Evaluations of Bone Tissue Integration to Pure and Alloyed Titanium Implants

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ABSTRACT

Purpose: This study was performed for comparisons of tissue integration to commercially pure (CP) and titanium-6-aluminum-4 vanadium (Ti-6-Al-4V) implants using various existing three-dimensional biomechanical and two-dimensional histomorphometrical techniques, and to monitor the loosening torque during in vivo removal torque (RTQ) test with a novel unit not used before in a pilot study in rabbits.

Materials and Methods: The implants were topographically characterized and inserted in femurs and tibiae of five rabbits (in total 40 implants, 20 per group). After 16 weeks, the implant integration was biomechanically evaluated by: (1) resonance frequency test, and (2) peak RTQ test and the graph from the monitoring curve. Biopsies of the implants in situ were processed to undecalcified cut and ground sections followed by light microscopical quantifications. Shear strength calculations were performed.

Results: Significantly higher mean value of RTQ ($p = .01$) and shear strength tests ($p = .03$) were observed for the CP titanium implants compared to Ti-6-Al-4V implants. The monitoring curve from the RTQ test demonstrated no differences in the shape or form that could provide further information about the differences in the implant-to-bone attachment.

Conclusions: The CP titanium implants showed increased RTQ and shear strength values compared to the Ti-6-Al-4V implants. The new tool of monitoring the RTQ curve could not demonstrate differences between the two materials. The exact influence of the implant materials on the surrounding tissues needs to be further investigated.

KEY WORDS: biomechanical test, bone, in vivo, surface topography, titanium, titanium alloy

Biomaterials research in animal models often involves various in vivo biomechanical tests, together with quantitative and qualitative histological evaluations. One of our biomechanical tests, that is, the removal torque (RTQ) test, represents a three-dimensional information about the bone apposition around an implant[1–3] and roughly reflects the shear strength.

Depending on the implant macro design, one specific biomechanical test may be more relevant than another. RTQ measurements on screw-shaped implants are used as an experimental in vivo evaluation or a comparison of, for example, different materials with similar surfaces or various properties, that is, materials, coatings, topographic, or chemical parameters that affect the interfacial bonding.[4–8] Significant differences between implants with different surfaces have been found with RTQ tests at one or several time points in a majority of studies.[4,6–8]

In general, in vivo biomechanical evaluations of animal research samples are performed with various tools that are tissue destructive and may therefore not be used in the clinical situation. There are but a few non-destructive biomechanical tests available, and one existing method that has been applied on animal research[9–11] and in the dental clinic[12,13] is the resonance frequency analysis (RFA). This test reflects the implant stability in the bone bed and is related to the marginal bone height,

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stiffness of the bone, and the length of the transducer, the latter which can be compensated for.[9]

Medical devices made of commercially pure (CP) titanium and titanium-6-aluminum-4 vanadium (Ti-6-Al-4V) are commonly used both in the orthopedic and dental clinic. The choice of Ti-6-Al-4V as a material for orthopedic implants is mostly because of its mechanical and physical properties being superior compared to CP titanium.[14] A suggested disadvantage with the titanium alloy material may be the release of products that increase the ion leakage which may influence the surrounding tissues.[15] Animal studies have demonstrated improved bone anchorage with CP titanium implants compared to Ti-6-Al-4V implants biomechanically and histologically after 6 and 12 months. Aluminum ion leakage was suggested as one possible explanation to the results.[6]

The purpose of the present pilot study was to compare the tissue integration of CP and Ti-6-Al-4V implants by using various existing three-dimensional biomechanical and two-dimensional histomorphometrical techniques, and to monitor the loosening torque during in vivo RTQ test with a novel unit not used before.

MATERIALS AND METHODS

Implants and Surgical Technique

In the present pilot study, a total of 40 screw-shaped implants ($n = 20$ per group) with a total length of 8 mm and a diameter of 3.75 mm were manufactured by turning rods of either CP titanium (grade 1) (Edstraco AB, Stockholm, Sweden) or the Ti-6-Al-4V alloy (grade 5) (Edstraco AB). The top (2 mm) of the implants was square headed, enabling the attachment of a pin for biomechanical tests and had an inner hole with a diameter of 2.0 mm designed to fit the screw that attached the transducer for the RFA to the implants. The implants were ultrasonically degreased in trichlorethylene and rinsed in absolute ethanol (2×) followed by drying and autoclaving.

