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Original Research Article

Extraction of nano-hydroxyapatite (Nano-HAp) from sheep molar teeth for nanodentistry

Ashraf Heidaripour [1] *, Asal Khazaei [2]

[1] *Department of Chemistry, Khajeh Nasir Toosi University of Technology, Tehran, Iran*
[2] *Research Center Karaj Moallem, Karaj, Iran*

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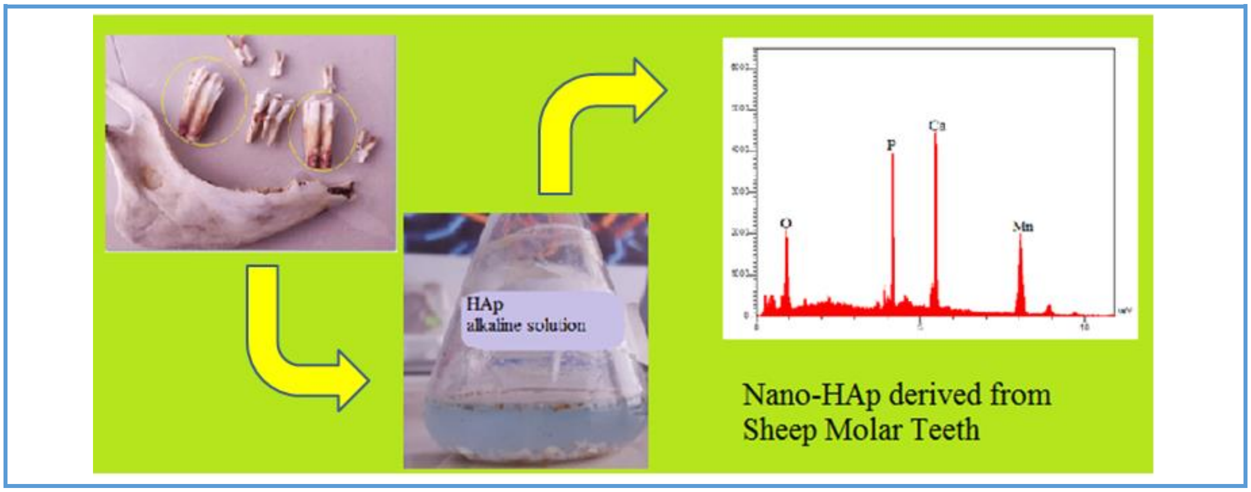
ABSTRACT

Nano hydroxyapatite (Nano-HAp) is one of the nanoparticles that have attracted the attention of researchers due to its biological similarity with dental and bone materials. Recently, Nano-HAp has been used to nanodentistry to treat dental diseases and has been very effective. The origin of the preparation of these biological nanoparticles was firstly from cow bone. In this research, Nano-HAp was prepared from sheep molar teeth. Extraction of Nano-HAp from teeth is a new and innovative idea. In this way, the HAp powder in sheep molar teeth was separated from the rest of the dental material, which includes fatty material and dental connective tissue, and then using ultrasonic waves, the HAp material was converted into Nano-HAp. The Nano-HAp powder was studied with the help of ultraviolet visible (UV-Vis) spectroscopy to prove its existence. The morphology and size of Nano-HAp were examined by Field Emission Scanning Electron Microscope (FESEM) and its chemical component was confirmed with Energy-dispersive X-ray spectroscopy (EDS).

Corresponding author, email: aheidari@mail.kntu.ac.ir (A. Heidaripour).

Graphical Abstract



Introduction

The expansion of nanotechnology in the field of dentistry is called "nanodentistry". With nanotechnology and controlling the size and morphology of particles, the desired materials in dentistry are prepared [1-3]. However, research in nanodentistry field is on the first step and needs to be progressed with new and biocompatible materials. It is possible to use nano-dental additives in prostheses, tooth reconstruction, orthodontics, and nanomedicine [3,4].

As shown in Figure 1, one of the branches of nanodentistry is related to the treatment of periodontitis by nanomedicine. The nanomedicine is placed in a patch inside the periodontal pocket, which continuously releases the medicine in the affected area. Treatment and management of dental defects is done with the help of various nanoparticles such as ZnO, TiO_2, CuO, MgO, etc. Recently, Nano-HAp has been evaluated as a nanomedicine and has shown positive results in nanodentistry [1,2].

