EFFECT OF HA-COATING AND HF ETCHING ON EXPEREMENTAL ZIRCONIA IMPLANT EVALUATION USING IN VIVO RABBIT MODEL

by

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INTRODUCTION

Dental implants have been used for replacing missing teeth and rehabilitating fully edentulous patients for decades. From the first machined surface root-form titanium implant introduced in the 1970s[1], newer designs and materials used on the implants have been developed throughout the years. Of more than 400 different implant systems on the market, the screw type titanium implants with various surface treatments are by far the most commonly used clinical design. Titanium implants are made from either commercially pure titanium (cpTi) or titanium alloys. Titanium alloys are mainly composed of Ti6Al4V. Both titanium materials exhibit a characteristic grayish color, which may sometimes result in an unpleasant esthetic outcome. Newer materials have been investigated to replace titanium for dental implants. Zirconia is a material which exhibits good biocompatibility and has been evaluated as a replacement for titanium. A previous study conducted by our group[2] demonstrated that machine-surfaced zirconia implants showed similar biomaterial property as machine-surfaced commercially pure (cp) titanium implants, both in bone-to-implant contact (BIC) and bone area (BA) surface between the threads. Additional surface modifications are being utilized on implant surfaces currently in clinical use which can enhance osseointegration of dental implants. We would like to utilize two of these surface modification techniques, hydroxyapatite (HA) and hydroflouric acid (HF) treatment, on zirconia implants and compare the result with commercially pure titanium implants treated in the same fashion.

PURPOSE OF THE STUDY

The objective of this study was to evaluate the *in vivo* performance of two different surface modifications on zirconia implants and compare them to commercially pure titanium implants treated with the same modifications. This study will be a pilot study

examining the osseointegration of zirconia implants treated with either HA or HF. The zirconia implants will be compared with cpTi implants treated in the same manner. We would like to compare the data with our previous study: zirconia and cpTi implant without any surface modification. A rabbit tibia model will be utilized for this study. Although animal tibia cannot fully represent the condition in a human oral cavity, we would like to collect preliminary data of these two surface modified zirconia implants for future investigations.

NULL HYPOTHESIS

1. Surface treatments on zirconia using HA-coating and HF etching do not increase the BIC and RT when compared to ZrO implant without surface modification (will be compared to the data from Dr. Daniel Shin's study).
2. There is no difference in BIC and RT in HA-coated and HF etched zirconia implants compared to Ti implants receiving the same surface treatments.

ALTERNATIVE HYPOTHESIS

We hypothesize that, when tested in the rabbit tibial model for six weeks, the HA-coated and HF-etched zirconia implants will show higher BIC and RT compared to the results we previously obtained for noncoated zirconia implants.

REVIEW OF LITERATURE

Titanium has been used in the dental implant industry for the past 40 years since Dr. Brånemark first introduced titanium dental implants[1]. Titanium implants can be divided into commercially pure titanium (cpTi) and titanium alloys. Titanium alloys are mainly composed of Ti6Al4V. Some important characteristics of titanium permitted it to be widely used as the choice of biomaterial for dental implants. Titanium is biocompatibile, nontoxic and nonallergenic[3]. Nevertheless, several drawbacks have been mentioned in the literature for using titanium as dental implants. The high concentration of titanium detected in surrounding tissue suggested that titanium particles can be released by fretting and wear debris from the implant surface. Weingart *et al.*[4] cautioned about the potential for spreading these particles into adjacent lymph nodes, which may cause further immunologic reactions in living beings. However, there have been no cases of local or systemic reactions to titanium reported. Another important potential drawback of titanium is that the unwanted grayish color of the implant sometimes can show through thin gingival or mucosal tissue around the cervical area of the implant restoration. If gingival recession develops after implant placement or restoration, the unpleasant color of the titanium fixture will become visible[5-6]. This potential drawback may jeopardize the esthetic outcome for implants placed in the esthetic zone and thus researchers have been searching for an alternative biomaterial with better esthetic outcomes.

Recently, attention has been focused on zirconia (ZrO) ceramic as a suitable implant material due to its high modulus of elasticity, high mechanical strength, minimal reaction (biocompatibility) and better color mimicking of the natural tooth[7-12]. In a finite element analysis, Kohal *et al.*[12] demonstrated that the magnitude and stress distribution were similar

between yttrium-partially stabilized zirconia implants and commercially pure titanium (cpTi) implants. Both cpTi and zirconia implants showed favorable and nondestructive stress distribution after loading. In a previous study from our group[2], comparing zirconia (ZrO) and cpTi threaded implants with machined smooth surfaces, we found no statistically significant differences between the ZrO and cpTi implant groups in terms of bone-to-implant contact (BIC) and removal torque (RT). The amount of mineralized surface (MS; bone area/BA) at the four-most coronal threads showed statistically significant differences between the two groups and the ZrO implant group was preferred.

Implant surface modification:

Titanium implants with a machined surface, such as the ones tested in our previous study, however, they are no longer commonly used in clinical practice. Most current commercial titanium implants include various surface treatments to facilitate and enhance osseointegration rates. For example, increasing surface roughness has been proposed to increase the rate of osseointegration and biomechanical fixation of titanium implants[13-14]. Modifications of dental implant surface microstructure can be achieved with either additive or subtractive methods. The additive methods include titanium plasma-spraying (TPS), plasma-sprayed hydroxyapatite (HA) coating and biomimetic CaP coating; the subtractive methods include blasting with ceramic particles, acid-etching and anodization[15]. In terms of the mechanisms, the roughness of the titanium implants was considered to be one of the parameters that affect the rate and quality of osseointegration[15]. With two different surface treatment modalities to create different topographies on zirconia implants, Sennerby *et al.*[16] demonstrated significantly higher (4-5 times) removal torque in the treated groups

compared to the group that had non-treated zirconia implants. Research pertaining to the two most common surface modifications, HA coating and acid etching, are reviewed in the following sections.

Hydroxyapatite (HA):

HA has a chemical formula $Ca_{10}[PO4]_6[OH]_2$ and is also known as calcium hydroxide phosphate[17]. Biological HA has a 1.67 Ca/P ratio[18] and is close to the Ca/P ratio of human enamel (Ca/P =1.58) and bone[19]. About 67% of the mineralized bone content is composed of HA, and thus HA was thought to be a suitable material to stimulate bone healing and to improve the rate of osseointegration in the initial stage following implantation[19-27]. Also with the plasma-sprayed HA coating, the surface roughness of machined and polished surface cpTi implants can be increased from 0.22± 0.01μm to 1.06± 0.24μm[15]. Although several techniques were proposed to adhere HA to titanium implants[19], only the plasma-spraying coating technique has been successfully used on commercial implants[15]. This plasma spray technique leaves a thickness of HA greater than 30μm on the surface[28]. Due to its advantages, the use of biological HA as an implant surface coating thus became popular. In a recent study by Le Guehennec *et al.*[29], the BIC of large-grit, sand blasting and acid-etched (SLA) implants were similar to the octacalcium phosphate (a form of calcium phosphate different from HA in Ca/P proportion) implants, and both of them were significantly greater than that from a grit-blasted surface. Despite the high bonding strength between the HA and surrounding bone, several problems arose when HA coated implants were used clinically[30-31]. The biodegradability of HA was known to be high after 14-15 years of implant loading[32-33]. HA degradation is intimately linked to the stability of the coating which may be critical to the implant success rate. The HA coatings are resorbed in

body fluids by ion exchange and/or degraded by osteoclasts and osteoconductive processes[34-37]. During the resorption process, the de-lamination of the HA coating from the implant surface was often reported. The bonding between the plasma-sprayed HA coating and underlying metal appears to be mechanical, thus a rough underlying surface is needed to increase the bonding strength of the HA[38]. On the other hand, several long-term clinical reports have shown that HA-coated implants revealed good osseointegration after 14 years of loading, even when HA resorption occurs. Iezzi *et al.*[33] retrieved two HA-coated implants with the surrounding bone *en bloc,* in which the implants were removed due to a prosthetic component fracture. Complete resorption of the HA coating was found in most of the Ti implant surface (46% and 68% for the two implants, respectively) and the percentage of BIC for the HA-coated surface was 35% and 13% for the two implants. Adding the BIC of the bone-titanium surface, implant 1 yielded a total BIC of 60% and implant 2 yielded a total BIC of 28%. Even with the aforementioned negative effect of the plasma-sprayed HA coating, meta-analysis reviews by Lee *et al.*[30] and Esposito *et al.*[39] revealed that the success rate of such implants were not inferior to others. In order to exploit the benefit of HA, new techniques including PLD (plused laser deposition, a physical vapor deposition technique), sputter coating, IBAD (Ion beam assisted deposition, a vacuum deposition technique), ESD (electrostatic srpay deposition, generation of an aerosol out of organic solvents containing CaP) and biomimetic deposition have recently been studied to deposit CaP coatings onto titanium implant surfaces. With the newer techniques applied, a thinner CaP coating layer can be formed on the implant surface and can solve the problems that were associated with a thicker CaP coating. With the animal

studies using these techniques, the CaP-coated implants showed a favorable bone response when compared to non-coated implants[40].

