Biomechanical and histological evaluation of a new zirconia implant in a canine model

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Introduction / Review of Literature

The oral rehabilitation and reconstruction of occlusion in completely and partially edentulous patients using dental implants is a scientifically accepted and well documented treatment modality. Pure titanium and titanium alloy implants with either smooth or rough surfaces have demonstrated high success rates in a variety of clinical indications.[1-3] These materials are in widespread use because of their biocompatibility, favorable biomechanical properties, and high corrosion resistance.[4] However, the esthetic outcome of restorations supported by titanium implants may be compromised if the dark gray color of the implant shows through a thin peri-implant mucosa or if the implant fixture becomes visible following soft tissue recession.[5] Oates et al. investigated the long-term changes in soft tissue height on the facial surface of dental implants. One hundred and six ITI implants were evaluated in 39 patients. All implants were placed in maxillary and mandibular anterior regions. Clinical assessment of the soft tissues on the mid-facial aspect of the implants was performed over a 2-year period, at 3 and 6 month intervals, following placement of the final restoration. Overall, on the facial aspect of 61% of the 106 implants there was 1 mm or more of soft tissue recession. The mean loss in tissue height was 1.6 mm after 24 months. These results suggest that there is potential for significant changes in soft tissue levels after completion of restorative therapy. [6] Another study by Rasperini et al. compared crestal bone changes at teeth and implants in periodontally healthy and compromised patients over 10 years. Sixty patients with a previous history of periodontitis and 60 periodontally healthy patients were included in this study. Comparisons were made in changes of radiographic bone levels around implants and adjacent teeth at time of implant crown delivery and after 10 years.

Teeth demonstrated a significantly more stable radiographic bone levels compared with adjacent dental implants (mean bone level change: 0.44 mm teeth;: 2.28 mm implant). [7] These soft and hard tissue changes around implants might be caused by peri-implant inflammation with bacterial invasion and incorrect tooth brushing trauma. Additionally the differences in soft tissue components at peri-implant mucosa level may result in less susceptibility to those factors. Berglundh et al, and Buser et al, investigated the epithelial and connective tissue attachment to implant surfaces in dogs. The established peri-implant soft connective tissue demonstrated fewer fibroblasts and diminished vascularity compared to the connective tissue around natural teeth. Also, orientation of the connective tissue fibers around implants were parallel and there was no insertion of connective tissue fibersinto the implant fixtures. [8-10] Sculean concluded that the established peri-implant soft connective tissue resembles a scar tissue in composition, fiber orientation, and vasculature. [11]. There is less vascularity and lower turn-over rate of new collagen fiber generation might make the peri-implant mucosa more susceptible to bacterial invasion as well as mechanical trauma from improper tooth brushing techniques. This recession around implants is of great concern especially in the esthetic regions. Jung et al. investigated color changes of soft tissues caused by restorative materials in vitro. Zirconia did not induce visible color changes in 2.0 and 3.0 mm thickness of peri-implant mucosa. On the other hand, titanium showed the most prominent color changes except where there was 3.0 mm t of peri-implant mucosa thickness. [12] Since 75% of population have normal or thin gingival biotypes, the majority of individuals would be at high risk of color changes at the site of titanium implant prostheseis.[13] Using a zirconia implant fixtures and abutments in anterior esthetic regions would be able to reduce the risk of esthetic complications during long-term post implant therapy due to the natural tooth color of zirconia fixtures and abutments.

Another potential advantage of using zirconia implant fixtures might be greater biocompatibility compare to titanium implants. Some authors suggested potential health hazard from titanium particles in the tissue and regional lymph nodes from metallic ion release, as well as possible allergic reactions. [14, 15] Placing permanent metal dental implants in allergic patients can induce type I or type IV hypersensitivity reactions. Several symptoms have been described, from skin rashes and implant failure, to non-specific immune suppression. Sicilia evaluated the presence of titanium allergy by the anamnesis and examination of patients, together with the selective use of cutaneous and epi-cutaneous testing, in patients treated with or intending to receive dental implants of such material [16].

Titanium allergy can be detected in dental implant patients, even though its estimated prevalence is low (0.6%). A significantly higher risk of positive allergic reaction was found in patients showing a post-op allergy compatible response.[16] Olmedo et al. investigated the tissue response of human oral mucosa adjacent to titanium cover screws with biopsy. Forty-one percent of mucosa biopsies exhibited metal particles in different layers of the tissue sections. The size of titanium particles detected in peri-implant tissue ranged from 0.9 ± 0.7 to 3 ± 2 μm. Immunohistochemical study confirmed the presence of macrophages and T lymphocytes associated with the metal particles. Microchemical analysis revealed the presence of titanium in the particles. [17] Released titanium particles can induce immune responses in human cells and play an important role in bone resorption at the interface of bone and implants. [18] Irshad conducted a study to determine the in vitro inflammatory responses of peri-implant granulation tissue fibroblasts to titanium particles with conjunction of *P. gingivalis* in vitro. Titanium oxide particles and *P. gingivalis*, individually, can induce pro-inflammatory responses in peri-implant granulation tissue fibroblasts. Additionally, titanium oxide particles and viable *P. gingivalis* enhanced the gene expression and production of TNF-α by peri-implant granulation fibroblasts. These result suggested that released titanium particles from implant fixtures, abutments, and cover screw might initiate and exaggerate peri-implant tissue inflammation. Consequently, the inflammation may result in marginal bone loss as well as soft tissue recession around the implants. [19]

Because of these disadvantages, biocompatible and enamel-colored ceramic implants have been considered as an alternative implant material. Since the color is similar to the natural teeth, it can be utilized in anterior esthetic regions even in thin tissue biotypes. Zirconia (yttria-stabilized tetragonal zirconia polycrystal: Y-TZP) has been proposed as an alternative to metallic alloys due to its high flexural strength (900-1200 MPa), favorable fracture toughness (K_{IC} 7-10 MPa-m$^{1/2}$), and satisfactory Young's modulus of elasticity (210 GPa). [20] In orthopedics, zirconia has been extensively utilized as a material for femoral ball-heads in total hip replacements since 1980's.[21] In dentistry, zirconia has been used for implant fixtures, abutments and as a framework of fixed dental prostheses.[22, 23] Biologically, zirconia implant fixtures have been studied in both in-vitro and in-vivo experiments for implant osseointegration and soft tissue response. [24-27] Zirconia implant fixtures are already commercially available in the United States and in some countries in Europe and Asia. However, since zirconia implant fixtures are relatively new, there are few clinical studies available evaluating their use and effectiveness. Kohal investigated one-piece zirconia implants in a one-year retrospective study. A total of 65 patients received a one-stage implant surgery with immediate temporization. The implant body and transmucosal collar of the ceramic implants were roughened using the ZiUniteTM surface technology (Nobel Biocare, Gothenburg, Sweden). The cumulative survival rate of this ceramic implant was comparable to the reported survival rates of titanium implants that had been immediately restored. However, the frequency of increased radiographic bone loss (>2 mm) after 1 year was considerably higher around the zirconia implants as compared to conventional two-piece titanium implants. [28] Gahlert evaluated the use of Z-Look 3 dental implants (Z-Systems) which is laser modified surface treatment for up to three years. All implants were loaded and in function during the evaluation phase. Overall, 30 implants were lost due to lack of osseointegration (n = 17) or fracture (n = 13). The diameter-reduced implants showed the lowest survival rate (59.5%) compared to the implants with a diameter of 4.0 mm or 5.0 mm. The survival rate for diameters of 3.25 mm was significantly lower than that for diameters of 4.0 mm. The estimated cumulative survival rate up to 3 years demonstrated a survival probability of 82.4% for all types of implant diameters and failures. [29]

Material composition and surface topography of a biomaterial are the key factors in osseointegration and stabilizing bone levels. The aim of sandblasting on titanium implants is to increase the irregularity of the surface of the implant on the micrometer scale range with aluminum oxide, and TiO_2 particles. Sandblasting surfaces allow the adhesion, proliferation, and differentiation of osteoblasts. However, fibroblasts were found to adhere with greater difficulty. Also, a dual acid-etched technique has been proposed to produce a micro-textured surface in the sub-micrometer and nanometer scale range. It was proposed to modify the implant surface without leaving the residues found after the sandblasting procedure, to avoid the non-uniform treatment of the surface, and to control the loss of metallic particles from the body of the implant. However, modified surface resulting from blasting (to produce a macro-texture) was followed by acid etching (to produce final micro-texture). The combined surface treatment tends to promote greater osseous contact at earlier time points compared with any of the single surface treatments. Sandblasting and acid etching is the current standard surface treatment for dental implants. Gahlert et al. compared hydrofluoric acid-modified zirconia implants (ZrO) to acid-etched sandblasted titanium implants (Ti-SLA) regarding bone-implant contact (BIC) ratio and peri-implant bone density. The implants were placed in mini pig maxillae for 4, 8, and 12 weeks. The results showed no significant differences in osseointegration between the ZrO and Ti-SLA implants at each time point. [29] Langhoff et al. compared BIC between sandblasted and acid etched titanium implants and zirconia implants placed in sheep iliac bone for 2, 4, and 8 weeks. There were no differences in BIC measurements from the zirconia implants and titanium implants. [30] Bormann et al. evaluated removal torques of micro-structured zirconia and Ti-SLA implants in miniature pigs. There were no statistically significant differences between the two materials after a time period of 4 and 12 weeks.[31] However, Gahlert's study compared zirconia implants that received only acid etching to titanium implants receiving both sandblasting and acid-etching. [29]

We suspect that an additional sandblasting surface treatment besides acid-etching may further improve the in vivo performance of a zirconia implant. However, the potential advantage of a zirconia implant receiving both sandblasting and acid etching surface treatments has never been evaluated. Although Langhoff's study compared titanium and zirconia implants that were both sandblasted and acid-etched, the study was conducted on iliac bone where cortical bone is much thicker than in alveolar bone. [30] An intra-oral model is needed to confirm the differences in the in vivo performance of zirconia and titanium implants receiving both sandblasting and acid etching surface treatments.

Purpose of Study

The purpose of this study was to determine the histomorphometric and biomechanical properties of zirconia implants manufactured by Shofu Inc. with a sand-blasted and acid-etched surface treatment, compared to that of the titanium implants from the same manufacturer with a sand-blasted and acid-etched surface treatment in a canine model. Specifically, the bone-implant contact, removal torque, and mineral apposition rate of the two groups were studied and compared.

Materials and Method

1. *Experimental Design*

Implant fixtures: Two types of implants were tested in this study.

Shofu thread type ZrO_2 (blast + acid): Ø3.5 x 9.2mm

Shofu thread type Ti (blast + acid) Ø3.5 x 9.2mm

An external hex design was used in both implants to facilitate removal torque analysis. All Shofu dental implants were provided by Shofu Inc.

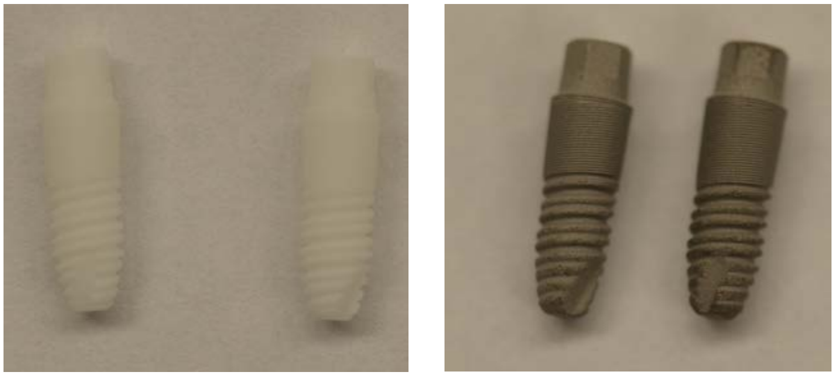


Fig. 1. Implant fixtures. Left: ZrO_2, Right: Ti

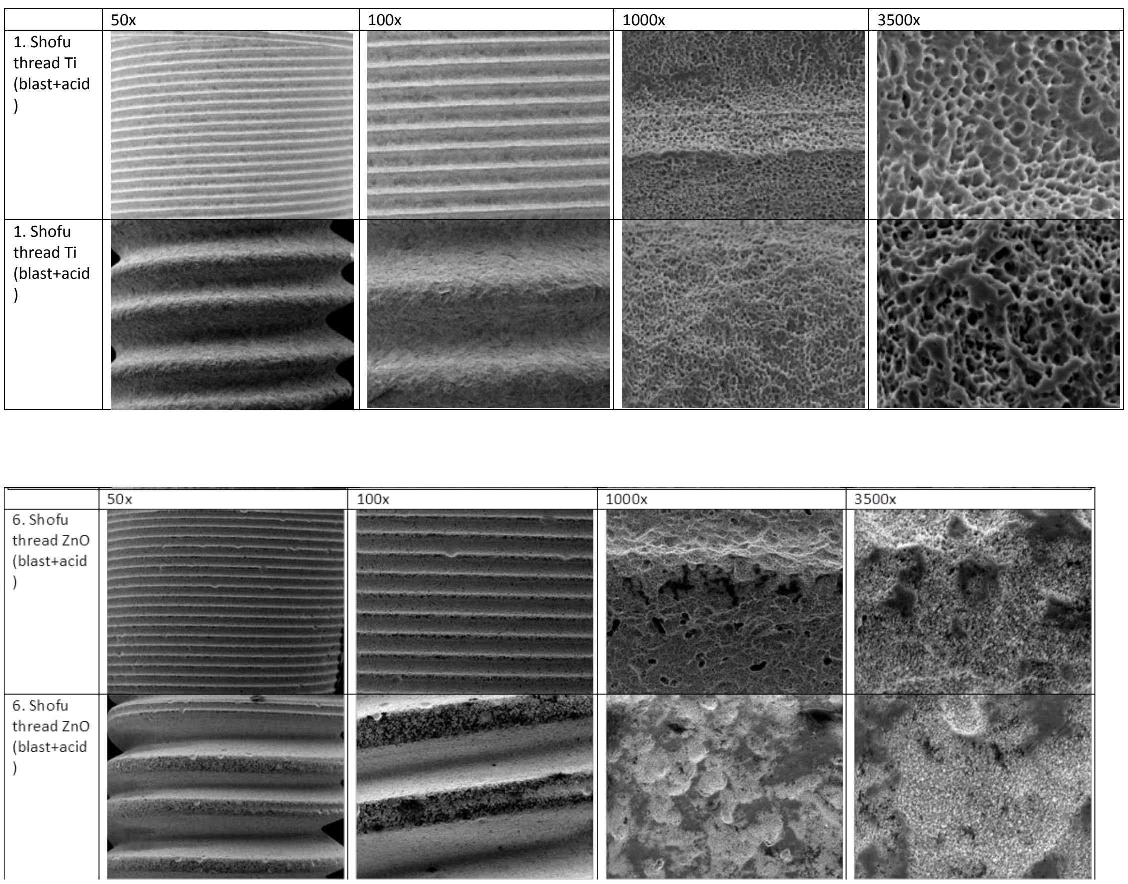
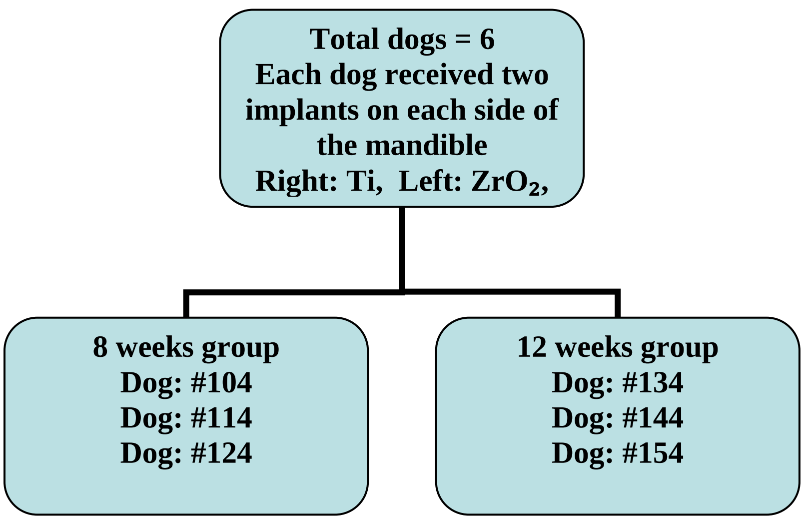


Fig. 2. SEM image for each implant fixture (Images taken from previous study).

Following the experimental design provided by Shofu Inc., a total six dogs were used. The dogs were divided into 2 groups. The first three dogs were used for 8 weeks healing period after implant placement. The next three dogs were used for 12 weeks healing period post implant procedure. Each dog received 2 ZrO_2 implants on the left side and 2 Ti implants on the right side of the mandible. The mesial implant from each side was used for histological evaluation, and the distal implant from same side was used for biomechanical evaluation.

Table. 1. Study design.



2. *Animal*

Six beagle dogs were used in this study. The animals were 1-2 years of age at the beginning of the experiment. All dogs were acclimated for a period of 1 week and housed in pairs in environmentally controlled rooms in Indiana University's AALAC accredited animal care facility. All dogs were fed standard dog chow containing 1.2% calcium, 1.0% phosphorus, and 851 IU/kg vitamin D3. Water was provided ad libitum.

3. *Surgery*

The surgeries were divided into two stages: extraction and implant placement.

<u>Extraction</u>

The surgeries were performed under general anesthesia. A local nerve block was used. The gingival attachment on the tooth was first detached using a periosteal elevator. The premolars were bisected by high-speed diamond burs. A dental elevator was wedged between the proximal and distal section to loosen the proximal and distal halves of the teeth. Each half was extracted carefully using dental forceps. A 4-0 Vicryl resorabable suture was used to close the gingiva. The extraction sites were allowed to heal for 8 weeks. Buprenorphine (0.3mg/ml) 0.005-0.02 mg/kg IM was injected 8-24 hours post operatively as an analgesia agents.

Soft diet was provided for the first few days after the extraction and the extraction sites were checked daily until wounds healed. There were no signs of infection and all extraction sites healed without any incident.

<u>Implant placement</u>

After 8 weeks of healing, the implants were placed by Dr. Tien-Min Chu with assistance from Dr. Yusuke Hamada, a resident at Graduate Periodontics Department experienced in dental implant placement. Two implants of the same type were placed in each side of the mandible, one side receiving zirconia implants and the contralateral side receiving titanium implants, following the experimental design provided by Shofu Inc. The first implant was placed at 5 mm away from the mesial surface of the first molar. The second implant was placed 10 mm mesial to the first implant.