HAp and derivatives

According to Figure 2, HAp is a natural component of enamel, dentin and bone with chemical formula, $Ca_{10}(PO_4)_6(OH)_2$ [5]. HAp-like compounds make up approximately 65% of bone tissues. The overall composition of HAp is slightly changes in different bone parts, which is due to the presence of elements such as Cu, Mg, Mn, and Fe (colored chart in Figure 2). Therefore, color and properties of HAp differ from one bone to another [6-9]. The HAp compounds can be used instead of calcium phosphate cements (CPM) and calcium silicate cements (CSC) that are traditionally used in dentistry. The addition of HAp to CSC is an alternative solution to increase the volume of apatite without reducing the amount of calcium hydroxide (CH) [5,10-11]. The research have shown that making changes in the structure and atomic ratio of HAp and replacing Ca with other elements can expand the scope of application of HAp material. For example, the partial replacement of Ca in HAp with Mn increases the thermal resistance of HAp and changes its color into blue [12-13]. The use of nanoparticles with their special capabilities can expand the specialized field of nanodentistry [14-16].

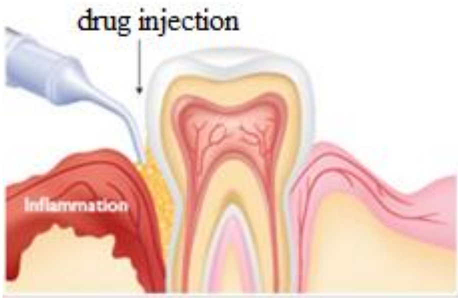


Figure 1. Drug injection in the periodontal area.

Preparation of HAp powder derived from cow bone

Bovine bone-derived hydroxyapatite (BHA) powder was produced firstly by Ratnaya [5]. Briefly, bovine condyles were cut into 3-5 cm cubes, and cooked for 2 hours. Then cubes were immersed in 0.1 M NaOH solution for 12 hours at 70 °C. In this way, fat and binding materials were dissolved in the alkaline solution and separated from the HAp material, and then the HAp powder was separated from the solution by filtration [5].

Nano-HAp

Nano-HAp is prepared from HAp materials with natural or synthetic origin. The use of Nano-HAp as a nanomedicine is more effective in oral and dental treatments. The UV-Vis spectrum of Nano-HAp has low intensity but broad absorption from short wavelengths of 190 nm to long wavelengths of 800 nm [5]. The Nano-HAp is promising in the future of health sciences and alloplastic grafting materials [6,7]. Also, Nano-HAp extracted from sheep femur bone has been investigated as a natural source of HAp and has shown good results in nanomedicine [8]. However, no reports were found on the use of HAp with origin of sheep teeth. Therefore, this issue is a suitable field for research. It seems that HAp extracted from sheep teeth has more potential for use in nanodentistry due to its biological similarity [9].

Experimental

Materials and Instruments

The materials include sheep molar teeth (Figure 3), 0.1 M NaOH solution, distilled water, and ethyl alcohol. The laboratory equipment used include: vCLEAN1-L13 ultrasonic cleaner bath with voltage of 220V and 50 Hz as a source of ultrasonic waves, spectrophotometer NDNM model, and optical microscope model: AC85-240V, and also FESEM and EDS model: VEGA\\TESCAN.

Preparation Method of HAp

In this research, Ratnaya's method was used to extract HAp from sheep teeth [5]. Initially, a piece of sheep's jaw bone was placed in the open air for a week until it was completely dry and its unpleasant smell disappeared, and then the teeth were pulled out of the jawbone. Three molar teeth were completely crushed in the mortar. The value of 2 g of the resulting tooth powder was poured into a flask containing 20 mL of 0.1 M NaOH solution and stirred for 12 hours at a constant temperature of 70 °C. To prevent the volume of the solution from decreasing, distilled water was added drop by drop to the solution. Over time, the color of the solution changed to light blue-violet. After that, the tooth powder was separated from the alkaline solution by filtration. It was washed several times with distilled water to neutralize its pH. Then the separated powder was completely dried in the oven with 100 °C.

