Research Article

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Incorporation and In Vitro Application of Hydroxyapatite with Silver and Titanium Dopants Synthesized by Wet Chemical Method

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ABSTRACT

Hydroxyapatite (HAP), a prominent Calcium Phosphate Bioceramic, has been widely used in different fields of Medicine, mainly in Orthopedic and Dentistry. In the present report, HAP was synthesized using two different dopants, Silver (Ag) and Titanium (Ti), in four different concentrations at 100^0C using the Wet Chemical Method. The resultant samples were characterized by X-Ray Diffraction (XRD) and Energy Dispersive X-ray spectrum (EDS). The in vitro investigation was demonstrated under Antimicrobial Activity using Staphylococcus aureus and Escherichia coli bacteria.

Keywords: Antimicrobial Activity; EDS; in vitro; Hydroxyapatite; XRD; Wet Chemical.

1. INTRODUCTION

Technological advances in the medical arena coupled with far-reaching changes in people's lifestyles and food habits have lengthened the average life expectancy of an individual considerably. In today's world, filled with a substantial aging population and with the availability of affordable hi-tech medical services, there is a corresponding increase and clinical demand for bone replacement and repair among the elderly and aged. In this context, the design and development of advanced nano biomaterials for the above-mentioned potential medical applications are one of the most challenging problems faced by modern material engineering today. Bone is a ceramic, organic composite consisting of collagen (20%), Calcium Phosphate (69%), and Water (9%). Calcium Phosphate is an attractive biomedical material owing to its excellent biocompatibility and non-toxicity. There are many phases of Calcium Phosphate that represent resorbable and non-resorbable ceramics and cement. Calcium Phosphate is thus available in different forms exhibiting different crystal structures (with different Ca/P ratios) as Hydroxyapatite, Octacalcium Phosphate, Tricalcium Phosphate, Dicalcium Phosphate Dihydrate, and Dicalcium Phosphate. Of the above, Hydroxyapatite (HAP) bio-ceramic is one of the important basic materials used in bone implant surgery (Daculsi, 1998; Ferraz *et al*. 2004). Due to its advantages, such as high biocompatibility, bioactivity, and very good adaptation under *in vivo* conditions, it is widely applied to fill bone defects in Orthopedic, Maxillofacial Surgery as well as Stomatology, including Dentistry. Due to its low load-bearing capacity and brittle nature, as well as low fracture toughness, the applicability of HAP is limited in Clinical Orthopedics and Dentistry.

This serious handicap of Hydroxyapatite can, however, be rectified in two ways. The most common procedure is to use hot processing and hot isocratic processing techniques for synthesis. An alternative economical technique to obtain a highly dense HAP body is by incorporating additives or dopants during powder processing (Haresh M. Pandya, 2012; Anitha and Haresh M. Pandya, 2013). In the present work, Silver and Titanium were added as doping materials to enhance Hydroxyapatite's mechanical as well as biological properties and applications.

2. EXPERIMENTAL PROCEDURE

Silver doped Hydroxyapatite $Ca_{10-x}Ag_x(PO_4)_6(OH)_2$ with X= 0.1, 0.5, 1, 1.5wt% were prepared by setting the ratio [Ca+Ag]/P = 1.67. Silver Oxide and Calcium hydroxide were dissolved in deionized water to obtain 100 ml [Ca+Ag] solution. This is made to stir for 30 minutes. Then Orthophosphoric acid solution was added to the above solution dropwise. Again the product solution was made to stir for 1hr.

The pH of the solution was made to maintain at 10 using aqueous ammonia. The above final solution was aged under the ice for 22 hrs. After aging, the subsequent

solution was washed with double distilled water several times to remove impurities, if any. The above obtain precipitate was then centrifuged and dried at 100 ^{0}C using a hot air oven.

The same procedure was followed for Titanium doped Hydroxyapatite taking Titanium Oxide as the Titanium precursor.

The obtained samples were characterized by X-Ray Diffraction (XRD) and Energy Dispersive X-ray spectrum (EDS). The *in vitro* investigation was demonstrated under Antimicrobial Activity using *Staphylococcus aureus* and *Escherichia coli* bacteria.

3. CHARACTERIZATION TECHNIQUES

3.1 X-ray Diffraction (XRD)

The Phase analysis of the synthesized samples was done by X-ray diffraction. The pattern was registered in the 2theta range from 0 to 70^0 with a 0.05 scanning step size. Crystallite size was calculated using Debye's Scherer Formula (Sanosh *et al.* 2009; Zhang Li *et al.* 2005)

$$D = 0.91\lambda/\beta\cos\theta$$

Where λ is the wavelength of the X-ray in Å, β is the full width at half maxima value, and θ is the diffraction angle. The lattice parameter a and c value is determined through the hkl peaks positions of apatite from XRD patterns according to the formula.

$$1/d^2 = 4/3(h^2+hk+k^2)/a^2 + l^2/c^2$$

Where d is the lattice distance obtained from XRD results.

