

The Effects of Dicalcium Phosphate Dihydrate-coated Titanium Implants on Bonding to Bone in Ovariectomized Rats

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Running title

The Effects of Dicalcium Phosphate Dihydrate-coated Implants on Bonding to Bone.

Abstract

The aim of this study was to evaluate the effects of dicalcium phosphate dihydrate (DCPD)-coated titanium implants on bonding to bone under osteoporotic conditions. We measured the bone mineral density (BMD) using peripheral quantitative computed tomography (pQCT) densitometry and evaluated the bone-implant shear force using push-out tests in ovariectomized rats. Twenty-week-old female rats underwent ovariectomy, and DCPD-coated and uncoated implants were inserted intramedullary into the right and left femurs, respectively, eight weeks after operation. Both the femora and right tibia were retrieved four weeks after implantation. In the 16 operated rats, the BMD in the right tibia was measured while the bilateral femora underwent mechanical push-out tests. The total and cancellous BMDs (\pm standard deviation) were 566.0 ± 26.3 and 114.5 ± 38.2 mg/cm³, respectively. The bone-implant shear force of the DCPD-coated implants was higher than that of the uncoated implants ($p < 0.05$). Our findings suggest that DCPD coating may improve fixation to the bone, even in the presence of osteoporosis.

Keywords

Dicalcium phosphate dihydrate (DCPD), Osteoporosis, Peripheral quantitative computed tomography (pQCT), Bone-implant shear force

Footnotes

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Introduction

Since the advent of total hip arthroplasty (THA), a cementless femoral stem has been commonly used, and good long-term results have been reported. Various designs have been developed, and the use of porous metal surfaces, in particular, has enabled biological fixation to be achieved through ingrowth of bone¹⁻⁴). Along with biomechanically optimized implant designs and bioactive coatings for enhanced bone ingrowth, the indications of the cementless stem have been gradually expanded to include elderly patients with impaired bone quality and limited healing capacity⁵⁻⁹). However, low bone mineral density (BMD) and age-related geometric changes of the proximal femur have been shown to affect the initial stability and delay osteointegration to the cementless femoral stems in THA¹⁰). Therefore, the development of a cementless stem that will confer implant stability under osteopenic conditions is necessary.

As a bioceramic coating material for prosthetic implants, hydroxyapatite (HA) has been commonly used and is known to accelerate bone healing and enhance the biological fixation of implants due to its biocompatibility and osteoconductive potential. A clinical meta-analysis based on 12 studies showed that an HA coating was able to reduce the incidence of femoral osteolysis, although there was no statistical difference in the femoral stem survival rate between those with an HA coating and those without¹¹). In their experimental study, Søballe et al. showed that the osteoconductive potential of HA-coated

implants was greater than that of non-HA-coated implants under osteopenic conditions¹²⁾. They also showed that there was no significant difference in the stability of HA-coated implants between osteopenic and non-osteopenic conditions¹²⁾. However, Hara et al. reported that the stability of HA-coated implants under osteopenic conditions was inferior to that of non-osteopenic conditions using rats¹³⁾.

As an alternative bioceramic coating material, dicalcium phosphate dihydrate (DCPD) has been developed for use with medical and dental implants due to its biocompatibility and ability to bond directly to bone. Chow et al. reported that DCPD dissolved about three times faster than HA in their *in vitro* study¹⁴⁾. In a pig study, the DCPD coating was substituted by bone without giant cell reaction until three months later¹⁵⁾. The traditional titanium porous coated implant is known for enabling rigid mechanical fixation. If the DCPD coating is added to this implant, we expect a greater osteoconductive potential, thereby increasing the mechanical strength of the bone-implant interface. To our knowledge, however, no study has assessed whether or not a DCPD coating improves bone-implant fixation under osteoporotic conditions.

In this study, we evaluated the bone-implant shear force of DCPD-coated titanium implants in ovariectomized rats to confirm the effects of DCPD under osteoporotic conditions.