The surgeries were performed under general anesthesia. A local nerve block was used. Incision on the implant sites were performed using a #15 blade to gain access to the alveolar bone. Following full-thickness mucoperiosteal f\lap reflection, implant osteotomies were prepared at 1000 rpm using a twist drill, followed by a pilot drill provided by Shofu Inc. Final drilling was performed at 1000 rpm to 1 mm above the top marking band on the cortical drill. A new cortical drill was used for each dog. A tap drill was used at 20 rpm. Finally, the ZrO_2 and titanium implants were placed to an insertion torque of 15 N-cm using a slow speed hand-. In order to adjust the depth of the implants, hand wrench was used to torque both types of implants. The shoulder of the implants was placed at the level of the crestal bone. After implant placement, the oral mucosa over the implants was sutured to cover the implants. The implant sites were allowed to heal and osseointegrate for 8 or 12 weeks before retrieval. Fluorescent dyes of alizarin red (20mg/kg, IV), calcein green (5mg/kg, IV), and xylenol orange (90mg/kg) were injected at 3, 2, and 1 weeks before specimen retrieval.

After the designated healing period, the animals were euthanized by an intravenous injection of a sodium pentobarbital derivative (0.22 mg/kg Beuthanasia-D Special). Bilateral mandibles were retrieved. To avoid the variations arising from the differences in cortical thickness at distal and mesial osteotomy sites, we assigned all implants at the mesial end to be subjected to histomorphometric analysis and histological examination, while all implants at the distal end were used to perform removal torque analysis. The mesial samples were stored in formalin for 48 hours and then serially dehydrated using 70-100% ethanol for tissue histological analyses. The distal samples were stored in saline and stored at 4º C until testing.

4. Histological and Histomorphometric Analysis

After ethanol dehydration, histological samples were embedded in polymethylmethacrylate (PMMA) without decalcification according to standard procedures.

Fig. 3. Retrieved histological samples embedded in PMMA.

A microtome Leica SP 1600 was used to carefully section the embedded specimen at intervals of 80 µm parallel to the long axis of the implant in a medio-lateral direction. Sections were then glued to plastic and further ground down to 70 µm with silicon carbides abrasive papers. The sections were then stained with toluidine blue. The slices were evaluated at 4x using a computer-based histomorphometric system (ImagePro®, Media Cybernetics, Inc. Acton, MA) under a light microscope (Nikon Eclipse 50i, Tokyo, Japan) equipped with a charge-coupled distributor camera and connected to a computer (Dell Optiplex GX280, Round Rock, Texas, USA). The samples were analyzed by the computer-based histomorphometric system ImagePro®. All measurements were performed for the right and left aspects of the implant sections and mean values and standard deviations of each variable were calculated for the implant groups. For the micro thread area, the entire length of the thread that has bone contact was included in the measurement for Bone Implant Contact (BIC) and Bone Area (BA). For the macro threads areas, BIC and BA were measured in the first four threads only since large variation in the mandible anatomy occurred below the fourth thread (Fig. 4). Both BIC and BA were measured on the right side and left sides of an implant fixture.

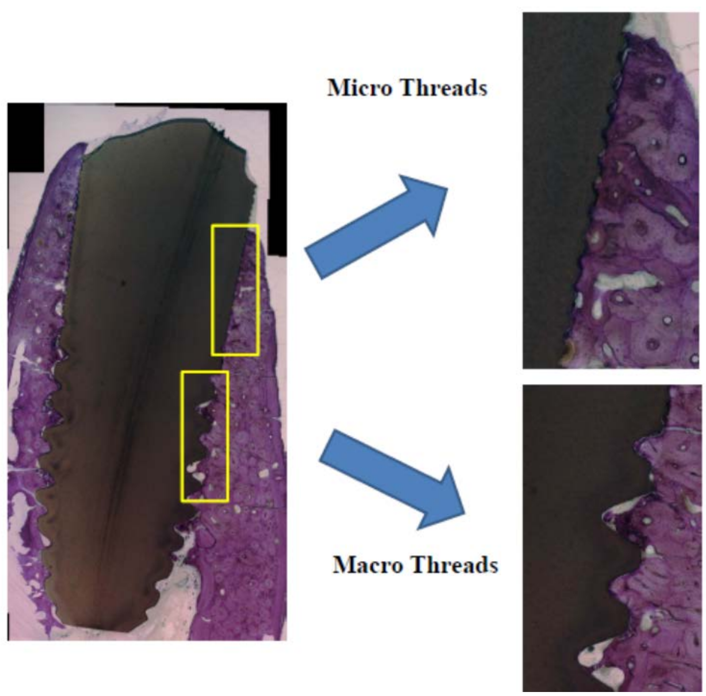


Fig. 4. Measurements for thread type implants were made in two areas: micro threads and macro threads areas for both BA and BIC.

Under fluorescent light microscopy, fluorochrome analysis was performed on unstained sections and examined using ultraviolet illumination. The histologic section through the middle of the implant was chosen for analysis. As mentioned previously, each dog received intravenous injections of alizarin red (20mg/kg) 3 weeks, calcein green (5mg/kg) 2 weeks, and Xylenol orange (90mg/kg) 1 week prior to sacrifice. Mineral apposition rate (MAR) was calculated by measuring the distance between the edges of alizarin red, calcein green, and xylenol orange then divided by the number of days (7 days) (Fig 5). MARs at 4 time points (5-6, 6-7, 9-10, 11-12 weeks following implant placement) were therefore evaluated in μm/day. The calculations were done from an average of 5 osteons randomly chosen on each side of the implant. Since fluorescent markers were found at the inferior border of the mandible, the length of stained cortical bone was measured and then divided by the length of the entire cortical bone to obtain bone forming area (BFA).

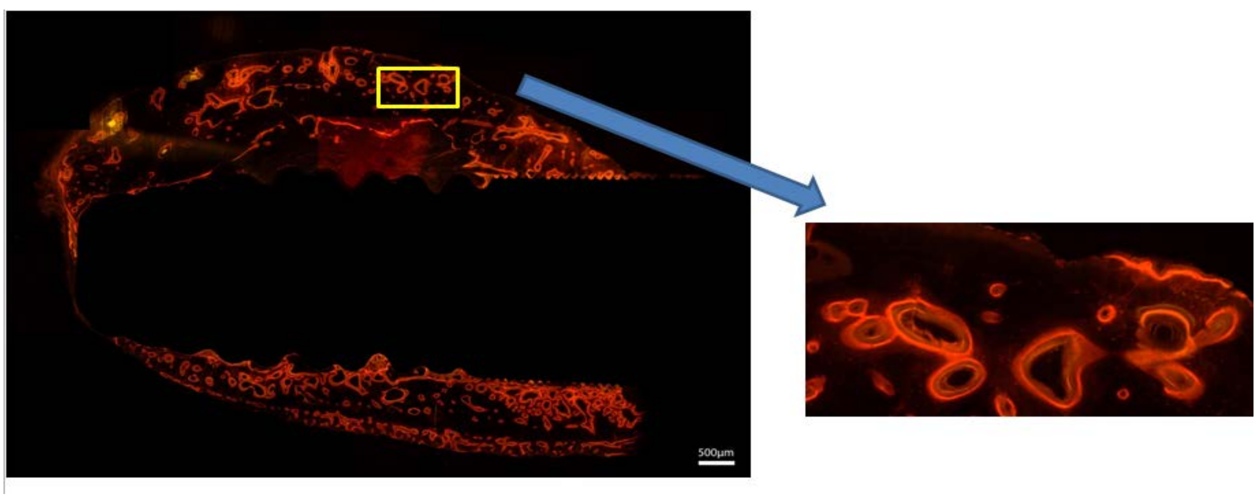


Fig. 5. Measurements for mineral apposition rate (MAR).

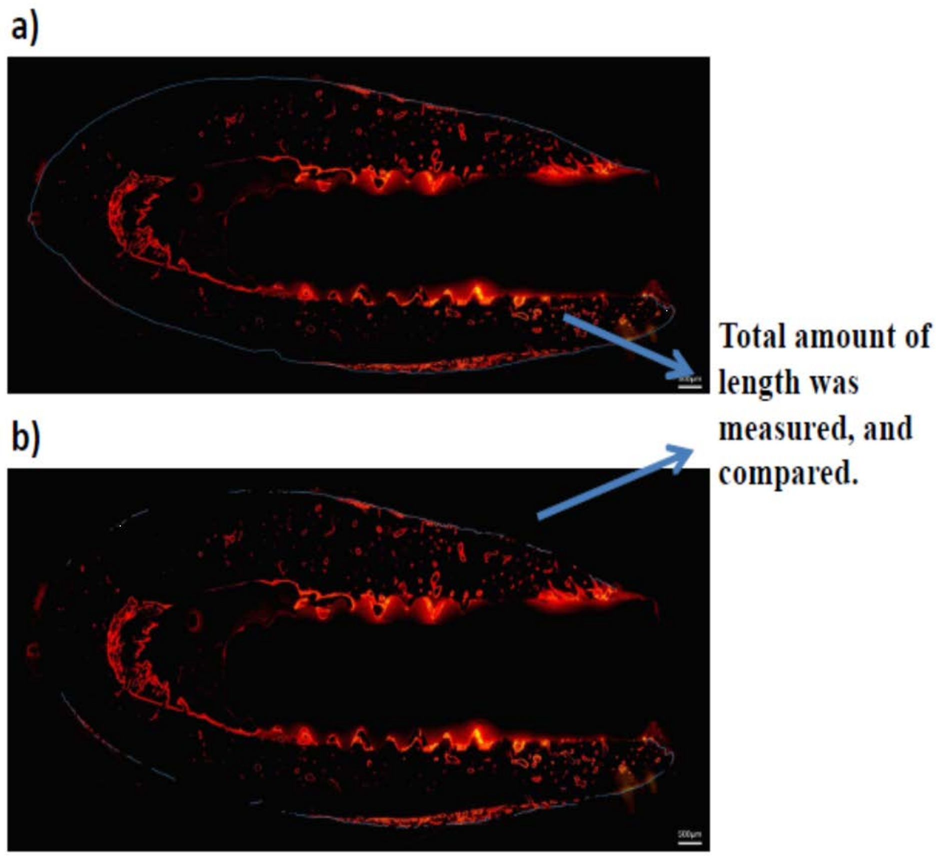


Fig.6. Bone forming area a) Total length, b) Reflected area.

5. *Removal Torque (RTQ) test*

For removal torque analysis, the head of the mesial end implant was fitted and secured into the actuator of the removal torque apparatus. The system consists of a digital force gauge (BGI Force Torque Gauge, Mark-10 Corp. Copiague, NY, USA) (Fig.7) and torque sensor (STJ100, Mark-10). The removal torque apparatus was connected to special computer software (Mesur Gauge, Mark-10) to record the maximal torque value. The axis of the implant was aligned with the axis of the testing machine. A slow, gradual increase of torque (1^{o}/sec) was applied counterclockwise until implant loosening occurred. The torque required to loosen the implant from bone was registered by the computer. Six parameters were registered as shown in the following (Fig. 8).

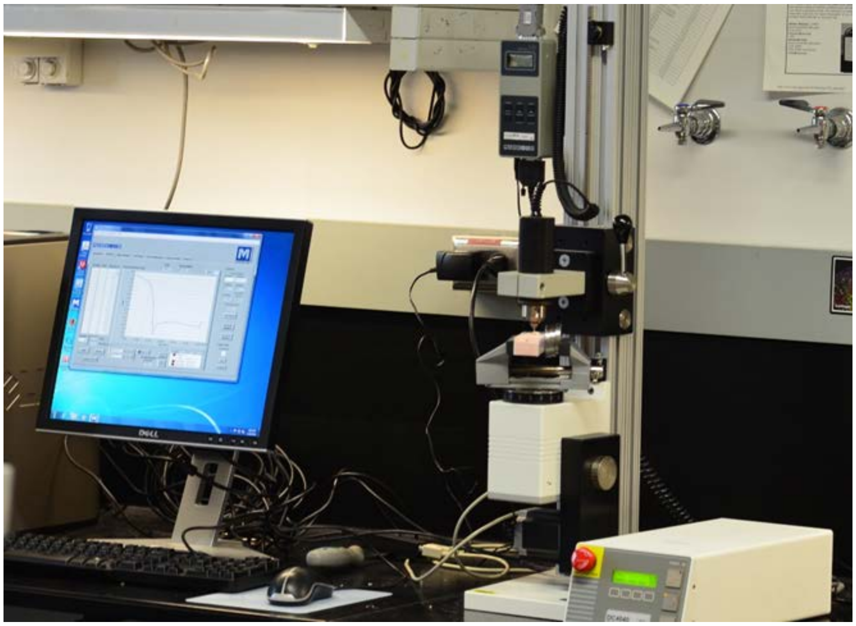


Fig. 7. Digital force gauge.

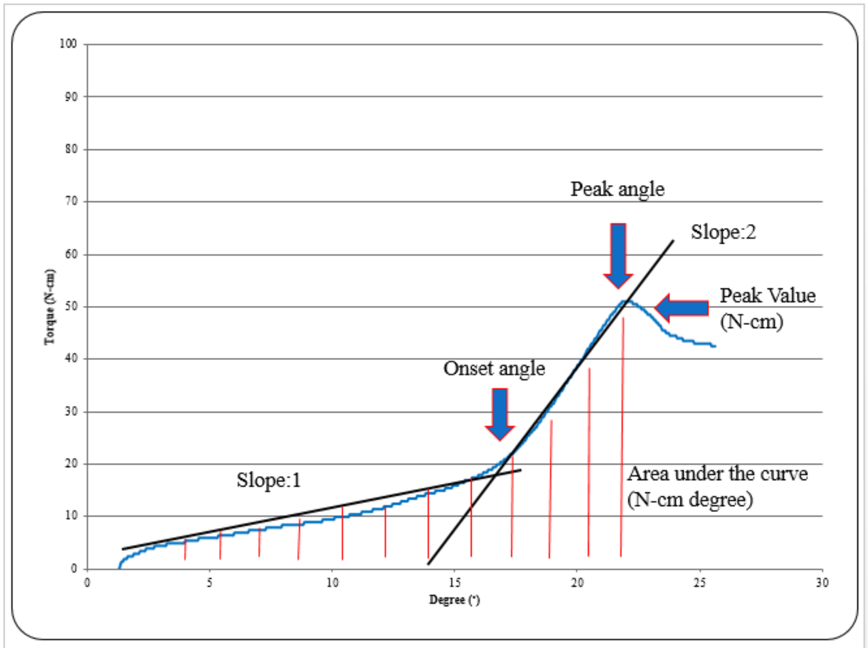


Fig. 8. Parameters for removal torque measurements.

From the torque-angle curve, it seems that the implant went through different phases of biomechanical stress. The first stage involved a lower modulus area as indicated by the lower slope, followed by a higher modulus area where a higher slope is seen. Finally the bone-implant interface failed as indicated by a sharp drop in the torque value (9 out of 12) or a large change in the slope (3 out of 12). The highest torque where bone-implant interface failed was registered as the removal torque as commonly used in the dental implant literature. We also registered the onset angle where the sharp change in between the first and the second slope occurred, as well as the peak angle where the peak torque (9 out of 12) or the second sharp turn of slope (3 out of 12) occurred. Finally, we calculated the area under the curve from the start to the peak angle. We are not exactly sure about the meaning of the initial lower slope, though it is observed in all curves. The peak angles show how many degrees the implant can be turned before the bone-implant interface failure occurred. The area under the curve shows the energy required for bone-implant interface failure to occur.

6. *PerioTest®*

The Periotest® system (Periotest®, Medizintechnik Gulden e.K.) (Fig. 9) was originally designed to quantify the dampening effects by the periodontal ligament surrounding the tooth, as a measure of mobility. It is a hand-held device with a metal bar that is attracted to the tooth by an electromagnet, giving an audible signal and showing the measurement digitally on a scale from -8 (low mobility) to 50 (high mobility) PTV units. (Garcia 2009). The lower the value, the greater the stability or dampening effect from the measured implant or tooth. The measurements were followed according to the manufacturer's instructions. Periotest values were measured three times on each implant, and the average measurement was used as a final value.

Table 2. Interpretations of the periotest.

Measuring Natural Teeth

Clinical degree of tooth loosening	Periotest Value Range
0	- 08 bis +09
I	+10 bis +19
II	+20 bis +29
III	+30 bis +50

Measuring Dental Implants

Periotest Value Range	**Interpretation**
-8 to 0	Good osseointegration; the implant is well integrated and pressure can be applied to it.
+1 to +9	A clinical examination is required: the application of pressure on the implant is generally not (yet) possible
+10 to +50	Osseointegration is insufficient and no pressure may be allowed to act on the implant

Fig. 9. Periotest M®.

7. Statistical Analysis

Mean histomorphometric and biomechanical values were calculated and recorded along with their standard deviations for each implant, in each dog. Statistical analyses were performed using paired t-test. A random effect was used to allow correlation between the implants from the left and right sides from each dog. Fixed effects analysis were included for implant type, healing period, and the implant-by-healing interaction. A 5% significance level was used for all tests. Distributions of the measurements were checked and transformations of the data (logarithmic, square root, rank, etc.) were used where necessary.

Results

Implant placement

For both Shofu thread type ZrO_2 and Shofu thread type Ti implants, the osteotomy sites were first marked with a marking drill, followed by drilling with a twist drill, a 3.0 mm pilot drill and a 3.5 mm pilot drill at 1,000 rpm. The osteotomy sites were then enlarged by a 3.5 mm cortical drill to the upper line of the top band at 1,000 rpm. A 3.5 mm tap drill was then used at 20 rpm to prepare the implant sites. The implants were then inserted with an implant driver at 20 rpm to about 2 mm above bone. Finally the implants were torqued with using an implant wrench. Since the connection part of those implants' design was changed from internal hex to external hex, external hex area was left supracrestally (Fig. 10). All drillings were done under copious saline irrigation. Due to the high bone density in dog mandibles, the implants were placed by hand using the wrench at an insertion torque higher than 35 N-cm. The mucogingival flap was primarily closed with a mucoperiosteum releasing incision on the buccal side.

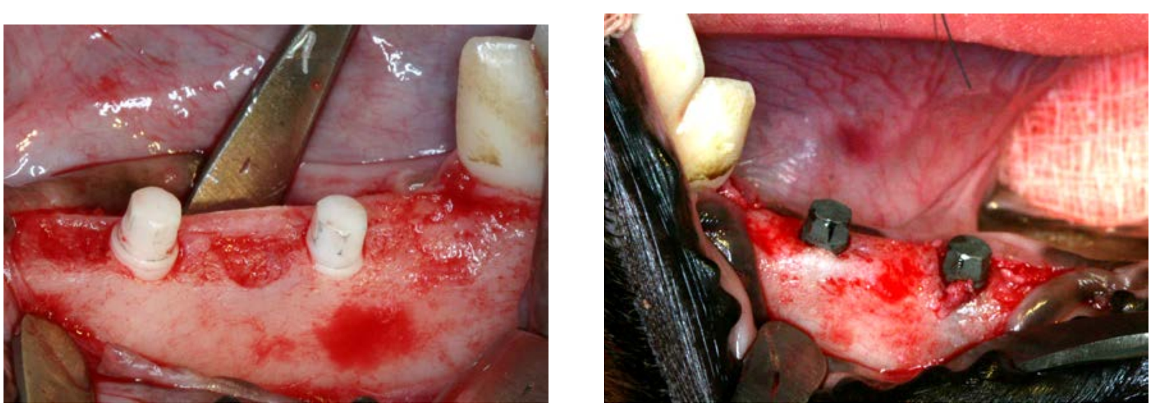


Fig. 10. Implant placement. Left side: Shofu ZrO_2 type, right side: Shofu Ti type.