Acid etching:

Etching the implant surface with strong acid such as HCL, H_2SO_4, HNO_3 and HF has also been commonly used for commercial implants, in order to increase surface roughness. The acids create porosity on the implant surface with a size ranging from 0.5 to 2 μm in diameter[41-42]. Acid-etched surfaces were reported to increase cell adhesion and bone formation, thus enhancing the osseointegration[43-46]. HF has been routinely used for etching restorative ceramics to increase bonding surface due to its dissolution ability[47-48]. However, the effect of HF on zirconia seems to be limited[49-57]. Due to the high crystalline content of zirconia material, hydrofluoric acid etching resulted in an unsatisfactory resin bond to zirconia[58]. The authors investigated the topography of a 9.5% HF treated zirconia ceramic surface and reported the surface roughness(Ra) for HF-treated group was 5.23±0.9 nm which was still considered to be as smooth as the non-treated zirconia ceramic (6.94± 1.3 nm). In an *in vitro* study by Della Bona *et al.*[59], the authors used 9.5% HF to treat alumina/zirconia ceramic for 90 seconds and used scanning electron microscopy (SEM), backscattered imaging (BSI), electron dispersive spectroscopy (EDS) and stereology to quantify and qualify the characteristics of the surface microstructure. The surface roughness (Ra) for the HF-etching zirconia ceramic surface yielded 231 ± 14nm and the peak-to-valley difference (Rt) was calculated to be 24.2 ± 3.1μm. Although the evidence showed no advantage of using HF to increase bonding strength on zirconia ceramics, there has been no study evaluating the BIC, RT and osseointegration of HF treated zirconia implants.

Recently Dr. Ito at Matsumoto University developed a method to coat the zirconia implant with hydroxyapatite using a sol-gel process. The purity of the HA coating was confirmed by x-ray diffraction. He also modified the zirconia implant surface using HF etching. Both surface treatments have the potential to enhance the bone-to-implant response in these implants. In this project, we seek to evaluate the effects of these surface treatments on zirconia implants in terms of the bone-to-implant contact and removal torque using a rabbit tibial implantation model. These experimental groups will be compared to the titanium control groups that receive the same surface modification. In total, four groups will be tested: HA-coated Ti, HA-coated zirconia, HF etched Ti and HF etched zirconia. The results will also be compared to those of our prior study[2] using Ti and ZrO implants without any surface modifications.

MATERIALS AND METHODS

ANIMALS

Twelve healthy New Zealand white rabbits (Myrtle's Rabbitry, Inc., Thompson Station, TN) weighing between 3.0 kg to 3.5 kg were utilized in this 6-week *in vivo* implant investigation. Prior to the surgery, the rabbits were housed in a standard cage and allowed to acclimate to their environment for a period of at least 7 days so as to ensure their health and stability. *En bloc* samples were obtained at the end of the study for histological, histomorphometric, and mechanical testing. The Institutional Animal Care and Use Committee (IACUC) at Indiana University, Indianapolis, Indiana, USA approved the study (Indiana University IACUC approval DS0000886).

IMPLANTS

A total of four different implant types were tested in this study. Threaded zirconia implants with HA coating (Test 1) and zirconia implants with an HF-treated surface (Test 2) were used and compared against the same size of titanium implants treated in identical fashion (control 1 and control 2). All implants measured 3.5 mm at the thread diameter and 7.0 mm in total length. All of implants were provided by Matsumoto Dental University. In each animal, two test implants (either test 1 or test 2) and two control implants (either control 1 or control 2) were randomly assigned to the right and left medial tibial diaphysis. For each different type of implants, twelve implants were tested in total.

SUGICAL PROCEDURE AND IMPLANT INSERTION

The surgeries were all performed under aseptic conditions. Antibiotic prophylaxis (Baytril 4 mg/kg SC, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, KS) was administered to all animals prior to the surgery. Initial sedation and induction was performed with a combination of Acepromazine/Torbugesic (Ace 0.6 g/kg + Torb 0.75

mg/kg) and maintained by Isoflurane (3.5% at 1.5 l/m) via gas mask until completion of the surgical procedure. Vital signs were monitored throughout the surgery. Towels and a heating blanket were used to maintain the intra-operative temperature of the animals. Following onset of anesthesia, the lower limbs of the animals were shaved, washed, and prepped with a combination of betadine (povidone-iodine; PVPI) and alcohol, and covered underneath surgical drapes. The medial tibial diahyses were exposed bilaterally via a 4-5 cm incision line at the lateral tibial diaphysis area through the skin and fascia. The size of the implants used in this study was approximately 20-30% of the diameter of the tibia and the implants were placed approximately 11 mm apart from each other. The superior implant placement was at least 10 mm away from the head of the tibia. Two zirconia implants and two titanium implants with the same surface treatment (either HA coating or HF etched) were randomly assigned to each side. The osteotomy was performed starting with a 2.0 mm round bur and continued with twist drills consecutively. The osteotomy site was prepared to a diameter of 3.5mm with copious of 0.9% saline irrigation. All implants were sterilized by autoclave prior to the surgery. The implants were then inserted into the tibia with only the implant shoulder exposed. The incision wound was closed with 5-0 Vicryl sutures (polyglactin 910, Ethicon, Inc. (Johnson & Johnson Company), Somerville, NJ) where the fascia and skin were sutured in separate layers. Primary closure was obtained for all wounds. The same procedure was repeated on the contralateral side.

POST-SURGICAL PROCEDURES

Post-surgical recovery was monitored for any possible complications. All animals were placed on postoperative antibiotics (Baytril 4 mg/kg SC for 4 days) and a postoperative analgesic (Meloxicam 0.2 mg/kg SC once daily for 2 days). The animals

were allowed full weight-bearing activity, water, and rabbit chow (Ralston Purina, Richmond, Indiana) *ad libitum*. Animals were checked daily until the surgical wound totally healed. Body temperature, hydration status, activity, food and water consumption, and conditions of operative sites were examined. Alizarin red, calcein green and xylenol orange (Sigma, St. Louis, MO) were injected at 20 mg/kg, 10 mg/kg and 10 mg/kg through intramuscular injection at 3 weeks, 2 weeks and 1 week prior to sacrifice, respectively.

Six weeks following implant placement, the animals were euthanized with an overdose of pentobarbitalum (Solfoton, ECR Pharmaceuticals, Richmond, VA) and natricum (Aventis Pharmaceuticals, Inc., Bridgewater, NJ) intravenously; lung puncture was performed in order to insure their death.

Implants were removed en bloc with the surrounding bone. A total of 48 samples (12 implants for each test and control groups) were obtained. Upon retrieval, 24 specimens (6 samples for each group) were embedded undecalcified in PMMA (poly methyl methacrylate), stained with toluidine blue and examined under histomorphometry to quantify the implant-to-bone contact. Another 24 samples were kept in 0.9 % saline and evaluated using removal torque analysis to assess the strength of the implant-to-bone interface.

SPECIMEN RETRIEVAL AND HISTOLOGICAL PREPARATION

Implants were removed *en bloc* with the surrounding bone at 6 weeks after implantation. A total of 48 samples (12 implants for each test and control group) were obtained. Upon retrieval, 24 specimens (6 samples for each group) were fixed and dehydrated for 48 hours in 90-percent ethanol solution and then embedded undecalcified in PMMA to be used for histomorphometry to quantify the bone-to-implant contact. Another

24 samples were kept in 0.9% saline and were evaluated using removal torque analysis to assess the strength of the bone-to-implant interface. The PMMA embedded specimens were then sectioned. For the titanium implants groups, about 50-80 μm thick sections were cut parallel to the long axis of the implant using a rotating microtome. For the zirconia groups, the specimens were first cut to 500 μm thick then glued to a plastic slide at one side. The whole sections were then grounded close to 100 μm later on. One slide from each sample was placed on glass slides (for the Ti groups) and stained with toluidine blue (Sigma).

HISTOLOGICAL AND HISTOMORPHOMETRIC ANALYSIS

The stained slices were photograghed at 5x, 10x and 20x and then evaluated at 10x and 20x using an automated histomorphometry system (Bioquant, Nashville, TN) under a microscope (Nikon Eclipse 50i, Tokyo, Japan). Total thread length and the length in direct contact with bone were measured at the first 3 threads (both on the right and left side of the implant in the tibia, i.e. all threads were measured on the ground sections). BIC were obtained by dividing the total thread length by the length in direct contact with bone from the platform to the third thread.

The left unstained sections from each sample were examined with ultraviolet illumination light for fluorochrome analysis under the fluorescent light microscopy. Osteons with all three clear fluorescent markers were photographed and measured at 20x. Mineral apposition rate (MAR) was calculated by measuring the distance between the edges of xylenol orange, calcein green and alizarin red then divided by the number of days (7 days). The mineral apposition rate was calculated in μm/day units. If all three clear

fluorescent markers could be found at the inferior border of the tibia, the distance between the edges of each marker were measured and the MAR were calculated as previously described.