Materials and Methods

Animal experimental design and surgical procedures

This study protocol was approved by the Fukuoka University Animal Care and Use Committee. A total of 21 female Wistar rats were purchased from Japan SLC, Inc. (Shizuoka, Japan) and maintained in separate plastic cages under normal conditions (22-26 °C; air humidity 55%-60%; 12-h light/dark cycle) with free access to food and water. At 20 weeks of age, the rats (mean weight: 199.9 ± 7.1 kg) underwent bilateral ovariectomy under general anesthesia induced by intraperitoneal injection with medetomidine hydrochloride (Domitol; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) at a dose of 0.375 mg/kg, midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan) at a dose of 2 mg/kg, and butorphanol (Vetorphale; Meiji Seika Pharma Co., Ltd.) at a dose of 2.5 mg/kg, as described previously¹⁶. Eight weeks after ovariectomy, implants were inserted into the bilateral femora of the rats via a medial parapatellar approach under similar anesthesia. After lateral dislocation of the patella via a 2-cm skin incision, a 1.6-mm-diameter hole was drilled into the medullary cavity of the femur.

Cylindrical titanium implants (18 mm long and 1.4 mm in diameter) coated with DCPD were inserted into the right femora, while implants without the DCPD coating were inserted into the left femora. The implant was a sandblasted Ti6-Al4-V rod and the thickness of the DCPD coating with electrochemical deposition was 20 μ m. The implants were manufactured

by Aesculap AG (Tuttlingen, Germany).

Two rats died after ovariectomy and one rat died after the implantation procedure. The remaining 18 rats were euthanized with an overdose of anesthesia inhalation using isoflurane (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), and the specimens were retrieved 4 weeks after the implantation procedure. Two rats (one in the right and one in the left femur) had malpositioned implants and were thus excluded from this study. The remaining 16 rats were used for mechanical testing and BMD measurement.

Peripheral quantitative computed tomography densitometry

The cross-sections of the proximal right tibiae were scanned using a peripheral quantitative computed tomography (pQCT) system (XCT Research SA+, version 6.20 C; Stratec Medizintechnik GmbH, Pforzheim, Germany). This system uses a 50-kV/0.3-mA X-ray source. On a scout view of the proximal tibia, a scan line was manually placed so that the cross-sectional slice passed 3 mm distal to the growth cartilage. The scan duration was 10 minutes, and the voxel size was $0.12 \times 0.12 \times 0.46$ mm. The total and cancellous BMDs were recorded using the pQCT software program.

Mechanical testing

Mechanical testing was conducted to evaluate the bone-implant shear force of the cancellous

bone by pushing out the implants to the condylar of the femur, as described previously^{13, 17, 18}.

The implants were cut at the proximal end, fixed into a plastic tube (diameter: 10 mm), and embedded in polymethyl methacrylate (PMMA). After PMMA fixation, the specimens were further cut until the area 5 mm from the proximal end of the implant was exposed (Fig. 1a).

The shear force of the bone-implant interface was measured using the EZ-Test pressure device (EZ-LX; Shimadzu Corporation, Kyoto, Japan) (Fig. 1b). The implants were displaced at a constant rate (6.0 mm/min), and the peak force was recorded with the TRAPEZIUMX software program (Shimadzu Corporation) (Fig. 2).

Statistical analyses

For statistical analyses, the data were analyzed using the GraphPad Prism software program, version 5.0 (GraphPad Software, San Diego, CA, USA). The mean peak forces and standard deviation (SD) for the DCPD-coated and uncoated implants were calculated, and the differences were analyzed using the Mann-Whitney test. Significant differences were defined as values of $p < 0.05$.

Results

pQCT densitometry

The total and cancellous BMDs were 566.0 ± 26.3 and 114.5 ± 38.2 mg/cm³, respectively (Fig. 3), values which were similar to those obtained by pQCT densitometry in previous studies in rats^{17, 19}.

Bone-implant shear force

The mean bone-implant shear forces of the DCPD-coated and uncoated implants in the ovariectomized rats were 72.6 ± 21.2 and 33.5 ± 10.7 N, respectively. The DCPD-coated implants showed a significantly higher bone-implant shear force than the uncoated implants ($p < 0.05$) (Fig. 4). No cement fractures were detected between the bone and PMMA after mechanical testing.

