Materials sciences And Engineering, (MSE'22)

Study of the Bioactivity of Hydroxyapatite Elaborated

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Keywords:
Hydroxyapatite
SBF
Biological response
Osteoconductivity

ABSTRACT

This work presents a study on the elaboration and characterization of Hydroxyapatite obtained from bovine bone calcined at 900°C for 3 hours with a particle size of around 125 µm and sintered at 1100°C. A prepared biological solution (Simulated Body Fluid, SBF), to study the biological response of our elaborated powder.
We carried out a characterization by scanning electron microscopy (SEM) in order to observe the formation of the apatite layer during the immersion time.

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1. Introduction

The use of metals and polymers for biomedical applications has faced the problem of poor bonding of prostheses and surrounding tissues. The analysis then moved on to ceramic materials, known for their hardness, chemical inertness, and wear resistance. However, they are considered delicate, have fragmented characteristics, and are very vulnerable to small defects.

Since it was pointed out that calcium phosphate plays an essential role in the inorganic phase of hard tissues, bones, and teeth, hydroxyapatite has been used effectively for many years to replace and incorporate bone tissue [1].

Calcium phosphate hydroxyapatite (HA) with the chemical formula $Ca_{10}(PO_4)6(OH)_2$ belongs to the crystallographic family of apatites [2]. Hydroxyapatite can be obtained naturally from biological sources or waste such as mammalian bones (cattle, camels and horses [3-5]) and from a marine source such as fish bone and scales [6], oyster shells, chicken egg bones, and shells, and corals from the source of Eg-shell [7].

A large number of studies of synthetic HA have clarified its biological behavior in the body, and it has been shown to have excellent biocompatibility, biological activities and osteoconductivity to promote bone growth and bind directly to the bone. Mediated by fibrous tissues in the bones [8].

For these reasons, synthetic hydroxyapatite has been used clinically as a bone substitute for the repair of bone defects [9]. However, new bone formation was limited with HA alone due to a lack of osteogenicity and osteoincitement.

To replicate calcification in the body, i.e. apatite formation, in vitro, simulated body fluid (SBF) with ion concentrations and pH equal to that of human plasma [10-11].

In this study, we elaborated a naturally sourced hydroxyapatite from bovine bone HA sintered at 1100°C and immersed in SBF solution at different times (1, 5, and 14 days) to assess their biological activities to promote new bone formation in vitro and in vivo.

2. Experimental methods

2.1. Preparation of bovine bones

Initially, bovine bones (Figure 1) were boiled to remove flesh, fat, and removal of any adhering macroscopic impurities. After washing and cleaning, the bones were heated in an oven at 100°C for 3 hours to remove moisture. To avoid blackening due to soot during heating, the bones were cut into small pieces about 10 mm thick and heated at 400°C for 2 h to allow evaporation of organic substances.

Figure 1 Powder preparation cycle, a) Bovine bones, b) Small pieces of bone, c) Bones heated at 400°C, d) Bones calcined at 900°C.

Then, the powder with a particle size of 125 µm is calcined at a temperature of 900°C. The powder was compacted into pellets of 20 mm in diameter and 2 mm thick, using a SPECAC 25T hydraulic press (Figure 2). The pellets were subsequently sintered at 1100°C with a ramp rate of 10°C/min.

Figure 2 Pellets before sintering.

2.2. Preparation of the SBF solution

We put 800 ml of demineralized water in a 1000 ml bottle then we placed a magnetic bar at a temperature of 36.5°C, after we dissolve the reagents one by one according to the order indicated in Table 1:

Table 1 Shows the order, reagents and quantity for the preparation of SBF [12].

Ordre	Reactifs	Quantity
1	NaCl	8.035 g
2	$NaHCO_3$	0.335 g
3	KCl	0.225 g
4	$K_2HPO_43H_2O$	0.231 g
5	$MgCl_26H_2O$	0.311 g
6	1M−HCl	39 ml
7	$CaCl_2$	0.292 g
8	Na_2SO_4	0.072 g
9	TRIS	6.118 g
10	1M–HCl	1.5 ml

To fully reproduce the living medium, not only synthetic plasma is needed, but also the normal temperature of the human body. For this, an oven set at a temperature of 37°C is used.

3. Characterization

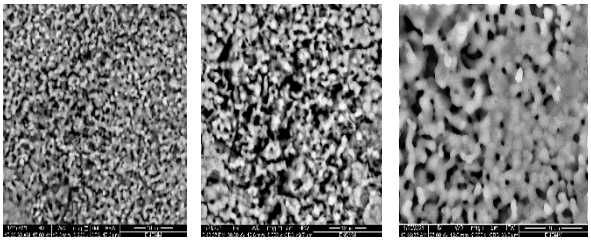
The pellets are immersed in the SBF at 37°C. for various durations ranging from one to 14 days. Before characterizing the immersed pellets, they must be rinsed with acetone and then air-dried. Just after drying, we carried out a characterization by scanning electron microscopy (SEM) in order to observe the formation of the apatite layer during the immersion time (Figure 3).

Figure 3 Bioactivity Assay Cycle (SBF)

Figure 4. Presents the SEM micrographs of the pellets sintered at 1100° C, and immersed in SBF after 1, 5 and, 14 days. We observed a surface covered by apatite crystals forming a uniform phosphocalcic layer.

At an immersion time of one day in SBF, apatite growth was observed, which became more pronounced after an immersion time of 5 days. However, the formed appetites were observed in different shapes and sizes.

EDS analyses show regions very rich in Calcium (Ca) and Phosphorus (P) which represent the main elements of hydroxyapatite with the existence of other elements such as Sodium (Na), Magnesium (Mg), (Figure 5).



(a) (b) (c)

Figure 4 SEM morphologies of the pellet surface after immersion in SBF, a) 1 day, b) 5 days, c) 14 days.

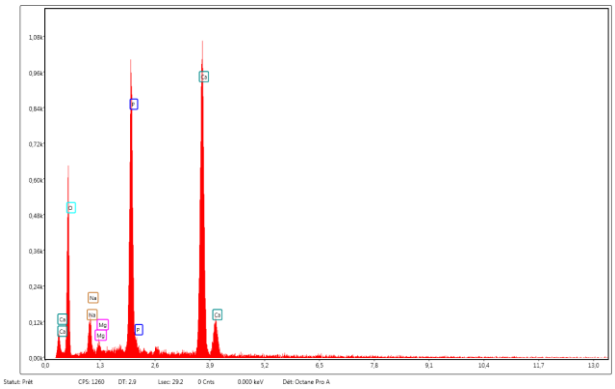


Figure 5 EDS analyses of the pellet surface after 5 days of immersion in SBF.

4. Conclusion

In this study, the effect of the immersion time of the elaborated hydroxyapatite in the biological solution was studied. As analyzed by SEM and EDX

- all samples formed apatite crystals on their surfaces after 5 days of immersion in SBF
- A homogeneous apatite layer after longer immersion times in SBF solution.

5. References

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