3.2 Energy Dispersive X-ray Spectrum (EDS)

The chemical compositional analysis was performed with an Energy Dispersive X-ray spectrum. The elemental analysis will reveal the composition of the ingredients present in the synthesized sample.

3.3 Antimicrobial Susceptibility Test

Antimicrobial Activity was tested for the prepared samples against two bacterial strains, *Staphylococcus aureus* and gram-positive bacteria and *Escherichia coli* and gram-negative bacteria by Agar well diffusion using DMSO as the buffer solution (Kalli Lin *et al.* 2007; Fathi and Hanifi, 2007).

The antimicrobials present in the test are allowed to diffuse out into the medium and interact in a plate freshly seeded with the tested organisms. The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Petri plates containing 20 ml Muller Hinton medium were seeded with 24 hours culture of bacteria strains. Wells were cut, and 20 microliters of the sample were added. The plates were then incubated at 37 ^{0}C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

4. RESULTS & DISCUSSION

4.1 Phase Analysis

XRD patterns of Silver and Titanium doped Hydroxyapatite with various concentrations were shown in fig. 1. All the patterns were verified with the standard value. There are in good agreement with the standard JCPDS value with card number 09-0432. There is only a small change in the increase or decrease in the intensity due to the addition of the dopants (Masato Wukamura *et al.* 2003; Anmin Hu *et al.* 2007).

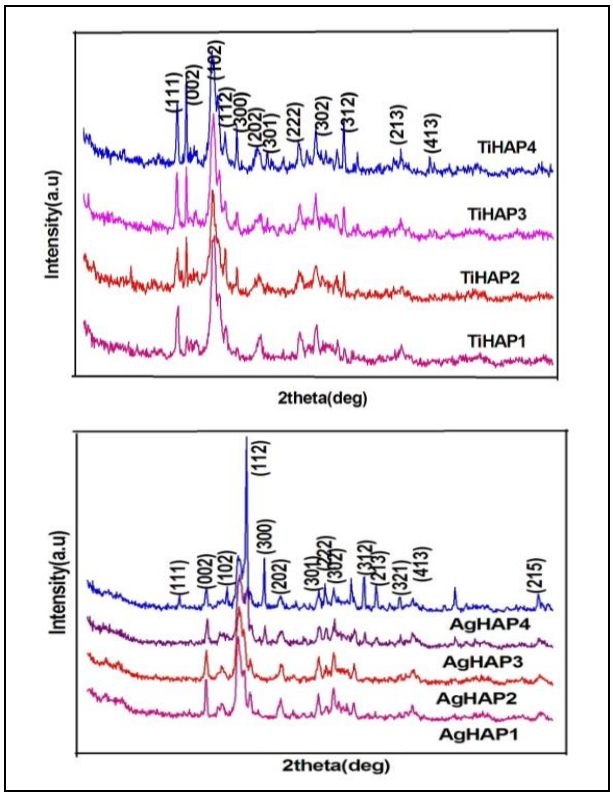

Fig. 1: XRD Spectra for Silver and Titanium doped HAP.

The crystallite size ranges from 68-14nm for Silver doped Hydroxyapatite and 24-19nm for Titanium doped nano Hydroxyapatite. Thus the crystallite size decreases with an increase in the concentration of the dopant. The lattice parameter and unit cell volume of the hexagonal structured Ag-doped HAP decreases with an increase in the concentration, whereas for Ti-doped HAP, it increases with the increase in the concentration. The crystallite size, lattice parameter, and unit cell volume of Ag and Ti-doped HAP were tabulated in table 1.

4.2 Elemental Analysis

EDS spectrum for Ag and Ti-doped HAP are shown in fig. 2&3. EDS spectrum confirms the presence of Calcium, Phosphor, Oxygen, and Silver for Ag-doped HAP and Calcium, Phosphor, Oxygen, and Titanium for Ti-doped HAP (Haresh M. Pandya and Anitha, 2015; Rameshbabu *et al.* 2007). The substitution of dopant in the place of Calcium was clearly proven in this study. The composition of Silver and Titanium gets increases as the concentration from 0.1 to 1.5wt% gets increases.

Table 1. XRD calculation for Ti and Ag-doped HAP.

