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Cleibson Oliveira de Lima, André Marcorin de Oliveira, Edson Silva Filho, Laís Chantelle, Maguy Jaber, Maria Gardênia Fonseca

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1 Zn-doped mesoporous hydroxyapatites and their antimicrobial  
2 properties

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4 Cleibson Oliveira de Lima,<sup>a</sup> André L. Menezes de Oliveira,<sup>a</sup> Laís Chantelle,<sup>a</sup> Edson C.  
5 Silva Filho,<sup>b</sup> Maguy Jaber,<sup>c</sup> Maria Gardênia Fonseca<sup>a,\*</sup>

6  
7 <sup>a</sup>*Universidade Federal da Paraíba, Núcleo de Pesquisa e Extensão LACOM, 58051-*  
8 *085, João Pessoa, Paraíba, Brazil.*

9 <sup>b</sup>*Interdisciplinary Laboratory for Advanced Materials-LIMAV, Universidade Federal*  
10 *do Piauí, Teresina, 64049- 11 550, Piauí, Brazil.*

11 <sup>c</sup>*Sorbonne Université, Laboratoire d'Archéologie Moléculaire et Structurale, Institut*  
12 *Universitaire de France (IUF), CNRS UMR 8220, UPMC – Tour 23, 3ème étage,*  
13 *couloir 23-33, BP 225, 4 place Jussieu, 75005 Paris, France.*

14 [\\*mgardennia@quimica.ufpb.br](mailto:mgardennia@quimica.ufpb.br)

15 Phone/Fax +55833216-7433

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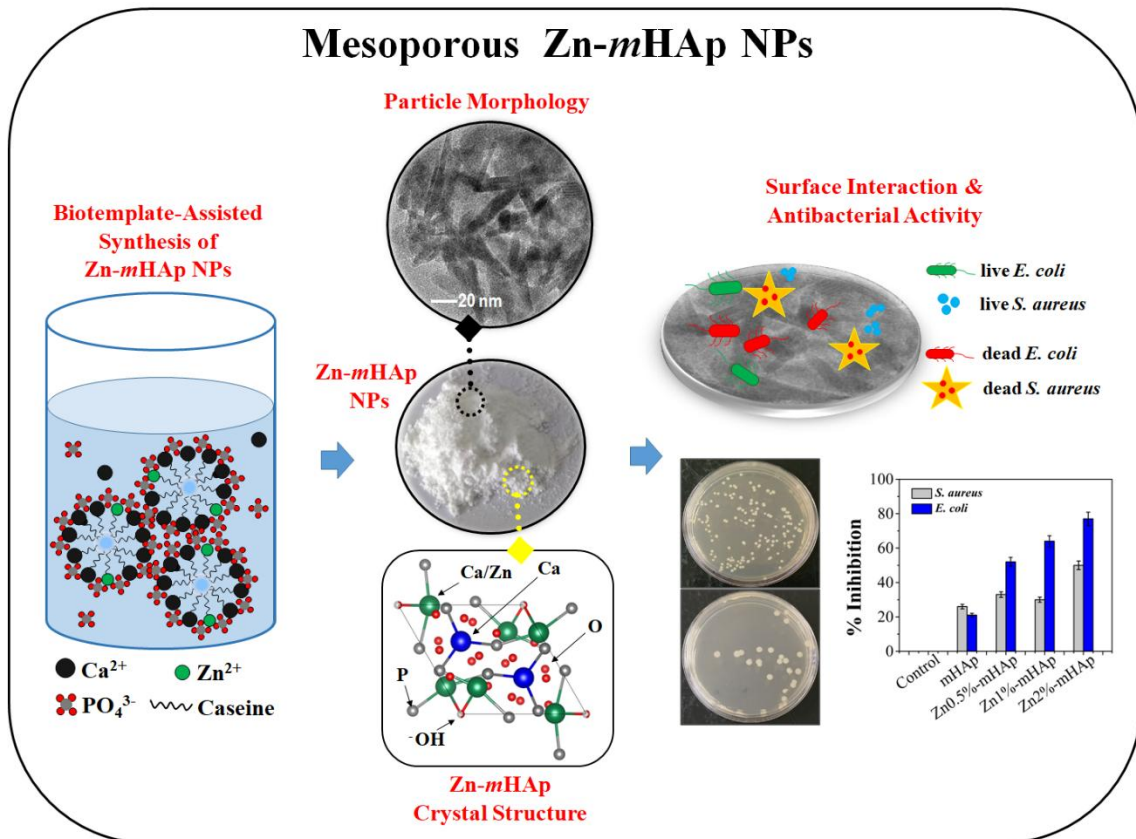
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1 **Graphical abstract**



2

3

4 **Highlights**

5

6 Pure and Zn-doped mesoporous hydroxyapatites were synthesized via casein  
7 biotemplating

8 Formation of mesoporous biomaterials is strongly dependent on biotemplating and Zn  
9 doping

10 Zn-rich mesoporous hydroxyapatites showed a high surface area of  $182 \text{ m}^2 \text{ g}^{-1}$

11 Antimicrobial activity of the hydroxyapatites depended on their  $\text{Zn}^{2+}$  content and  
12 mesoporous surface

13 Zn-hydroxyapatite showed the strongest inhibitory effect against Gram-positive bacteria  
14 stain.

15

1 **Abstract**

2 Recently, zinc-based materials have gained immense attention as antimicrobial agents.  
3 In this study, zinc-doped mesoporous hydroxyapatites (HAp)s with various Zn contents  
4 were prepared by co-precipitation using a phosphoprotein as the porous template. The  
5 use of the phosphoprotein as the porous template resulted in the formation of zinc-  
6 doped mesoporous HAp)s (mHAp)s with large pores and specific surface area ( $182 \text{ m}^2 \text{ g}^{-1}$ ),  
7 as indicated by the nitrogen adsorption/desorption measurements. The formation of  
8 the zinc-doped HAp)s was confirmed by various analytical techniques such as X-ray  
9 diffraction, Fourier transform infrared spectroscopy, transmission electron microscopy,  
10 and X-ray photoelectron spectroscopy. The biomaterials prepared in this study were  
11 used as antimicrobial agents against gram-positive (*Staphylococcus aureus*) and gram-  
12 negative (*Escherichia coli*) bacteria. The Zn2%-mHAp sample showed the maximum  
13 bacterial inhibitory concentrations of  $50 \pm 5\%$  and  $77 \pm 5\%$  for the gram-positive and  
14 gram-negative bacteria, respectively. The antibacterial activity of the mHAp samples  
15 depended strongly on their  $\text{Zn}^{2+}$  content. Thus, the use of a biotemplate and  $\text{Zn}^{2+}$  ions is  
16 an efficient approach for the formation of novel HAp-based biomaterials with promising  
17 antibacterial properties. This synthesis approach will pave a new pathway for the  
18 functionalization of other materials for different biomedical applications.

19

20

21 **Keywords:** Hydroxyapatite; biomaterial; zinc; template; antibacterial.

22

## 1. Introduction

The emergence of bacterial strains resistant to the multiple antibiotics used worldwide is a huge health challenge, as bacteria are a major cause of chronic infections and mortality. Therefore, the development of novel efficient antimicrobial agents is imperative [1]. Both inorganic and organic materials and their combinations have been used as antimicrobial agents against gram-positive and gram-negative bacteria. Among the various antimicrobial inorganic materials investigated till date, oxides such as titanium oxide [2,3], silver oxide [4], and zinc oxide [5–7] and phosphates such as pure hydroxyapatite (HAp) [8], HAp doped with different cations including zinc [9], silver [10–12], cerium [13,14], strontium [15], copper [16], and titanium [17] have been extensively investigated.

HAp ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is an inorganic biomaterial with great potential for biomedical applications such as in dentistry because of its biocompatibility, bioactivity, and osteoconductivity [18]. The biological and physicochemical properties of synthetic HAp can be improved through various chemical modification processes such as cationic and anionic doping [19,20]. The dopants incorporated into the HAp network can affect its crystallinity, particle morphology, surface charges, dissolution/reabsorption rates, densification, mechanical resistance, and thermal stability [21].  $\text{Zn}^{2+}$  is one of the most promising dopants used to modify the properties of HAp. This is because  $\text{Zn}^{2+}$  is present in bones; acts as a cofactor for enzymes; and contributes to the metabolism of proteins, carbohydrates, and lipids [19,22–24].

Hence, various studies have been carried out to prepare Zn-doped HAp for application as antimicrobial agents [24–26] such as in bone grafting, coatings on metallic implants because of their high biocompatibility and bioactivity, in bone

1 mineralization [27–29], and for improving the adhesion between osteoblasts. The  
2 properties of HAp for orthopedic and dental applications have also been investigated  
3 [30,31]. For example, Mg<sup>2+</sup>-, Zn<sup>2+</sup>-, Sr<sup>2+</sup>-, and Si<sup>4+</sup>-doped HAp/chitosan composites are  
4 obtained by co-precipitation and are used for biomedical applications [21]. It has been  
5 reported that Zn<sup>2+</sup>-doped HAp/chitosan promotes *in-vitro* cell proliferation and is a  
6 promising material for bone implants. The Zn<sup>2+</sup>-doped apatite coating on titanium rods  
7 used as bone implants induces fibroblastic proliferation, *in-vitro* osteoblastic  
8 proliferation, and differentiation around the implant and reduces the infections in bone  
9 fixation pin linings [29].

10 In a previous study, Tank et al. [32] synthesized nano Zn<sup>2+</sup>-doped HAps with  
11 approximately 1, 3, and 5 mol% Zn<sup>2+</sup> by co-precipitation mediated by a nonionic  
12 surfactant (Triton X-100) and were tested against *Staphylococcus aureus* (*S. aureus*),  
13 *Micrococcus luteus*, *Bacillus cereus*, *Shigella flexneri*, and *Pseudomonas aeruginosa*  
14 [32]. The authors evidenced that Zn<sup>2+</sup>-doped HAps showed good antimicrobial activity  
15 against gram-positive bacteria, but no meaningful efficiency was observed against  
16 gram-negative bacteria. In the research conducted by Ofudje et al. [9], Zn<sup>2+</sup>-doped  
17 HAps with 5, 10, 15, and 20 mol% Zn<sup>2+</sup> were prepared via co-precipitation also exhibit  
18 excellent antibacterial activity against *Escherichia coli* (*E. coli*). The antibacterial  
19 activity of HAps increased with an increase in the Zn<sup>2+</sup> concentration [9]. More recently,  
20 Ullah et al. [33] reported the synthesis of Zn<sup>2+</sup>- and Sr<sup>2+</sup>-codoped HAps using a  
21 hydrothermal method. These HAps showed efficient antibacterial activity against *S.*  
22 *aureus* and *E. coli*. They also show increased proliferation, fixation, and cell adhesion  
23 compared to pure HAp [33].

