Strength of bond to bone and cytotoxicity of sintered bodies of hydroxyapatite/zirconia composite particles

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Regarding sintered bodies of hydroxyapatite (HAP)/zirconia (PSZ) composite particles prepared by covering the surface of HAP particles with PSZ particles, their strength of the bond to bone after implanting them into an organism and their cytotoxicity were evaluated. Cytotoxicity tests were conducted by the colony formation method. Cytotoxicity was not observed in the sintered bodies of HAP/PSZ composite particles, or in sintered monolithic HAP body and sintered monolithic PSZ body. The strength of bond between a sintered body and a bone was evaluated by measuring the shear strength at the interface between them after a fixed period following implantation of a sintered body into a rabbit femur. In all cases of the sintered monolithic HAP body, and the sintered bodies of HAP/PSZ composite particles and monolithic PSZ body, when the implantation period increased, the shear strength of the sintered body/bone interface tended to increase. In particular, this tendency was very high in the case of sintered bodies of HAP/PSZ with weight ratios of 1/1.0 and 1/1.5, the shear strength for each reaching 20 MPa 12 months after implantation.

1. Introduction

Ceramics presently used as substitute materials for hard tissues of organisms are alumina, partially stabilized zirconia (PSZ), hydroxyapatite (HAP) and tricalcium phosphate (TCP). Although these ceramics are inferior in terms of workability or fracture toughness to metallic materials or polymer materials, they are superior to these materials in terms of biocompatibility. In particular, alumina and PSZ have sufficient mechanical strength; therefore, they are frequently used as materials for artificial bones and artificial roots of teeth [1, 2]. On the other hand, although HAP and TCP have limited use due to their insufficient mechanical strength as substitute materials for hard tissues of organisms, they have an excellent property of chemically bonding to bones after being implanted in an organism, thus they attract attention as potential biomaterials. The property of directly bonding to the bone is not observed in alumina and PSZ.

In order to prepare a biomaterial with both advantages of HAP and PSZ, several studies on binary systems of sintered bodies of HAP/PAZ have been carried out. One of these studies involved coating HAP on the surface of a sintered PSZ body. A sintered body consisting of an HAP layer is formed on the PSZ surface by coating the surface with a mixed slurry of calcium metaphosphate and tetracalcium phosphate, followed by heating [3]. Another involved coating HAP on the surface of a sintered PSZ body by the radio-frequency thermal plasma process [4]. In such studies, preparation of a biomaterial with excellent mechanical strength and the property of chemically bonding to bones has been attempted. However, in both of the above cases, problems involving the chemical change of HAP to other calcium phosphates and the peeling of the coat layer from the PSZ surface prevent the practical utilization of such biomaterials. There are other reports stating that sintered bodies, which are mechanically stronger than the sintered monolithic HAP body, can be obtained by sintering HAP powder and PSZ powder after mixing them by the conventional

method [5–9]. However, because the particles of HAP and PSZ used in these studies are small, the contact area among them is large. As a result, their chemical change is accelerated during sintering, so that the possibility of HAP changing to TCP is very high, as well as of the crystal system of PSZ changing from tetragonal to cubic [10–14]. These chemical changes are disadvantageous because the probability of decreasing the mechanical strength of sintered bodies is high. In addition, when conventional powder mixing methods are adopted, such as the dry mixing and wet mixing methods, the homogeneity of the mixed powder is not enhanced, which increases the frequency of contact among fine HAP particles. Furthermore, by these mixing methods, mixed particles are likely to separate again from each other in the succeeding process.

The important factor to consider in improving the mechanical strength of sintered bodies is that when sintered bodies are produced, direct contact among HAP particles should not occur and HAP particles should independently exist within the three-dimensional network structure of the sintered PSZ body. In addition, for suppressing chemical changes of two components, it is considered effective to enlarge the particles of either component to decrease contact areas among identical components. In this research, using the dry impact blending method [15, 16], which differs from conventional methods, composite particles were prepared by coating the surface of spherical HAP particles of about 9.3 μm mean diameter with PSZ particles of about 0.1 μm mean diameter, and the substitute material for hard tissues of organism was prepared by sintering the powders of these composite particles. The wettability [17], chemical change [18], fine structure and mechanical strength [19] of the obtained sintered composite bodies were already reported. In this paper, we report the strength of bond to bone and cytotoxicity of the sintered composite bodies.

2. Materials and methods

2.1. Preparation of sintered bodies of HAP/PSZ composite particles

HAP powder (spherical particle, mean particle diameter, 9.3 μm; Ca/P mole ratio, 1.67; Mitsubishi Material Co., Ltd.) and PSZ powder (mean particle diameter, 0.1 μm; Y_2O_3 content, 3 mol %; Tosoh Co., Ltd.) were used. The spherical HAP particles were coated with PSZ particles using a Hybridization System NHS-1 type (circumferential velocity of rotor, 60 m/s; treatment time, 3 min; Nara Machinery Co., Ltd.). The composite particles obtained and PSZ particles were sintered by hot-pressing (argon atmosphere; pressure, 30 MPa; temperature, 1350 °C; treatment time, 15 min; size of sintered body, $50 \times 50 \times 16\,mm^3$). The sintered monolithic HAP body was prepared by hot-pressing at 1150 °C (other sintering conditions were the same as above).

2.2. Evaluation of cytotoxicity

In this research, the cytotoxicity of the sintered composite body was evaluated by the colony formation test [20, 21] using V79 cells derived from the lung of a Chinese hamster. V79 cells (JCRB0603) were cultured and incubated in CO_2 atmosphere in Eagle's MEM containing 10% bovine fetus serum (JRH Biosciences). In the cytotoxicity test, Eagle's MEM medium containing 5% bovine fetus serum was used. The evaluation of cytotoxicity by the colony formation test was carried out using sintered bodies T-1, T-2 and T-5 (Table I). The cytotoxicity of T-3 and T-4 was considered to be almost the same as that of T-2, and therefore not evaluated. For comparison, cytotoxicity tests were carried out using the following: (1) high-density polyethylene (HDPE) film as a negative control material, (2) polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC, Food and Drug Safety Center) as a standard material showing strong cytotoxicity, (3) polyurethane film containing 0.25% zinc dibutyldithiocarbamate (ZDBC, Food and Drug Safety Center) as a standard material showing weak cytotoxicity.

