Al-Azhar Journal of Dentistry

Manuscript 1653

Restorative Dentistry Issue (Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

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REVIEW

Bioactivity and Antibacterial Effect of Zinc Doped Hydroxyapatite as Bone Scaffold

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Abstract

Bone defects remain a challenge for orthopedic treatment. At present, there are various treatment methods by drugs and by using many bone grafts. Bone graft and bone transport have emerged as the primary clinical strategy for treating the disorder of the bones. New concepts and approaches for treating bone abnormalities have emerged recently as a result of a quick development of scaffold materials and bone tissue engineering. Because of its high biocompatibility and resemblance to the natural apatite found in human bone, hydroxyapatite (HA) has been the most extensively utilized substance in the biomedical area, but HA still has several shortcomings regarding to its mechanical qualities and bacterial contamination due to the adhesion of bacteria to the scaffold surface. These HA problems are considered as impediment of traumatology and orthopedics that inhibit both antimicrobial action and bone repair. By adding trace elements to the HA material; this changing the material's characteristics, and eventually maintaining bone growth without scaffold failure, these shortcomings can be mitigated.

Keywords: Bone scaffold, Hydroxyapatite, Zinc containing hydroxyapatite

1. Introduction

In addition to being a dynamic, self-repairing organ, bone is a connective tissue mostly made of minerals. Large bone abnormalities, however, cannot be effectively mended if they are brought on by trauma, cancer, congenital conditions, or infection. Therefore, the treatment of these abnormalities requires both surgical intervention and bone substitutes. Allografts contain growth factors and bone morphogenic protein stimulate mesenchymal stem cells differentiation, that encourage the production of bone progenitor cells. Allogenic transplants, however, could not be accepted [1,2].

Bone repair is a complicated process that involves regeneration and self-healing to return the bone to its original mechanical and structural characteristics. Trace elements, like calcium and magnesium ions, are crucial in this process because they control the growth and differentiation of different cells, including mesenchymal stem cells, osteoblasts, and osteoclasts, during the bone repair stage. They also promote the release of growth factors and angiogenesis. A multitude of components must work in concert, with the use of biomaterials and trace elements having significant clinical significance [3].

Hydroxyapatite (HA) is a bio-ceramic items that are family-owned by calcium phosphate ceramic. HA has many advantages such as biocompatibility, bioactivity, osteoconductive and ability to create a solid connection with the osseous tissues. This correlation makes HA is an important research subject for bone and dental anomalies [2]. These important characteristics are due to its chemical similarity to human bone. However, HA has low fracture toughness, poor mechanical strength, inadequacies in structural integrity, and high solubility. Many attempts were made to improve the structural integrity of HA by manufacturing the material with different fabrication techniques, as well as the integration of alternative elements using doping or substitution [4,5].

Zinc is required for activity of more than three hundred enzymes involved in bone metabolism. Its mode of action is similar to that of copper ions, and it likewise demonstrates antibacterial activity. Zinc ions were first added to the HA molecule because of its antimicrobial characteristics. Additionally, the

Received 28 August 2024; accepted 14 October 2024.
Available online 8 February 2025

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https://doi.org/10.58675/2974-4164.1653

proliferative characteristics of osteoblasts are directly impacted by zinc ions, and osteoclasts are inhibited from resorbing bone [6].

The primary objectives of this review were to outline the characteristics and synthesis techniques of HA as it pertains to bone scaffolds. Additionally, it illustrates the significance of functionalization through zinc doping of HA.

2. Patients and methods

2.1. Review design

Studies included in the current review were selected from papers and reviews published from 2020 to 2024 were assessed with the primary objective of evaluating the properties of HA alone or combined with zinc ion. Only papers and reviews published in the English language were selected.

2.2. Criteria for inclusion and exclusion

(i) The criteria for inclusion include
   (a) Literature about materials for HA scaffolds.
   (b) The most recent research or articles from reputable journals in the same subject were chosen.

