

Mineralization of Hydroxyapatite over Collagen Type I

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INTRODUCTION: Bone is a specialized form of connective tissue, that similar to others connective tissues, consists of cells and extracellular matrix. The difference between bone and others tissues, is the mineralization of the matrix, which provide the bone hardness for support and protection. The composition of the mineral matrix in cortical bone is ~70% of Hydroxyapatite and ~30% organic (largely collagen). In order to develop bone substitutes, Hydroxyapatite (HAp) has been intensively studied from de viewpoints of biocompatibility, bioactivity and osteoconductivity similar to bone, and collagen has also been studied for the osteoinductive properties and bioresorbability [1]. To achieve properties that are similar to the properties of bone, it is necessary to mimic not only the composition of the natural bone but also its structure, where HAp nanocrystals align their c-axes along collagen fibers. In the present investigation HAp-collagen composites have been made by two different methods of synthesis. The orientations of the structures have been observed by electron diffraction patterns.

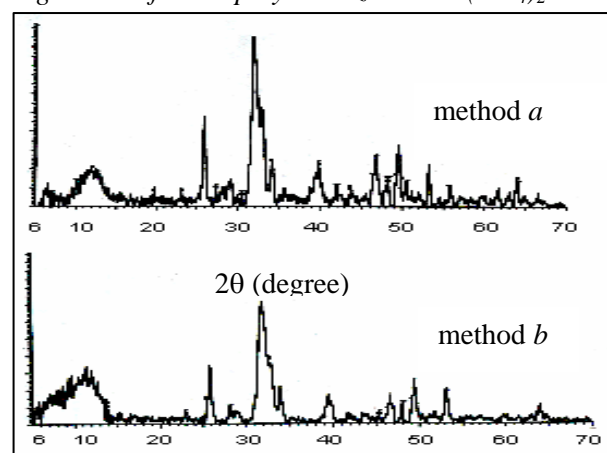
METHODS: Two different methods were used to produce the Hap/collagen composite. The starting materials used for the first reaction (method a) were $(\text{NH}_4)_2\text{HPO}_4$ and $\text{Ca}(\text{OH})_2$, with a ratio Ca/P 1,67. A $\text{Ca}(\text{OH})_2$ aqueous suspension and a solution $(\text{NH}_4)_2\text{HPO}_4$ with collagen type I were gradually added into a reaction vessel with a pH ~10,5. The weight ratio of HAp/collagen was fixed at 80/20. Then, the precipitate was aged at 40°C for 24 h, the pH was gradually lowered to 8 by centrifugation at 10000 rpm. Finally the solid collected was dried at 60°C under vacuum. The second reaction (method b) was made with H_3PO_4 aqueous solution in presence of collagen type I and $\text{Ca}(\text{OH})_2$ suspension with a final pH 8,5 following the same procedure described above[2].

Characterization of HAp/collagen was made by X-ray diffraction (XRD) analyses. In preparing samples for transmission electron microscopy (TEM) observation, the dried powder was re-suspended in distilled water; a drop of the suspension was placed on a collodion-coated and carbon-reinforced copper electron microscope grid. After air drying, the sample was observed. The self-assembly phenomena of the HAp crystals that

formed on the bio-organic templates were assessed using electron diffraction patterns.

RESULTS: Figure 1 shows the results of XRD measurements of both composites (a and b). The HAp peaks are broad, implying the nanocrystalline size of HAp. Figure 2 shows the TEM micrograph, where the mineral formed by the method a with $(\text{NH}_4)_2\text{HPO}_4$ have a crystal size of 10 nm width and 30 nm length and figure 3 shows the TEM micrographs of the synthesis by method b with H_3PO_4 and a crystal size of 10 width and 55 nm length.

Fig.1: XRD for HAp synthesized with $(\text{NH}_4)_2\text{HPO}_4$



(method a) and with H_3PO_4 (method b).

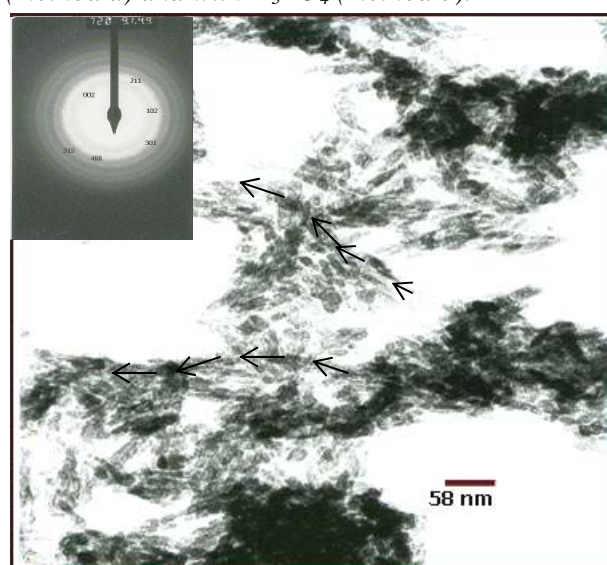


Fig. 2: TEM micrograph and electron diffraction pattern of HAp/Coll by method a.

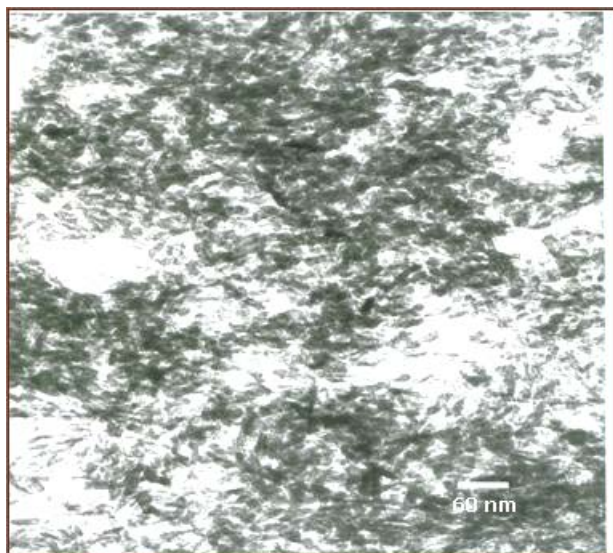


Fig. 3: TEM micrograph of HAP/Coll by method b.

DISCUSSION & CONCLUSIONS: The nucleation of Hydroxyapatite through the chemical interaction with collagen by two different methods of synthesis has been examined. The method *a* is not exactly a biomimetic synthesis, but the crystal size corresponds with that reported in literature for natural bone, the arrows show that crystals follows a oriented distribution over collagen fibrils, although diffraction pattern did not show any preferential orientation, perhaps it could not be clearly observed due to the large amount of HAP crystals present. Method *b* was made with an almost neutral pH, the crystals size is larger than the obtained with the first method although this size is still in good agreement with that of natural bone.

It has been under discussion for decades whether collagen acts as a promoter or inhibitor of the mineralization; however, recent studies point more in the direction that collagen has no effect on the mineralization[3]. In this work we could prove that an interaction exists between collagen and hydroxyapatite, and this was possible without the presence of noncollagenous proteins.

REFERENCES: ¹ S-H. Rhee, J. Tanaka (1998) *J. Am.Ceram.Soc.*,81:3029-31, ²S. Itoh, M. Kikuchi, et al (2002) *J Biomed. Mater. Res. (Appl. Biomater.)*,**63**:507-15. ³A.L. Boskey (1996) *Connective Tissue Res.*,**35**:357.

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