REMOVAL TORQUE TEST

Twenty-four specimens were used to examine the mechanical properties of the implants-bone contact and were stored in saline at 4°C until testing. RTQ (in N-cm) was measured on an axis-alignment table. The peak removal torque was measured with a digital torque gauge (Chatillon®, Ametex Inc. FL, USA). The machine was connected to a computer and the results were recorded using a measurement software (Nexygen DF, Ametex Inc). The calluses formed around the implant shoulder area were removed prior to the testing with carbide bur and high speed hand piece. The implant shoulders were secured by the beaks of the machine during the measurement. The peak removal torque force was registered when a sudden drop (>20%) in the torque resistance was detected by the digital torque gauge.

VARIATION BETWEEN MEASUREMENTS

The measurement of the BIC and bone area (BA) under the light microscope and the Bioquant system were done two times for the same specimen in the same day. A third measurement was done on the same specimen about 1 week following the 1st and 2nd measurements to evaluate the variation between measurements. Variation was evaluated from the standard deviation of the three measurements in the same sample at three time points.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Systat program (version 12). The bone-to-implant contact ratios, bone area and maximum removal torque were analyzed using two-way ANOVA with implant type and surface treatment type as the two main effects. The comparison of the data of the zirconia implant groups in this study and Shin *et al.* was analyzed using t-test while the comparison of the data of the titanium implant groups between the two studies was analyzed using one-way ANOVA with surface treatment as the main effect. A Tukey post-hoc test was used to compare between groups. Differences were considered significant at $p < 0.05$.

RESULTS

IMPLANT CHARACTERISTICS

Both the ZrO-HA and Ti-HA implants were coated under the same condition by Matsumoto Dental University. The average dimensions of the implants provided were showed in Table 1 & Figure 1. No data was provided on the thickness of the HA by Matsumoto Dental University. However, the SEM observation shows that the HA-coatings were predominantly in the grooves of the threads (Figure 2a and 2c). The average thread diameter for all four groups ranged from 3.10± 0.07 mm to 3.56± 0.03 mm with the Ti-HF group having the narrowest diameter.

The data of the surface roughness of the HF-etched implants showed that there is no significant difference between the two HF-etched groups (Table 2 and Diagram 1). The surface roughness (Ra) decreased from 0.35± 0.03 μm to 0.30± 0.03 μm for the ZrO group but increased from 0.28± 0.01 μm to 0.31± 0.03 μm for the Ti group.

CLINICAL OBSERVATIONS

All rabbits showed uneventful healing throughout the 6-week experimental time period. No sign of infection or inflammation around the implants was found during the 6 weeks study period.

HISTOLOGY

At gross examination of the light microscopic toluidine blue sections, callus formation was found in close proximity to the implant at the most coronal portion. Callus was also found continuing to extend to the thread spaces (Figures 3) to fill the gap between the osteotomy in the cortical bone and the implant surface. The new forming bone could be

distinguished from the original cortical bone by its woven appearance different from compact laminate original cortical bone. The toluidine blue staining caused the newer forming calcified structure (bone) to stain in blue compared to a more pinkish staining original cortical bone (Figure 4). For some specimens, soft tissue down growth could be found between the original cortical bone and the implant surface (Figure 5). In a majority of the sections in the Ti-HF group, separation of the bone with the implant was observed which left a gap between the bone and the implant surface. The separation and gap was due to the cutting/grinding procedure, thus the bone-to-implant contact and bone area measurement by using the outline of the PMMA which embedded the tissue was still viable. One of the specimens in this group lost the implant and the bone tissue adjacent to the implant during processing, thus it was excluded from the study.

For the Ti-HA group, separation of the HA coating could be seen between the threads (Figure 6). No foreign body reaction was associated with the HA particles, however, which appeared to be detached from the coating. The separated HA particles accumulated mostly in the valley of the thread with few particles congregated at the peak of the tread. For those slides which were found to have gaps between the bone and the implant surface, the separation gap was mostly found between the implant and the HA-coating (Figure 7). The measurement of BIC and bone area was still viable for this group using the method mentioned above. Among the 6 slides in this group, one of the slide showed decreasing bone density and unorganized bone structure in the original cortical bone area. Soft tissue down growth was found in this slide and extended to the first 2 threads on both sides of the implant. It was hypothesized that the rabbit might have some osteo-dysformative or

osteoporotic condition which led to minimum bone formation seen around the implant (Figure 5).

In the ZrO-HF group, separation between the bone and the implant was not commonly seen unlike that in the Ti-HF group. Within one of the slides in this group, debris from the processing procedure was found between the 2nd and 3rd thread space. The debris superimposed the underlying specimen which made the measurement impossible in this thread space.

The measurement of BIC and bone area in the ZrO-HA group was not possible due to the debris found in the majority of the specimens (Figure 8). Upon the discovery of the debris between the thread spaces in this group of slides, the slides were sent for further treatment to remove the debris. The special treatment included soap, water and soaking in 70% ethanol followed by some judicious cleaning with a pick while viewing the slide under a stereomicroscope. The slides were rechecked under light microscopy immediately after each treatment and the debris was still presented after the toluidine blue staining. It was suspected that the debris was between the specimen and the underlying plastic slide and this might be caused during the cutting and gluing procedures. No attempt was made to remove the implant specimen for further treatment due to the thin nature of the prepared slide.

VARIATION BETWEEN MEASUREMENTS

The variation ranged from 0.65% to 13.99% for the BIC in the Ti implant group, from 0.22% to 5.07% for the BA in the Ti implant group. The variation ranges were 0.67% to 8.78% and 0.06% to 1.88% for BIC and BA measurement in the ZrO implant group,

respectively. All measurements show standard deviation of less than 10% in the means of the three measurements done at three time points in the same sample. The mean values for all measurements in the following represent the average of all three measurements for all samples in each group.

BONE TO IMPLANT CONTACT

Results of the bone to implant contact analysis are shown in Table 3. The BIC for Ti-HA in the first three threads was approximately 57.78 ±18.22 % when all six samples were included. When the suspected osteoporotic sample was excluded, the BIC increased to 63.69 ±12.4%. The Ti-HF group demonstrated a mean BIC of 46.41 ±14.58%. The BIC for the ZrO-HA group was unable to be measured due to the debris contamination. The BIC for the ZrO-HF group was approximately 47.41 ±14.05% when excluding the slide with contamination at the left side. When including the data of the right side of the contaminated slide, the BIC turned out to be 47.59 ±12.58%. The change between including and excluding the sample was very minimal, indicating the consistency between data. Analyzing using two-way ANOVA, there were no statistical differences ($p>0.05$) between the Ti-HA, Ti-HF and ZrO-HF groups. However, statistically significant difference was found between the Ti-HA group and the smooth surface Ti group from Shin *et al*.. A statistically significant difference was also found between the Ti-HF group and the smooth surface Ti group.

BONE AREA

Results of the bone area (BA) analysis are shown in Table 3. The BA for Ti-HA was approximately 64.85 ±19.7 % when all samples were included. When the suspected

osteoporotic sample was excluded, the BA increased to 72.57 ±6.24%. The Ti-HF group demonstrated a mean BA of 74.91 ±4.18%. The BA for the ZrO-HA group was unable to be measured due to the debris contamination mentioned above. The BA for the ZrO-HF group was approximately 79.99 ±4.31% when excluding the slide which was also found to have debris contamination at the left side. When including the data of the right side of that contaminated slide, the BA turned out to be 78.97 ±4.6%. %. The change between including and excluding the sample was very minimal, indicating the consistency between data. Analyzing using one way ANOVA, there was statistical difference ($p<0.05$) between the Ti-HA and ZrO-HF groups, with ZrO-HF at statistically significant higher BA than Ti-HA. No other differences were detected.

MINERAL APPOSITION RATE

The fluorescent markers of the osteons and periosteum are demonstrated in Figures 9 and 10. The results of the mineral apposition rate analysis (MAR) are shown in Tables 4 and 5. The average MAR for the osteons in the Ti-HA group demonstrated 7.68 ±1.45 μm/day in week 4 and 7.41± 0.93μm/day in week 5. The average osteon MAR for the TI-HF group were approximately 10.98 ±1.89 μm/day in week 4 and 7.75 ±1.87 μm/day in week 5. The average MAR for the osteons in the ZrO-HA group demonstrated 10.08 ±1.25 μm/day in week 4 and 8.13± 1.73μm/day in week 5. The average osteon MAR for the ZrO-HF group were approximately 11.26 ±1.58 μm/day in week 4 and 6.88 ±1.33 μm/day in week 5. The average periosteum MAR for the Ti-HA group were 11.96 ±6.08 μm/day in week 4 and 12.27± 3.81μm/day in week 5; they were 9.61 ±1.21 μm/day and 8.24 ±1.02 μm/day for the Ti-HF group, respectively. The average periosteum MAR for the ZrO-HA group were

9.62 ±4.65 μm/day in week 4 and 10.32± 2.64μm/day in week 5; they were 11.15 ±3.36 μm/day and 10.9 ±3.0 μm/day for the ZrO-HF group, respectively. Analyzing the data using two-way ANOVA, there were not statistical differences in the MAR in the periosteum among all groups. However, there were statistically significant differences in the MAR measured in the osteons from the Ti-HF and the ZrO-HF groups. In both groups, there was statistically significant higher MAR at 4 weeks than at 5 weeks, indicating faster bone forming rate at 4 weeks, then slowed down at 5 weeks. The same observation was not found in either the Ti-HA or the ZrO-HA group. The results seem to indicate the effect on the HF-etched surface on the MAR in osteons next to the implants. However, more study is needed to clarify the actual mechanism that led to our observation in this study.

