articles in English describing materials in the form of coatings, cements, or scaffolds were included. Works that did not describe the synthesis or biological effects of modifications and non-peer-reviewed conference publications were excluded.

2. Structure and properties of hydroxyapatite

Hydroxyapatite (HAp) is a material often used as a bone substitute due to its chemical properties similar to natural bone. Bone consists of several main components: mineral phase (69 wt. %), organic matrix (22 wt. %) and water (9wt.%) (Dorozhkin, 2012; Kattimani et al., 2016). Hydroxyapatite with the sum formula $Ca_{10}(PO_4)_6(OH)_2$ shows great similarity to the inorganic component of bone. Therefore, it can act as an excellent bone substitute. It has a Ca/P ratio of 1.67. Among calcium phosphates, it is one of the most stable and less soluble bioceramics. Pure HAp powder is a white powder, but naturally occurring hydroxyapatite can range in color from yellow through green to brown. It should be noted that the mechanical properties of HAp depend on such characteristics as porosity, density and crystal size, among others (Dubok, 2000; Hench & Thompson, 2010). Hydroxyapatite also has excellent biological properties. Due to the calcium content of its structure, it can increase the concentration of local Ca^{2+}, which in turn contributes to the activation of osteoblast proliferation and

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simultaneous bone growth and differentiation of mesenchymal stem cells (MSCs) (R. Zhao et al., 2017)secreted by pathologic osteoblasts, had a smaller crystal size and lower crystallinity than that of the normal. To date, little is known regarding the interaction of synthetic hydroxyapatite nanoparticles (HANPs. It exhibits non-immunogenic properties, biocompatibility, bioactivity, good bone conductivity, biological affinity, osteoconduction or osteointegration which shows it as an ideal material for bone tissue repair (LeGeros, 2008; Murugan & Ramakrishna, 2004; Oliveira et al., 2017; Szcześ et al., 2017). The surface of hydroxyapatite (HAp) promotes adhesion, proliferation and differentiation of osteoblast cells, and new bone tissue is formed by the gradual replacement of material by bone derived from surrounding, living tissues. HAp scaffold structures can also act as cytokine carriers, demonstrating the ability to bind and locally accumulate bone morphogenetic proteins (BMPs) in vivo (Ohgushi et al., 1993). During regeneration, an important aspect is the interaction of apatite with biological tissues. It is important to understand the body's response to hydroxyapatite. Biologically inert materials do not interact with the environment in any beneficial way, nor do they release harmful substances. The bioactive material dissolves slightly, but before it interacts with tissues in an atomic way it forms biological apatite. This treatment affects the formation of chemical bonds directly with bone tissue. The material, which is bioresorbable at first, dissolves which makes it possible to locate new tissue in surface irregularities. Despite these advantages, the material in question has some disadvantages, such as brittleness and easy aggregation, which make it difficult to obtain satisfactory HAp-based materials. In order to increase the attractiveness of hydroxyapatite in biomedical applications, it is possible to modify it by adding various ions. The crystal structure of hydroxyapatite allows the replacement of Ca^{2+}, PO_4^{3-} and OH^- ions with other ions. Figure 1 shows the crystal structure of hydroxyapatite and possible substitutions of this structure (Arcos & Vallet-Regí, 2020; Safarzadeh et al., 2019).

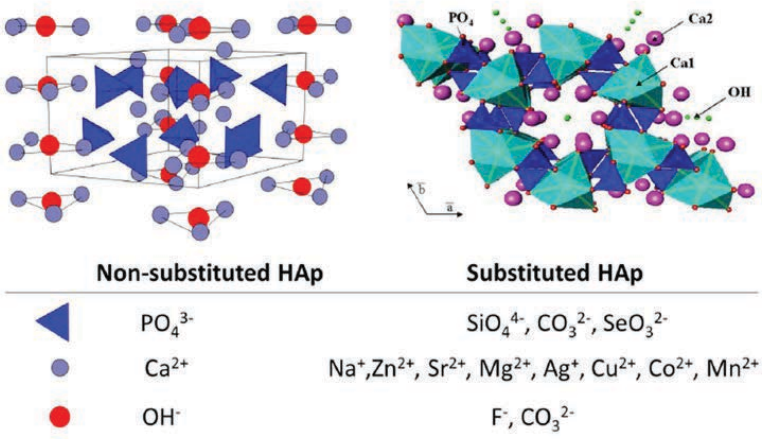

Fig. 1. The crystal structure of hydroxyapatite and possible substitutions of this structure (adapted from Shi et al., 2021)

