Preparation and Characterization of Biological Hydroxyapatite (HAp) Obtained from Tilapia Fish Bone

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Abstract
Increased amount of fishery by-product waste from tilapia fish and concerned over reducing undesirable environmental impact leads to conduct of this study. This study performs biological HAp extracted from tilapia fish bones which were collected from fishery factory as discard waste. Therefore, fish bone can be as cheap sources to produce HAp and can be crucial contributions in medical application. Based on this objective, fish bones of tilapia have been cleaned after boiled and milled for 1 h performed fine powder. The powder was calcined at temperature 800°C and 1000°C for 3h respectively. The XRD and TGA result revealed the presence derived HAp and coherent with Joint Committee on Powder Diffraction Powder Standard data. SEM results shown polygon particle shape obtained from HAp powder. The findings present a promising future regarded on producing high added value product from fishery waste beneficial to medical application.

Keywords: Tilapia fish bone, hydroxyapatite

Introduction
The medical implant technology has growth rapidly nowadays and enrolled as crucial part in healing human tissue defect. Hydroxyapatite was found as biocompatibility, biodegradable and osteoconductive material widely used in medical implant or orthopaedic surgery in healing tissue defect [1]. Hydroxyapatite is an inorganic substance occurred in bone where its chemical formula is Ca_{10}(PO_4)_6(OH)_2 with stoichiometric ratio of Ca/P is 1.67. The chemical composition of hydroxyapatite was proven established in animal experiments and it has shown as biocompatible and bioactive material. Nevertheless, hydroxyapatite recently can be found in natural sources such as fish bone [2], fish scale [3], and cuttlefish [4]. Natural hydroxyapatite is believed to have better metabolic activity and more dynamic response to the environment than the synthetic.

Experiment Method
Frozen tilapia fish bone were collected from fishery factory located in Perak as a by product waste. Bones were directly boiled in hot water for 1 h. Later, it were washed with tap water and brushed to remove adherent fish meat and impurities. Thereafter, bones were dried under the sun light for 2 days. The dried bone afterwards had been calcined in furnace at different temperatures 800°C and 1000°C with heating rate of 5°C/min in air and cooled naturally in furnace. Bones were heated for 3 h of each increment of temperatures. Next, calcined samples were milled by using ball mill machine for 1 min to produce in powder form. The powder morphology, mineralogy and composition were characterized by used of X-ray diffraction (XRD), Scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDX), and thermal gravimetric analysis (TGA) machines.
Result and Discussion
SEM analysis indicated that the obtained calcined powders are composed polygon shape particles. There were significant different observed on morphology crystals between 3 examined samples. The representative morphology of those samples is presented on Figure 1. Both calcined powders were conjectured to have different crystal size. The crystal sizes were predicted to increase with respect to the temperature. Therefore, calcined powder (1000°C) was observed to have larger particles grain size than calcined powder at 800°C. According to Figure 2, the existed of HAp phase and purity were confirmed from XRD analysis where it was founded to have similar composition compared with HAp (JCPDS-01-1008). The derived HAp was believed to be obtained in thermal process due to the tendency of particles to crystalline and agglomerates at high temperature. The main components of the powders obtained are calcium and phosphorus, where an average of Ca/P molar ratio around 1.757 which higher compared to stoichiometric of HAp in human bones around 1.67. Higher Ca/P molar of the powder was suspected happened due to the existing of B-type carbonate hydroxyapatite where the carbon ions were occurred to substitute the phosphate ions. However, occurrences of B-type carbonate hydroxyapatite can be significant in stimulate cell attachment and collagen production due to the low attraction of osteoblast cell in human trabecular reported for A-type apatite.

Conclusion
Tilapia fish bone proved contained hydroxyapatite which is crucial in medical augmentation or regeneration of human defected tissue. Calcined fish bone powders with high temperatures were able to produce HAp stoichiometric closely to standard HAp. Even though, the Ca/P molar ratio obtained from calcined samples are higher than 1.67 stoichiometric of HAp, it still believed to bring beneficial in stimulating bone in growth. Hence, fish bones was identified as cheap source available to produce HAp and sustainable

Fig 1: SEM micrograph of surface morphology of tilapia bone powder at different calcinations temperature.

Fig 2: Comparison of XRD patterns for tilapia bone powder.

Reference