# Novel Bioceramic Production via Mechanochemical Conversion From Plate Limpet (*Tectura scutum*) - Shells

Ahmet Talat Inan[1,2], Oguzhan Gunduz[2,3], Yesim Muge Sahin[4], Nazmi Ekren[5,2], Serdar Salman[2,3], Joshua Chou[6], Besim Ben-Nissan[7], Hasan Gokce[8], Faik Nuzhet Oktar[9, 2]

[1]Department of Mechanical Engineering Faculty Technology Marmara Univ., Istanbul, Turkey [2]Advanced Nanomaterials Research Laboratory, Faculty Technology, Marmara Univ., Istanbul, Turkey
[3]Department of Metallurgy. & Mater. Engineer. Faculty Technology, Marmara Univ., Istanbul, Turkey[4]Biomed. Engineer. Dept., Engineer. & Architecture Faculty, Istanbul Arel Univ., Istanbul, Turkey
[5]Department of Electrical and Electronic Engineering Faculty Technology Marmara Univ., Istanbul, Turkey[6]Faculty of Med. & Molecular Biosciences, The Univ. of Technology, Sydney, NSW, Australia
[7]Chemistry and Forensic Sciences Dept., The Univ. of Technology, Broadway, Sydsney, NSW, Australia [8]Prof. Dr. Adnan Tekin Mater. Sci. and Produc. Tech. Applied Res. Centre, Istanbul Tech. Univ.,Istanbul, Turkey
[9]Bioengineer. Dept., Engineer. Faculty, Marmara Univ., Istanbul, Turkey

## Abstract

Calcium phosphates are very important biomaterials for orthopaedic and dental applications. Hydroxyapatite (HA) is one of the important phases used for grafting. Those are produced from synthetic and natural sources with various methods. Especially nano-bioceramics can be produced through calcitic and aragonitic structures (i.e. mussel shells, sea snail shells, land snail shells and sea urchin shells). The plate limpet shells were used. The plate limpet is a gastropod, a soft-bodied invertebrate (an animal without a backbone) that is protected by a very hard, flattened conical shell. In this study the Plate Limpet (Tectura scutum) shells were obtained from a local gift store in Istanbul. The habitation of these limpets broadens from south Alaska down to California - Mexico. First the exact $CaCO_3$ content was measured with thermal analysis (DTA/TGA). Here in this study agitation was carried out on a hot-plate (i.e. mechano-chemical processing). First the temperature was set at 80 ° C for 15 min. Then equivalent amount to CaO $H_3PO_4$ was added dropwise for HA phase formation and the reaction was set on a hotplate for 8 hours. The dried sediments HA part was divided into 2 groups. One group was sintered to 835 ° C and second group to 855 ° C. Here x-ray diffraction and scanning electron microscope (SEM) studies were performed. From the study various HA phases and TCP phases were obtained. A previous study done with Atlantic Deer Cowrie encourages nanobioceramic production from natural sources. This study proposes that mechanochemical agitation with very simple way for producing nano-sized calcium phosphates for future bioengineering scaffold applications.

## Introduction

A biomaterial is a synthetic or natural material suited to replace or treat natural body tissues and organs on connection with biological systems. It traces its history to more than 2000 years ago, when Romans, Chinese, and Aztecs used gold for dental applications [1]. It was investigated that Mayans had used nacre as false teeth more than 4000 years ago [2]. Biomaterials, which were also biocompatible, have since been modified, improvised, and precisely allowed to improve body functions and replace injured tissues. In a recent report the global biomaterial market is estimated to reach $88.4 billion by 2017 from $44.0 billion in 2012, growing at a Compound Annual Growth Rate (CAGR) of 15% [1].

Hydroxapatite (HA) biomaterial is a very popular material for various reconstruction applications in the human body. HA usually is produced from synthetic reagents with time consuming methods. Nowadays the production of natural HA's are very popular, because of having small ingredients such as Mg, F and others. The most preferred way for HA productions, treating natural tissues (human or bovine originated) with diluted hydrochloric acid (HCl), which demineralizes the tissues. But this way could be dangerous for surviving, which can cause death (i.e. from bovines: bovine spongiform encephalopathy) [3-4]. Other popular method is calcinating of various tissues (human-bovine-bones, dental structures such as dentine [5-6] and enamel [6-7] and fish scales [8]) at 800-850 ° C.