REMOVAL TORQUE TEST

The peak removal force for all four groups is shown in Table 3. The peak removal force for the Ti-HA group ranged from 12.50 Ncm to 44.34 Ncm. Within this group, only three measurements were obtained while the other three failed to show any result on the measurement software. The peak RT for the Ti-HF group ranged from 21.22 Ncm to 27.39 Ncm with an average RT peak force of 24.39 Ncm. All measurements in this group were obtained without difficulties. Fracture of the implant head was noted in five out of six samples in the ZrO-HA group. The only peak RT measurement was noted to be 12.83 Ncm in this group. In the ZrO-HF group, one peak RT measurement went to 37.21 Ncm but the implant head sheared after removal was observed. Two implant head fractures were noted in this group and one implant showed no result on the measurement software during testing. The other two measurements were 6.32 Ncm and 10.46 Ncm. The photos of implant head fractures are shown in Figure 11. Compared to the data of machined-surface Ti implant

done by Shin *et al.*[2], there was a statistically significant increase in the removal torque in the Ti-HF group ($p<0.05$).

FIGURES AND TABLES

FIGURE 1. Picture showing the dimension and measurement of the implant. All implants have identical thread spacing
A: the body diameter of the implant screw.
B: the outer diameter of the implant screw

Average±SD	ZrO	ZrO-HA	ZrO-HF	Ti	Ti-HA	Ti-HF
A (mm)	2.85±0.02	3.05±0.02	2.84±0.06	2.82±0.01	3.06±0.04	2.70±0.02
B (mm)	3.54±0.05	3.48±0.04	3.55±0.00	3.54±0.03	3.56±0.03	3.10±0.07

TABLE 1. Average dimension of each different implant groups. A indicates the body diameter of the implant screw. B indicates the outer diameter of the implant screw.

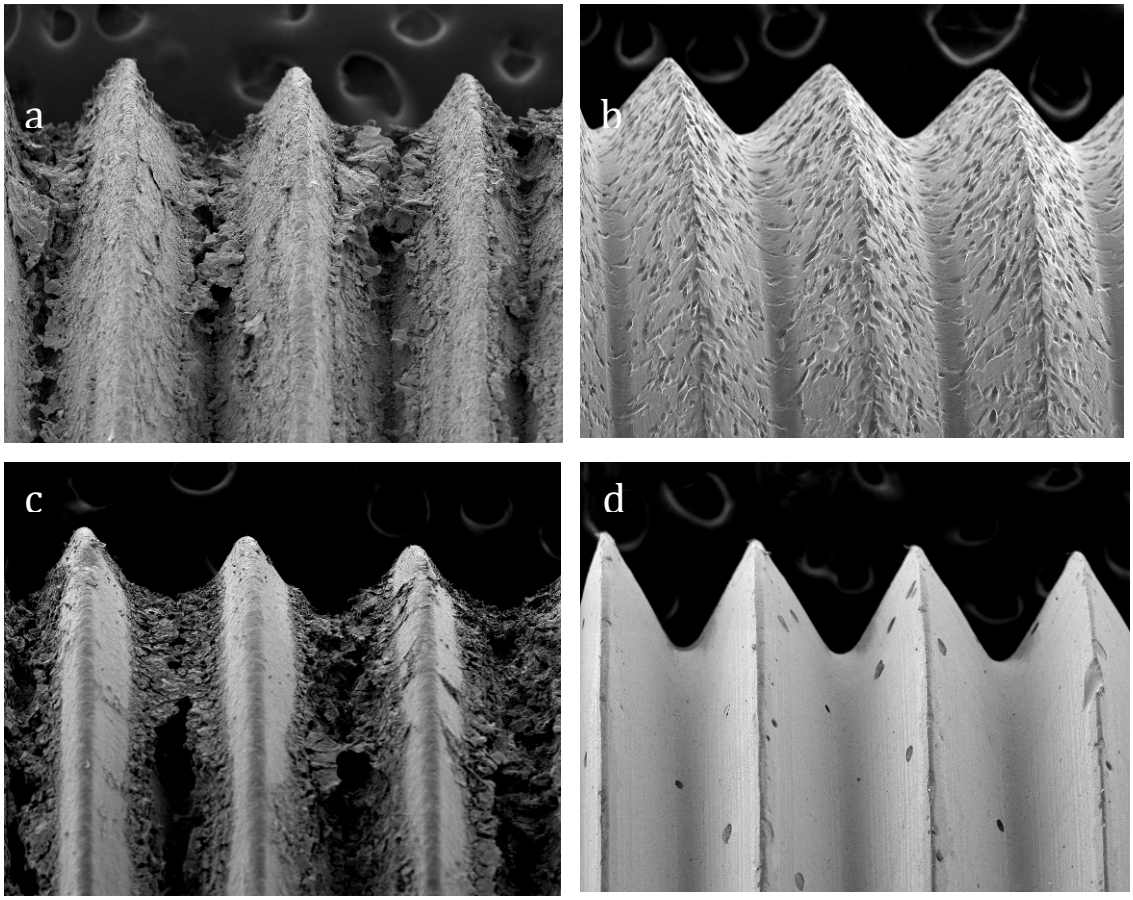

FIGURE 2. SEM images of studied implants at X100 magnification. a. Ti-HA; b. Ti-HF; c. ZrO-HA; d. ZrO-HF. Noted the HA particles were found mostly in the groove of the thread (valley) in a and c.

Ra	AS	Etching
Ti	0.28±0.01	0.31±0.03
ZrO2	0.35±0.03	0.3±0.03

TABLE 2. The surface roughness (Ra, in μm) of the HF-etched implants provided by the manufacturer. Values displayed as mean ± standard deviation

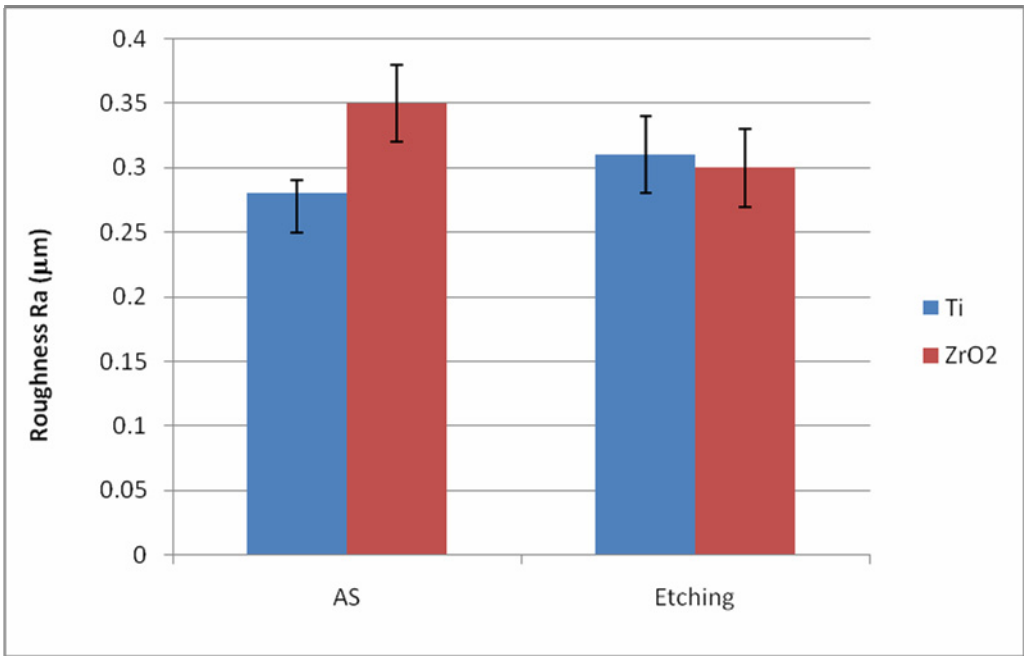

DIAGRAM 1. Chart showing the comparison of the roughness (Ra) between the non-etched implant surface (AS) and the etched implant surface.

