

## SUCCESSFUL PRESSURE SINTERING OF HYDROXYAPATITE-COLLAGEN COMPOSITE

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Conventionally, hydroxyapatite sintering has been aimed at stiff, stable apatite due to chemical bonding with osseous tissue. However, direct bonding of sintered hydroxyapatite with bone may easily be destroyed under severe repeated loading just like osseointegration of metal because of differences in elastic moduli from each other. Therefore, development of stiff, stable hydroxyapatite makes no sense just as in the case of bioinert metal for biomechanical substitution. The merit in medical application of synthesized hydroxyapatite is to provide materials easily utilized and remodeled for surrounding living osseous tissue<sup>1-5</sup>. Collagen and calcified substance are essential for bone remodeling. It is well known that the apatite phase found in living bodies exists as very small crystallites which are bonded with organic, high-molecular polymers such as collagen. The biological apatite phase also contains bonded water, carbonate ions, and many other inorganic minor components. If it is possible to prepare an implant made of hydroxyapatite-collagen composite similar to biological apatite, the biological effects of the implant may be different from those of conventionally sintered hydroxyapatite.

### 1. INTRODUCTION

Sintering of calcium-deficient hydroxyapatite has already been reported, which bonded water at the calcium-deficient site, and was sintered up to fully dense bodies at 300°C under a pressure of 600 MPa<sup>1</sup>. In the presence of collagen in an aqueous phase, we tried to synthesize the hydroxyapatite by means of reaction between an aqueous solution of phosphoric acid and a calcium-hydroxide suspension. A diluted collagen solution was mixed with an aqueous solution of phosphoric acid and was poured slowly into a calcium-hydroxide suspended aqueous phase. No collagen was found in the mother liquid thus formed. All collagen in the solution was found to be collecting in the precipitate. Five hundred grams of commercially available

collagen solution was diluted up to 8 liters and mixed with 0.6 mole of phosphoric acid. The CaO was crushed into fine powder and mixed with water. The Ca (OH) aqueous suspension thus formed was mixed, and the collagen-phosphoric acid mixed solution was added slowly. The precipitate was filtered and partly dried until suitable water content formed. Then, it was mounted in a metal capsule. The capsule was evacuated and sealed by welding. It was then kept for 8 hrs at 200 MPa at 40°C. The resulting apatite-collagen composite was 1.75g/ml in density, 2 GPa in Young's modulus, and 6.5MPa in compression strength. The specimen could be cut by a knife, and was stable against immersion in water. The physical property of this type of complex may change according to composition and treating conditions. The sample was implanted in dogs and histologically evaluated.

## 2. MATERIALS AND METHODS

### 2.1. Comparative histopathological studies on conventional stoichiometric hydroxyapatite and new type hydroxyapatite sintered by high pressure gas technique

For a preliminary experiment, the following studies were carried out. Conventionally sintered stoichiometric porous hydroxyapatite plates and dense new type plates of stoichiometric and nonstoichiometric hydroxyapatite were implanted in the bone and muscle of a dog to compare histological tissue reactions for 8 weeks. For conventional stoichiometric hydroxyapatite, porous hydroxyapatite plate (40% porosity) made by ASAHI Optical Co. Ltd. was used. Dense new type of stoichiometric and nonstoichiometric hydroxyapatite were sintered in the National Institute for Research in Inorganic Materials. Undecalcified polished specimens for SEM and EPMA were made. They were then observed and compared.

### 2.2. Experiments on pressure sintering of apatite-collagen composite

Five hundred grams of commercially available collagen solution (concentration 2 wt%, isoelectric point 9, pH3) were diluted up to 8 liters and mixed with 0.6 mole of phosphoric acid. The CaCO<sub>3</sub>, 1 mole was kept at 900°C in air for 10 h. The CaO thus formed was crushed in a mortar into fine powder and mixed with 3 liters of water. The Ca(OH)<sub>2</sub> aqueous suspension thus formed was mixed vigorously, and collagen-phosphoric acid mixed solution was slowly added at room temperature to the aqueous suspension. In this case, the mixing ratio of collagen to hydroxyapatite was 1 to 10. The precipitate thus formed was filtered and partly freeze-dried until the water

content of the precipitate became suitable for sintering. Then, the precipitate was mounted in a metal capsule. The capsule was evacuated and sealed by welding, after which it was kept for 8 h at 200 MPa, 40°C.