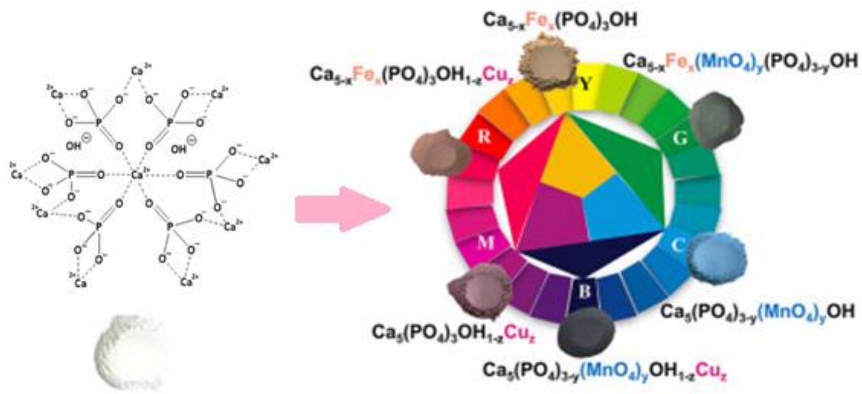


Figure 2. Chemical structure of HAp, also doped-HAp in colored chart [9].

Figure 3. Sheep molar teeth as a source of Nano-HAp

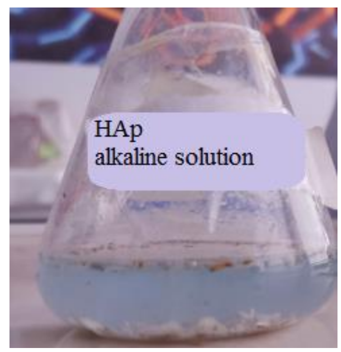


Figure 4. HAp powder dispersed in NaOH solution

Preparation of Nano-HAp via ultrasonic waves

The value of 1 g of prepared HAp powder was mixed with 100 mL of distilled water in a beaker and on the stirrer. The HAp powder was dispersed in the solution as fine particles which were insoluble in water and a suspension was formed, and then 10 mL of ethanol was added to the mixture and stirred for 30 min. Then the value of 2 mL was separated from the upper part of the suspension and subjected to UV-Vis spectroscopy analysis. After that, the suspension was subjected to ultrasonic waves with full power 50 Hz during 15 min. Each 5 min, 2 mL of the suspension was separated and subjected to UV-Vis spectroscopy analysis (samples 1, 2 and 3).

Results and Discussion

Study of UV-Vis spectroscopy of Nano-HAp

As can be seen in Figure 5, UV-Vis spectroscopy of HAp suspension, before and after the application of ultrasonic waves, showed that the spectra are in full agreement with what has been reported in the literature, that is, a broad absorption spectrum from 190 to 800 nm without obvious absorption [5]. The UV-Vis spectroscopy of HAp before application of ultrasonic waves shows an absorption maximum of 0.64 at 235 nm. However, after 5 min application of ultrasonic waves, the absorption intensity reaches more than 1.48. By increasing the ultrasonic time to 10 and 15 min, the maximum absorbance reaches 2.32. These observations show an increase in the Nano-HAp concentration by 3.625 times in the HAp suspension prepared with ultrasonic irradiation.

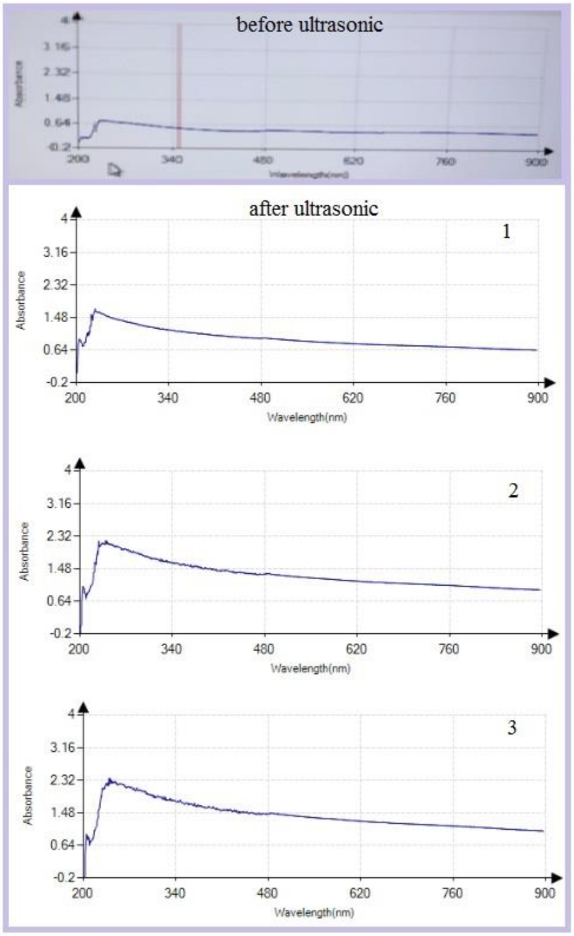


Figure 5. UV-Vis spectroscopy of Nano-HAp extracted via ultrasonic waves during different times

The EDS elemental analysis of Nano-HAp powder

Nano-HAp powder prepared by ultrasonic waves was examined under photo microscope with 40 magnifications. According to Figure 6A, the powder has layered structure. Also, in Figure 6B, the FESEM image confirms the two-dimensional and layer-by-layer structure of Nano-HAp. These observations are in full agreement with the reports in the literature [5-10].