(ii) Exclusion criteria:
   (a) Exclusion standards: more than 5 years old and repeated studies
   (b) Unavailable full texts, abstract-only papers, and duplicated papers were not eligible.

2.3. Data sources and search strategies

Three databases were used for searching the articles (Medline, Scopus, and Science Direct). The databases were chosen due to their comprehensive coverage and easy accessibility. The online databases were performed using the following search Key words were: HA, Bone scaffold, zinc containing HA.

2.4. General aspect of hydroxyapatite bone scaffold

2.4.1. Physical and mechanical properties

Hydroxyapatite $Ca_{10}(PO4)_6(OH)_2$ is a naturally occurring calcium phosphate ceramic group which is present in human bone. It represents between 60 and 65 % of bone composition. This material has been placed within the bone tissues engineering because of its Ca/P ratio, which is close to that of human bone (1.67). Furthermore, its strong osteoconductive, biocompatibility, and bioactivity are also well-known. However, due to the lack of particular trace ions that are typically present in the osseous tissue's chemical structure and support bone formation, manufactured HA has poorer bone regeneration [7,8].

To successfully apply HA in bone tissue engineering (BTE), it must, first and foremost, give the growing tissues with appropriate mechanical support while maintaining adequate mechanical strength until full regeneration and new tissue development. Furthermore, many factors affect the mechanical properties of sintered HA such as pores shape, pores connection, grain size, and the material density, by adjusting the sintering temperature, all of these properties can be altered.

In this regard, the porosity and elastic deformation as determined by young's modulus values, were enhanced while the compressive strength and density of HA were enhanced by raising the sintering temperature decreased [5]. Furthermore, the use of HA sintered at high temperatures is restricted, particularly because of its extreme brittleness. The resultant HA is highly prone to minute defect formation, gradual crack propagation, and ultimately, ceramic failure. Moreover, the production of big grains was seen at sintering temperatures greater than 900 °C, high crystallinity higher biocompatibility, reduced porosity and low specific surface area [9].

Crystallization represents an additional asset that has an excellent effect about the application of HA. Higher abrasion resistance and a higher molecular weight of HA are reinforced by enhanced crystallization. As a result, as the bone's nanoscale size increases, its tensile strength increases. The size of the crystallites is also influenced by how closely the scaffold resembles the natural osseous tissues, which increase the abrasion resistance and resist the load during scaffold implantation [10].

Ultimately, poor hardness, brittleness, unfavorable tribological reaction, and fracture toughness. All of the most recent research findings indicated that the qualities of synthetic HA and osseous tissue varied significantly, despite their comparable chemical characteristics. Despite that, the most advantageous aspect of HA is its capacity to accept both cationic and anionic substituents, enhancing their characteristics for a variety of uses. With this subject, it is necessary to alter the characteristics of HA in a way that will enhance cell proliferation and avoid failure [11,12].

2.4.2. Hydroxyapatite sources

In addition to human teeth and bone (allograft), the source of HA is obtained from animal bone or other sources (xenograft) in inorganic and organic forms. Inorganic sources such as rocks containing

phosphate. While organic sources are obtained from chicken egg shells, fish bone, cow bone and clam shells. HA may be also made with the synthetic substances used within the sol–gel technique. However, based on the results of many research it has been conducted that, HA made from natural materials is better in terms of crystal size, biocompatible and nontoxic to cells, besides that it is also more economical in terms of raw materials [13].

2.5. Hydroxyapatite synthesis methods

Synthetic HA can be obtained through two principal methods: top-down and bottom-up approaches. The top-down technique is established by successive cutting or slicing the bulk of the materials to obtain nanoparticles. However, As an illustration in the bottom-up approach, consider the building of the material atom by atom, or molecule by molecule, or cluster by cluster, starting from the bottom. Most published study findings rely on the bottom-up approach to derive HA [14].