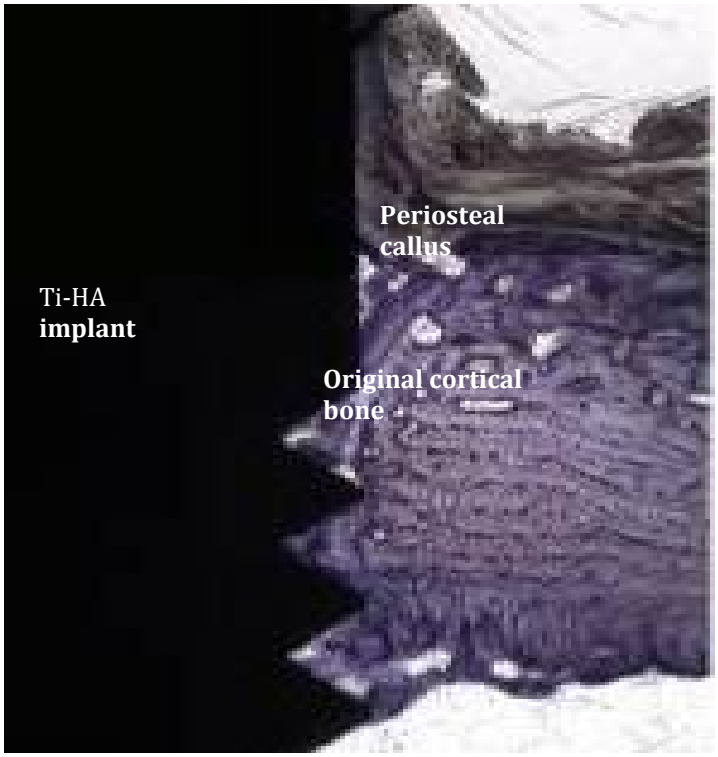

FIGURE 3. Picture showing periosteal callus overgrowth on Ti HA-coated implant under 2.5X magnification. No signs of inflammation or soft tissue encapsulation around implants.

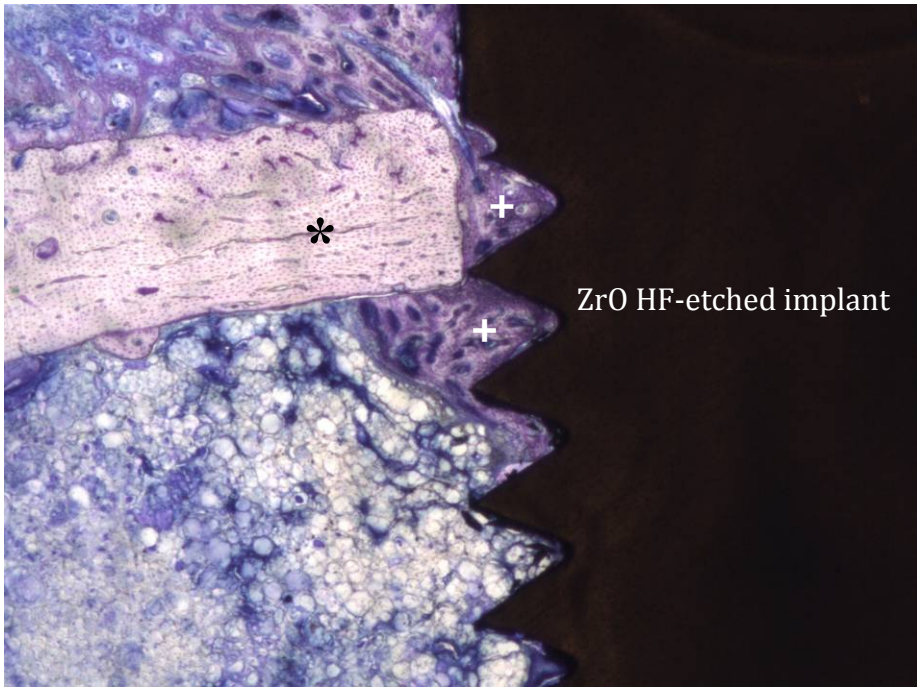

FIGURE 4. Picture showing original cortical bone (*, pink) and new bone layer (+, blue) formed between implant and the original cortical bone. ZrO HF-etched implant at 2.5X magnification.

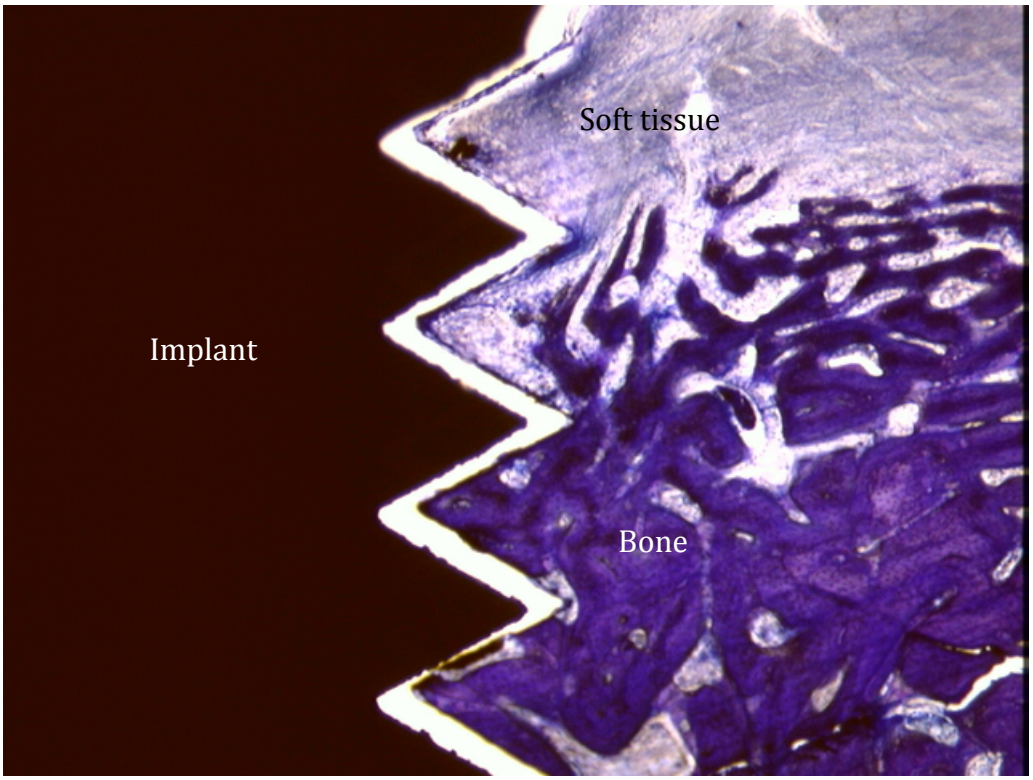

FIGURE 5. Picture showing soft tissue down growth in between the Ti HA-coated implant and original cortical bone under 2.5X magnification. Gap was found between the bone and the implant. The gap is expected to have formed during the histological preparation process. Gap existing before the histology process would have plastic filled in the gap.

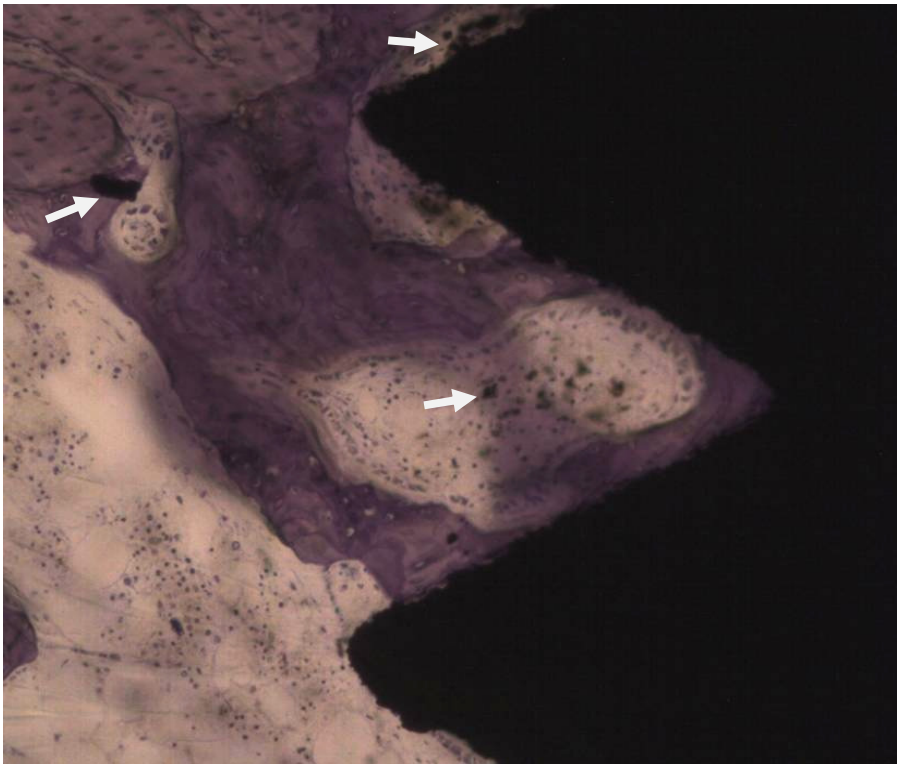

FIGURE 6. Picture showing separated HA particles (white arrows) into the tissue surrounding the Ti HA-coated implant at 10X magnification