24 There is no consensus on the type of antibacterial action mechanism prevalent in  
25 nanoparticulate materials, including HAp-based biomaterials. However, three principal

1 mechanisms have been reported for antimicrobial action in the presence of  $Zn^{2+}$ -  
2 containing nanoparticles. According to the first mechanism, the  $Zn^{2+}$  ions bind to the  
3 proteins in the bacteria and deactivate them. The second mechanism proposes that the  
4 interaction of  $Zn^{2+}$  ions with the bacterial membrane leads to structural changes in the  
5 biomaterial and an increase in its permeability, resulting in the death of the  
6 microorganisms. According to the third mechanism, the interaction between the  $Zn^{2+}$   
7 ions and microbial nucleic acids interrupts the replication of microorganisms [34,35].  
8 However, the other antimicrobial actions include damage to the plasma membrane and  
9 cell wall, inhibition of electron transport, blocking cell division, and production of  
10 reactive oxygen species (ROS) with the functional groups of proteins and nucleic acids  
11 such as mercapto ( $-SH$ ), amino ( $-NH$ ), and carboxyl ( $-COOH$ ), which can impair  
12 enzymatic activity, alter the cell structure, and affect the normal physiological  
13 processes, and thus inhibit the growth of the microorganisms, causing even cell death  
14 [36,37]. In addition, electrostatic, van der Waals, hydrophobic, and ligand-receptor  
15 interactions can occur between the bacterial cells and nanoparticles. Ligand-receptor  
16 interactions have been proposed as the dominant antimicrobial action mechanism in the  
17 presence of weak repulsive electrostatic interactions [25].

18 The synthesis of HApS doped with different ions has been extensively investigated.  
19 In addition, the use of a template can lead to the formation of mesoporous HApS with  
20 different surface areas, particle sizes, and pore volumes, and hence can expand the range  
21 of their applications [38–40]. Despite the number of reported studies concerning HAp  
22 materials, the synthesis of  $Zn^{2+}$ -doped mesoporous HAp using a biotemplate and their  
23 use as antimicrobial agents has not yet been reported. In relation to applications in  
24 biomedical field, mesoporous materials are important for drugs delivery systems [41].

1 In addition, mesoporous materials can be used as carriers of larger biological molecules [38]  
2 and scaffolds for bone tissue regeneration [41].

3 Motivated by the above-mentioned works and in order to develop novel HAp-based  
4 biomaterials with specific characteristics and properties, in this study, we synthesized  
5 for the first time, Zn<sup>2+</sup>-doped mesoporous HAp biomaterials via co-precipitation using a  
6 phosphoprotein (casein) as the template. The effect of the low amount of Zn<sup>2+</sup> content  
7 (0.5, 1, and 2 mol%) on the structural and textural features of the biomaterials was  
8 investigated. The antibacterial activity of the biomaterials for gram-positive (*S. aureus*)  
9 and gram-negative (*E. coli*) bacteria were investigated. Unlike previous studies  
10 concerning Zn-doped HAp, biomaterials with mesoporous characteristics with large  
11 surface area were obtained in the present work. It is also important to mention that the  
12 use of casein as a biotemplate for the synthesis of Zn<sup>2+</sup>-doped mesoporous HAp may  
13 still enhance the bioactivity and biocompatibility of the synthetic mHAp systems  
14 obtained in the present work. The findings of this study will pave a new pathway for the  
15 synthesis of novel HAp-based materials and other mesoporous biomaterials for different  
16 biological applications.

## 17 **2. Experimental**

### 18 **2.1. Chemicals**

19 Ammonium phosphate ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, Merck, 99%), calcium chloride  
20 (CaCl<sub>2</sub>·2H<sub>2</sub>O Sigma-Aldrich, 93%), zinc nitrate (Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, Vetec, 97%), casein  
21 from bovine milk (Reagen, 99%), and sodium hydroxide (NaOH, Vetec, 97%) were  
22 used as received without further purification. Used casein is a mixture of  
23 phosphoproteins that contains all of the common amino acids, and any purification or



1 isolation of specific type was performed prior to use. Deionized water was used in all  
2 the procedures.

3

## 4 **2.2. Synthesis of pure and doped mesoporous HAps**

5 Mesoporous HAp (mHAp) was prepared according to a previously reported  
6 method [38]. Initially, a 0.2 mol L<sup>-1</sup> NaOH solution was prepared and 1.25 g of casein  
7 was added to 250 mL of this solution. The resulting solution was mechanically stirred at  
8 1200 rpm for 1 h at 30 °C to form a white suspension. Then, the agitation speed was  
9 decreased to 200 rpm and 250 mL of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and CaCl<sub>2</sub>·2H<sub>2</sub>O were  
10 simultaneously added to the system at 2 mL min<sup>-1</sup>. The amounts of calcium and  
11 phosphate used in the synthesis procedure were 0.056 and 0.033 mol, respectively. The  
12 resulting white precipitate was aged for 24 h at 30 °C, and the solid part was recovered  
13 by filtration and was then washed with distilled water until a negative chloride test was  
14 achieved. Finally, the solid was dried at 100 °C in a furnace for 24 h followed by  
15 calcination at 500 °C for 12 h in an O<sub>2</sub> atmosphere at a heating rate of 2 °C min<sup>-1</sup> in  
16 order to eliminate the casein template. The obtained sample was labeled as mHAp.

17 The same procedure was used for the synthesis of the zinc-doped HAps by  
18 adding a zinc nitrate solution to the system simultaneously with the addition of the  
19 calcium and phosphate salts. The Zn<sup>2+</sup> doping amount was varied (0.25, 0.5, and 1.0  
20 mmol L<sup>-1</sup>). The zinc-doped samples were labeled as Zn<sub>x</sub>-mHAp (where  $x = 0.5, 1, \text{ and } 2$   
21 mol%).

## 22 **2.4. Characterization**

23 The powder X-ray diffraction (XRD) patterns of the samples were recorded on a  
24 Shimadzu diffractometer 6000 model equipped with CuK $\alpha$  monochromatic radiation ( $\lambda$

1 = 0.154 nm) and operating at 30 kV and 30 mA. The XRD patterns were recorded over  
2 the  $2\theta$  range of  $5\text{--}50^\circ$  with a step size of  $0.02^\circ$  at a scan rate of  $0.05^\circ\text{min}^{-1}$ .

3 The lattice parameters of the samples were determined using the (0 0 2), (1 0 2),  
4 (2 1 0), (2 1 1), (3 1 0), and (2 2 2) Bragg diffraction reflections of the hexagonal crystal  
5 structure of HAp (ICDD 00-009-0432). The crystallite size of the samples was  
6 calculated using the Scherrer equation as follows:

7

$$D = \frac{0.9 \times \lambda}{\beta \times \cos \theta} \quad (1)$$

8 where  $D$  is the average crystallite size,  $\lambda$  is the wavelength,  $\theta$  is the diffraction angle,  
9 and  $\beta$  is the line broadening at half the maximum intensity (full width at half maximum,  
10 FWHM) of the peak after subtracting the instrumental line broadening.

11 The Fourier transform infrared (FTIR) spectra of the samples were recorded on  
12 an IR Prestige-21 Shimadzu spectrophotometer in the transmittance mode using the KBr  
13 pellet method. For each spectrum, a set of 30 consecutive scans were collected over the  
14 wavenumber range of  $4000\text{--}400\text{ cm}^{-1}$  at a resolution of  $4\text{ cm}^{-1}$ .

15 The transmission electron microscopy (TEM) images of the samples were  
16 obtained using a FEI-Tecnaï G2 Spirit Biotwin microscope operating at 120 kV. The  
17 samples were suspended in isopropyl alcohol and were then deposited on 400 mesh  
18 copper grids covered with an ultrafine carbon layer with a thickness of 2–3 nm. The  
19 high-resolution TEM (HR-TEM) analysis of the samples was carried out using a JEOL  
20 100CX microscope operating at 200 kV. The TEM images were processed using ImageJ  
21 software (version 1.53a). To generate the particle size distribution histograms of the  
22 samples, approximately 250 particles were counted. For the HRTEM analysis, the fast  
23 Fourier transform (FFT) and inverse fast Fourier transform (IFFT) of the samples were

1 calculated in order to isolate the dots from the noise to recompense the microscopy  
2 images. Thus, a clear image was obtained, providing the crystallographic planes from  
3 which the corresponding  $d$  spacing was calculated.

4 The nitrogen adsorption/desorption measurements of the samples were carried  
5 out on an ASAP 2420 Micrometrics system. The specific surface areas of the samples  
6 were calculated using the Brunauer-Emmett-Teller method [42]. The pore diameters and  
7 volume distributions of the samples were measured using the Barret-Joyner-Halanda  
8 method [43]. The thermogravimetry (TG) curves of the samples were recorded on a  
9 Netzsch STA 449F3 instrument. For the TG analysis, 10 mg of the samples were  
10 transferred into alumina crucibles, which were then heated to 1000 °C in a nitrogen  
11 atmosphere at a flow rate of 50 mL min<sup>-1</sup> and a heating rate of 10 °C min<sup>-1</sup>.

12 The X-ray photoelectron spectroscopy (XPS) profiles of the samples were  
13 obtained using a VSW HA-100 spherical analyzer with an AlK $\alpha$  radiation ( $h\nu = 1486.6$   
14 eV). The high-resolution spectra of the samples were obtained at a constant analyzer  
15 pass energy of 44 eV. The surface charging was corrected for all the spectra, shifting  
16 them in relation to the C1s line at 284.6 eV, and the curve fitting was performed using a  
17 Gaussian line shape by subtracting the Shirley background.

18

### 19 **2.3. *In-vitro* antimicrobial activity**

20 The direct contact method was used to investigate the antibacterial activity of the  
21 biomaterials according to a previously reported procedure [44]. Mueller Hinton agar  
22 was used as the growth medium. The growth medium was hydrated using 36 g of  
23 medium per 1000 mL of distilled water. After the hydration process, the Mueller Hinton  
24 agar solution (pH 7.3  $\pm$  0.1) was heated until the agar dissolved completely. The

1 solution was then transferred to an autoclave and heated to 121 °C for 15 min. After  
2 plating, the culture medium was subjected to the sterility test in a microbiological oven  
3 for 24 h.

4 The test was carried out on a mixture of 2000  $\mu\text{L}$  of inoculum in  $10^{-4}$  CFU $\text{mL}^{-1}$   
5 of *S. aureus* (ATTC 25923) and *E. coli* (ATTC 25922) and approximately 2000  $\mu\text{g}$  of  
6 the biomaterials (mHAp and Zn $x$ -mHAp). An aliquot of 200  $\mu\text{L}$  was withdrawn and  
7 spread vertically, horizontally, and diagonally over the growth medium with a Drigalski  
8 loop on the Petri dish. The plate was kept in a microbiological oven for 24 h. Then, the  
9 colony-forming units (CFUs) were counted. The tests were performed in triplicate for  
10 each of the biomaterials. Positive bacterial growth control (0.85% saline) was prepared  
11 to compare and verify the viability of the strains.

12

### 13 3. Results and discussion

#### 14 3.1. Textural properties

15 The textural properties of the samples were investigated by carrying out their  $\text{N}_2$   
16 adsorption/desorption measurements (**Figure SM1, Table 1**). All the samples prepared  
17 using casein showed type-IV isotherms with type-H1 hysteresis at  $P/P_0 = 0.75$  and 1.0,  
18 which are characteristic of typical mesoporous materials having regular pores with  
19 cylindrical or polyhedral shapes and open edges [45].