Topographic surface analyses of three implants from each group were performed with an optical interferometer (MicroXam, PhaseShift, Tucson, AZ, USA). Areas of 310×410 μm were measured. On each implant, three tops, three valleys, and three flanks were analyzed. A Gaussian filter was used with a size of 50×50 μm to separate roughness from waviness. The following surface parameters were evaluated: Sa value = the arithmetic mean of the absolute values of the surface departures from a mean plane within the sampling area; Sds value = density summits, that is, number of peaks per unit area; and Sdr value = the ratio between the developed surface area and a flat reference area.[2,16] A mean value was calculated for each implant group.

Implant insertion was made under aseptic conditions in five adult New Zealand White rabbits.

The rabbits were anesthetized by intramuscular injections of fentanyl and fluanisone (Hypnorm Vet., Janssen, Saunderton, England) at a dose of 0.5 mL/kg body weight and intraperitoneal injections of diazepam (Kabi Pharmacia, Helsingborg, Sweden) at a dose of 2.5 mg per animal. Local anesthesia with 1.0 mL of 5% Xylocaine® (Astra Zeneca, Södertälje, Sweden) was injected into the surgery area. The skin of the rabbits was shaved and carefully washed with a mixture of 2% iodine and 70% ethanol prior to surgery. Analgesic was given postsurgically with a dose of 0.5 mL Temgesic® at a concentration of 0.3 mg/mL (Reckittt and Coleman, Hull, England) subcutaneously. One implant was inserted in each condyle region in femur, and three implants of the same material in each tuberositas tibiae region in both hind legs of the rabbits. A similar number of implants from each implant material were used. The implants in tibia were allowed to penetrate the first cortical layer only. One leg harbored CP titanium implants ($n = 20$) and the other leg received Ti-6-Al-4V implants ($n = 20$). Follow-up time was 16 weeks.

The animals were sacrificed with an intravenous overdose of 10 mL of pentobarbital (100 mg/mL, Apoteksbolaget, Malmö, Sweden). This study was approved by the local animal ethical committee.

Biomechanical Evaluations: RFA and RTQ Test

The implant integration was evaluated biomechanically by two tests:

1. RFA was performed on all implants at the day of sacrifice, that is, 16 weeks. The RFA test is a nondestructive method that enabled measurements of the stability of the implant in the bone bed.[9] A specially designed transducer was attached to the implant with a small screw. The transducer was vibrated by exciting a piezoceramic element. The frequency value, in hertz, is received through a computer.

Prior to sacrifice, the resonance frequency was measured under anesthetics. The resonance frequency in hertz was converted into the implant stability quotient (ISQ) unit with a calibration curve of the used transducer at Osstell, Integration Diagnostics AB, Sävedalen, Sweden.

2. At sacrifice, all implants were subjected to the RTQ test. This test is performed with an electronically controlled equipment involving a strain gauge, and does not allow man-made errors, that is, neither a too rapid nor a too slow torque is applied. The RTQ test itself provides a direct reading of the tissue-to-implant bonding. The torque necessary to loosen the implant from the bone bed, hence a destructive test, is registered and the received value in newton-centimeters is roughly reflecting the interfacial shear strength.[6] The RTQ values (Ncm) may later be converted to shear strength data (N/mm^2)[17] (see the following).

In this study, software was applied and connected to the RTQ equipment that made it possible to monitor each measurement on a screen, where the increasing strength required to loosen the implant from the bone bed was registered and saved as a graph. The graphs were analyzed with respect to shape of the curve, peak value, and changes related to bone type and materials of the implants.

Calibration of the RTQ Equipment. Calibration was performed prior to usage. The setup includes a lever arm with a length of 22 cm that is connected horizontally to the RTQ jig, and a free hanging mass of 0.5006 kg is attached to the outer end of the lever arm. The applied torque is then $0.5006 \times 9.81 \times 22 = 108$ Ncm (the acceleration of free fall). Six measurements were taken, and the mean value of these was 107.5 Ncm. The difference between the applied torque as calculated (108 Ncm) and the measured value 107.5 Ncm, standard deviation 0.8 (range 107–109) was less than ±0.5 Ncm and regarded as acceptable.

