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 REVIEW

A Review on Biomimetic Hydroxyapatites for Biomedical Applications

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Abstract

This review provides an overview of characteristics on nano hydroxyapatite, HAP, with an emphasis on the improvement of its properties for biomedical applications, on the basis of our original research in the context of the state of the art. We consider the biological effects inspired by the role of HAP and physiological essential elements in the metabolism, development and regeneration of bone. The employment of multiple strategies to tackle the multi-substitution in the HAP lattice, resulting in multi-substituted hydroxyapatites, ms-HAPs, is likely to be accompanied by improvements of HAP properties as biomimetic hydroxyapatites for bone substitutes and dental cements for biomedical applications. The obtained nanostructured innovative biomaterials are briefly characterized by various physical and chemical methods. Due to the excellent capacity of HAP and ms-HAPs to adsorb various ions and biomolecules, like antimicrobial agents, they are major carriers for infection therapy. Also, we demonstrated that HAP is very efficient for the heavy metal removal from wastewater, such as industrial and mine water.

Keywords: hydroxyapatite, substituted hydroxyapatites, XRD, thermal treatment, morphological characterization.

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Introduction

Natural bone is the only tissue that can regenerate without the formation of any scar tissue due to osteoclasts, osteoblasts and osteocytes, which are important in bone function. Osteoclast cells absorb old bone lining the medullary cavity and osteoblasts, by intramembranous ossification, produce young bone tissue under the periosteum.

This modeling process takes place during bone growth. Throughout life, the bone undergoes a remodeling process, in which the absorption of old or damaged bone tissue takes place at the same interface where osteoblasts produce new bone to replace the resorbed one. About 5-10% of bone mass is replaced annually, a high percentage of which is due to physical exertion, injuries, etc. With the inability to produce optimal bone mass, excessive bone resorption, or an inadequate response to increased resorption during the bone remodeling process, the skeleton acquires a fragility characteristic of osteoporosis [1]. This can be aided with the use of various materials, the majority based on calcium phosphates.

Among these phosphates, synthetic hydroxyapatite (HAP, $C_{10}(CaPO_4)_6(OH)_2$) has been the subject of numerous studies due to its biocompatibility, bioactivity and chemical similarity to the inorganic phase in natural bone (around 60 to 70% hydroxyapatite) and its thermodynamic stability in body fluids [2, 3].

Hydroxyapatite is increasingly used for biomedical applications, especially concerning the repair of bone defects, coatings on metallic implants, dental applications and drug delivery systems [4-22].

Of course, all intrinsic properties of stoichiometric HAP can be improved upon by incorporating several divalent metal ions into its structure, such as silicon [23, 24], strontium [25, 26], zinc [27-29] and magnesium [30, 31].

Thus, hydroxyapatite also gains the properties of the substitution ions, where Si is crucial in bone calcification and is known to stimulate osteoblasts [23, 24]; Sr reduces bone resorption and improves bone formation [9]; Mg plays an important role in skeletal development [30] and Zn has a prominent role in biological functions, having the ability to stimulate bone regeneration and increase bone density while reducing bone loss [27, 29, 32].

Due to the high stability of the hydroxyapatite structure, a large part of literature focuses either on a single substitution [33-84] or on co-substitutions [85-102]. Recently, triple and quadruple substitutions are also presented using various physiological essential elements [103-109].

Considering all of the above, this review focuses on the improvement of hydroxyapatite properties due to its substitution with various elements resulting in biomimetic hydroxyapatite and its medical applications, with an emphasis on our research.

Several types of hydroxyapatite were synthesized through a wet precipitation method by using a pilot equipment [24, 26, 29, 110], namely unsubstituted hydroxyapatite, HAP1; complex hydroxyapatite (triple-substituted with Mg, Zn and Si), HAP2; complex hydroxyapatite with 5 wt% Sr (tetra-substituted HAP), HAP3; and complex hydroxyapatite with 10 wt% Sr, HAP4; all compositions are shown in Table 1.