All surgeries were uneventful. During the surgery, it was found that the mandibles of some animals were too narrow and a few implants were partially exposed in the top part of implant on the buccal side of the ridge. This is why in some histology sections, the implants do not have bone coverage on one side. The age and species of the animal were chosen to be consistent with our first implant study with Shofu Inc.

<u>Failure of osseointegration</u>

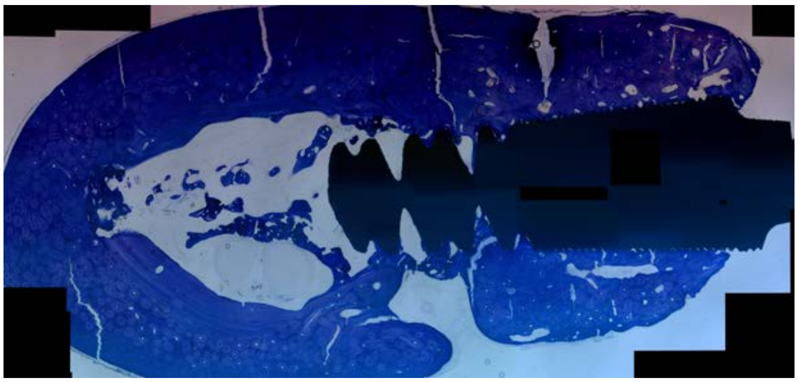
One Shofu thread type Ti implant in dog #134 did not integrate and no data was obtained from that implant. The reason is because the cortical drill used at that time got dull very quickly from the very dense dog bone and probably excessive pressure was applied (with excessive heat generation) when using the cortical drill. This may have caused bone necrosis in the implant site. The other Shofu thread type Ti implant in dog #134 achieved osseointegration well at the time of retrieval. The integrated implant was used for removal torque test.

<u>Histological and Histomorphometric Analysis</u>

The unit of length of the histological specimens was Pix according to computer-based histomorphometric system ImagePro®. A 500 μm ruler was placed on the histology slide. With image pro software it was measured 374.34 pixels. As a result, it was calculated as 1 pixel =1.335 μm.

8 week Shofu thread type Ti

#104Ti:



1) Bone Implant Contact (BIC), Bone Area (BA).

Bone implant contact

			Right						**Left**			
		Micro			Macro			Micro			Macro	
Dog/ imp	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**
104/ Ti	3836.15	2392.97	**62.38%**	3631.29	1508.46	**41.54%**	3246.03	2505.46	**77.19%**	3686.37	2432.12	**65.98%**

Bone Area

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
104 Ti	70291	47598	67.72%	265343	79293	29.88%	59018	50362	85.33%	286278	148675	51.93%

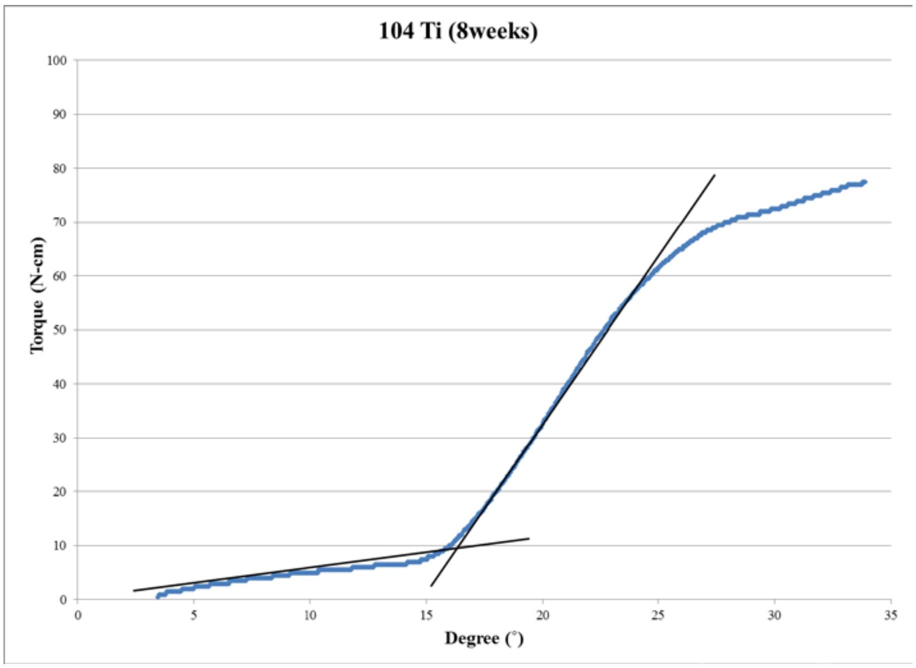
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
104/ Ti	1.99258	2.20656		1.66989	1.92278
Avg	2.09957			1.796335	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
104/ Ti	21727.59	8007.35	**36.85%**

4) Removal Torque Test (RTQ)

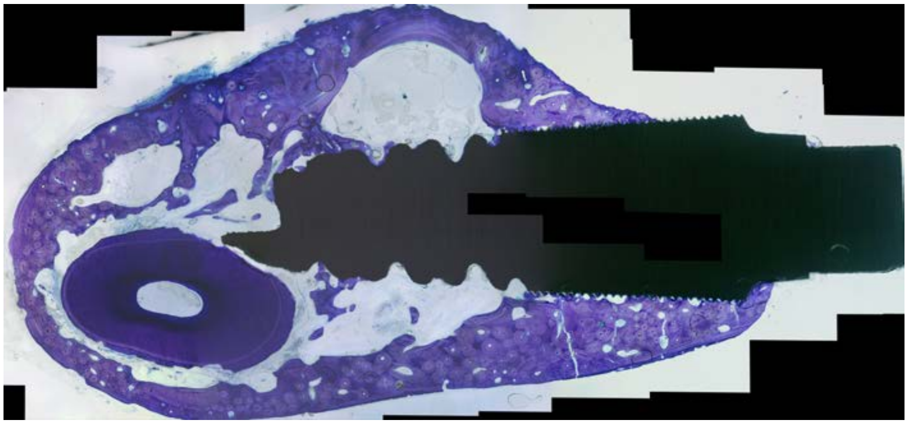


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
104 Ti	15.723	24.385	59	347.425	y = 0.5652x - 0.7917	y = 6.1673x - 90.286

5) Periotest value; PTV

Dog/imp	Medial			Distal		
104/Ti	-2.4	-0.9	-2.4	-2.2	-2.6	-1.9
Avg	**-2.066666667**					

#114Ti:



1) Bone Implant Contact (BIC), Bone Area (BA)

Bone implant contact

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/ imp	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
114/ Ti	2395.96	1184.16	**49.42%**	1256.07	1021.46	**81.32%**	3653.83	1241.38	**33.97%**	1411.47	445.28	**31.55%**

Bone Area

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
114 Ti	45156	23028	51.00%	172454	48600	28.18%	58094	28192	48.53%	157023	2153	1.37%

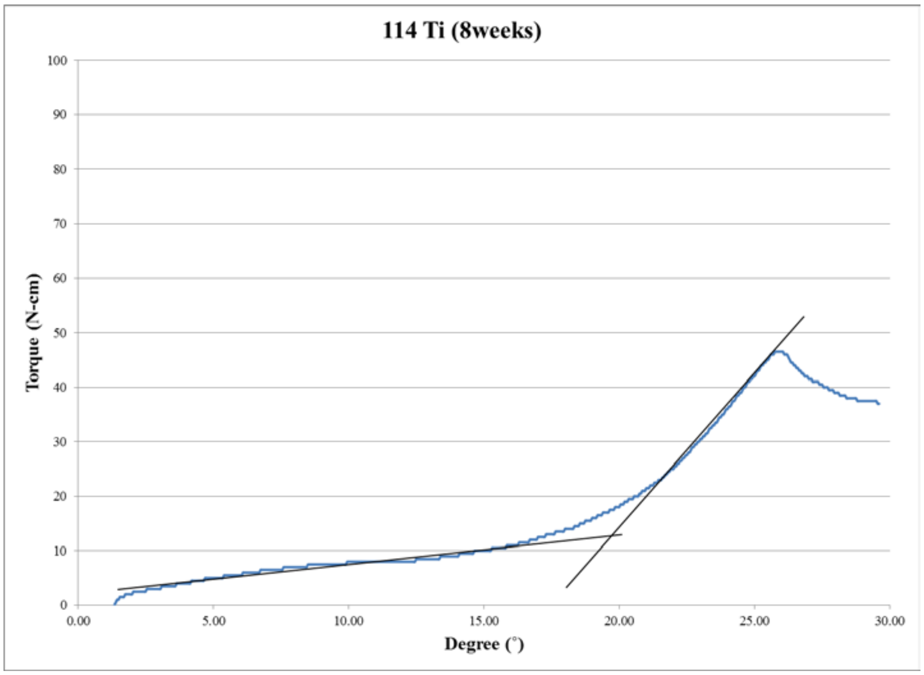
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
114/Ti	2.2428	2.08451		2.07879	1.85069
Avg	2.163655			1.96474	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
114/ Ti	19877.61	7140.74	**35.92%**

4) Removal Torque Test (RTQ)



	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
114 Ti	19.94	26.04	46.5	337.575	y = 0.5561x + 1.8708	y = 5.6709x - 99.771

5) Periotest value; PTV

Dog/imp	Medial			Distal		
114/Ti	-3.4	-2.4	-1.4	-1.3	-2.3	-2.1
Avg	**-2.15**					

#124Ti:

1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

			Right						Left			
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
124/ Ti	3015.78	1978.17	**65.59%**	2323.59	782.57	**33.68%**	1869.59	1005.61	**53.79%**	1443.08	869.8	**60.27%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
124 Ti	54914	15616	28.44%	142594	47081	33.02%	35416	10952	30.92%	179761	18011	10.02%

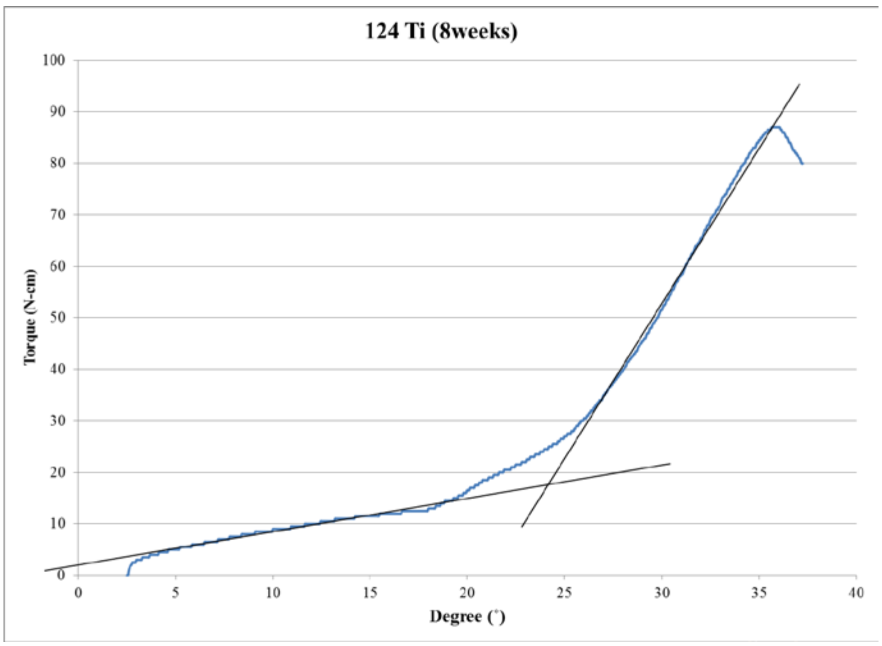
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (μm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
124/Ti	2.01242	2.81952		1.58217	2.21038
Avg	2.41597			1.896275	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
124/ Ti	16881.32	3417.12	**20.24%**

4) Removal Torque Test (RTQ)



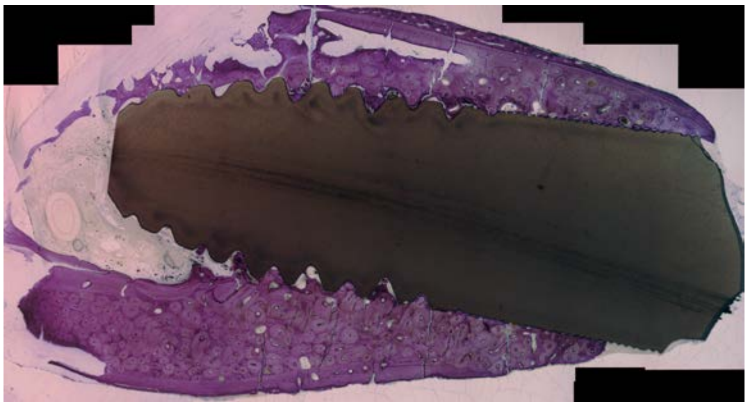
	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
124 Ti	24.55	36.05	87	890.85	y = 0.6325x + 2.0782	y = 6.3328x - 137.59

5) Periotest value; PTV

Dog/imp	Medial			Distal		
124/Ti	-3.7	-2.4	-2.3	-1.3	-1.2	-1.9
Avg	**-2.133333333**					

8 weeks Shofu thread type ZrO_2

#104 ZrO_2



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/ imp	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**	Screw (px	Bone (pxl	**BIC (%)**
104/ Zr	2757.32	2277.57	**82.60%**	2566.42	1395.68	**54.38%**	2381.91	1630.29	**68.44%**	2620.71	1355.07	**51.71%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
104 Zr	29823	17074	57.25%	136984	112199	81.91%	27491	17249	62.74%	174060	118697	68.19%

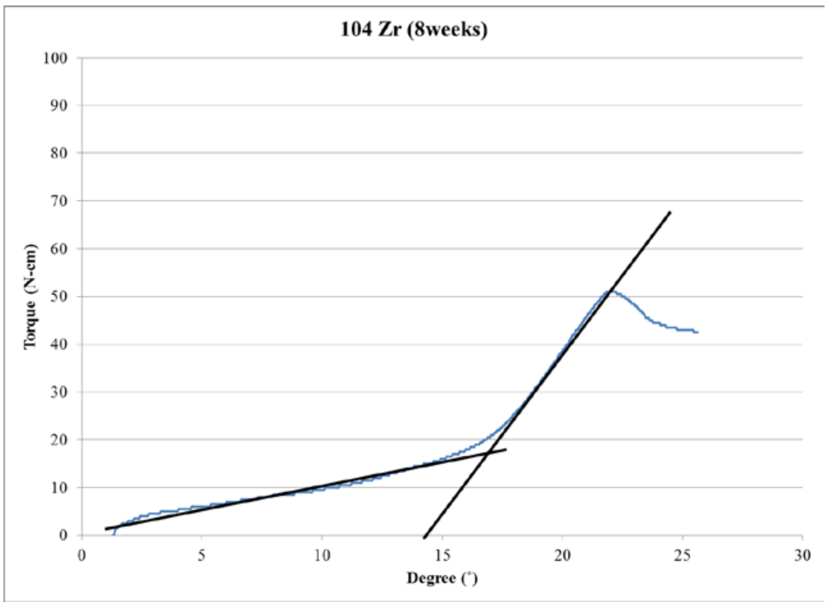
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
104/Zr	2.04338	1.93613		2.26797	1.72863
Avg	1.989755			1.9983	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
104/ Zr	23503.3	13352.33	**56.81%**

4) Removal Torque Test (RTQ)

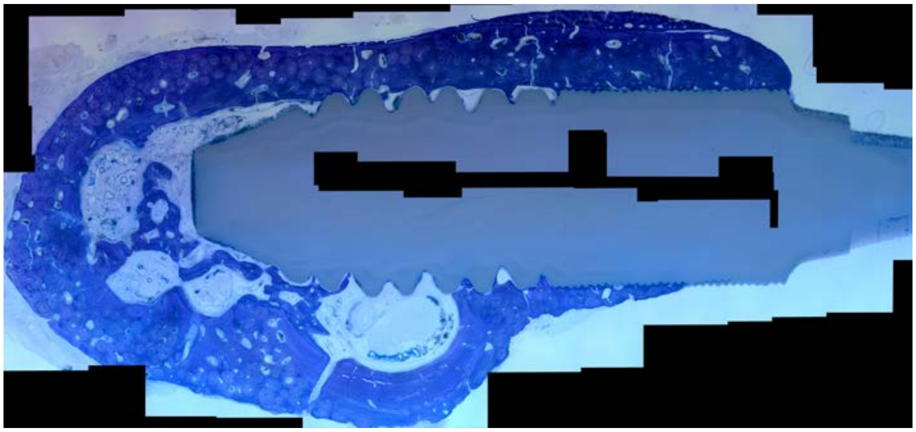


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
104 Zr	16.429	22.229	51	342.073	y = 0.9248x + 1.076	y = 6.5794x - 92.815

5) Periotest value; PTV

Dog/imp	Medial			Distal		
104/Zr	-4.8	-4.6	-3.1	-4.7	-4.6	-4.5
Avg	**-4.383333333**					

#114 ZrO_2



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/imp	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw(pxl	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
114/ Zr	2929.66	2580.06	**88.07%**	2714.31	888.69	**32.74%**	1881.78	1540.59	**81.87%**	2640.86	1384.88	**52.44%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
114 Zr	25212	23481	93.13%	152474	45038	29.54%	34553	18816	54.46%	180391	73165	40.56%