The preparation of HA powders via solid-state reactions, hydrothermal techniques, sol–gel procedures, microemulsion, emulsion, and chemical precipitation have accounted for the majority of the researches, to increase the usefulness and effectiveness of each of these synthesis techniques; Countless researchers have studied them in great detail over a long period. These methods have been chosen because they are easy to use and reasonably priced. However, the resultant material may be deficient in several necessary ions, such as Na, Sr, Mg, Si, K, and Fe [15].

The best way to synthesis HA is using the usual solid-state method since the final nanoparticles retain the shape of the phosphate precursor particles. As an alternative, the chemical precipitation approach produces porous nanoparticles by reacting precursor materials with an aqueous solution at low reaction temperatures. Because it's easy to use and inexpensive, the precipitation approach is another recommended way for creating very pure nanosized HA. Precursor selection, synthesis method, and processing parameters (reactant addition rate during reaction, aging duration, and reaction temperature) all affect the characteristics of the resultant HA [16].

Another technique for HA synthesis is 'co-precipitation' that received in a lot of research. This process of synthesis produces low-crystalline particles with a variety of morphologies [17]. Wet chemical precipitation is also regarded as one of the most widely used techniques because of its affordability, ease of use, and ability to produce large amounts of pure product at a low reaction temperature. This method is reliant on the growth-agglomeration–nucleation–aggregation pathway. The process starts with the creation of acidic (P source) and alkaline (Ca source) solutions, followed by their reaction. The phases of precipitation, filtration, drying, and, in the end, heat treatment come next [18].

It has been demonstrated that the materials produced using this technique have improved surface absorption, mechanical qualities, and bone regeneration. However, faster, more effective synthesis techniques with less time for preparation are needed [19]. Yet, because harsh chemicals and hazardous solvents are used in typical synthesis processes, there is a risk of toxicity and environmental harm, particularly water and air pollution [19].

In addition, scientists have shown that HA that is produced normally lacks significant biological qualities. In light of this, eco-friendly synthesis techniques for HA have been effectively created using natural resources (eggshells, fish bones, seashells, and cow bones) since they are nontoxic, readily available, and less expensive. To improve their quality and expand their application areas, additional research has been done recently on the extraction and purification of HA. The ability to preserve some advantageous trace elements from the synthetic material's natural source is another benefit of this approach. Moreover, improvements can be made to mechanical and thermal processes [20,21].

2.5.1. Zn^{2+}-doped hydroxyapatite

There are three locations in the crystal structure where HA can be doped. These include doping at the OH-site, at the location of PO_4^{3-} ions, or at Ca^{2+} ions. It has been observed that doping these sites with different ions increases the applicability of HA in the biomedical area and in BTE. Additionally, it has been demonstrated to enhance bone density development, encourage osteocytes proliferation, and prevent the formation of bone tumors [22].

In addition, the total outcome of doping ions is altered based on the synthesis methods, conditions, and kind of doping applied to the materials to achieve the required attributes for the intended usage. The solubility, crystal morphology, lattice parameters, crystallinity, grain size, concentration, and kind of ionic doping agent, all affect the microstructure, thermal stability, and solubility of HA [23].

Different ions have demonstrated their impact and efficacy in BTE and are utilized as doping agents for HA [24]. Furthermore, lattice characteristics gradually drop as doping concentration

increases in HA structures due to the substitution of doping ions. Moreover, doping agents decrease crystallinity and restrict the development of HA particles [25].

Doping ions a trace element associated with bone metabolism that is present in osseous tissues. Zinc (Zn^{2+}) also possesses antioxidant properties and is essential for several metabolic processes, such as the metabolism of carbohydrates, the digestion of proteins, and blood coagulation. It also has a substantial impact on the immune system. Zinc has been linked to increased bone formation via supporting osteoblast cell development and differentiation, according to a number of studies. These properties imply that using it with HA might boost BTE bioactivity and release of ions that are bioactive and can start biological processes [26].

