Cobalt Incorporation into Hydroxyapatite by a Simple Ion Exchange Technique and Its Effect on Physical Properties

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Abstract

Synthetic hydroxyapatite (HA) is developed for biomedical applications. To modify HA property, ion incorporation into HA is of interest recently because the structure of HA permits ion substitution/adsorption. The aim of this study was to synthesize and characterize the physical properties of cobalt (Co)-incorporated HA (HACo) using simple soaking procedure.

Different concentrations of Co were incorporated into HA (40-8,000 μM) via a simple soaking method. The resulting samples were uniaxially pressed into the disc form and sintered at 1100 oC. The discs were characterized for material properties including color, weight, diameter, surface microstructure, and surface roughness.

The color of unsintered HA and HACo were white and pink color, respectively. After sintering, the color of HACo turned to grey color. The color changes with Co concentrations. In general, the discs showed decrease in diameter and increase in weight loss after sintering and with increasing Co concentrations. HACo 8000 showed the highest loss in both diameter and weight. HACo did not exhibit difference in surface microstructure from HA. HACo 4000 possessed the greatest surface roughness.

The material characteristics were altered after Co incorporation. These data provided information that should be considered when developing biomedical materials.

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Introduction

Hydroxyapatite (HA) has extensive application due to its biocompatibility and ability to respond to physicochemical environment.[1,2]

HA is a good candidate for bone grafting material since it resembles mineral component of tooth and bone.[3] Besides bone augmentation, synthetic HA has been applied for the coating of implants or acting as fillers in bone or teeth.[4] Furthermore, the use of synthetic grafting material such as HA reduces the risk of donor morbidity[5] and has no limitation for availability. The use of the HA bone substitute was found to enhance alveolar bone regeneration when used for sinus augmentation but not for periodontal bone defects.[5]

Aforementioned above, the use of pure HA remains limited. It can be further developed in order to use as a treatment of choice for alveolar ridge preservation[5] or for other applications. HA properties and characteristics can be improved because the structure of HA allows a wide range of modifications. The crystal structure of HA accepted various types of substitutions, adsorptions, or binding, which allowed for tailoring material properties.[6-9] Such alteration could influence the physicochemical and biological characteristics of the biomaterials.[7-10] For example, bisphosphonate-bound HA particles engulfed by macrophages could alter cell viability.[10] Carbonate-substituted HA was developed for an implanted ceramic biomaterial. HA with high carbonate content had an inductive effect on osteoclast resorption through an osteocyte-like cell activity.[7] Iron-doped HA

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nanoparticles exhibited paramagnetic properties, which is contrasted with the diamagnetism of pure HA.[9] The synthesis of HA that contains substituted/adsorbed ions or drug binding has lately attracted a lot of interest.

Metal ions at low doses could be used as therapeutic agents and can potentially be applied in tissue engineering biomaterials to alter the material properties.[11] Although much work has been done in HA doping with several types of metal ions, cobalt-incorporated HA (HACo) using a simple soaking method has not been widely studied. Co is naturally found in the human body as a fundamental component of vitamin B12 and other co-enzymes called cobalamins.[12] Thus, this study focused on the characterization of the physical and surface properties of HACo. HA and Co-incorporated HA were prepared at various concentrations and the effects of incorporation on material characteristics including color, weight, diameter, disc surface, and surface roughness were assessed. The knowledge might lead to the prediction and understanding of their physicochemical and biological performance.

Materials and methods

Hydroxyapatite and cobalt-incorporated hydroxyapatite synthesis

Hydroxyapatite (HA) powder (Hap-200) was obtained from Taihei Chemical Industrial Co. To attain cobalt (Co)-incorporated HA, HA powder was subjected to a simple soaking procedure modified from previous studies.[9, 13] Salt solutions of the cobalt chloride ($CoCl_2$•$6H_2O$; Sigma) were prepared at the concentrations of 40 μM, 400 μM, 4000 μM, 8000 μM. HA powder at an amount of 1 g was then soaked in 100 ml of prepared Co solutions and stirred under moderate stirring for 1 h at room temperature. The powder was collected by filtration, washed by deionized water and dried in hot air oven at 100ºC.

To prepare a disc-shaped specimen, 0.28 g of powder was loaded into a 10-mm diameter mold and uniaxially pressed using a bench top laboratory manual press (Caver, M3853) at a pressure of 2 MPa. The obtained specimen had a thickness of approximately 2 mm. All discs were sintered at a temperature 1100 °C for 6 hours using a ramp rate of 5°C per minute in a chamber furnace (CM, 1610FL). HA sample was used as control. HACo samples at various concentrations were designated as HACo 40, HACo 400, HACo 4000, HACo 8000.