TABLE I Sintered bodies of HAP/PSZ composite particles prepared in the present study

Sample	HAP/PSZ		
	Weight ratio	Mole ratio	Volume ratio
T-1	1/0	1/0	1/0
T-2	1/1.0	1/8.1	1/0.52
T-3	1/1.5	1/12	1/0.78
T-4	1/2.0	1/16	1/1.0
T-5	0/1	0/1	0/1

Sintered bodies T-1, T-2 and T-5 were crushed to obtain particles of 100 μm diameter and sterilized using high-pressure steam for 20 min at 121 °C prior to use. Extraction was carried out by adding 20 ml of the medium to 2.0 g of sample. The mixture was then allowed to stand for 24 h at 37 °C. The supernatant obtained was referred to as 100% extract. The HDPE film (2.0 g), 1.0 g of polyurethane film containing 0.1% ZDEC, and 2.5 g of polyurethane film containing 0.25% ZDBC were each added into 20, 10, or 25 ml of the medium, and each of the mixtures was extracted in the same way as described above. The extracts were diluted with a fresh medium to prepare samples with different extract concentrations. A suspension with a V79 cells at a concentration of 10^3 particles/ml was prepared, and 0.1 ml (number of cells 100) of this suspension was dispensed into a well of a plate (diameter 35 mm) containing 2 ml of the medium. On the following day, the medium in the well was discarded, and replaced with a medium containing the extract. The medium was again discarded on the seventh day of cell inoculation, and after the colony was fixed using methanol, Giemsa staining was carried out. The number of colonies per well was counted, and the number of colonies in 100% fresh medium was determined to be 100, and relative colony formation efficiency for each concentration of material extract was calculated.

2.3. Evaluation of strength of bond to bone

The sintered bodies were implanted into the femurs of rabbits, and after a fixed period the bonding conditions between the sintered bodies and the bones were

examined by a push-out test on the sintered body and the bone, and by light transmission microscopy (LTM) and scanning electron microscopy (SEM) of the sintered body/bone interface.

2.3.1. Implantation of test piece

The sintered body was processed to a cylindrical shape with 3 mm diameter and 10 mm length to form a test piece for implantation. The surface of the test piece was smoothed by a #1000 abrasive paper. The surface roughness of the test piece was measured by a Surftest analyzer (Mitsutoyo Co., Ltd.). The test piece was subjected to ultrasonic cleaning with acetone for 5 min and successive dry-heat sterilization for 40 min at 160 °C. For the animal experiment, female Japanese domestic white rabbits of 3.5 kg weight were used. Each rabbit was subjected to general anesthesia by intravenous injection of 0.57 ml/kg body weight sodium pentobarbital (Abbot Laboratories), and the fur of the thigh on both sides was shaven and the exposed parts were disinfected. A suitable dose of epirenamine (Fujisawa Pharmaceutical Co., Ltd.) as a local anesthetic was injected into the target site of operation (outside of the thigh), and an incision was made on this site. The periosteum was removed to expose the external femur.

Next, using a drill with a bit of 3.1 mm diameter, three through-holes (10 mm distance) were made on both femurs, in a direction vertical to the external surface. Physiological saline solution was continuously poured on drilled parts for cooling, in order to prevent the degradation of the bone by overheating. The six test pieces were implanted in each rabbit such that about 0.5 mm of the upper end of the test pieces protruded from the bone surface. After implantation, the muscle layer and skin were each sewn up. Implantation periods were 1 month, 3 months, 6 months, and 12 months, and six test pieces for each of the five kinds of sintered bodies were implanted for each period. Two test pieces for LTM and SEM observations were implanted for each period.

2.3.2. Push-out test

Both femurs of the rabbit that was sacrificed by intravenous injection of an overdose of sodium pentobarbital were removed. Following soft X-ray photography, the implanted test pieces were cut into cross sections using a diamond belt saw. The push-out test was carried out using a standard test machine (Instron 4467). A thermostat bath was installed on the crosshead of the test machine and phosphate-buffered saline solution (PBS) at 40 ± 0.5 °C was continuously circulated in it to keep the temperature of the bones similar to that of the rabbit and to prevent the direct exposure of the bones to air. A cylindrical test piece was placed on the base seat of the thermostat bath, and the tip of the test piece was placed in contact with the tip of the push-out rod of the test machine. The load was increased at a crosshead speed of 0.5 mm/min, and the maximum load at which the test piece was pushed out was denoted as the push-out strength. The contact area between the test piece and the bone was obtained based on the obtained soft X-ray image, and the push-out strength per unit area of the sintered body/bone interface was obtained and defined as the shear strength (MPa) of the sintered body/bone interface.

2.3.3. LTM observation

Each rabbit was administered 30 mg/kg body weight tetracycline and 15 mg/kg body weight calcein by intravenous injection 2 weeks and 1 week before the sacrifice, respectively. Both femurs of the rabbit that was sacrificed by intravenous injection of sodium pentobarbital were removed. Following soft X-ray photography, the implanted test pieces were cut into cross sections and fixed with 70% ethanol followed by Villanueva staining. Subsequently, the pieces were dehydrated in an ethanol series of 50, 60, 70, 80, 90, and 100%, and degreased by dipping in 100% acetone. The degreased samples were embedded in PMMA resin. The embedded samples were cut into about 0.5-mm-thick sections, polished up to about 20 μm thickness on a glass slide using a precision polishing machine (Maruto Co., Ltd., ML-180), and then observed under a microscope. The bone stained with tetracycline and calcein was observed by radiation-type fluorescence microscopy. The clearance between the interface stained with tetracycline and the interface stained with calcein was measured in three places, and the mean value was divided by 7. The quotient obtained was denoted as calcification velocity (μm/d). The results of Villanueva staining were observed by LTM under natural light.