Preparation of Specimen and Histomorphometric Evaluation

The implants with surrounding tissue were removed en bloc and immersed in 4% neutral buffered formaldehyde. All samples were processed to be embedded in light-curing resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Preparation of undecalcified cut and ground sections was performed with the EXAKT® (EXAKT Apparatebau GmbH & Co., Norderstedt, Germany) sawing machine and grinding equipment[18] to a thickness of 10 to 15 µm according to internal guidelines at the laboratories of Biomaterials/Handicap Research, Göteborg University, Göteborg, Sweden. The sections were stained in toluidine blue mixed with pyronin G prior to quantitative and qualitative evaluation in light microscope. The computer-based histomorphometry was performed using a Leitz (Ernst Leitz GmbH, Wetzlar, Germany) Microvid® equipment connected to a PC. These measurements were performed directly in the eyepiece of the light microscope using a 10× objective and a zoom of 2.5×. For the bone length, 1.6× objective was used.

The following parameters were measured: (1) bone length (mm) along the threaded part of the implants; (2) percentage of bone area in the inner threaded region in all threads (bone area) and a selection of the three best consecutive threads in the femur samples and three best consecutive threads in the cortical region (area 3 best) from the tibia samples; and (3) percentage of bone area in the outfolded thread region = mirror image area, for the corresponding three best inner threads.[1]

Shear Strength Calculations

The RTQ values (Nmm) from the measurements in vivo and the bone length (mm) measurements performed on the cut and ground sections from each RTQ-loosened implant were used for calculations of the mean shear strength (N/mm^2). The formula: $T/\pi \times d \times rl \times l$ was applied where $T = \text{RTQ}$ (Nmm), $d =$ mean diameter of the implant (3.45 mm), $rl =$ lever arm/radius (1.725 mm), and $l =$ implant length in bone tissue (mm).[17,19]

Statistical Analysis

Calculations of mean values for each implant, followed by computation of group mean number and standard deviation, were carried out for all measurements by using the SPSS 11.5 program.

Wilcoxon signed-rank test was used for paired comparisons within each animal; $p \leq .05$ was considered significant.

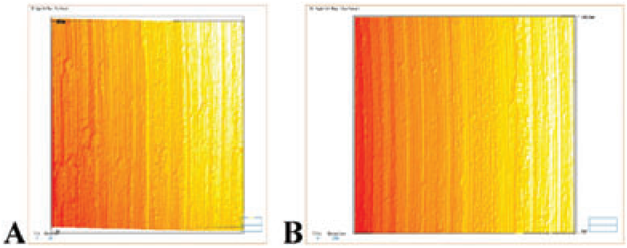

Figure 1 An illustration of the surface topography of the implants. *A*, A flank measured on a commercially pure titanium implant. *B*, A flank measured on a titanium-6-aluminum-4 vanadium implant.

RESULTS

Implants

The surface characterization of the implants demonstrated similar values for the Sa and Sds mean values between the two implant types (Figure 1). The variation between the two groups was highest for the Sdr mean value (Table 1).

RFA

The mean value of resonance frequency at sacrifice demonstrated no significant difference between the CP titanium and the Ti-6-Al-4V implants (Table 2).

RTQ Test

The RTQ test demonstrated a significantly higher mean value for the CP titanium implants compared to the Ti-6-Al-4V samples. (Mean numbers are presented in Figure 2.)

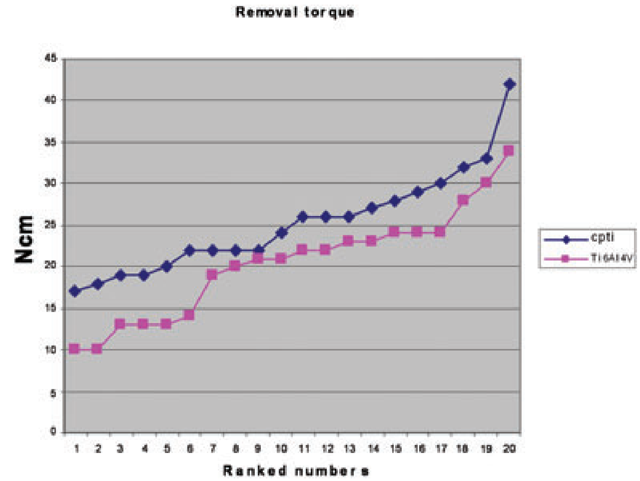

Figure 2 The graph illustrates commercially pure (CP) titanium (cpti) and titanium-6-aluminum-4 vanadium (Ti-6-Al-4V) removal torque (RTQ) data as ranked numbers. This way of presenting the data shows the relation between the materials. Significant differences ($p = .01$) in RTQ data were obtained between CP titanium compared to Ti-6-Al-4V implants after 16 weeks of healing in rabbit bone.