Characterization of hydroxyapatite and cobalt-incorporated hydroxyapatite

Physical characteristics

The color of samples before and after sintering was estimated by taking the photographs of the samples using the same acquisition parameters and visually gauged. Weight and diameter of the samples were measured by analytical balance (Mettler Toledo) and vernier caliper (Absolute Digimatic), respectively. Fifteen discs per sample were determined. Difference in weight and diameter between nonsintered and sintered samples were then calculated and presented in % difference.

Scanning electron microscopy (SEM)

The surface microstructure of the sintered samples was determined by SEM (JCM-6000) at 5 kV accelerating voltage and photographed at 1,000x and 5,000x magnifications. Prior to observation, the samples were mounted on stubs with a conductive carbon tap and sputter-coated with gold.

Atomic force microscopy (AFM)

AFM was used for the surface roughness (Ra) analysis of the samples. Imaging was performed with semiconductor laser at the wavelength 830 nm (pixel 512, line 256) attached to cantilevers (SiDF3) with scan rate 0.25 Hz (Hitachi, model 5500M). Three discs per sample (three different areas on each specimen) were analyzed.

Statistical analysis

Statistical analyses were performed by using GraphPad Prism Version 9.0 (GraphPad Software Inc.). Quantitative data were expressed as mean ± standard deviation. Normality and distribution of the data were tested by Shapiro-Wilk. For the results comparison among groups, one-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test. Statistical significance was considered when $p<0.05$.

Results

Physical characteristics of hydroxyapatite and Co-incorporated hydroxyapatite discs: color, dimension, and weight

The unsintered HA disc had white color. After incorporation of Co, the color of the discs turned to pinkish color and the color saturation increased with increasing Co concentration (Figure 1A). After sintering, no change in color was noted for HA but the color of HACo turned to

gray shade and the color saturation also increased with increasing Co concentrations similarly to the unsintered samples (Figure 1B).

Figure 1

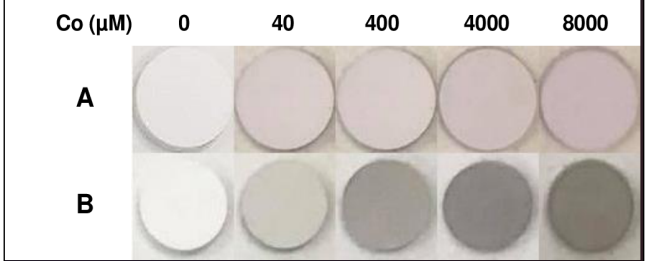

Figure 1. Color of hydroxyapatite and Co-incorporated hydroxyapatite disc color. (A) Discs before sintering. (B) Discs after sintering.

Table 1 shows the diameter and weight of the HA and HACo samples before and after sintering. The nominal diameter and weight of the unsintered discs after mold pressing were 10 mm and 280 mg, respectively. All samples showed a decrease in diameter and weight after sintering regardless of the amount of Co concentration. However, increasing Co concentrations tended to increase the weight loss after sintering. The sintered HACo 8000 showed the highest weight loss at 12.5 ± 1.56 % (Table 1). However, all discs retained the typical flat top and bottom surfaces.

Samples	Diameter (mm)		Diameter difference (%)	Weight (mg)		Weight loss (%)
	Before sintering	After sintering		Before sintering	After sintering	
HA	10	8.93 ± 0.03 ***	10.68 ± 0.29	280	263.8 ± 1.01 ***	5.79 ± 0.36
HACo 40	10	8.81 ± 0.04 *** ***	11.86 ± 0.42 ***	280	261.07 ± 2.25 ***	6.76 ± 0.8
HACo 400	10	8.90 ± 0.06	10.99 ± 0.61	280	260.8 ± 3.05 * ***	6.86 ± 1.09
HACo 4000	10	8.83 ± 0.04 *** ***	11.7 ± 0.41 ***	280	250.27 ± 3.58 *** ***	10.6 ± 1.28 ***
HACo 8000	10	8.81± 0.04 *** ***	11.9 ± 0.36 ***	280	245.13 ± 4.36 *** ***	12.5 ± 1.56 ***

*** vs control; +++ vs before sintering
% difference between before and after sintering

Table 1 Dimensional changes of hydroxyapatite and Co-incorporated hydroxyapatite discs.

