Effect of zinc content on physicochemical characteristics and biological activity of synthesized Zn-doped hydroxyapatite

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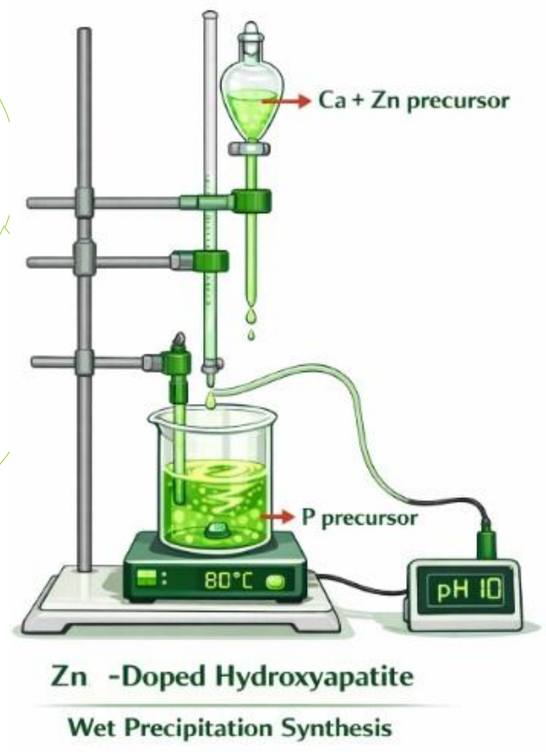
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Introduction

- Hydroxyapatite (HAp) is a widely used bioceramic material in bone regeneration due to its **excellent biocompatibility, osteoconductivity, and chemical similarity to natural bone mineral**.
- However, **pure HAp exhibits limited osteoinductivity, low mechanical strength, and insufficient antibacterial activity**, which restricts its effectiveness in load-bearing and infection-prone applications.
- Incorporation of **dopant ions** such as Zn^{2+} into the HAp lattice has been shown to **enhance biological functionality** by influencing cellular responses and material properties.
- Zinc is an essential trace element in bone metabolism and plays a positive role in:
  - ✔ promoting osteoblast proliferation and differentiation, enhancing osteogenic activity;
  - ✔ inhibiting osteoclast resorption, contributing to improved bone formation;
  - ✔ exhibiting antibacterial effects.

Experimental



- Structural, morphological, chemical, and thermal properties were characterized by XRD, SEM, ICP–OES, FT-IR, and TGA.
- In vitro biological performance was evaluated using MG-63 cells and representative Gram-positive and Gram-negative bacterial strains.
- Cell metabolic activity was quantified via resazurin-based fluorescence assay.



Results

XRD:

- ✔ All samples exhibit hexagonal hydroxyapatite as the primary phase;
- ✔ Crystallite size decreases with Zn substitutions, from 27 nm (HAp) to 24.8 nm (2% Zn-HAp) and 15.1 nm (10% Zn-HAp);
- ✔ The values of lattice parameters “a” and “c” decreased when the Zn concentration in the samples increased;
- ✔ No secondary phases detected.

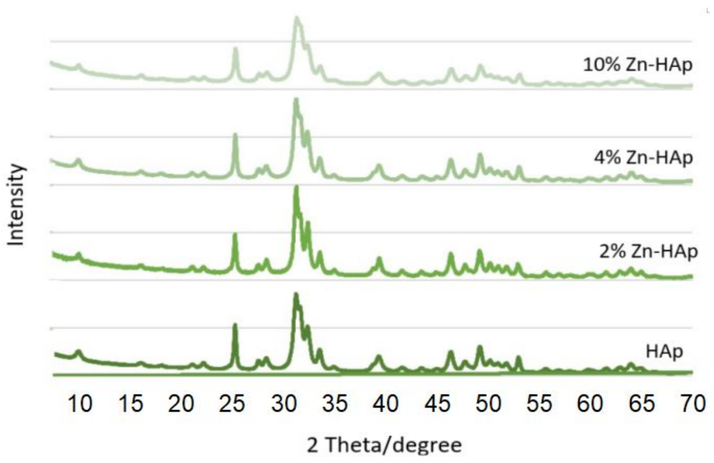


Figure 1. XRD patterns of Zn-doped hydroxyapatite

ICP-OES:

- ✓ Confirms zinc incorporation (2–7%) with (Ca+Zn)/P ratios close to stoichiometric HAp, indicating structural stability.