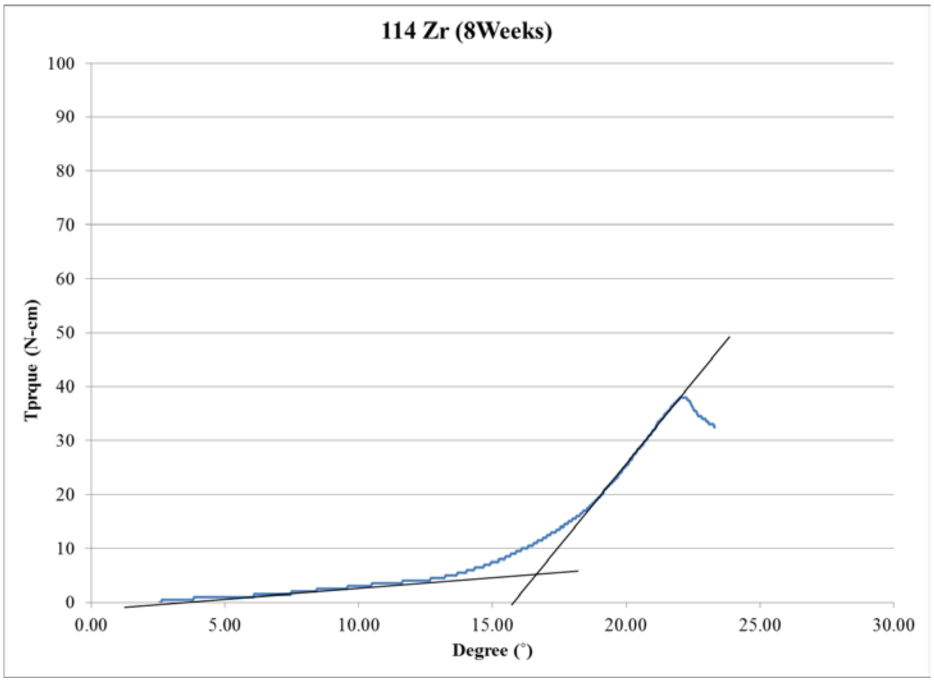
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
114/Zr	N/A	N/A		N/A	N/A
Avg					

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
114/ Zr	N/A	N/A	

4) Removal Torque Test (RTQ)

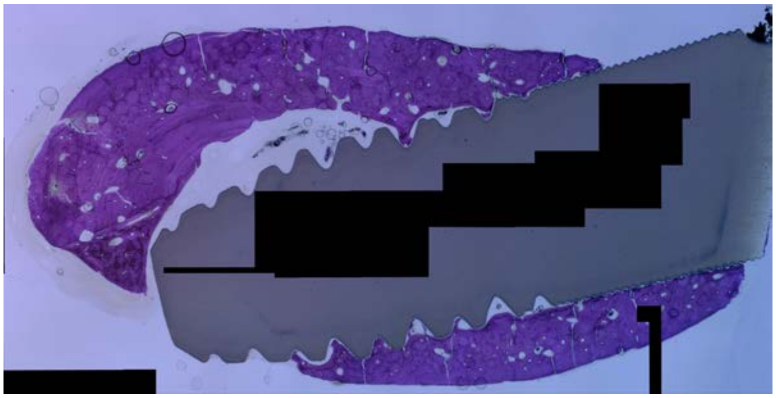


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
114 Zr	16.7	22.25	38	179.3	y = 0.3995x - 0.985	y = 6.0402x - 95.135

5) Periotest value; PTV

Dog/imp	Medial			Distal		
114/Zr	0.2	-0.2	-0.2	-3.6	-2.7	-3.8
Avg	**-1.716666667**					

#124ZrO_2:



1) Bone Implant Contact (BIC), Bone Area (BA)

B IC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
124/ Zr	1394.11	749.1	**53.73%**	1887.39	614.58	**32.56%**	2387.28	2038.14	**85.37%**	2830.85	1176.33	**41.55%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
124 Zr	6576	2264	34.43%	138598	57186	41.26%	22524	11765	52.23%	197579	96154	48.67%

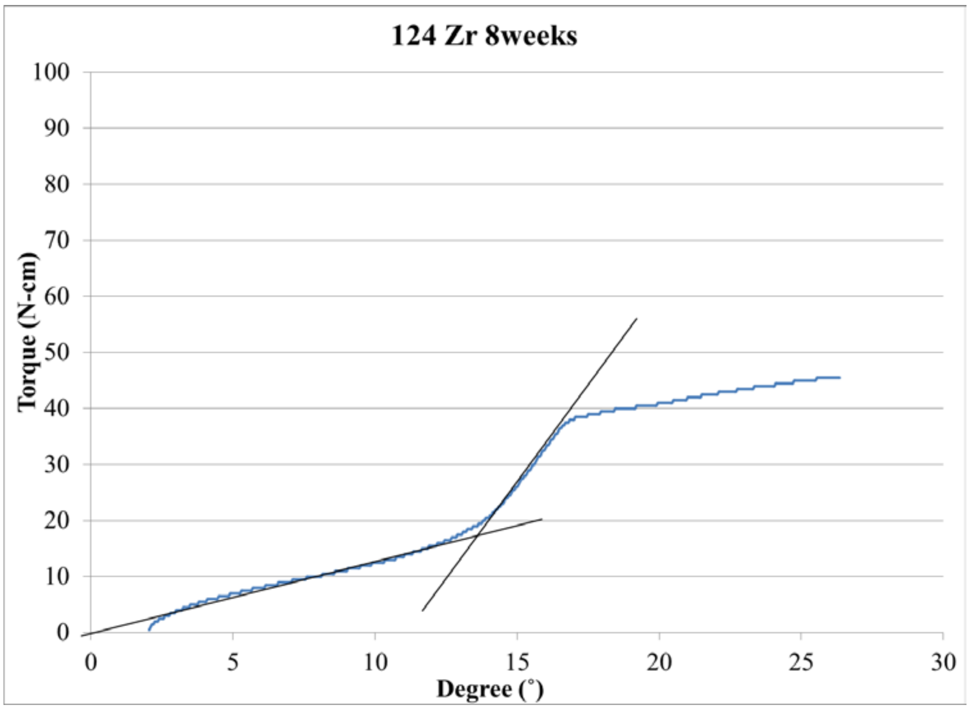
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (5-6w)			**Green-Orange(6-7w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
124/Zr	2.2428	2.70662		1.92087	2.39804
Avg	2.47471			2.159455	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
124/ Zr	16660.29	11171.29	**67.05%**

4) Removal Torque Test (RTQ)



	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
124 Zr	13.8	17.38	38.5	230.988	y = 1.2487x + 0.2138	y = 5.8288x - 60.772

5) Periotest value; PTV

Dog/imp	Medial			Distal		
124/Zr	0.6	1.3	-2.2	-1.7	-2.6	-2.6
Avg	**-1.2**					

12 weeks Shofu thread type Ti

<u>#134Ti</u>

An implant failed/ No histology was available.

1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/Hist/imp	Screw	Bone	**BIC (%)**	Screw	Bone	**BIC (%)**	Screw	Bone	**BIC (%)**	Screw	Bone	**BIC (%)**
134/ Ti						Missing						

BA:

1pxl = 1.335µm	Right				Left			
	Micro		Macro		Micro		Macro	
Dog/ imp	Bone (pxl)	**BA (µm)**	Bone (pxl)	**BA (µm)**	Bone (pxl)	**BA (µm)**	Bone (pxl)	**BA (µm)**
134/Ti				Missing				

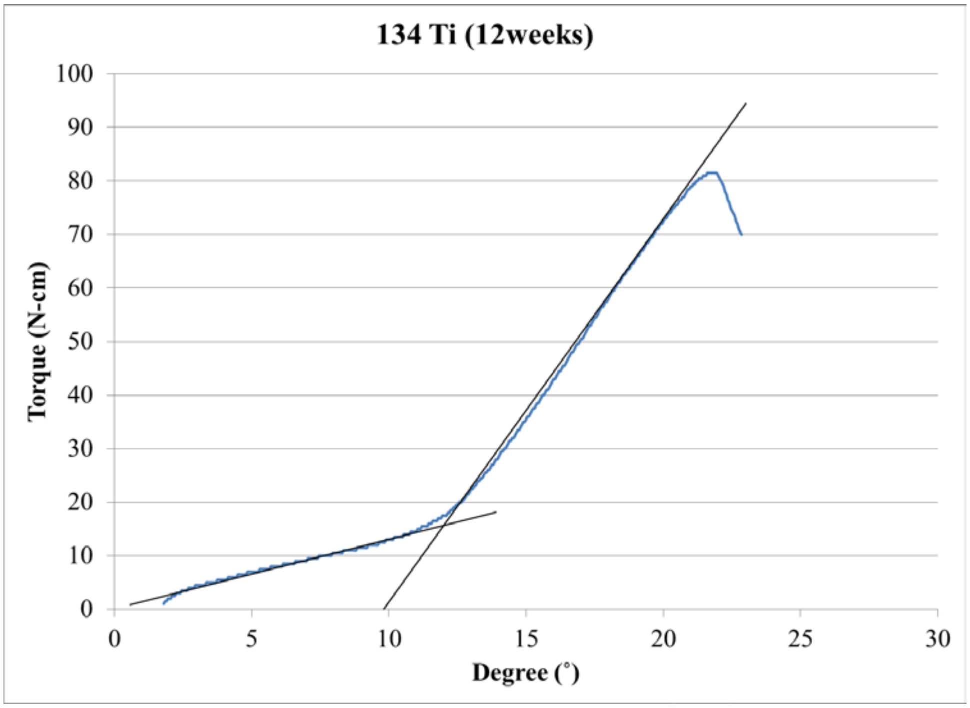
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (9-10w)			Green-Orange(10-11w)	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
134/Ti	N/A	N/A		N/A	N/A
Avg					

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
134/ Ti	Missing		

4) Removal Torque Test (RTQ)

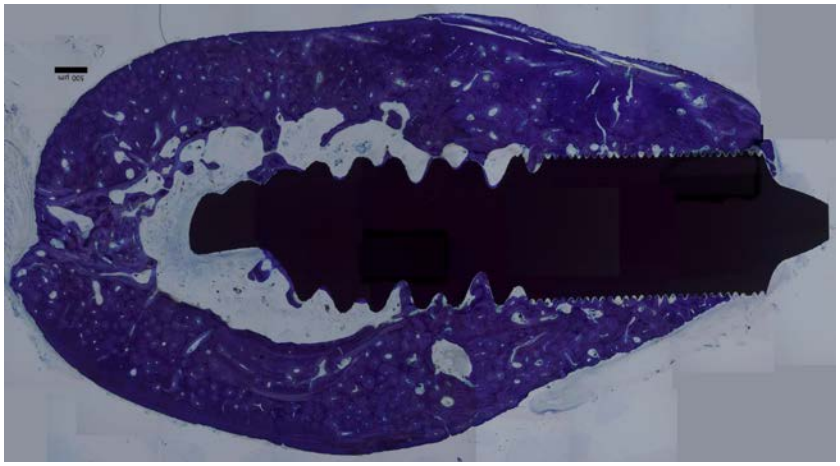


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
134Ti	12.047	21.947	81.5	585.763	y = 1.195x + 0.8295	y = 7.1463x - 71.204

5) Periotest value; PTV

Dog/imp	Medial			Distal		
134/Ti	4.3	4.8	5.2			
Avg	**4.766666667**					

#144Ti:



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
144/ Ti	3854.45	2445.04	**63.43%**	2869.97	1689.16	**58.86%**	3495.49	1290.13	**36.91%**	3071.1	738.51	**24.05%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
144 Ti	85027	43859	51.58%	223155	61310	27.47%	80055	46399	57.96%	278144	157342	56.57%

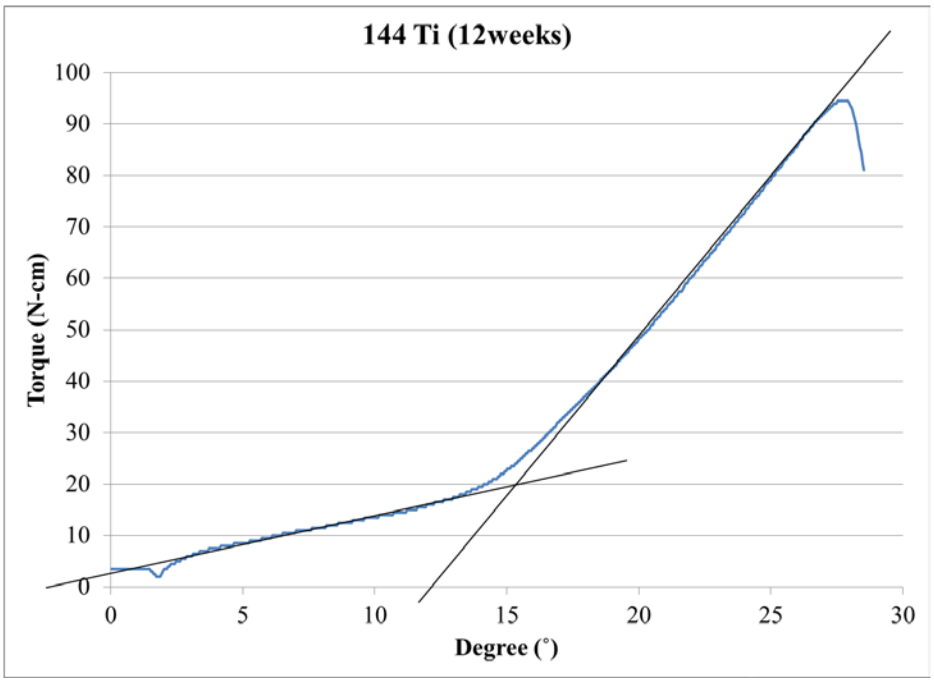
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (9-10w)			**Green-Orange(10-11w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
144/Ti	1.96092	1.78661		2.01966	1.48833
Avg	1.873765			1.753995	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
144/Ti	21362.08	3415.59	**15.99%**

4) Removal Torque Test (RTQ)

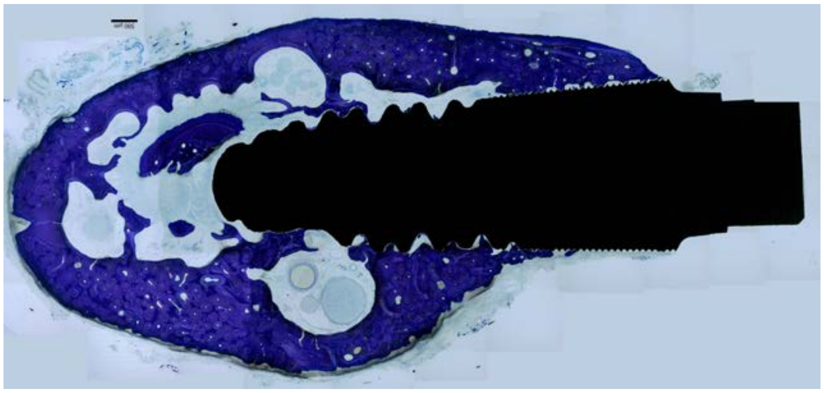


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
144Ti	15.223	27.923	94.5	917.23	y = 1.1365x + 2.4328	y = 6.1721x - 75.158

5) Periotest value; PTV

Dog/imp	Medial			Distal		
144/Ti	1.4	0.6	0.6	-1.7	-1.8	-1.8
Avg	**-0.45**					

#154Ti:



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	BIC	Screw (px	Bone (pxl)	BIC	Screw (px	Bone (pxl)	BIC	Screw (px	Bone (pxl)	BIC
154/ Ti	3641.04	1097.92	**30.15%**	2553.57	764.29	**29.93%**	1073.7	605.39	**56.38%**	2385.5	1463.73	**61.36%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
154 Ti	55317	15990	28.91%	178234	38241	21.46%	20909	10317	49.34%	156002	86866	55.68%

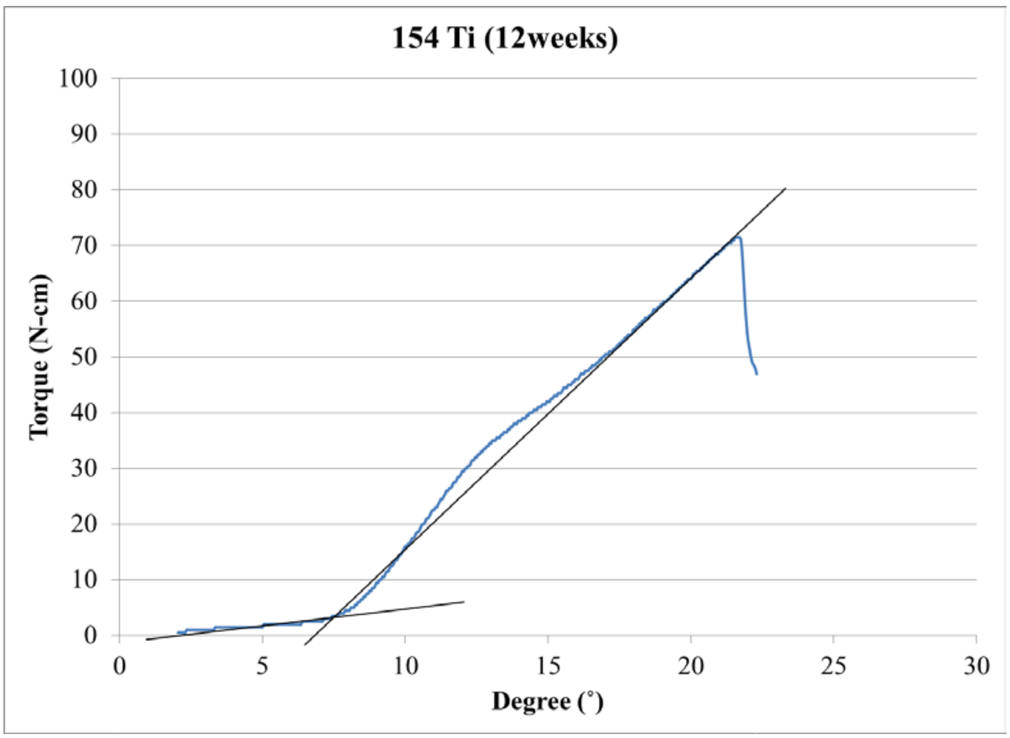
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (μm/day)					
	Red-Green (9-10w)			**Green-Orange(10-11w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
154/Ti	1.93155	2.57884		1.6779	2.1955
Avg	2.255195			1.9367	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
154/Ti	21993.46	6160.49	**28.01%**

4) Removal Torque Test (RTQ)



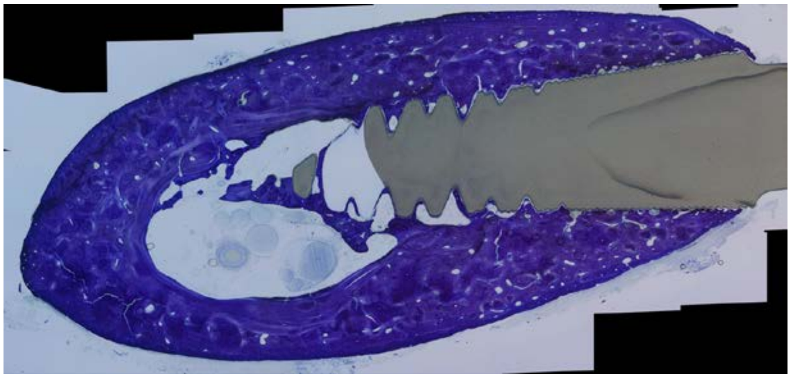
	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
154Ti	12.15	21.7	71.5	564.137	y = 0.4746x - 0.5295	y = 4.3181x - 22.394