Table 1. Chemical compositions of hydroxyapatite samples, where HAP = HAP1; HAP-1.5%Mg-0.2%Si-0.2%Zn = HAP2; HAP-1.5%Mg-0.2%Si-0.2%Zn-5%Sr = HAP3; HAP-1.5%Mg-0.2%Si-0.2%Zn-10%Sr = HAP4

Substitution element (wt%)					Theoretical formula
Sample	Mg	Zn	Si	Sr	
HAP1	0	0	0	0	$Ca_{10}(PO_4)_6(OH)_2$
HAP2	1.5	0.2	0.2	0	$Ca_{9.36}Mg_{0.61}Zn_{0.03}(PO_4)_{5.93}(SiO_4)_{0.07}(OH)_{1.93}$
HAP3	1.5	0.2	0.2	5	$Ca_{8.76}Mg_{0.63}Zn_{0.03}Sr_{0.58}(PO_4)_{5.93}(SiO_4)_{0.07}(OH)_{1.93}$
HAP4	1.5	0.2	0.2	10	$Ca_{8.12}Mg_{0.65}Zn_{0.03}Sr_{1.2}(PO_4)_{5.93}(SiO_4)_{0.07}(OH)_{1.93}$

All synthesized hydroxyapatites were characterized both in their precipitated paste form and powdered form after sintering at different temperatures. This is of particular importance due to the different applications they might have.

1. Hydroxyapatite pastes

The hydroxyapatite paste is a suitable bone substitute in filling bone defects in dentistry and orthopedics, leading to a minimally invasive surgery. Also, HAP as paste can be added to toothpaste composition for a good remineralization of tooth enamel or be used in 3D printing to create ceramic scaffolds with predetermined characteristics [5, 111].

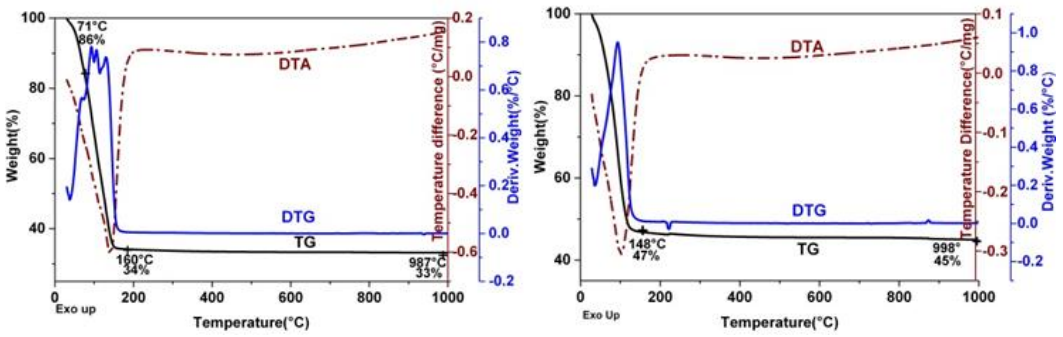

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Figure 1. Thermal curves (TG, DTG, and DTA) for 2 pastes: a) HAP1; b) HAP4, where TG is the thermogravimetric curve; DTG is the first derivative of TG curve; DTA is the differential thermal analysis curve

For biomedical applications requiring hydroxyapatite pastes, their thermal stability and water content are crucial parameters. Therefore, the representative thermal behavior of two pastes HAP1 and HAP2, with different compositions given in Table 1, are presented in Figure 1 [112]. The pastes, that were previously

stored in airtight containers, were analyzed through heating, up to 1000 ^{o}C in an air atmosphere.

Figure 1 presents the thermal curves for stoichiometric hydroxyapatite (a) and multi-substituted hydroxyapatite pastes (b) for HAP-1.5%Mg-0.2%Si-0.2%Zn-10%Sr. The TG curve records the mass loss with the increase in sample temperature. DTA curve measures the temperature difference between the hydroxyapatite sample and the reference, while being subjected to an identical thermal treatment.

The thermal behavior of both HAP and ms-HAP pastes is similar in the temperature range of 30-1000 ^{o}C. The difference between them comes in the form of mass loss, around 66% for stoichiometric HAP and 53% for the multi-substituted one. For both samples, the largest percentage of mass loss happens between 30 ^{o}C and 200 ^{o}C temperature range and can be attributed to water loss due to its high content in pastes. The DTA data indicate an endothermic transformation for the two samples at around 160 °C. From 200 to 1000 ^{o}C, the mass loss is negligible, being around 1-2% for both samples. Above 200 °C up to 1000 °C the thermogravimetric curves became parallel to one another and with temperature axis.

Further, Figure 2 presents the SEM images for both samples, namely stoichiometric and multi-substituted hydroxyapatite pastes. The morphology of the two pastes is somewhat similar, with agglomerated structures comprised of spherically-shaped particles.