20 **Table 1.** Textural properties for pure and zinc-doped mesoporous HAp.

Sample	$\text{SSA}_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	$V_p^*$ ( $\text{cm}^3 \text{g}^{-1}$ )	$D_p^*$ (nm)
mHAp	$138 \pm 3.0$	0.55	14.7
Zn0.5%-mHAp	$180 \pm 2$	0.49	10.9

Zn1%-mHAp	170 ± 3.0	0.49	11.5
Zn2%-mHAp	182 ± 4.0	0.50	11.1

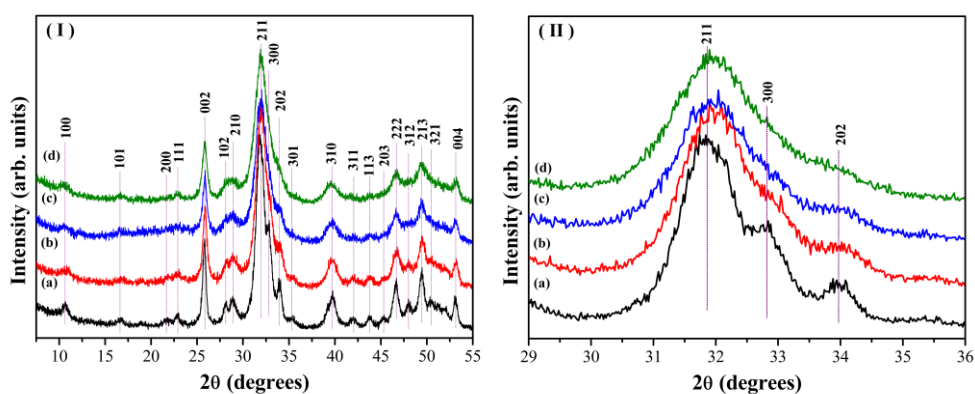
\*V<sub>p</sub> and D<sub>p</sub> are volume and diameter of pore, respectively.

The HAp prepared in this study showed a specific surface area (SSA) of 138–182 m<sup>2</sup> g<sup>-1</sup>, which is higher than those reported previously for HAp. In a previous study, Pure mHAp and HAp coated with iron oxide (HAp-Fe<sub>2</sub>O<sub>3</sub>) were synthesized using the F-127 triblock copolymer as the template and sucrose [46]. The resulting pure HAp and HAp-Fe<sub>2</sub>O<sub>3</sub> samples showed the SSA values of 141 and 148 m<sup>2</sup> g<sup>-1</sup>, respectively [46]. On the other hand, mHAp synthesized using vitamin C as template shows an SSA of 62–88 m<sup>2</sup> g<sup>-1</sup> [47]. HAp synthesized using CTAB as template shows a low specific surface area of 62 m<sup>2</sup> g<sup>-1</sup> [48]. In addition, mHAp prepared via the co-precipitation method using casein as template shows an SSA of 54–106 m<sup>2</sup> g<sup>-1</sup> [38]. Zn-doped HAp prepared via co-precipitation with no template shows an SSA of 99–115 m<sup>2</sup> g<sup>-1</sup> [23].

As the pore size distribution of a material is closely related to its total area, it is an important parameter for investigating the structural properties of porous materials. As can be observed from the inset of **Figure SM1** and **Table 1**, the average pore diameter of the HAp prepared in this study varied from 10.9 to 14.7 nm. This indicates that the addition of Zn<sup>2+</sup> to the HAp structure as well as the use of the template favored the formation of small and uniformly distributed pores (**Figure SM1**) as compared to the previously reported mHAp with the pore sizes of 3.17–5.8 [49] and 5.4–12.2 nm and an SSA of 46.5 m<sup>2</sup> g<sup>-1</sup> [50].

### 3.2 XRD patterns

1 The XRD patterns of the pure and zinc-doped HAp are shown in **Figure 1**. The  
 2 diffraction patterns of the synthesized samples showed broad diffraction peaks, which  
 3 are characteristic of nanostructured materials. The diffraction peaks of the samples  
 4 could be indexed to the hexagonal phase of HAp with the  $P63/m$  space group. In  
 5 addition, no secondary phases were detected in the XRD patterns of the Zn-doped  
 6 HAp, indicating the formation of stable  $Zn_x$ -HAp compounds irrespective of the  $Zn^{2+}$   
 7 content. This is consistent with the results obtained in a previous study [32].



9  
 10 **Figure 1.** (I) XRD patterns and (II) magnified XRD patterns over the  $2\theta$  range of 30–  
 11  $36^\circ$  for (a) mHAp, (b) Zn0.5%-mHAp, (c) Zn1%-mHAp, and (d) Zn2%-mHAp.

12  
 13 The presence of  $Zn^{2+}$  cations in the mHAp structure resulted in an increase in the  
 14 long-range structural disorder, as evidenced by the broadening of the (211) reflection  
 15 peak, which merged with the reflections corresponding to the (112) and (300) planes.  
 16 The calculated FWHM values indicated that an increase in the amount of  $Zn^{2+}$  resulted  
 17 in an increase in the long-range structural disorder of the samples (**Figure SM2**). This  
 18 resulted in a lower decrease in the crystallite size (calculated using the Scherrer  
 19 equation) of all the  $Zn_x$ -mHAp samples. This behavior can be attributed to the increase

1 in the number of nucleation sites due to the presence of  $Zn^{2+}$  cations in the solution. The  
 2 increase in the number of nucleation sites inhibited the growth of crystallites, which  
 3 also decreased the crystallinity of the material. These results are consistent with those  
 4 reported previously [51–54]. In addition, the casein micelles acted as a template to  
 5 control the pore distribution and particle growth. This significantly affected the  
 6 crystallite growth of the pure and Zn-doped HAp samples. A similar phenomenon has  
 7 been reported previously for pure HAp.

8 In order to further investigate the structural properties of the  $Zn_x$ -mHAp samples  
 9 and the effect of the  $Zn^{2+}$  doping amount on the structure of HAp, the lattice parameters  
 10 of the samples were calculated and are listed in **Table 2**. As expected, the addition of  
 11  $Zn^{2+}$  to mHAp caused changes in its lattice parameters,  $a$  and  $c$ , as well as the unit cell  
 12 volume,  $V$ . As the ionic radius ( $r$ ) of  $Ca^{2+}$  ( $r_{Ca^{2+}} = 1.00$  in coordination number (CN) 6)  
 13 is larger than that of  $Zn^{2+}$  ( $r_{Zn^{2+}} = 0.74$ , CN = 6) [55], the substitution of  $Ca^{2+}$  by  $Zn^{2+}$  is  
 14 favorable for the formation of the HAp structure and results in an increase in the lattice  
 15 parameters, as observed in this study [51,52,54]. For instance, X-ray absorption fine  
 16 structure measurements have revealed that  $Zn^{2+}$  cations preferentially occupy the  $Ca^{2+}$   
 17 sites in Zn-doped HAp [51]. Compared to standard HAp, the  $Zn_x$ -mHAp samples  
 18 showed increased lattice parameters and unit cell volume because of the use of casein as  
 19 the template in the synthesis process.

20 **Table 2.** Lattice parameters of the synthesized pure and Zn-doped mHAp solids.

Solid	Lattice parameters (Hexagonal)		Unit cell volume
	<b>a (nm)</b>	<b>c (nm)</b>	<b>V (nm<sup>3</sup>)</b>
Standard HAp*	0.941	0.688	52.80
mHAp	0.944 ± 0.008	0.688 ± 0.013	53.12

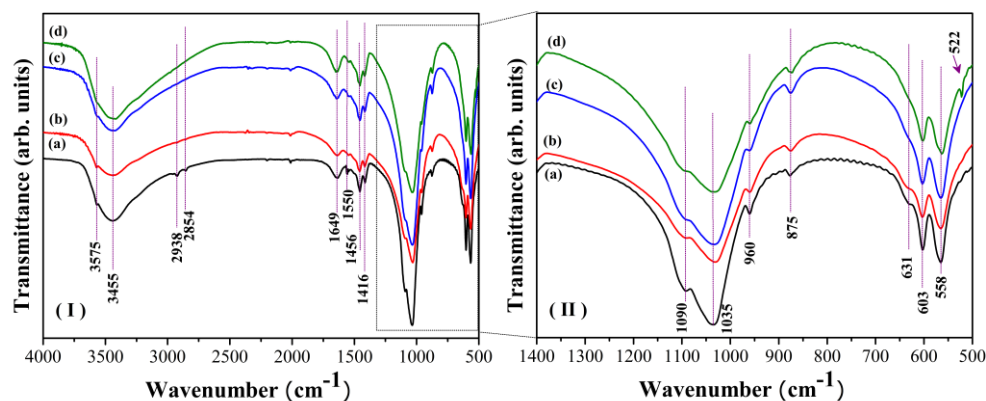
Zn0.5%-mHAp	$0.942 \pm 0.014$	$0.684 \pm 0.024$	52.59
Zn1%-mHAp	$0.944 \pm 0.012$	$0.686 \pm 0.022$	52.91
Zn2%-mHAp	$0.947 \pm 0.020$	$0.683 \pm 0.034$	53.09

1 \*ICDD 00-009-0432

2

### 3 3.2. FTIR spectroscopy

4 The FTIR spectra of the mHAp and Zn<sub>x</sub>-mHAp samples are shown in **Figure 2**,  
5 and the assignments of the IR bands are summarized in **Table SM1**.



6

7 **Figure 2.** Infrared spectra in the (I) 4000–400 cm<sup>-1</sup> and (II) 500–1400 cm<sup>-1</sup> regions for  
8 the (a) mHAp, (b) Zn0.5%-mHAp, (c) Zn1%-mHAp, and (d) Zn2%-mHAp samples.

9

10 The FTIR spectra of the samples showed bands characteristic of HAp in the  
11 region below 1100 cm<sup>-1</sup>. This is consistent with the results reported previously  
12 [38,56,57]. The bands at 1090, 1035, and 960 cm<sup>-1</sup> can be attributed to the asymmetric  
13 deformation of PO<sub>4</sub><sup>3-</sup> and the stretching of P-OH in HPO<sub>4</sub><sup>2-</sup>, while the band at 875 cm<sup>-1</sup>  
14 corresponds to the P-O(H) deformation [38,48,58]. Bands at 603 and 558 cm<sup>-1</sup> are  
15 assigned to PO<sub>4</sub><sup>3-</sup> vibrations and P-O(H) asymmetric deformation in HPO<sub>4</sub><sup>2-</sup>,



1 respectively [59]. The additional bands at 3575 and 3455  $\text{cm}^{-1}$  can be attributed to the  
2 OH stretching of the structural groups in HAp and the OH groups of the adsorbed water  
3 [60]. The OH deformation band of the water molecules was also observed at 1649  $\text{cm}^{-1}$   
4 [61]. Considering that the samples were synthesized in the presence of casein, the  
5 appearance of low-intensity bands at 2938 and 2854  $\text{cm}^{-1}$  in the case of the mHAp  
6 sample can be attributed to the C-H asymmetric and symmetric stretching of casein  
7 [38], respectively. The band at 1550  $\text{cm}^{-1}$  corresponds to amide II resulting from the  
8 combination of the C–N stretching and N–H deformation [62]. The other bands  
9 observed at 1456 and 1416  $\text{cm}^{-1}$  correspond to the  $\text{CH}_2$  and C-OH deformations,  
10 respectively. These results confirm the presence of casein in the samples [38].