2.3.4. SEM observation

Each rabbit was subjected to general anesthesia by intravenous injection of sodium pentobarbital, followed by perfusion ischemia with physiological saline from the abdominal aorta to the lower limbs, and successively subjected to perfusion fixation with a solution containing 2.0% glutaraldehyde and 4.0% paraformaldehyde (0.2% cacodyl buffer solution, pH 7.4). Then, both femurs were removed. Following soft X-ray photography, parts of the implanted test piece were cut into cross sections, and immediately dipped in glutaraldehyde–paraformaldehyde solution for fixation. The samples after postfixation were dehydrated and degreased in the same manner as described above, and embedded in PMMA resin. The embedded samples were sliced into about 1-mm-thick sections. The cut surface was polished using a precision polishing machine, ultrasonically cleaned in distilled water, and then dried in vacuum. Finally, carbon was deposited on the sample surface for SEM observation, which was carried out using a HORIBA S-4500 scanning electron microscope at an acceleration voltage of 5 kV.

3. Results and discussion

The compositions of sintered composite HAP/PSZ bodies, which were evaluated in terms of cytotoxicity and strength of bond to bone in this experiment, are shown in Table I. T-2, T-3 and T-4 are sintered composite bodies, T-1 is a sintered monolithic HAP body, and T-5 is a sintered monolithic PSZ body.

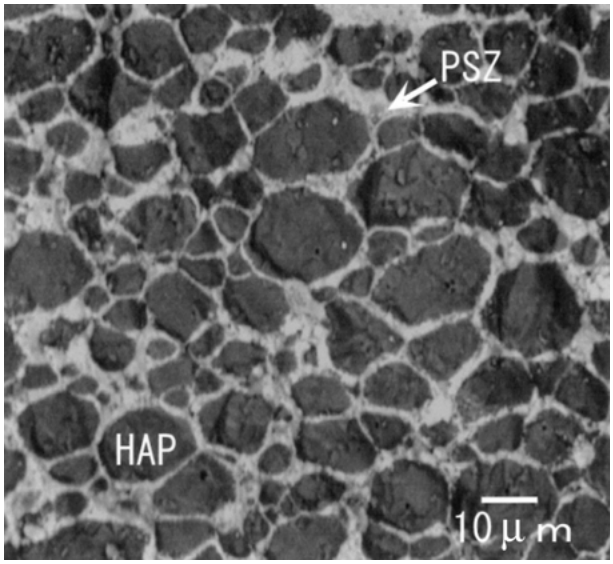

Figure 1 SEM image of a cross section of sintered composite body T-2.

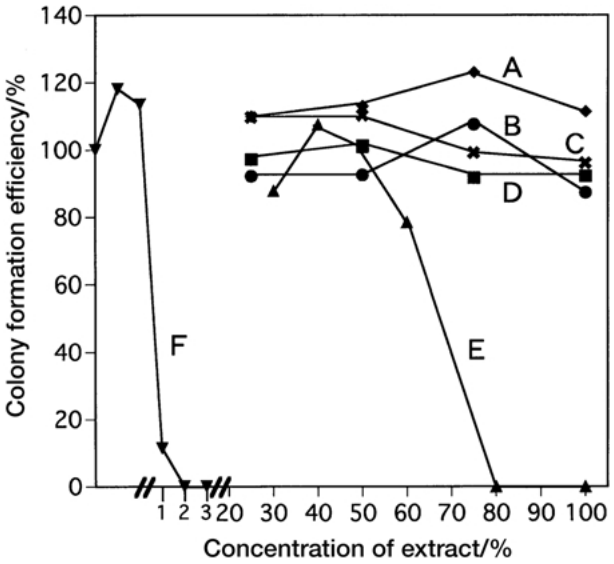

Figure 2 Cytotoxicity of sintered bodies of HAP/PSZ composite particles. A:T-1; B:T-2; C:T-5; D:HDPE; E:ZDBC; F:ZDEC.

Although T-2, T-3 and T-4 respectively contain 50, 40 and 33 wt % HAP (the density of HAP is 3.16 g/cm^3 and that of PSZ is 6.05 g/cm^3 for PSZ), they contain 66, 56 and 49 vol % HAP. The sintered bodies used in this experiment were prepared in blocks that were cut into smaller pieces, which were then polished into appropriate shapes and sizes depending on their use. The HAP area ratio on the surface (percentage of total HAP area relative to the total surface area) of the sintered bodies is the same as the volume percentage.

The sintered composite bodies in this experiment were prepared by first preparing HAP/PSZ composite particles by coating spherical HAP particles with PSZ particles, and then sintering them. For this reason, spherical HAP particles are not in contact with each other inside the sintered composite body, but independently exist in the three-dimensional network structure of PSZ. The SEM image of a cross section of sintered composite body T-2 is shown in Fig. 1. Black parts are HAP and white parts are PSZ. Apparently, PSZ phases are considered to have bonded to the rough surface of HAP and the so-called anchoring effect seems to have occurred. The falling-off of spherical HAP particles from PSZ phases did not occur at all in any operations in this experiment.

3.1. Cytotoxicity

Extracts of sintered bodies T-1, T-2 and T-5 at any concentrations did not disturb the colony formation of V79 cells as did the HDPE extract used for comparison (Fig. 2). In Fig. 2, line A stands for T-1, line B for T-2, line C for T-5, and line D for HDPE. The concentration of the polyurethane film (containing 0.1% ZDEC) extract, which was used as the standard substance with strong cytotoxicity, required to cause a 50% disturbance of the colony formation was 0.8% (line F). On the other hand, the concentration of the polyurethane film (containing 0.25% ZDBC) extract, which was used as the standard substance with weak cytotoxicity, required to cause a 50% disturbance of the colony formation was 67% (line E). These values depended on the cytotoxicity strength of each standard material. Both HAP and PSZ are already confirmed to be biologically safe [22–24]. Even though the existence of calcium zirconium oxide was not confirmed by X-ray diffraction analysis in this study, there is a possibility for it to form in very small quantities in the interface of HAP/PSZ, and the cytotoxicity of this compound has not been examined. In this experiment, even if the above chemical change had occurred, it was shown that sintered composite HAP/PSZ bodies are not cytotoxic under the conditions used in this experiment.