5) Periotest value; PTV

Dog/imp	Medial			Distal		
154/Ti	-0.9	-0.8	-1.2	-1.8	-1.7	-1.2
Avg	**-1.266666667**					

12 weeks Shofu thread type ZrO_2

#134 ZrO_2



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

	Right						Left					
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
134/ Zr	3180.56	1972.6	**62.02%**	4174.61	2986.37	**71.54%**	2846.19	2504.23	**87.99%**	3426.09	1750.98	**51.11%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
134 Zr	24227	4866	20.09%	293637	213814	72.82%	49830	24853	49.88%	221183	103994	47.02%

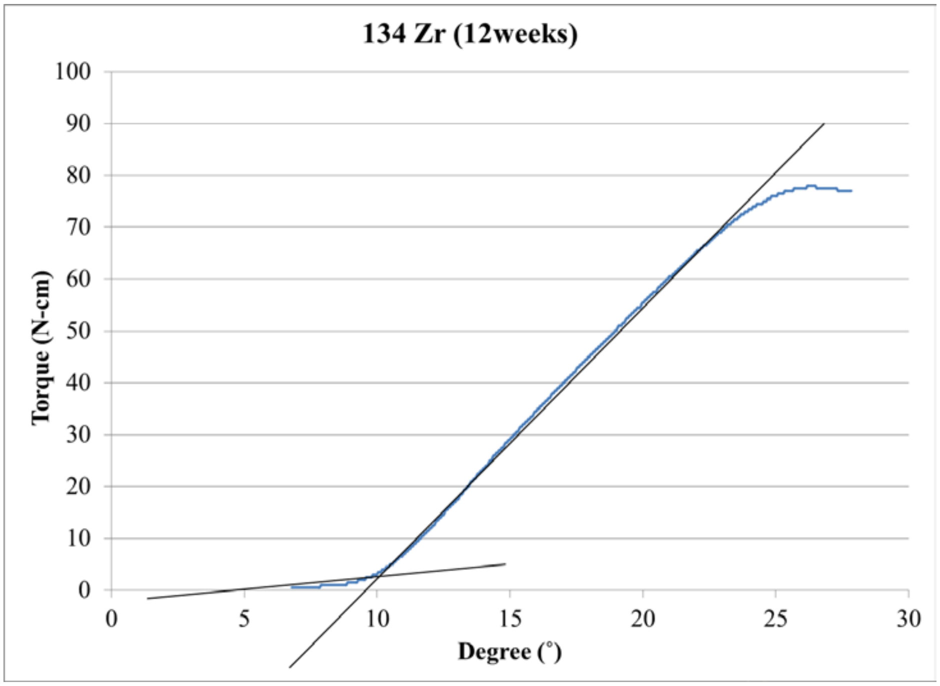
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (9-10w)			Green-Orange(10-11w)	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
134/Zr	2.41559	2.50332		2.01127	2.16041
Avg	2.459455			2.08584	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
134/ Zr	22112.77	9895.13	**44.75%**

4) Removal Torque Test (RTQ)

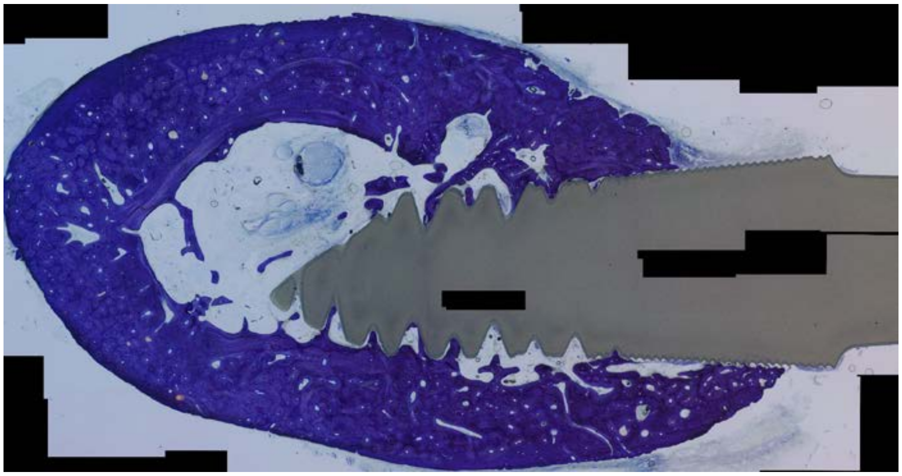


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
134Zr	9.987	26.337	78	741.524	y = 0.1739x - 0.4954	y = 4.9262x - 45.124

5) Periotest value; PTV

Dog/imp	Medial			Distal		
134/Zr	-3.5	-5	-3.7	-1.8	-1.5	-1.4
Avg	**-2.816666667**					

#144 ZrO_2



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

			Right						**Left**			
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
144/ Zr	931.92	617.82	**66.30%**	3096.56	1414.11	**45.67%**	2467.05	731.73	**29.66%**	3229.91	1772.76	**54.89%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
144 Zr	8802	7262	82.50%	220489	127348	57.76%	44402	19417	43.73%	216244	70235	32.48%

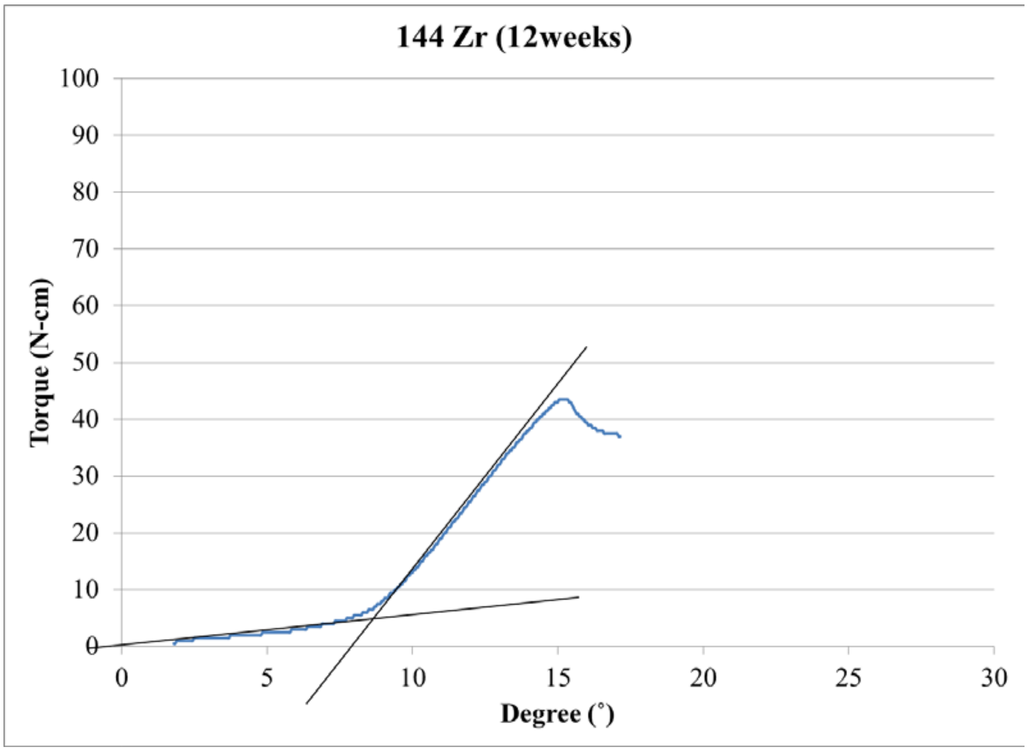
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (μm/day)					
	Red-Green (9-10w)			**Green-Orange(10-11w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
144/Zr	2.65207	2.42932		2.32214	2.22144
Avg	2.540695			2.27179	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
144/ Zr	21446.67	6399.59	**29.84%**

4) Removal Torque Test (RTQ)

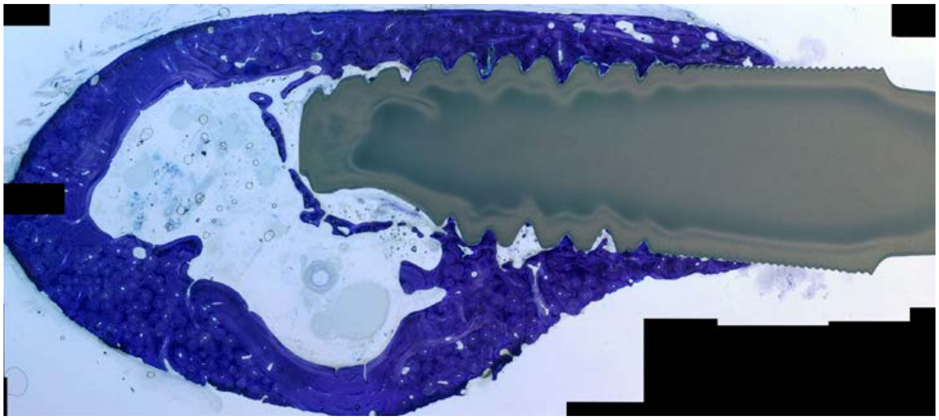


	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
144Zr	8.592	15.342	43.5	191.62	y = 0.6341x - 0.6145	y = 6.0884x - 47.486

5) Periotest value; PTV

Dog/imp	Medial			Distal		
144/Zr	-1.5	-0.7	-1.6	-2.2	-2.1	-1.8
Avg	**-1.65**					

#154 ZrO_2



1) Bone Implant Contact (BIC), Bone Area (BA)

BIC:

			Right						**Left**			
		Micro			Macro			Micro			Macro	
Dog/Hist/	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**	Screw (px	Bone (pxl)	**BIC (%)**
154/ Zr	1545.11	525.99	**34.04%**	2823.16	2195.62	**77.77%**	1773.11	1207.26	**68.09%**	2629.87	1319.52	**50.17%**

BA:

	Right						Left					
	Micro			Macro			Micro			Macro		
Dog / Imp	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)	Total (pxl)	Bone (pxl)	BA(%)
154 Zr	19279	9871	51.20%	147367	119363	81.00%	10583	5499	51.96%	198784	94595	47.59%

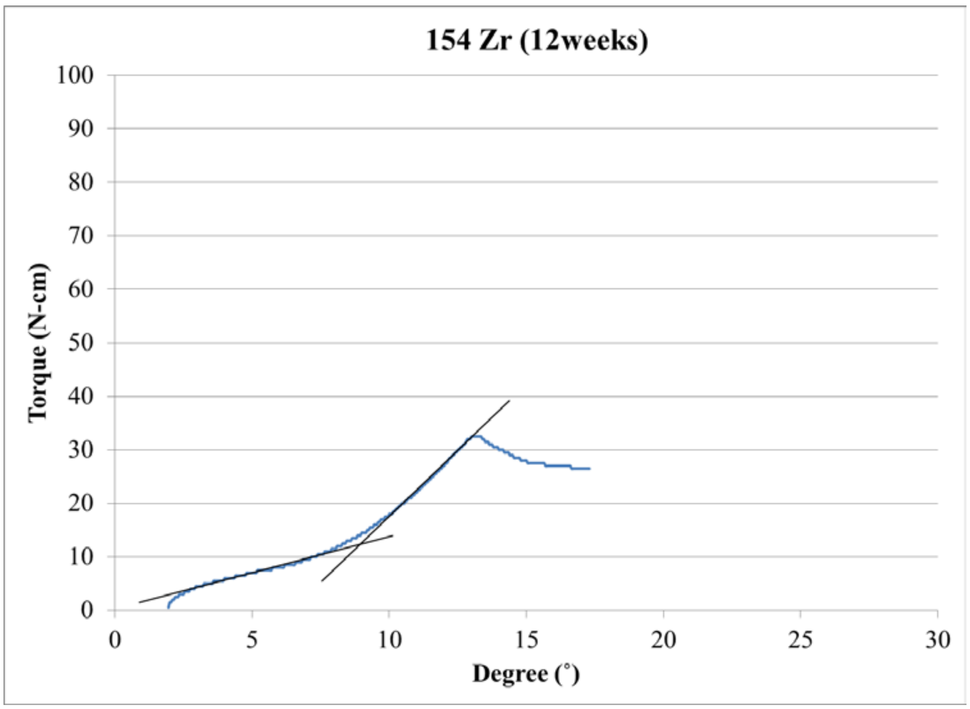
2) Mineral Apposition Rate (MAR)

avg Mineral appositon rate (µm/day)					
	Red-Green (9-10w)			**Green-Orange(10-11w)**	
Dog/Hist/imp	**Right**	**Left**		**Right**	**Left**
154/Zr	2.40453	2.50751		2.21152	2.11388
Avg	2.45602			2.1627	

3) Bone Forming Area (BFA)

Dog/imp	Cortical length(pxl)	Total of turned over length (pxl)	%
154/Zr	24841.81	11198.72	**45.08%**

4) Removal Torque Test (RTQ)



	onset degree (°)	Peak degree (°)	Peak Value (N-cm)	Area under the curve (N-cm degree)	Slope:1	Slope:2
154Zr	8.7	13.35	32.5	156.238	y = 1.376x - 0.0154	y = 4.7397x - 29.686

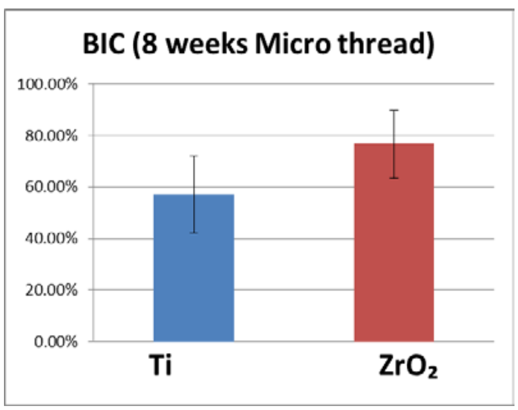
5) Periotest value; PTV

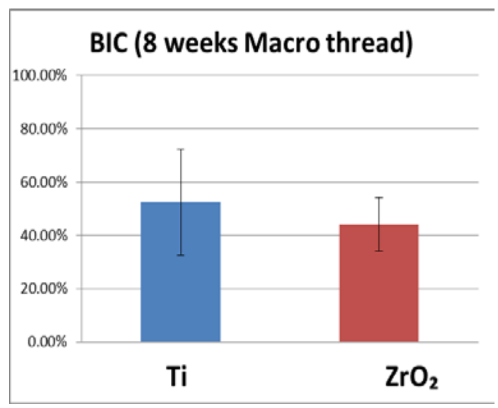
Dog/imp	Medial			Distal		
154/Zr	-1.4	-3.4	-3.5	-2.5	-3.5	-3.6
Avg	**-2.983333333**					

Summary of 8 weeks results

a) Bone implant contact (%).

BIC(%)	Micro		Macro	
8 weeks	Ti	ZrO_2	Ti	ZrO_2
	62.38%	82.60%	41.54%	54.38%
	77.19%	68.44%	65.98%	51.71%
	49.42%	88.07%	81.32%	32.74%
	33.97%	81.97%	31.55%	52.44%
	65.59%	53.73%	33.68%	32.65%
	53.79%	85.37%	60.27%	41.55%
Avg	**57.06%**	**76.70%**	**52.39%**	**44.25%**
SD	14.89%	13.14%	19.93%	10.00%



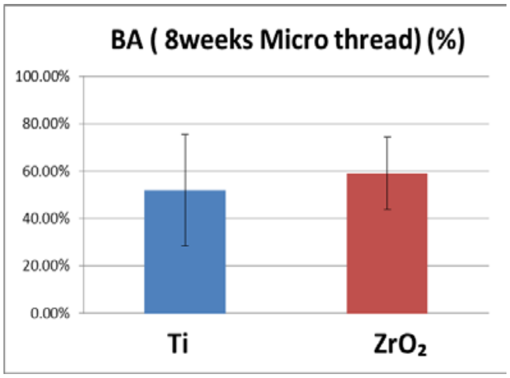


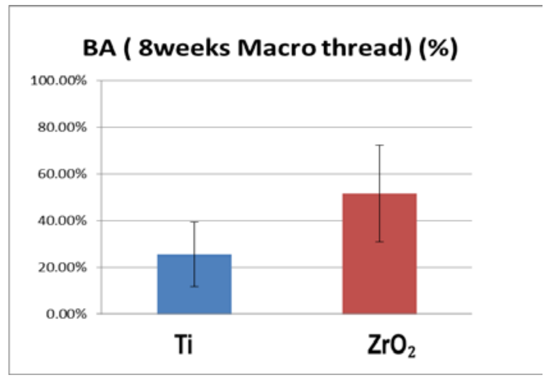
Total three implants with two sides (right and left) from each group were analyzed. The average bone implant contact in the micro thread region of titanium and zirconia were 57.06%, 76.70% respectively. In the macro thread regionss, Mean BIC was 52.93% and 44.25% with titanium and zirconia, respectively. Compared to our results in our first implant project completed in 2013, there were no statistical differences between the previous study and this current study in the BIC of zirconia implants placed for 8 weeks. The BIC for the Ti implants in this current study was statistically lower than the results in our previous study.

In this current study, there were no statistical differences in BIC in both micro and macro thread regions between titanium and zirconia implants at 8 weeks. ($p>0.05$)

b) Bone Area (%).

Bone Are (%)	8 Weeks		
Shofu Thread Ti (Blast +acid) Micro	76.52%	49.76%	29.68%
Shofu Thread Ti (Blast +acid) Macro	40.91%	14.08%	21.52%
Shofu Thread ZrO_2 (Blast +acid) Micro	60%	73.79%	43.33%
Shofu Thread ZrO_2 (Blast +acid) Macro	75.05%	35.05%	44.96%



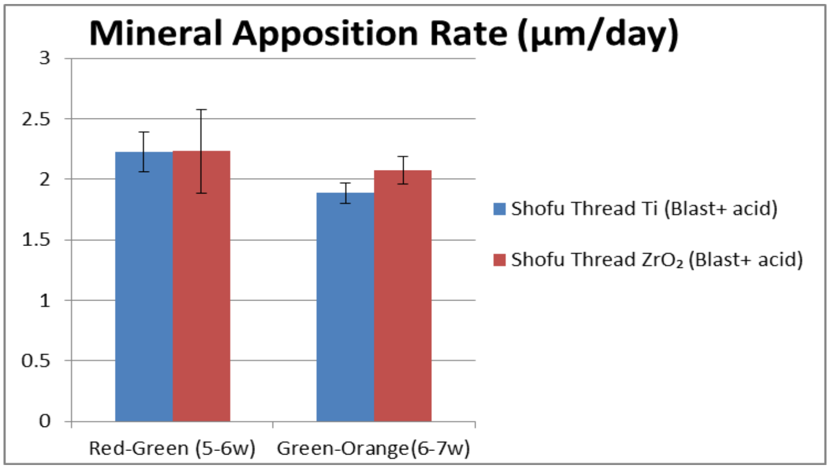


Total three implants in each group were evaluated. The mean bone area in micro thread regions with titanium and zirconia were 51.99% and 59.04%, respectively. In the macro thread areas, BA was 25.5%, and 51.69% for titanium and zirconia, respectively. Compared to our results in the first implant project completed in 2013, there were no statistical differences between the previous study and this current study in the BA of both zirconia and titanium implants.