There is also an another popular method producing HA and related phases from calcitic – aragonitic structures such as corals [9], sea shells [10], sea snails shells [11], sea urchins shells [12], cuttle fish bones [13], egg shells [14], land snail shells [15] and with many other. Hydrothermal treatment is very popular, but a special equipment is needed which works with high pressure [16]. Instead of this expensive and complicated equipment a hot-plate stirrer [10] (with heat application on) or an ultrasonic cleaner (needed with heat application) can be used. These mechanochemical methods are much more economic and less time consuming.

The aim of this study is to produce bioceramic particles based on nano level using simple mechanochemical transformation via hot-plating from the plate limpet shells. Characterization of HA derived from plate limpet shells as aragonite and calcite sources lead to the evaluation of the product and the method.

## Materials and Methods

Empty plate limpet shells were collected from a local gift store in Istanbul. The shells were washed, cleaned and dried. Photograph of the plate limpet shell samples is shown in Fig. 1. Afterwards they were crushed to small particles with a hand mortar and ball billed down to <100µm particles and sieved through a <100µm sieve.

**Fig. 1.** Photograph of the plate limpet shells

The preparation method was carried out according to one of our previous study [17]. Differential thermal and gravimetric analyses (DTA/TGA) of plate limpet shells were conducted at heating rate of 20 $^0$C/min between 50 and 1000 $^0$C under stream flow of nitrogen gas (Perkin Elmer Diamond TG -TDA). For HA batch, the required volume of an aqueous $H_3PO_4$ solution was calculated in order to set the stoichiometric molar ratio of Ca/P equal to 1.667 for HA material. Process temperature was set to 80° C for 15 min. After the titration of the equivalent amount of reagent grade $H_3PO_4$ into the prepared solution. The solution was subjected for agitating mechanochemically on a simple hot-plate stirrer for 8 hours duration. After the process the sediments were dried. Processed HA materials were calcined at 835 and 855 $^0$C (for additional 4 hours). The processed limpet originated bioceramics x-ray diffraction (XRD) and scanning electron microscopy (SEM) studies were carried out.

## Results and Discussion

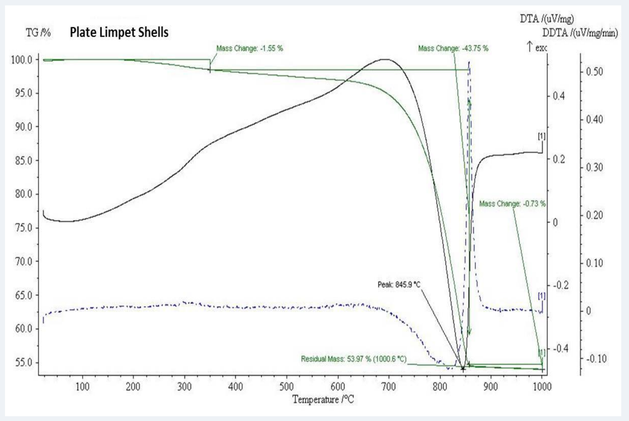


**Fig. 2.** DTA/TGA analyses of raw plate limpet shells.

The DTA and TG curves for the coral powders after drying are shown in Fig.2. Fig.1 shows the weight loss of 43.75% due to thermal decomposition of calcium carbonate to calcium oxide and carbon dioxide at 845.9 $^0$C, which is the main composition of plate limpet shells. The weight loss due to organic matter was 1.55%.

