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CALCIUM PHOSPHATE FORMATION FROM SEA URCHIN - (BRISSUS LATECARINATUS) VIA MODIFIED MECHANO-CHEMICAL (ULTRASONIC) CONVERSION METHOD

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This study aims to produce apatite structures, such as hydroxyapatite (HA) and fluorapatite (FA), from precursor calcium phosphates of biological origin, namely from sea urchin, with mechano-chemical stirring and hot-plating conversion method. The produced materials were heat treated at 800 °C for 4 hours. X-ray diffraction and scanning electron microscopy (SEM) studies were conducted. Calcium phosphate phases were developed. The SEM images showed the formation of micro to nano-powders. The experimental results suggest that sea urchin, *Brissus latecarinatus* skeleton could be an alternative source for the production of various mono or biphasic calcium phosphates with simple and economic mechano-chemical (ultrasonic) conversion method.

Key words: calcium phosphates, mechano-chemical method, sea urchin, ultrasonic

INTRODUCTION

Calcium-phosphate-based ceramics have been used successfully for more than 40 years to replace and augment damaged or degenerative hard tissues. Bone itself has a composite structure comprised of biogenic hydroxyapatite (HA) nano-crystals organized throughout a continuous organic matrix [1]. Hydroxyapatite has a general chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. It can be produced either synthetically or from naturally derived resources. HA is used in orthopedic and dental graft applications but it is normally surgically applied only on non-load bearing applications due to the relatively poor mechanical properties.

There is a great interest not only in developing ways of processing HA synthetically but also in understanding of the theoretical aspects of their *in vitro* and *in vivo* behavior under functional loading.

Currently there is an ongoing research on the synthesis methods of calcium phosphates from natural materials such as: natural composites [2], bones, dentine [3], enamel, and natural waste materials, such as eggshells, shells of some marine mollusks such as sea urchins [4, 5], mussels, sea and land snails [6] and cuttlefish bones. Some other materials of biological origin were calcined (i.e. presintered) under ambient temperature, e.g. 850 °C (i.e. bovine bone, human dentine [5], and human enamel, for HA powder preparation. Another process for HA preparation is the transformation of aragonitic structures (such as corals) with hydrothermal treatment, hot-plating, and ultrasonication [4].

In this study, sea urchin, *Brissus latecarinatus* (Figure 1), was chosen to be the aragonite source to transform to calcium phosphate phases, via heating and ultrasonication methods. The sea urchin was first named by Leske in 1778, which is widely distributed in Indo-west Pacific Ocean, except Arabian Sea and Australian coast. It is also seen in Hawaii [4]. Simple chemical methods, like heating and ultrasonication, are very economical techniques for the production of calcium phosphates. Agaogullari *et al.* [4] produced in a previous study monetite and other calcium phosphates which were precursors of HA. Kel *et al.* also produced monetite, fluorapatite and calcium phosphates from land snail shells via heating and ultrasonication methods [6]. Ozyegin *et al.* reported a similar monetite production from a sea snail (from Turkey, Black Sea) with the same method [7].

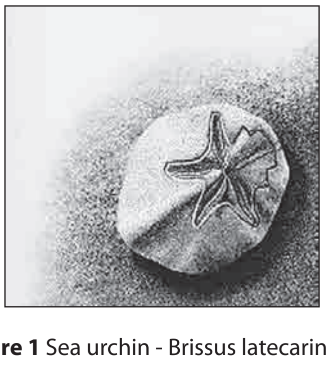

Figure 1 Sea urchin - Brissus latecarinatus

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MATERIALS AND METHODS

Heating and ultrasonic stirring methods were applied to produce powders of calcium phosphate bioceramics which can be applied in tissue engineering as bone graft materials. Marine structures are very versatile and readily available. For this purpose *Brissus latecarinatus* shells were obtained from a local shop in Istanbul, Turkey.

According to differential thermo gravimetric analysis (DTA/TG), the exact CaO content of urchin was determined [4]. The suspended raw powders (in distilled water) were heated on a hot-plate while an ultrasonic mixing was applied. The temperature (in both cases) was set at 80 °C. Then, appropriate (according to the amount of $CaCO_3$ in the sea urchins) amount of H_3PO_4 solution was added into the suspension. The reaction lasted 2 hours (both for hot-plate and ultrasonication methods). Then, the liquid part was evaporated in an incubator at 100 °C for 24 hours. The dried sediment was collected for further treatment and analyses.

