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Synthesis and structure of eggshell hydroxyapatite bone implant

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ABSTRACT

Atiyah AG, AL-Falahi NH, Farhan FK., Synthesis and structure of eggshell hydroxyapatite bone implant, Onl J Vet Res., 22 (6):486-491, 2018. We describe synthesis and structure of an hydroxyapatite implant from avian eggshell waste by hydrothermal sintered heating with H_20 and H_3P0_4 solutions calcinated at 1200°C. The resultant powder was compressed to a hydroxyapatite implant, then characterized by X-Ray Diffraction, Energy Dispersive X-ray spectroscopy and scanning electron microscopy. We found the implant to resemble features of pure hydroxyapatite with favorable calcium/phosphorus ratio.

Key words: Hydroxyapatite, Eggshell, Bioactive materials.

INTRODUCTION

Hydroxyapatite is commonly used in orthopedic and dental surgery (de Groot, 1993). Hydroxyapatite and related calcium phosphate materials have been widely used for bone regeneration or substitution due to their similarity to the inorganic phase of bone (Szurkowska and Kolmas, 2017). Natural hydroxyapatite is biocompatible with bone due to similar physical and chemical characteristics (Hench, 1998; Ghomi et al., 2011). The ratio of calcium to phosphate (C/P) in bone is 1.5 to 1.7 (de Groot, 1993).

Natural hydroxyapatite can be synthesized from eggshells, coral, fish bone, chicken and bone (Baba et al., 2013). Eggshells are 3-layered structures with an outer cuticle, inner spongy and lamellar layers of protein fibers, bonded with calcium carbonate crystals (Sanosh *et al*., 2009). The shell constitutes 11% of total weight of an egg constituted by 94% calcium carbonate, 1% calcium phosphate, and 5% other organic matter (Sanosh *et al*., 2009). Eggshell are good source for synthesis of hydroxyapatite as it contain high percentage of calcium carbonate (Rivera *et al*.,

1999). We describe synthesis and structure of eggshell hydroxyapatite for bone implant.

MATERIALS AND METHODS

Steps for fabrication of the eggshell hydroxyapatite implant are illustrated in Figure 1 below. First, uncrushed eggshells were cleaned in deionized water, boiled for 30 min and oven dried at 100c^{o} for 30 min as described by Khandelwal *et al*. (2016). The shells were then crushed to powder in an electrical crushing machine and calcinated in a muffled furnace (Prothrom, Turkey) at 1200˚C for 2 hrs. Carbon dioxide in the eggshell is converted into calcium oxide:

$$CaCo_2 \rightarrow CaO + CO_2$$

The calcinated egg shell powder is then placed in a beaker and dispersed in distilled water, wherein CaO transforms to $Ca(OH)_2$ as shown below:

$$CaO + H_2O \xrightarrow{\Delta} Ca(OH)_2 + Heat$$

Orthophosporic acid solution (Ridel, Turkey) of 0.6M was added to $Ca(OH)_2$ solution, to calibrate pH of the solution to 8.5 monitored with a PH meter (AD1000-Germany). This forms a precipitate as shown below:

$$10\ Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

The solution was kept for 48 hours at ambient temperature to consolidate the precipitate and formation of hydroxyapatite. The precipitate was kept at 100˚C to dry in an oven and then calcinated at 1200˚C for 2 hours in a muffled furnace, which formed a white crystalline powder with HA. The powder was then compressed in an electromechanical compression device (MTI-40MPA-USA) to form the hydrroxyapatite implant as shown below in Figure 1.