3.2. Strength of bond to bone

All the test pieces used in the implantation experiment were smoothed by a #1000 abrasive paper to obtain the same surface roughness (R_a) for all pieces. However, an exactly the same R_a could not be achieved because the physical properties of each material were different. R_a of the curved surface of the sintered body test pieces used in the implantation experiment is shown in Fig. 3. R_a was measured 8 mm along the long axis of the cylindrical test piece. As shown in Fig. 3, R_a was measured at three points (center and both ends) and their average was taken as R_a of a test piece. Although the same polishing treatment was applied to test pieces T-1–T-5, differences in R_a were observed following this treatment. Compared with R_a of 0.39 μm for T-1, a test piece of sintered monolithic HAP body, R_a for T-5, a test piece of sintered monolithic PSZ body, was small at 0.17 μm, and the R_a values for test pieces of the sintered composite HAP/PSZ bodies were between these two values. Among the three sintered composite bodies T-2, T-3, T-4 with different HAP contents, a difference in R_a was not observed. It seems that because the sintered monolithic HAP body has a small toughness value, polishing did not decrease R_a. Because the sintered monolithic PSZ body has a much larger toughness value than HAP, R_a seemed to have decreased in comparison. For three kinds of sintered composite HAP/PSZ bodies, which consist of two

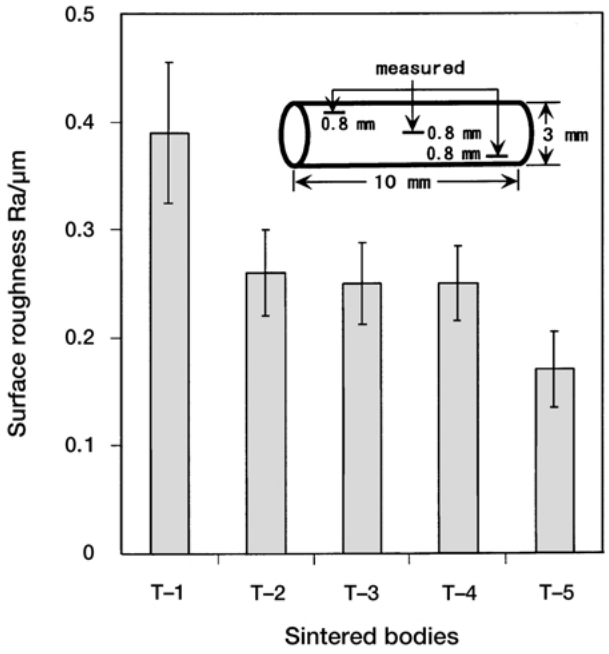

Figure 3 Surface roughness (R_a) of the sintered body test pieces used in the implantation experiment.

components (HAP and PSZ), their R_a values were reasonably between those of T-1 and T-5.

A photograph taken during the operation to implant a cylindrical test piece into the femur of the rabbits, which shows the condition of the three test pieces implanted at 10 mm intervals, is shown in Fig. 4. Through-holes are drilled on the bone, and the tips of the test pieces protrude slightly from the side not seen in the photograph. A soft X-ray image of the femur which was implanted with sintered bodies T-2, T-3 and T-4 and then removed 12 months after implantation is shown in Fig. 5. It was confirmed that at the contacting areas between the test pieces, and the bone and the peripheral parts, calcification and formation of new bone have proceeded and it has nearly the same soft X-ray image as that of the original bone. The shear strength of the sintered body/bone interface, which was measured by the apparatus shown in Fig. 6, is shown in Fig. 7. Before measurement, incision lines shown in Fig. 6 were made on the bone, and

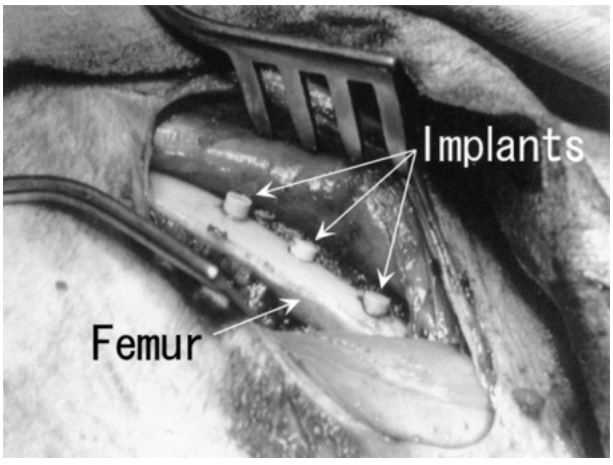

Figure 4 Photograph taken during the operation to implant the cylindrical test pieces into the femur of the rabbit.

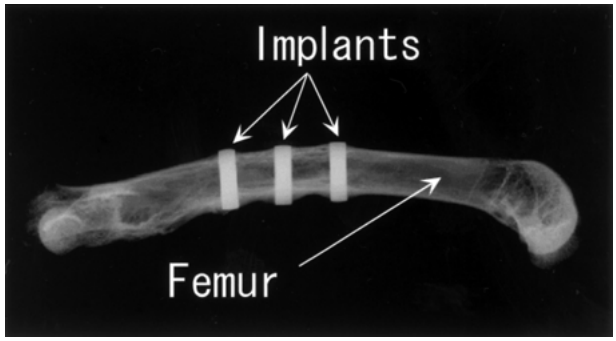

Figure 5 Soft X-ray image of the femur and implants (12 months after implantation).