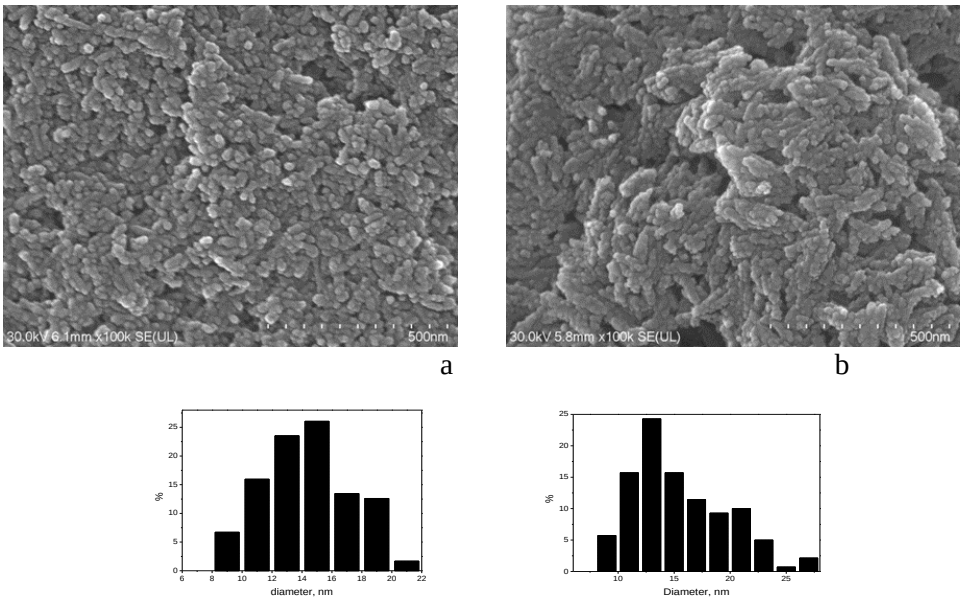

Figure 2. SEM images and histograms for two pastes: stoichiometric HAP1 (a) and multi-substituted HAP4 (b).

The average diameters of nanoparticles are around 14 nm, for both pastes. However, the pastes have a slightly different particle arrangement. This can be explained through the different water content present in these pastes as well as their chemical compositions. All HAP and ms-HAP nanoparticles are fairly well defined but do have a tendency to form clusters.

2. Hydroxyapatites powder

Powder is probably the most used form of synthetic hydroxyapatite. Although like pastes, powders can also be added as a remineralization agent in toothpastes or even bone cements, the focus has been bone implantology. However, in order to create adequate implants or scaffolds of certain dimensions several factors have to be taken into account, the most crucial being the stability of the powder at higher temperatures that are required when forming ceramics.

Figure 3 present the TG curves for hydroxyapatite powders both in their lyophilized form with lower crystallinity (a) and thermally treated at 300 ^{o}C, with a higher crystallinity (b).

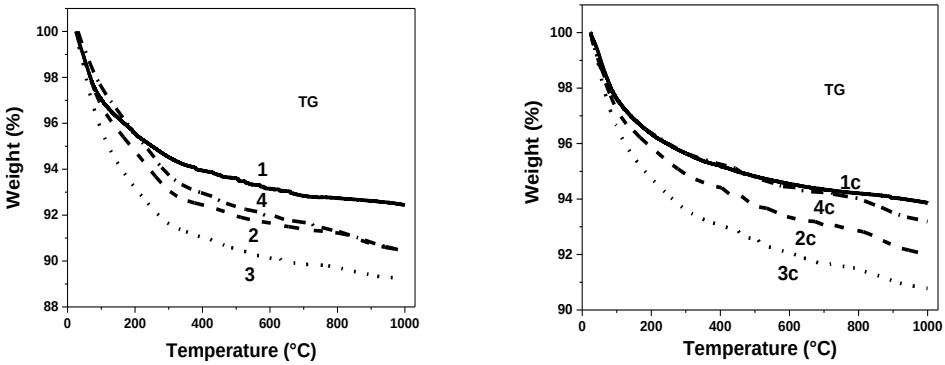

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Figure 3. TG curves for lyophilized (HAPs) powders (a): HAP1 (1); HAP2 (2); HAP3 (3); HAP4 (4) and lyophilized (HAPs) powders, calcined at 300 ^{o}C for 1h (b): HAP1 (1c); HAP2 (2c); HAP3 (3c) and HAP4 (4c).

