The effect of hydroxyapatite coating on the bonding of bone to titanium implants in the femora of ovariectomised rats

T. Hara, K. Hayashi, Y. Nakashima, T. Kanemaru, Y. Iwamoto

From Kyushu University, Fukuoka, Japan

We have studied the effect of hydroxyapatite (HA) coating in 15 ovariectomised and 15 normal rats which had had a sham procedure. Twenty-four weeks after operation, HA-coated implants were inserted into the intramedullary canal of the right femur and uncoated implants into the left femur. The prostheses were removed four weeks after implantation. Twelve specimens in each group had mechanical push-out tests. Sagittal sections of the other three were evaluated by SEM.

The bone mineral density (BMD) of the dissected left tibia was measured by dual-energy x-ray absorptiometry. The difference in BMD between the control and ovariectomised tibiae was 35.01 mg/cm² (95% CI, 26.60 to 43.42). The push-out strength of the HA-coated implants was higher than that of the uncoated implants in both groups (p < 0.0001), but the HA-coated implants of the ovariectomised group had a reduction in push-out strength of 40.3% compared with the control group (p < 0.0001).

Our findings suggest that HA-coated implants may improve the fixation of a cementless total hip prosthesis but that the presence of osteoporosis may limit the magnitude of this benefit.

Materials and Methods

Experimental design and operative procedure. We used thirty 12-week-old female Wistar King rats weighing between 243 and 282 g. They were housed and tested at the Institute of Laboratory Animals, Faculty of Medicine, Kyushu University. All the rats were sedated with ether and anaesthetised by peritoneal administration of pentobarbital (40 mg/kg body-weight). They were then divided into two groups. One had bilateral ovariectomy using standard veterinary procedures and the other a sham operation which consisted of exposure and palpation of the ovaries without removal. At 24 weeks after this operation HA-coated and uncoated implants were inserted into the medullary canal of the right and left femora, respectively, in both groups, after reaming the intercondylar notch of each distal femur with a 1.5 mm drill. Each animal therefore acted as its own control. Four weeks later, all the rats were killed and the femora with the implants and the left tibiae were removed. Radiographs of all the femora were taken using SOFTEX-C-SM (SOFTEX Co, Osaka, Japan) so that the shape and bone density of the right and left femora could be compared to reduce any effect of asymmetry. If the latter was present the animal was rejected. We performed a mechanical push-out test on the implants of 12 rats and evaluated specimens from the other three in each group with respect to the pattern of trabecular bone and new bone around the implant using SEM. The bone mineral density (BMD) of the dissected left tibiae was measured by dual-energy x-ray absorptiometry (DEXA).
Implants. Both implants were made from cylindrical titanium rods 23.0 mm in length and 1.4 mm in diameter. One was sand-blasted without HA coating and the other was coated with HA using a flame-spray technique. The thickness of the coating was 20.0 µm and the crystallinity 55% with the amorphous layer composed of calcium phosphate. The Ca/P ratio was 1.66. The surface roughness (roughness average) of the HA-coated and uncoated implants was 4.3 µm (SD, 0.6) and 4.3 µm (SD, 0.5), respectively. All implants were sterilised in an autoclave which did not affect the HA coating.

DEXA study. The dissected left tibiae were fixed in 75% alcohol and the BMD of the metaphyseal area, exactly 5mm distal to the proximal end of the tibiae, was measured using DEXA (ALOKA Dchroma Scan DCS-600; ALOKA Co, Tokyo, Japan).

Mechanical tests. After removal of soft tissue, all the femora were refrigerated in gauze dampened with physiological saline. They were cut at the proximal end of the implants and the area 2 mm from the distal end of the implants was exposed. Each femur was fixed in a wooden base (thickness, 9 mm; width, 19 mm; length, 30 mm; diameter of the central hole, 4.5 mm) with cement (Fig. 1). These specimens were then placed on an X-Y slide-support platform which allowed them to self-align so that only a vertical load was applied to the implant. The shear strength of the bone-implant interface was measured using an Instron-type apparatus (Autograph-2000A; Shimazu, Kyoto, Japan) operated in stroke-control mode at a constant displacement of 0.5 mm/min. A vector of the push-out force was applied parallel to the long axis of the implant. The peak load was obtained before failure to provide a measurement of the bone-implant attachment strength.

