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Transition metal doped hydroxyapatites: from chemistry to antibacterial properties

Arjak Bhattacharjee[1a]

Department of Ceramic Technology, Government College of Engineering and Ceramic Technology, Kolkata 700010, W.B., India

Abstract. Musculoskeletal disorders are one of the leading causes of disability. Currently, 1.3 billion population across the globe are suffering from various musculoskeletal disorders (MSK) and it imposes a total of $ 136.8 billion annual burden to the US economy. Current treatment options consist of pain medication and surgical intervention by inserting implants and scaffolds on the affected site. Hydroxyapatite [HA, $Ca_{10}(PO_4)_6OH_2$] based implants are widely preferred for tissue engineering applications due to their compositional similarities with human bone. However, post-surgical infections on the implant surface often cause implant failure and need to be rectified by three times costlier revision surgery. The flexible crystal chemistry of hydroxyapatite allows transition metal substitution both in the cationic and anionic sites due to the ionic radius differences between calcium and first row transition metals. Transition metal substitutions incorporate exiting biological and antibacterial properties to HA. This paper will summarize the available literature reports which describe the crystal chemistry of first row transition metal incorporation in HA and the resulting antibacterial properties.

Keywords: Hydroxyapatite, transition metal doping, Antibacterial efficacy, crystal chemistry

1. Introduction and historical background

1944! It was the story of devastated Europe during world war two. A Jew young man was able to escape from the holocaust and took refuge in Institute of soil research to continue the studies of Apatite minerals which he had started in Scandanavia [1-3]. Figure 1is a tribute to that young man, named Victor Goldschmidt, who is known as the father of modern geochemistry. Apatites (A10(MO4)6X2) are a diverse family of materials which have attracted wide attention in the scientific community due to their broad application as pigments, catalyst, energy materials, materials for the remediation of hazardous waste and as a bio-ceramics [4-8]. The general chemical formula of apatite is A10(PO4)6X2 where the A cation can be an alkaline earth metal

[a] **Present address:** *School of Mechanical and Materials Engineering, Washington State University, Pullman, WA, USA*

[1] Email: arjak.ceramic16@gmail.com

Sr, Ca or Ba and the X anion can be OH^- ion or Cl^- ion, which represents hydroxyapatite, and chlorapatite, respectively. V. Goldschmidt was the first one in history who explained apatite structures by thorough study of deposits in Scandinavia. That time apatite structure was represented by the general notation [Ca4][Ca6][(PO4)6][F]2, which represents that it is formed CaO6 units and those are joined together with PO4 networks which make a structure similar to that of a hexagonal honeycomb extending in the c direction [9-11].

Why could bone take up fluorine selectively even from dilute solutions? This was one of the most wondering questions to scientists of that era. A careful examination of apatite structure gave a satisfactory answer to this question and the following conclusion was reached based on the observations and experimental results. (1) apatite structure is a channel structure with partitions having corner-connected CaO_6 and PO_4 polyhedra; (2) bond-length criteria are fulfilled with filling of these channels by Ca and anions (OH^-, F^-); and (3) change in channel length happens with variation of ionic radii. Keeping these factors in consideration, better stability of Fluroapatite can be explained by the best fit of fluorine atom in apatite structure which is the basic science behind the treatment of dental cavities by altering fluorine content [12,13].