The specimen thus formed was examined by both 5 MHz sound velocity measurement and compression strength measurement using INSTRON model 1123. The cross head speed was 0.5mm/min. during the measurement. Sintered apatite-collagen composites were implanted in dorsal muscles of a dog; 8 weeks after implantation, specimens were recovered, prepared, and observed.

### 3. RESULTS

3.1. Studies by SEM observation on comparison of conventional hydroxyapatite and new type hydroxyapatite sintered with high pressure gas technique revealed the following:

Conventionally sintered porous hydroxyapatite was observed as amorphous in the cutting surface. Therefore, the grain could not be detected (Figures 1, 2).

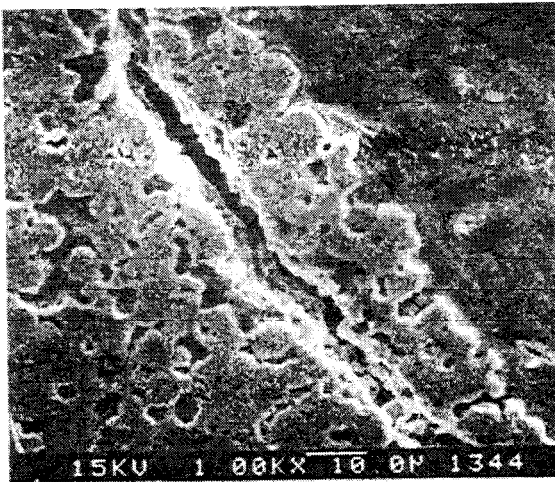


FIGURE 1

FIGURE 1

Fusion of conventionally sintered hydroxyapatite with newly formed bone tissue-low magnitude.

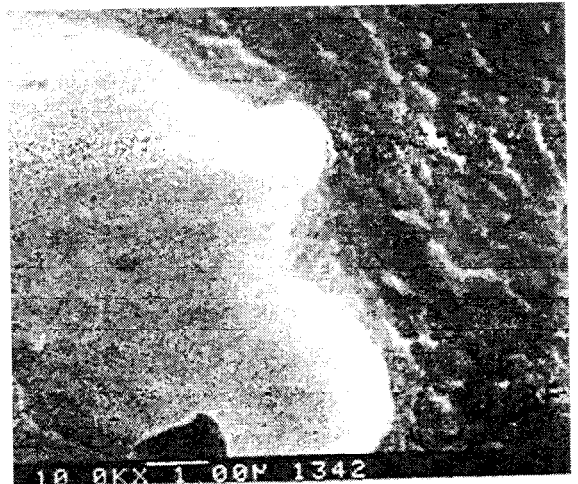


FIGURE 1

FIGURE 2

Fusion of conventionally sintered hydroxyapatite with newly formed bone tissue-high magnitude. Sintered hydroxyapatite appeared amorphous without grains.

Porous hydroxyapatite plate and newly formed bone fused well (Figure 1, 2). However, conventionally sintered apatite plate did not fuse with fibrous tissue (Figure 3). In the interface between the porous apatite plate and fibrous tissue, Ca. 2 $\mu$ m space was observed, wherein some small spots of connection of tissue and apatite could be observed (Figures 1-3). On the contrary, dense apatite plates sintered with high pressure gas technique fused with soft tissue firmly (Figures 4-7).

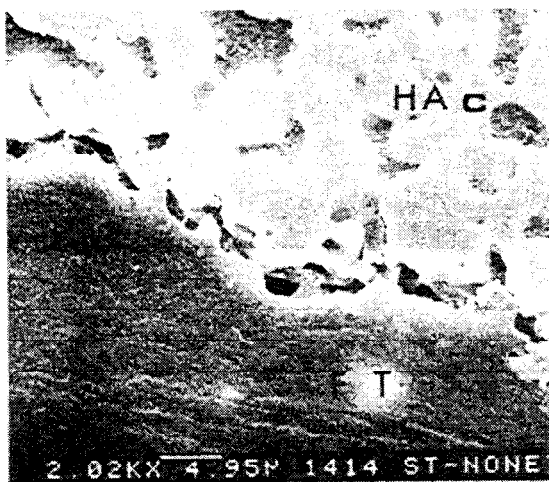


FIGURE 3

FIGURE 3

Fusion of conventionally sintered hydroxyapatite with fibrous tissue. Ca 2 $\mu$ m cleavage in interface between ceramics and soft tissue can be seen.