Because zinc is found in both bone and tooth enamel and affects the metabolic processes involved in bone formation, substitution of zinc in HA structures has attracted a lot of attention. Zn ions were added, and this enhanced osteoblast production, biomineralization, and bone formation. However, bone density was decreased due to the loss of zinc in the osseous tissues [27]. According to recent findings; the amount of doping material present determines the antibacterial activity of zinc in ceramic materials. Other authors observed that resistance against *Staphylococcus aureus* and *K. pneumoniae* can be attained with a low concentration of Zn in the HA structure [28].

As the chemical co-precipitation approach was used to create Zn-doped HA scaffold, successful demonstrations of their bioactivity and antibacterial activity against *S. aureus* and *Escherichia coli* were made [29]. Additionally, several researchers employed the produced zinc-doped HA biomaterials as antibacterial agents against both gram-negative (*E. coli*) and gram-positive (*S. aureus*) pathogens. The Zn 2 % HAp sample revealed that the highest concentrations of bacterial inhibitory agents for Gram-positive and Gram-negative bacteria were 50 ± 5 % and 77 ± 5 %, respectively. Zn^{2+} concentration was a major determinant of the HAp samples' antibacterial activity [30,31].

Zinc content has shown advantageous for osteoclast formation in another recent review. Because the coating's has greater Zn content, which has antibacterial properties, and the bacterial adhesion was prevented or reduced. Conversely, a zinc deficit may result in osteoporosis and protracted bone repair [32]. Additionally, it was demonstrated that the Zn-doped HA coating increased osteogenic differentiation and bacterial inhibition in addition to producing increased corrosion resistance on the implant's surface. In contrast to pure HA coatings, the doped coating effectively prevented bacteria while simultaneously enhancing bone marrow mesenchymal stem cell cellular adhesion and differentiation [33].

Moreover, other writers synthesized HA nanoparticles from cortical bone of cows and mechanically increased their property by alloying them with zinc. The outcomes showed that whereas HA nanoparticles greatly increased cell proliferation and alkaline phosphatase activity, they had no detrimental effects on stem cell behavior. Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were the targets of the antibacterial test, which demonstrated the antibacterial activity of HA nanoparticles [34]. Zinc-doped HA nanoparticles (ZnHA NPs) with varied zinc contents (0–20 per mol) were created in another investigation. Size, shape, content, and antibacterial activity of ZnHA NPs were evaluated, with 15 mol% ZnHA NPs demonstrating the ideal ratio of antibacterial activity to cytocompatibility [35].

2.6. Kinetic analysis of zinc ion release

All of the Zn-HAp/cell scaffolds showed the ability to release zinc ions from solution in phosphate buffered solution (PBS) at 37 °C based on their release profiles. During the first three days of immersion, the Zn-HAp/cell released Zn ions quickly. Nevertheless, as the reaction achieved equilibrium over the course of the reaction period, the amount of Zn ions released from the cell load scaffold's surface decreased, and eventually, after five days of immersion, no discernible release was found [36]. The highest concentrations of zinc ions that were released by 5 %, 10 %, 15 %, and 20 % zinc-HAp were 1.434, 2.012, 2.331, and 2.780 mg/g, respectively. Furthermore, it was observed that the quantity of Zn ions released rose in proportion to the Zn ions contained in the Zn-HA/cell load scaffolds. The HA nanocomposite's rate of dissolution in the buffer solution at a pH of 7.4 is dependent on how crystallinity changes; a decrease in crystallinity causes the rate of dissolution to increase [37]. According to the study's findings, the more Zn ions that are present on the HA, the faster the Zn-doped HA composite released, with values ranging from 20 to 15 %–10 % to 5 %. Higher rate of dissolution in phosphate buffer solution corresponds with a less crystalline structure, as revealed by the XRD examination. However, the least Zn-doped HA exhibited a slower rate of release because of their increased crystallinity, which allowed them to dissolve more slowly in phosphate buffer solution [38].