There are several types of ion substitutions that take place in the crystal structure, namely OH^- ions can be replaced by F^- and CO_3^{2-} ions, while calcium cations can be easily substituted by strontium, magnesium or zinc ions, and PO_4^{3-} is most often replaced by CO_3^{2-} and SiO_4^{4-}. With these modifications, we obtain HAp doped with ions (Arcos & Vallet-Regí, 2020). The introduction of different ions affects many properties of hydroxyapatite i.e. crystallization, degradation, mechanical or biological properties (Porter et al., 2003). Ion doping can positively contribute to the regeneration of damaged bone (Driessens et al., 2002; Webster et al., 2002)K+ and Na+ so that in an open system they are transformed into calcium deficient hydroxyapatite Ca9(HPO4.

3. Mechanisms and strategies for modifying hydroxyapatite

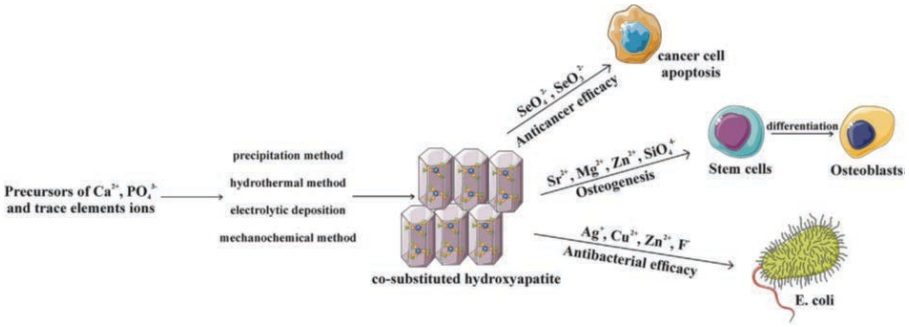

Fig. 2. Schematic diagram of the synthesis methods for ion-substituted HAp and the influence of certain ions on its properties (adapted from Ressler et al., 2021)

In recent years, researchers have focused their attention on the susceptibility of hydroxyapatite to substitution with therapeutic ions in order to improve its properties. Partial replacement with ions such as Ca^{2+}, PO_4^{3-}, or OH^- is aimed at improving mechanical, thermal, and physicochemical parameters. There are several ion substitution strategies. The most well-known is ion substitution in three main locations of the crystal lattice. Due to its hexagonal structure and chemically similar composition to bone, isomorphic substitution is possible. This means that calcium cations can be replaced by other ions such as Mg^{2+}, Zn^{2+}, Sr^{2+}, Cu^{2+}, Ag^{+}, Fe^{3+}. Phosphate anions can also be replaced by CO_3^{2-} (carbonation), SiO_4^{4-}, VO_4^{3-}. The third possible substitution is the OH hydroxyl group, which can be partially substituted with F^-, Cl^-, CO_3^{2-}, and NO_3^- ions. The condition enabling substitution is that the dimensions of the crystallites and the charges of the substituting ions are similar to those of the substituted ions(Sobczak-Kupiec & Wzorek, 2010). Hydroxyapatite has two sites for cations in its structure, namely Ca(I), which prefers substitution by smaller cations (e.g. Mg^{2+}), and Ca(II), which is occupied by larger ions, e.g. Sr^{2+}(Boanini et al., 2010). Hydroxyapatite can be doped with therapeutic ions using precipitation, hydrothermal, sol-gel and mechanochemical methods. The most common substitution method is the so-called wet method, i.e. carried out in an aqueous solution.

