Title: Pedicle screws with thin hydroxyapatite coating improve fixation at the bone- implant interface in the osteoporotic spine: An experimental study of porcine model

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Running Title: Thin HA coating improves PS fixation in the osteoporotic spine

Note:
We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

Structured Abstract

Object: Instrumentation failure by loosening of pedicle screws (PSs) in osteoporosis is a serious problem in spinal surgery. A thin hydroxyapatite (HA) surface coating by a sputtering process was recently reported as a promising method providing bone conduction around the implant with few concerns about breakage of the coating layer. We evaluated the biomechanical and histological features of bone-implant interface (BII) of PSs with thin HA coating in vivo osteoporotic porcine spine model.

Methods: Three different types of PSs (STs: untreated, BLs: sandblasted, and HAs: HA-coated) were implanted into the thoracic and lumbar spine (T9-L6) of 8 mature porcine (6 ovariectomized: osteoporosis group, 2 sham-operated: control group). The spines were harvested from the osteoporosis group at 0, 2, 4, 8, 12, and 24 weeks after PS placement and from the control group at 0 and 24 weeks. Bone mineral density (BMD) was measured by peripheral quantitative computed tomography. Histological evaluation of BII was conducted by Bone Volume/Tissue Volume (BV/TV) and Bone Surface/Implant Surface (BS/IS) measurement. The strength of BII was evaluated with extraction torque testing.

Results: BMD decreased significantly in the osteoporosis group ($p<0.01$). HAs exhibited the greatest mean extraction peak torque at 8 weeks, HAs and BLs exhibited significantly greater mean torque than STs at 12 weeks ($p<0.05$). BS/IS was significantly higher for HAs than for STs after 2 weeks ($p<0.05$), and bonding between the bone and the implant surface was maintained until 24 weeks with no detachment of the coating layer. On the other hand, BV/TV was not significantly higher for HAs than for BLs or STs except at only 4 weeks.

Conclusions: PSs with thin HA coating by a sputtering process strengthen the bonding at the bone-screw interface, which may improve early implant fixation in spinal surgery for osteoporosis patients. However, the absence of increased bone mass around the screw remains an issue, improving bone quality by osteoporosis treatment may be necessary to prevent fractures around screws.

Introduction:

As we have entered an era of aging society, spinal instrumentation surgery for patients with osteoporosis is rapidly increasing [5]. Pedicle screws (PSs) provide the strongest biomechanical anchor in spinal instrumentation surgery and transpedicular fixation is now the gold standard of spinal reconstruction for deformities, traumas, tumors, and degenerative conditions [8,12]. However, loosening of PSs that causes postoperative correction loss or pseudarthrosis has been particular evident in patients with osteoporosis and a major concern in spinal surgery [10]. Most of clinical studies reported a low loosening rate, in some cases less than 1 %, when considering standard posterior fixation with PSs and rods with anterior support in non-osteoporotic patients [9]. On the other hand, Wu et al. in a prospective, randomized study reported 48 loosened PSs in a total of 464 screws in osteoporotic patients [22]. PSs fixation remains a challenge for patients with severely osteoporotic spines. Previously reported methods for improving the anchoring strength of PSs into fragile bone include using expandable titanium PSs and augmentation of fixation with polymethylmethacrylate [22,23]. However, these methods have not come into general use due to the technical complexity and reported adverse consequences such as neurovascular damage caused by pedicle fracture and cement leakage.

In fields such as titanium dental implants and total hip arthroplasty, a hydroxyapatite (HA) surface coating is believed to stimulate bone ingrowth at the bone-implant interface (BII), and many basic studies and clinical applications have shown that it is a powerful means of improving implant fixation [17,20]. The ideal stable state of titanium implants within the bone is that of osseointegration, in which lamellar bone formed as a result of bone remodeling around the implant is in close contact with the implant without any gaps [2]. HA surface coating is used for many titanium implants as a method of accelerating osseointegration even in fragile bone [17,20]. Reported drawbacks of the conventional HA surface coating include breakage of the coating layer that results in the detachment of the implant from the host bone [14]. As a solution for the coating layer failure, a sputtering process in which extremely thin surface coating of HA of approximately 1 μm thickness enables to prevent BII detachment while still providing bone conduction around the implant has recently been developed [18,19]. We hypothesized that a thin HA surface coating produced by the sputtering process would also enhance implant fixation strength even in osteoporotic fragile bones. The aim of this study is to investigate a mechanical and histological state of the BII of PSs with thin HA coating using in vivo porcine osteoporosis experimental models.