Discussion

The present study showed that the bone-implant shear force of DCPD-coated implants was significantly higher than that of uncoated implants in ovariectomized rats.

With the increase in the mean life expectancy, patients requiring THA are frequently elderly and often have osteoporosis. Considering the prognosis of cementless THA, the integrity of the bone-implant interface is the main determining factor predicting the implant survival²⁰⁾. Bone resorption around the periprosthetic area and consequent shifting of the implant are negative factors affecting the long-term outcome^{17, 20)}. The bone-implant interface in the metaphyseal area is generally filled with cancellous bone. Because ovariectomized rat models are associated with loss of cancellous bone, animal study models based on ovariectomized rats provide conditions similar to that of a postmenopausal osteoporotic bone host^{17, 21)}. In our study, the total and cancellous BMDs in the ovariectomized rats were 566.0 ± 26.3 and $114.5 \pm 38.2 \text{ mg/cm}^3$, respectively, values which were similar to those obtained in previous studies using ovariectomized rats^{17, 19)}.

Calcium phosphate coatings were first described in 1986 and have been shown to produce a significant early effect on bone tissue formation with a porous coating^{22, 23)}. The DCPD coating is a resorbable calcium phosphate ceramic that absorbs without giant cell reactions until three months later¹⁵⁾. It continuously dissolves into calcium and phosphate ions in a 1:1 ratio²⁴⁾. This continuous dissolution of DCPD helps keep the pore coating open

for bony ingrowth. Chen et al. reported that the titanium porous surface with and without the DCPD coating exhibited *de novo* bone formation on the implant surface as early as two weeks after implantation. At this time-point, osteoblasts were observed around the new bone on both the implant surface and adjacent host bone²⁵). The osteoconductive characteristics and *in vivo* behavior of DCPD have been previously investigated in animal models²⁶). Gottlander et al. showed that the bone-to-implant contact with the calcium phosphate coating was greater than that of an uncoated control under normal conditions at four weeks of follow-up²⁷). Similarly, in our study using ovariectomized rats, the bone-implant shear force of the DCPD-coated implants was 2.2 times that of the uncoated implants four weeks after implantation. This finding suggests that the DCPD coating may improve the bone-implant shear force, even under osteopenic conditions.

Of note, another experimental study using rats with HA-coated implants was conducted by Nakamura et al. and showed an even higher shear force of about 122 N at 4 weeks in the ovariectomized group¹⁷). The HA coating might be superior in initial bone-implant fixation strength. HA is well known as a nonresorbable bioceramic coating with a strong osteoconductive effect and a gap-filling ability at bone-HA contact. However, Reigstad et al. reported that the fixation pattern differed between HA and calcium phosphate coatings, with a sharper but time-constrained increase in fixation for the HA-coated implant, and a slower but more steadily increasing fixation pattern for resorbable calcium phosphate-coated implants²⁸).

Despite the encouraging results with the DCPD coatings in the present study, the long-term effects of the biodegradation of this coating are still unknown. Additional studies should therefore investigate the long-term stability of the DCPD coating.

Several limitations associated with the present study warrant mention. First, the BMD of sham-controlled rats was not included in the present study. However, the total and cancellous BMDs in our study were similar to those obtained on pQCT densitometry in previous studies in rats^{17, 19}). Therefore, the rats in the present study were considered to be osteoporotic. Second, we did not evaluate the bone-implant shear force under normal conditions. Although the DCPD coating significantly improved the bone-implant shear force under osteoporotic conditions, it was unclear how the shear force was improved compared with normal conditions.

In conclusion, our findings suggest that DCPD coating improves the implant fixation, even under osteoporotic conditions.