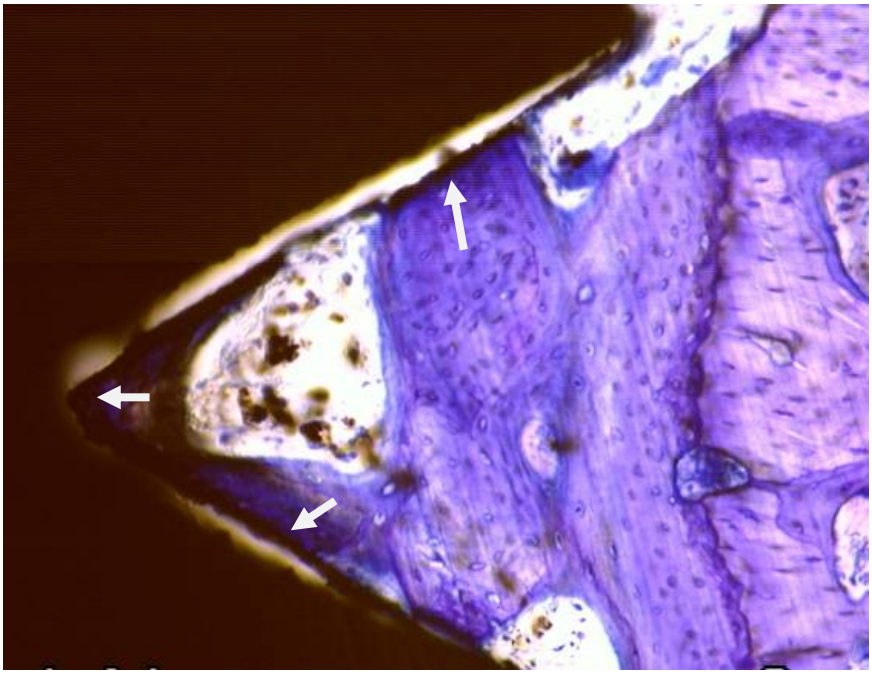

FIGURE 7. Picture showing the gap observed in between the implant and the HA coating. Arrows indicate the HA particles separated from the implant surface. HA particle can be found scattering in the surrounding tissue without sign of infection or inflammation. HA-coated Ti implant at 20X magnification.

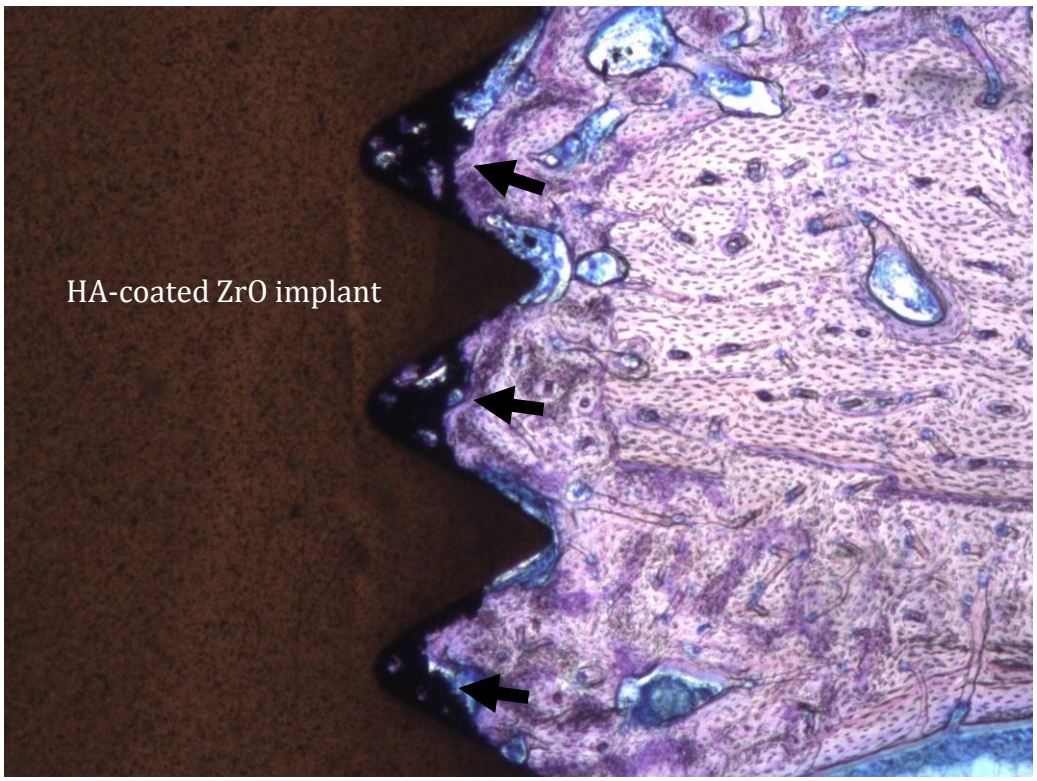

FIGURE 8. Picture showing the HA particles forming smear/debris accumulated in the thread areas around HA-coated ZrO implant at 5X magnification. Arrows indicated the HA debris.

Mean ±SD	Ti-HA	Ti-HF	ZrO-HA	ZrO-HF
BIC (%)	57.78±18.22%	46.41±14.5%	N/A	47.59±12.58%
BA (%)	64.85±19.7%¶	74.91±4.18%	N/A	78.97±4.6%¶
RTQ (N-cm)	N/A	24.39±2.58*	N/A	N/A

Table 3. Table showing the bone-to-implant contact (BIC), bone area (BA) and removal torque (RTC) of all implant groups. The data for the HA-coated ZrO implants were unavailable due to the debris. SD: standard deviation; N/A: not available.

* $p<0.05$, data showing statistically significant difference when comparing the data of machined-surface Ti implant done by Shin *et al.*[2]

¶ $p<0.05$, statistically significant difference between the value of these groups

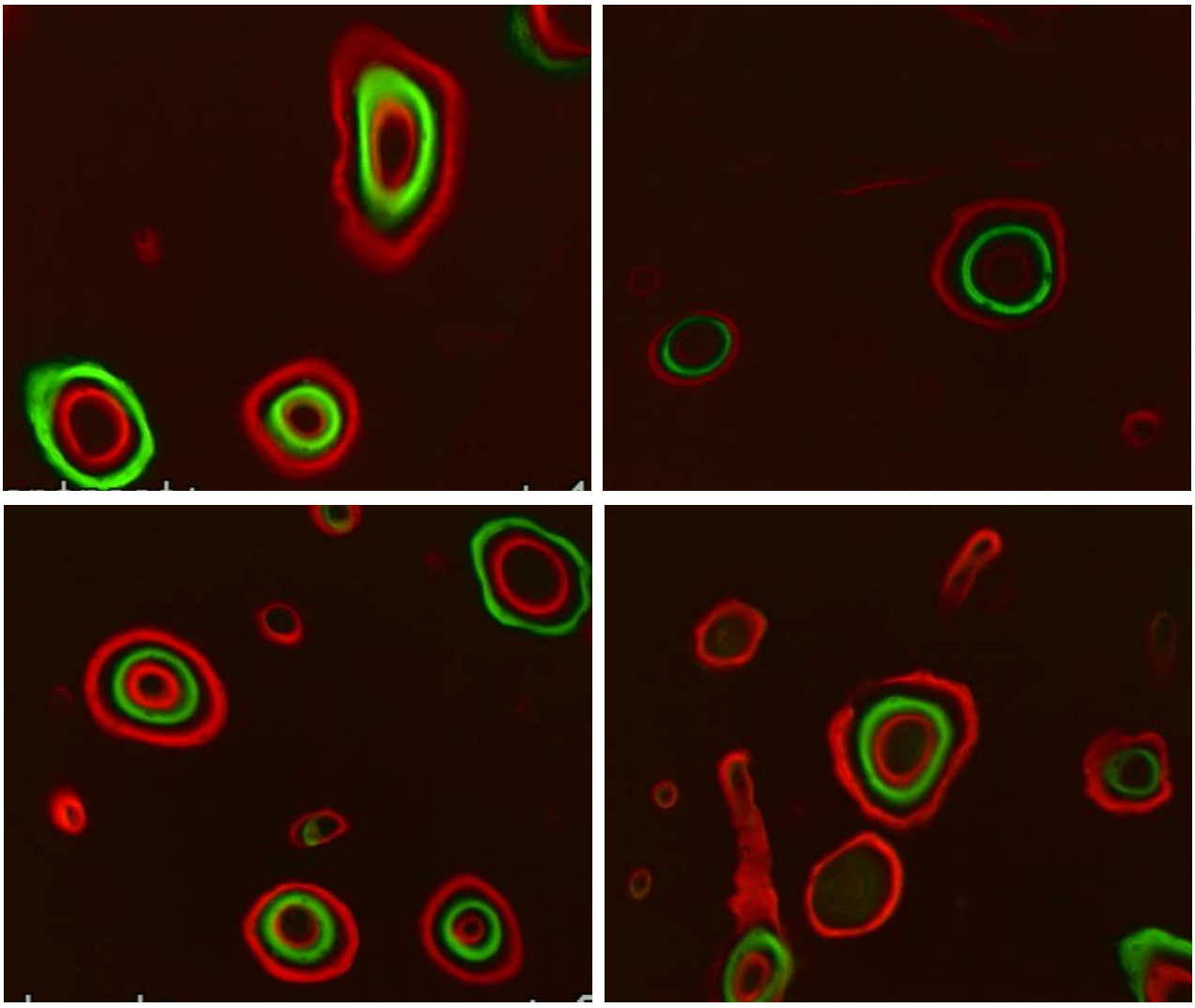

FIGURE 9. Fluorescent microscopy images (20X) were used to measure the mineral apposition rate. The fluorochrome labels used to label the osteon were Oylenol (orange, inner circle), calcein (green) and Alaziran (red, outer circle).