Materials and Methods:

Implant materials

Monoaxial PSs made of titanium alloy (Ti-6Al-4V, Showa Ika, Co Ltd. Toyohashi, Japan) of diameter 4.75 mm and length 30 mm were used. Sandblasted PSs (BLs) were produced by sandblasting these PSs to create microscopic irregularities on the titanium surface of 0.48–1.38 μm in size, and HA-coated PSs (HAs) were then produced by coating the surface of BLs with approximately 1 μm of HA by the sputtering process. The sputtering process followed that reported by Ozeki et al., comprising the application of a voltage between the BLs and HA powder in a vacuum into which argon gas has been introduced, causing the ionized argon gas to collide with the HA powder so that the ejected HA powder forms a thin film on the BLs [19]. Three different types of

PSs were used in this experiment: HAs, BLs, and standard PSs (STs) that had not undergone any surface treatment as controls (Figure 1).

The animal model

This study was approved by the Animal Experiment Committee at our institution (Experiment No. 0670). Eight 4-month-old female Clawn miniature pigs were used, 6 of which were used to generate an osteoporosis model following the method of Mosekidle et al. by feeding them a low-calcium diet (0.75% Ca) for 6 months from age 4 months, performing ovariectomy (OVX) at age 10 months, and feeding them a low-calcium diet for a further 6 months [15]. Two control miniature pigs were reared on a normal diet (0.90% Ca) for 6 months from age 4 months, underwent a laparotomic sham operation only at age 10 months, and were then reared for a further 6 months.

Surgical Procedure

PS placement surgery was performed at age 16 months. After ensuring an adequate level of anesthesia, the thoracic and lumbar spine was subperiosteally exposed via a midline posterior approach. The point of insertion and trajectory of PSs were determined with reference to the vertebral bone specimens obtained from the same species. The three types of screws (STs, BLs, and HAs) were inserted randomly into a total of 11 vertebrae between T10 and L6. In order to avoid screw loosening on the BII, all PSs were placed independently in each vertebra and not interconnected with rods (Figure 2). Postoperative X-rays were taken to verify the positions of PSs (Figure 3).

Postharvest Procedure

The osteoporosis model animals were also fed a low-calcium diet after PS insertion, and 1 animal each was euthanized and the spine harvested after 0, 2, 4, 8, 12, and 24 weeks. The spines of the control animals were similarly harvested after 0 and 24 weeks. The PSs harvested at these periods were referred to as the 0W, 2W, 4W, 8W, 12W, 24W, Control 0W, and Control 24W Group. After harvest of the spine, each vertebra was split vertically along a line connecting the center of the spinous process and the center of the vertebral body using an oscillating saw. Vertebrae sustained damage such as pedicle fractures and cortical breaches of PS were excluded. Vertebrae T10 and T11 were fixed in 10% formalin for histological investigation and a total of 9 vertebrae (T12-T14, L1–L6) were immediately frozen at –25ºC for biomechanical testing.

Measurement of bone mineral density

The 5 vertebrae (T5–9) were fixed in 70% ethanol immediately after harvesting. The bone mineral density (BMD) was measured using the peripheral quantitative computed tomography (pQCT) (XCT, Stratec GmbH, Pforzheim, Germany). The analytical region was an area of 4 x 6 mm in the central portion of the vertebral body. The average bone density in this area (mg/cm3) was determined.

Biomechanical Testing

The frozen specimens (T12-L6) were defrosted to room temperature. Using metallic rods and resin, each specimen was fastened to a jig such that the screws were perpendicular. Torsional screw extraction was performed using a

torque meter (PT-1950J, PROTEC, Kawasaki, Japan) at a uniform rate (360º /min) to a maximum excursion 1440º. The torque value and angular displacement were measured, and peak extraction torque (Nm) was defined as the peak torque value in the initial linear region of the torque–angular displacement curve.

Histologic analysis

On the cross-sectional surface passing the longitudinal axis of pedicle screw, a hard tissue polishing sample was prepared. After cutting the specimen and embedded with methyl methacrylate resin, the observation surface was polished with a micro grinding machine (BS-300CL, EXAKT, Hamburg Germany) for mirror polishing. The slices with 30-40μm thickness were stained with hematoxylin/eosin (HE) and toludine blue (TB) stains.