3.1. Functional behavior of ion-substituted hydroxyapatite as a "smart" biomaterial

Hydroxyapatite modified with therapeutic ions exhibits multifunctional properties, which allows it to be classified as a smart material due to its ability to respond dynamically to its surrounding environment. It should be emphasized that the incorporated ions change the structural and physicochemical properties, but more importantly, they are able to activate specific biological pathways that adapt to local tissue conditions. Examples include copper and zinc ions, which have antibacterial and angiogenic effects, while strontium ions stimulate bone proliferation and mineralization, and cobalt ions trigger reactions leading to increased vascularization (Bose et al., 2013; Khosrowshahi et al., 2021; O'Neill et al., 2018). This effect of ions makes ion-doped hydroxyapatite a "smart" biomaterial capable of producing multiple biological effects simultaneously, such as promoting osteogenesis while preventing inflammation, for example, after implant placement. Furthermore, studies indicate that specific synthesis conditions can influence the kinetics of therapeutic ion release, enabling controlled delivery of ions over time (Arcos & Vallet-Regí, 2020; Mehrabi et al., 2020). The term "smart biomaterial" used in this article refers to biochemical adaptability, which manifests itself in the ability of the biomaterial to maintain proper cell behavior and regenerate tissue regulated by the ionic environment.

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4. Biocompatybility and Safety Consideration

From a regulatory standpoint, medical materials—including coatings and implants that release therapeutic ions—must be evaluated in accordance with ISO 10993, the standard for the biological safety of medical devices. This standard requires a series of tests, including cytotoxicity, irritation, hypersensitivity (sensitization), systemic toxicity, as well as tests for ion release/extraction under conditions similar to physiological conditions (37 °C, specific surface area to extract volume ratio).

According to ISO 10993-12, which deals with the preparation of samples and extracts, it is essential to maintain strictly defined extraction conditions to ensure the comparability and repeatability of test results. Among other things, this standard specifies the method of sample surface preparation, the sample-to-medium volume ratio, and the incubation conditions.

For metallic materials, a conversion factor of 200 mg of material per 1 ml of medium is most commonly used, which allows for a representative concentration of substances released from the surface during incubation (37 °C, 24–72 h) to be obtained. However, for ceramics and ceramic coatings, such as hydroxyapatite (HA) or its ion-substituted derivatives, the standard recommends that the sample-to-medium ratio refer to the surface area of the sample rather than its mass. The standard ratio is 6 cm^2 of surface area per 1 ml of extraction medium.

When the ceramic material is in the form of powder (e.g., bioactive hydroxyapatites, phosphate glasses, or metal oxides with bioceramic properties), it is impossible to determine the exact surface area. In such cases, ISO 10993-12 allows the use of a ratio of 100 mg of powder per 1 ml of medium, which ensures adequate contact surface area and representativeness of the extract. Extraction is usually carried out at a temperature of 37 ± 1 °C for 24 ± 2 hours, in an aqueous, saline, or cell culture medium (e.g., DMEM, MEM, PBS) environment, depending on the type of biological test planned. Extracts prepared in this way form the basis for further in vitro biological evaluation, especially cytotoxicity tests, which allow the impact of potentially released ions or degradation products on cells to be assessed.

These assessments ensure that released ions remain below cytotoxic thresholds, which is particularly important for ions such as Cu^{2+}, Co^{2+}, and Zn^{2+} that may induce oxidative stress or inflammation when present at excessive concentrations.

In addition, ISO 10993 emphasizes the need to consider the form of the material, since each exhibits a distinct release profile and biological exposure level. In the case of porous, powdered, or nanocrystalline materials, the specific surface area plays a key role in the speed and quantity of ions passing into the medium. Therefore, for ion-substituted hydroxyapatites (e.g., Cu-HAp, Zn-HAp, Sr-HAp), the use of appropriate extraction conditions is essential to accurately reflect the actual processes occurring in the physiological environment.