The X-ray diffraction analysis was conducted to detect the formation of calcium phosphates. The produced powders were fired at 800 °C for 4 hours to promote the formation of other calcium phosphates.

X-ray diffraction and SEM (JEOL 590) studies were conducted in the final products. For the SEM images, the produced powders were dispersed in acetone, ultrasonically stirred, and dropped on metallic stoppers and then coated with platinum.

RESULTS AND DISCUSSION

Nanostructured materials, such as HA, can be obtained by many ways [7]. Natural species of sea origin, such as corals and nacres, always attract special interest in biomaterials science and technology.

Their bio-mineralization mechanism has been extensively investigated and documented.

The production of HA from natural aragonite via hydrothermal transformation is a known technique since 1970 s when corals from the Pacific Ocean were transformed into HA-scaffolds that featured great similarities to the mineralized structure of bones. Corals are among a number of marine species that are in danger of diminishing due to the global warming and climate change. However a new generation of artificially grown corals is now available in commercial quantities.

The general structure of sea urchin is shown in Figure 2a gives the top view of *Brissus latecarinatus*. Another SEM image (Figure 2b) was taken from the side of the fracture surface. This image shows a very porous structure with a pore size ranging between ~10 μm and ~50 μm. Figure 3 shows the X-ray diffractogram of the raw sea urchin *Brissus latecarinatus*. The peaks match well the patterns of $(Ca,Mg)CO_3$ (JCPDS 00-043-0697).

In Figure 4, as shown in the SEM, the interconnected pores ranging from 2 μm to 30 μm are observed. In Figure 5, some typical calcium carbonate (aragonite or calcite) crystals are clearly seen in the powdered raw material.

In Figure 6 morphological changes of the crystals after the transformation with hot-plate and ultrasonication methods and firing at 800 ^{o}C for 4 hours (Figures 6 a and 6 b, respectively) are observed which have particle sizes ranging between 1-5 μm.

Figure 7 compares X-ray diffractograms of the sea urchin in hot-plated (Figure 7 a) and ultrasonicated forms (Figure 7 b).

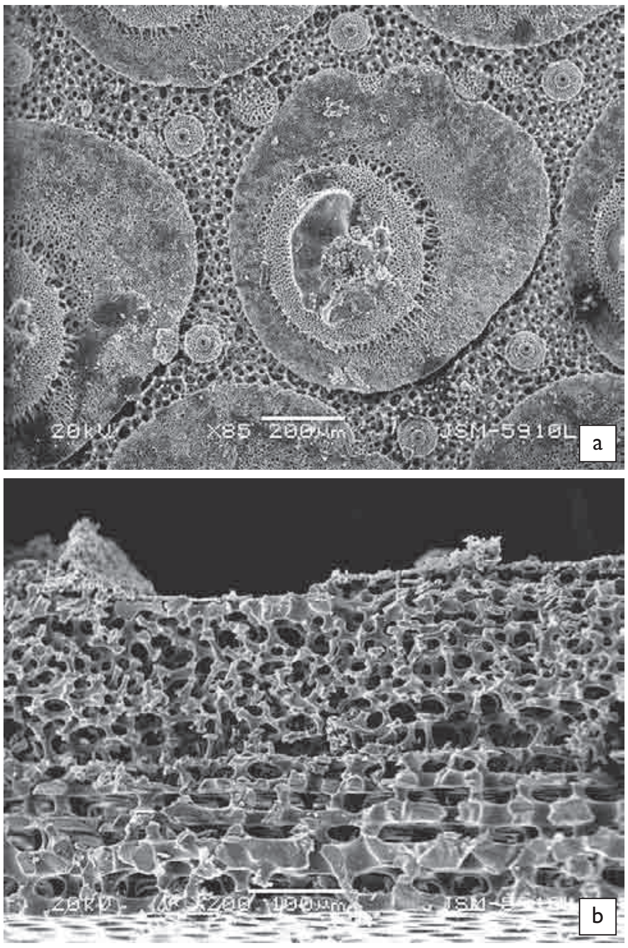

Figure 2 SEM images of (a) the top-view of the surface of a sea urchin at x 85 magnification and (b) fracture surface of the sea urchin's walls at x 200 magnification.