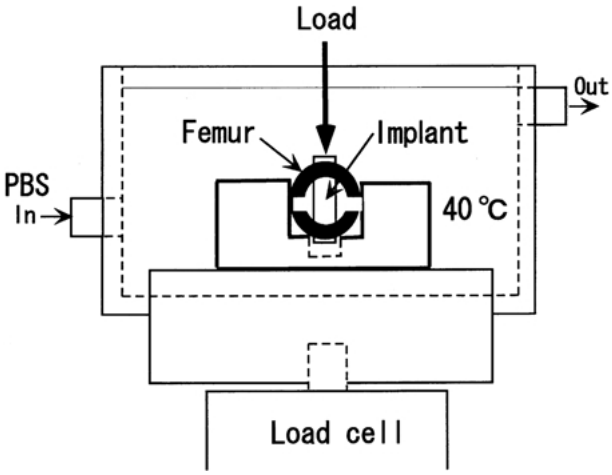

Figure 6 Schematic representation of the push-out test method.

shear strength was separately measured on both sides of the sintered body/bone interface.

Fig. 7 shows a chart indicating the implantation period in the abscissa axis and the shear strength in the ordinate axis. The Wilcoxon test was carried out at a significance level 5% for the data in Fig. 7. There were significant differences in shear strength between the sintered bodies tested except between T-2 and T-3, T-2 and T-4, T-3 and T-4, T-4 and T-5 (1 month after implantation), T-3 and T-4 (3 months after implantation), T-2 and T-3 (6 months after implantation), T-1 and T-2, and T-2 and T-3 (12 months after implantation). Regarding the shear strength at 1 month after implantation, while the average shear

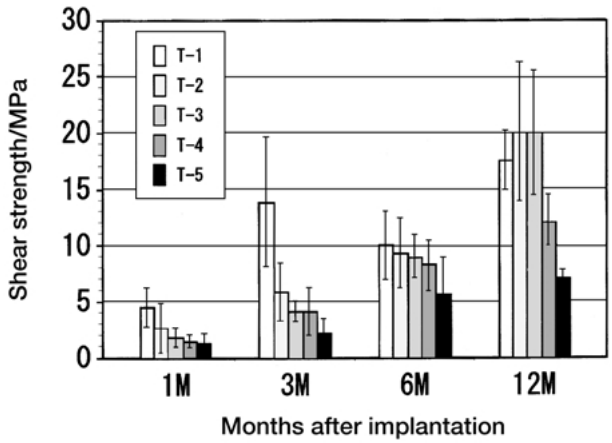

Figure 7 Shear strength of the sintered body/bone interface as a function of implantation period.

strength of T-1 was 4.4 MPa, those of T-2, T-3, T-4 and T-5 were less than 2.5 MPa. The shear strengths of the sintered body/bone interface for all sintered bodies were very small, which clearly shows that the bonding ability between a sintered body and a bone is very small at 1 month after implantation. However, in the case of sintered body T-1 which consists of HAP alone and sintered bodies T-2, T-3 and T-4 which contain HAP and other components, results shows that bonding between a test piece and a bone increases with time. A typical example is shown in Fig. 8. Apparently, the gap between a test piece and a bone is partially embedded under what seems to be a new bone. At 3 months after implantation, the shear strength of T-1 increased to 13.8 MPa, but those of the others were less than 6 MPa, indicating the difference between T-1 and the other sintered bodies. The SEM image of the T-1/bone interface at 3 months after implantation is shown in Fig. 9. The gap between a sintered body and a bone was observed to be completely embedded. Even in T-2 and T-3 at 3 months after implantation, the gap between a sintered body and a bone

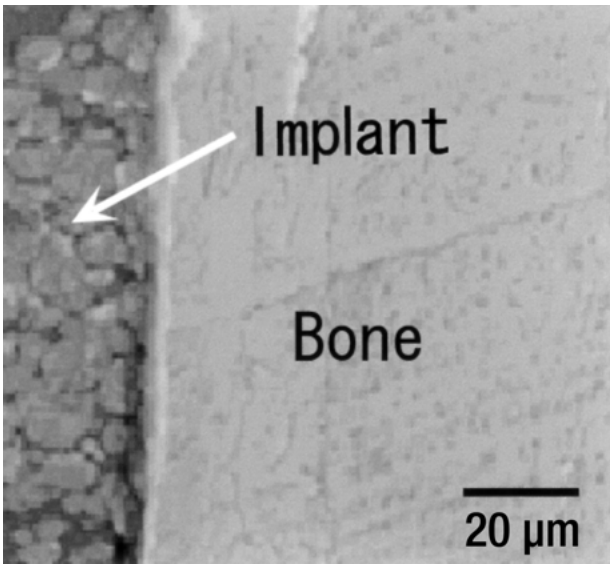

Figure 8 SEM image of the T-2/bone interface at 1 month after implantation.

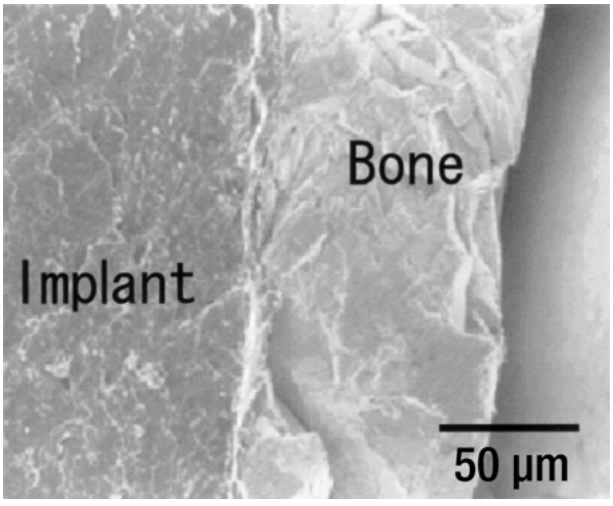

Figure 9 SEM image of the T-1/bone interface at 3 months after implantation.