The EDS elemental analysis confirms the extraction of Nano-HAp (Figure 7). Also, the presence of manganese was confirmed with a percentage of over 18%. According to Figure 2, the light blue-violet color observed in the HAp suspension indicates the presence of the intermediary element Mn with an oxidation number of 8 in the structure of Nano-HAp. Accordingly, the presence of MnO_4 is proved in the structure of Nano-HAp extracted from sheep molar teeth. This new achievement in nano-dentistry can lead to the preparation of nanobiological materials compatible with the body and suitable for the mouth and teeth environment.

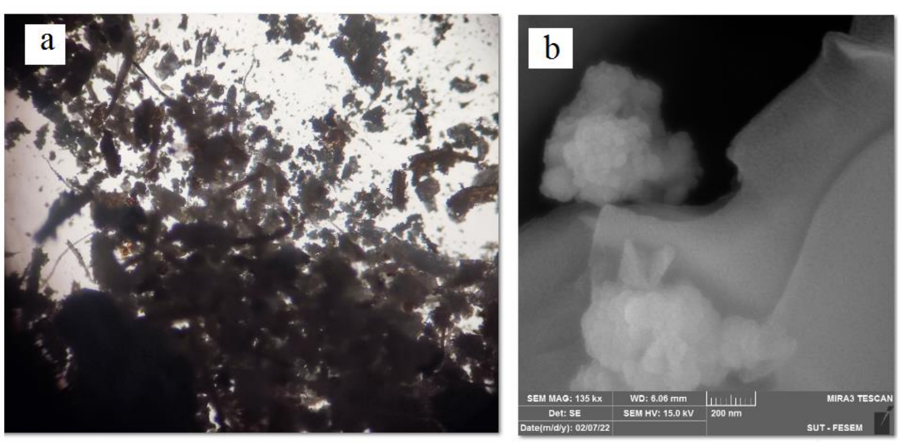


Figure 6. a) Optical microscope and b) FESEM Image of Nano-Hap.

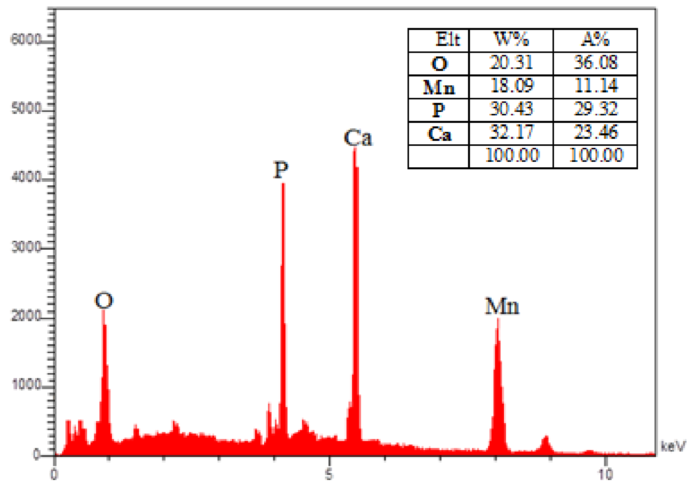


Figure 7. EDS of Nano-HAp, with Atomic and weight percentages.

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Conclusion

The extraction of Nano-HAp from sheep molar teeth was successfully done, which is completely new and innovative. In this research, HAp was extracted from the sheep molar teeth by Ratnaya method, and then HAp was transformed into Nano-HAp using ultrasonic wave's radiation. With the help of the colored chart of HAp compounds, it was predicted that the HAp extracted from the sheep molar teeth contains some amounts of Mn. This assumption is completely consistent with what was obtained from elemental analysis of EDS studies. Examining the images of optical microscope and FESEM showed a layered nanostructure. The UV-Vis spectroscopy of the HAp suspension showed a spectrum that confirms the correct preparation of the nanostructure. This innovative research can be an introduction to further study on the materials preparation needed for nanodentistry from natural animal tooth samples.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Orcid

Ashraf Heidaripour : 0000-0003-0223-5383
Asal Khazaei : 0009-0005-3422-6918

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