It should be emphasized that ions with beneficial biological effects can be toxic above certain concentration thresholds, hence the need for careful evaluation in vitro and in vivo. Compliance with ISO 10993 is essential for translating ion-substituted hydroxyapatites into clinical use, ensuring that therapeutic benefits such as osteogenesis, angiogenesis, and antibacterial activity are achieved without compromising biocompatibility (Reeve & Baldrick, 2017; Sharma & Luthra, 2023) differences in proposed biocompatibility assessments or test methods lead to confusion and inefficiencies in generating the package of supporting nonclinical data. Areas covered: Modifications to available guidance for biological safety testing of medical devices, as described by the International Organisation for Standardisation (ISO.

5. Overview of therapeutic ions and their functions

Copper

Copper is one of the chemical elements that can be incorporated into the structure of hydroxaypatite. This element is distinguished by two oxidation levels - Cu(II) and Cu(I) (Noori et al., 2024). Copper is one of the most important micronutrients for humans and has a special function in maintaining the body's balance. Its deficiency can cause serious conditions such as anemia, pancytopenia and neurodegeneration (Manto, 2014; Myint et al., 2018; Tahir et al., 2022). This element plays an indispensable role in the skeletal system. An important issue is the reduced mineral content of the bones, which translates into poorer bone tissue strength as a result of a deficiency of the element in question. This can quickly lead to osteoporosis and other disorders of the skeletal system. Copper acts as a cofactor for superoxide dismutase, which contributes to the elimination of free radicals during bone resorption (Dollwet & Sorenson, 1988; Habibovic & Barralet, 2011)particularly copper metabolism, as an important component of normal bone metabolism in maintenance and repair. Literature published since Rademacher's early (1. Copper is also essential for extracellular matrix synthesis and angiogenesis (Sen et al., 2002).

Copper plays an important role in the repair and regeneration processes of tissues and organs, especially bones and skin. Thanks to its properties - such as stimulating cell differentiation, promoting angiogenesis and inhibiting bacterial growth - it is a valuable micronutrient that effectively accelerates tissue healing (Hu, 1998; Kargozar et al., 2021). An important function that can be attributed to copper is its ability to release bacteria, if released gradually and in the right concentration. The antimicrobial effect that copper possesses is due to a number of mechanisms, i.e. disruption of the integrity of the bacterial membrane, production of free radicals or alteration of the structure of bacterial proteins and enzymes (Vincent et al., 2018). It should be noted that excess copper can contribute to the production of free radicals, which in some cases causes inflammation. Therefore, the concentration of copper must be optimized to properly target bacteria and at the same time without harming human cells (Noori et al., 2022)some studies have indicated that it inhibits osteogenesis at high concentrations. On the other hand, L-arginine (Arg. A study was conducted to investigate the potential of copper embedded in the hydroxyapatite structure. Copper ions exhibit antibacterial potential, and studies show that bacteria experience "contact killing" on Cu^{2+} surfaces (Mokhtari et al., 2017; Mokhtari & Wren, 2019; Weiss et al., 2023). Copper ion-subsidized hydroxyapatite has also been used to remove heavy metals from soil and water, mainly arsenic (Liu et al., 2010). This ability could potentially be useful in terms of dissolving heavy metal ions that may appear on the surface of orthopedic implants. The addition of copper ions to bone cements has also been confirmed to improve osteoblast proliferation, as well as their viability. Moreover, copper contributed to the formation of microvessels (Ewald et al., 2012; Finney et al., 2009; Gérard et al., 2010)biological modification based on delicate protein factors like extracellular matrix proteins or growth factors is subject to a number of shortcomings like the need for storage below room temperature and cost of production. The aim of this study was to investigate ionic modification as an alternative bioinorganic route for implant modification. Although it is known that Cu(II.

