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論文内容の要旨

[Introduction]

To apply hydroxyapatite (HAp) as a substrate for soluble factors or cells for bone tissue engineering purpose, the modification of its physico-chemical properties is a pre-requisite. Since, acidic amino acids (negatively charged) have the well documented affinities to HAp; we assumed that HAp modified with negatively charged amino acids would have the potentiality to carry the positively charged growth factors, such as basic fibroblast growth factor (bFGF) and bone morphogenetic protein (BMP). Based on this idea, HAp was synthesized in the presence of various concentration of aspartic acid-Asp (HAp-Asp). Physico-chemical properties including crystal morphology and protein adsorption was investigated in this study.

[Methods]

HAp particles were synthesized by using wet chemical precipitation method. $Ca(CH_3COO)_2 \cdot H_2O$ and $NH_4H_2PO_4$ was added to acetate buffer solution under controlled temperature and pH. Asp (0 - 0.5 mol/l) was dissolved in the acetate buffer solution. The obtained particles (HAp-Asp) were washed with distilled water and Phosphate Buffered Saline (PBS), and then dried for 72 h at 80°C.

Obtained HAp-Asp was investigated with X-ray diffraction analysis (XRD) for crystal analysis, transmission electron microscopy (TEM) for crystal morphology, and fourier transform infrared spectroscopy (FTIR) and simultaneous thermogravimetric analyzer (DTG) for Asp incorporation in the obtained HAp. The nitrogen gas adsorption was measured for understanding the specific surface area of the HAp-Asp particles by Flowsorb II 2300 (Micrometrics, Norcross, GA, USA). Then, adsorption of model protein, which is acidic (bovine serum albumin-BSA, pI = 4.7) and basic (cytochrome C, pI =10.2), onto the HAp-Asp was measured by using BCA protein assay. Also, Asp adsorption/desorption onto normal HAp was investigated using FTIR.

[Results and Discussion]

XRD results indicated that the crystallinity of HAp gradually decreased including a decrease in peak heights in (002) and (300) plane and increase in half value breadths with the increase of Asp concentration during HAp-Asp synthesis. The 002 peak in HAp-Asp shifted to higher angle position which indicated that Asp had led the lattice misfit of HAp crystals. The lattice constants in both a and c axis of HAp-Asp changed on increasing Asp concentration compared to HAp. TEM images showed that the HAp mean crystal size decreased when Asp was added to its synthesis solution from 1795 (±6.55) x 279 (± 1.92) nm (Length x Width, HAp) to 246 (±1.16) x 32 (±0.23) nm (HAp-Asp with 0.25 mol/l Asp). FTIR results confirmed the presence of Asp in HAp-Asp crystal. DTG analysis showed that the weight loss in HAp-Asp was gradually increased with an increase in the concentration of Asp. Moreover, the weight loss profile of Asp from HAp-Asp did not reflect a similar pattern to that of Asp alone. The desorption study showed that desorbed amount of Asp from HAp-Asp was extremely low whereas Asp was almost completely desorbed from HAp with adsorbed Asp. These results together indicated that Asp inhibited the crystal growth of HAp-Asp in both a and c axis; and Asp was incorporated in HAp crystals, though a small amount of Asp was trapped between HAp crystals.

An increase in Asp concentration during HAp synthesis promoted an increase in the adsorption of both proteins onto HAp-Asp, though the adsorbed amount of the positively charged protein (cyto c) onto HAp-Asp was higher than that of the negatively charged protein (BSA). This suggests that the increase in protein adsorption onto HAp-Asp was due to the increase of negatively charged sites of HAp-Asp. To confirm this, HAp-Asp particles blocked by NH_4^+ (positively charged ion) was prepared and protein adsorption onto NH_4^+ blocked HAp-Asp particles was investigated. Cyto c adsorption onto HAp-Asp blocked by NH_4^+ was significantly attenuated compared to that onto normal HAp-Asp, while the adsorbed amount of BSA onto HAp-Asp blocked by NH_4^+ slightly decreased compared to that onto normal HAp-Asp. These results indicated that the synthesized HAp-Asp increased the selective loading capacity of molecules, especially the positively charged ones.

In conclusion, HAp synthesis in the presence of Asp leads the incorporation of the acidic molecules in the HAp crystals that alters the surface characteristic, which shows a selective loading capacity of positively charged protein.

論文審査の結果の要旨

本研究では、ハイドロキシアパタイトにアミノ酸による物理･化学修飾を行うことにより、細胞や増殖因子のキャリア材料としての有用性を高めることを目指

した。

その結果、アミノ酸の修飾により、アパタイト材料表面の化学的特性を制御できること、特に酸性アミノ酸はアパタイト結晶構造内に取り込まれ、高い等電点を有する塩基性タンパク質などの分子の選択的吸着性を高めることを明らかにした。

硬組織の誘導や細胞接着において、多様な塩基性タンパク質が寄与することから、本研究の結果は、新しい硬組織再生用生体材料の開発に有効な知見であり、博士（歯学）に値するものと認める。