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Transmission electron microscopy analysis of hydroxyapatite nanocrystals from cattle bones

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ABSTRACT

In this present study, hydroxyapatite which was obtained from cattle bones has been heat treated at temperature 400 °C and 600 °C. The microstructure after the treatment has been studied in detail using Transmission electron microscopy (TEM) and X-ray diffraction techniques. The TEM results indicate that natural bone consists of collagen and hydroxyapatite nano-crystals which are needle shaped. The heat treatment influences the crystallinity and growth of these hydroxyapatite nano-crystals known as 'crystal maturation' or 'crystal ageing'.

1. Introduction

Bone comprises of two major phases: organic phase consisting of collagen and the inorganic phase consisting of hydroxyapatite nanocrystals [1]. Collectively, they form 95% of the dry bone weight [1,2]. The remaining consists of some organic noncollagenous proteins and amorphous inorganic salts [1]. All these phases together make a tough material.

Recently, most research are focussed on the structural morphology and synthesis of replicate materials [2]. Due to some of its superior known properties such as good biocompatibility, bioactivity, and osteoconductivity, hydroxyapatite finds its application in medical field [3]. In addition, due to its adsorption properties, it is being used in removing pollutants from waste waters [4]. Bone apatite consists of poorly crystalline structure along with some ionic substitutions [1,5]. Apart from this, carbonate ion is found abundant in the bone mineral. It plays a major role in the crystal stability since its substitution at the phosphate position can distort the lattice structure [5]. It is a very well-known fact that the natural bone powder is poorly crystalline and after thermal treatment, the crystallinity increases. This crystallinity is due to the hydroxyapatite nanocrystals which are majorly oriented towards their c-axis [6] and are arranged in a parallel array. When the crystal ages, the size, shape, composition, and the crystal lattice structure change rapidly [7].

This present study gives an overview of the entire journey of these hydroxyapatite nanocrystals before and after thermal treatment. Cattle (bull and bovine) bones were selected for the study. The temperature for the study has been chosen as 400 °C and 600 °C. It is an attempt to study the changes occurring during the crystal ageing at molecular and nanoscale level.

2. Experimental section

2.1. Preparation of bone char

The precursor for the bone char has been prepared as described in our previous studies [8].

The precursor was then carbonised at the temperatures 400 °C and 600 °C for 2 h respectively. These temperatures were chosen for detailed study since at 400 °C there is loss of carbon, water and volatiles and at 600 °C, the organic matrix gets completely removed [9]. Pyrolysis of the samples took place in a rectangular muffle furnace with a limited supply of air and the heating rate was set to 10.2 °C/min.

X-ray Diffraction (XRD) was used to study the phase composition of the samples before and after the heat treatment. The X-ray spectra was analysed using BRUKER D2 PHASER operating with Cu-Kα (λ = 1.54184 Å) radiation at a current of 10 mA and an accelerating voltage of 30 kV. The data was collected with a step count of 0.02° in the range of 2θ = 10–70°.

Transmission Electron Microscope (TEM) was used to study the crystal orientation of the samples prepared. The TEM micrographs

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Fig. 1. Colour change during heat treatment. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

were obtained using Tecnai F20 operating at 200 kV. For the TEM analysis, the samples were prepared by dispersing a very small amount of it in ethanol under ultrasonication. After this a drop of the diluted sample was placed on the copper grid coated with a thin carbon film.

3. Results and discussion

3.1. Colour of the bone char

In the bone, the collagen fibres form an organised matrix within which the nanocrystals of the hydroxyapatite are located. The amorphous phase of the mineral is dominant in early bone and as the bone matures, the crystallinity of the apatite increases [5]. Also, the colour of the charred samples is an indication of the bone being altered due to the heat treatment [10]. The raw bone samples obtained were pale yellow in colour. When these samples were charred at 400 °C, the colour changed to black and at 600 °C, the colour changed to light grey as shown in Fig. 1.

3.2. XRD analysis

The XRD studies were performed on the raw bone powder and charred samples as shown in Fig. 2. The XRD patterns show that both raw bone powder and charred samples are hydroxyapatite (HAP). Strong peaks are observed at (002) which is also the c-axis [11] of HAP and the other peaks are merged at (211), (112), and (300) planes. The XRD pattern does not show changes after raw bone powder has been treated at 400 °C for 2 h. However, the peaks of HAP become sharp with increasing the thermal treatment temperature to 600 °C, indicating the crystallinity of HAP phase increased. The morphologies and structure of HAP have been further investigated by the TEM techniques.

The average size of hydroxyapatite is estimated by the Debye–Scherrer equation:

$$\tau = \frac{K\lambda}{\beta \cos\theta}$$

where τ is the mean crystal size, K is the shape factor taken as 0.9, λ is the X-ray wavelength in nm, β is the line broadening at the half maximum intensity (FWHM) on the 2θ scale in radians and θ is the Bragg angle of the peak in degrees.

The average crystal size [9] along the (002) plane is as shown in Table 1.

3.3. TEM analysis

Fig. 3 shows the TEM results of the raw bone powder. Fig. 3 (a) and (b) shows the bright field (BF) and dark field (DF) images of raw bone

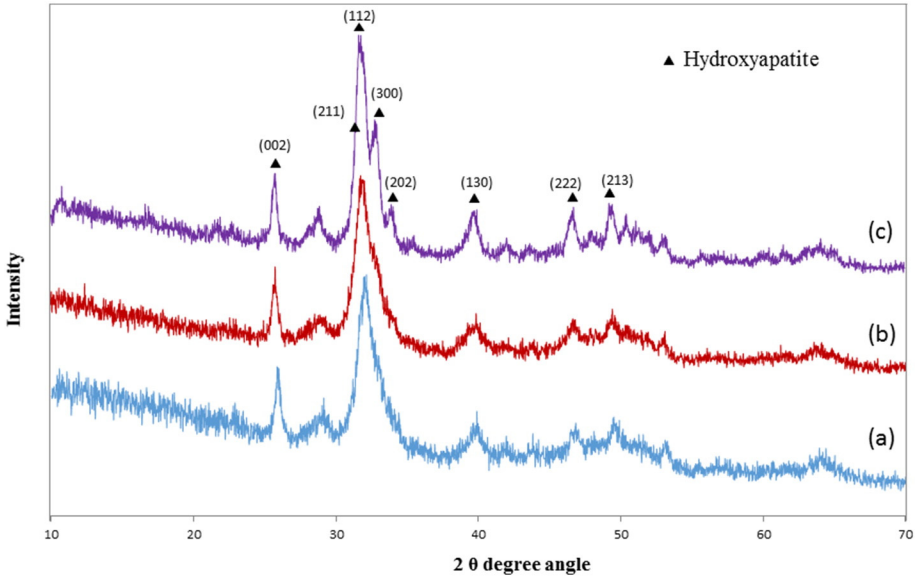

Fig. 2. XRD patterns of (a) raw bone powder and bone char at (b) 400 °C and (c) 600 °C.