After testing, we confirmed that the cement between the bone and wood had not fractured in all cases.

SEM study. Specimens which had not been tested mechanically were fixed in 75% alcohol, dehydrated in graded alcohols, and embedded in polyester resin. Each was cut in the sagittal plane on a slicer (Maruto Co, Tokyo, Japan) using a diamond blade. The surfaces of these specimens were observed by SEM in backscatter mode.

Statistical analysis. Differences in the values of the BMD between the ovariectomised and the control groups were calculated using Student’s unpaired t-test. The mean push-out strengths and standard deviations (SD) for the HA-coated and uncoated implants were calculated for each group. The effects of insertion of the HA-coated implants and those which were not coated in the control and ovariectomised groups, were analysed statistically using analysis of variance (ANOVA). Differences between the treatment groups were calculated using Scheffe’s multiple comparison test and were considered to be significant at p < 0.05.

Results

One rat died after the sham operation. No signs of infection were seen in any of the rats. There was no asymmetry and no difference in bone density between the right and left implanted femora as assessed by SOFTEX-C-SM. All implants were well seated in the medullary canal (Fig. 2).

DEXA study. The mean BMD of the control and ovariectomised groups was 151.56 mg/cm² (SD, 12.48) and 116.55 mg/cm² (SD, 9.05), respectively (Table I). The difference in BMD between the control and ovariectomised tibial bone was 35.01 mg/cm² (95% CI, 26.60 to 43.42).

Mechanical test. In the control group, the bone-implant attachment strength of HA-coated and uncoated implants was 27.45 kgf (SD, 4.58) and 2.98 kgf (SD, 1.62), respectively. In the ovariectomised group, the strengths of HA-coated and uncoated implants were 16.39 kgf (SD, 2.07) and
0.81 kgf (SD, 0.08), respectively (Fig. 3). HA-coated implants showed a significantly higher bone-implant attachment strength than uncoated implants in both the control and ovariectomy groups.

**Table 1.** The BMD (mg/cm²) in both groups as measured by DEXA

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<tr>
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<th>Control</th>
<th>Ovariectomised</th>
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<tr>
<td>168.89</td>
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<td>144.60</td>
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<td>death</td>
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<tr>
<td>Mean</td>
<td>151.56 (SD, 12.48)</td>
<td>116.55 (SD, 9.05)</td>
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The bone-implant attachment strength (kgf) for each group (OVX-Ti, ovariectomy + implant; C-Ti, control + implant; OVX-HA, ovariectomy + HA coating; C-HA, control + HA coating; error bar = 1 SD).

**Figure 4**

Photomicrographs of sagittal sections which had not been mechanically tested. Figure 4a – Control group/HA-coated implant. The new bone covering the surface of the HA coating was continuous and well connected to trabecular bone. Figure 4b – Ovariectomised group/HA-coated implant. The new bone covering the surface of the HA coating was continuous, but had fewer connections to trabecular bone. Figure 4c – Control group/uncoated implant. The new bone covering the surface of the titanium was intermittent. There was abundant trabecular bone around the implant. Figure 4d – Ovariectomised group/uncoated implant. The new bone covering the surface of the titanium was intermittent and thin with decreased trabecular bone around the implant (C, cortex; T, trabecular bone; I, implant; the bars in the lower right corner = 200µm; × 32).
and ovariectomised groups (p < 0.0001). No significant differences were observed in uncoated implants in either group, but HA-coated implants had a reduction of 40.3% in bone-implant attachment strength in the ovariectomised group as compared with the control group (p < 0.0001).

No cement fractures were detected between the bone and wood after mechanical testing.

**SEM study.** In the ovariectomised group, the metaphyseal trabecular bone density had decreased and there was a loss of connectivity compared with the control group (Fig. 4).

The new bone covering the surface of the uncoated implants was intermittent in both groups. In ovariectomised rats, it was very thin and had less connectivity with the surrounding trabecular bone. The HA-coated implants were circumferentially covered with new bone from metaphysis to diaphysis in both groups. Good connectivity between the new bone covering the HA and trabecular bone was observed in the control group. At the diaphyseal contact area between implant and endosteal bone, no significant differences were seen in the ovariectomised group compared with the control group with either type of implant (Fig. 4).