Histological evaluation of BII was carried out qualitatively and quantitatively. Qualitative evaluation was done on the TB stained sample in which woven bone was deeply stained [4]. For the quantitative evaluation of osseointegration around the pedicle screws, rectangular areas (1.0 mm x 1.1 mm) covering a valley and the adjacent threads were selected as a region of interest (ROI). Bone Volume/Tissue Volume (BV/TV) and Bone Surface/Implant Surface (BS/IS) were measured in each ROI (Figure 4) [21]. For measurement of BV/TV, image processing software, Image J (NIH, MD, USA) was used, and for measurement of BS/IS, image analysis software, Win ROOF (MITANI Co, Fukui, Japan) was used.

Statistical Analysis

For statistical analysis, the Tukey-Kramer HSD test and the Steel-Dwass test was used. The level of significance in cases with "significant differences among the groups" was defined as $p<0.05$. On the other hand, the level of significance in cases with "no significant differences among the groups" was defined as $p>0.20$.

Results:

Measurement of bone mineral density

All 8 experimental animals tolerated the surgery, with no major complication. Table 1 shows the BMD (pQCT) values for each individual. All members of the osteoporosis group had significantly lower BMDs than Control 24W Group.

Biomechanical testing

A total of 134 specimens were used for biomechanical testing and details were listed in Table 2. According to the peak extraction torques reported in Table 3, the mean value was significantly higher for HAs than for BLs and STs in 8W Group. In 12W and Control 24W Group, the value was significantly higher for HAs and BLs than for STs, and in 24W Group, the value tended to be higher for HAs than for STs with marginal significant difference (HA: $p = 0.076$). There is no regular pattern of progression of the extraction torques over time. The peak extraction torque observed having a limit value in this testing was around approximately 1.8 Nm, this was the estimated reaching plateau in 8W for HAs.

Histological evaluation

1. Qualitative analysis

In 0W Group, trabecular bone around the implant was disrupted and fragmented as a result of fracturing caused by screw insertion (Figure 5-a). In 2W Group, most of the fragmented bone had been resorbed in all samples (Figure 5-b). For HAs in 2W Group, some parts of the implant surface were observed covering with bone-lining cells comprising a single layer of osteoblasts, and some of the cells on the implant side formed woven bone (Figure 5-c), but such findings weren't observed at the surface of BLs and STs in 2W Group. The implant surface of HAs was covered with bone-lining cells comprising a single layer of osteoblasts, and some of the cells on the implant side formed woven bone (Figure 5-c). In 4W Group, newly developed lamellar bone was observed at the BII of HAs with direct contact between the bone and the implant (Figure 5-d), but no such increase in areas of bone substance was seen in regions adjacent to STs (Figure 5-e). In 24W Group, the formation of lamellar bone in close contact with HAs was apparent (Figure 6-a). There were few such areas with STs, and numerous soft tissue interpositions were present in the gaps between the bone and the implants (Figure 6-b). For BLs, although there was some formation of lamellar bone, this was far less than that for the HAs (Figure 6-c). At locations further away from the implants, fatty marrow conversion associated with osteoporosis was present in all of the samples, with no clear difference among the different types of screws.

2. Quantitative analysis

Quantitative evaluation was carried out for all samples other than 0W and Control 0W samples, in which fragmented bone by PS insertion was still present around the implants. BS/IS was significantly higher for HAs than for STs in 2W, 4W, and 12W Group. In 8W, 24W, and Control 24W Group, the value was significantly higher for HAs and BLs than for STs. In all of the samples, the value was the highest for HAs, and in 24W and Control 24W Group, it was also significantly higher for HAs than for BLs (Table 4). BV/TV was significantly higher for HAs than for STs in 4W Group only. There were no significant differences among the values of BV/TV for the three different types of screws in any of the other samples (Table 5).

Discussion:

Coating implant surfaces with HA, which possesses bone conductivity, is a useful method of achieving mechanical stability at the BII that requires no special techniques and is comparatively safe [17,20]. Several studies have addressed the use of HA-coated PSs as a means of enhancing the fixation of PSs in fragile bone. Aldini et al. carried out a 16-week experiment using an osteoporotic sheep model [1], and Hasegawa et al. carried out a 10-day experiment using a canine osteoporosis model [11], with both finding that HA-coated PSs provided advantage uncoated PSs. The present study is the first to investigate the value of HA-coated PSs at multiple time points and over a long period from week 0 to week 24 in an osteoporotic spine model.