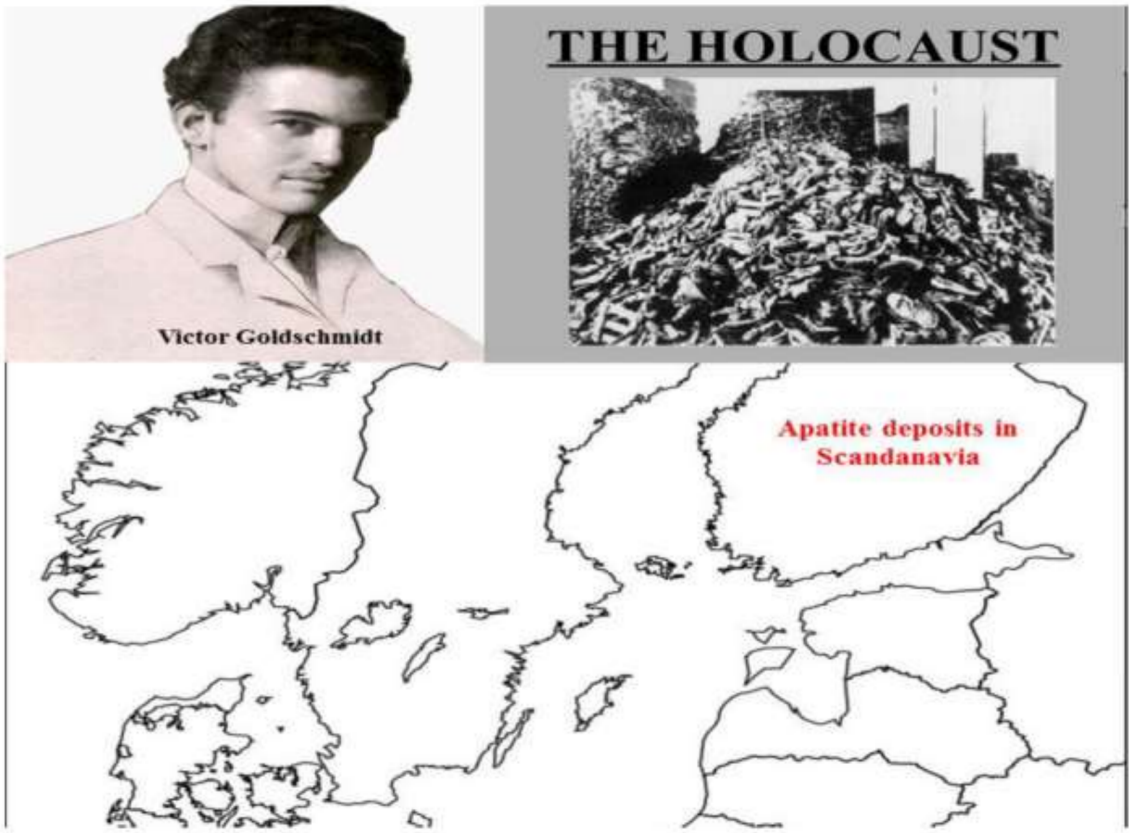


Fig. 1: History of research on apatite crystal chemistry

Calcium hydroxyapatite [HA, $Ca_{10}(PO_4)_6OH_2$], an important member of the apatite family is a suitable ceramic for orthopedic, dental, and maxillofacial repair [14–16] due to its hexagonal structure and Ca/P ratio of 1.67, that identical to bone apatite. To tailor the crystal chemistry of HA for specific applications (bioceramic, catalyst, pigments etc.), doping with several transition metals are a unique option [17,18]. Doping of HA by metals like Ag, Cu and Zn also improves its biological properties. However, to understand the doping mechanism of cations in HA structure and its effect on chemical and biological properties, it is very important to demonstrate the effect of processing techniques via solid state route, wet chemical routes and heat treatment cycleon doping mechanism [19-20].

2. The open debate about transition metal doping site in hydroxyapatite

Several contradictory reports are presented in literature regarding doping site of transition metals in HA structure. It can be concluded from the available literature that when sintering temperature is above 900 °C, Zn^{+2} ions not simply substitute the Ca site of HA instead they reside in the OH hexagonal channel [21]. Similar phenomena have been observed in case of Cu after heat treatment and quenching from 1100 °C [22]. But now the question comes, *why is this happening? Is it possible to incorporate transition metals in OH channel instead of Ca site?* A detailed investigation about at ionic radii parameters of concerned ions indicate that there is a large difference of ionic radii value (~25%) between Ca^{2+} and transition metal cations as listed in table 1. This is the primary reason why transition metals are not always substitutes in the cationic site of HA [23].

Element	Coordination no.	Ionic radius (Å)
Ca^{+2}	8 (octaheadral)	1.00
Mn^{+2}	8 (octaheadral)	0.67
Co^{+2}	8 (octaheadral)	0.65
Ni^{+2}	8 (octaheadral)	0.69
Cu^{+2}	8 (octaheadral)	0.73
Zn^{+2}	8 (octaheadral)	0.74

Table 1: Ionic radius comparison of Ca^{+2} and first row transition metals

Correlation of heat treatment parameters with Rietveld refinement data of doped samples revealed that below a temperature of 700 °C, Zn^{+2} incorporation happens in b-TCP structure of the biphasic calcium phosphate which leads to unit cell volume contraction. When the sintering temperature is above 900 °C, then b-TCP becomes unstable and Zn^{+2} incorporation happens in the interstitial 2b wyckoff position instead of the Ca site which leads to unit cell volume expansion of doped hydroxyapatite [24]. A similar observation has been found for Cu^{+2} when the sintering temperature reaches above 1100 °C [25]. However, Cu^{+2} show a tendency of reduction to Cu^{+1} when heated at that temperature. No such observation (tendency of reduction) had been found in case of Zn^{+2}. Figures 2 represents the crystal structure of pure HA with different crystallographic sites.