**Discussion**

Our findings indicate that HA coating on implants improves the strength of the attachment between the implant and bone even under osteoporotic conditions, but there is a limit to the effect of HA coating.

We used ovariectomised rats in an attempt to replicate the many events associated with the development of post-menopausal osteoporosis in women and the rapid decrease in the cancellous bone mass in the metaphyseal regions of the long bones. When implants are inserted into the medullary canal of the femur, the main region of contact between bone and implant is the metaphyseal trabecular bone and the diaphyseal endosteal bone. We therefore believe that this is a suitable model to evaluate the integrity between the implant and bone in osteoporotic conditions.

In our study, the ovariectomised rats had osteoporosis. This was shown by the reduction of BMD at the proximal metaphysis of the left tibia and the observed decrease in metaphyseal trabecular bone around the implants.

The two types of implant had the same degree of surface roughness but the diameter of the HA-coated implants was about 40 µm greater than that of the uncoated implants because of the thickness of the HA coating. The difference in the diameter between the two implants may have influenced the evaluation of the bone-implant attachment strength, but we believe that this can be ignored since the diameter of the reaming drill was 1.5 mm and there was a gap of about 0.1 mm between the implants and the reamed holes. All the implants had been inserted tightly into the medullary canal of the femur.

In evaluating mechanical testing, we used the kilogram force as the unit of bone-implant attachment strength instead of the megapascal. The reason for this is that, unlike the trans cortical model, the region of each implant in the femur was uniform but the bone which contacted the implant was not. In the proximal part of the femur, the implant was in contact with the diaphyseal endosteal bone, while in the distal part it was with the metaphyseal trabecular bone. We could not therefore justify dividing the bone-implant attachment strength by the exterior surface area of the implant.

All HA-coated implants were inserted into the right femur and all uncoated implants into the left. Ideally, half of the rats in each group should have had HA-coated implants in the left femur and uncoated implants in the right, and the BMD of the right and left femora of each rat should have been measured by DEXA and compared. However, we could not find any differences in bone density between right and left implanted femora in the rats using SOFTEX-C-SM. We therefore conclude that the differences in the attachment strength between bone and implant in HA-coated and uncoated devices in each group were not due to the side but to the effect of the HA coating.

We evaluated the sagittal sections of the three tested specimens in each group which had not been mechanically tested using SEM. It is impossible to analyse these images statistically with such small numbers, but similar findings from the three specimens in each group helped in the understanding of the results of the mechanical tests. Uncoated implants showed equally low bone-implant attachment strength in both groups. We suspect that this was the result of the low surface roughness of the implants, with no direct bonding between the titanium and bone. The HA-coated implants showed higher bone-implant attachment strength than uncoated implants in both groups. This was the result of circumferential covering of new bone around the implants and the direct bonding between HA coating and new bone. We also observed connections between the new bone around the HA-coated implants and the trabecular bone. There was a reduction of 40.3%, however, in the bone-implant attachment strength of the HA-coated implants in the ovariectomised group compared with the control group. There were no differences in the findings of SEM between the ovariectomised and control groups in the diaphyseal area, but there was decreased trabecular bone surrounding the HA-coated implants and a loss of connections between the trabecular bone and the new bone around the HA coating in the metaphyseal area in the ovariectomised group. We suspect that these observed decreases and losses in the metaphyseal area led to a corresponding reduction in the bone-implant attachment strength in the ovariectomised group.

There have been few studies on the effects of HA coating on the bonding of bone to implants in the osteoporotic state. Søballe et al. studied the ingrowth of osteopenic bone into titanium-alloy porous-coated implants with and without HA coating in dogs and concluded that HA-coated implants were not affected by the osteopenic condition. This is the only report in which the integrity between an...
HA-coated implant and osteoporotic bone has been evaluated mechanically. Our study suggests, however, that HA coating improves the fixation of the implant even in the osteoporotic condition but that osteoporosis decreases this effect.

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References