Sample	Crystallite Size	Lattice Parameter		Unit Cell Volume
		a	c	
AgHAP1	68nm	9.6514	6.8927	1662.29
AgHAP2	62nm	9.6232	6.8778	1649.01
AgHAP3	51nm	9.5026	6.8395	1598.98
AgHAP4	14nm	9.3070	6.1806	1385.96
TiHAP1	24nm	9.1295	6.7466	1455.83
TiHAP2	22nm	9.1645	6.7952	1477.58
TiHAP3	21nm	9.1950	6.8064	1489.89
TiHAP4	19nm	9.1967	7.0647	1547.03

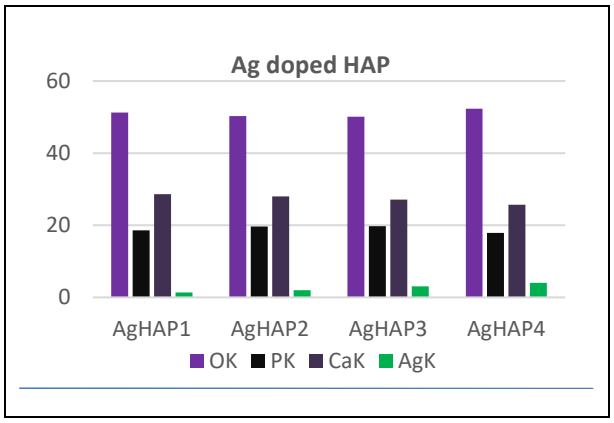

Fig. 2: EDS spectra for Ag-doped HAP.

4.3 In Vitro Analysis

Antimicrobial activity is a necessary step for the suitability of use age in the medical field. The zone of inhibition for Ag and Ti-doped HAP using the Agar well diffusion method was tabulated in table 2. The Zone of inhibition for Ag-doped HAP against *Staphylococcus aureus* bacteria ranges from 9-14mm and 14-16mm for *Escherichia coli* bacteria (Marcos Diaz *et al.* 2009; Anitha and Haresh M. Pandya, 2014; Kalaiselvi and Mathammal, 2016). Similarly, for Ti-doped HAP 14-27mm for *Staphylococcus aureus* bacteria and no zone of inhibition for *Escherichia coli* bacteria. Antimicrobial images of Ag and Ti-doped HAP were shown in fig. 4 & 5.

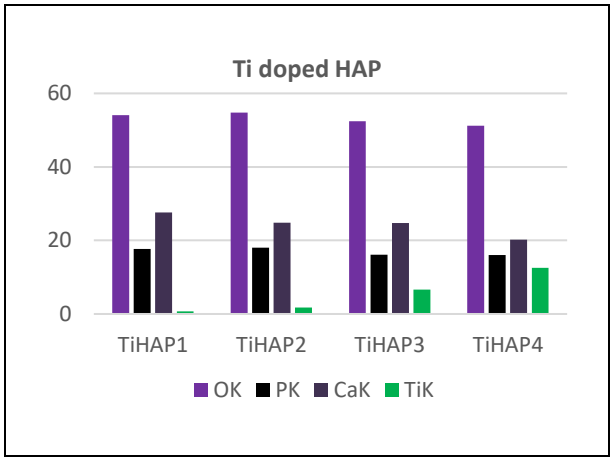

Fig. 3: EDS spectra for Ti-doped HAP.