The biological effect of copper is strongly dependent on its concentration. At low concentrations (0.01–0.1 mM), Cu^{2+} ions stimulate endothelial cell proliferation, collagen synthesis, and angiogenesis, and support osteoblast activity (Sen et al., 2002; Hu, 1998). However, concentrations above approximately 0.5 mM lead to the overproduction of reactive oxygen species (ROS) and increased oxidative stress, which can result in bone cell apoptosis (Noori et al., 2022; Kargozar et al., 2021). At values above 1 mM, marked cytotoxicity associated with mitochondrial membrane damage and protein denaturation is observed

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(Weiss et al., 2023). Therefore, safe copper-releasing materials should provide a controlled concentration below 0.1 μg/cm²/day, which allows for maintaining antibacterial activity without toxic effects (Mokhtari & Wren, 2019).

Research conducted by Alireza Noori et al. indicates that the controlled introduction of copper ions into hydroxyapatite effectively modulates the release of both Ca^{2+} and Cu^{2+} ions. It has been shown that there is a narrow range of Cu-HAp concentrations (approximately 100–200 mg/ml for Cu-HA 5%) that exhibits bactericidal activity without causing toxicity to eukaryotic cells. The addition of copper does not significantly affect the osteoconductivity of hydroxyapatite, but it clearly promotes endothelial cell migration, which is a key indicator of angiogenesis processes. In summary, doping hydroxyapatite with copper ions improves its biocompatibility and gives it antibacterial and proangiogenic properties (Noori et al., 2024).

Strontium

Strontium is a trace element found in limestone rocks, ocean water and a natural ingredient in food and beverages (Geng et al., 2016; Landi et al., 2007, 2008). Strontium is chemically and physically similar to calcium, making it a bone-seeking natural element. Most of the strontium content in the human body is found in bone tissue (Dahl et al., 2001). A number of clinical studies indicate the dual action of stable strontium ions (Sr^{2+}) to promote bone formation and reduce bone resorption (Arlot et al., 2008; Bain et al., 2009; Bonnelye et al., 2008). Because of the benefits that strontium ions can introduce, research has begun on the substitution of calcium ions with strontium ions in the hydroxyapatite structure. Such treatment stimulates osteoblast activity and inhibits osteoclast proliferation (Capuccini et al., 2008; Qiu et al., 2006). Studies conducted on poly(methyl methacrylate) bone cement confirmed that Sr-containing calcium phosphate has enhanced bioactivity and biocompatibility. This observation is due to the release of strontium ions, which contribute to osteoblast proliferation. It was also noted that this modification facilitates the precipitation of apatite, which affects the increased mechanical strength of the bone-implant interface (Zhang et al., 2011). It was concluded that the presence of strontium ions at the bone-implant interface could be promising for osteointegration, especially in cases of osteoporosis or low bone density (Cheung et al., 2005; Ni et al., 2006).

Cobalt

Cobalt is one of the trace elements that naturally occurs in the human body. In the human body, it is capable of forming complexes with proteins which translates into the formation of important metabolic compounds, including vitamin B12. Most of the cobalt is found in the liver, and excess is excreted by the kidneys (Kramer et al., 2014). Despite the fact that the element occurs naturally in the human body it is adapted to excrete moderate amounts of excess cobalt from the body (Khosrowshahi et al., 2021). Too much cobalt can be toxic (Safari-Gezaz et al., 2025). Cobalt ions have been confirmed to promote a hypoxia-like response and stimulate angiogenesis with osteogenesis, which may be useful in bone regeneration (Fan et al., 2010; Khosrowshahi et al., 2021). The introduction of cobalt ions into the structure of hydroxyapatite increases osteogenesis in vivo. However, it should be noted that despite the satisfactory effects on bone tissue regeneration, too high a concentration of the element ions can be toxic to cells in vitro and in vivo. Modification of ceramic materials with cobalt ions has wide applications in many areas of life, i.e. biomedicine, optical materials, environmental applications or supercapacitors (Azab et al., 2019; Hafeez et al., 2022; Vindhya & Kavitha, 2023). It was confirmed that cobalt-modified oxide layers on the titanium surface contributed to corrosion resistance and, moreover, improved biocompatibility while enhancing cell proliferation (Wu et al., 2024). Despite the potentially toxic effects of cobalt, the element plays an important

