Synthesis And Characterization Of Hydroxyapatite (HA) From Eggshells

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Abstract: In this research, a biomedical material Hydroxyapatite (HA) is synthesized from eggshells. Physico-chemical characteristics confirmed the synthetic material is completely pure. “In vitro” test was conducted by soaking of HA-powder samples in a simulated body fluid SBF. Obtained results after “in vitro” test showed the bioactivity of this synthetic material by the formation of a new bioactive hydroxyapatite (HA) layer on its surface. This apatite layer has a similar chemical composition with the mineral phase of human bone. It allows a chemical bonding between bio-implant and natural bone. Consequently, the bone architecture is repaired and restored.

Keywords: Eggshells; bioactivity; hydroxyapatite; “in vitro”; SBF.

1. Introduction

Hydroxyapatite (HA) Ca₁₀(PO₄)₆(OH)₂ is one of the few materials that are classified as bioactive, meaning that it will support bone in growth and osseous integration when used in orthopedic, dental and maxillofacial applications. Coatings of hydroxyapatite are often applied to metallic implants, especially stainless steels, and titanium alloys to improve the surface properties. Hydroxyapatite may be employed in forms such as powders, porous blocks and hybrid composites to fill bone defects [1-2].

The chemical, structural and morphological properties of synthetic HA can be modulated by varying the method and the conditions of synthesis. Classical methods for HA powder synthesis include direct precipitation, hydrothermal techniques, hydrolysis of other calcium phosphates, as well as solid-state reactions and mechano-chemical methods [3-4]. One of the most widely used methods is wet precipitation, where chemical reactions take place between calcium and phosphorus ions at a controlled pH and temperature of the solution. The precipitated powder is typically calcined at 400-600°C or even at the higher temperature in order to obtain a stoichiometric, apatite structure. In some cases, a well crystallized HA phase was only developed while approaching a sintering temperature of 1200°C. However, fast precipitation during phosphate solution titration (to calcium solution) leads to chemical in homogeneity in the final product. Slow titration and diluted solutions must be used to improve chemical homogeneity and stoichiometry of the resulting HA. Careful control of the solution condition is critical in the wet precipitation. Otherwise, a decrease of solution pH below about 9 could lead to the formation of Ca-deficient HA structure [4].

In this study, hydroxyapatite (HA) material from eggshells was synthesized using the precipitation method. “In vitro” experiment was effectuated by soaking of material powder in simulated body fluid (SBF). Properties of synthesized biomaterial were investigated by XRD and SEM methods.

2. Experimental procedure

2.1. Materials

Pure chemicals of Sigma-Aldrich Company: (NH₄)₂HPO₄, KH₂PO₄, H₃O, Na₂SO₄, MgCl₂, H₂O, HNO₃, HCl, NaCl, KCl, NaHCO₃, CaCl₂, and eggshells.

2.2. HA Powder Preparation

Eggshells were separated membranes, washed with distilled water and boiled for 30 minutes. After that, eggshells were crushed and put in the furnace to heat in 3 stages. In the first stage, eggshells were heated at 450°C and retained for 1 hour to remove the organic components. Next, the temperature was raised to 600°C during 1 hour to completely remove out organic components and impurities of the eggshells. The final stage, eggshells were heated at 900°C and kept for 30 minutes in order to decompose calcium carbonate mineral into calcium oxide (CaO) (Reaction 1).