There was no statistical difference in BA between titanium and zirconia implants on the micro threads ($p>0.05$). However, on the macro threads, a statistical difference was found ($P<0.05$) where zirconia implants showed higher BA at 8 weeks.

c) Mineral Apposition Rate (µm/day).

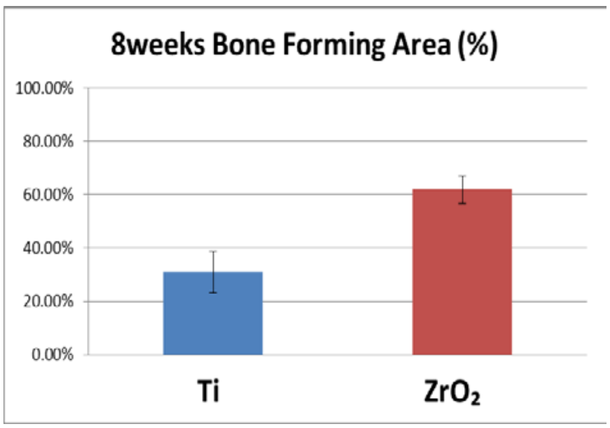
Mineral Apposition Rate (µm/day)	**Red-Green (5-6w)**	**Green-Orange(6-7w)**
Shofu Thread Ti (Blast+ acid)	2.2264	1.88578
Shofu Thread ZrO_2 (Blast+ acid)	2.23223	2.07888



In 5-6 weeks post implant placement specimens, mineral apposition rates were 2.2264 µm with titanium, and 2.2322 µm with zirconia implants. In 6-7 weeks post implant placement specimens, mineral apposition rates were 1.88578 µm with titanium, and 2.07888 µm with zirconia. Zirconia might stimulate more mineral apposition rate around implant at 6-7 weeks post implant installation. However, there were no statistical differences between titanium and zirconia implants in MAR at 5-6 week and 6-7 weeks post-implantation ($p>0.05$).

d) Bone Forming Area (%).

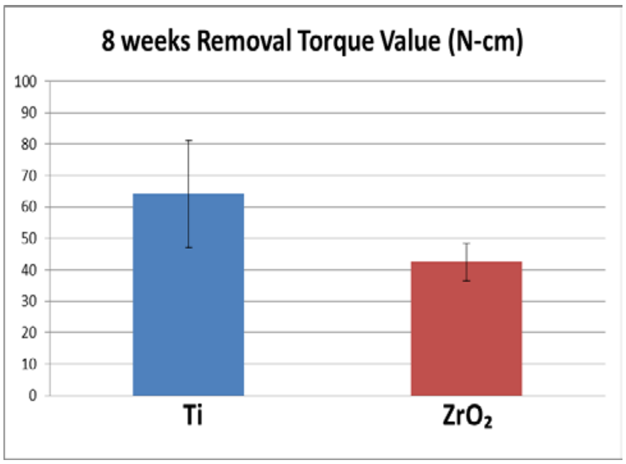
8weeks Bone Forming Area		
	Ti	Zr
	36.85%	56.81%
	35.92%	67.05%
	20.24%	
Avg	**31.01%**	**61.93%**
STD	**0.076217026**	**0.0512**



Since one implant from the zirconia goup was not available for intravenous fluorescent markers, three implants from titanium and two zirconia implants were evaluated. Average bone forming area with titanium implants and zirconia implants was 31.01% and 61.39% respectively. There was a statistical difference between titanium and zirconia implants in BFA at 8 weeks post-implantation where zirconia implants showed higher BFA at 8 weeks. ($p<0.05$)

e) Removal Torque Value (N-cm).

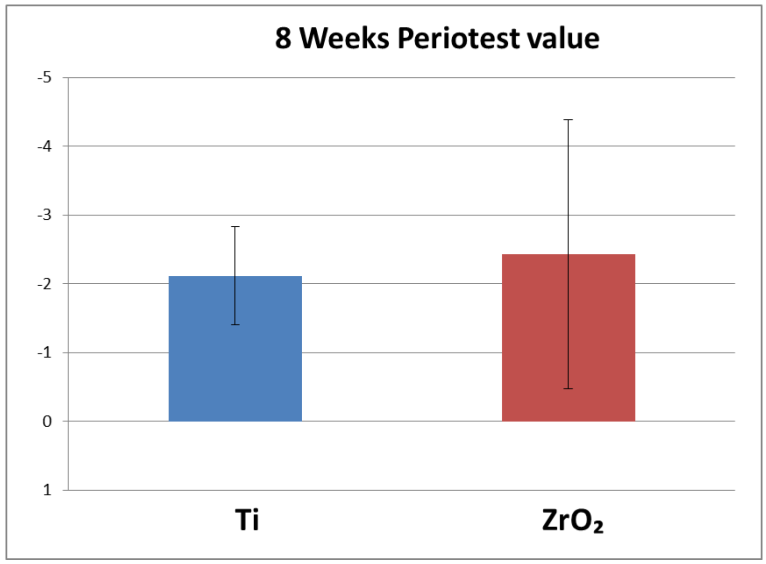
Removal Torque Value (N-cm)		
8 Weeks	Ti	ZrO_2
	59	51
	46.5	38
	87	38.5
Avg	**64.16667**	**42.5**
SD	16.93287	6.013873



Three implants from each group were examined for removal torque values. Removal torque values with titanium and zirconia implants at 8 weeks were 64.1N-cm, and 42.5N-cm, respectively. Even though the titanium implant group had higher removal torque than zirconia implants, these differences did not reach statistical significance between titanium and zirconia implants ($p>0.05$) at 8 weeks.

f) Perio test value (PTV).

8Weeks	Ti	Zr
	-2.4	-4.8
	-0.9	-4.6
	-2.4	-3.1
	-2.2	-4.7
	-2.6	-4.6
	-1.9	-4.5
	-3.4	0.2
	-2.4	-0.2
	-1.4	-0.2
	-1.3	-3.6
	-2.3	-2.7
	-2.1	-3.8
	-3.7	0.6
	-2.4	1.3
	-2.3	-2.2
	-1.3	-1.7
	-1.2	-2.6
	-1.9	-2.6
Avg	**-2.11667**	**-2.43333**
SD	**0.708872**	**1.955619**

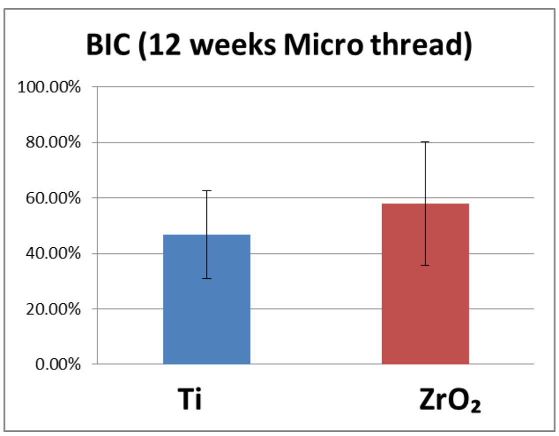


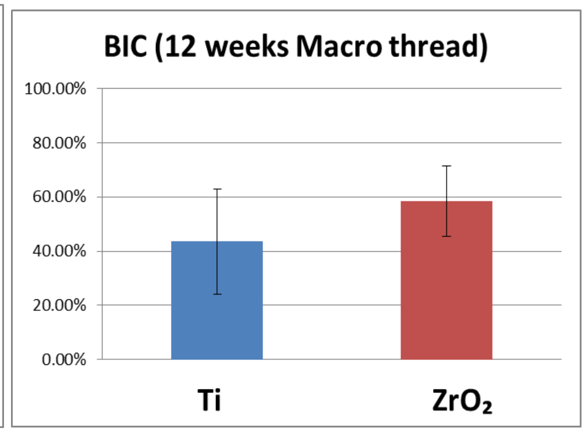
Six implants from each group, each implant was measured three times with Periotest®. Average Periotest values were -2.11 PTV in titanium implants, and -2.43 PTV in the zirconia implant group. There was no statistical difference between titanium and zirconia implants in their Periotest value ($p>0.05$) at 8 weeks.

Summary of 12 week results

a) Bone implant contact (%).

BIC (%)	Micro		Macro	
12 weeks	Ti	ZrO_2	Ti	ZrO_2
	63.43%	62.02%	58.86%	71.54%
	30.15%	87.99%	29.93%	45.67%
	36.91%	66.30%	24.05%	77.77%
	56.38%	29.66%	61.36%	51.11%
		34.04%		54.89%
		68.09%		50.17%
Avg	**46.72%**	**58.02%**	**43.55%**	**58.53%**
SD	15.74%	22.20%	19.30%	12.98%

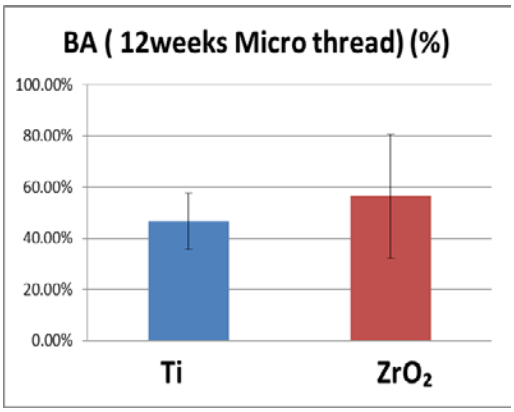


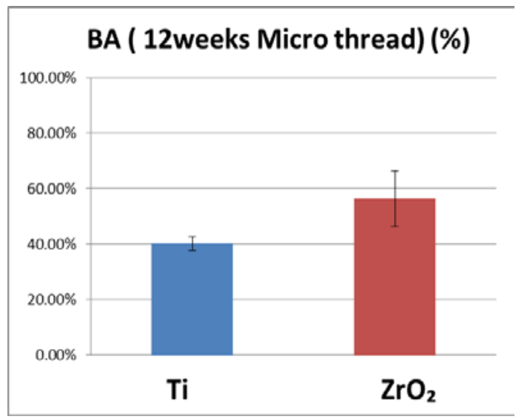


Since one implant from titanium group failed to achieve osseointegration, a total of two implants in the titanium group and three implants in the zirconia group with two sides (right and left) were analyzed. Average BIC in the micro threads was 46.72% and 58.02% with titanium and zirconia, respectively. In the macro threads, BIC was 43.55% and 58.53% for titanium and zirconia, respectively. There were no statistical differences in BIC in both micro and macro thread areas between titanium and zirconia implants at 12 weeks. ($p>0.05$)

b) Bone Area (%).

Bone Are (%)	12 weeks		
Shofu Thread Ti (Blast +acid) Micro	54.77%	39.12%	N/A
Shofu Thread Ti (Blast +acid) Macro	42.02%	38.57%	N/A
Shofu Thread ZrO_2 (Blast +acid) Micro	34.98%	82.50%	51.58%
Shofu Thread ZrO_2 (Blast +acid) Macro	59.92%	45.12%	64.29%

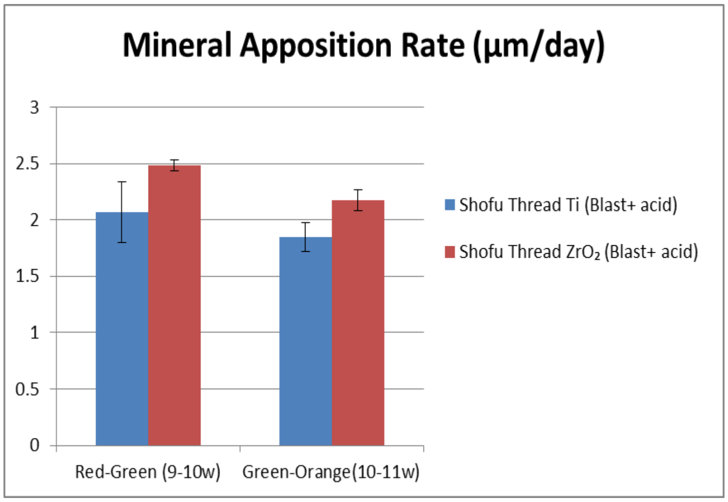




Total two titanium implants with and three zirconia implants were evaluated for BA at 12 weeks. Mean BA in the micro threads was 46.95% for , titanium and 56.35% for zirconia. In the macro threads, BA was 40.30% and 56.44% for titanium and zirconia, respectively. There were no statistical differences in BA in both micro and macro thread areas between titanium and zirconia implants at 12 weeks. ($p>0.05$)

c) Mineral Apposition Rate (µm/day).

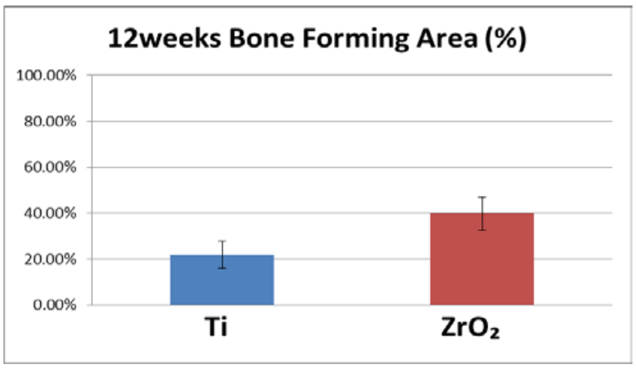
Mineral Apposition Rate (µm/day)	**Red-Green (9-10w)**	**Green-Orange(10-11w)**
Shofu Thread Ti (Blast+ acid)	2.06448	1.84533
Shofu Thread ZrO_2 (Blast+ acid)	2.48539	2.17344



In the 9-10 weeks post implant placement specimens, mineral apposition rates were 2.06448 µm with titanium, and 2.48539 µm with zirconia implants. In the 10-11 weeks post implant placement specimens, mineral apposition rates were 1.84533 µm with titanium, and 2.17344 µm with zirconia. There were statistical differences between titanium and zirconia implants where zirconia implants showed higher MAR at 10 weeks and 11 weeks. ($p<0.05$)

d) Bone Forming Area (%).

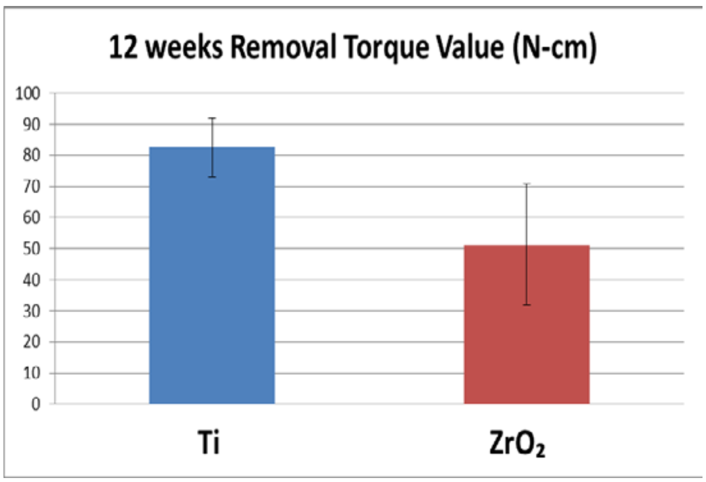
12 weeks Bone Forming Area		
	Ti	Zr
	15.99%	44.75%
	28.01%	29.84%
		45.08%
Avg	**22.00%**	**39.89%**
SD	**6.01%**	**7.11%**



A total of two titanium implants and three zorconia implants were evaluated. Mean bone forming area was 22.0% with titanium implants and 39.89% with zirconia implants. There was no statistical difference in BFA between titanium and zirconia implants at 12 weeks. ($p>0.05$)

e) Removal Torque Value (N-cm).

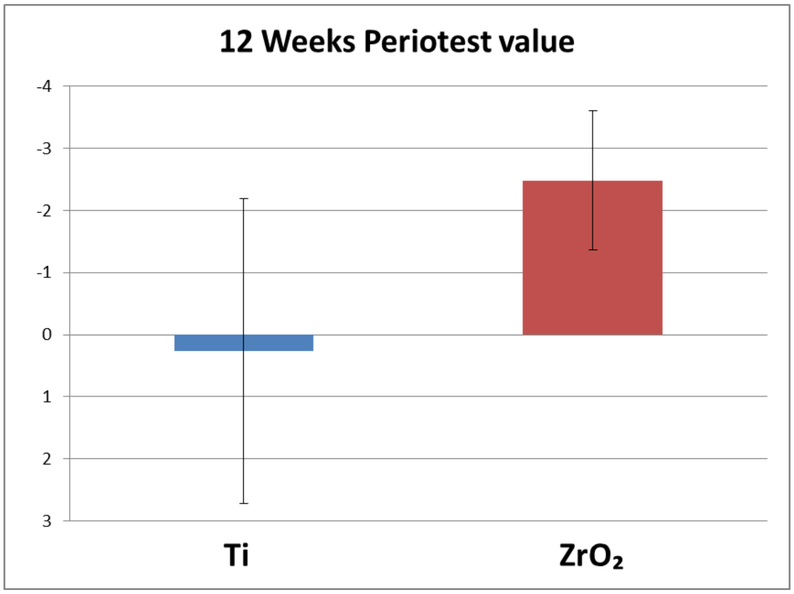
Removal Torque Value (N-cm)		
12 Weeks	Ti	ZrO_2
	81.5	78
	94.5	43.5
	71.5	32.5
Avg	**82.5**	**51.33333**
SD	9.416298	19.38356



Three implants from each group were examined for removal torque value. Removal torque value with titanium and zirconia implnts at the time of 8weeks were 82.5N-cm and 51.3N-cm, respectively. Although average removal torque with titanium implants was higher than the zirconia implant group, there was no statistical difference between titanium and zirconia implants in the RTQ value at 12 weeks. ($p>0.05$)

f) Periotest value.