The plasma spray technique is currently the most widely used method of HA surface coating. However, this technique produces a thick layer of 20-100 μm with internal defects and degradation of the HA [13], and reported clinical issues including breakage of the coating layer, bone-implant detachment, and inflammatory reaction [14]. Potential alternatives to the plasma spray method have been studied so far including flame spraying, thermal decomposition, and

sputtering. Ozeki et al. reported that a sputtering process enables the creation of a thin and precise HA surface coating only 1-μm thick, use of sputtering not only prevents breakage of the coating layer, but also speeds up the rate at which osseointegration is achieved [18,19]. This is the first study to investigate the value of PSs with thin HA coating by the sputtering process in osteoporotic spine.

The porcine osteoporosis model produced in this study significantly reduced BMD to 76–84% of the values in the control, suggesting that it is an adequate osteoporosis model for evaluating implant fixation in osteoporotic fragile bone. In the extraction torque tests in this osteoporosis model, after 4 weeks, the HA-coated PS tended to have a higher value than the standard PS; after 8 and 12 weeks, it was significantly higher, and this tendency was maintained until 24 weeks. This result showed that, even in fragile bone, PSs with thin HA coating showed enhanced integration at the BII after a waiting period of approximately 8 weeks, and that this integration at the BII was maintained until 24 weeks. On the other hand, the results of the extraction torque tests demonstrated no regular pattern of progression of the extraction torques over time. The peak extraction torque observed having a limit value in this testing around approximately 1.8 Nm, this may be due to fact that BII was surrounded by cancellous bone. If osteointegration of PS makes the BII stronger than the surrounding cancellous bone over time, the peak extraction torque reaches the plateau due to the occurrence of microfractures of surrounding cancellous bone.

Histologically, the BS/IS, indicating the ratio of close contact at the BII, was significantly higher for HA-coated PSs than for standard PSs after 2 weeks. Bonding between the bone and the implant surface was maintained until 24 weeks with no detachment of the coating layer or surrounding inflammation as shown in Figure 5-d and Figure 6-a. This histologic analysis supports the previous reports of thin HA coating by the sputtering process [18,19], and it conformed the results of the extraction torque tests. We also found out from the present study performed over multiple time points that the osteoconductivity of the HA coating shown on histological examination appeared some weeks earlier than the mechanical superiority of the HA-coated PSs in the extraction torque tests.

The blast-treated PSs showed higher peak extraction torque than did the standard PSs after 12 and 24 weeks. This result indicates that bone tissue invades the surface irregularities created by sandblasting, increasing the surface area of the BII and mechanically strengthening integration at this site, as described in previous studies [7]. In the histological evaluation, the blast-treated PSs showed a higher BS/IS ratio than the standard PSs after 8 and 24 weeks, and there was good formation of continuous lamellar bone in close contact with the PS, reflecting the previously reported fact that the irregular shape of the implant surface effectively increases protein adsorption and osteoblast adhesion [6]. A comparison of HA-coated and blast-treated PSs found that extraction torque tended to be higher for HA-coated PSs than for blast-treated PSs at 4 weeks and was significantly higher at 8 weeks, but that from 12 weeks onward, the value was similarly high for blast-treated PSs, and the significant difference disappeared. This result indicated that simply coating the surface of the PS with HA by the sputtering process, excluding the effect of its surface morphology, speeds up the rate at which osseointegration is achieved.

Other than after 4 weeks, there was no significant difference in the BV/TV ratio, which indicates the total volume of bone substance surrounding the PS, between any of the samples in the osteoporosis group. At 4 weeks, it was higher

for the HA-coated PSs than for the standard PSs owing to the effect of lamellar bone formation in close contact with the thread, but at locations further away from the thread, trabecular thinning was evident similar to that seen around the standard PSs as shown in Figure 5-d and Figure 5-e. This suggests that the bone conductivity of the thin-film HA coating was unable to overcome the effect of osteoporosis on the bone at locations not directly adjacent to the thread.