The graphs produced at the measurements had a similar appearance with both implant types although higher peak levels were observed for the CP titanium implants. There was no difference in the qualitative appearance of the graphs from femur implants compared to tibia implants. The increased torque that was applied on the implant was registered in the ascending line of the graph. The graph increased with increased load until the implant loosened from the bone bed at the peak value (Figure 3).

TABLE 1 The Results of the Topographic Surface Analysis Presented as a Mean Value ± Standard Deviation for Each Parameter

	Sa (μm)	Sds (1/μm^2)	Sdr (%)
Commercially pure titanium	0.65 ± 0.46	0.09 ± 0.03	10.3 ± 3.4
Titanium-6-aluminum-4 vanadium	0.53 ± 0.32	0.08 ± 0.05	7.7 ± 5.0

$n = 3$ implants per group, 9 measurements per implant.

TABLE 2 The Mean Values from the Biomechanical Tests and Calculations Are Presented ± Standard Deviation and Range Within Parentheses

Parameter	Commercially pure titanium ($n = 20$)	Titanium-6-aluminum-4 vanadium ($n = 20$)	Statistics
Resonance frequency (implant stability quotient)	68 ± 3 (62–74)	69 ± 3 (65–72)	ns
Removal torque (Ncm)	24 ± 6 (14–39)	19 ± 7 (7–34)	$p = .01$
Shear strength (N/mm^2)	2.9 ± 1.4 (1.4–5.5)	2.3 ± 0.7 (0.8–3.7)	$p = .03$

ns = not significant.

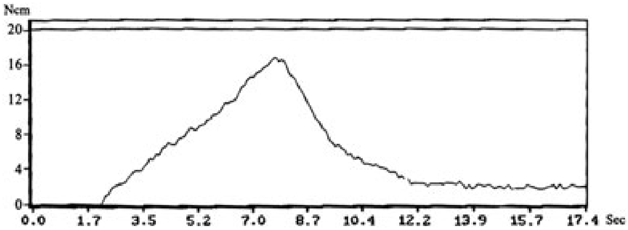

Figure 3 A typical graph obtained during removal torque testing a commercially pure titanium implant. The section of the curve on the left side of the peak value, before the loosening from the bone bed, represents the building up of the torque. The peak value represents the value when the implant releases from the bone bed. The right side illustrating the remaining resistance, for example, could be because of surface irregularities and fractured bone remaining on the implant surface.

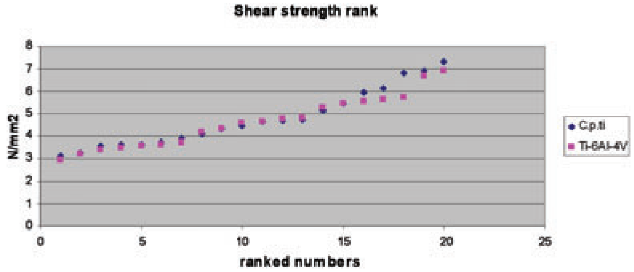

Figure 4 Shear strength rank and data. Significant differences ($p = .03$) in shear strength data (as deduced from removal torque data and length measurements) were obtained between commercially pure (CP) titanium (c.p.ti) compared to titanium-6-aluminum-4 vanadium (Ti-6-Al-4V) implants after 16 weeks of healing in rabbit bone. The graph illustrates the data ranking. The data ranking shows a relation between the data albeit the individual comparisons between the CP titanium and Ti-6-Al-4V implants have been used for statistical analysis ($p = .03$).

Shear Strength Calculations

The results from the shear strength calculations demonstrated a significant higher mean value for the CP titanium implants compared to Ti-6-Al-4V samples (see Table 2).

The data of the shear strength numbers are illustrated in rank form in Figure 4.

Histomorphometry and Qualitative Analysis

The histomorphometric evaluations and comparisons of the various measurements demonstrated no statistically significant difference between the two implant groups. The mean values of the length measurements were higher for the CP titanium implants compared to the Ti-6-Al-4V implants. However, the bone lengths, area, and mirror image measurements demonstrated a tendency toward higher mean values for the Ti-6-Al-4V implants (Table 3).

Histology: Qualitative Description

In general, there seemed to be similar tissue reactions around both materials. No differences could be observed on the light microscopic level (Figure 5).