Table 1. Chemical composition: content of elements and the molar ratio.

Sample	Ca, %	P, %	Zn, %	(Ca+Zn)/P ratio
2% Zn-HA	43.14	23.17	1.8	1.48
4% Zn-HA	38.49	19.92	1.97	1.54
6% Zn-HA	43.36	24.51	5.67	1.48
10% Zn-HA	40.41	20.59	5.13	1.63

TGA:

- ✓ Zn-doped hydroxyapatite undergoes three-stage weight loss, with the main decomposition occurring below 300 °C and good thermal stability thereafter, indicating that zinc incorporation does not significantly compromise high-temperature stability.



Figure 6. Thermogravimetric curve of Zn-doped hydroxyapatite

SEM:

- ✔ HAp nanoparticles present a spherical-like shape, and zinc incorporation does not alter their morphology.

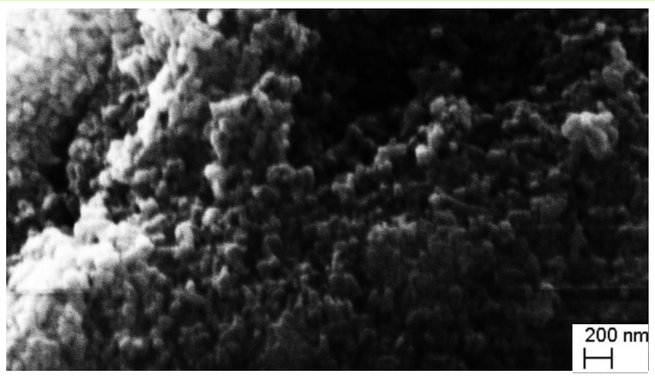


Figure 2. Scanning electron microscopic image of Zn-doped hydroxyapatite

FT-IR:

- ✔ Exhibit the characteristic bands of the CO_3^{2-}, PO_4^{3-}, and OH^- ions present in the apatite crystal lattice.
- ✔ Sintering reduces CO_3^{2-} and OH^- signals and sharpens peaks, indicating enhanced crystallinity and phase purity.

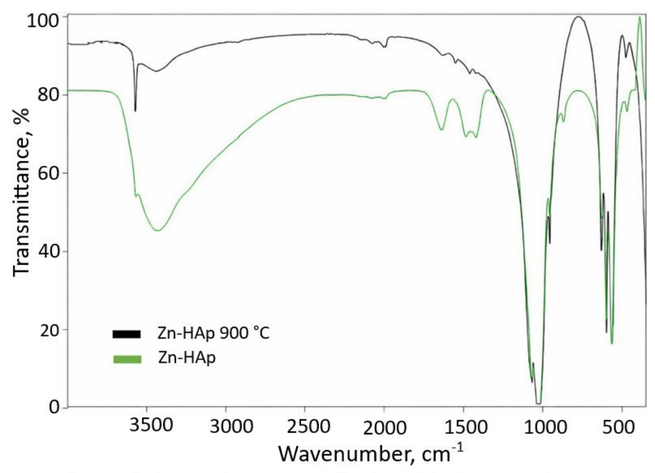


Figure 5. Infrared spectra of Zn-doped hydroxyapatite

Antibacterial effect:

- ✓ Zn-doped HAp did not exhibit strong antibacterial activity. A slight reduction in bacterial metabolic activity was observed at 24 hours; however, by 168 hours, inhibitory effects remained only in samples with 10% Zn.

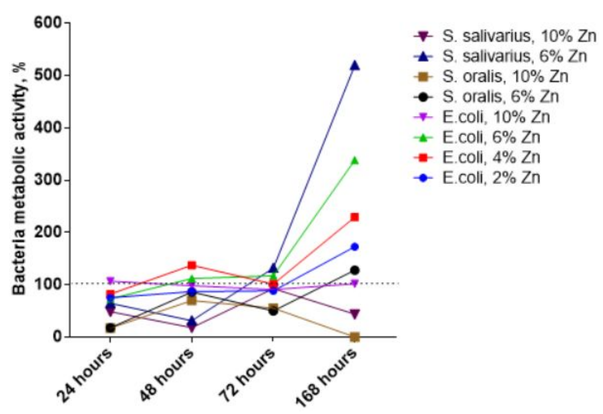


Figure 7. Antimicrobic activity of Zn-doped HAp

BET:

- ✔ Zn-doped HAp exhibits improved porosity and a higher surface area compared with the commercial sample.