Obtained calcium oxide was dissolved in nitric acid solution (HNO₃) (Reaction 2). This reaction is exothermic and was carried out in the fume hood. The reactive solution was concentrated by boiling over a flame. Next, reactive solution was filtered to remove impurities from the burning process. A Solution of Calcium nitrate Ca(NO₃)₂ was obtained. Above solution Ca(NO₃)₂ was dropped step by step in solution of diammonium phosphate (NH₄)₂HPO₄ to elaborate hydroxyapatite (HA) material. The mixture was stirred for 5 hours under a condition of pH =10. Obtained milky white product was washed...
with distilled water then put in an oven at 120°C during 8 hours. The collected powder was heated at 1000°C for 5 hours to crystallize totally hydroxyapatite (HA) material. HA synthesis process from eggshells is summarized as describing in Fig.1.

$$\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2$$ (1)

$$\text{CaO} + \text{HNO}_3 \rightarrow \text{Ca(NO}_3)_2 + \text{H}_2\text{O}$$ (2)

$$10\text{Ca(NO}_3)_2 + 6(\text{NH}_4)_2\text{HPO}_4 + 8\text{NH}_3\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 20\text{NH}_4\text{NO}_3 + 6\text{H}_2\text{O}$$ (3)

2.3. “In vitro” test

HA material was effectuated “In vitro” test by soaking of powder samples in simulated body fluid (SBF). Solution SBF was prepared in accordance to Kokubo’s protocol with minerals composition nearly equal to those of human plasma [5].

2.4. Physico-chemical characterization

To evaluate physico-chemical properties of HA powder before and after soaking in SBF solution, XRD and SEM analysis methods were employed. The crystalline phase of HA was investigated by X-Ray diffractometer (Bruker D8 Advance). Scanning Electron Microscopy (SEM) (Hitachi, Jeol 5) was used to observe and evaluate the morphological shape and particle size of the material.

3. Results and discussion

3.1. XRD analyses

Figure 2 shows X-ray diffraction diagram of the HA synthesized from eggshells. Sigma-Aldrich Company’s HA was used as the standard material. Synthetic HA revealed the characteristic peaks at 26°, 32°, 40°, 46.5°, 49.5°, 53.2° and 64° (2θ). These peaks correspond respectively to (002); (211); (310); (004) and (304) miller planes [6]. No strange peak was observed. The achieved results confirmed HA material synthesized from eggshells similar to the standard HA and it is completely pure. By using inexpensive raw materials as the precursors, we have successfully built a synthetic process to synthesize HA biomedical material with good quality.

The X-ray diffraction diagram of the HA material after 5 days of immersion in SBF solution is presented in Fig. 3. It was recognized that the peaks were extended in comparison with these one of initial HA. This illustrated the interactions between synthetic material and SBF environment. On the other hand, no strange peak was revealed. These obtained analyses confirmed the bioactivity and also biocompatibility of synthetic HA material after “In vitro” experiment. Following soaking times, the reactions between synthetic HA and SBF solution will lead to the destruction of crystal lattice structure of synthetic HA to form a new apatite layer. This new apatite layer plays a role as a connector between...
artificial material and natural bone in bone grafting technique.

3.2. SEM observation

The SEM images of synthetic HA are shown in Fig. 4. The surface morphology of material expresses uneven grain structure with gaps and pores. This structure facilitates the chemical interaction between material and biological fluid and leads to create a new HA layer on the surface, filling the gaps and pores, so artificial material pieces fastened and created links with the natural bone. These properties are highlighted via the SEM images of HA after 5 days of immersion in SBF solution (Fig. 5). The surface of material was changed in comparison with initial material. The tiny crystals formed on the surface of the material and filled the initial gaps and pores and created the tight surface. SEM observation and XRD data confirmed the formation of a new layer on the surface of HA after immersion in SBF solution.

4. Conclusion

In this study, from an inexpensive raw material, hydroxyapatite HA was synthesized successfully. "In vitro" experiment was conducted by soaking the material powder in simulated body fluid (SBF). XRD and SEM methods were used to investigate the characteristics of the synthetic material. The XRD analyses confirmed the HA synthesized from the eggshells completely pure and similar standard product. The obtained results from XRD and SEM highlighted the formation of a new mineral apatite layer on the surface of material after immersion in SBF solution. The results also confirmed the biocompatibility of synthetic material by the non-appearance of strange peaks on its XRD diagram after "In vitro" test.

References