Research has been conducted on the effectiveness of zinc-doped HAs as vehicles for the regulated release of ciprofloxacin, a drug with antibacterial properties against *S. aureus* and *Pseudomonas aeruginosa*, the two most common pathogens responsible for diseases of the bones and joints. They have demonstrated that the HA releases ciprofloxacin in tandem with the release of zinc ions, and that the antibacterial activity increases with increases in drug concentration and the quantity of Zn^{2+} ions employed [39,40].

Moreover, ciprofloxacin is released in higher amounts from hydroxyapatite doped with zinc ions than from 'pure' HA. When such a solution is used to treat bone tissue infections, it can shorten the course of treatment, enhancing its effectiveness, and lower the dosage of ciprofloxacin, which helps prevent the development of resistant strains. The cytotoxicity of zinc, which becomes apparent when the level surpasses 1.2 %, may restrict the usage of HAs doped with zinc ions, much as it does in the case of copper ions. Zinc's antibacterial effect works through the same method, which involves destroying cell membranes, inhibiting DNA replication, metabolic enzymes, and inhibiting metabolic enzymes. It is also as effective in terms of intervention with microbes [41,42].

2.6.1. Application of hydroxyapatite doped with ion in dentistry

The development of ion-doped HA for the treatment of various dental applications has been the focus of numerous investigations. Numerous writers have concentrated on the application of HA in this area since it is the primary tooth component that demonstrates a demineralizing impact on tooth enamel and can strengthen the link to the structure. Doped HA was the best option for addressing some abnormalities that can arise throughout the course of treatment because HA still has poor mechanical strength and bioactivity. Doping therefore helps the materials with improved antibacterial qualities to promote the formation of new bone and reduce inflammation, which is crucial for dental applications [34,43].

Orthodontic materials with doped ceramics like Zn^{2+} ions showed stronger antibacterial and remineralization effects than non-doped materials.

These doped materials also represent novel biomaterials for bone cavity restoration, serving as a cell matrix to promote the regeneration of injured tissue [43]. Furthermore, HA that had been doped with zinc was used to coat dental implants, providing better adhesion, ductility, and density and homogeneity. Furthermore, an elevation in the doping ion concentration enhanced biocompatibility by augmenting the osteoconductive process, cell adhesion, and proliferation [44].

2.7. Conclusion

The creation of an ideal material with exceptional biocompatibility, mechanical strength, and bioactivity has garnered a lot of attention due to HA production and its use in biomedical engineering. While pure HA has lower antibacterial activity and insufficient mechanical strength to be used in load-bearing application. Researchers have concentrated on developing ways to get around these drawbacks. Therefore, an effective method for creating HAp-based biomaterials with promising antibacterial properties is to use HA scaffold in conjunction with Zn^{2+} ions.

The best way to add trace elements to HA structures and change the material's characteristics without sacrificing the doping ions, so that increasing the biological performance through ion doping or substitution. Zinc is essential for bone remodeling and regeneration as well as for shielding osteoblasts from stress-induced apoptosis. The crystallinity and lattice properties of HA diminish with increasing zinc content, because zinc doped HA exhibits antibacterial activity and pro-regenerative qualities; it is advised that it be utilized as scaffold biomaterials with favorable prognosis and as a replacement for missing bone. Given that a high concentration may interfere with bioactivity or possibly cause toxicity.

2.8. Recommendations

Further research is still needed to determine the ideal concentration and kind of doping material. Furthermore, more research is still required to correlate the physicochemical characteristics, bioactivity, and production methods of HA for usage in BTE. As well as, because the majority of studies have mainly concentrated on the advancement of the synthesis techniques, the influence of the doping agents has not been thoroughly proven. This provides the opportunity to think about conducting additional research in the future to examine the synergy between different doping materials by functionalizing the generated HA with multiple dopants.

Ethics information

None.

Funding

This research didn't receive any grant from funding agencies in the public, commercial, or not

from profit sector. And This investigation was not funded by any agency.

Biographical information

None.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

I would like to thank Dr. Shahenda Ahmed, Lecturer of dental biomaterials, Al-Azhar university for her support and encouragement and Dr. Mohsena Ahmad for her brilliance in this work.

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