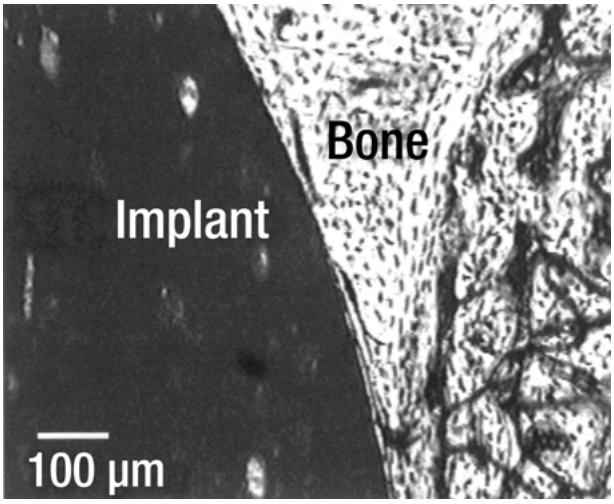

Figure 10 LTM image of the T-2/bone interface at 3 months after implantation.

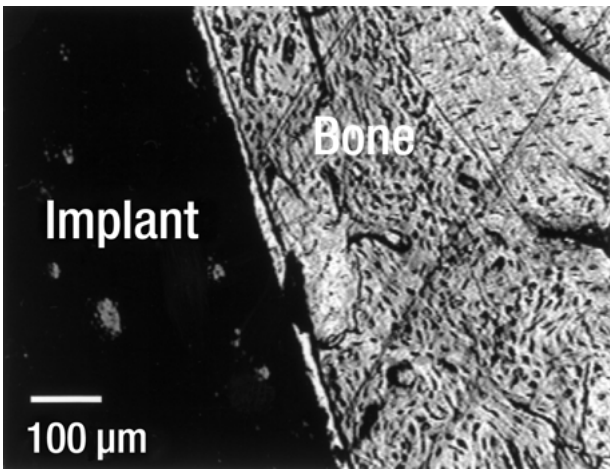

Figure 11 LTM image of the T-3/bone interface at 3 months after implantation.

was observed to be nearly completely filled with new bone, and the new bone and the sintered body are in directly contact (Figs. 10 and 11).

At 6 months after implantation, the shear strengths of T-2–T-5 increased by 1.6–2.6-fold compared with those at 3 months after implantation, but that of T-1 decreased by about 4 MPa. The SEM image of the T-1/bone interface at 6 months after implantation is shown in Fig. 12. T-1 and the bone directly bonded to each other, but damage occurs inside T-1. This damage is considered to have been caused by the force added to the test piece when the test piece was cut with a diamond belt saw during the preparation of the SEM observation sample. It is suggested that this was due to the small bending strength and fracture toughness of the sintered monolithic HAP body. At the same time, the image indicates that the bonding strength of the T-1/bone interface is strong as reported previously [25].

At 12 months after implantation, compared with the values taken at 6 months, the shear strengths of T-1–T-3 at the bone interface increased to a large extent, but the increase was small in T-4 and T-5. The SEM image of the T-5/bone interface at 12 months after implantation is shown in Fig. 13. A gap of about 5 μm exists between the bone and the test piece, and the existence of a fibrous

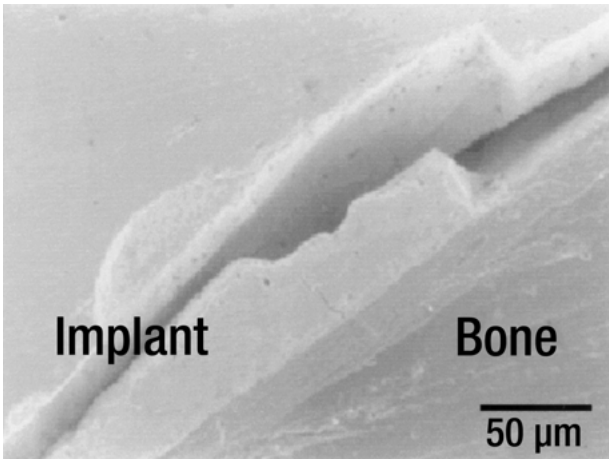

Figure 12 SEM image of the T-1/bone interface at 6 months after implantation.

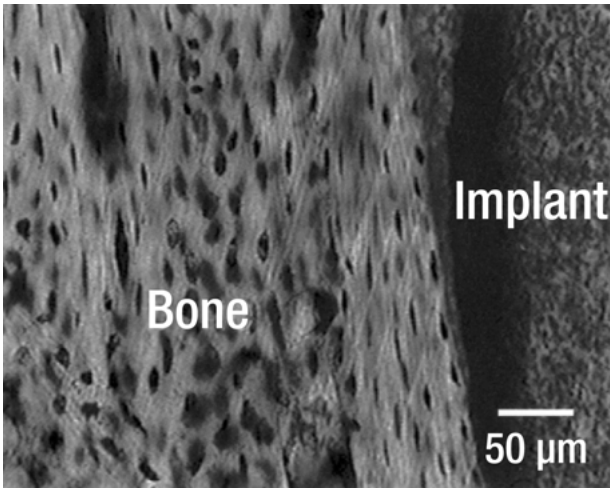

Figure 14 LTM image of the T-1/bone interface at 12 months after implantation.

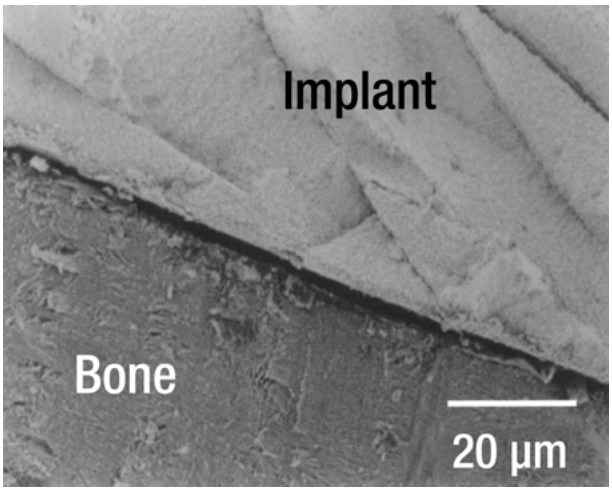

Figure 13 SEM image of the T-5/bone interface at 12 months after implantation.