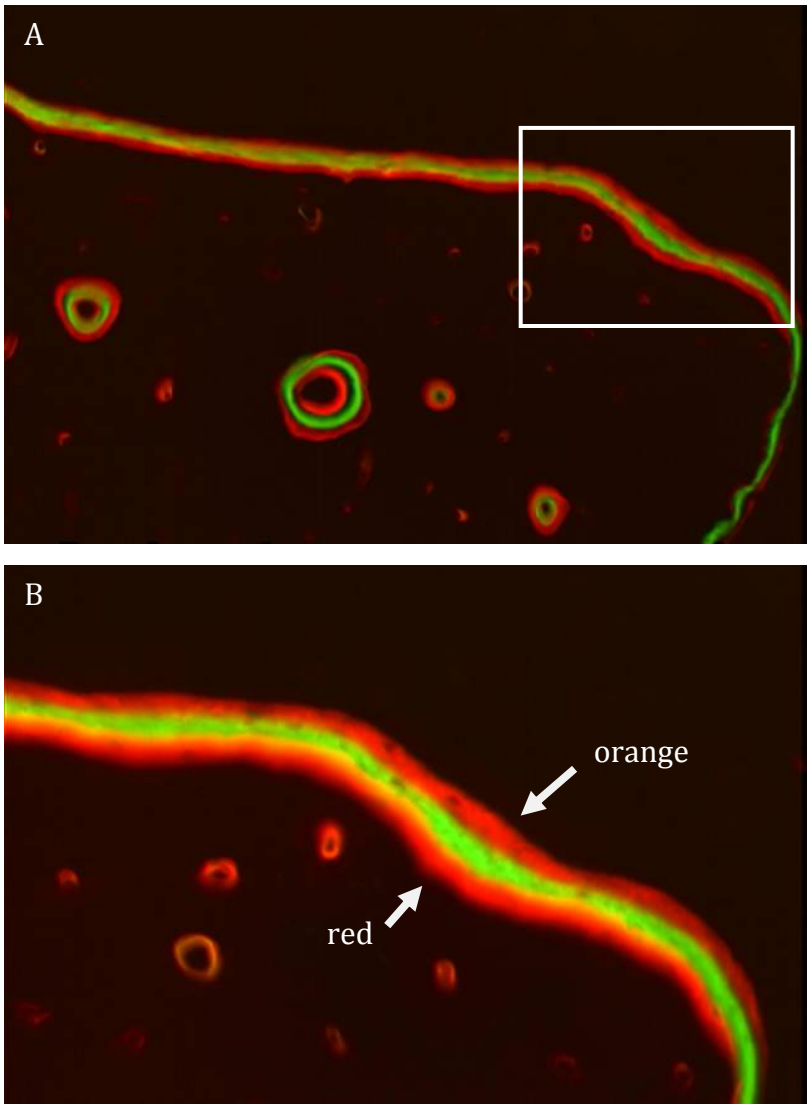

FIGURE 10. Fluorescent microscopy images were used to measure the mineral apposition rate at the periosteum. The fluorochrome labels used to label the periosteum were Oylenol (orange, the outer line), calcein (green) and Alaziran (red, inner line). A. photo showing the fluorescent image at 10X; B. phto of the framed region in A. at 20X.

MAR (Periosteum) (mm/day)	Ti-HA	Ti-HF	ZrO-HA	ZrO-HF
Week 4 **(Red-Green)**	11.96± 6.08	9.61 ±1.21	9.62±4.65	11.15±3.36
Week 5 **(Green-Orange)**	12.27 ±3.81	8.24 ± 1.02	10.32 ± 2.64	10.90± 3.00

TABLE 4. Periosteum mineral apposition rate (MAR, µm/day) measured in fluorescent samples. Values displayed as mean ± standard deviation (mm/day). No statistical significance was found between week 4 and week 5 for all four types of implants.

MAR (osteon) (mm/day)	Ti-HA	Ti-HF	ZrO-HA	ZrO-HF
Week 4 **(Red-Green)**	7.68± 1.45	10.98 ±1.89*	10.08 ± 1.25	11.26±1.58¶
Week 5 **(Green-Orange)**	7.41 ±0.93	7.75 ± 1.87*	8.13 ± 1.73	6.88± 1.33¶

TABLE 5. Osteon mineral apposition rate (MAR, µm/day) measured in fluorescent samples. Values displayed as mean ± standard deviation (mm/day).

*, ¶ $p<0.05$. Higher MAR value at week 4 than week 5 showing statistically significant difference

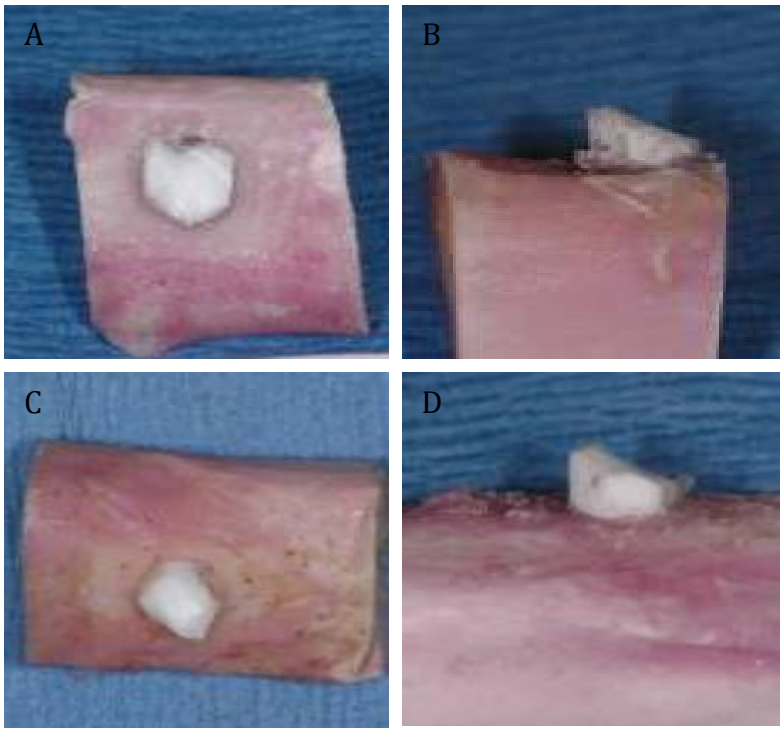

FIGURE 11. Picture showing the fracture on ZrO implants during the removal torque test. A&B: ZrO-HA; C&D: ZrO-HF.

DISCUSSION

Root form screw type dental implants have been widely utilized in clinical settings to restore/rehabilitate edentulous ridge spaces. Numerous clinical studies were done to prove that screw type cpTi implants with or without surface treatments showed a high success rate at 10 years following implantation[60-61]. To increase the rate of osseointegration and to increase the longevity of the implants in use in the human oral cavity, newer materials and surface treatments that may help to reach the goals have been studied. The previous study from our group using machined-surface ZrO implants and cpTi implants proved that ZrO showed similar *in vivo* performance as cpTi and can be possibly used as a substitute to replace cpTi to avoid the esthetic disadvantage. In this study, we tested the possibility of different surface treatments on ZrO implants and compared the histomorphometric results with cpTi implants treated in the same fashions. In this research project, we have obtained valuable information, and at the same time, encountered many challenges. We will use this section to discuss our findings as well as the challenges. We then seek to propose potential solutions to these challenges for future references.

General clinical observation

Under light-microscopy all four surface treatments used in the present study are proved to be biocompatible and allow direct bone-to-implant contact. No adverse effects were seen, and animals recovered normally after surgery. After 6 weeks of implantation, the implants were well integrated in the surrounding bone and showed new bone penetrating into the thread space.

The issue of measurement variation

Histomorphometric measurement requires human input to differentiate between soft tissue and bone in BIC and BA measurements. The human intervention is needed to identify the histological entity from their color, location and morphology. However, the variation from measurement to measurement has never been addressed in dental implant research in regards to the BIC and BA measurement. In this study, we have attempted to address this issue by conducting three measurements at two different time points on the same sample. Our preliminary data analysis shows that the variation as assessed by the standard deviation of the three measurements is less than 10%. We will consult a biostatistician for appropriate reliability statistics in the future.

The issue of comparing with literature data

When comparing our histomorphometric results with other studies[29, 62-68], we found large variation in the percentage of BIC and BA. One study reported a BIC of CaP-coated implants at 30.8% at 4 weeks when the cpTi implants showed only 9.2% BIC[66]. Sennerby *et al.* [67]reported the average BIC for the three best threads over the rabbit tibia was around 19% for a machined surface ZrO implant (values not given in the text; assumption from figures) at 6 weeks. With the most similar study design as our study, Park *et al.* [62] reported an overall mean of BIC around 69% for grit-blasted plus ion-beam-assisted deposition HA surfaced Ti implants at 6 weeks. However, direct comparisons between studies are difficult due to the different designs configured by different researchers. In fact, we understood the difficulties of comparing with literature data at the initiation of the project. We therefore chose to use the same implant geometry as we have used in our pilot study[2] with the intention of using the pilot study as our own comparison. We hope that through the use of the same implant geometry, animal model, implant duration, plus a multiple measurement

protocol, we can establish a reliable testing system for dental implants.