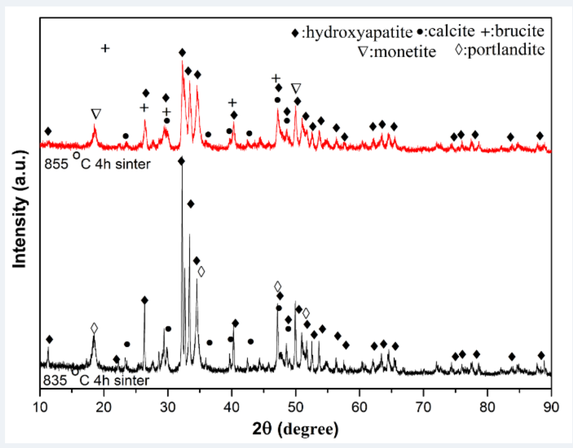


**Fig 3.** X-ray diffraction patterns of mechanochemically at 835° C, 4h and 855° C 4h sintered samples.

X-ray diffraction analyses of the raw plate limpet samples showed calcium magnesium carbonate (JCPDS card number 00-060-0473). The sample sintered at 835 $^0$C in air for 4 hours is revealed a major phase consists of following phases (Fig. 3); hydroxyapatite (JCPDS card number 98-004-0602) and portlandite (JCPDS card no: 98-006-3140). The minor phase is shown calcite (JCPDS card no: 98-002-1954).

The major phases, which were sintered at 855 $^0$C are the followings (Fig. 3): hydroxyapatite (JCPDS card number 98-004-0602), brucite (JCPDS card number 98-005-6733) and monetite (JCPDS card number 98-000-5364). The minor phase is detected as calcite (JCPDS card no: 98-002-1954).

The spectra provide a clear evidence of the appearance of crystalline phases both sintered at 835 and 855 $^0$C. At 835 $^0$C treated sample is a very interesting phase, which was developed as portlandite phase. It is a rare oxide mineral with the naturally developed form of calcium hydroxide $\{Ca(OH)_2\}$ bioceramic. It is also the calcium analogue of brucite phase ($Mg(OH)_2$). Apparently, the brucite phase was found very similar to natural bone mineral phase. Mg is a very important element for bone structure. The sintered plate limpet sample at 855° C was detected a brucite phase, which is the mineral form of magnesium hydroxide, with the chemical formula $Mg(OH)_2$.

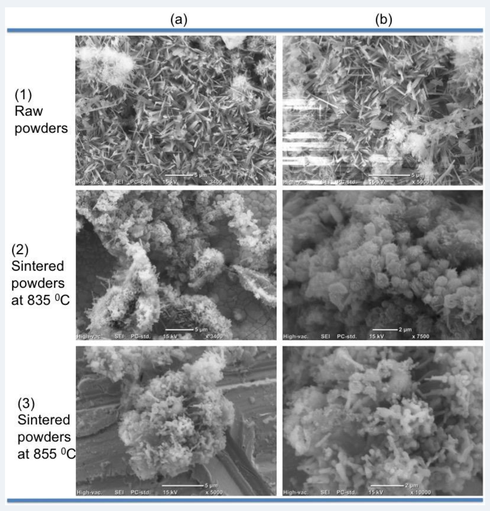


Fig. 4 1a-b shows fibers of structures on the surface. High magnifications image reveals (Fig. 4 1a) fibers as thin ribbons shape crystallite, which are consistent with aragonite or calcite materials. Fibers were approximately 2 µm long and average diameter of around 140-150 nm (Fig.4. 1b). After sintered at 835 $^0$C (Fig. 4 2a-b), the morphology was converted to small ball particles, which were measured less than 2 µm (Fig. 4 2b). Agglomerates were also observed as shown in Fig. 4 3a-b. From Fig.4 3b reveal thin rod fibers structures, which were sintered at 855 $^0$C in air for 4 h. In this agglomerated thin rod fibers were consistent with nano\micro structures less than 1 µm in diameter.

**Fig. 4.** Scanning Electron microscopy images (a) low and (b) high magnifications of (1) ZNSS raw powders, (2) ZNSS sintered powders at 865 $^0$C and (3) ZNSS sintered powders at 885 $^0$C.

## Conclusion

Hydroxyapatite powders were successfully produced from plate limpet shells by mechanochemical method. The brucite phase ($Mg(OH)_2$) was observed after sintered at 835 and 855 $^0$C as shown by XRD of final products from mechanochemical method. These materials can be a very economic and efficient producing in biomedical engineering applications.

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