11 In addition to the characteristic bands of HAp and casein, an additional band was  
12 observed at 522  $\text{cm}^{-1}$  in the spectrum of Zn2%-mHAp, attributed to the P-O $\cdots$ Zn and  
13 P $\cdots$ O-Zn bonds (P-O-Zn) [59][63]. This band was not observed in the spectra of the  
14 samples with less than 2% (mol concentration)  $\text{Zn}^{2+}$ . This is consistent with the results  
15 reported previously for Zn-doped mHAp of the type  $\text{Ca}_{10-x}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_2$  ( $0 \leq x \leq$   
16 70%) [52].

### 17 **3.4. TG analysis**

18 The TG curves of the samples are shown in **Figure SM3**, and the results are  
19 summarized in **Table SM2**.

20 All the HAp samples showed three mass loss events over different temperature  
21 ranges (**Table SM2**). The mHAp, Zn0.5%-mHAp, Zn1%-mHAp, and Zn2%-mHAp  
22 samples showed the first mass losses of 2.7%, 2.5%, 4.1%, and 3.4%, respectively,  
23 attributing to the loss of the adsorbed water [64]. These samples showed the second  
24 mass losses of 1.8%, 1.8%, 1.9%, and 2.1%, respectively, attributing to the OH

1 condensation and loss of the organic material associated with the remaining casein  
2 [38,56]. The presence of casein was also detected by FTIR spectroscopy, as discussed in  
3 the previous section. The samples showed the third mass losses of 1.0%, 1.5%, 1.9%,  
4 and 1.8%, respectively, attributing to the decomposition of HAp [65].

5

### 6 **3.5. TEM and HR-TEM analyses**

7 The TEM images of the samples are shown in **Figure SM4**. The morphologies,  
8 particle sizes, and porous structures of the samples were investigated from these images.  
9 **Figure 3** shows the HR-TEM images of Zn1%-mHAp.

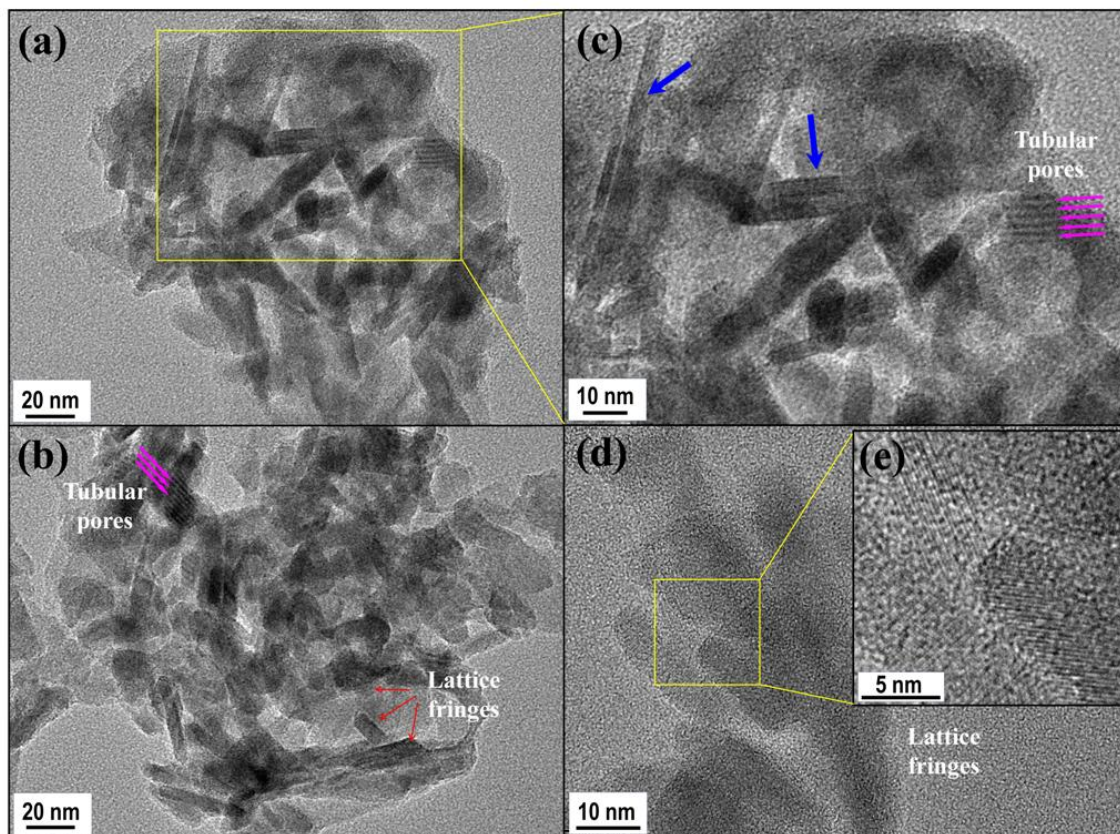
10 All the TEM images (**Figure SM4**) indicated the formation of rod-like  
11 nanoparticles smaller than 100 nm in size in the presence of agglomerate clusters. The  
12 mHAp, Zn1%-mHAp, and Zn2%-mHAp samples (**Figures SM4a–SM4c**) showed  
13 pores with disordered arrangements, which occupied some domains (indicated by the  
14 yellow arrows in **Figures SM4a and SM4c**). In addition, the Zn<sup>2+</sup>- samples (**Figures**  
15 **SM4b and SM4c**) showed mesoporous channels distributed parallel to the particles  
16 (indicated by the red dashed circles). The arrangement of these channels could be  
17 clearly observed from the HR-TEM images (indicated by the blue and purple arrows in  
18 **Figure 3**) of the Zn1%-mHAp sample. The less dense white stripes, through which the  
19 electron beam could easily pass, observed in the images represent the channels. In  
20 contrast, the darker stripes represent the walls of the HAp channels, which were dense  
21 and absorbed more electron beams during the analysis. The HR-TEM images (**Figure 3**)  
22 also show the lattice fringes of the Zn1%-mHAp sample, reflecting the periodicity of  
23 the atomic planes of the mHAp hexagonal structure [38,48], which caused the formation  
24 of tubular mesopores (as indicated by the purple arrows in **Figure 3**). Although the  
25 sample showed a disordered arrangement, its interplanar spacings could be calculated

1 (as indicated by the inset of **Figure 3**), as shown in **Figure 4**. This provided a better  
2 insight into the crystal structure of the synthesized Zn-mHAp biomaterials.

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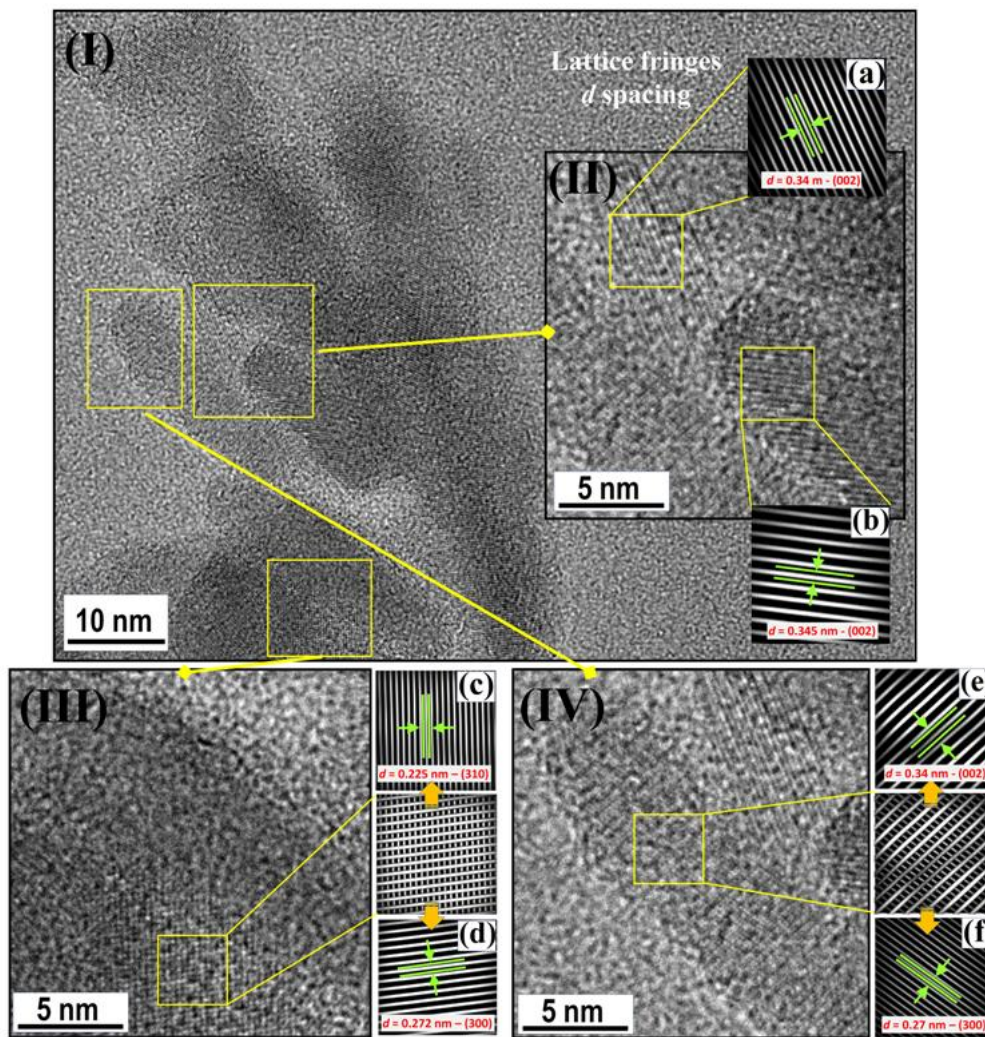
7 **Figure 3.** HR-TEM images (a, b) and high-magnification HR-TEM images (c, d) of  
8 Zn1%-mHAp showing tubular pores and lattice fringes. The inset (e) shows the clear  
9 image of the lattice fringes in this selected area.

10

11 The Zn1%-mHAp sample showed the interplanar distances ( $d$  spacing) of  
12 approximately 0.22, 0.27, and 0.34 nm corresponding to the (310), (300), and (002)  
13 crystallographic planes of the hexagonal HAp structure, respectively (**Figure 4**). This

1 indicates that the synthesized biomaterials were polycrystalline in nature. In addition, as  
 2 shown in **Figure 4**, [100] was the preferred direction for crystal growth in Zn1%-  
 3 mHAp. We believe that the mHAp, Zn0.5%-mHAp, and Zn2%-mHAp samples also  
 4 showed the same preferred crystal growth direction as they were synthesized using the  
 5 same concentration of the casein template.