References

- 1) Turner TM, Sumner DR, Urban RM, Rivero DP, Galante JO: A comparative study of porous coatings in a weight-bearing total hip arthroplasty model. *J Bone and Joint Surg.* 68-A(9): 1396-1409, 1986.
- 2) Eingartner C, Volkmann R, Winter E, Maurer F, Sauer G, Weller S, Weise K: Results of uncemented straight femoral shaft prosthesis after 9 years of follow-up. *J Arthroplasty.* 4 (15): 440-447, 2000.
- 3) Badhe NP, Quinnell RC, Howard PW: The uncemented Bi-Contact total hip arthroplasty. *J Arthroplasty.* 17(4): 896-901, 2002.
- 4) Keisu KS, Orozco F, Sharkey PF, Hozack WJ, Rothman RH: Primary cementless total hip arthroplasty in octogenarians. Two to eleven-year follow-up. *J Bone Joint Surg Am.* 83-A(3): 359-363, 2001.
- 5) Yammada H, Yasuhara Y, Henmi O, Morita M, Shiromoto Y, Kawano T, Kanaji A, Ando K, Nakagawa M, Kosaki N, Fukaya E: Cementless total hip replacement: past, present, and future. *J Orthop Sci.* 14:228-241, 2009.
- 6) Kelly SJ, Robbins CE, Bierbaum BE, Bono JV, Ward DM: Use of a hydroxyapatite-coated stem in patients with Dorr Type C femoral bone. *Clin Orthop.* 465: 112-116, 2007.
- 7) Meding JB, Galley MR, Ritter MA: High survival of uncemented proximally porous-coated titanium alloy femoral stems in osteoporotic bone. *Clin Orthop.* 468: 441-447,

2010.

8) Mäkelä KT, Eskelinen A, Paavolainen P, Pulkkinen P, Remes V: Cementless total hip arthroplasty for primary osteoarthritis in patients aged 55 years and older. *Acta Orthop.* 81: 42-52, 2010.

9) Thillemann TM, Pedersen AB, Mehnert F, Johnsen SP, Soballe K: Postoperative use of bisphosphonates and risk of revision after primary total hip arthroplasty: a nationwide population-based study. *Bone.* 46: 946-951, 2010.

10) Aro HT, Alm JJ, Moritz N, Mäkinen TJ, Lankinen P: Low BMD affects initial stability and delays, stem osseointegration in cementless total hip arthroplasty in women: 2-year RSA study of 39 patients. *Acta Orthop.* 83 (2): 107-114, 2012.

11) Chen YL, Lin T, Liu A, Shi MM, Hu B, Shi ZL, Yan SG: Does hydroxyapatite coating have no advantage over porous coating in primary total hip arthroplasty? A meta-analysis. *J Orthop Surg Res.* 28:10-21, 2015.

12) Søballe K, Hansen ES, Brockstedt-Rasmussen H, Hjortdal VE, Juhl GI, Pedersen CM, Hvid I, Bünger C: Fixation of titanium and hydroxyapatite coated implants in arthritic osteopenic bone. *J Arthroplasty.* 6: 307-316, 1991.

13) Hara T, Hayashi K, Nakashima Y, Kanemaru T, Iwamoto Y: The effect of hydroxyapatite coating on the bonding of bone to titanium implants in the femora of ovariectomised rats. *J Bone Joint Surg Br.* 81:705-709, 1999.