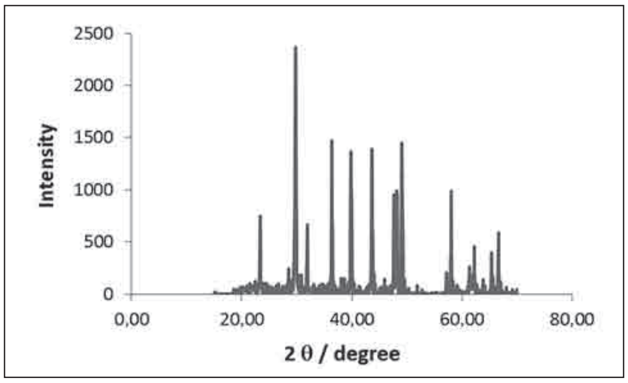

Figure 3 X-ray diffractogram of raw sea urchin, Brissus latecarinatus.

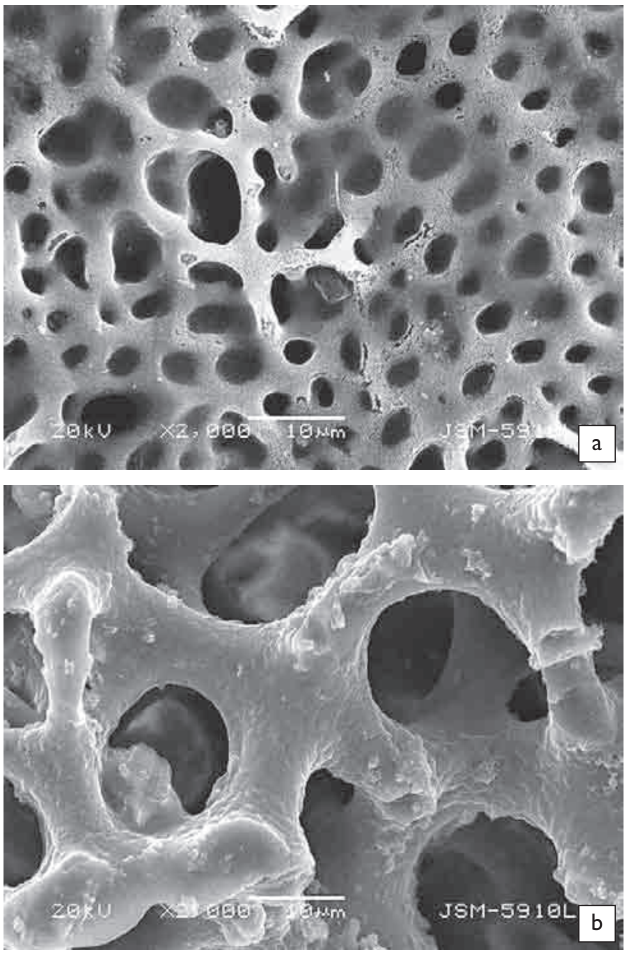

Figure 4 SEM images at (a) lower and (b) higher magnification from cross-sections of the sea urchin**.**

In the hot-plated material calcium pyrophosphate (JCPDS 00-009-0346) was formed. In the ultrasonicated samples, formation of a mixture of calcium pyrophosphate (JCPDS 00-009-0346) and calcium phosphate (JPDS 00-015-0177) can be suggested.

Calcium pyrophosphate ceramics are traditionally processed by firing secondary phosphates like brushite or monetite.