6



7

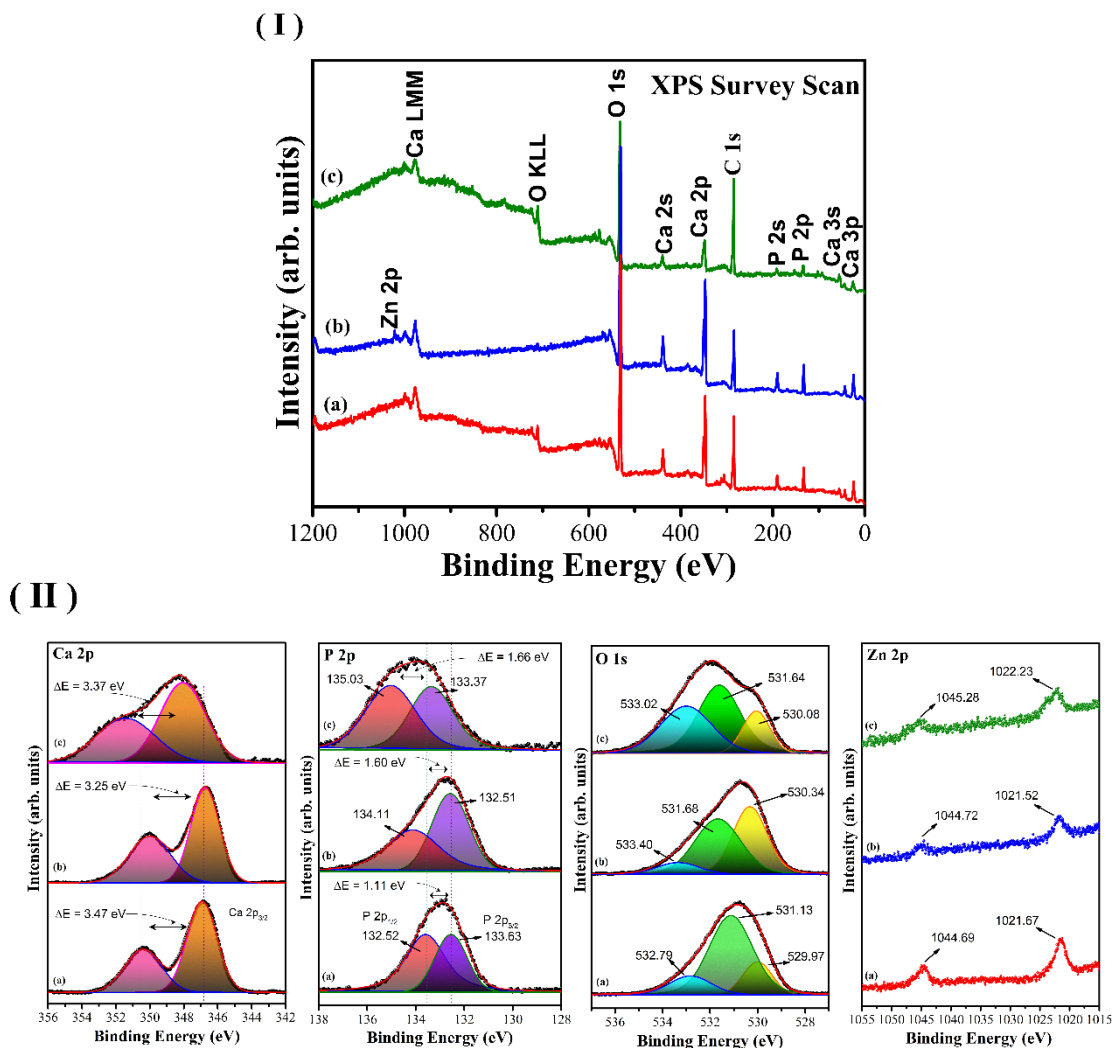
8 **Figure 4.** HR-TEM image (I) of Zn1%-mHAp and the three selected areas (I), (II), and  
 9 (III) in the HR-TEM images. Images of the selected areas in the HR-TEM image after  
 10 the IFFT treatment showing the crystal planes and their respective  $d$  spacing (a–f).

11

1 **3.6 XPS analysis**

2

3 The chemical nature and elemental composition of the surface of the samples  
 4 were investigated by carrying out their XPS analysis, and the XPS profiles of the  
 5 samples are shown in **Figure 5**. The survey and high-resolution spectra of the Zn0.5% -  
 6 mHAp, Zn1% -mHAp, and Zn2% -mHAp samples were recorded.



7

8

9 **Figure 5.** XPS survey profiles (I) for (a) Zn0.5%-mHAp, (b) Zn1%-mHAp, and (c)  
 10 Zn2%-mHAp and high-resolution XPS scans (II) for Ca2p, P2p, O1s, and Zn2p.

1

2           The survey spectra of the samples showed the presence of Ca, P, and O  
3 corresponding to the mHAp structure. In addition, Zn was detected in the doped mHAp  
4 samples. These results confirm the successful formation of the target materials.  
5 Furthermore, all the samples showed an intense C1s signal (see also **Figure SM5**)  
6 attributed to the remaining casein anchored on the surface of the synthesized solids, as  
7 also indicated by the FTIR and TG analysis results discussed earlier. It should be noted  
8 that the emission lines of mHAp tended to shift with the incorporation of Zn, especially  
9 at higher Zn<sup>2+</sup> concentrations (Zn2% -mHAp). The incorporation of Zn<sup>2+</sup> into mHAp  
10 changed its local surface structural properties.

11           It has been reported that the high-resolution Ca2p XPS profile of HAp shows  
12 two well-defined peaks corresponding to the Ca2p<sub>3/2</sub> and Ca2p<sub>1/2</sub> orbitals [9,66,67].  
13 These peaks were clearly observed in the present study. The high-resolution Ca2p peak  
14 of the biomaterials could be deconvoluted into two peaks, whose binding energies (BEs)  
15 varied according to the biomaterial composition. In the Ca2p spectrum of the Zn0.5%-  
16 mHAp sample, the Ca2p<sub>3/2</sub> and Ca2p<sub>1/2</sub> peaks were observed at 348.06 and 351.43 eV,  
17 respectively, and the spin-coupling energy ( $\Delta E$ ) was 3.37 eV. The Zn1%-mHAp (BE  
18 Ca2p<sub>3/2</sub> = 346.74 eV; BE Ca2p<sub>1/2</sub> = 349.99 eV and  $\Delta E$  = 3.25 eV) and Zn2%-mHAp (BE  
19 Ca2p<sub>3/2</sub> = 348.06 eV; BE Ca2p<sub>1/2</sub> = 351.51 eV and  $\Delta E$  = 3.45 eV) samples showed  
20 different BE Ca2p<sub>3/2</sub>, BE Ca2p<sub>1/2</sub>, and  $\Delta E$  values. The Ca2p photoemission lines of the  
21 samples shifted with an increase in the Zn<sup>2+</sup> content. In addition, the samples with high  
22 Zn<sup>2+</sup> contents showed broad and less-defined peaks. This confirms that the Zn<sup>2+</sup>/Ca<sup>2+</sup>-  
23 type substitution occurred in the mHAp lattice. These results are in good agreement  
24 with those reported previously [66]. In addition, X-ray absorption near edge structure,  
25 extended X-ray absorption fine structure, and density functional theory simulation

1 results have demonstrated that  $Zn^{2+}$  cations preferentially occupy the  $Ca^{2+}$  sites in the  
2 HAp structure [51]. Hence, the incorporation of  $Zn^{2+}$  strongly affects the local  
3 environment of the substituted atoms, as observed in this study.

4 The high-resolution P2p profiles of the samples did not exhibit well-defined  
5  $P2p_{3/2}$  and  $P2p_{1/2}$  components [66,67]. However, a single asymmetric peak formed by  
6 the superimposition of the two P2p components was observed. This asymmetric peak is  
7 characteristic of phosphate compounds such as HAp, and the two 2p components are  
8 associated with the phosphate groups and P-O-Ca bonds at the biomaterial surface [66–  
9 68]. Thus, the high-resolution P2p peak of the samples could be deconvoluted into  
10  $P2p_{1/2}$  and  $P2p_{3/2}$  peaks. The  $P2p_{1/2}/P2p_{3/2}$  peaks of the Zn0.5%-mHAp, Zn1%-mHAp,  
11 and Zn2%-mHAp samples were observed at 133.63/132.52, 134.11/132.51, and  
12 135.03/133.37 eV, respectively. The variation in the BE and the broadening of the  
13 photoemission lines of the 2p components of P can be attributed to the increase in the  
14 spin-orbit coupling energy ( $\Delta E$ ) with  $Ca^{2+}/Zn^{2+}$  substitution. The calculated  $\Delta E$  values  
15 for the Zn0.5%-mHAp, Zn1%-mHAp, and Zn2%-mHAp samples were 1.11, 1.60, and  
16 1.66 eV, respectively. This change can be attributed to the difference in the local  
17 environment of the phosphate groups such as  $Zn/Ca-PO_4-OH$  and  $P-O-Ca/Zn$  generated  
18 upon  $Zn^{2+}$  doping.

19 The samples with different  $Zn^{2+}$  contents showed different high-resolution O1s  
20 profiles. The O1s peaks of the samples could be decomposed into three distinct peaks.  
21 The first peak at 531.13–531.64 eV can be attributed to the structural oxygen ( $O_{stru}$ )  
22 associated with the P (P-O) bond of HAp. The second peak at 532.79–533.40 eV  
23 corresponds to the P-O-P bonds, while the third peak observed at 529.97–530.34 eV can  
24 be attributed to the oxygen-derived species chemically adsorbed on the surface of the  
25 samples ( $OH^-$ , CO and  $CO_2$ ) and the P-O-Ca/Zn interface [67,68].

1 As expected, the Zn2p peaks of the samples could be decomposed into two  
2 peaks corresponding to the Zn2p<sub>1/2</sub> and Zn2p<sub>3/2</sub> orbitals. The difference in the spin-orbit  
3 coupling energy ( $\Delta E$ ) for the Zn0.5%-mHAp, Zn1%-mHAp, and Zn2%-mHAp samples  
4 was 23.02, 23.20, and 23.05 eV, respectively, as calculated from their Zn2P XPS  
5 profiles. This indicates that Zn<sup>2+</sup> was predominant in all the samples. These results  
6 confirm the successful incorporation of Zn<sup>2+</sup> cations into the mHAp structure. The Zn<sup>2+</sup>  
7 cations were present at the surface of the samples and the P-O-Ca/Zn interface was  
8 created.

9 The XPS results were consistent with the FTIR results, which indicated the  
10 presence of the P-O···Zn and P···O-Zn bonds (P-O-Zn) in the samples, especially in the  
11 Zn2%-mHAp sample. The creation of these interfaces upon Ca<sup>2+</sup>/Zn<sup>2+</sup> substitution  
12 changed the local coordination of Ca, P, and O at the surface of the samples, as  
13 indicated by their high-resolution XPS profiles. Therefore, the substitution of Ca<sup>2+</sup> by  
14 Zn<sup>2+</sup> induced an electronic disturbance at the surface of mHAp, altering its surface  
15 properties and hence functionality.