- 14) Chow LC, Markovic M, Takagi S: A dual constant-composition titration system as an in vitro resorption model for comparing dissolution rates of calcium phosphate biomaterials. *J Biomed Mater Res.* 65B-2: 245-251, 2003.
- 15) Szmukler-Moncler S, Perrin D, Piattelli A, Scarano A: Evaluation of a soluble calcium phosphate coating obtained by electrochemical deposition: a pilot study in the pig maxillae. *Biological mechanisms of tooth eruption, resorption and replacement by implants:* 481-485, 1998.
- 16) Kawai S, Takagi Y, Kaneko S, Kurosawa T: Effect of Three Types of Mixed Anesthetic Agents Alternate to Ketamine in Mice. *Exp Anim.* 60(5):481-487, 2011.
- 17) Nakamura Y, Hayashi K, Abu-Ali S, Naito M, Fotovati A: Effect of preoperative combined treatment with alendronate and calcitriol on fixation of hydroxyapatite-coated implants in ovariectomized rats. *J Bone Joint Surg Am.* 90(4): 824–832, 2008.
- 18) Hayashi K, Fotovati A, Ali SA, Oda K, Oida H, Naito M: Prostaglandin EP4 receptor agonist augments fixation of hydroxyapatite-coated implants in a rat model of osteoporosis. *J Bone Joint Surg Br.* 87:1150-1156, 2005.
- 19) Cao DP, Zheng YN, Qin LP, Han T, Zhang H, Rahman K, Zhang QY: *Curculigo orchioides*, a traditional Chinese medicinal plant, prevents bone loss in ovariectomized rats. *Maturitas.* 58: 373-380, 2008.
- 20) Chen BL, Xie DH, Zheng ZM, Lu W, Ning CY, Li YQ, Li FB, Liao WM: Comparison of

the effects of alendronate sodium and calcitonin on bone-prosthesis osseointegration in osteoporotic rats. *Osteoporos Int.* 22(1): 265–70, 2011.

21) Tonino AJ, van der Wal BC, Heyligers IC, Grimm B: Bone remodeling and hydroxyapatite resorption in coated primary hip prostheses. *Clin Orthop Relat Res.* 467(2):478–84, 2009.

22) Ducheyne P, Raemdonck, WV, Heughebaert JC, Heughebaert M: Structural analysis of hydroxyapatite coatings on titanium. *Biomaterials.* 7: 97-103, 1986.

23) Ducheyne P, Beight J, Cuckler J, Evans B, Radin S: Effect of calcium phosphate coating characteristics on early postoperative bone tissue ingrowth. *Biomaterials.* 11: 531-540, 1990.

24) Alge DL, Goebel WS, Chu T-MG: Effects of DCPD cement chemistry on degradation properties and cytocompatibility: Comparison of MCPM/ β -TCP and MCPM/HA formulations. *Biomed Mater.* April; 8(2): 1-16, 2013.

25) Chen D, Bertollo N, Lau A, Taki N, Nishino T, Mishima H, Kawamura H, Walsh WR: Osseointegration of porous titanium implants with and without electrochemically deposited DCPD coating in an ovine model. *J Orthop Surg Res.* 6:56, 2011.

26) Bohner M, Theiss F, Apelt D, Hirsiger W, Houriet R, Rizzoli G, Gnos E, Feri C, Auer A, Rechenberg BV: Compositional changes of a dicalcium phosphate dehydrate cement after implantation in sheep. *Biomaterials.* 24:3463–3474, 2003.

27) Gottlander M, Johansson CB, Wennerberg A, Albrektsson T, Radin S, Ducheyne P: Bone

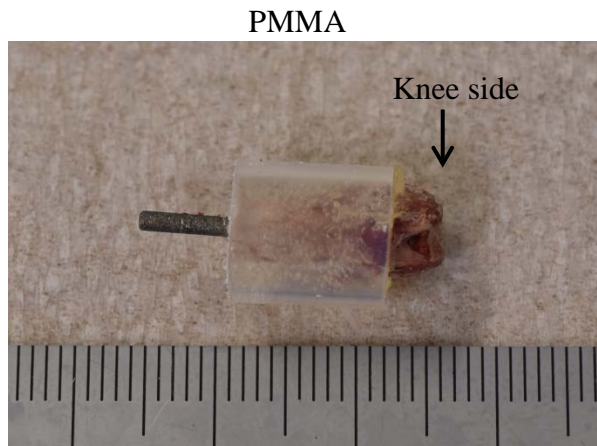
tissue reactions to an electrophoretically applied calcium phosphate coating. *Biomaterials*.

18: 551-557, 1997.