Femur Samples

Survey inspection of the area toward the “cartilage side” (facing the knee joint region) revealed some very thin trabeculae approaching the implant from this side and spreading out, ending with a thin bone shell formation around the implant. The opposite side of the screws had a thicker bone shell formation covering the surface resembling a “corticalization” on this side. Most samples revealed to be bone tissue integrated with rather thick bone shell coverage also in the apical/marrow region. Higher magnification revealed a mixture of mature and less mature bone with clear demarcation lines/cement lines and a few darker-stained tissue areas inside

TABLE 3 The Results from the Histomorphometry Presented with Mean Values ± Standard Deviation

Parameter	Commercially pure titanium ($n = 20$)	Titanium-6-aluminum-4 vanadium ($n = 20$)	Statistics
Bone length (mm)	4.7 ± 1.3	4.6 ± 1.1	ns
Bone area (%)	69 ± 14	70 ± 10	ns
Bone area 3 best (%)	80 ± 10	82 ± 5	ns
Mirror image (%)	76 ± 14	82 ± 14	ns

ns = not significant.

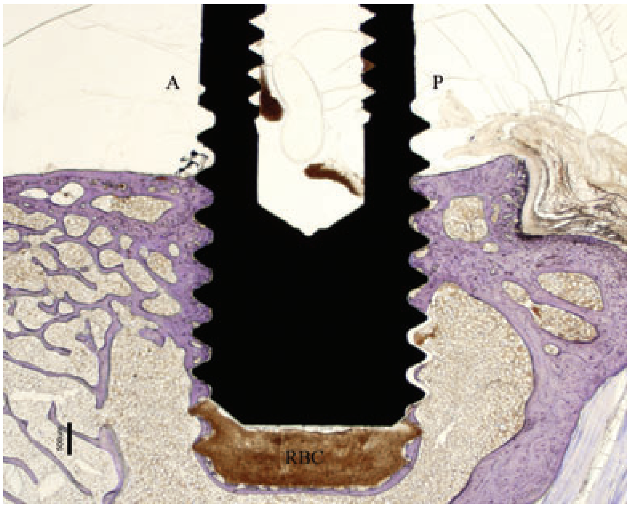

Figure 5 A survey picture of an undecalcified cut-and-ground section from a commercially pure titanium implant inserted in rabbit tibia for 16 weeks. The implant has been exposed to the removal torque (RTQ) test, and the original position of the implant bed can be seen in the lower part of the marrow cavity under the implant (note bone formation around the apical part of the implant). In this part, red blood cells (RBCs) can be observed as a result of the trauma after the RTQ. A = anterior side; P = posterior side.

lighter-stained bone. These areas resembled not fully remodeled bone (young osteocytes could be observed occasionally). Bone formation and remodeling cavities were clearly observed, and in these soft tissue areas macrophages could be revealed in close relation to the implant surfaces.

Tibia Samples

The positions between the CP titanium and the Ti-6-Al-4V implants were similar, that is, the screws were inserted in the same level irrespective of left or right leg.

The anatomical variation between the insertion sites, that is, the proximal, mid, and distal placement of the implants demonstrated differences within the very same animal, not only in original cortical bone thickness but also in geometry. The anterior side revealed more bone trabeculae than the posterior side. The proximally positioned implants also had the greatest amount of trabeculae on the anterior side compared to the posterior side (having a rather thin cortex). The further distal in the tibial tuberositas, the greater thickening of the cortex, but still the anterior side revealed trabeculaes.

DISCUSSION

The results of the present study demonstrated significantly higher mean RTQ and shear strength values for the CP titanium implants compared to Ti-6-Al-4V implants. The graphs from the RTQ test demonstrated differences in peak levels; however, the graphs provided no further information about the differences of the implant-to-bone attachment. There were no differences between the two implant types with respect to the resonance frequency test or histomorphometric evaluations.

The surface analysis revealed that both implant types were minimally rough (ie, Sa < 1 μm),[20] and that the Sa, Sds, and Sdr parameters had mean values with small differences between the two implant groups. As yet it is unknown to what extent a difference of one specific roughness parameter affects the RTQ results. However, Wennerberg and colleagues[5] have demonstrated that a rough implant resulted in increased RTQ values compared to a smooth implant surface.

The results of the present study confirm the results from an earlier study in which CP titanium implants were compared to Ti-6-Al-4V implants. That study showed higher RTQ values for the CP titanium implants after 3 months.[21] In yet another animal study, CP titanium implants were compared to Ti-6-Al-4V implants, with rather similar surface roughness values (Sa, 0.74 and 0.58; Scx, 8.73 and 8.48; Sdr, 1.22 and 1.16). After 1, 6, and 12 months, significantly higher RTQ values were demonstrated for the CP titanium implants. However, histomorphometrical differences were not significant in that study.[6]

The RFA test of the present study demonstrated no significant difference between the two implant groups. The RFA test has been demonstrated to be related to the stiffness of the bone and the height of the transducer. Earlier studies have been able to demonstrate differences in resonance frequency when comparing turned and blasted implants.[9]

The significant difference in mean values with the RTQ test and shear strength calculations, between the CP titanium implants and the alloy implants, indicates that there may be differences in bone attachment at the interface level.