The uncalcined powders all have a similar behavior up to 1000 ^{o}C, where three series of mass loss can be observed, namelly between 25-200 ^{o}C, 200-800 ^{o}C and 800-1000 ^{o}C. This is in accordance with the behavior of the previously discused pastes with the same compositions. This can also be said for the thermally treated powders in Figure 3, the TG curves being even arranged in the same order as the untreated samples. The only major difference here between the lyophilized HAP samples and the ones lyophilized and then subjected to a thermal treatment at 300 ^{o}C is the lower mass loss for the latter. This can of course be

attributed to the higher content of water in the lyophilized powders, water that is lost in the furnace for the thermally treated hydroxyapatites. Nevertheless, these HAPs are quite stable at 300 ºC.

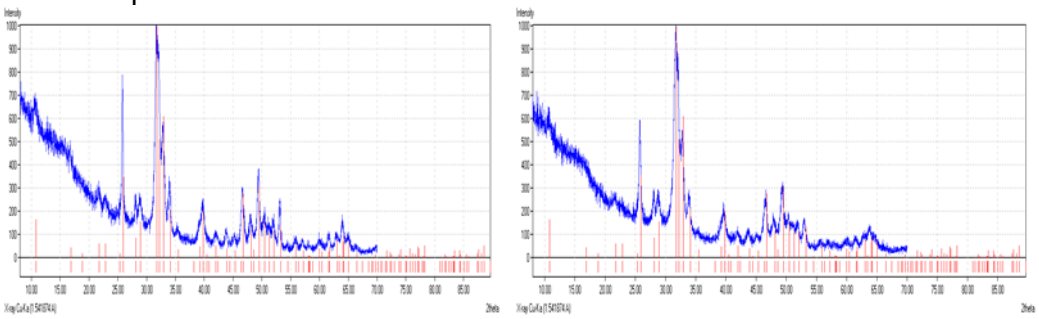

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Figure 4. XRD patterns HAP1 (a) and HAP4 (b) that were lyophilised and thermally treated at 300 ºC for 1h; compared with standard PDF patterns 74-0566 of stoichiometric HAP (red lines).

The XRD patterns for stoechiometric HAP1 and multi-substituted HAP4 samples that were lyophilized and thermally treated at 300 ºC are given in Figure 4. As it can be observed, for HAP1 all peak positions are in agreement with those for stoechiometric hydroxyapatite (standard PDF patterns 74-0566). This is also the case for HAP4 but with the presence of a slight lower angle shift in the 2θ positions. This can of course be explained by the Sr substitution into the hydroxyapatite lattice.

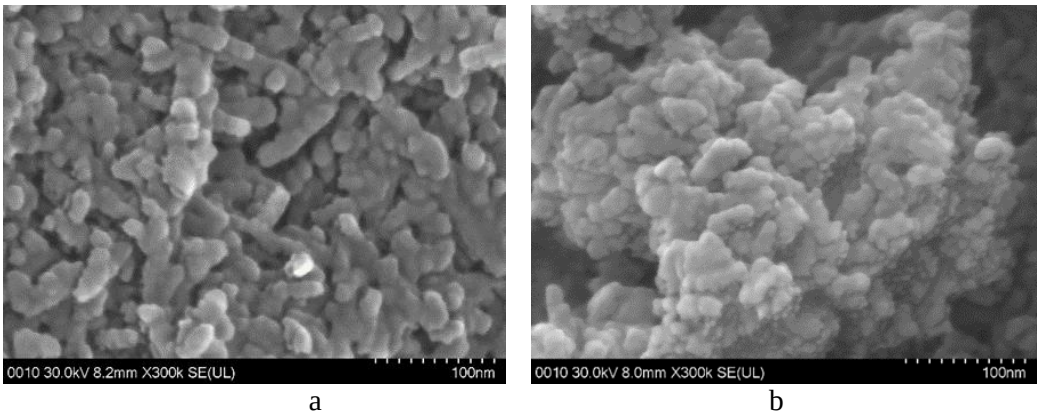

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Figure 5. SEM images for stoichiometric HAP1 (a) and multi-substituted HAP4 (b) powders, lyophilised and calcined at 300 ºC for 1h

The morphology of particles was also studied in the case of HAP powders after calcination at 300 ºC for a 1h, This is presented in Figure 5 for HAP1 (a) and HAP4 (b). A porous structure can be distinguished, with a slightly different

packing of particles for HAP1 as opposed to HAP4. This can be of course dependent upon the composition of the hydroxyapatite.