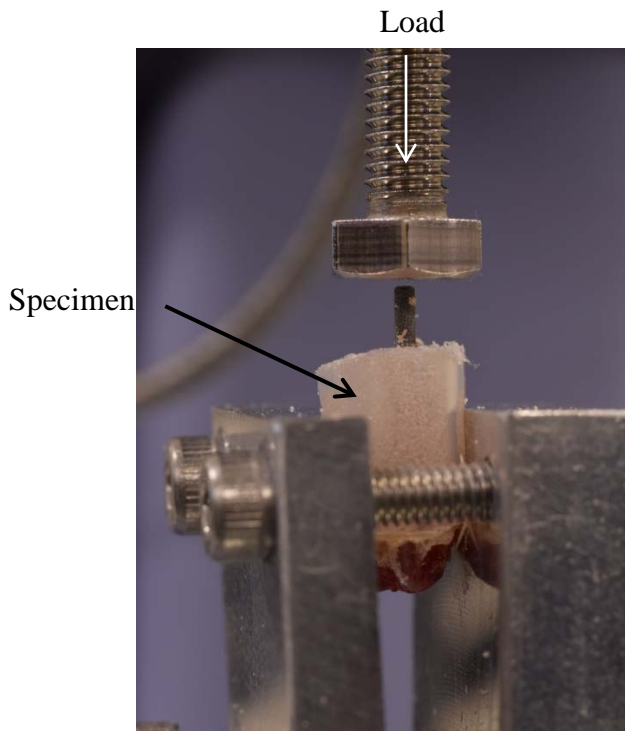
28) Reigstad O, Johansson C, Stenport V, Wennerberg A, Reigstad A, Røkkum M: Different patterns of bone fixation with hydroxyapatite and resorbable CaP coatings in the rabbit tibia

at 6, 12, and 52 weeks. *J Biomed Mater Res*. 99B:14-20, 2011.

Figure 1(a)

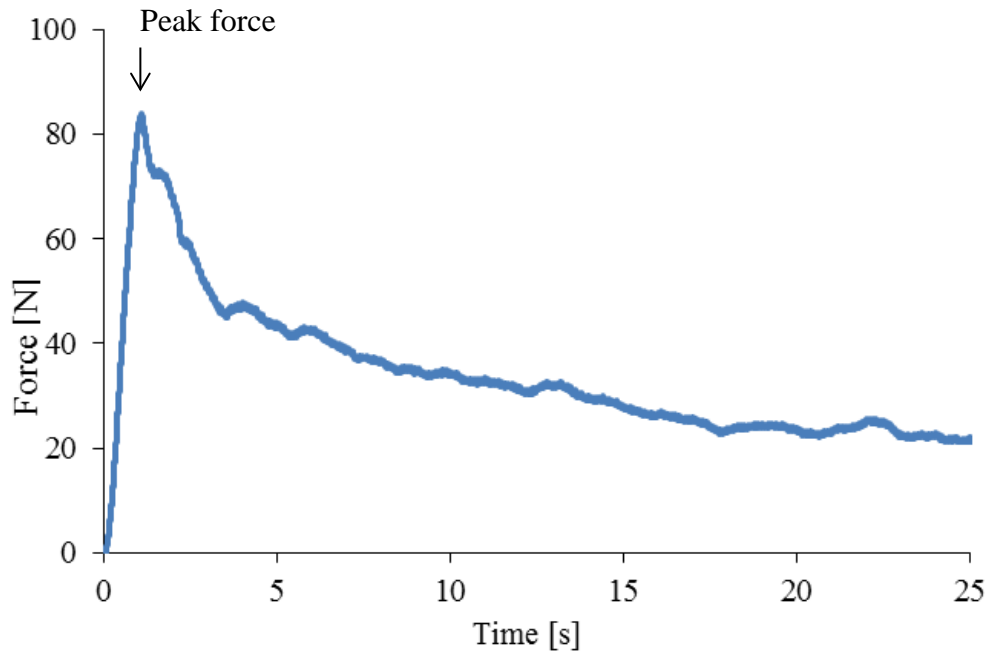


(b)



Push-out test. (a) The femoral segment was potted in PMMA using a flat-bottomed cylindrical mold. (b) A potted test specimen was set with the testing machine. The implant axis was set parallel to the pushing-out loading direction.