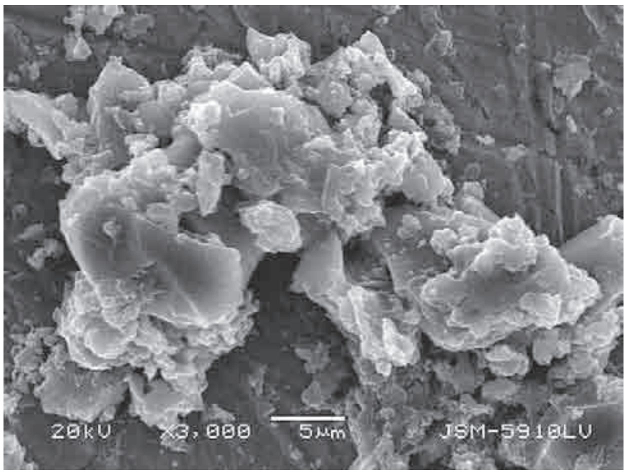

Figure 5 SEM image of raw (not transformed) crushed powder from the sea urchin.

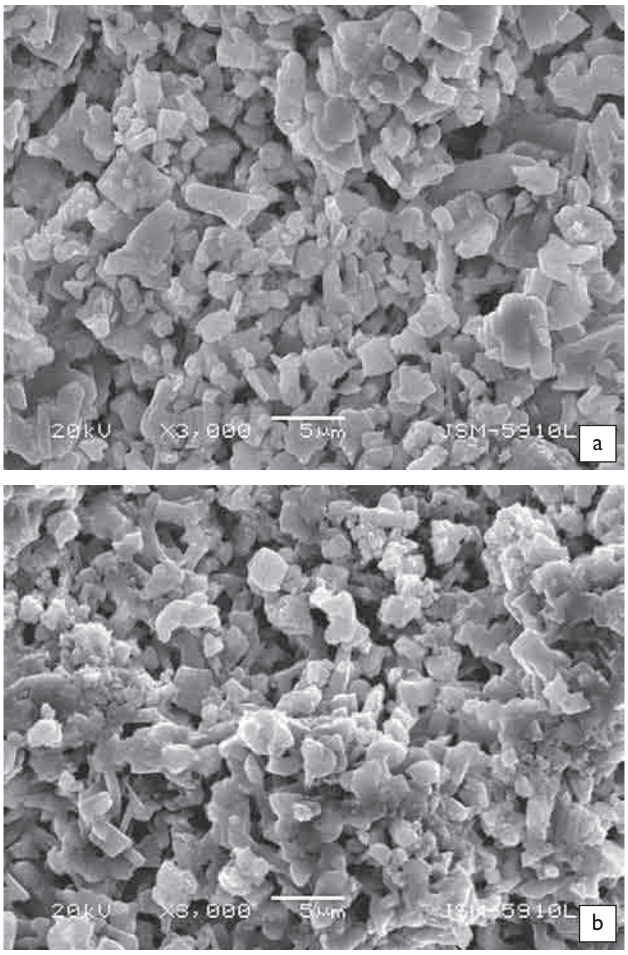

Figure 6 Particles, which were produced by hot-plating (a), and ultrasonication (b) methods, after firing at 800 °C for 4 hours.

CONCLUSIONS

Natural marine structures are very promising and attractive candidates for producing meso, micro and nano-powders of calcium phosphate bioceramics for medical applications. The methods used in this study, which efficiently combine hot-plate and ultrasonication, are safe, simple, and economic for calcium phosphate biomaterial production.

According to the X-ray diffraction analysis, the calcium pyrophosphate was the only phase in hot-plating process when compared to ultrasonication.

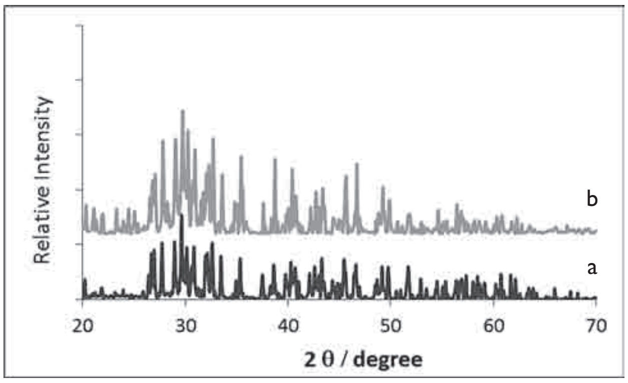

Figure 7 X-ray diffractograms of sea urchin powders, (a) hot-plated and (b) ultrasonicated

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Note: The responsible translator for English language is Ö. Faruk CANTEKİN, School of Foreign Languages, Gazi University, Turkey