Surface microstructure by scanning electron microscopy

SEM micrographs depicted the coalescence and dense contacts between granules and micropores were distributed throughout. Generally, no substantial difference in the surface microstructure was observed among all samples (Figure 2). However, HACo 400 and HAC0 4000 seemed to possess higher microporosity than other samples (Figure 2).

Figure 2

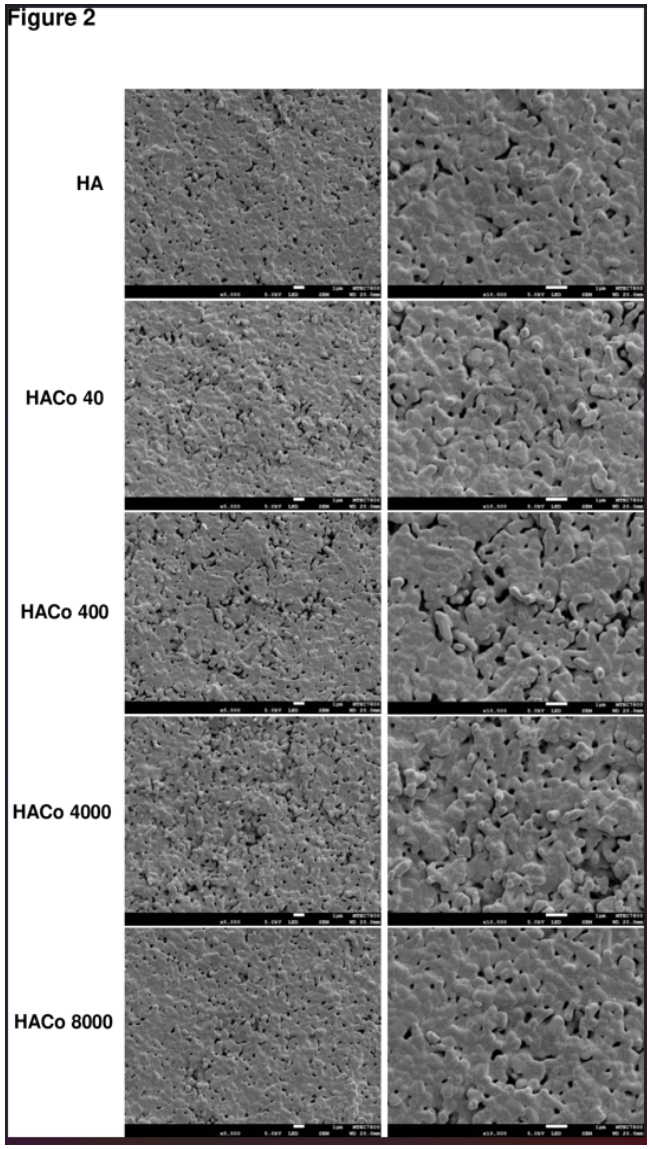

Figure 2 Scanning electron microscope demonstrates disc surfaces. Left panel; the disc surface at magnification x5,000. Right panel; the disc surface at magnification x10,000. Bar = 1 μm.

Surface roughness by atomic force microscopy

Images of unpolished surfaces of the sintered discs were also taken using AFM, allowing the observation of the roughness of these samples in 3 dimensions (Figure 3A). The measured average roughness (Ra) values of HA, HACo 40, HACo 400, HACo 4000, and HACo 8000 surfaces were 1.46 ± 0.52, 1.56 ± 0.41, 1.91 ± 0.27, 3.11 ± 0.38, and 1.47 ± 0.75 μm, respectively. The surface roughness of HACo 4000 was significantly higher than those of HA, HACo 40, and HACo 8000 (Figure 3B).

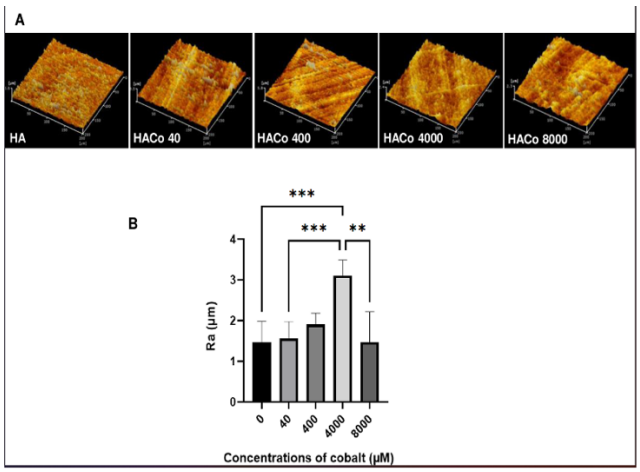

Figure 3 Atomic force microscopy demonstrates disc surface roughness. (A) 3-D micrographs of hydroxyapatite and Co-incorporated hydroxyapatite disc. (B) Ra of the disc surfaces (µm).