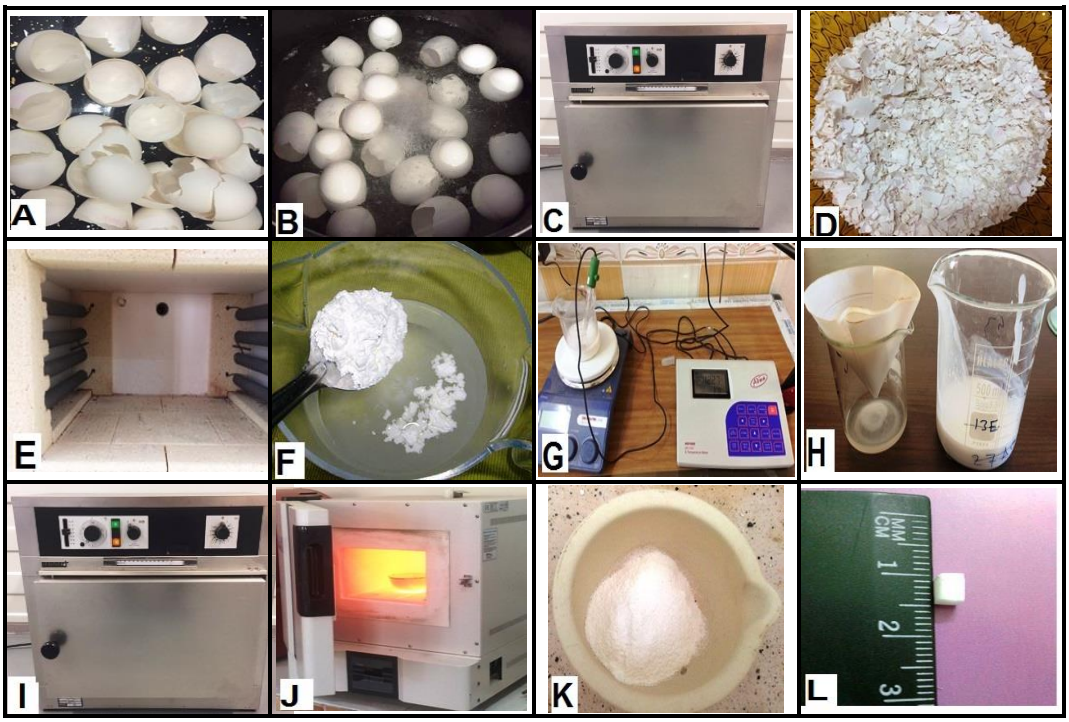

Figure 1. Synthesis of eHA (A), Cleaning and washing of egg shells (B), Boiling of egg shells in distilled water for 30 min (C), Drying in oven at 100C^{o} for 30 min. (D) Crushing egg shells (E), Calcination at 1200 c^{o} for 2 hr. (F) Dispersion of powder in water (G), Addin drops of H_3PO_4 to adjusted PH to 8.5 (H),

Aging for 48hr. (I) Dray in oven 100c for 30 min. (J) Calcination at 1200 c^{o} for 2 hr. (K) Egg shell HA powder formation. (L) Egg shell HA implant.

Phases and purity of the eggshell hydroxyapatite powder were determined by X-ray diffraction (Crystalloflex-diffractometer, D-500, Siemens, Germany), at 63kV and 8 mAs. Scanning electron microscopy (Tuscan Vega 3rd Generation - England) was used to visualize surface morphology and approximate pore sizes of implant. Samples were subjected to gold spattering prior to electron microscopy to provide conductivity. Energy dispersive X-ray analysis was performed to identify calcium, phosphate and oxygen in the implant (Li-yun C *et al.,* 2005).

RESULTS

X-Ray diffraction of the eHA powder samples were analyzed within 2 theta (2θ) range from 20^{o} to 60^{o} with intensity peaks ranging 0 to 1000^{o}. Figure 2 shows that at 1000°C, X-ray diffraction showed hydroxyapatite at peaks 27.6, 29.8, 30.1, 30.7 and 40.4 representing crystalline phases (ICPD, Card number 9-0432) with other phases such as calcium oxide.

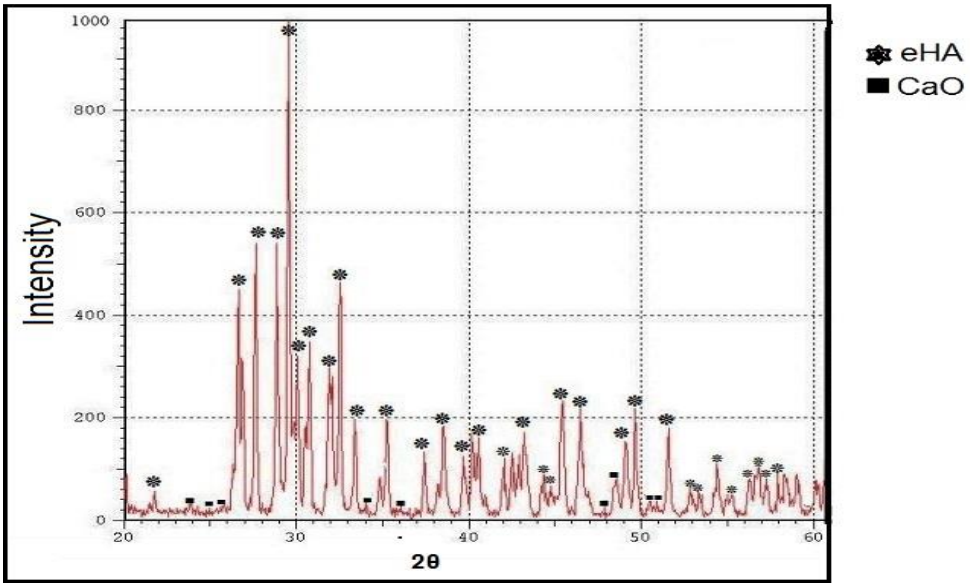

Figure 2. X- ray diffraction of eggshell hydroxyapatite intensities at scattering angles of 2θ.