3. Applications of hydroxyapatites

Due to its properties, synthetic hydroxyapatite holds a large range of applicability either as a unique material or as part of composites. Although, the major focus in hydroxyapatite research has been related to bone substitutes and implants, in the following some other applications of HAP will be presented.

3.1. Hydroxyapatites used as carriers for antibacterial agents

Probably one of the most common further processing of hydroxyapatite is its sintering into a type of ceramic that could be further used as orthopedic implants. Taking into account that, although the risk is relatively low (approx. 5%), orthopedic surgery infections do happen, and there is a need for a material or a composite that could tackle multiple issues at the same time. A more localized, postoperative administration of agents with antimicrobial properties would significantly reduce both the cost and duration of treatment. However, a prevention of such infections would be much more preferable.

Taking this into account, in the following, some results concerning hydroxyapatite ceramic scaffolds under the form of disks, loaded with antimicrobial agents will be presented. Two types of stoichiometric HAP with different degrees of crystallinity (one calcined at 450 ^{o}C and another at 850 ^{o}C) were pressed into disks and then, further sintered into ceramics at a higher temperature (900^{o}C). The prepared ceramics were characterized in terms of porosity (using the Archimedes method).

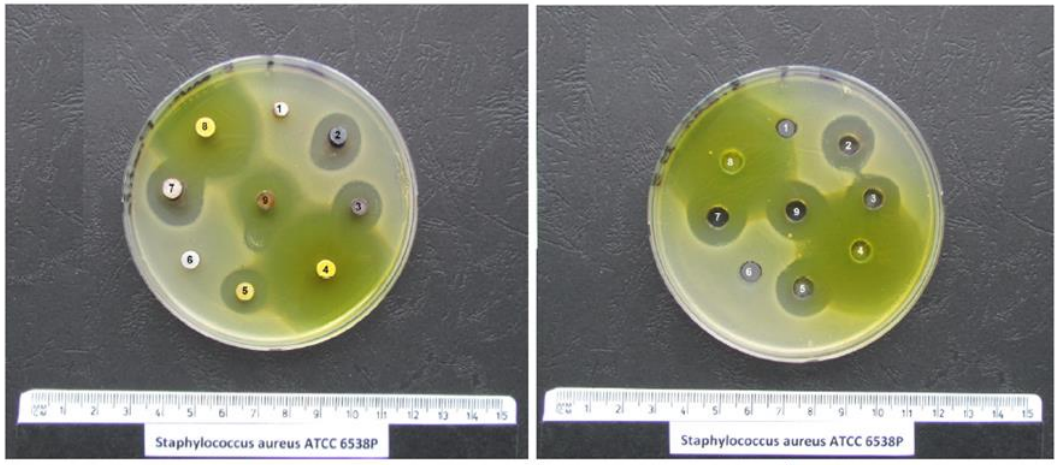
Here, porosity is an important parameter when it comes to further loading the ceramics with active substances, as it increases the specific surface allowing solutions to penetrate inside the ceramic and not just linger on the surface. Table 2 presents the apparent porosity for the ceramic disks obtained from the 2 hydroxyapatite powders, HAP1-450 and HAP1-850. It can be observed that in these cases the porosity is quite high.

All ceramics were then loaded with silver ions (by means of $AgNO_3$) and nitroxoline (5-nitro-8-hidroxichinoline). While silver ions are already known to have an antibacterial property, nitroxoline was fully chosen for its capacity to combat biofilm infections. The loaded ceramic samples were tested for their antimicrobial activity using a Staphylococcus aureus strain, one of the most prevalent causes of orthopedic infections.

Table 2. Apparent porosity of HAP1-450 and HAP1-850 ceramic disks. Hydroxyapatite was calcined at 450 ^{o}C (HAP1) and 850 ^{o}C (HAP2)

Ceramic disk	Sample	Apparent porosity P_a (%)
HAP1-450	1	47.67
	2	47.01
	3	54.73
HAP1-850	1	46.39
	2	48.12
	3	44.56

Figure 6 shows the inhibition areas after 24 hours for both the loaded disks and for the solutions in which the disks were submersed in, $AgNO_3$, nitroxoline (NHQ) or a combination of both.



a. b.

Figure 6. Inhibition areas for Staphylococcus aureus in presence of ceramic disks loaded with antimicrobial solutions (a), or in presence of antimicrobial solutions in wells (b). Samples are numbered as follows: 1 for HAP1/water and 6: HAP2/water, each as control; 2 and 3: HAP1/$AgNO_3$; 7: HAP2 /$AgNO_3$; 4: HAP1/NHQ; 8: HAP2/NHQ; 5: HAP2/NHQ + $AgNO_3$; 9: HAP1/NHQ + $AgNO_3$.