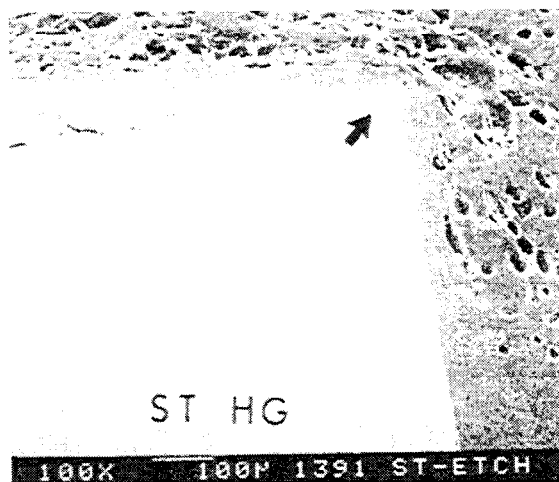


FIGURE 4

FIGURE 4

Stoichiometric hydroxyapatite sintered with high pressure gas technique was implanted in soft tissue. Excellent fusion could be obtained.

Both new types of apatite (stoichiometric and nonstoichiometric) were observed constructed with ultras-small grains by SEM. Nonstoichiometric apatite of high pressure gas technique was observed having very weak fusion with soft tissue. Almost all parts of the fusion were disrupted by artifact of specimen treatment.

3.2. The results of pressure sintering of the apatite-collagen composite were as follows:

After pressure treatment, the metal capsule made from lead was removed. In every run, the specimen was slightly yellow and solid. When the water content of a specimen during pressure treatment was about 10 wt%, for example, the resultant solid specimen was stable in air, but unstable

in water. When the specimen was immersed in liquid water, it broke vigorously into small pieces.

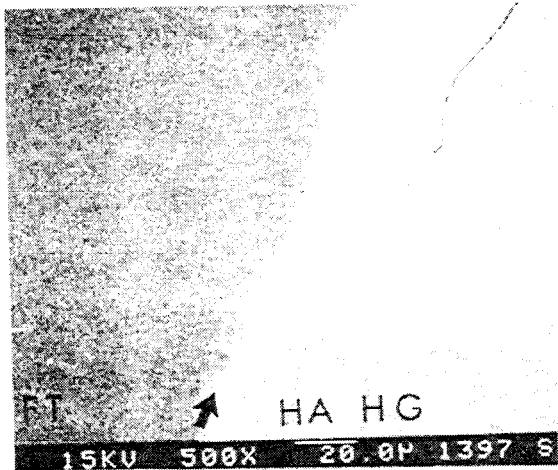


FIGURE 5

FIGURE 5: Interface between stoichiometric hydroxyapatite made of high pressure gas technique and fibrous tissue-lower magnitude. Excellent fusion could be obtained.

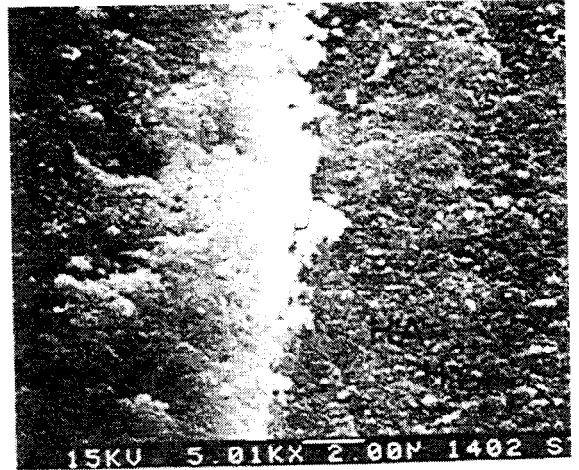


FIGURE 6

FIGURE 6: Interface between stoichiometric hydroxyapatite made of high pressure gas technique and fibrous tissue-higher magnitude. Excellent fusion could be obtained.



FIGURE 7

FIGURE 7: Hydroxyapatite-collagen composite sintered with high pressure technique under conditions of water at room temperature.