The issue of implant placement and measurement

The measurement location of our study was modified from the previous study. Shin *et al.*[2] measured from the 1st tread to the 4th thread while our study measured from the platform to the 3rd thread. The difference in measurement methods was the result of different implant positions. From the histomorphometric figures from Shin *et al.*[2], thicker cortical bone was noted as well as the position of the implant being placed more coronal to the original cortical bone than that from our study. The cortical bone was in contact with the implant starting at the first thread in that study. While in this current study, the implants were placed with the implant platforms level with the adjacent cortical bone and the bone contact starts with the platform. When future studies are designed, the implant placement should be more standardized. Also the measurement methods should be altered to measure the continuous 3 threads close to the cortical bone to lessen the discrepancy of different implant levels. We will consider re-measuring all slides in this study using a standard measurement protocol in the future.

The issue of sample thickness

Some drawbacks of using the histomorphometrical evaluation should be mentioned in this present study. The percentage of BIC was measured on only 1 histology slide per implant sample, which gave us limited information in this specific region only and may not represent the entire implant surface[69]. However, this is an inherent limitation of analyzing BIC with the histological method. X-ray microtomography has been studied for non-

invasive osseointegration assessment and showed high correlation with the histomorphometric data[62]. Obtaining 3D distribution of the BIC will require an advanced CT methodology not yet available at our facility. Although whether the direction of the sample preparation affects the result of measuring direct bone-to-implant contact has been questioned in the past, no differences were found. Rebollal *et al*.[70] recently published that BIC measured using the longitudinal or transverse histologic sections showed no statistically significant differences. One more point worth mentioning is the histology slide thickness. Typical undecalcified bone sections are in the thickness of 30-50 μm. The ground section thickness of the zirconia implants in this present study was around 100 μm. Further thinning of the sample was found to result in sample cracking or implant pop-out. The thicker slides may result in overestimation of the BIC and BA value.

The issue of surface roughness

The purpose of different surface modification was to increase the surface roughness thus increase the bone-to-implant contact[71], while the other study[65] debuted the concept and claimed the advantage created by HA can be offset by the implant design, e.g., screw type implant. This may explain the result found in our study. Wennerberg and Albrektsson[72] suggested that not all rough surfaces provide better bone integration. The optimal surface topography should be a Ra value between 1 to 1.5 μm. In a review by Shalabi *et al.*[73], the authors stated that even with heterogeneous evidence, the available data supported the positive relationship between bone-to-implant contact and surface roughness. The Ra value of the HF-etched Ti and ZrO implants used in our study was around 0.3 μm, which was lower than the optimal value and was almost similar to the non-treated surface (0.28 and

0.35 μm). As a result, using HF as a surface treatment agent may not be appropriate either for Ti or ZrO implants to create an optimal roughness. Still, the similar surface roughness number between the Ti and ZrO samples provided by the company is not consistent with our SEM image. We will measure the roughness number again in our lab in the future.

The issue of implant duration

Gottlander *et al.*[66] suspected that HA coating stimulates the osseointegration rate, when comparing to machined surface, at an early stage (4 weeks) and may lose this superiority at a later time (6 months). The advantages of the rougher surface may become less important with time when the non-surface-treated implants are stabilized. The animals in our study were sacrificed at 6 weeks; this may account for the non-statistically significant result when comparing to no surface treatment group.

The issue with removal torque

Greater removal torque value was measured in the Ti-HF implant group when comparing to the previous study from our group. RT measurement has been applied to clinical situations to interpret the success of osseointegration and bone-to-implant contact. Implant surface modifications affect the torque value on early bone healing[74] but whether it represents the BIC was unclear. A study by Johansson *et al.*[75] demonstrated a significantly higher RT value but found no difference in BIC when comparing the commercially pure niobium and titanium implants. Due to the similar roughness profile of the implants, the authors hypothesized that a more positive biocompatibility of the niobium material led to the result. In our study, higher RT as well as higher BIC was found in the Ti-HF group,

even with similar surface roughness as the machined-surface Ti group. This might suggest that HF etching created a more biocompatible surface in an early healing period.

The issue with HA coating

In the histology samples of all HA-coated implants, we observed HA particles scattering to the surrounding living tissue. It is unknown whether the separation of the HA particles was due to the sample preparation procedure or the de-lamination during the healing period. The newer sol-gel technique used to deposit the HA particles in our study may require further examination to validate its bonding strength to other material surfaces. However, the bond between HA to the substrate is purely mechanical[76], which makes it difficult to prevent any HA separation. Others also hypothesized that the hydrolysis and bone remodeling may contribute to HA separation[77]. Although no sign of infection or inflammation was observed around the scattered HA particles, attention should be paid to these scattered HA particles. Complications were reported with HA particles separated in total hip arthroplasy[78]. Studying the possible cause of destructive osteoarthritis, Alwan *et al.*[79] demonstrated that HA crystals stimulate the release of bone resorbing agent, prostaglandin E2 (PGE2), in a mouse model. Whether the dislodged HA particles will create further complications in human subjects in the long term requires further study. In the mean time, with the available evidence in implant dentistry, the success rate of the HA-coated implant appears comparable to other implant systems.

SUMMARY AND CONCLUSIONS

In this rabbit study, we demonstrated the *in vivo* biocompatibility of the two different surface treatments, HA-coated and HF-etched, on both titanium and zirconia implants. The implant surfaces tested in this study showed successful osseointegration property without any foreign body reaction or inflammation in the rabbit model. The displacement of the HA particles on the ZrO implants made histomorphometric measurement impossible. In all three other implant types, there were no statistically significant differences in their BIC. Statistically significant difference in BIC was found in our preliminary analysis between the Ti-HA group versus the smooth surface Ti group and between the Ti-HF group versus the smooth surface Ti group. In the RT study, the result of Ti-HF implants showed statistically significant higher value when comparing to the result of machine-surface cpTi implants in our previous study. Similar to the literature findings, the results demonstrated the importance of surface coating on mechanical stability of the implants. Several research challenges arose in this study and we will look for potential solutions to overcome these technical issues in future studies.

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ABSTRACT

EFFECT OF HA-COATING AND HF ETCHING ON EXPEREMENTAL ZIRCONIA IMPLANT EVALUATION USING IN VIVO RABBIT MODEL

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The objective of this study was to evaluate the *in vivo* performance of the hydroxyapatite (HA) coating and hydrofluoric acid (HF) etching zirconia (ZrO) implants and to compare the result with titanium (Ti) implants treated in a similar manner.

A total of four different implant types were tested in this study. Threaded zirconia implants with HA coating (Test 1) and zirconia implants with HF-treated surfaces (Test 2) were used to compare to the same size of titanium implants treated in identical fashion (control 1 and control 2). All implants measured about 3.5 mm at the thread diameter and 7.0 mm in total length. Each rabbit received two zirconia and two titanium implants treated in the same manner (either HA-coated or HF-etched). The samples were implanted into the

rabbit tibias and retrieved at 6 weeks. Upon retrieval, 24 specimens (6 samples for each group) were fixed and dehydrated. The samples were then embedded undecalcified in PMMA for histomorphometry to quantify the bone-to-implant contact (BIC). Another 24 samples were kept in 0.9% saline and were evaluated using removal torque (RT) analysis to assess the strength of the implant-to-bone interface.

The histomorphometric examination demonstrated direct bone-to-implant contact for all four groups. HA particle separation from the implants surface was seen in a majority of the HA-coated samples. No signs of inflammation or foreign body reaction were found during examination. Due to the HA particle smear contamination in the ZrO-HA group, no data was collected in this group. The mean BIC at the first three threads of the Ti-HA, Ti-HF and ZrO-HF were 57.78±18.22%, 46.41±14.55% and 47.41±14.05%, respectively. No statistically significant difference was found pair-wise among these three groups. When comparing the BIC data with the machined-surface implants, a statistically significant difference was found between the Ti-HA versus Ti implant group and the Ti-HF versus Ti implant group. The mean bone area (BA) at the first three threads for Ti-HA, Ti-HF and ZrO-HF showed statistically significant difference ($p<0.05$) between the ZrO-HF and Ti-HA groups, favoring the ZrO-HF group. The value of the peak removal force could only be collected from the Ti-HA group during the removal torque test. The mean RT value for the Ti-HA group was 24.39±2.58 Ncm. When comparing the RT result with our pilot study using machined-surface implants, the Ti-HA group showed statistically significant ($p<0.05$) higher values than the machined-surface Ti implants.

The result of this study proves the *in vivo* biocompatibility of all four implant types tested. In the three measurable implant groups, the histomorphologic analysis showed comparable osseointegration properties in this animal model.

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