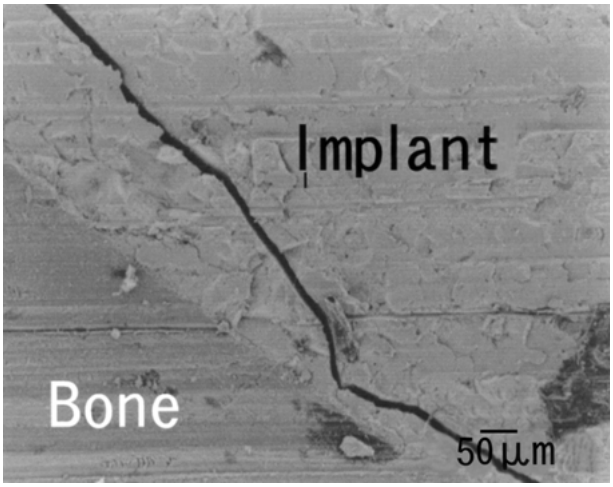

Figure 15 SEM image of the T-1/bone interface at 12 months after implantation.

bonding tissue is not observed. The shear strength of the T-5/bone interface at 12 months after implantation was smaller than those of the other sintered bodies, but considering the value of 7.1 MPa, it is reasonable to consider that peeling occurred due to the force exerted during preparation of the test piece for SEM observation, rather than due to the clearance existing during implantation. When the test piece was in the body of the rabbit, the test piece and the bone (new bone) were probably in direct contact with each other. This inference coincides with Fukuda's report [26]. It was observed that the bonding of the T-1/bone interface at 12 months after implantation was firm and therefore peeling did not likely occur at this site, but rather damage inside T-1 had likely occurred (Figs. 14 and 15).

It is clear that except for T-1 which consists of HAP alone, the shear strength of the sintered body/bone interface for all sintered bodies increases as the implantation period advances. The shear strength of the T-1/bone interface at 6 month was decreased, which is now under examination. At 1, 3 and 6 months, the shear strength of the T-1/bone interface was larger than or equivalent to those of the other sintered bodies; however, at 12 months, although it was considerably larger than those of T-4 and T-5, it was smaller than those of T-2 and

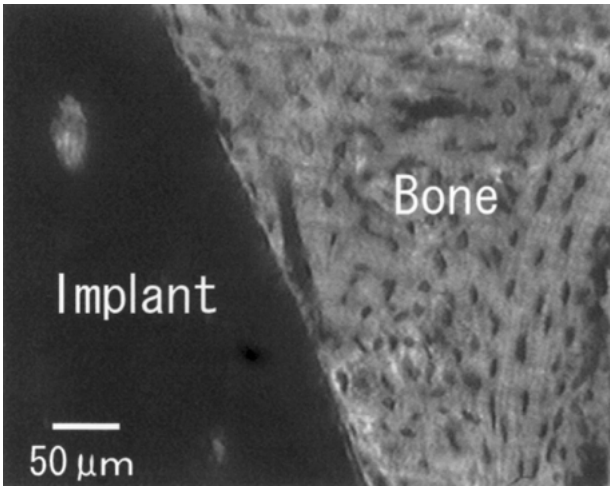

Figure 16 LTM image of the T-2/bone interface at 12 months after implantation.

T-3. T-5 is a sintered body consisting of PSZ alone and has poor bonding except in terms of the anchoring effect due to surface roughness of the HAP particle. Although the shear strength of 7.1 MPa of the T-5/bone interface was obtained at 12 months after implantation, this value

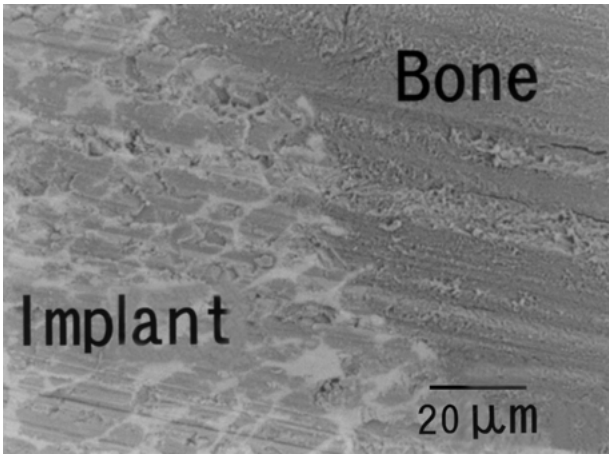

Figure 17 SEM image of the T-2/bone interface at 12 months after implantation.

may be attributed to the above-mentioned anchoring effect.

The shear strength of the T-4/bone interface was smaller than that of T-2 or T-3/bone interface, which may be attributed to T-4 having a 1/1.0 volume ratio of HAP/PSZ and 51% of its surface being occupied by PSZ, which does not exhibit good bone bonding. At 12 months after implantation, the shear strength of the T-1/bone interface was slightly smaller than those of T-2 and T-3/bone interfaces. The reason for this is that T-1 has a low fracture toughness, hence damage occurred inside the sintered body near the interface when the shear force was applied on the sintered body/bone interface. Typical LTM and SEM images of the T-2/bone interface at 12 months after implantation are respectively shown in Figs. 16 and 17. The images suggest that T-2 and the bone are firmly bonded; in addition, due to the excellent mechanical strength of T-2, damage is unlikely to occur inside T-2.