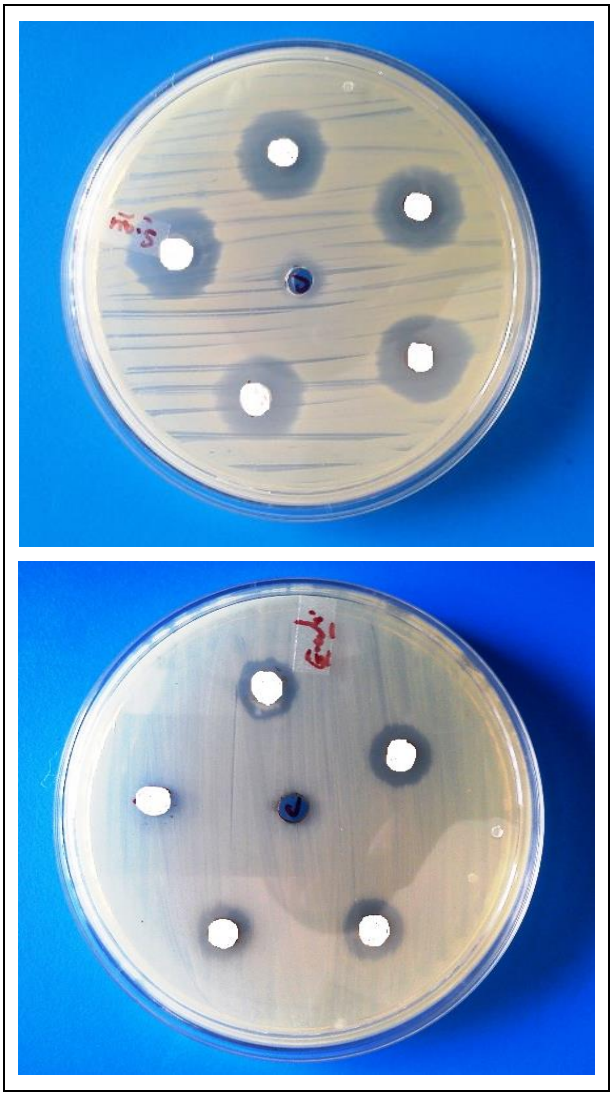

Fig. 4: Antimicrobial Image of Ag-doped HAP for *Staphylococcus aureus* and Escherichia *coli* bacteria.

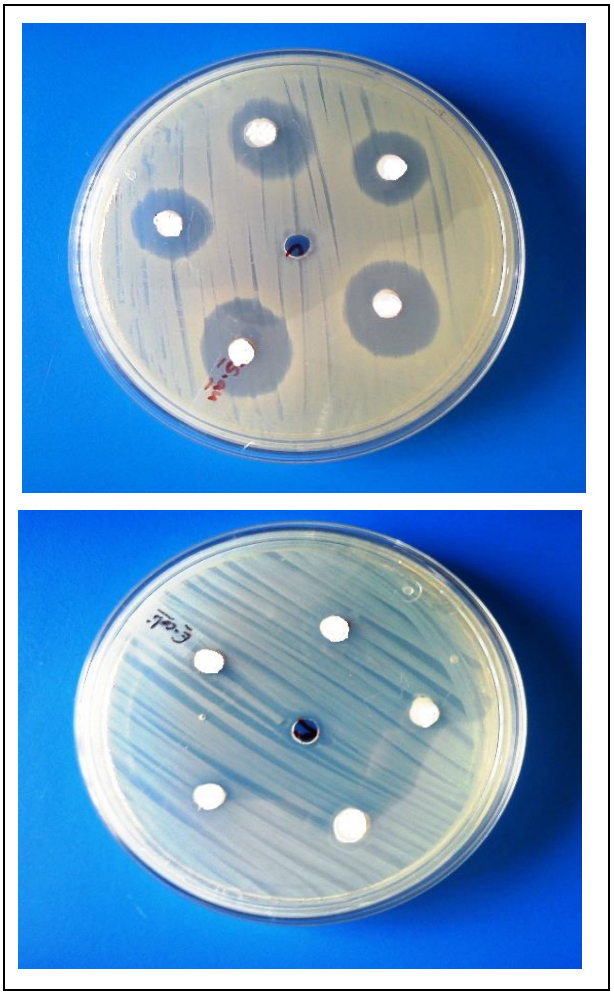

Fig. 5: Antimicrobial Image of Ti-doped HAP for *Staphylococcus aureus* and *Escherichia coli* bacteria.

Table 2. Antimicrobial activity of Ag & Ti-doped HAP

Sample	Zone of Inhibition in mm	
	S.aureus	E-Coli
AgHAP1	14	9.5
AgHAP2	14	12
AgHAP3	14.2	12.1
AgHAP4	16	14.2
TiHAP1	15	-
TiHAP2	16.3	-
TiHAP3	18	-
TiHAP4	21	-

5. CONCLUSION

XRD pattern for Ag and Ti Doped HAP matches well with the standard value. The crystallite size decreases with an increase in the concentration for Ag, and Ti-doped HAP where the lattice parameter and unit cell volume decrease with the increase in the concentration for Ag-doped HAP whereas it increases with an increase in concentration for Ti-doped HAP. The change in the increase or decrease in the intensity of the XRD pattern implies that the dopants were incorporated in the HAP but did not alter the structure of the HAP EDS confirms the presence of all elemental compositions. Thus the XRD and EDS prove that the prepared sample was Hydroxyapatite.

Antimicrobial activity for Silver doped Hydroxyapatite shows zones of inhibition for *Staphylococcus aureus* and Escherichia *coli* bacteria. But Titanium doped Hydroxyapatite shows the zone of inhibition for *Staphylococcus aureus* bacteria and no inhibition rate for *Escherichia coli* bacteria. Even though the Zone of inhibition rate for Ti-doped HAP is high compared to Ag-doped HAP, this Ag-doped HAP affects *Staphylococcus aureus* and *Escherichia coli* bacteria. Thus Silver doped Hydroxyapatite has high infection reducing tendency than Titanium doped Hydroxyapatite.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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