role in the formation of blood vessels in bone tissue. Studies have shown that the introduction of cobalt ions into scaffolds made of bioactive glass induces the expression of genes responsible for the production of pro-angiogenic proteins, which is done by stabilizing the HIF-1α factor, associated with the cellular response to hypoxia. Thus, the main action of cobalt in the context of bone tissue regeneration is to stimulate the process of angiogenesis. A key aspect in the use of cobalt in biomedical materials is the precise choice of its concentration - so as to achieve the maximum proangiogenic effect while limiting potential cytotoxicity (Mehrabi et al., 2020; O'Neill et al., 2018).

The effect of cobalt is dose-dependent and has a narrow therapeutic range. At low concentrations (0.01–0.05 mM), Co^{2+} ions activate HIF-1α, mimicking hypoxic conditions, which leads to increased VEGF expression and stimulation of angiogenesis and osteogenesis (Fan et al., 2010; Khosrowshahi et al., 2021). However, at concentrations above 0.1 mM, a decrease in cell viability and an increase in oxidative stress are observed (Safari-Gezaz et al., 2025). Prolonged exposure to cobalt can also be harmful in vivo – levels above 50 µg/L in body fluids are considered potentially toxic (Kramer et al., 2014). Therefore, the Co^{2+} content in implant materials should be strictly controlled so as not to exceed the safe threshold, ensuring an angiogenic effect without the risk of toxicity.

In the context of biological safety for cobalt-releasing materials, it is worth referring to the toxicity assessment in accordance with the standardized ISO 10993 protocols. According to ISO 10993-5, a biomaterial is considered non-cytotoxic if cell viability does not decrease by more than 30% compared to the control (i.e., ≥70% survival), with the preparation of extracts and incubation conditions specified in ISO 10993-12 (medium, temperature 37 °C, defined volume to mass ratio of the sample, time).

An example of this can be found in studies conducted on composites with the addition of cobalt and curcumin, which indicate that cytotoxicity assessment confirms the biocompatibility of doped scaffolds that meet the criteria of ISO 10993. It was found that HA scaffolds doped with CoO and MnO_2 together with curcumin have a combined effect that enhances antibacterial properties, bioactivity, and mechanical stability (Pilli et al., 2025).

Zinc

Zinc is mainly found in bones in vivo and has an important function in the metabolism of bone formation. It has a positive effect on bone development, as it promotes osteoblast proliferation and contributes to the production of osteocalcin and accelerates the maturation of the bone matrix (Hoppe et al., 2011; Kabir et al., 2021; Shi et al., 2021; Uysal et al., 2021). Modification of hydroxyapatite with Zn^{2+} ions affects its properties by increasing bioactivity, osteogenesis capacity and introducing anti-inflammatory and antimicrobial activities (Chopra et al., 2020; Martinez-Zelaya et al., 2019; Toledano et al., 2021). The study indicates that the addition of zinc to hydroxyapatite promotes the formation of an apatite layer in a simulated physiological environment. Moreover, the apatite layer increased with zinc content, indicating that the addition of Zn^{2+} promotes mineralization (Jallot et al., 2005). The element in question is known for its very good antibacterial properties. Over the years, zinc has been confirmed to have an inhibitory effect on Gram-positive and Gram-negative bacteria. It also exhibits antimicrobial properties that inhibit the growth of fungi, i.e. candida albicans(de Lima et al., 2021; Predoi et al., 2019; Sergi et al., 2018). The modification of hydroxyapatite with zinc in biomedical applications is found to exhibit antimicrobial properties, as well as affect the regulation of immune cells, which may contribute to the functional value of these materials.