Discussion

By developing a biomaterial, the appropriate biophysical characteristics should be screened and characterized. This is because such biophysical properties can impact the biological behavior of the biomaterial. Incorporation of a molecule into the material is an interesting way to change the biomaterial properties. In this study, we incorporated Co into HA and found that incorporation of Co altered HA gross physiologic structure and surface property.

HACo was prepared via soaking commercial HA powder into Co solution (also known as ion exchange method).[13] It was shown that HA particles had the ability to uptake Co ion from the solutions.[2] By using the soaking method, the presence of Co in HACo powder could be verified by energy dispersive x-ray spectroscopy.[13] The color of HA and HACo were notably different. Since Co solution had pinkish color, such color change of HACo discs after soaking suggested incorporation of Co into HA. The color change after Co doping into HA has been reported by Kulanthaivel, S., et al.[14] This group also describes that color intensity is directly proportional to the doping concentration, indirectly indicating Co doping in HA.[14]

Sintering is intended to give bioceramic its stability and bulk mechanical properties.[15] After sintering, the color of HACo changed from pinkish shade to grayish shade. It was postulated that the color change was a result of an oxidation of Co to cobalt oxide. Appearance of cobalt oxide impurity was seen when sintered at temperature 800 - 1,100 ºC.[16] Intense color of HA after Co incorporation and sintering is a concern if applied in the esthetic area such as the anterior area with thin gingival biotype. The high concentration of Co-incorporated material might not be suitable for implantation in the esthetic area.

Shrinkage and weight loss of both HA and HACo samples were visible after the sintering process. Shrinkage of HA dimensions in the forms of particle and pellet has been reported when being heated at temperature higher than 780 ºC.[17-19] The reduction of dimensions could be a result of decreased surface area[20] and the evaporation of water.[19] Sintering enhanced densification of HA particles by bonding between the particles via their necks.[15] The weight loss percentage was apparent when sintering above 1000 ºC.[19] This weight loss was probably caused by water loss.[21] The percentage of weight loss varies between studies, possibly due to the sintering temperature and the synthesis of HA powder.[19, 21]

Impurity of HA could further enhance weight loss after sintering. The loss of HA/MgO weight is higher than that of HA after sintering.[22] Decrease in diameter and weight with increasing Co concentrations were notable. These data showed that Co incorporation influenced these parameters. It has been shown that selected additives added to calcium phosphate bioceramics can enhance densification.[23, 24] However, the mechanism is still unclear. Doping of HA with cobalt chloride leading to smaller crystal size compared with pure HA has been reported.[14] It was possible that this occurrence could affect the dimension of the samples.

In this study, HA particles coalesced and consequently established the microporosity on the surfaces. Incorporation of Co increased surface roughness of HA in a micron range. The surface morphology of a biomaterial has a great influence on bioactivity and responses of cells as well as protein adsorption onto the substrates.[25-29] A short review of the effects of surface structure on bone cells is reviewed. Attachment and differentiation of osteoblasts were more effective on microrough HA surfaces (Ra = 2 µm) than smoother surfaces (Ra = 1 µm).[25] Proliferation and detachment of human bone marrow cells also depended on the surface roughness of the HA.[28] The submicron-scale and

micrometer-scale topographical features of the substrates supported osteoclastogenesis and activated the resorption of an osteoclast[26, 27] Surface topography influenced cell–substrate interactions and played a role in cytoskeleton organization of the cells.[30] However, the effect of HA and HACo surface roughness on bone cells is still unclear and further study is needed.

HA and HACo are promising materials for a variety of biomedical applications such as bone grafting material, drug delivery, and etc. It is noticeable that incorporated element grants material new properties. From the material point of view, properties of the biomaterial provided information into which direction the biomaterials could be developed and could be suitable for applications. We acknowledge that this study is limited and challenges still remain. Since the materials are developed to use in human body, future studies should investigate the release of Co from the samples and the effects of these samples on cell responses including biocompatibility and cell functions.

Conclusions

This study showed that the simple soaking procedure of HA into Co solution could produce Co-incorporated HA. The HACo samples possessed alteration in color, disc dimension, weight, and surface roughness. All these characteristics should be taken into consideration in the development of materials for biomedical applications.

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Declaration of Interest

The authors report no conflict of interest.

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