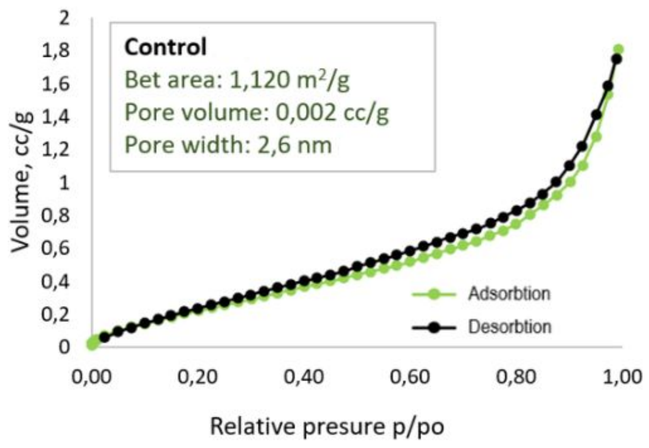


Figure 3. N_2 adsorption–desorption isotherm of commercial control

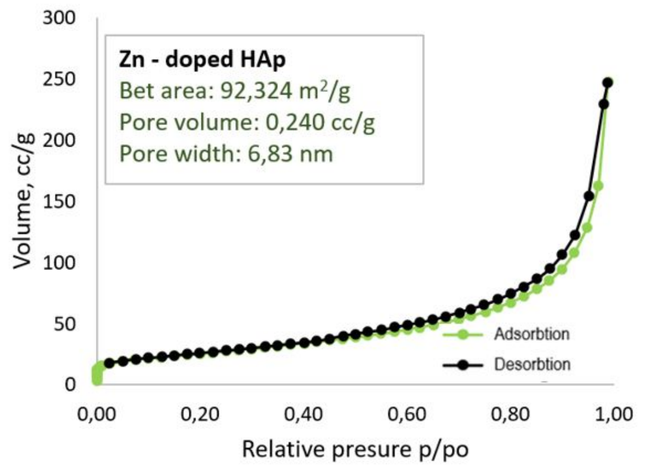


Figure 4. N_2 adsorption–desorption isotherm of Zn-doped hydroxyapatite

Cytotoxicity:

- ✓ Zn-doped HAp demonstrates excellent biocompatibility, with increasing zinc concentrations enhancing cell metabolic activity over time, and no cytotoxic effects observed.

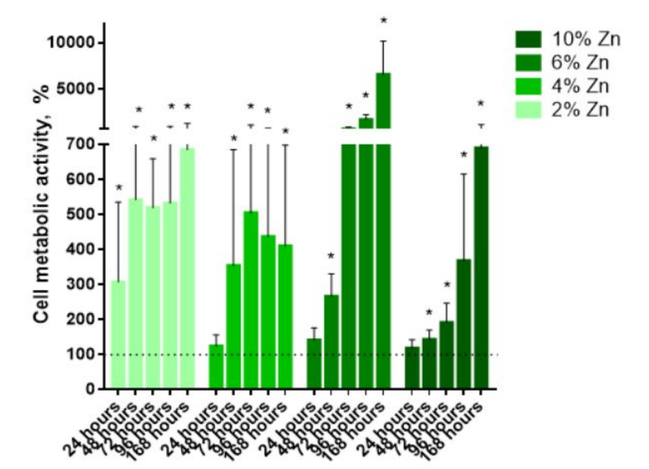


Figure 8. Cytotoxic activity of Zn-doped HAp. *p<0.05

Conclusion

Zinc-doped hydroxyapatite was successfully synthesized with confirmed zinc incorporation, resulting in improved properties compared to commercial materials. Ongoing *in vitro* studies and extended analyses are being conducted to further confirm its potential in bone regeneration.

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