Table 3 better presents the exact inhibition area in mm. The control samples of HAPs in water (1 and 6) do not produce any antibacterial effect as opposed to

the samples loaded with nitroxoline (4 and 8) which produce the highest one. This is comparable to the results related to the individual solutions (Figure 6b). Samples 2, 3 and 7, namely HAPs loaded with just silver ions produce a smaller inhibition area when compared to their counterpart solutions. This can be explained by the interaction of Ag^+ with the ceramic disk, leading to a slow release of the silver ions.

Table 3. Inhibition areas for the samples presented in Figure 6. Labels are the same as in Figure 6.

Sample	**Inhibition areas (mm)**								
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***
Disks	-	15	15	>30	14	-	17	>30	18
Solutions	-	18	18	>30	17	-	18	>30	18

Conversely, samples 5 and 9 corresponding to the ceramic disks loaded with both nitroxoline and silver ions lead to inhibition areas smaller than that of those loaded with nitroxoline (3 and 8). However, a comparative result is shown between samples 5 and 9 and those with just the silver ion solution. This can be explained by the fact that the silver ion solution was the last one the ceramic disks were in contact with.

This study is important as it offers a different approach to administrate antimicrobial agents against orthopedic infections. By loading these agents even in low concentrations on the implant at the surgery would prevent a potential infection.

3.2. Dental cements

Hydroxyapatite has been the objective of many studies regarding dentistry. Its ability to promote osteoconduction, bonding with teeth and forming a hermetic seal, make it an ideal material for endodontic purposes. Its bioactivity is quite close to the crystalline and amorphous phases present in the structure of enamel thus, many studies have analysed its addition to certain fillers [113]. Also, Portland cement has long since been employed as a dental material due to its ability to set at a physiological temperature combined with its low cost. Previous studies have proven this cement to be biocompatible [114-117] and its main component, tricalcium silicate to be able to induce cell proliferation and hydroxyapatite build-up [118].

Considering that commercial endodontic cements have quite a wide range of setting times, anywhere from a few minutes (2-3 minutes for EndoChe Zr) to a few hours (4h for Trioxident) or even days and weeks (3+ weeks for Roth 801,

Elite) [119-122] there is a need to obtain a material that offers an adequate amount of work time for dentists but does harden in fast enough for patients.

With this in mind, in the following, some results regarding Portland cement enriched with hydroxyapatite will be presented. Two types of nano hydroxyapatite powder were used, a stoichiometric HAP and a Zn-substituted HAP, both synthesized through a wet precipitation method. Their compositions are presented in Table 4.

Table 4. Composition in stoichiometric HAP and Zn substituted HAP (HAP-Zn)

Nanomaterial	Zn (wt%)	Theoretic formula
HAP	0	$Ca_{10}(PO_4)_6(OH)_2$
HAP-Zn	5.0	$Ca_{9.217}Zn_{0.783}(PO_4)_6(OH)_2$

Each nanomaterial was mixed together with Portland cement following the compositions in Table 5. Sample S0 acts as the control sample and is formed only of Portland cement. The setting time was studied for all samples using a simple Vicat apparatus and a constant consistency water of 87 ml. The experiments were performed at 2 temperatures, namely at room temperature (22 ^oC) and at a temperature that mimics that of the human body (37 ^oC).

Table 5. Experimental compositions of endodontic cement

Material / Sample	HAP [wt %]	HAP-Zn [wt %]	Portand cement [wt %]
S0	-	-	100
S1	3	-	97
S2	-	3	97
S3	5	-	95

As it can be observed from Table 6 the setting time at room temperature for both endodontic samples containing 3wt% nanomaterial, namely stoichiometric HAP for S1 and Zn-substituted HAP for S2, decreases significantly when compared to the standard S0. In addition, a reduction to almost half of the initial Portland cement setting time can be observed for sample S3 containing 5 wt% stoichiometric HAP.

At 37 ^{o}C a similar behavior for all samples is encountered. Firstly, the setting time for S0 decreases by 15 minutes. Then, the same pattern as above can be applied with the lowest setting time, almost half of the standard, being observed for sample S3. This of course can be explained by the higher hydroxyapatite content when compared to samples S1 and S2.