16 High-resolution C1s XPS profiles of the samples were also obtained (**Figure**  
17 **SM5**). The profiles showed three peaks at 284.0, 286.1, and 288.2 eV, attributing to the  
18 sp<sup>2</sup> and sp<sup>3</sup>-hybridized carbon (C=C and C-C), hydroxyl groups (C-OH), and carboxyl  
19 (O-C=O, C=O) groups [66,67], respectively. The high intensity of the C1s peaks can  
20 be attributed to the casein remaining after the synthesis process. Moreover, the signals  
21 corresponding to the C=O and C-OH bonds can be attributed to the CO<sub>2</sub> and hydroxyl  
22 groups adsorbed on the surface of the samples.

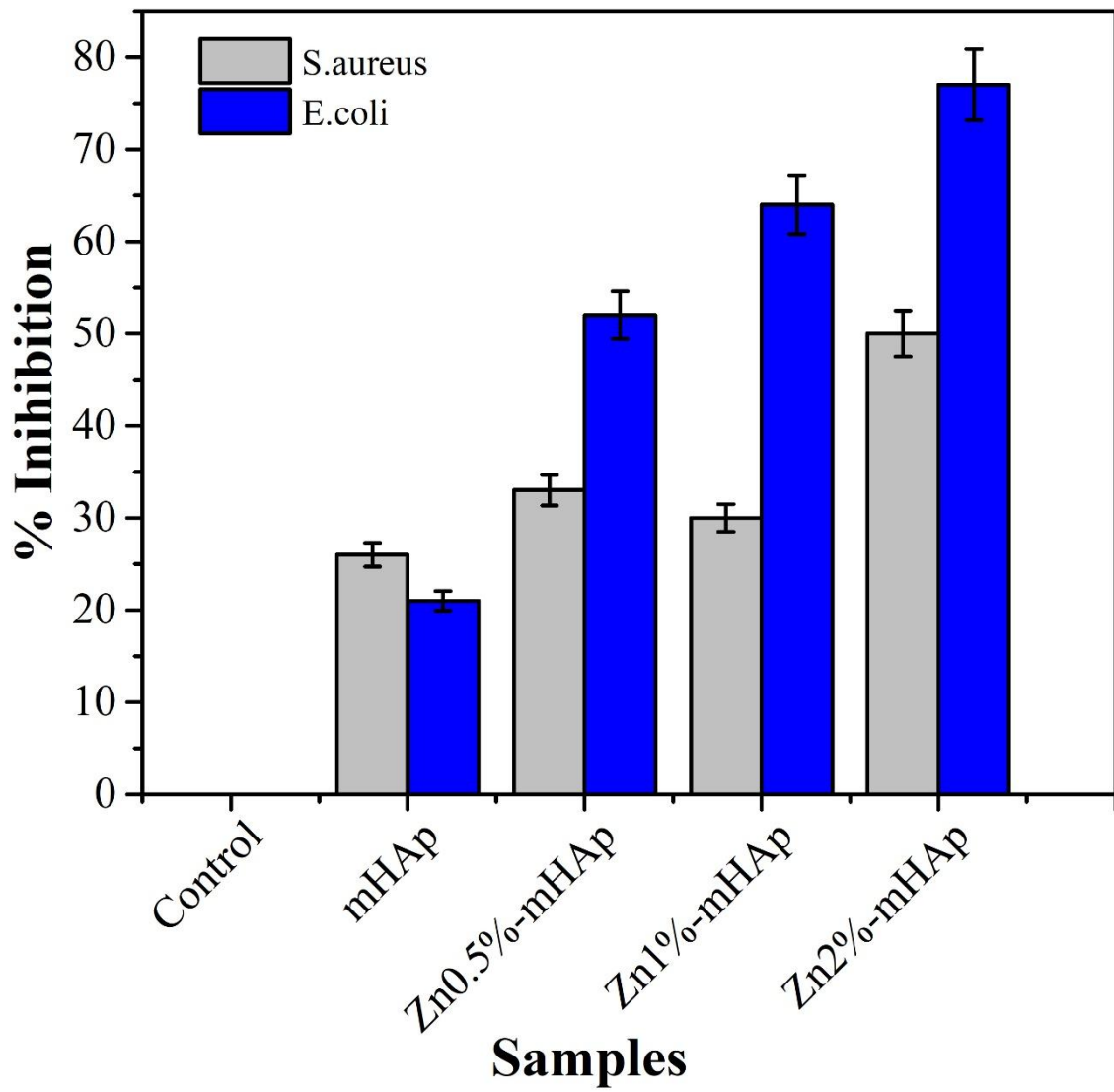
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### 24 **3.7. Antimicrobial activity**



1 **Figure SM6** shows the images of the Petri dishes with *S. aureus* and *E. coli*  
2 bacteria after using the mHAp and Zn<sub>x</sub>-mHAp samples as the bacterial growth  
3 inhibitors. The results obtained by the direct contact method are shown in **Figure 6**.

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9

1 **Figure 6.** Bacterial growth inhibitory concentrations of the mHAp and Zn $x$ -HAp  
2 samples.

3

4 The pure mHAp sample showed a growth inhibition of 26% and 21% for *S.*  
5 *aureus* and *E. coli*, respectively. These results can be related to the surface  
6 characteristics of the mHAp, which consisted of hydroxyl groups (a strong oxidizing  
7 agent) and the remaining casein on the surface. It is well-known that mHAp is  
8 hydrophilic and interacts with the hydrophilic groups of the bacterial cell wall, which is  
9 composed of a thick layer of peptidoglycan (a carbohydrate-conjugated amino acid  
10 copolymer) [69]. Similar behavior has been reported by Tank *et al.* [32] for *S. aureus*  
11 using pure nano-HAp. The antimicrobial activity of pure HAp against *S. aureus* and *E.*  
12 *coli* bacteria has also been investigated using the paper disc method [19]. The lower  
13 activity of HAp against *E. coli* can be attributed to the composition of the hydrophobic  
14 outer membrane of this bacterium. As the *E. coli* membrane is composed of  
15 amphipathic molecules such as lipopolysaccharides and phospholipids, it shows poor  
16 interaction with the hydrophilic surface of HAp. Thus, HAp shows poor growth  
17 inhibition for *E. coli* [69–71].

18 All the Zn $^{2+}$ -doped mHAp samples showed higher antibacterial activity than  
19 pure mHAp, and the antibacterial efficiency of the Zn $^{2+}$ -doped mHAp samples  
20 increased with an increase in the Zn $^{2+}$  content. For example, the Zn2%-mHAp showed  
21 the highest antibacterial activity with a maximum bacterial inhibitory concentration of  
22 50% for *S. aureus* and 77% for *E. coli* (**Figure 6**). In fact, the best antibacterial  
23 performance of the Zn2%-mHAp sample can be attributed to the Zn $^{2+}$  cations present on  
24 its surface (as indicated by the XPS analysis results), which improved the surface  
25 charge properties of this material.

1           Various factors are responsible for the inhibition of bacterial growth by Zn<sub>x</sub>-  
2 HAp biomaterials. One of these factors is the particle size. The mHAp, Zn1%-mHAp,  
3 and Zn2%-mHAp samples showed the average particle sizes of 42, 27, and 26 nm  
4 (**Figure SM7**), respectively. In addition, the presence of mesoporous channels is  
5 important for the diffusion of species. These factors are fundamental and affect the  
6 antibacterial activity of Zn<sub>x</sub>-HAp biomaterials [72].

7           Despite the number of studies concerning the antibacterial activity of pure and  
8 Zn-doped HAps against gram-positive and gram-negative bacteria, different efficiency  
9 has been observed [9,32,33]. The different behavior observed among the materials  
10 might be attributed to the particle characteristics and, especially, to different surface  
11 properties, which can be strongly influenced by the type of surfactant and the amount of  
12 Zn<sup>2+</sup> used to prepare the materials. In our study, lower amount of Zn<sup>2+</sup>-content were  
13 incorporated to the HAp matrix in comparison to the previous reports. Moreover, Tank  
14 *et al.* [32], prepared Zn-HAps using a nonionic surfactant (Triton X-100), known to be  
15 toxic and can play a role in the bacteria inhibition growth. [73–75]. In the present work,  
16 casein phosphoprotein was used as a biotemplate to prepare Zn-mHAp biomaterials. As  
17 a result, its use as template in the synthesis of mesoporous materials for applications in  
18 biological systems cannot only enhances biocompatibility, but also can eliminate  
19 problems of toxicity of materials prepared in the presence of synthetic-type templates  
20 such as Triton X-100.

21           The results discussed thus far suggest that using casein (a natural  
22 phosphoprotein) as a template and Zn<sup>2+</sup> as a dopant is an efficient approach to  
23 synthesize novel HAp-based biomaterials with tailored properties for specific  
24 applications.

## 25 **Conclusion**

1 Monophasic nanoparticles of pure and Zn<sup>2+</sup>-doped mHAp were successfully  
2 synthesized by the co-precipitation method using casein as the template. The structural,  
3 textural, morphological, and biological characterizations of the samples confirmed the  
4 incorporation of Zn<sup>2+</sup> into the HAp matrix. The use of the casein template allowed the  
5 control of the surface properties of mHAp by increasing its porosity, which improved its  
6 interaction with the bacteria through the active sites. The antimicrobial activities of the  
7 Zn<sup>2+</sup>-doped mHAp against gram-positive and gram-negative bacteria were investigated.  
8 It was found that the antibacterial efficiency of the Zn<sup>2+</sup>-doped mHAp depended  
9 strongly on the amount of Zn<sup>2+</sup> cations incorporated into the mHAp structure and their  
10 presence on the HAp surface, as indicated by the XPS analysis results. The  
11 antimicrobial tests demonstrated the potential of the Zn<sup>2+</sup>-doped HAp samples for  
12 biomedical applications, especially in the control of bacterial infections in bone repair  
13 or dental prosthesis.

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1 **Figure Captions**

2 **Figure 1.** (I) XRD patterns and (II) magnified XRD patterns over the  $2\theta$  range of 30–  
3  $36^\circ$  for (a) mHAp, (b) Zn0.5%-mHAp, (c) Zn1%-mHAp, and (d) Zn2%-mHAp.

4 **Figure 2.** Infrared spectra in the (I)  $4000\text{--}400\text{ cm}^{-1}$  and (II)  $500\text{--}1400\text{ cm}^{-1}$  regions for  
5 the (a) mHAp, (b) Zn0.5%-mHAp, (c) Zn1%-mHAp, and (d) Zn2%-mHAp samples.

6 **Figure 3.** HR-TEM images (a, b) and high-magnification HR-TEM images (c, d) of  
7 Zn1%-mHAp showing tubular pores and lattice fringes. The inset (e) shows the clear  
8 image of the lattice fringes in this selected area.