3. Effects of Copper doping in HA

3.1 Effect on HA crystal chemistry

As discussed in the earlier sections, Cu can go both in Ca site of HA structure as well as in OH channel depending on the preparation techniques. According to the available literature, if copper doping in HA is performed in solid state reaction route followed by quenching from 1100 °C, Cu goes in OH channels retaining the hexagonal crystal structure with space group *P*63/*m* [21]. Logically it can be concluded from table 1, due to the smaller size of Cu^{+2} (~0.73 Å) in comparison to Ca^{+2} (~1.00 Å), unit cell volume reduction will happen for substitution of Cu^{+2} in Ca^{+2} position of HA structure. However, opposite observation was found after heat treatment and quenching from 1100 °C (it leads to incorporation of Cu in OH channel of HA instead Ca site). substitution in OH

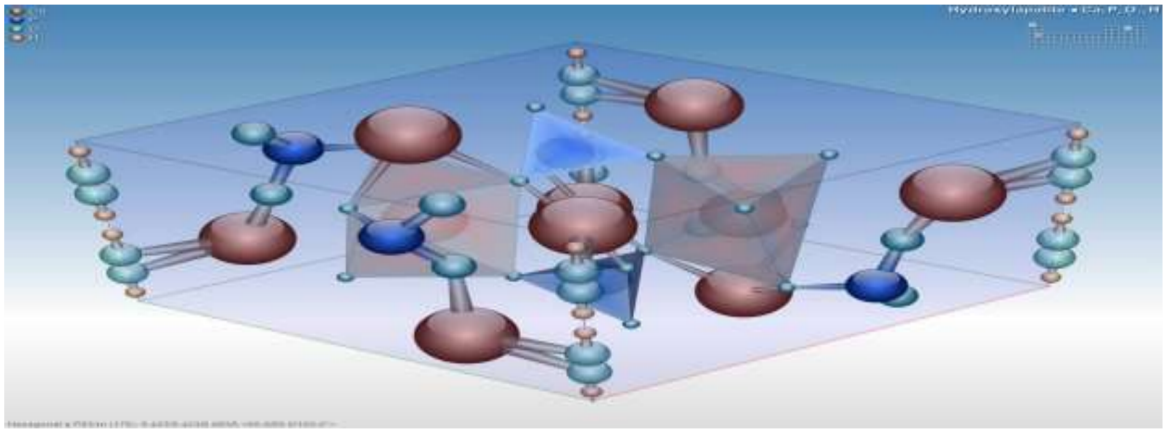


Fig. 2: Crystal structure model of HA [26]

channel. A previous work reports the Xray absorption spectroscopy (XAS) result of Cu doped HA containing Cu in OH channels. It is previously reported that heat treatment over 1100 °C leads to reduction of Cu^{+2} to Cu^{+1} (peak around 8990 eV) [21]. Due to the mixed oxidation states of Cu in HA, it results in O-Cu-O chromophore with bright pink color [27]. The presence of these chromophores makes Cu doped HA, a potential candidate for use as an inert non-toxic pigment that is stable even at high temperatures.

3.2 Effects on biological properties of HA

Due to its involvement in several metabolic processes, Cu is an essential micronutrient of several living organisms. However, due to its ability to form reactive oxygen species, the cytotoxicity limit of Cu is an important factor that needs serious attention [28]. The antimicrobial properties of copper were known since ancient times and examples can be found in ancient Indian literatures where uses of Cu as drinking water sterilizing metal has been reported [29.30]. Figure 3 represents various uses of Cu in different parts of India.

Fig. 3: Uses of Cu in different parts of India

There are various competing theories to explain the antibacterial mechanism of Cu. Although no theory can explain it with perfection [30]. The most accepted theory is that Cu causes reduction potential change of bacterial cell membrane followed by its penetration inside the cell. Cu has the ability to forms reactive oxygen species as well as it can bond with the thiol, amine and carboxyl groups present inside the cell. Combinations of these two effects cause cell

membrane dysfunction which is the major cause of cell death. Several researchers also demonstrated that Cu forms bond with the DNA of bacterial cell causing DNA damage and leads to cell death [31,32]. Variable oxidation states of Cu are also an important factor to determine its antimicrobial efficiency. It has been reported that Cu^{+1} is more toxic to bacteria than Cu^{+2} [21]. A previous work also reports that due to leaching of Cu from HA structure (~0.082 ppm to 0.73 ppm in PBS solution for a doping level of 1 wt. % and 10 wt. % respectively), Cu doped HA shows zone of inhibition ranging from 0.2 cm to 0.5 cm [33]. However, most antimicrobial studies of Cu doped HA were performed after substituting Cu in Ca site of HA and confirming Cu^{+2} oxidation state. One recent study concludes that Cu can also show antibacterial property after getting incorporated inside the hydroxyl channels.