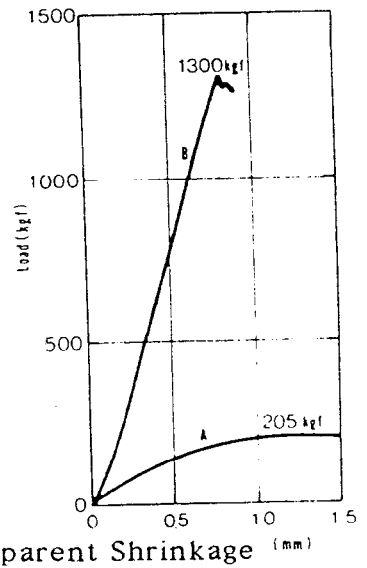


FIGURE 8

FIGURE 8: Load-displacement relation in compression strength test. A: collagen-apatite composite, 22mm in diameter and 50mm in length. B: apatite powder compact, 23mm in diameter and 50mm in length. Both are sintered at 40°C, 200MPa, in the presence of liquid phase water.

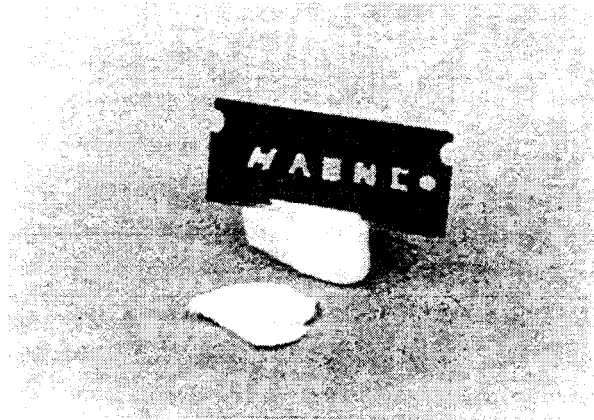


FIGURE 9

FIGURE 9

Collagen-apatite composite could be cut by razor blade.

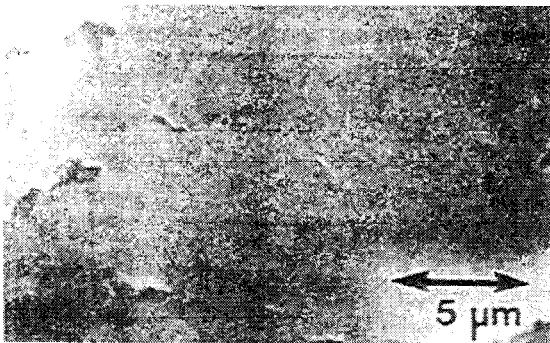


FIGURE 10

FIGURE 10

Blade-cut surface of collagen-apatite composite.

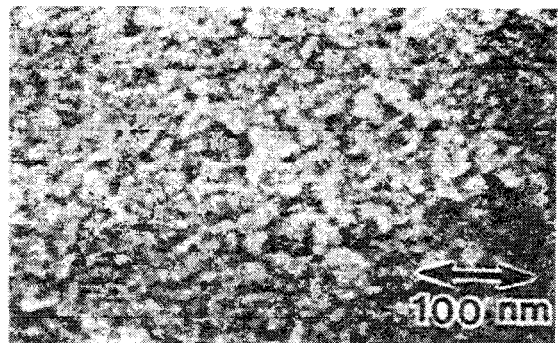


FIGURE 11

SEM image.  $\times 5,030$ .

FIGURE 11

Blade-cut surface of collagen-apatite composite.

SEM image.  $\times 203,000$ .

When the water content was near 50 vol%, the pressure-treated specimen was stable in liquid water. The solid feature of the specimen was unchanged during a period of one month of immersion in water at room temperature. The results of the compression test are shown in Figure 9. The results were compared with apatite powder compact treated under the same conditions as

those of the apatite-collagen composite, i.e., 200 MPa, 40°C, and 8 h of run duration, and the presence of liquid water. The apparent density of the apatite powder compact thus obtained was 2.1g/ml, and was hard and brittle. In this case, without collagen, the load-displacement curve in Figure 9 was almost a straight line until it broke, while in the case of the collagen-apatite composite, the load-displacement line in Figure 9 showed small and varying gradients, indicating large deformation of the specimen. These results coincided with the sound velocity measurement, from which 2 GPa of Young modulus and 6.5 MPa in compression strength were calculated. The collagen-apatite composite could be cut with a razor blade (Figure 10). The blade-cut surface is shown in Figure 11. The average size of the apatite crystal was 10 nm in diameter and 40 nm in length.