The calcification velocity (μm/d) that was obtained by calculating the measured value based on the distance between two lines, which was formed by staining the parts about 20 μm distant from the sintered body/bone interface using tetracycline and calcein, is shown in Fig. 18. It was predicted before the experiment that as HAP content increases and as the period after operation is shortened, calcification velocity increases, or that calcification velocity reaches the maximum after a fixed period following operation. However, as the figure indicates, it is not confirmed based on the difference in the HAP content of the sintered body and implantation period that calcification velocity changes with a characteristic tendency. The average calcification velocity for all measuring points shown in Fig. 18 was

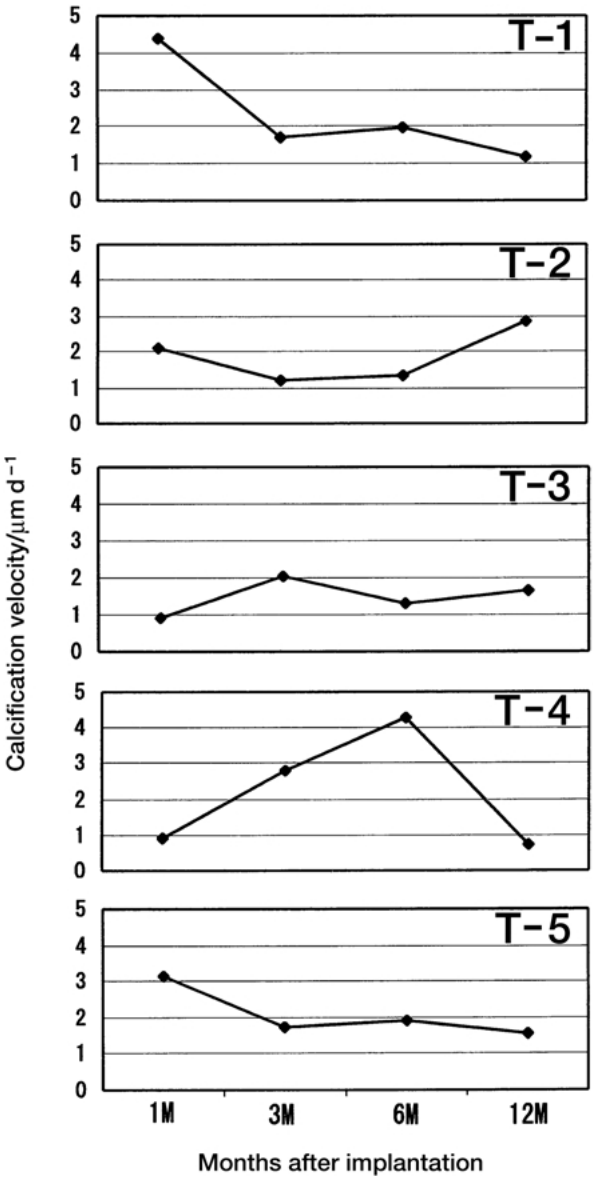

Figure 18 Calcification velocity in the vicinity of the sintered body/bone interface as a function of implantation period.

2.0 μm/d. Regarding calcification velocity, we consider that a detailed confirmation test is necessary for obtaining the accurate values.

The mechanical strength of the sintered bodies used in this study is shown in Table II [19]. It is clear that the mechanical strength of the sintered bodies markedly increases with the increase in the PSZ content, and the increase in their fracture toughness is particularly significant. The largest defect of the sintered monolithic HAP body preventing its use as a substitute material for the hard tissue of organisms is that its fracture toughness is much less than that of bone. While the fracture

TABLE II Mechanical strength of sintered bodies of HAP/PSZ composite particles

Sample	Bending strength (MPa)	Compressive strength (MPa)	Fracture toughness ($MPam^{1/2}$)	Modulus of elasticity (GPa)
T-1	139	825	0.9	118
T-2	173	1218	2.6	150
T-3	200	1322	3.1	157
T-4	203	1501	3.3	164
T-5	790	2959	4.8	209

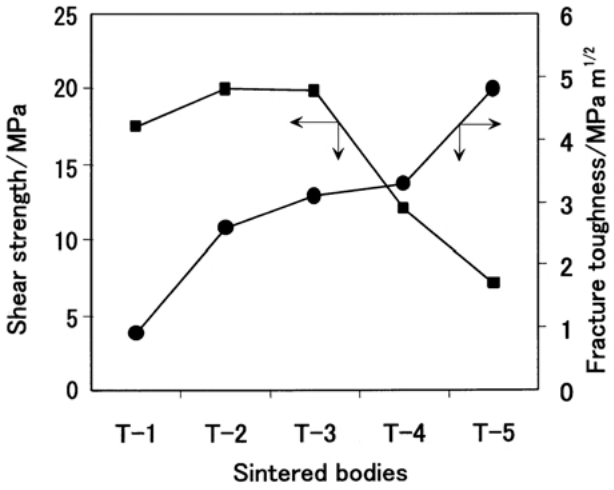

Figure 19 Shear strength of the sintered body/bone interface and fracture toughness of the sintered bodies.

toughness of human cortical bone is 2.2–4.6 MPam$^{1/2}$, that of sintered monolithic HAP body is 0.9 MPam$^{1/2}$. Fig. 19 shows the type of sintered body in the abscissa axis, and the shear strength of the sintered body/bone interface and fracture toughness in the left and right ordinate axes, respectively. Fig. 19 shows clearly that the sintered body with the optimum HAP/PSZ weight ratio taking into consideration both the strength of bond to bone and the fracture toughness is T-3, whose weight ratio is 1/1.5 (volume ratio = 1/0.78).

4. Conclusions

As a result of the cytotoxicity test carried out by the colony formation method on sintered composite bodies consisting of HAP and PSZ prepared in this study, it was confirmed that the sintered bodies are not cytotoxic. As a result of evaluation of the strength of bond between the bone and the sintered composite bodies, which were implanted into rabbit femurs for various periods, by measuring the shear strength of the sintered body/bone interface, it was clarified that sintered composite bodies with an HAP/PSZ weight ratios 1/1.0 and 1/1.5 show equivalent or larger shear strength than sintered monolithic HAP body at 12 months after implantation. The sintered composite body that is excellent in terms of both mechanical strength and the strength of bond to the bone was shown to have an HAP/PSZ weight ratio of 1/1.5.

Acknowledgments

The authors would like to thank Mr K. Ai and Mr M. Yokouchi, Material Engineering Division, Kanagawa Industrial Technology Research Institute, for helpful discussions. One of the authors (T. M.) would like to thank Miss N. Nemoto, Electron Microscopy Center, School of Medicine, Kitasato University, for cooperation in SEM. Authors would like to thank Mrs N. Handa and A. Kudo, School of Allied Health Sciences, Kitasato University, for their assistance with push-out test.

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Received 11 April 2001
and accepted 30 October 2002