Figure 3 illustrates scanning electron microscopy of the hydrioapatite implant at 500x and 1000x magnification. Crystal in the implant can be seen with agglomerates of irregular particles with spherical shapes between random irregular pores of 10-50µm.

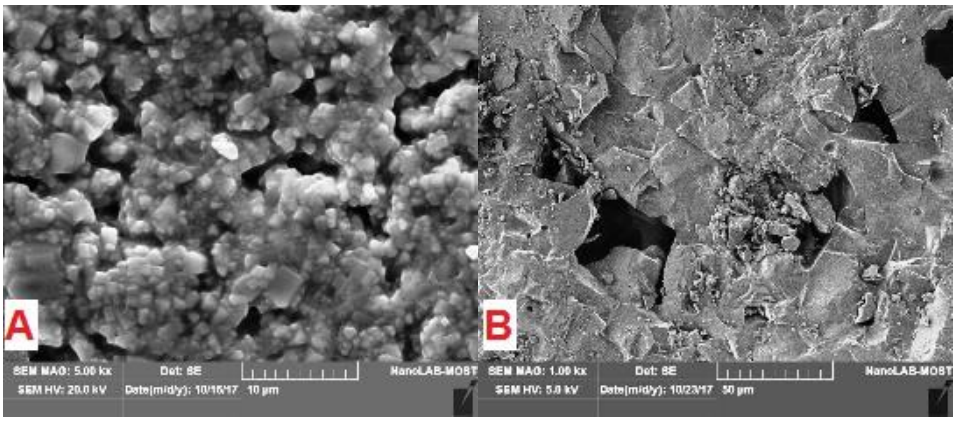

Figure 3. Scanning electron microscopy images show structural morphology of eggshell hydroxyapatite at (A) 500× (B) 1000×.

Table 1 lists chemical analysis determined by energy-dispersive X-Ray spectroscopy of the implant with calcium (Ca), phosphorus (P), and oxygen (O_2) atomic weights and a Ca/P ratio of 1.56 , (35.56 % and 22.77 % respectively).

Table (1): Energy-Dispersive X-Ray Spectroscopy of eHA implant

Elements	Weight %	Atomic %
O	41.67	63.42
P	22.77	17.56
Ca	35.56	19.02
Total	100.00 %	100.00 %

DISCUSSION

X-Ray diffraction of the implant showed sharp narrow peaks suggesting that the amorphous eggshell calcium hydroxide was converted to crystalline hydroapatite with less organic and carbonate material as described by Scalera et al. (2013). Le and Radlazi (2014) maintained that stirring speed and setting time play an important role in the purity in eggshell hydroxyapatite because it allows diffusion of ions of the reactants (Ca^{+2}, Po_4, H^+ and OH^-) with each other, and complete transformation of amorphous calcium hydroxide to crystalline forms.

By microscopy we found a porous grainy crystal structures which became thicker and aggregated and bonded at higher temperature (1200c^o). This allowed formation of pores between the crystals with clusters of agglomerate appearing rounded to spherical. Wu et al. (2015) found that the average size of hydroxyapatite particles gradually increased from 100c^o to 1200c^o.

By X-Ray spectroscopy we found mainly Ca and P in implant material together with minor elements from eggshell similar to that commonly found in natural bone as described by Roudan et al., (2017). In the present study, the Ca/P molar ratio of our eggshell hydroxyapatite was about 1.56, close to the stoichiometric theoretical value for hydroxyapatite of 1.67 (Dadhich et al. (2013). Changes in the Ca/P ratio can be

controlled during processing by adding phosphoric acid solution to calcium hydroxide to calibrate to pH 8.5 (Dadhich et al. (2013).

Processing temperature has a crucial role in Ca/P ratio in hydroxyapatite with higher temperatures forming more crystalline bone substitute (Li-yun et al., 2005). Crystallinity dictates behavior of bone matrix in vivo or the behavior of biodegradable material in to biological fluids and samples with a greater molar ratio Ca/P are more stable, crystalline and less soluble; whereas lower temperature of synthesis leads to greater solubility in hydroxyapatite (Li-yun et al., 2005).

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