#### 4. DISCUSSION

New type hydroxyapatite apatite sintered with high pressure gas technique proved to have excellent histocompatibility with fibrous and osseous tissue. Fusion of apatite to soft tissue is the most important property of any biomaterial. It was found that the stoichiometric hydroxyapatite became nonstoichiometric *in vivo*. On implantation of hydroxyapatite, it has been shown that the bond formation between the implanted hydroxyapatite and tissue of living bodies needs some induction period, about 4-5 weeks. In hard tissue of living bodies, it has been shown that the hydroxyapatite phase is complex in chemical composition. It is, therefore, doubtful that this induction period is long enough to change the implanted hydroxyapatite. It has been shown that stoichiometric hydroxyapatite is one of the thermodynamically stable phases of the system CaO-P<sub>2</sub>O<sub>5</sub>-H<sub>2</sub>O. But for nonstoichiometric hydroxyapatite, no stable field has been given. This means that the solubility of stoichiometric hydroxyapatite in aqueous solution is lower than other metastable phases such as nonstoichiometric hydroxyapatite. Due to this character, sintered stoichiometric hydroxyapatite can keep its shape long-term in living bodies, being an excellent material for implantation. From this experiment, it was found that stoichiometric hydroxyapatite sintered bodies change their composition to nonstoichiometric in living bodies. It seems important that the shape of implanted sintered bodies not be changed in spite of their compositions being changed. Sintering temperature suitable for hydroxyapatite is known to be 1200±100°C. Under a pressure of 200 MPa, a fully dense sintered specimen can be obtained at 800°C. Presence of water in the system lowers

the sintering temperature of hydroxyapatite. In a stream of steam at 300°C and atmospheric pressure, grain growth of hydroxyapatite can be found, and also a powder compact of hydroxyapatite shows small shrinkage in volume due to its sintering.

Previously, we reported that calcium-deficient hydroxyapatite, which has bonded water at the calcium-deficient site, sinters up to full density at 300°C under a pressure of 600 MPa. At that time, 300°C was the lowest limit to obtain a stable sintered specimen. At 200°C or lower, full density could not be obtained and strain remained in the pressed powder by treating it at 600 MPa. It was found that such an obtained specimen, placed in air, gradually broke into small pieces. When specimens with remaining strain were dipped into water at room temperature, they broke vigorously into small pieces within several seconds. The water content in these apatite specimens was determined. It was found that these specimens were intensely dried. The bonded water molecules in the calcium-deficient site had been partly lost. In the present experiment, therefore, wet apatite powder was used for the pressure treatment. The pressure-treated apatite specimen was thus obtained by pressure treatment at room temperature. It seemed obvious that in liquid water, the strain in the apatite phase due to the pressure treatment could be released at room temperature. This phenomenon led us to the further possibility of pressure-sintering of hydroxyapatite mixed with organic compounds at room temperature.

These results also suggest that the higher pressures make it possible to obtain a collagen-apatite composite of higher density, as well as higher strength at room temperature.

#### REFERENCES

- 1) K.HIROTA, K.NISHIHARA and H.TANAKA, Pressure sintering of apatite-collagen composite, *Bio-Medical Materials and Engineering*, 3(3):147-151, 1993
- 2) A.HUC, R.ALLARD and J. BEJIUI, Osteogenic collagen-hydroxyapatite-glycosaminoglycan composition for replacement of bone matrix, *Fr. Demande FR 2,585,576, Appl. 85/12,053; 30 July 1985*
- 3) B.D.KATTHAGEN and H.MITTEIMEIER, Bone regeneration with collagen-apatite, *Adv. Biomater.* 6:39-44, 1986
- 4) H.KIMURA, H. SUH,H and M.OKAZAKI, A study of the apatite-collagen composites Part 1: Disintegration properties and biological responses, *Dent. Mat. J.* 10: 46-57, 1991
- 5) K.HIROTA and Y.HASEGAWA, Preparation of calcium-deficient hydroxyapatite-metal composite, In: Vincenzini, P., ed., *Ceramics in substitutive and reconstruction surgery*, New York: Elsevier Science Publishers B.V.: 137-145, 1991