Production of Apatite from Snail Shells for Biomedical Engineering Applications

Naziem Elkren1,2, Oguzhan Gunduz1,2, Sibel Celik1,2, Bilge Ayata3,2, Yesim Muge Sahin4, Joshua Chou5, Besim Ben-Nissan6, Serdar Salman1,2, Hasan Gokce7, Faik Nuzhet Oktar1,2

1Department of Electrical and Electronic Eng., Faculty Technology Marmara Univ., Istanbul, Turkey
2Advanced Nanomaterials Research Lab., Faculty Technology Marmara Univ., Istanbul, Turkey
3Department of Metallurgy, & Mater. Engineer., Faculty Technology Marmara Univ., Istanbul, Turkey
4biomed. Engineer. Dept., Engineer. & Architecture Faculty, Istanbul Arel Univ., Istanbul, Turkey
5Faculty of Med. & Molecular Biosciences, The Univ. of Technology, Sydney, NSW, Australia
6Chemistry and Forensic Sciences Dept., The Univ. of Technology, Sydney, NSW, Australia
8Bioengineer. Dept., Engineer. Faculty, Marmara Univ., Istanbul, Turkey

Abstract
Bioceramics is very important application for dental and orthopedic procedures. Beside all these normal procedures traffic accidents are requiring increasing number of grafts, prostheses and orthosis applications. Bioceramics can be produced from local and natural sources with various methods. Those can be produced from various bone structures through calcination (at high temperatures) or with diluted hydrochloric acid (HCl) application & freeze drying. Beside these methods calcite and aragonite structures like from sea shells and egg shells bioceramics production can be realized through mechanochemical processing via a simple hot-plate or ultrasonic equipment. A fresh water snail shell (Zebra Nerite Snail – Neritina natatorlis) was prepared as bioceramic production source. The resulting hydroxyapatite (HA) powders were obtained without any impurities. At two varying temperature of 865 and 885°C the snail shells was transformed to HA bioceramics. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential thermal analysis (TG/DTA) were evaluated.

Introduction
Calcium phosphate-based bioceramics such as HA have received much attention owing to their high biocompatibility, ability to be utilized as synthetic osteoconductive scaffolds in bone and with numerous clinical applications [1]. Beside orthopedic applications those bioceramics are also in use in various dental surgery applications (i.e. graft material for bone regeneration, filling material and dental implants) and aesthetic surgery applications such as facial, breast and body contouring applications. Also with increasing in traffic accidents biomaterials are gaining a big role for human health reconstruction in the field of orthopedic application.

Aging of the world population and increasing living standards have also boosted the increasing development of the orthopedic industry. Emerging materials, high technologies, applications and procedures will challenge the future market as reported in Ace Business Report [2].

HA based on biomaterials can be produced with various methods from synthetic and natural sources. One of the basic techniques is convention precipitation method from reagent grade chemicals. This method is a time consuming method. Natural species of sea origin, such as sea shells, coral and snail shells always receive much attention in biomedical engineering applications. Freeze drying and with diluted HCl treatment are also conventional ways for HA graft provision however prion diseases such as mad cow and related diseases can be very dangerous, risky and deadly [3-4]. The calcification of natural materials (which posses calcium phosphate at high temperature) of sea shells to human [3], bovine [4], sheep [5], chicken bones [6] HA, human-bovine-sheep tooth structures were used as obtaining of biomaterials such as dentine [7-8] and enamel [9-10] Natural HA structures can be produced very economically. HA biomaterial can also obtain from fish scales [10]. Lately natural HA is produced from calcium and aragonite structures with different methods mechanochemically using ultrasonic equipment [11-12] or a hot-plate stirrer [12].

Calcite and aragonite process material were converted to HA and other related phases the shells of Zebra Nerite Snail (Neritina natatorlis), which was used (Fig.1). Zebra Nerite Snail shells are approximately 8 and 16 mm in diameter [13].

The aim of this presented study is to obtain HA powders from snail shells using mechanochemical method via hot-plate. Characterization of HA transformed from snail shells sources follow to the evaluation of the final product, which can be used in biomedical engineering.

Materials and Methods
The shells of Zebra Nerite Snail were used (Fig. 1). Empty zebra nerite snail shells (ZNSS) were obtained from a local gift store in Istanbul – Turkey. ZNSS were washed under tap water possible from dirt remains. Cleaned ZNSS were soaked into distilled water, washed again and dried. After drying shells were ball grinded and sieved through 100 µm sieve.

Results and Discussion
The DTA and TG curves for the ZNSS samples after drying are revealed in Fig. 2. Fig. 2 reveals the weight loss of 43.04% owing to thermal decomposition of calcium carbonate to calcium oxide and carbon dioxide at 861.6°C, which is the main composition of ZNSS. The weight loss owing to organic matter was 1.55%. The weight loss along with endothermic peak at 700-870°C indicates the decomposition of calcite reaction. It is confirmed that the thermal analysis ZNSS mainly contains CaCO3 along with small amount of magnette and other organic matters.

Fig. 3. XRD analysis of the raw powders of ZNSS after ball milling and ageing.

Fig. 4. Scanning electron microscopy images (a) low and (b) high magnifications of (i) ZNSS raw powders, (ii) ZNSS sintered powder at 865°C and (iii) ZNSS sintered powder at 885°C.

SEM micrographs of the surface of the raw powders are shown in Fig. 4 a-b. It is clear from Fig.4 a-b that ZNSS consists mainly of calcite which is the most stable calcium carbonate. Agglomerates were also clearly observed as revealed in Fig. 4. The calcite particles are very large which is about 3μm x 3μm in size in square form and thin layer laminar prismatic form on each other. After sintering, the morphology was converted to nanoparticles which are about 60-100nm in Fig. 4 a-b, 3a-b.

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The production method, differential thermal and gravimetric analyses (DTA/TGA) of ZNSS were conducted at heating rate of 20°C/min between 50 and 1000°C under stream flow of nitrogen gas (Perkin Elmer Diamond TG-TDA). According to a previous study [15]

Discussion
The production procedure X-ray diffraction (XRD) and scanning electron microscopy (SEM) studies were conducted.

Fig. 1. Photograph of Zebra Nerite snail shells (Neritina natatorlis).

Fig. 2. Photograph of Zebra Nerite snail shells (Neritina natatorlis).