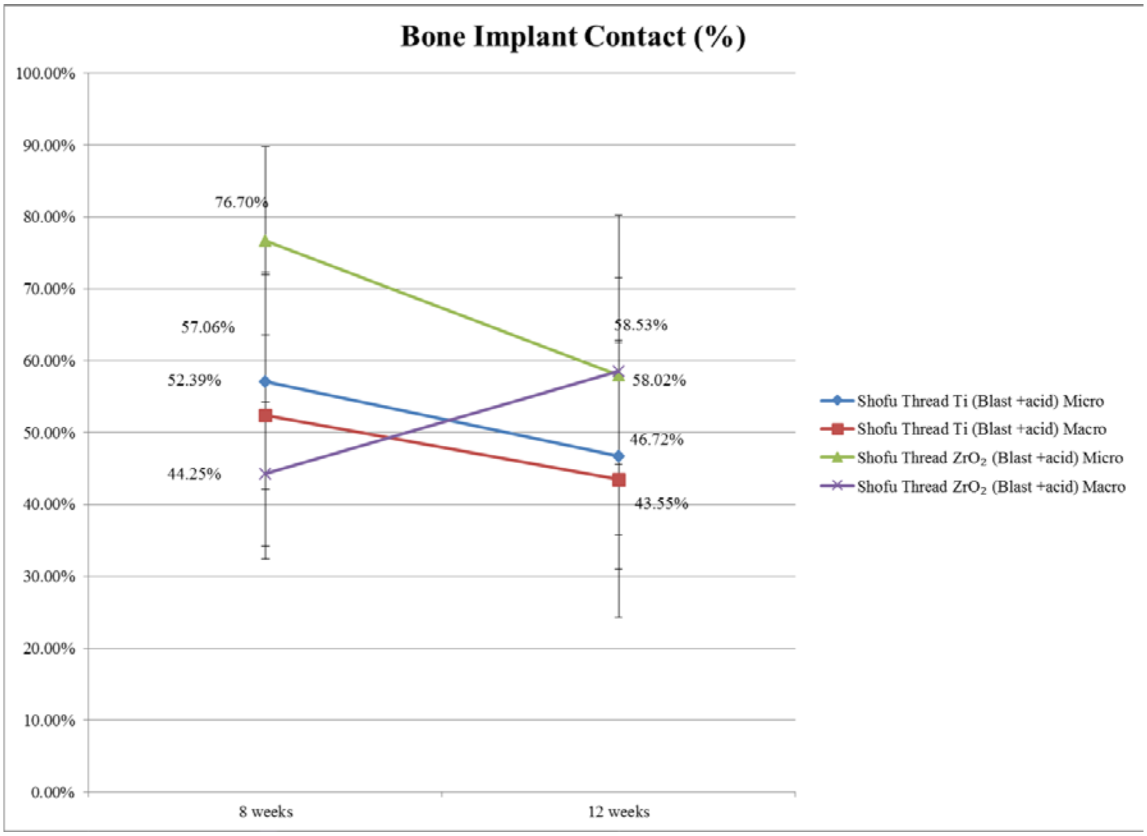
12Weeks	Ti	Zr
	4.3	-3.5
	4.8	-5
	5.2	-3.7
	1.4	-1.8
	0.6	-1.5
	0.6	-1.4
	-1.7	-1.5
	-1.8	-0.7
	-1.8	-1.6
	-0.9	-1.8
	-0.8	-2.1
	-1.2	-2.2
	-1.8	-1.4
	-1.7	-3.4
	-1.2	-3.5
		-2.5
		-3.5
		-3.6
Avg	**0.266667**	**-2.48333**
SD	**2.449671**	**1.115671**



Six implants from each group were evaluated with Periotest, with each implant measured three times with Perio test®. Average Periotest values were 0.267 PTV in titanium implants, and -2.483 PTV in in zirconia implants. Zirconia implant group demonstrated higher mean PTV, and there were statistical differences for PTV in both micro and macro thread groups between titanium and zirconia implants. ($p<0.05$)

Discussions

Bone Implant Contact



BIC(%)	**Micro**		**Macro**		**BIC (%)**	**Micro**		**Macro**	
8 weeks	Ti	ZrO_2	Ti	ZrO_2	**12 weeks**	Ti	ZrO_2	Ti	ZrO_2
	62.38%	82.60%	41.54%	54.38%		63.43%	62.02%	58.86%	71.54%
	77.19%	68.44%	65.98%	51.71%		30.15%	87.99%	29.93%	45.67%
	49.42%	88.07%	81.32%	32.74%		36.91%	66.30%	24.05%	77.77%
	33.97%	81.97%	31.55%	52.44%		56.38%	29.66%	61.36%	51.11%
	65.59%	53.73%	33.68%	32.65%			34.04%		54.89%
	53.79%	85.37%	60.27%	41.55%			68.09%		50.17%

(P>0.05 in all groups)

Putting all results together, in general, the BICs decreased from 8 to 12 weeks with the exception of the macrothread area in the ZrO_2 implants. These decreases might be the result of the remodeling activities around the implants that occurred between 8 and 12 weeks. It was observed that at 12 weeks, ZrO_2 implants have higher averaged BIC than Ti implants in both the micro- and macro-thread area, although the differences were not statistically significant. A larger sample size would be needed to definitely determine actual differences between groups. The percentage of BIC of ZrO_2 implants from the current study was compared to other study. (Fig.11) Gahlert study used acid-etched implants with placed in the maxilla in a miniture pig model. Langhoff et al. used SLA implants placed in the illiac crest in sheep, and Shin et al. used non-coated zirconia implants in rabbits. The results were not directly compared because of differences in implant surface treatment and the animal model used. However, the SLA surface treatment might improve BIC and the BIC might be highere with in areas of higher bone density.

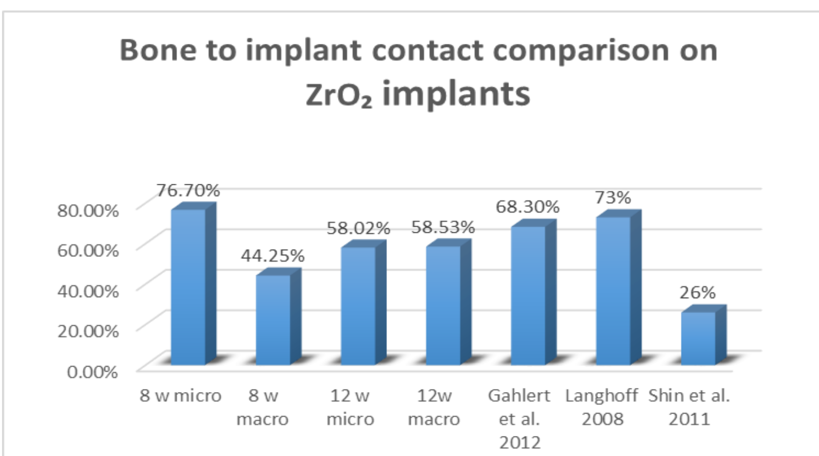
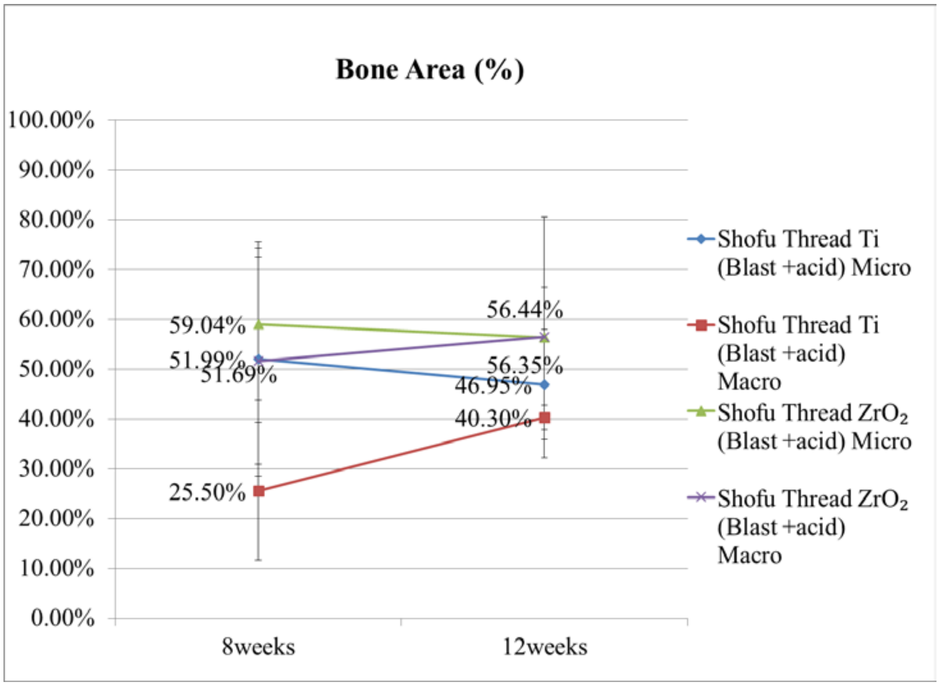


Fig. 11. Bone to implant contact comparison on ZrO_2 implant.

Bone Area



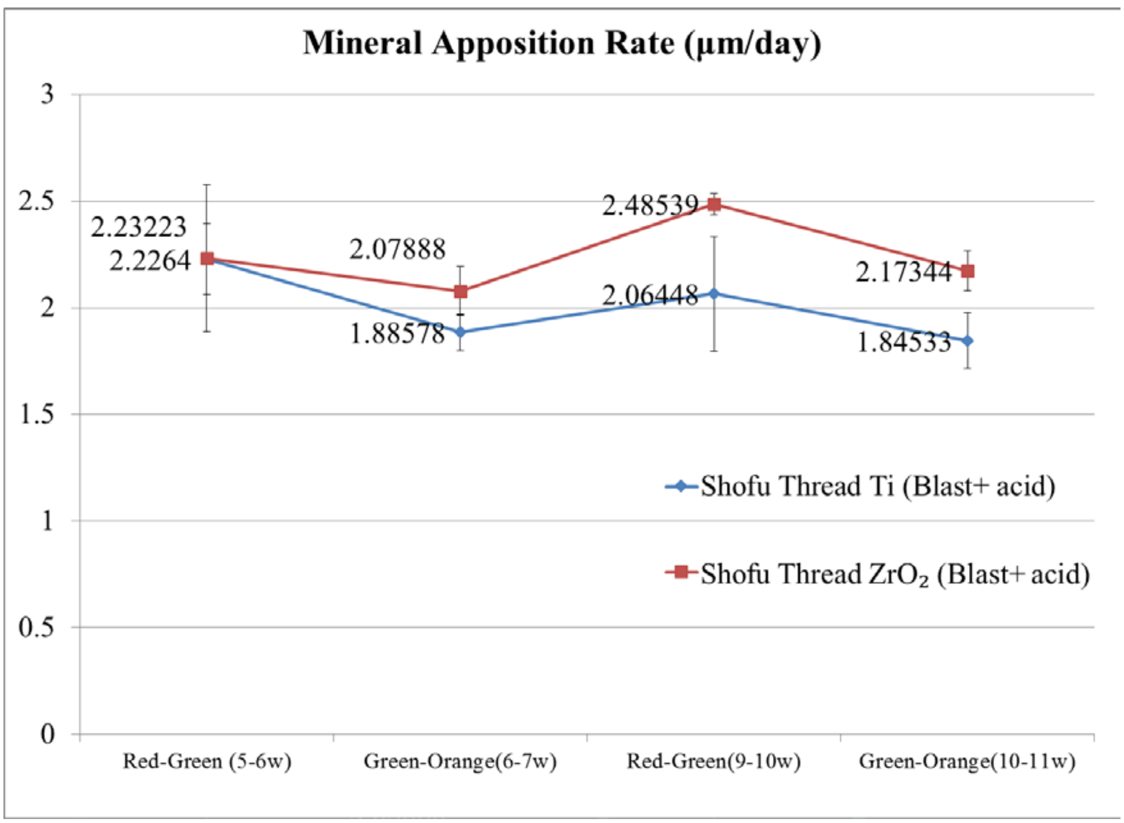
Bone Are (%)	8 Weeks			12 weeks		
Shofu Thread Ti (Blast +acid) Micro	76.52%	49.76%	29.68%	54.77%	39.12%	N/A
Shofu Thread Ti (Blast +acid) Macro	40.91%	14.08%	21.52%	42.02%	38.57%	N/A
Shofu Thread ZrO_2 (Blast +acid) Micro	60%	73.79%	43.33%	34.98%	82.50%	51.58%
Shofu Thread ZrO_2 (Blast +acid) Macro	75.05%	35.05%	44.96%	59.92%	45.12%	64.29%

In both titanium and zirconia implants, there was increase in BA in the macro thread area from 8 to 12 weeks. However, BA decreased in the micro thread area from 8 to 12 weeks for both implant types. The decrease in bone area at the micro threads might be due to bone remodeling. The BA increase in the macro thread area from 8 to 12 weeks might be due to the larger areas between the thread that require longer time for bone fill in this region. At 8 and 12 weeks, ZrO_2 implants showed higher mean BA than Ti implants in both the micro- and macrothread area.

Mineral Apposition Rate (MAR)

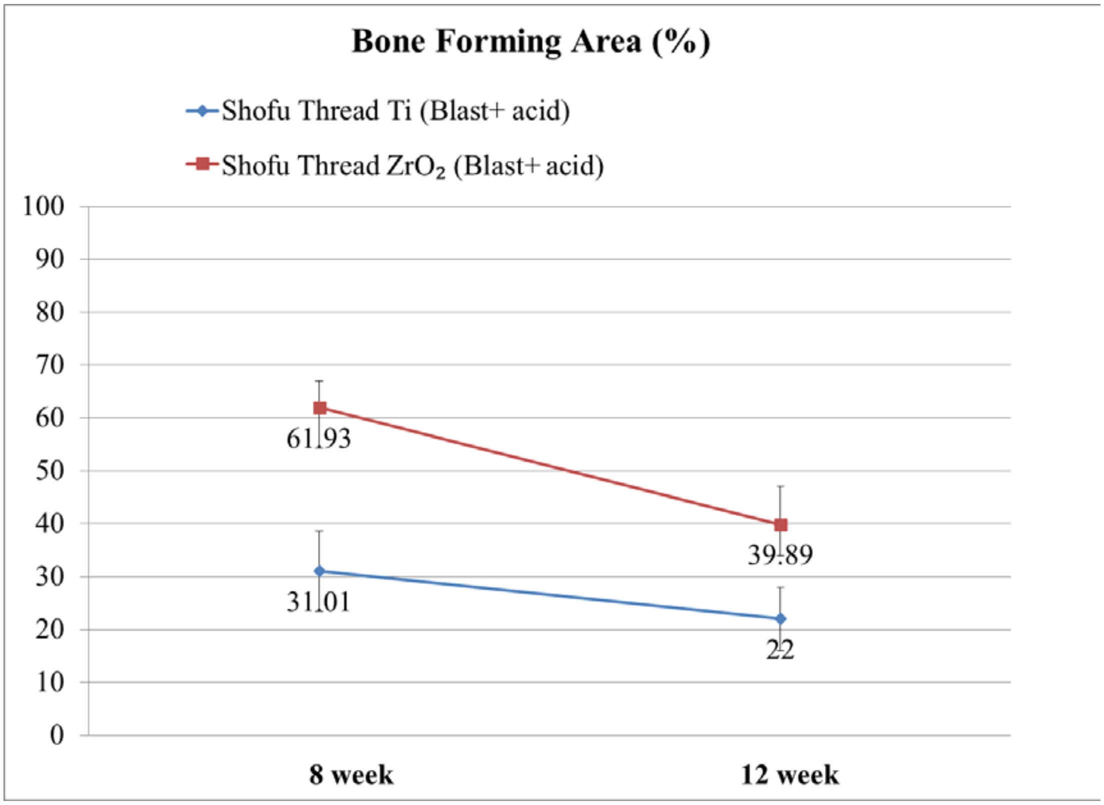
Shofu Thread Ti (Blast+ acid) avg Mineral appositon rate (µm/day)				
	Red-Green (5-6w)	**Green-Orange(6-7w)**	**Red-Green(9-10w)**	**Green-Orange(10-11w)**
	2.09957	1.79634	1.87377	1.75396
	2.16366	1.96474	2.25520	1.93670
	2.41597	1.89628		
Avg	**2.22640**	**1.88578**	**2.06448**	**1.84533**
SD	0.1673	0.0847	0.2697	0.1292

Shofu Thread ZrO_2 (Blast+ acid) avg Mineral appositon rate (µm/day)				
	Red-Green (5-6w)	**Green-Orange(6-7w)**	**Red-Green(9-10w)**	**Green-Orange(10-11w)**
	1.989755	1.9983	2.459455	2.08584
	2.47471	2.159455	2.540695	2.27179
			2.45602	2.1627
Avg	**2.23223**	**2.07888**	**2.48539**	**2.17344**
SD	0.3429	0.1140	0.0479	0.0934



There was a higher mineral apposition rate around zirconia implants at 10 and 11 weeks. The higher mineral apposition rate might be a result of biomechanical or chemical difference. It might be that the larger difference in elasticity between bone and zirconia stimulates the bone to respond with a faster deposition rate to stabilize the interface between bone and zirconia. It can also be that the chemical nature of zirconia stimulates a higher mineralization activity in osteoblasts around the zirconia implant due to its surface chemistry and/or topology. Further experiments will be needed to clarify the mechanism. Nonetheless, the higher BA found around in zirconia implants were consistent with the higher MAR around these implants.