9 **Figure 4.** HR-TEM image (I) of Zn1%-mHAp and the three selected areas (I), (II), and  
10 (III) in the HR-TEM images. Images of the selected areas in the HR-TEM image after  
11 the IFFT treatment showing the crystal planes and their respective  $d$  spacing (a–f).

12 **Figure 5.** XPS survey profiles (I) for (a) Zn0.5%-mHAp, (b) Zn1%-mHAp, and (c)  
13 Zn2%-mHAp and high-resolution XPS scans (II) for Ca2p, P2p, O1s, and Zn2p.

14 **Figure 6.** Bacterial growth inhibitory concentrations of the mHAp and Zn $x$ -HAp  
15 samples.

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1 **Supporting information for**

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3 **Zn-doped mesoporous hydroxyapatites and their antimicrobial**

4 **properties**

5

6 Cleibson Oliveira de Lima,<sup>a</sup> André L. Menezes de Oliveira,<sup>a</sup> Laís Chantelle,<sup>a</sup> Edson C.

7 Silva Filho,<sup>b</sup> Maguy Jaber,<sup>c</sup> Maria Gardênia Fonseca<sup>a,\*</sup>

8

9 *<sup>a</sup>Universidade Federal da Paraíba, Núcleo de Pesquisa e Extensão LACOM, 58051-*

10 *085, João Pessoa, Paraíba, Brazil.*

11 *<sup>b</sup>Interdisciplinary Laboratory for Advanced Materials-LIMAV, Universidade Federal*

12 *do Piauí, Teresina, 64049- 11 550, Piauí, Brazil.*

13 *<sup>c</sup>Sorbonne Université, Laboratoire d'Archéologie Moléculaire et Structurale, Institut*

14 *Universitaire de France (IUF), CNRS UMR 8220, UPMC – Tour 23, 3ème étage,*

15 *couloir 23-33, BP 225, 4 place Jussieu, 75005 Paris, France.*

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1 **Table SM1.** Band assignments in the FTIR spectra of the mHAp and Zn<sub>x</sub>-HAp  
 2 samples.

Wavenumber (cm <sup>-1</sup> )	Assignment
3575	ν (structural O-H)
3455	ν OH of the water
2938	asymmetric ν(C-H)
2854	symmetric ν(C-H)
1649	δ(O-H) of the adsorbed H <sub>2</sub> O
1550	2 <sup>nd</sup> amide (C-N and N-H)
1456	symmetric and asymmetric ν(C-H)
1416	C-OH
1090	ν(P-O) of the PO <sub>4</sub> <sup>3-</sup>
1035	ν(P-O) of the PO <sub>4</sub> <sup>3-</sup>
960	ν(P-O) of the PO <sub>4</sub> <sup>3-</sup>
875	δ(P-O(H)) of the HPO <sub>4</sub> <sup>2-</sup>
603	δ(P-O) of the PO <sub>4</sub> <sup>3-</sup>
558	δ(P-O(H)) of the HPO <sub>4</sub> <sup>2-</sup>
522	PO <sub>4</sub> <sup>3-</sup> / Zn-O

3 ν – Stretching, δ – bending vibrations

4

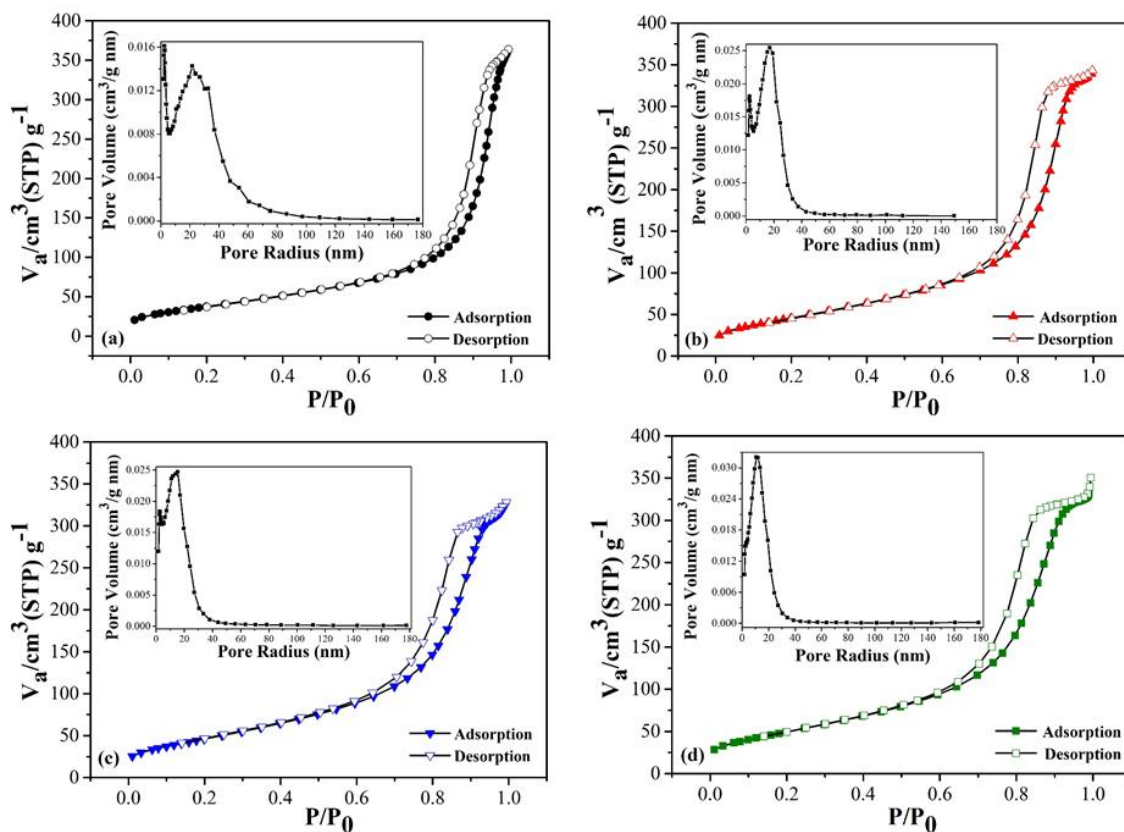
1 **Table SM2.** Summary of mass losses and temperature intervals for thermal  
 2 decomposition events of the synthesized samples.

Sample	Event	Mass loss (%)	Temperature (°C)
mHAp	I	$2.7 \pm 0.4$	26-164
	II	$1.8 \pm 0.1$	164-540
	III	$1.0 \pm 0.1$	540-949
Zn0.5%-mHAp	I	$2.5 \pm 0.1$	26-173
	II	$1.8 \pm 0.1$	173-513
	III	$1.5 \pm 0.1$	513-905
Zn1%-mHAp	I	$4.1 \pm 0.2$	26-172
	II	$1.9 \pm 0.1$	172-513
	III	$1.9 \pm 0.1$	513-883
Zn2%-mHAp	I	$3.4 \pm 0.2$	27-170
	II	$2.1 \pm 0.1$	170-518
	III	$1.8 \pm 0.1$	518-967

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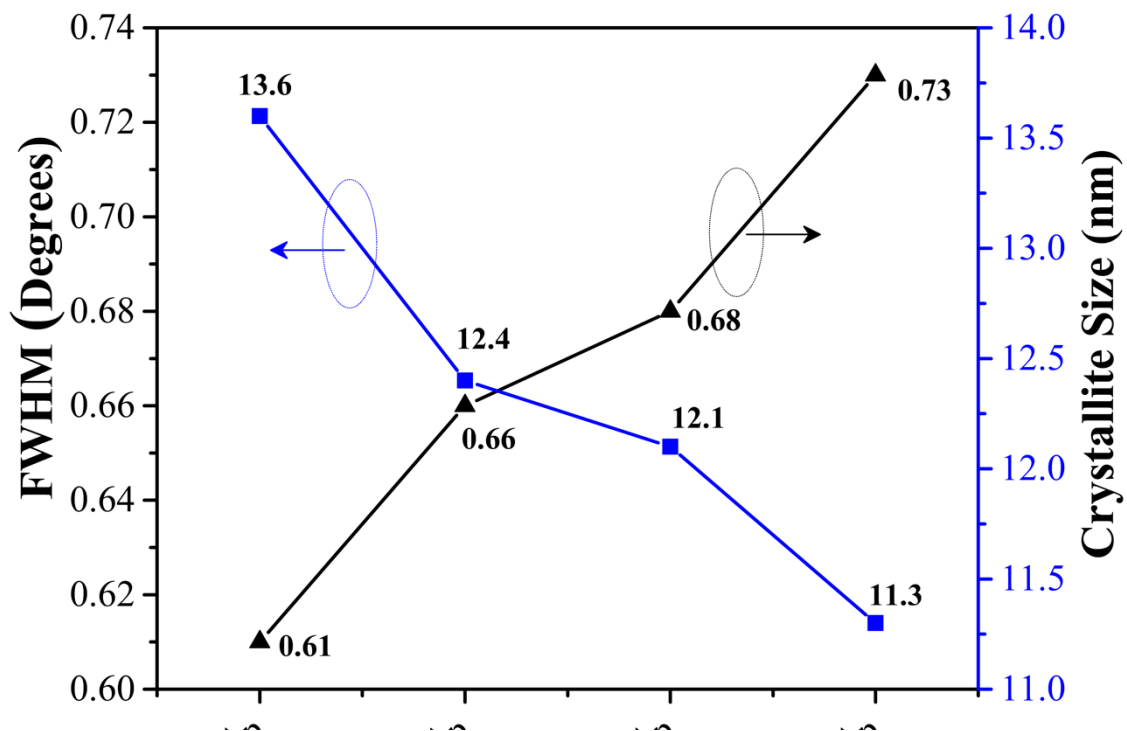
- 1 **Figure SM1.** N<sub>2</sub> adsorption/desorption isotherms for (a) mHAp, (b) Zn0.5%-mHAp, (c)  
2 Zn1%-mHAp, and (d) Zn2%-mHAp. The insets show the corresponding pore size  
3 distribution.



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- 1 **Figure SM2.** Relationship between the FWHM and average crystallite size for the
- 2 synthesized samples.