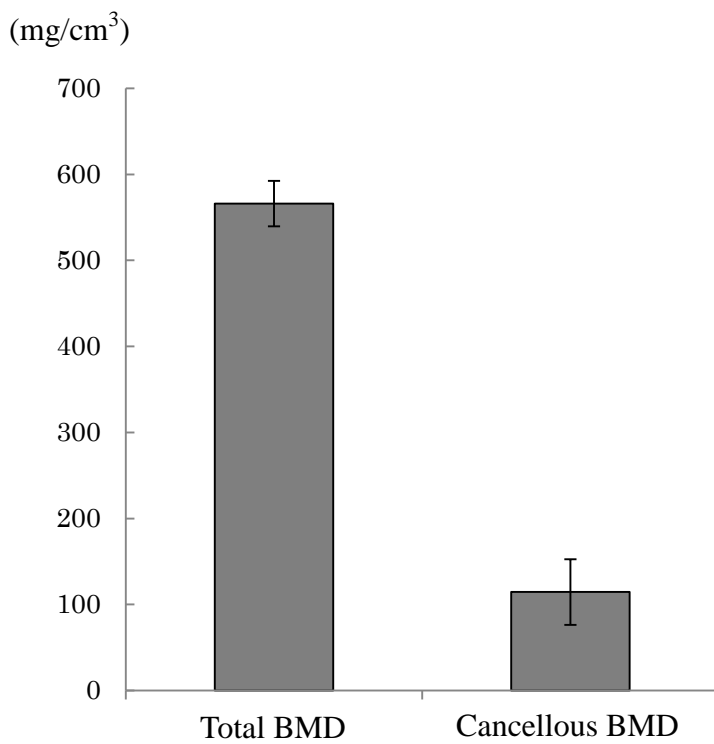
Figure 2



The representative timeline of the shear force during the process of pushing out the implant.

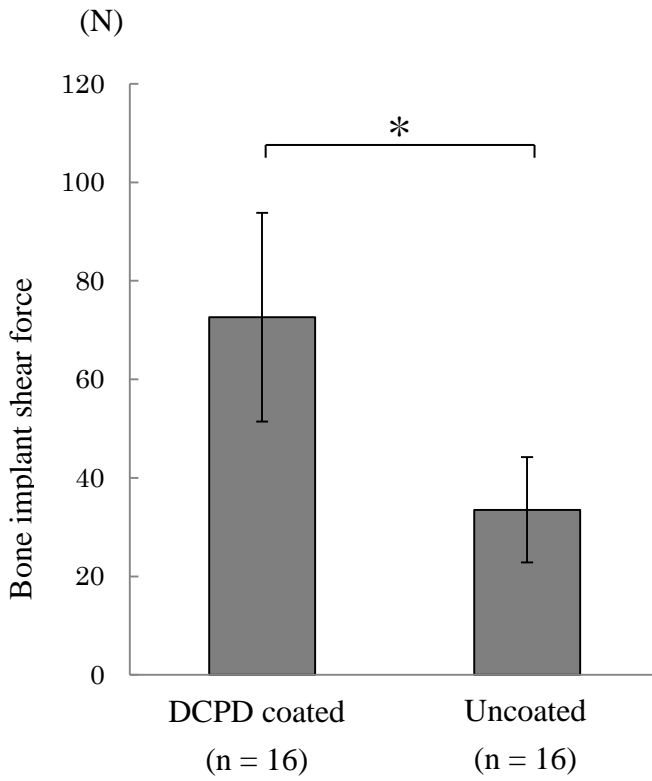
The peak force was determined as the ultimate compressive force (N) necessary to push out the implant.

Figure 3



The total and cancellous bone mineral density (BMD) of the tibia were measured using peripheral quantitative computed tomography (pQCT). The data are expressed as the mean (bars) and standard deviation (error bars).

Figure 4



The bone-implant shear forces of the DCPD-coated and uncoated implants four weeks after implantation. The data are expressed as the mean (bars) and standard deviation (error bars). A significant difference is indicated by the * ($p < 0.05$, the Mann-Whitney test).