Several studies have demonstrated that the three-dimensional RTQ analysis correlated positively to the two-dimensional bony-contact measurements after 6 to 12 weeks of follow-up time.[1,22] However, because all implants in the present study were exposed to the RTQ test and the interface subsequently had been disrupted, bone-to-implant contact measurements could not be performed. The histomorphometric evaluation of the bone area within the threads demonstrated no difference between the CP titanium and Ti-6-Al-4V implants. A similar bone area result was reported by Johansson and colleagues[6] and Han and colleagues[7] comparing titanium and titanium alloy in rabbit bone for 1, 6, or 12 months with significant differences with the RTQ test.

According to the results in the literature and the present study, the RTQ test (three-dimensional in vivo test) appears to be a more sensitive tool in the evaluation of implants with different surface properties compared to histomorphometry (one central section) but also the resonance frequency test. This has also been demonstrated by Sul and colleagues[23] who reported significant differences with the RTQ test, but not with the resonance frequency test on turned implants with different oxide thicknesses.

In the literature, the curvature of graphs from RTQ tests has been presented by Buser and colleagues[24] and Li and colleagues.[25] A plasma–sprayed implant surface resulted in a steeper curve compared to a sandblasted and a turned surface when biomechanically tested in the bone bed of pigs after 3 months.[23] However, in that study, the angle of the implant rotation was presented in the x-axis, and the torque (Ncm) was shown on the y-axis. In the present study, the elapsed time (seconds) was shown on the x-axis and the applied force (Ncm) on the y-axis. This may be one explanation to differences in the shape of RTQ curves from the various studies.

One factor that has been discussed as a reason for Ti-6-Al-4V implant failure is ionic leakage and corrosion products.[21,26] A porous-coated Ti-6-Al-4V dental implant was compared with a threaded turned CP titanium implant in a dog model. After 18 months, the threaded CP titanium implant demonstrated significantly higher bone length in contact with the implant surface compared to the Ti-6-Al-4V implant.[27] However, that study compared implants with entirely different surface morphologies which may have an impact on the outcome of the study. Ion leakage has been evaluated in a few studies. One study demonstrated that both titanium and titanium alloy implants, inserted for 12 weeks in a similar model as in this paper, revealed an ion leakage of 100 ppm of titanium (outside both CP titanium and Ti-6-Al-4V samples), and 50 ppm of alumina outside the alloy samples only, as measured by secondary ion mass spectrometry (SIMS).[28] Wennerberg and colleagues[29] demonstrated that there were no correlations between increased roughness (CP titanium implants blasted with 25, 75, 250 μm with Al_2O_3 particles) and ion release, measured with SIMS 10 μm from the interface, this with implants in rabbit bone after 12 weeks or 1 year of follow-up. The topography parameter Sa, obtained with an optical interferometer, demonstrated values of 1.27, 1.43, and 2.21 μm in that study.

In a study by Dorr and colleagues,[30] levels of metal ions in the fibrous capsule around human orthopedic implants were found to be 1.5 ppm (Ti), 2.0 ppm (Al), and 0.2 ppm (V) in the fixed implants, and 7.6 ppm Ti, 2.4 ppm Al, and 0.7 ppm V in the implants with osteolysis. The levels were measured with atomic absorbance spectrophotometry. As there are titanium alloy implants in the dental market, it is of importance to perform studies on corrosion and compare CP titanium implants to alloy implants in vivo. In vitro studies indicate that bone-forming cells may be inhibited by corrosion products from Ti-6-Al-4V.[31–33]

In conclusion, the CP titanium implants demonstrated significantly higher biomechanical results, that is, RTQ and shear strength values compared to Ti-6-Al-4V screws after 16 weeks of insertion in rabbit bone. The RTQ graphs had similar shape irrespective of bone type and material although with higher peak levels for the CP titanium. The increased attachment of the bone to the CP titanium implants could not be detected with RFA or light microscopic histomorphometrical bone area and bone length measurements. The exact influence of the implant materials on the surrounding tissues needs to be further investigated.

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