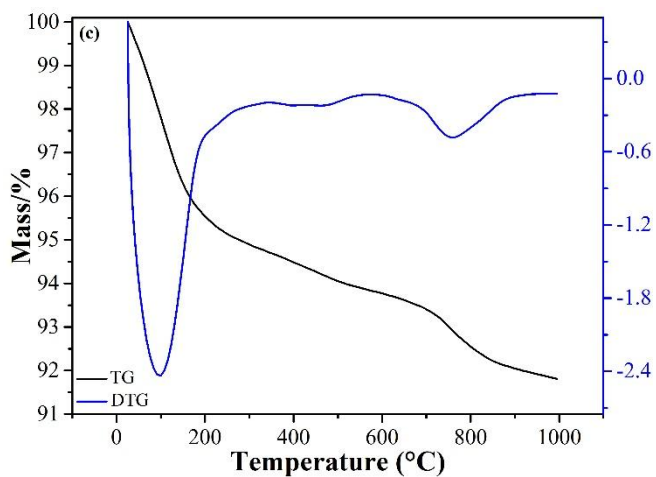
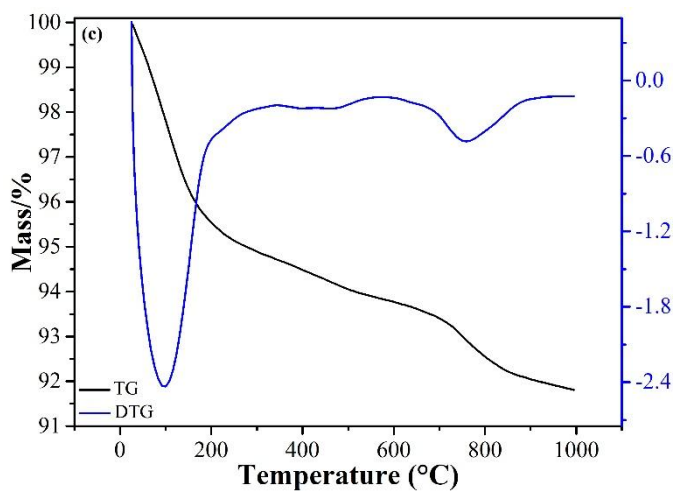
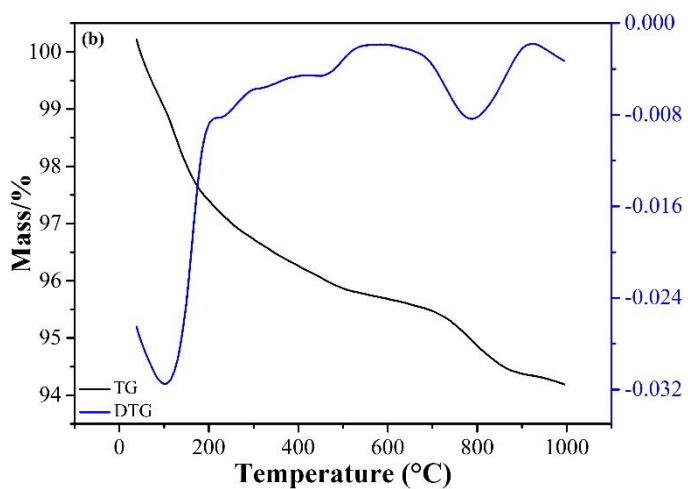
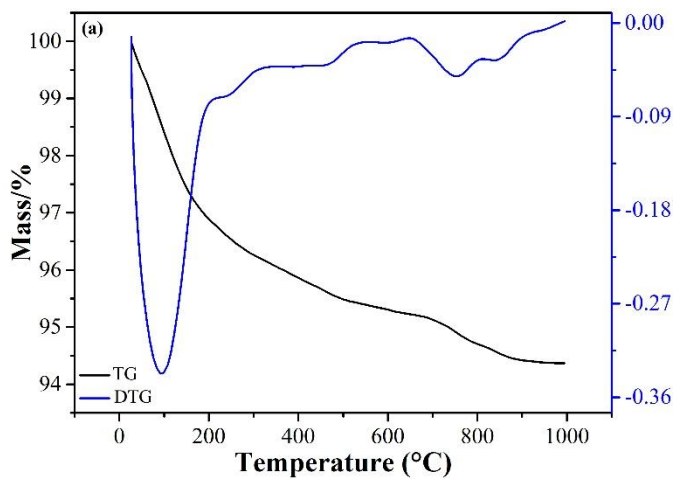


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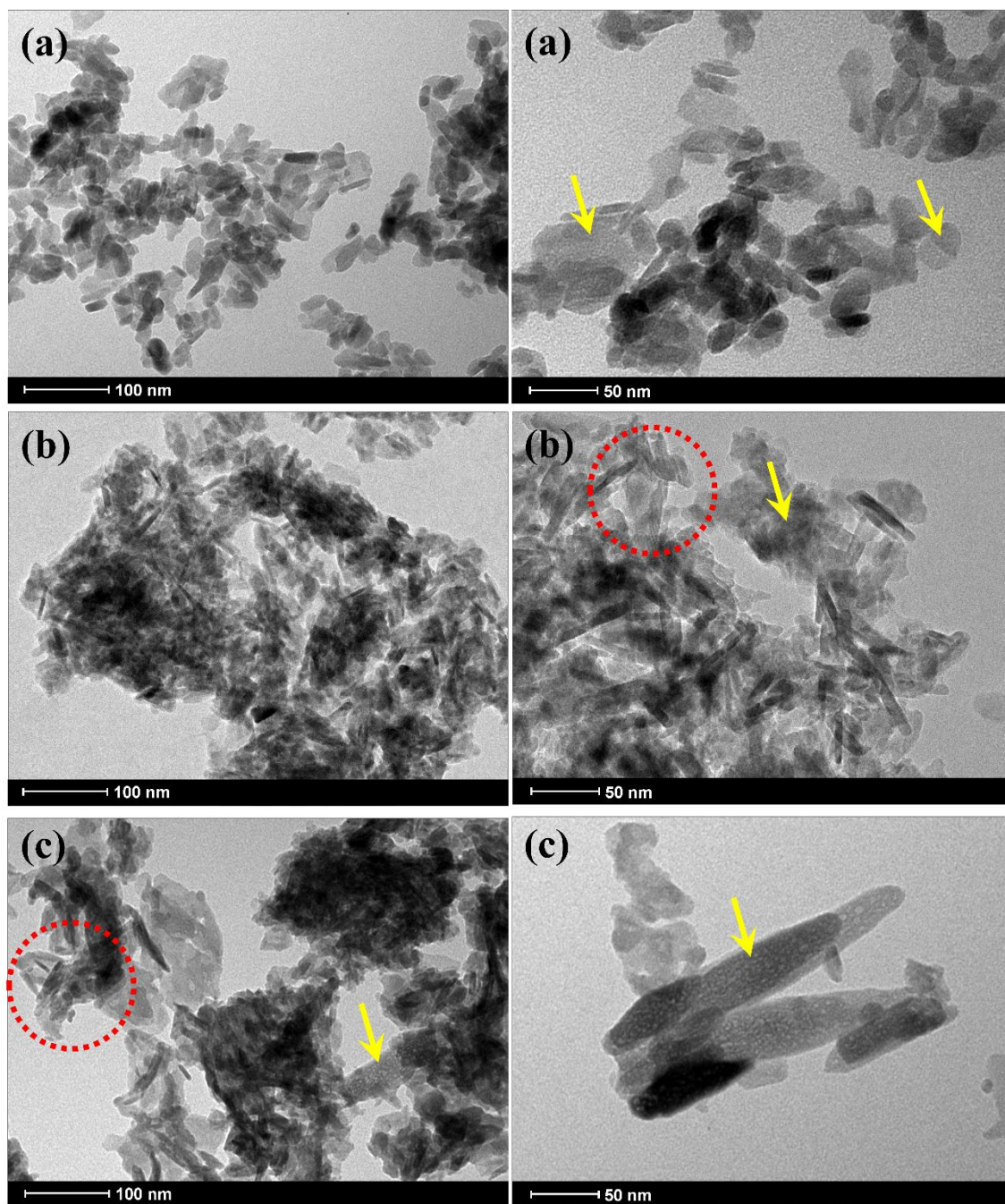
1 **Figure SM3.** TGA (DTG) curves of the (a) mHAp, (b) Zn0.5%-mHAp, (c) Zn1%-  
2 mHAp and (d) Zn2%-mHAp.



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4

1 **Figure SM4.** TEM images of (a) mHAp, (b) Zn1%-mHAp and (c) Zn2%-mHAp.



2

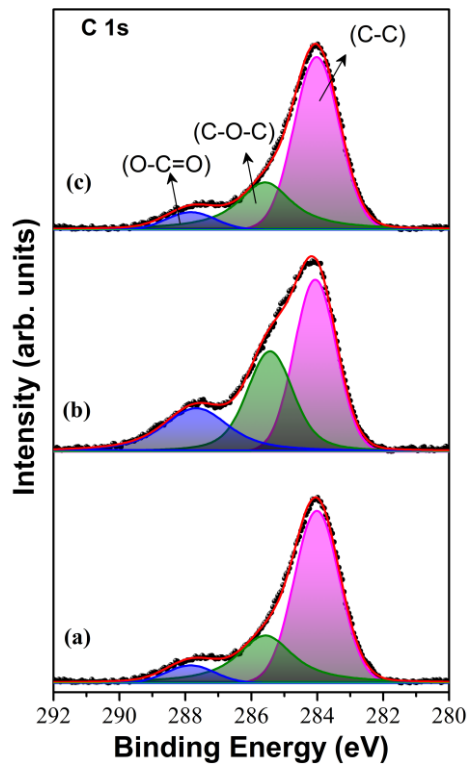
3

4

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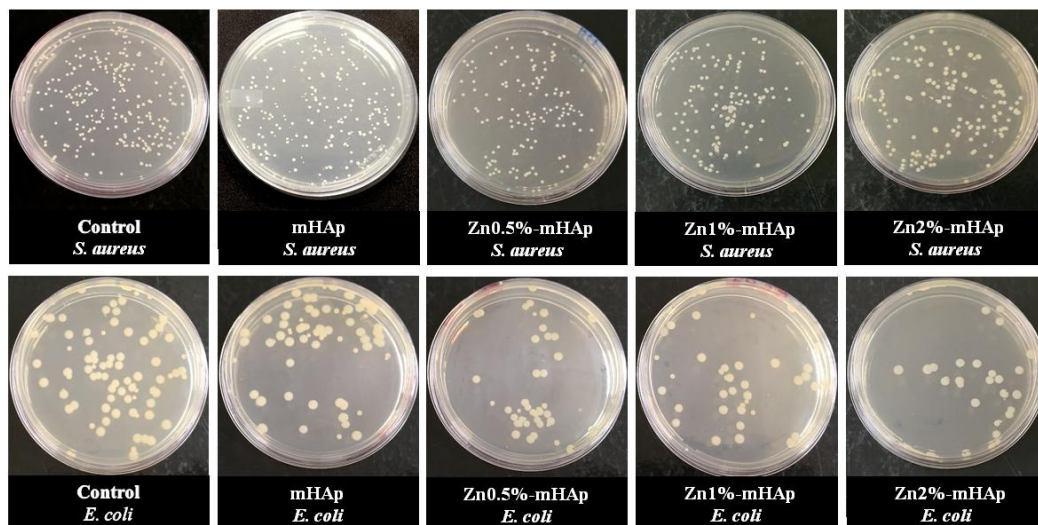
6

- 1 **Figure SM5.** High resolution XPS C1s scans for (a) Zn0.5%-mHAp, (b) Zn1%-mHAp
- 2 and (c) Zn2%-mHAp.



- 3
- 4

1 **Figure SM6.** Inhibitory effect of mHAp on *S. aureus* and *E. coli*.



2

3

1 **Figure SM7.** Histograms of the particle size distribution for (a) mHAp, (b) Zn1%-  
2 mHAp and (c) Zn2%-mHAp.