Table 6. Setting time for Portland cement and experimental endodontic samples

Sample	Consistency water (ml)	Setting time (min)	
		22 °C	37 °C
S0	87	85	70
S1	87	55	45
S2	87	55	45
S3	87	45	35

While studies regarding the incorporation of hydroxyapatite into Portland cement with endodontic purposes can be improved upon, the presented work does show that HAP can be an important component of such mixtures, having the ability to lower the setting time. For dentistry, Zn substituted hydroxyapatite is specifically noteworthy when it comes to the property of Zn ions to exhibit antimicrobial effects against several bacterial and fungi strains.

3.3. Hydroxyapatite used for removal of toxic elements

Hydroxyapatite can be used in a variety of ways that are not related to implants or the medical field. Several studies report on HAP having an efficient heavy metal removing capacity from aqueous solutions [123-125]. However, while this is true, this property can depend quite heavily on the nature of heavy metal ions, their charge, concentration in which they are present, diameter, and of course on the properties of the water itself as in pH values or temperature [123, 126].

In the following some results concerning the removal of highly toxic heavy metals from wastewaters will be presented. This study holds a special importance as the wastewater was collected from Roșia Montană, a heavily polluted area. As it can be seen from Table 7, the initial concentration of some heavy metals (Al, Fe, Mn, Zn) way surpasses the limit values imposed by law for discarded wastewater. All adsorption experiments were carried out employing a

HAP/wastewater ratio of 10g/100ml. After 100 minutes, all concentrations of heavy metals were drastically reduced way under the imposed limit.

Table 7. Metal removal from mine wastewaters using nano HAP powder; results are given as mean ± confidence interval for n = 3 and 95% confidence level

Metal	Initial concentration c_0, mg/L	Final concentration c_e, mg/L	Removal degree, % [a]	Limit values for wastewater, mg/L
Al	313 ± 37	1.9 ± 0.6	99 ± 12	5 (STAS 9411-83)
Fe	92 ± 9	0.9 ± 0.6	99 ± 10	5 (SR ISO 6332-96)
Mn	190 ± 5	0.81 ± 0.48	100 ± 3	1 (SR ISO 6333-96)
Zn	14.4 ± 0.9	0.18 ± 0.08	99 ± 6	0.5 (SR ISO 8288:2001)

[a] the removal degree and its confidence interval were calculated by a concentration difference and pooled standard deviation

Overall, these experiments show that hydroxyapatite can be successfully used to treat heavily contaminated water for a significantly low cost. This is important as water is a crucial parameter in a sustainable development. However, also due to this development (manufacturing industries, mining, paper production plants, leather tanning, explosives, fertilizers, just to name a few) large bodies of water are contaminated with heavy metals. Easily able to enter the human body following the food chain [123, 127] these heavy metals lead to a large variety of health concerns with some being potentially deadly [123, 128, 129].

4. *In vitro* generation of hydroxyapatite by forsterite scaffolds

As it has been stated in the above material, synthetic nano hydroxyapatite closely mimics the inorganic phase of the human bone, thus making it the ideal material in orthopedics. Although, synthetic HAP has been used for orthopedic implants for some time, the viability of an implant is dependent on the various processes that take place at its interface with human bone.

This need for sustainable and rapid bone integration has led to attempts to approximate its composition with biomimetic hydroxyapatite. This can be achieved by using porous forsterite (Mg_2SiO_4) ceramics due to their ability to trigger the production of new bone by the human body. The bioactive property of forsterite highly depends on the ions in its composition, both essential minerals that have been proven to help the young bone remineralization and the gain of bone mass [130-136].

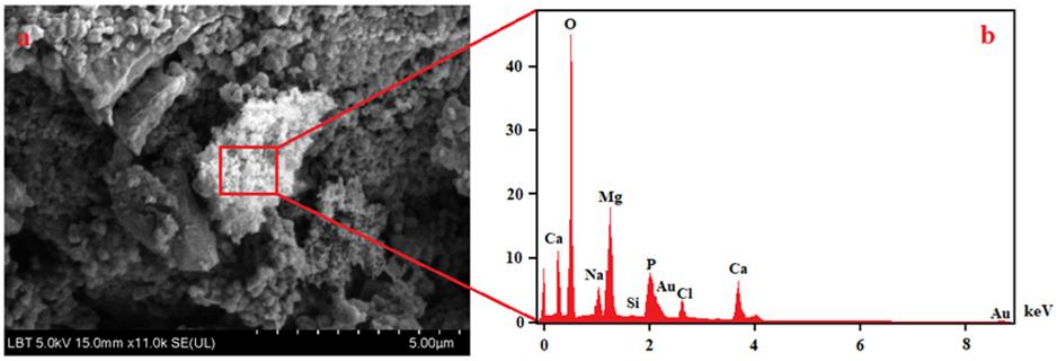


Figure 7. SEM image (a) and EDS spectrum (b) for forsterite ceramic, FC-1400, sintered at 1400 ºC, after 3 months of immersion in simulated body fluid, SBF