Zinc ions exhibit beneficial biological effects within a narrow concentration range. At low doses (0.01–0.05 mM), Zn^{2+} stimulates osteoblast proliferation, increases osteocalcin expression, and promotes extracellular matrix mineralization (Hoppe et al., 2011; Toledano et al., 2021). Antibacterial and anti-

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inflammatory effects are also observed in this range. However, concentrations above 0.1 mM cause increased oxidative stress and decreased cell viability (Predoi et al., 2019). In the case of coating materials, a Zn content above 1 wt% may lead to reduced biocompatibility (Chopra et al., 2020). Therefore, it is crucial to maintain controlled zinc release kinetics, ensuring antibacterial and osteoinductive effects without cytotoxicity.

The effect of zinc on the biological activity of hydroxyapatite is highly dose-dependent. At concentrations of 1–50 μM, Zn^{2+} ions optimally stimulate osteoblast proliferation and differentiation, while at higher concentrations, inhibition of osteogenesis and a decrease in cell viability are observed (Kurzyk et al., 2023). It has been shown that too much Zn^{2+} can cause oxidative stress and apoptosis, so the zinc content in HAp should not exceed 1% in order to maintain a balance between osteogenic activity and biological safety (Kubiak-Mihkelsoo et al., 2025; Shi et al., 2021; Wang et al., 2023). In a study by Kazimierczak et al., the cytotoxicity of zinc-doped nanohydroxyapatite (Zn-HA) was assessed in accordance with ISO 10993-5 using an indirect method (extract test). Materials with different Zn^{2+} ion contents were subjected to a 24-hour extraction process in culture medium at 37 °C, maintaining the medium volume to sample weight ratio recommended in the standard. The results showed that samples containing 0.03 mol Zn per mol HA (approx. 0.2 wt. %) were non-toxic to human osteoblasts, as confirmed by high cell viability (> 90%) compared to the control. At the same time, the material with this zinc content showed over 99.9% bactericidal efficacy against *Staphylococcus epidermidis* and *Escherichia coli*. The authors emphasized that at higher Zn^{2+} contents, the risk of toxicity increases, therefore low doping (approximately 0.2 wt.%) is an optimal compromise between antibacterial activity and biocompatibility. This study confirms that the use of protocols compliant with ISO 10993-5 enables a reliable assessment of biological safety and comparability of results in terms of zinc ion toxicity (Kazimierczak et al., 2022).

Magnesium

Magnesium is a very well-known element. This cation owes its popularity to properties such as supporting bone mineralisation through the activation of osteoblasts and osteoclasts. What is more, it inhibits nucleation and hydroxyapatite growth, and stabilises more acidic precursors (G et al., 2022; Kumta et al., 2005; Li et al., 2025). As is widely known, magnesium is an essential element for the formation and maintenance of bones and teeth, and its presence in hydroxyapatite can improve osteoblast activity and osseointegration (Chen et al., 2022). In the adult human body, over 50% of Mg 2+ ions are stored in bones and constitute 0.7% of their total mass. According to the US Food and Drug Administration, the recommended dose for adults is between 320 and 420 mg (Welch et al., 2017). Its deficiency negatively affects bone metabolism, reducing bone density and thus increasing the risk of fractures. The presence of magnesium increases the level of osteoprotegerin, which has a positive effect on bone resorption (Cao et al., 2018; X. Zhao et al., 2022). Magnesium-doped hydroxyapatite exhibits modified mechanical and biological properties that favour its use in bone tissue engineering. The addition of magnesium alters the crystal structure by reducing grain size, increasing microporosity and causing distortions in the crystal lattice(Alanis-Gómez et al., 2024; Bhatnagar et al., 2024).