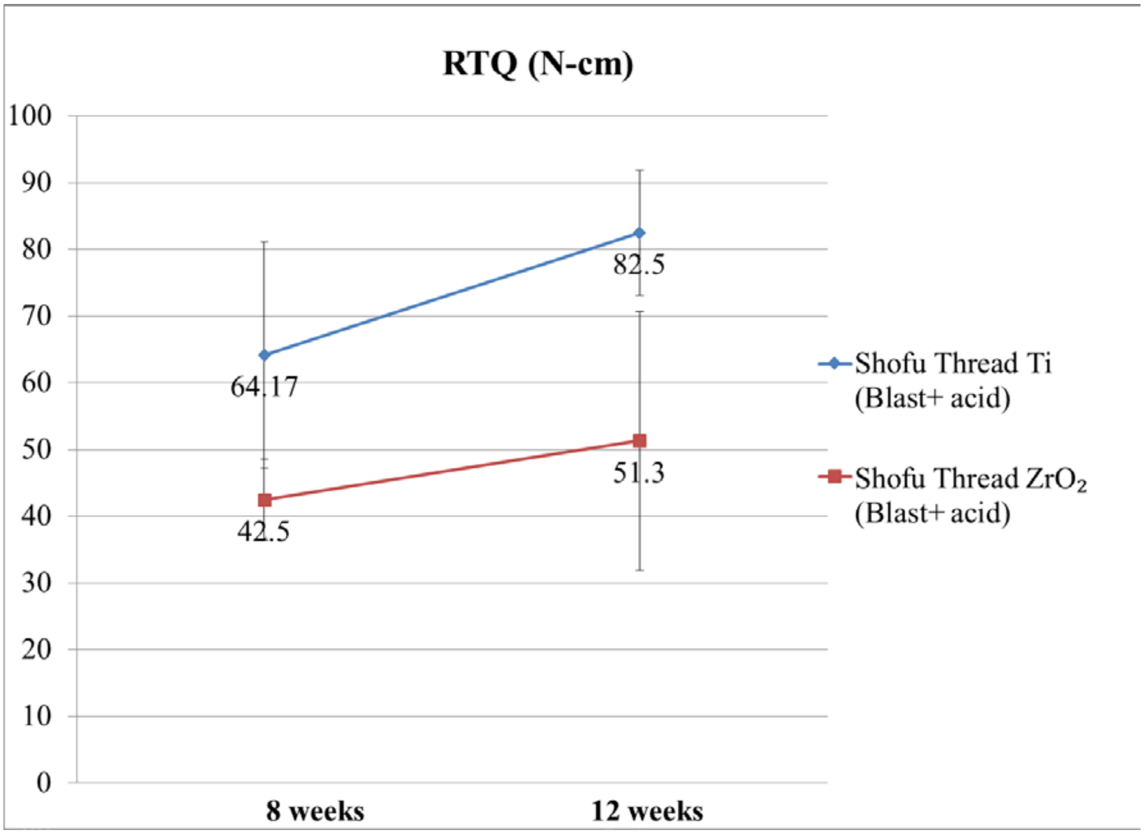
Bone Forming Area



Bone Forming Area (%)	8Weeks				12Weeks		
Shofu Thread Ti (Blast+ acid)	36.85%	35.92%	20.24%		15.99%	28.01%	N/A
Shofu Thread ZrO_2 (Blast+ acid)	56.81%	67.05%	N/A		44.75%	29.84%	45.08%

The bone forming area represents areas of bone that had been remodeled. The decrease of BFA from 8 to 12 weeks seemed to indicate that the remodeling activity decreased during this four week period. The BFA was still higher in zirconia implant groups than in the titanium implant group, and the larger difference between the stiffness of zirconia and that of bone may have contributed to the higher remodeling rate.

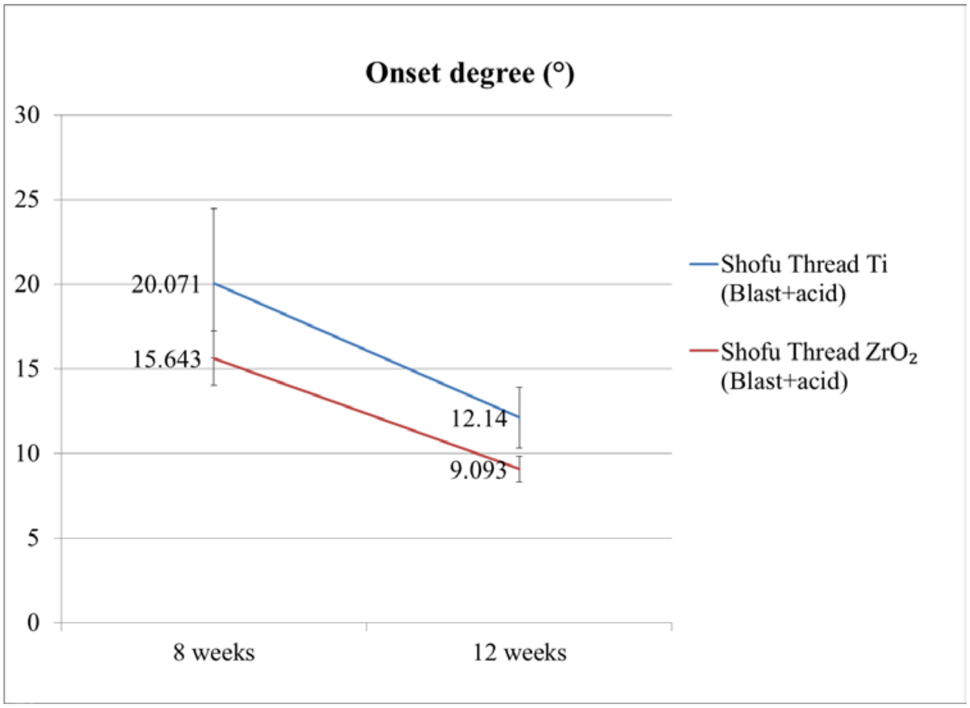
Removal Torque Value



Removal torque (N-cm)	8weeks				12weeks		
Shofu Thread Ti (Blast+ acid)	59	46.5	87		81.5	94.5	71.5
Shofu Thread ZrO_2 (Blast+ acid)	51	38	38.5		78	43.5	32.5

The removal torque increased with time which is within our expectations. The mean removal torque of zirconia implants was lower than titanium implants although there was no statistical significance difference between the groups.

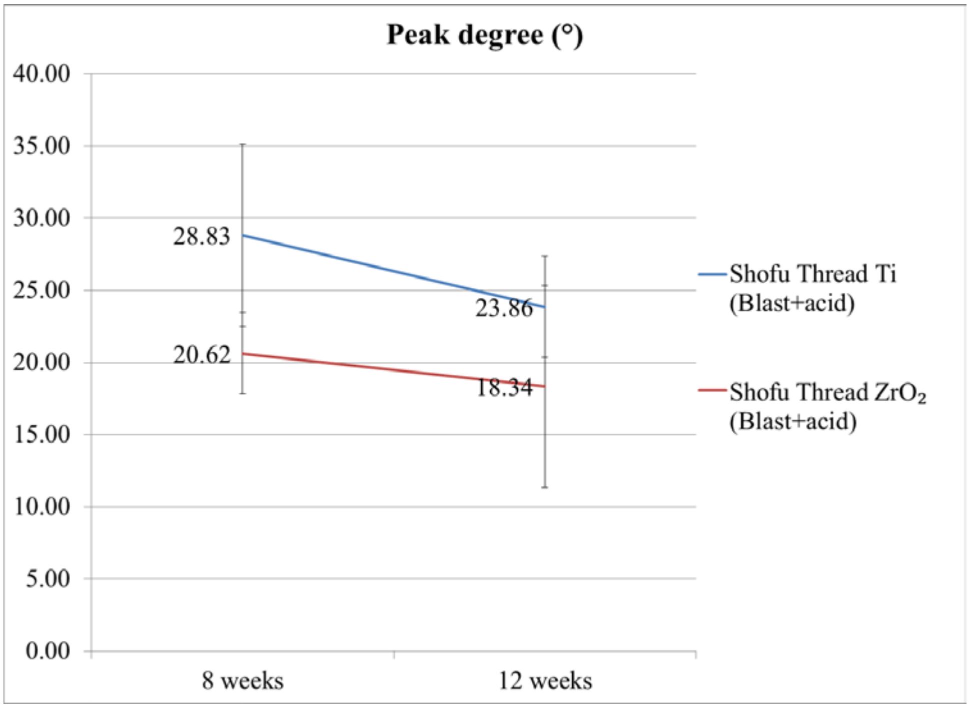
Onset degree



Onset degree (°)	8 weeks	12 weeks
Shofu Thread Ti (Blast+acid)	20.071	12.14
Shofu Thread ZrO_2 (Blast+acid)	15.643	9.093

The onset degree was defined as cross point of slope 1 and slope 2 on removal torque measurement. It decreased from 8 to 12 weeks. This seemed to indicate that the bone-implant interface became more mineralized from 8 to 12 weeks, and that it took less displacement to start the second higher modulus slope. The fact that the mean degrees of onset for zirconia implants was lower than that for titanium implants, which would seem to indicate that the bone-implant interface was more rigid in zirconia implants than in titanium implants. There was no a statistical difference between titanium and zirconia implants at 8 or 12 weeks for onset degree.

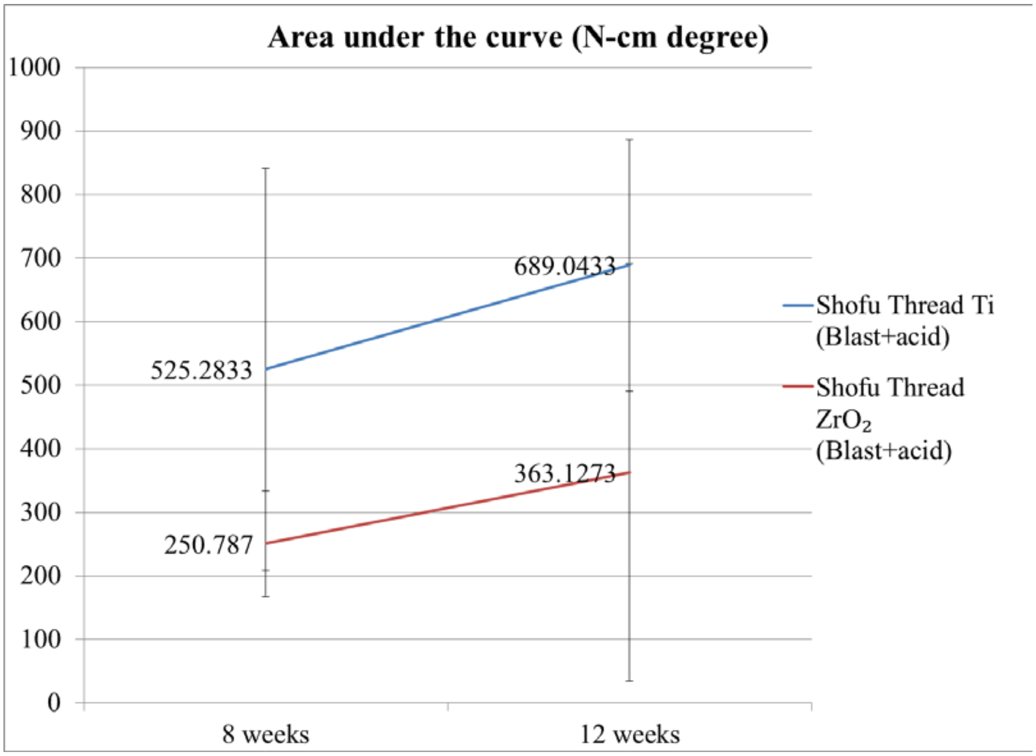
Peak degree



Peak degree (°)	8 weeks	12 weeks
Shofu Thread Ti (Blast+acid)	28.83	23.86
Shofu Thread ZrO_2 (Blast+acid)	20.62	18.34

The peak degree was defined as a degree when implant was completely detached from bone surface and it decreased from 8 to 12 weeks. This seemed to indicate that the bone-implant interface became more mineralized from 8 to 12 weeks and that it took less displacement to cause failure at bone-implant interface. The fact that peak onset degrees of zirconia implants were lower than titanium implants, would seem to indicate that the bone-implant interface was more rigid in zirconia implants than in titanium implants. There was no statistical difference between titanium and zirconia implants at 8 or 12 weeks.

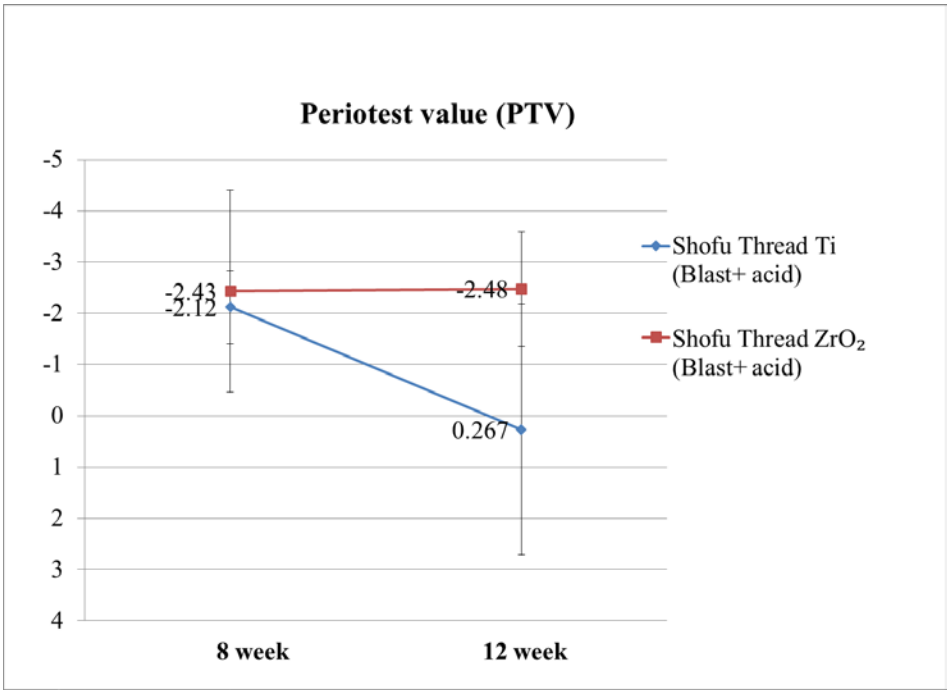
Area under the curve



Area under the curve (N-cm degree)	8 weeks	12 weeks
Shofu Thread Ti (Blast+acid)	525.283	689.043
Shofu Thread ZrO_2 (Blast+acid)	250.787	363.127

The area under the curve increased from 8 to 12 weeks. This indicates that it required more energy to break the bone-implant interface from 8 to 12 weeks. The fact that area under curve for zirconia implants was lower than titanium implant, which seemed to indicate that the bone-implant interface was not as tough for zirconia implants as compared to titanium implants. There was no statistical difference in the area under the curve between the two groups at 8 or 12 weeks.

Periotest Value



Perio test valuse (PTV)	8weeks				12weeks		
Shofu Thread Ti (Blast+ acid)	-2.07	-2.15	-2.13		4.76	-0.45	-1.27
Shofu Thread ZrO_2 (Blast+ acid)	-4.38	-1.72	-1.2		-2.81	-1.65	-2.98

The mean Periotest value increased from 8 to 12 weeks for the titanium implants, but stayed about the same for zirconia implants. Though the results seemed to indicate that there was no change in zirconia implant stability while the titanium implants became less stable, the results should be viewed with caution. Since it was a handheld device, we found large variation in the values depending on the angle and distance of how the operator held the device when making the measurement. Also, it was expected that the physical property of the implant material (zirconia versus titanium) could have a strong impact on the measured damping effect. We therefore did not further pursue the analysis of this set of data. Additionally, the sample size made it difficult to draw any conclusions. Resonance frequency analysis (RFA) was the another option for non-invasive measurements of implant stability. PTV and RFA value were not able to show a positive and direct relationship between bon-implant contact, removal torque value, and clinical implant survival/success rate. Further investigation will be needed to evaluate the PTV, and RFA values to determine the usefulness of these instruments in assessing implant stability/osseointegration.

Summary and Conclusion

In this study, we had evaluated the histomorphometric and biomechanical properties of zirconia and titanium implants (both treated with a sand-blasted and acid-etched surface) manufactured by Shofu Inc. in a canine model. We had to first point out that there is limited statistical power in this study due to the limited number of samples tested. The comparisons between titanium and zirconia implants in many test areas were based on the trends observed from the mean values in each test. The comparisons therefore should be treated as observations, but not as final conclusions. Nonetheless, the trends were consistent: for both biological responses (histological analysis) and biomechanical behaviors (removal torque analysis). In the histological analysis, it was observed that zirconia implants seem to induce a faster and larger bone response surrounding the implants, as indicated by the higher mean BIC, BA, MAR, and BFR. In the removal torque analysis, it was observed that the bone-implant interface seems to be more rigid and brittle in nature as indicated by the lower mean onset angle, peak angle, area under curve and RTQ. Periotest® values (PTV) were not consistent as we expected. Since there was a lot of variability of values of these type of non-destructive implant stability examinations. Further investigation will be needed to determine the predictability and relationship between BIC, removal torque value, Osstell® (RFA) and Periotest® value to non-invasively evaluate the stability (and presumed osseointegration) of dental implants in daily clinical settings. Within the limited number of samples tested in this study, there was no difference between the BIC and RTQ of zirconia and titanium implants after 8 and 12 weeks of implantation, however, it appeared that the bone stimulation around zirconia implant was faster than titanium implants.

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Abstract

Background: Currently titanium implant fixtures are considered as a gold standard because of their biocompatibility and their clinical success rates have been well documented. The esthetic outcome of restorations supported by titanium implants may be compromised if the dark gray color of the implant shows through a thin peri-implant mucosa or if the implant fixture becomes visible following soft tissue recession. Also titanium might cause allergic reactions. For these reasons, zirconia implants have been considered as alternative materials because of their white color, high material properties and biocompatibilities. Still, further investigations are necessary to confirm the in-vivo performance of these implants.

Purpose: The purpose of this study is to determine the histomorphometric and biomechanical properties of zirconia implants manufactured by Shofu Inc. with a sand-blasted and acid-etched surface treatment, compared to that of the titanium implants from the same manufacturer with a sand-blasted and acid-etched surface treatment in a canine model.

Material and Methods: Six beagle dogs (1-2 years old) will be used in this split mouth trial. After 8 weeks following extraction of the second to fourth mandibular premolars, zirconia implants (experimental group) and titanium implants (control group) were placed on the each side of mandible. At 8 weeks and 12 weeks after implant placement, the animals were sacrificed, and implants were removed in block sections, and histological and histomorphometric analyses were measured. Specifically, the bone-implant contact (BIC), bone area (BA), removal torque (RTQ), mineral apposition rate (MAR), bone

forming area (BFA), and Periotest value (PTV) of the two groups were studied and compared.

Results: At 12 weeks post operatively, one Shofu thread type Ti implant were not integrated. Over all failure implant was 0/12 in zirconia group, and 1/12 in titanium group. In 8 weeks samples, only statistical differences were higher BA (p=0.02) in macro threads area and BFA (p=0.02) in zirconia implants group than titanium implants group. In 12 weeks group, zirconia implant group showed higher MAR at 9-10 and 10-11weeks time frame (p=0.02, and 0.04 restectively), and PVT value (p=0.01) than titanium implants group. Removal torque value increased in both titanium and zirconia group with time. Average of removal torque value showed higher in titanium implants than zirconia implants, but the differences were not statistically significant in both 8 weeks (Ti; 64.16±16.93 N-cm, ZrO_2: 42.5±6.01 N-cm : p=0.247) and 12 weeks (Ti; 82.5±9.41 N-cm, ZrO_2: 51.3±19.38 N-cm: p=0.16). In the removal torque analysis, it is observed that the bone-implant interface seems to be of more rigid and brittle in nature as indicated by the lower averaged onset angle, peak angle, area under curve and RTQ.

Conclusion: Zirconia implants group showed higher value of BA with macro thread and BFA in 8weeks and MAR at 9-10, 10-11 weeks period, and PVT in 12 weeks post operatively. Within the limited number of samples tested in this study, there is no difference between the BIC and RTQ of zirconia and titanium implants after 8 and 12 weeks of implantation.

Curriculum Vitae

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