Figure 7 presents a SEM image (a) coupled with EDS spectrum (b) for forsterite ceramic (e.g., sol-gel derived forsterite) powder sintered at 1400 ^{o}C. The FC-1400 was immersed in SBF for 3 months. As it can be observed from Figure 7, after the immersion period a spot with different morphology appears on the surface of the forsterite ceramic, confirmed by the EDS analysis to be hydroxyapatite. These results recommend Mg_2SiO_4 as a good alternative to hydroxyapatite for bone implants. This could be especially important in the case of metallic implants where forsterite would be used as a coating. Here, by facilitating HAP formation on its surface, it would lead to a better osseointegration process, leading the body into easily accepting the metallic implant.

5. Nanoscale interactions

Hydroxyapatite has been proven to be biocompatible being even used clinically and in various commercial products. However, the fact that it is synthesized in nano form still raises some questions in regards to its potential side effects related to the interaction of nanoparticles with the living cell. The detrimental effects of nanomaterials on the living cell have gained a lot of attention especially concerning their application in medical fields.

However, the nanoscale interactions are part of a less researched area of nanoscience in spite of countless nanoparticles being studied for targeted drug delivery and the necessity of certain drugs to cross the blood-brain barrier [137-141] or penetrate cell membranes to approach the nucleus [142-148]. Considering that the cell membrane is a phospholipid bilayer barrier [149-151] it is very important to understand the interaction of nanoparticles with various self-assemblies of organic molecules such as monolayers [152-200], bilayers [201-203] or Langmuir-Blodgett layers [204-209], which are frequently used as models of biological membranes.

Crossing the cell membrane can be done by endocytosis when the nanoparticle is encapsulated in vesicles or by passive penetration through the membrane. Nevertheless the interaction of nanoparticles with a cell membrane is dependent on the physicochemical properties of said nanoparticles. These relate to type, shape, size, composition, crystalline structure, and surface characteristics (charge, type of targeting functional groups, coatings) of nanoparticles [210].

However, the cell type and the chemical composition of cell membrane jointly with cell cycle leading for example to cell division [211] are also important [212]. With so many variables to be taken into account, studies on the interaction of nanoparticles, even biocompatible ones like hydroxyapatite, with the human cells are of crucial importance in developing a better understanding at the nanoscale interactions. The future research will help to gain knowledge on the interaction of nanoparticles with various models of biological membrane and healthy cells, and also with cancer cells, exploring nanoparticles used for targeted drug delivery [213-222] to better design innovative nanomaterials for biomedical applications.

Conclusions

Hydroxyapatite has a multitude of applications. This review focuses on research regarding some biomedical applications of nano HAP and biomimetic (multi-substituted) HAPs. While most materials present on the market are based on stoichiometric hydroxyapatite much effort has been employed towards improving its innate properties.

Thus, many studies focus on ionic substitutions in the HAP network using physiological essential elements. Some of these elements are of particular importance as they play an important role in the development and regeneration of bone tissue. Consequently, single or multi-substitutions into the hydroxyapatite lattice would allow HAP to not only keep its innate biocompatible properties but also inherit those of all substituting ions, which will be eventually sustained release in vivo. Various applications of hydroxyapatite and biomimetic HAPs have also been discussed as bone substitute and coatings on metallic implants for osseointegration and enhanced fracture healing.

Due to its high adsorptive capacity for various molecules or ions coupled with its biocompatibility make hydroxyapatite and ms-HAPs ideal vehicles for targeted drug delivery. The specific example of HAP loaded with nitroxoline and silver ions was discussed in this review and has potential applications in infection therapy.

Also, this adsorptive property of hydroxyapatite turns it into a good candidate for heavy metal removal from wastewaters. Of course, the example provided on Roșia Montană mine wastewater might bring the same principle that

can be applied in the medical field when dealing with the ingestion of toxic substances and detoxification by using nanomaterials. Definitely, in vivo research is still needed to develop innovative nanomaterials for clinical applications.

Acknowledgments

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