Multi-ion systems

In recent years, there has been growing interest in multi-ion systems, in which two or more therapeutic ions are simultaneously introduced into the hydroxyapatite structure. It has been shown that this approach can lead to synergistic effects, combining the beneficial properties of individual elements – for example, Sr^{2+} and Ag^{+} together enhance osteogenic activity and antibacterial effects. As indicated

by the conducted studies, the addition of silver alone has shown toxic effects, manifested by poor cell morphology and cell death, as well as an almost complete loss of ALP functional activity. The addition of strontium ions to Ag-HA coatings was able to effectively counterbalance these negative effects and improve performance compared to samples with a pure HA coating (Fielding et al., 2012).

In vitro bioactivity experiments have shown that HA compositions with high Sr and Zn concentrations exhibit excellent bone-like apatite layer formation properties. In contrast, high Ag and F concentrations showed limited bioactivity. Furthermore, the additives also increased the sintered density of HA and improved grain size (Reger et al., 2019).

On the other hand, doping hydroxyapatite with Mg^{2+} and Sr^{2+} ions improve its mechanical properties and affects osteoblast function. In turn, the Co^{2+} + F^{-} system promotes angiogenesis and osteogenesis, while the combination of Zn^{2+} + F^{-} can improve corrosion resistance and also influences the bone regeneration process (Arcos & Vallet-Regí, 2020).

It should be noted that double or multi-ion doping of HAp can lead to synergistic effects, such as a simultaneous increase in osteoinductive and antibacterial activity, but at the same time requires precise control of the composition due to possible antagonisms and disturbances in the crystal lattice structure.

However, it should be emphasized that the effects of multi-ion substitutions are strongly dependent on the ratio of ions and their total concentration – some combinations may lead to crystal lattice disturbances or excessive solubility of the material. Therefore, research on such systems should be conducted based on standardized ISO 10993 protocols, including control of extraction conditions, temperature, and time, which allows for a reliable assessment of toxicity and biological effects.

6. Conclusions

Literature reports indicate that there is growing interest in doping hydroxyapatite with ions as an alternative path to bone formation and regeneration. The introduction of elements into apatite ceramics produces the desired effects. Hydroxyapatite modified with therapeutic ions is a promising group of materials for implant and regenerative applications. Analysis of available data indicates that the biological effect depends directly on the type of ion introduced, its concentration, and the form of the material in which it is used—whether as a coating, cement, or scaffold. The properties of apatites can be manipulated depending on the type of ions added, which opens up a wide range of possibilities.

Cu^{2+} ions exhibit antibacterial and proangiogenic effects, with their beneficial effects observed in a narrow range of concentrations below cytotoxicity thresholds.

Co^{2+} activates the cellular response to hypoxia by stabilizing the HIF-1α factor, which leads to the induction of angiogenesis, but its effect is highly dose-dependent and requires precise control.

Zn^{2+} plays an important role in osteogenesis processes, and at the right level of doping, it gives the material anti-inflammatory and antibacterial properties.

Sr^{2+}, in turn, stimulates osteoblast proliferation and reduces bone resorption, improving osteoconductivity and implant-tissue integration.

Mg^{2+} promotes mineralization and has a beneficial effect on microstructure, increasing the bioactivity and mechanical properties of the material.

In the case of multi-ion systems, synergistic effects are observed, such as a simultaneous increase in osteoinductive and antibacterial activity (e.g., Sr^{2+} + Ag^{+}), but the effects obtained are strongly dependent on the ratio and total concentration of ions. Excessive content can lead to disturbances in the crystal lattice or an increase in HAp solubility.

The key to a valuable and detailed characterisation of a given modification

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of hydroxyapatite with ions is in vitro and in vivo analysis. Both *in vitro* and *in vivo* test results confirm that appropriately selected ion substitutions can significantly improve the biological properties of hydroxyapatite. At the same time, the interpretation of the effects requires consideration of factors such as porosity, crystallite size, and heat treatment, which influence the kinetics of ion release.This is important in order to select the appropriate proportions and degree of substitution, which will allow the appropriate biological properties to be found in human stem cells. Controlled ionic modification of calcium phosphate ceramics enables the design of "smart" materials that combine bioactivity, osteoconductivity, angiogenesis, and antibacterial activity.

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