To prevent loosening of PSs inserted in osteoporotic fragile bone for clinical purposes, not only must the bone-implant interface be firmly anchored, but the quality of the bone surrounding the PS must be improved to avoid the occurrence of microfractures. The drugs currently used to treat osteoporosis, bisphosphonates, which inhibit bone resorption, and parathyroid hormone (PTH), which promotes bone formation, may help to increase the volume of bone substance around the PS and prevent fractures [16]. Using the PSs with thin HA-coating in conjunction with osteoporosis drugs may further improve PS fixation in fragile bone, but further studies will be required to verify this.

This study has some limitations. First, although this study investigated the value of HA-coated PSs at multiple time points in a porcine osteoporosis model, owing to the limited scale of the facility and bioethical considerations, we used only one animal to provide samples at each time point. In addition to the small sample size, individual differences between experimental animals may also have influenced the outcome. Second, we used only extraction torque tests in biomechanical testing of PSs in the study.In a 1998 study, Brancemark et al. stated that extraction torque tests are the best way of evaluating the interface mechanics between the implant and surrounding tissue, whereas pull-out tests are more strongly affected by the properties of the bone surrounding the implant rather than the interface [3]. Given the limited number of samples, we used only extraction torque tests to concentrate on the evaluation of the mechanical stability of the interface. Third, we didn't perform CT of the harvested spine after screw placement. Vertebrae sustained damage such as pedicle fractures and cortical breaches of PS were excluded macroscopically. The damage we couldn't detect may also have influenced the outcome of biomechanical testing. Finally, we performed stand-alone insertion of the PSs to eliminate the effect of spinal movement on BII after PS insertion. In clinical spinal instrumentation surgery, the PSs are connected by rods or plates, and spinal fusion and/or deformity correction surgery are also performed; thus, this experimental model did not fully reproduce the clinical situation. Further studies under conditions closer to actual clinical practice will be required before the PSs with thin HA coating can be brought into clinical use. The results of the present study suggest that stand-alone placement of PSs prior to definitive stabilization surgery may be possible alternative method of spinal reconstructive surgery for osteoporotic spine.

Conclusion:

In an in vivo experimental study using a porcine model of postmenopausal osteoporosis, PSs with thin HA coating by the sputtering process provided stronger fixation at the bone-screw interface where the screw and bone substance are in close contact, suggesting that they may have the potential to reduce the incidence of complications caused by loosening of PSs in spinal instrumentation surgery. However, the absence of increased bone mass around the screw remains an issue, improving bone quality by osteoporosis treatment may be necessary to prevent fractures around screws.

Disclosure:
We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Conflict of Interest:
Dr. Taneichi has received Speaker's Bureau from Century Medical Inc. No funding was provided for any portion of this research product. All other authors report no conflict of interest concerning the materials and methods used in this study or findings specified in this paper.

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Figure Legend:

Figure 1 Implant materials.
Three different types of PS were used in this experiment: standard PS (ST) that had not undergone any surface treatment as control, BL, and HA.

Figure 2 Surgical Procedure.
All PSs were placed independently in each vertebra and not interconnected with rods.

Figure 3 Postoperative X-rays were taken to verify the positions of pedicle screws.
a. AP view.
b. Lateral view.

Figure 4
a. Region of interest (ROI) (1.0mm×1.1mm)
b. Tissue volume (TV): The area except screw thread (horizontal line)
c. Bone volume (BV): The area of the bone (vertical line)
d. Implant surface (IS): The total length of the implant (band with horizontal line)
e. Bone surface (BS): The length of bone in contact with the implant (band with vertical line)

Figure 5 Qualitative analysis of bone-implant interface a. HAs in 0W Group: trabecular bone around the implant was disrupted and fragmented as a result of fracturing caused by screw insertion.
b. HAs in 2W Group: most of the fragmented bone had been resorbed.
c. HAs in 2W Group (TB staining): the implant surface was covered with bone-lining cells and some of the cells formed woven bone.
d. HAs in 4W Group: newly developed lamellar bone was observed at the BII with direct contact between the bone and the implant.
e. STs in 4W Group: no increase in areas of bone substance was evident in regions adjacent to the implant

Figure 6 Qualitative analysis of bone-implant interface in 24weeks
a. HAs: the formation of lamellar bone in close contact with the implant was apparent.
b. STs: numerous soft tissue interpositions were present in the gaps between the bone and the implants.
c. BLs: there was some formation of lamellar bone.
At locations further away from the implants, fatty marrow conversion associated with osteoporosis was present in all of the samples, with no clear difference among the different types of screws