4. Effects of Zinc doping in HA

4.1 Effect on HA crystal chemistry

Available literature on Zn doped HA suggests that without any heat treatment operation in wet chemical route, Zn^{+2} is incorporated in Ca site of HA [24]. However, heat treatment at 1100 °C results incorporation of Zn^{+2} in the hexagonal channel of HA structure at the 2b Wyckoff site and forms O–Zn–O bonding [22], which results to a solid solution of the formula $Ca_{10}Zn_x(PO_4)_6(OH)_{2-2x}O_{2x}$. Incorporation of Zn^{+2} in OH channel happens due to the more stable HA phase at 1100 °C thanb-TCP. Insertion of Zn^{+2} is similar to that of Cu^{+2} but Zn^{+2} does not show any change of oxidation states as shown for Cu.

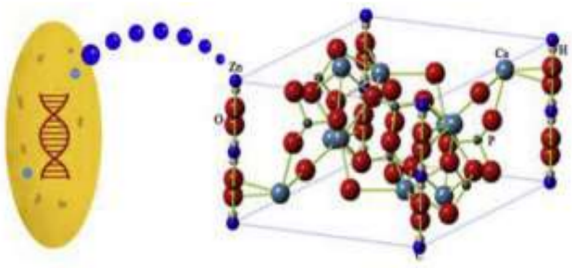
4.2. Effect of Zn doping on Biological properties of HA

It also been proved that Zn^{+2} shows antimicrobial properties against a wide range of bacteria (*S. aureus, E. coli*, *S. epidermidis*) and other microorganisms (*c. albican*). Most studies of Zn doped HA have been performed after substituting Zn in Ca site of HA structure [34]. Nowdays it is of utmost importance to understand the effect of Zn substitution site on the antibacterial efficiency of Zn doped HA. One recent work report that Zn substitution in the OH channels does not show any antibacterial effects against E. coli and S. aureus due to restricted Zn leaching by the formation of O-Zn-O bonding. Figure 4 represents the antibacterial mechanism of Zn doped hydroxyapatite after substitution in different doping sites.

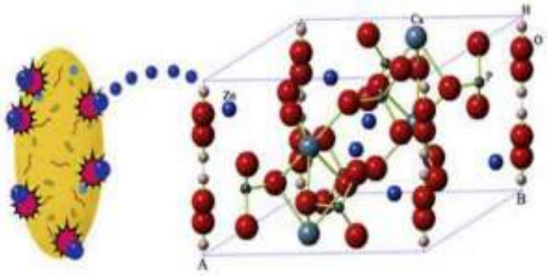
5. Summary and future trends

This paper summarizes that, (1) The substitution site of Cu and Zn in HA structure can be altered by altering the processing parameters (heat treatment cycle, doping composition in solid state and wet chemical processing route etc.). For doping Cu in HA by solid state route, there is a confirmation that Cu goes into the HA channel after heat treatment. (2) Most of the antibacterial

Cu doped HA are prepared by wet chemical route confirming the substitution of Cu in Ca site of HA. Antibacterial efficiency of Cu substituted HA [Cu in OH channel of HA] prepared by wet chemical route also demonstrates antibacterial efficacy. In contrast, the Zn doped HA shows antibacterial efficacy only when Zn replaces the Ca site of the HA. (3) Zn can be doped in HA by wet chemical route and there is evidence to confirm that incorporation of Zn in OH channel of HA structure happens heat treatment at 1100 °C, but this substitution leads to restricted Zn leaching and no antibacterial efficacy due to the formation of O-Zn-O binding. Future works in this field can be directed to (a) to test the cytotoxicity of Cu and Zn doped HA with human osteoblast cells for monitoring the maximum safety doping limit of Cu and Zn in HA (b) to find out which method of synthesis (between solid state and wet chemical) is better for production of less toxic Cu and Zn doped HA (c) to characterize the potential of prepared materials for using as a bone implant by in vivo testing in mouse models (d) based on the results of in vivo testing, patient specific scaffold can be prepared by the doped HA powder, using 3D printing technique (e) after analyzing 3D printing results carefully, obtained scaffolds can be utilized for low load bearing defect repair such as spinal fusion.



Restricted bacterial killing after thermal treatment and Zn substitution in OH channel



Enhanced bacterial killing after Zn substitution in Ca site of HA

Fig.4.

Schematic representing enhanced bacterial killing after Zn incorporation in Ca site of HA, whereas, Zn substitution in the OH channels leads to no bacterial killing due to restricted Zn leaching by the formation of O-Zn-O bonding [22].

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